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.

**3263-Plat**

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

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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 the mammalian excitatory amino acid transporter EAAC1 (vSGLT), the sodium-sulfate symporter VSLT, 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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**3266-Plat**

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

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Glutamate transporters regulate synaptic concentrations of L-glutamate to prevent excitotoxicity in nerve cells. Current crystal structures of GltPh, an asexual 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 dithiothreitol. Once treated with the oxidizing agent copper(II)(1,10-phenanthroline)3, however, activity ceased. Importantly, this inhibition was potentiated under conditions expected to promote the inward-facung 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 repeat 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.

**3267-Plat**

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

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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 GlmR 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 support the model comprising the extracellular gate of glutamate transporters, and that Na+ binding opens and stabilizes the extracellular protein thereby allowing for amino acid substrate binding.