combines stepwise targeted motion and long, unrestrained equilibrations. The structural inferences regarding intermediate steps in the modeled translocation path agree well with those from MP-MD and sTMD computations, including the observation of intermediate trimer asymmetry (average monomer- monomer RMSD<4Å), relative domain movements, and protein-protein interface changes. A new finding is that the TM3-4 loop, previously suggested to play an essential role in GltPh’s substrate transport, undergoes remarkable changes in both conformational and dynamic properties during transition from the outward- to inward-facing end states. Some structural differences between results from the present path calculation and findings from MP-MD are identified in the TM1-TM5 and HP1/2,TM7 region (<5Å), TM3 (<3Å) and TM3-4 loop (<8Å). Together, these computational modeling studies have produced specific predictions amenable to experimental testing, e.g. with FRET/ EPR and cross-linking experiments, in the form of predicted residue-specific proximity and accessibility along the translocation path.

1025-Plat
A Sodium-Sensitive Salt Bridge in the Na⁺/H⁺ Antiporter NhaA
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The transmembrane protein NhaA from Escherichia coli is a prototypical sodium/proton antiporter. It enables the bacterium to grow under high salt conditions when homologous proteins in eukaryotes are involved in pH and cell volume regulation. A number of acidic and basic residues have been shown to be essential for the transport of one sodium ion for two protons but the mechanistic details of their involvement have not been fully determined. In particular, a highly conserved lysine residue (Lys300) near the center of the membrane had so far been only given a possible indirect role in the transport mechanism. We present a new atomic resolution structure of the inward facing conformation that shows a novel salt bridge between Lys300 and the conserved Asp163. Microsecond molecular dynamics simulations indicate that the salt bridge is sensitive to the presence of a sodium ion that spontaneously binds to the conserved aspartate residue 164. The simulations show how binding of sodium ion can be coupled to a structural change which might trigger a conformational change to an outward facing conformation. Taken together, the structural and simulation data generate a new hypothesis for how Lys300 could be directly involved in proton transport.

1026-Plat
Identification of Amprenavir, Quinidine and Loperamide Kinetic Parameters for P-gp Transporter in Caco-2 Confluent Cell Monolayer
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P-glycoprotein (P-gp) is a member of the ATP binding cassette (ABC) family of proteins that has been extensively studied due to its ability of multidrug resistance and for causing clinically important drug-drug interaction (DDI). Structural knowledge and functional knowledge of transport kinetics in physiological relevant system have been intensively studied for a molecular understanding of P-gp activity. Using the mass action kinetic model without imposing the steady-state assumptions, previous studies have identified the kinetic parameters for a series of P-gp substrates in MDCKII-hMDR1 confluent cell monolayer. However, little is known whether this model can be extended to other cell lines to study P-gp. Here, we applied our model to another widely-used P-gp expressing cell line, human colon adenocarcinoma (Caco-2), and successfully fitted the elementary rate constants of P-gp for substrates amprenavir, quinidine and loperamide as well as P-gp surface density. Furthermore, the fitted rate constants of above drugs in Caco-2 cells are similar to those in MDCKII-hMDR1 cells. Our results suggest that the mass action kinetic model can identify P-gp rate constants in human cell line, Caco-2 cells and that this model can be used to predict and characterize P-gp pharmacokinetics in vivo.