inter-residue interactions but it remains largely unknown which interactions are crucial for the signal transduction events that mediate conformational changes during transport. We have inferred such interactions by detecting coevolving pairs of amino-acid positions from aligned ABCC protein sequences using several current methods, which vary in their correction for phylogenetic relatedness, and their assumptions about how amino-acid substitutions occur. We evaluated the performance of these methods by testing their ability to predict side-chain contacts given a 3D structural model. Since the heterogeneity of amino-acid substitution rates generates correlations between positions and thus undermines accurate detection of coevolving pairs, we partitioned pairs into classes based on the rate of amino-acid substitutions at both positions of each pair. We then optimized the performance of the methods in three sequential steps: removal of the most redundant sequences from the alignment to correct for phylogenetic artifacts, logical combination of methods in proportion to their individual performances, and adjustment of the coevolution score of each pair according to the performance associated with that pair's substitution rate class. We verified that performance was improved by these procedures, in comparison to results obtained with each method separately and without partitioning pairs by substitution rate. As expected, many of the detected coevolving pairs bridge consecutive turns in alpha-helices in the structural model. Others, however, link distinct structural elements or domains, and so might transduce signals during the protein's conformational cycle. Intriguingly, some of the positions identified have been implicated in cystic fibrosis.
[NIH-DK51767]

1954-Plat
Mechanism of Sugar Transport by a Phosphoenolpyruvate-Dependent Phosphotransferase
Yu Cao, **Elena J. Levin**, Matthias Quick, Ming Zhou.
Columbia University, New York, NY, USA.
The phosphoenolpyruvate-dependent phosphotranferase system (PTS) is responsible for the uni-directional, phosphorylation-coupled uptake of sugar in bacteria. The multicomponent PTS differs from other carbohydrate uptake systems in that active transport is driven by coupling the translocation of the ligand across the membrane with its concomitant covalent modification to prevent efflux. Crystal structures have been solved previously for the numerous cytoplasmic proteins involved in the PTS pathway, but a complete understanding of the system has until now been hampered by the lack of any structures for the integral membrane component EIIC. The EIICs are a large, diverse family of carbohydrate transporters that selectively bind a cognate sugar, translocate it across the inner membrane, and assist in the phosphorylation reaction. In order to better understand how these functions are carried out, we have solved the 3.3 Å structure of an ortholog of the transporter ChbC. This member of the glucose EIIC superfamily is specific for the uptake of N.N'-diacetylchitobiose, a component of chitin and important food source in a number of pathogens. The structure shows that ChbC possesses a novel fold, and has led us to propose a mechanism for how the transporter couples phosphorylation and transport of its substrate. We are now attempting a variety of functional assays to characterize the sugar selectivity of the transporter, as well as to elucidate the mechanism of phosphorylation and transport.

1955-Plat
Crystal Structure of a Trimeric Sodium/Aspartate Symporter with Protomers in Mixed Outward- and Inward-Facing States
Gregory Verdon, Olga Boudker.
GltPH is a bacterial trimeric sodium-coupled aspartate transporter homologous to human glutamate transporters that catalyze the uptake of the neurotransmitter from the synaptic space. Crystal structures of GltPH in the outward- and inward-facing conformations suggest that a ''transport'' domain carries ions and aspartate across the membrane by sliding along an immobile ''trimerization'' domain. To explore the range of motions of the transport domain, we have designed pairs of cysteine mutations, which upon cross-linking capture the transport domain in distinct positions relative to the membrane and the trimerization regions. Remarkably, cross-linking results suggest that the transport domain is able to move significantly further towards the cytoplasm compared to what could be inferred from the previously determined structure of the inward-facing state. To further understand the structural changes in the transporter, we have crystallized and determined a low-resolution structure of one of these cross-linked mutants. Strikingly, we found that the cross-link broke and that the three protomers relaxed to an assortment of inward- and outward-facing conformations. Two monomers show conformations nearly identical to that of the published inward-facing state. The third protomer assumes an outward-facing conformation. Remarkably, this position of the transport domain is significantly more inward compared to the previously reported outward-facing conformation. Combined, our data suggest a highly dynamic nature of the transport domain, which explores a range of orientations relative to the trimerization domain. Furthermore, we provide the structural evidence supporting the hypothesis that the three protomers function independently within the trimeric transporter. Finally, because the two inward-facing protomers are not constrained by cross-links, we conclude that the observed position of the transport domain likely corresponds to an energetic minimum.

