ion-releasing state and the modeled ion-binding state, confirming that it is more difficult to release the substrate in the presence of the Na^+ ion in its binding site.
Furthermore, local transition of the Na^+ binding site from an ion-releasing state to an ion-binding state in our constrained simulation induced significantly global conformational change in the protein, specifically, partial opening of the periplasmic side and closing of the cytoplasmic side, thus, capturing for the first time large-scale conformational changes between the inward-facing and outward-facing states in the transport cycle of this secondary transporter.

3579-Pos
Modeling of the Inward-Facing State of LeuT and Dynamics of the Outward-To-Inward Transition
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Leucine transporter (LeuT) is a bacterial aminoacid transporter belonging to the neurotransmitter:sodium symporter family. An 'alternating-access model'is proposed for LeuT function, wherein the transporter alternates between outward-facing and inward-facing states. While multiple crystal structures of LeuT bound with substrate and inhibitors have been reported, only the outward-facing state is known. The inward facing state, and the dynamics of the outward-to-inward transition remain undescribed.
Several transporters from different families report a structural fold similar to the basic 'LeuT fold'indicating the significance of this fold in transporter function. These structures include some in the inward-facing state. Exploiting this information, we have generated a model for the inward-facing state of LeuT. Since the inward-facing structure employed for modeling had very low sequence similarity, and moderate structural similarity to LeuT, a combination of several techniques was required for model generation. A detailed modeling approach was adopted, including sequence- and structure-based approaches, combined with molecular dynamics techniques, such as targeted MD. The final model retains the secondary structural features of LeuT and the substrate/ion binding sites, while adopting an inward-facing state.
We have also employed this model to study the dynamics of outward-to-inward transition of LeuT. The behavior of bound substrate and ions during this transition was recorded and shows interesting features relevant to the transport mechanism. Water permeation was monitored with the progress in transition. The main structural elements of LeuT involved in this transition are described. This study thus presents a model of the inward-facing state of LeuT and a description of a possibly general transport mechanism in transporters adopting the LeuT fold.

3580-Pos
Molecular Determinants of the Stoichiometry of Transport in GlpT
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The glycerol-3-phosphate/phosphate antiporter GlpT belongs to the Major facilitator superfamily (MFS), the largest group of secondary active membrane transporters. Stoichiometry of transport in organophosphate/phosphate antiporters has been a long-standing question. Experimental studies performed on hexose-6-phosphate transporter (UhpT), a close homolog of GlpT, suggested that the exchange stoichiometry might be regulated by pH in these antiporters. The crystal structure of GlpT, although devoid of a bound substrate, seemed to contradict with ''variable stoichiometry'' hypothesis, featuring a ''single'' putative binding site with two arginines (R45 and R269). The ''putative'' binding site also involves a histidine residue (H165) whose titration state have been suggested to play a key role in transport. We have previously identified one of the arginines (R45) as the binding site residue, but our simulations showed no indication of binding to the other arginine (R269). In order to examine the capacity of GlpT for binding two substrates simultaneously, and to investigate the molecular basis of the ''variable stoichiometry'' model, we have performed an exhaustive set of MD simulations in which binding of a second substrate to GlpT was simulated. The simulations, performed at different titration states of the substrates (Pi & G3P) and of H165, indicate that the GlpT binding site can, indeed, accommodate two substrates simultaneously upon protonation of H165 and R269 is the preferential binding residue for the second substrate. Moreover, combining of the trajectories of MD simulations with pKa calculations based on continuum electrostatics, we also analyzed the substrate-induced changes of the titration state in the binding site. Our results further indicate that H165 might act as a ''pH sensor / stoichiometric switch'' in addition to coordinating the substrate. Our findings might represent a general mechanism for transporters with ''variable stoichiometry''.

3581-Pos
Conformational Changes in the ApcT Amino Acid Transporter: Monte Carlo Normal Mode Following
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Amino acid/polyamine/organocation (APC) transporters belong to a large family (~250) of secondary transport proteins that catalyze bilayer translocation of a broad range of substrates. Monte Carlo Normal Mode Following [Miloshevsky & Jordan, *Structure* **14**, 1241 (2006); **15**, 1654 (2007)] is used to explore possible conformational change mechanisms in a proton-dependent APC transporter, ApcT, a bacterial homologue from *Methanocaldococcus jannaschii* [Shaffer *et al.*, *Science* **325**, 1010 (2009)]. ApcT was captured in an inward-facing apo state. Gating is initiated by global counter-torsions of the intracellular and extracellular domains of ApcT around the pore axis, with the extracellular half rotating clockwise and the intracellular half anticlockwise, and vice versa. The domain motions are highly concerted and cooperative. The stationary plane relative to which counter-torsion occurs passes through the center of ApcT parallel to the membrane. Intracellularly, overall rotation of the peripheral helices (TM7, TM5, TM8, TM3, TM4, TM9, TM10, TM11 and TM12) reconfigures TM6a significantly and TM1b slightly. These helices alternately approach and separate from the opposed peripheral TM10 and TM11, affecting the intracellular mouth. TM6a and TM1b move toward the protein's perimeter and become buried inside the protein. Loops and small helices on the intracellular surface of ApcT undergo large-scale rotations. Extracellular motion is similar. Overall peripheral helix rotation affects TM1a significantly and TM6b slightly, displacing them from and collapsing them onto TM8 and TM5. TM8 and TM5 alternately undergo large-scale bending near their midpoints. Normal mode following along the lowest-frequency eigenvector(s) reveals details of the gating transition in the ApcT transporter.

3582-Pos
The Allosteric Role of Ion Binding in the Functional Mechanisms of Transporters With LeuT Fold
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Recent crystallographic studies revealed that five transporter families without much sequence similarities among them have similar structure folds to LeuT, a bacterial neurotransmitter:sodium symporter (NSS). The LeuT fold is characterized by an internal two-fold structural pseudosymmetry. Interestingly, the transport cycle of at least some members of each of these families is dependent on a sodium gradient across the membrane. Remarkably, the role of sodium is mimicked by a proton in others. We report our computational findings focusing on the LeuT conformations with various combinations of bound substrate and ions, performed in the context of on-going collaborative studies utilizing electron paramagnetic resonance (EPR) spectroscopy and single-molecule fluorescence (smFRET) to identify dynamic details of the mechanism. The resulting mechanistic implications from the study of LeuT are generalized to two other transporter families, the sodium:solute symporter (SSS) and amino acid-polyamine-organocation (APC) transporter, using comparative molecular dynamics simulations. These comparative studies lead to the proposal of a set of common structure-function-dynamic elements recognizable in the conformational transitions of the transporters with LeuT-fold.

Membrane Domains & Lipid Dynamics

3583-Pos
Neuroligin-1 Oligomerization Induces Cell Morphology Changes Via Lipid Domain Nucleation
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Thus far, neuroligin-1 (Nlg-1) has been known as a post-synaptic adhesion-signaling membrane protein involved in initiating synaptic contact, and in triggering presynaptic differentiation in a neurexin-expressing axon. Here, we are reporting that nlg-1 might also play a role in neuron morphology changes. Indeed, when nlg-1 was coexpressed in HEK-293 with psd-95, a scaffolding protein which binds nlg-1 PDZ domain, we have observed extensive cell morphology changes. Co-transfected cells exhibited long expansions resembling dendritic branches, as well as a significant increase in cell surface area. However, nlg-1 dimerization mutant did not lead to any major changes in morphology suggesting that nlg-1 multimerization was required.