topologically equivalent 5-helix repeats. Importantly, we show that these changes result in the opening and closing of water-filled pathways that reach from either side of the membrane into the proton binding site, which is found at the interface between repeats, half-way through the membrane. More specifically, the transition between the L T, and O states of the functional cycle represents a change from inward to outward to inward-facing conformations. Interestingly, our analysis also shows that the structural changes associated with this alternating-access mechanism differ in the proton-free (L to T) and proton-loaded (T to O) portions of the cycle, a feature that might be shared by other ion-coupled secondary transporters. Finally, we propose a mechanism of strict coupling between transmembrane and periplasmic domains, and thus between proton-import and drug-efflux.

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Physics of Multidrug Efflux through a Biomolecular Complex

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Insertion and release of a solute into and from a vessel comprising biopolymers is a fundamental function. A typical example is found in a multidrug efflux transporter. ''Multidrug efflux'' signifies that solutes such as drug molecules with diverse properties can be handled. In earlier works, we showed that the spatial distribution of the solute-vessel potential of mean force (PMF) induced by the solvent plays imperative roles in the insertion/release process. The PMF can be decomposed into the energetic and entropic components. The entropic component, which originates from the translational displacement of solvent molecules, is rather insensitive to the solute-solvent and vessel inner surface-solvent affinities. This feature is not shared with the energetic component. When the vessel inner surface is neither solvophobic nor solvophilic, the solvents within the vessel cavity and in the bulk offer almost the same environment to any solute, and the energetic component becomes much smaller than the entropic component. Our idea is that the multidrug efflux can be realized if the insertion/release process is accomplished by the entropic component. However, we have recently argued that the entropic release is not feasible as long as the vessel geometry is fixed. Here we consider a model of TolC, a cylindrical vessel possessing an entrance at one end and an exit at the other end for the solute. The spatial distribution of the PMF is calculated by employing the three-dimensional integral equation theory with rigid-body models whose behavior is purely entropic in origin. We show that the entropically inserted solute can be released by a continuous variation of the vessel geometry which forms a time-dependent entropic force continuing to accelerate the solute motion to the exit. Solutes with a wide range of sizes are entropically released using the same vessel-geometry variation.

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Structure and Function of a Phosphorylation-Coupled Saccharide Transporter

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The bacterial phosphotransferase system (PTS) drives unidirectional transport of saccharides across the inner cell membrane and signals the intracellular availability of these sugars. Directionality of transport is accomplished through phosphorylation of the sugar by the soluble protein enzyme IIB (EIIB) while it is bound to the component of the PTS responsible for translocation, the integral membrane protein enzyme IIC (EIIC). The first crystal structure of an EIIC was recently reported[1]. The N,N'-diaceylchitobiose transporter, a homolog of *E. coli* ChbC, was crystallized in a state in which the bound substrate is not solvent accessible from either side of the membrane. In order to further characterize the EIIC conformational changes needed to translocate the sugar molecule across the bilayer and covalently modify the sugar, we have taken several approaches. We cloned and screened 91 ChbC homologs with the goal of solving structures that assume different solvent accessbility to the bound substrate. We found 11 homologs that are suitable for crystallization, 7 of which contain an EIIB domain. Molecular dynamics simulations, as well as mutational studies, were conducted to investigate motions of domains that are responsible for substrate translocation and phosphorylation.

1 Cao, Y.; et al., Crystal Structure of a phosphorylation-coupled saccharide transporter. *Nature***2011**, 473 (7345), 50-54.

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Functional Coupling of a Urate-Anion Exchanger URAT1 and Sodium-Dependent Anion Transporter SMCT2 on a PDZK1 Scaffold; Proposal of "Transportsome" for Urate-Transport

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Transporters utilize electrochemical gradients across membranes to translocate their substrates through the membranes. Many eukaryotic plasma membrane symporters transport substrates in a Na^+-dependent manner by using an electrochemical Na^+ gradient across plasma membranes, while exchangers couple the movement of a substrate against its electrochemical gradient with the movement of another substrate down its electrochemical gradient in the opposite direction. Although the Na^+ gradient is ubiquitous and stable, the electrochemical gradients of substrates for exchangers usually depend on cells and are affected by cellular conditions. The concentrations of intracellular substrates limit the accumulation of extracellular substrates thorough exchangers. To overcome the constraint, exchangers need to couple with other proteins that help accumulation of substrates around exchangers. We have been proposing that such functional coupling of transporters is general and constitutes the functional unit of transport that we call ''transportsome''. Here, we show one of the examples. Proteomics analysis following co-immunoprecipitation revealed that urate-anion exchanger URAT1 and Na^+-dependent anion transporter SMCT2 formed a complex through scaffold protein PDZK1 in the apical membrane of renal proximal tubules. FRET experiment clearly indicated that PDZK1 assembled URAT1 and SMCT2 in living cells. To elucidate functional coupling of the transporters, URAT1 and SMCT2 were overexpressed in insect cells, purified and reconstituted into proteoliposomes in the presence or absence of PDZK1. The initial rate of urate-uptake was increased in the presence of Na^+ when co-reconstituted with PDZK1 while Na^+ did not alter the rate without PDZK1. These results suggest that SMCT2 generate an electrochemical gradient of the substrate which drives the urate-uptake via URAT1 located in close proximity to SMCT2 with PDZK1 scaffold. Taken together, the transporters and the scaffold protein formed Transportsome for urate-transport.

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Experimentally Defined Structural Model of the Human Proton-Coupled Folate Transporter

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Folate cofactors, Vitamin B9, play crucial roles in more than a hundred one-carbon metabolism reactions in mammalian cells. Humans cannot synthesize folates *de novo* and absorption through the diet is the only source of this vitamin. The proton-coupled folate transporter (PCFT) mediates this uptake in the upper small intestine by a pH-dependent process. PCFT also transports folates into the central nervous system. Point mutations in the PCFT gene cause Hereditary Folate Malabsorption (HFM), with associated hematological and neurological defects due to impaired folate transport. Certain solid tumor cell lines express high levels of PCFT mRNA. Importantly, the overall expression of PCFT is limited to only certain tissues in humans. Therefore, PCFT is a molecular target for specific delivery of anti-folate chemotherapeutic agents to tumor cells. Unfortunately, the success rate of anti-folate agents to reach clinical use is very low due to their side-effects. A structural model of PCFT can aid the development of specific anti-folate agents for their PCFT-targeted delivery and thus minimize their side-effects. To optimize and verify an initial structural model of PCFT, we applied a wide array of experimental approaches: solvent accessibility profiling through substituted cysteine accessibility scanning, studying the helix packing, and assaying the functionality of PCFT mutants. We complemented the experimental data with theoretical approaches for structure prediction such as homology modeling/threading, ligand docking and an extensive review of other secondary transporters and folate transport proteins. With these combined approaches, we have developed a structural model of PCFT that accurately reflects our experimental observations. This model forms a basis to understand the impacts of HFM mutations, and to develop folate analogues for folate deficiency intervention. Additionally, it will aid the design of PCFT structure-based anti-folate agents for the treatment of cancer.