1956-Plat
Enhanced Substrate Release from a TRAP Transporter Binding Protein by Remote Modulation of its Intrinsic Conformational Dynamics
Fabrizio Marinelli[1], Sonja I. Kuhlmann[1], Ernst Grell[1], Ralf Bienert[2], Hans-Jörg Kunte[2], Christine Ziegler[1], José D. Faraldo-Gómez[1].
[1]Max Planck Institute of Biophysics, Frankfurt am Main, Germany, [2]Federal Institute for Materials Research and Testing, Berlin, Germany.
The tripartite ATP-independent periplasmic (TRAP) transporter TeaABC enables *Halomonas elongata* to uptake ectoine, a compatible solute protective under osmotic stress. TeaABC consists of three proteins: the Na^+-coupled transporter TeaC; the transmembrane protein TeaB; and the periplasmic binding-protein TeaA. Like the binding proteins in ABC transporters, TeaA contains two globular domains, in-between which the substrate binds with high affinity. This notwithstanding, TeaA must somehow release ectoine upon interaction with TeaC. By analogy with similar transport systems, it is plausible that release is facilitated by a conformational change in TeaA, induced by TeaC. However, direct evidence and structural detail of such a mechanism are still unknown.
Here, we use experimental and theoretical methods to demonstrate that modulation of the conformational dynamics of TeaA, as may occur by association with TeaC, results in an enhanced propensity for ectoine release. First, a novel crystal structure of apo TeaA reveals a pronounced domain rearrangement and opening of the binding cleft, relative to the known substrate-bound structure. Extensive molecular simulations and free-energy calculations are then employed to elucidate the mechanism by which the conformational dynamics of TeaA is modulated not only by ectoine binding and release, but also by structural elements remote from the binding cleft. This insight allows us to design a triple mutation whose influence on the dynamics of the protein may resemble that of the transporter domain TeaC, while not directly affecting the binding site. Calorimetric studies of mutant and wild-type TeaA demonstrate how this conformational modulation results in a reduction of its substrate affinity. Altogether, this data provides compelling support for the view that ectoine release from TeaA onto TeaC may be induced by the latter through a mechanism of conformational modulation of the former.

1957-Plat
Conformational Space and Dynamics of Sodium-Coupled Symporters
Oliver Beckstein, Mark S.P. Sansom.
University of Oxford, Oxford, United Kingdom.
Secondary transporters utilize the free energy stored in the sodium gradient to move a solute against a concentration gradient. The transport process involves a sequence of conformational changes that exposes the substrate and ion binding sites alternatively to the extracellular and the intracellular compartment. At least three conformational states are required for this "alternating access model": An outward facing open state, connecting ion and substrate binding site to the outside; an occluded state where ion and substrate are buried inside the protein; and an inward facing open state that allows egress of substrate into the cytosol. We recently published a study of these three crystallographically defined states in the prokaryotic nucleobase:sodium symporter Mhp1 [1], which has a similar fold to many other transporters such as LeuT, vSGLT or BetB. We showed that alternating access can be understood as a sequence of multiple gating events. Here we present long, micro-second molecular dynamics simulations that further explore the conformational space available to the gating elements. They reveal that the two "thin gates", formed by the ends of helices TM5 and TM10 move on the 100 ns time scale. The state of the thin gates is coupled to the "thick gate" (formed by a 30 degree rotation of the "hash" domain relative to the "bundle"). The extracellular thin gate can only open if the thick gate is in the outward facing conformation, a synchronization necessary for the alternating access model. The protein also transitions spontaneously into a fourth "inward-facing occluded" state. Comparison to simulations of LeuT and vSGLT indicates that the gating elements in those transporters can function in similar manner to Mhp1. Our results suggest a structural model of the transport cycle in sodium-coupled symporters.
[1] T. Shimamura et al., Science, 328 (2010) 470-473.