Lactose permease (LaY) has become a model system for monitoring substrate transport across the lipid bilayer. As a result of considerable experimental and structural biology, the understanding of substrate specificity and affinity, as well as a mechanism for symport have been postulated. We have now monitored LaY structural dynamics in a lipid environment for 10 microseconds by using molecular dynamics simulations of a recent mutant LaY crystal structure trapped in a novel occluded state with bound high-affinity substrate. On this timescale, the sugar molecule exits the protein and re-enters. Therefore, the accompanying dynamics provide important clues regarding substrate specificity. In particular, Phc27 and neighboring lipid molecules assist in directing the sugar molecule to its binding position. In addition, simulations of substrates with different binding affinities enabled characterization of the structural framework governing substrate affinity in LaY.

1548-Pos Board B500

Defining the Conformational States in an Mfs Transporter, FucP

Joon Lee, Philip C. Biggin.

Department of Biochemistry, University of Oxford, Oxford, United Kingdom.

The plethora of X-ray crystal structures in the Major Facilitator Superfamily has provided a series of snapshots that define the alternating access mechanism for ligand transport. A sticking point, however, is that there is not yet a series of conformational states observed from one organism to support the proposed mechanism of transport. This is a problem that Molecular Dynamics (MD), using atomistic simulation, is ideally suited for to help improve the overall working hypotheses of how conformational changes in transmembrane helices occur during ligand transport.

FucP is the only X-ray crystal structure in an outward facing conformation and it is a L-fucose/H+ symporter, much like LaY. The resting state for this protein is thought to be accessible to the periplasm (outward facing) and the protonation of E135 in the central, solvent-accessible cavity along with the binding of the transported ligand, L-fucose is what destabilizes the conformation, causing a movement to the inward-open state, allowing ligand transport into the cytoplasm.

Using MD simulation, the conformational changes FucP undergoes on ligand binding can be characterized and related to the specific roles of each of the 12 transmembrane helices. In particular helices 1 and 4 are believed to be pivotal to proton translocation via residues D46 and E135 [1], with E135 also involved in L-fucose binding. On protonation, the interaction between E135 and Y365 (helix 10) is destabilized and this is investigated using both biased and unbiased MD simulations to better define the degree to which the protein is ‘open’.


1549-Pos Board B500

The Human Proton-Coupled Folate Transporter: Determination of Conformation and Identification of the Folate-Binding Pocket

Swapnaeta Date, Cheng-Yen Charles Chen, Yudong Chen, Michaela Jansen. Cell Physiology and Molecular Biophysics, Texas Tech University Health Sciences Center, Lubbock, TX, USA.

Folate cofactors play crucial roles in hundreds of reactions in cells including DNA and protein synthesis. The human proton-coupled folate transporter (PCFT) is the only means of absorption of dietary folates in humans. PCFT expression and function is associated with many disorders including hereditary folate malabsorption, neural tube defects, Down syndrome, cancer, heart diseases, Alzheimer’s and Parkinson’s disease. Upregulation of PCFT expression in tumor cells is of significant consideration for development of PCFT-targeted chemotherapeutic agents. However, much is not known about the structure and function of PCFT, which contributes to the low clinical success rate of folate-based agents. To address this gap in the knowledge we performed extensive Cys-mutation studies. We analyzed 40 residues towards the extracellular face of PCFT, 35 positions towards the cytoplasmic face of PCFT and 28 positions along the proposed folate-binding pocket of PCFT. Based on the accessibility studies of the extracellular face of PCFT we determined loop-helix boundaries of this face and identified the glycerol-3-phosphate transporter (PDB#1PW4) and tripeptide-proton symporter (PDB#4AP5) as the best templates for modeling PCFT. Based on the accessibility studies of the cytoplasmic face of PCFT, we identified loop-helix boundaries of this face. Here we show that our accessibility studies support the hypothesis that PCFT is present predominantly in an inward-open conformation in the absence of substrate (pH 7.5 and no folate acid). We also show that the folate-binding pocket of PCFT is formed by residues present in PCFT transmembrane helices I, IV, V, X and XI. Our results are of high significance in understanding the details of folate-homeostasis mechanisms and in design of PCFT-targeted therapeutic and diagnostic agents.