Simulating membrane-bound polypeptides

Using molecular dynamics (MD) to explore the conformational dynamics of sufficiently large polypeptides often requires an impractical length of computational time in order to adequately sample transitions of interest. This is due to the relative infrequency of conformational changes over timescales on the order of femtoseconds to microseconds. Thus, sampling a significant number of the transitions between distinct states of a given protein presents itself as a challenging computational problem. A variety of techniques, such as dynamic importance sampling (DIMS) MD[1], have been developed to overcome this limitation and produce physically plausible trajectories between putative metastable states[2][3][4][5]. However, these methods were tested on soluble proteins and not membrane-bound proteins, leaving open the question of their performance on the latter. We employed a variety of these path generating and sampling methods on the inward-facing to outward-facing transition of the membrane-bound benzylhydantoin symporter Mhp1, as well as our Path Similarity Analysis[6] to directly compare the generated transitions. In addition, we examined whether Mhp1 transitions simulated with an implicit membrane model differ significantly from transitions simulated with a membrane-less generalized Born implicit model.

Hydantoin permease in Microbacterium liquefaciens

The hydantoin permease Mhp1 from Microbacterium liquefaciens is a nucleobase-sodium symporter, a member of the NCS1 family, and it shares a five-helix inverted repeat architecture with other members of the superfamily of LeuT-like transporters. It's postulated that Mhp1 behaves according to the alternating access model of transport, allowing it to both conserve the free energy stored in the ionic gradient and couple it to the movement of solutes across the cell membrane.

This alternating mechanism manifests as the movement of regions of the protein, dubbed "gates," toward and away from relatively stable "bundle" domain. The so-called “thick gate” regulates the passage through the centre of the membrane. In Mhp1 it consists of the hash motif (formed by helices TM3, TM4 and their inverted-repeat counterparts TM8 and TM9; see Fig. 1A, C) that can rotate by about 30° on an axis parallel to TM3 relative to the four-helix “bundle” (TM1, TM2 and TM6, TM7). The resulting large conformational change switches Mhp1 from an outward facing conformation to an inward facing one. “Thin gates” are formed by the N-termini of the pseudo-symmetry related helices TM5 and TM10 and the linker to each preceding helix. The extracellular (EC) thin gate (TM10; Fig. 1B) governs access to the substrate binding site from the periplasmic medium while the intracellular (IC; Fig. 1D) gate fulfills the symmetrical role of controlling the pathway to the cytosol.

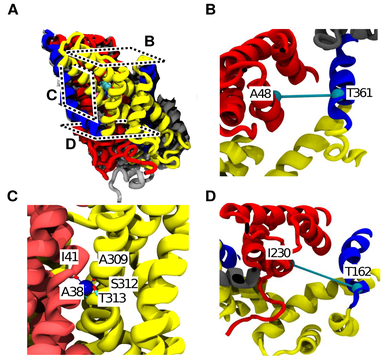


Figure 1. The three gates in Mhp1. **A**: Mhp1 in the membrane. The hash motif (helices 3, 4, 8, 9) is shown in yellow, the bundle (helices1, 2, 6, 7) in red, flexible (thin gate) helices 5 and 10 in blue, and C-terminal helices 11 and 12 in gray. The views on the gates (B–D)are indicated by broken rectangles. **B**: extracellular thin gate (formed by TM10). **C**: thick gate, quantified by the distance across the Na_2 sodium binding site. **D**: intracellular

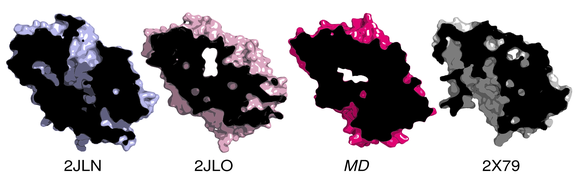


Figure 2. Conformational states of Mhp1. Cuts through a plane perpendicular to the membrane reveal the ligand binding cavity inside the transporter. The sequence of conformational changes corresponds to the alternating access model where access to the binding siteis switched from the extracellular (top) to the intracellular (bottom) side. An occluded state occurs for the outward facing occluded conformation (crystal structure 2JLO) and is observed in simulations of the inward facing conformation (MD). 2JLN: crystal structure ofthe outward facing open state.

Sampling and simulation methods

Dynamic Importance Sampling (DIMS)[1]

The DIMS algorithm, implemented in the CHARMM program, is a biased molecular dynamics sampling methodology. Every step taken towards a target structure in configuration space is immediately accepted by DIMS. For every step taken away from a target structure, DIMS will accept this step with some probability determined by a soft-ratcheting parameter (figure 4). This biasing allows DIMS simulations to avoid becoming trapped in metastable states for long periods of time while also promoting the physically plausible sampling of transition paths.
The DIMS simulations presented here employ the generalized Born model with a smoothed switching function (GBSW) to implicitly model the solvent and membrane around Mhp1. The GBSW accomplishes this by approximating the linearized Poisson-Boltzmann equation and applying a smoothing function at the dielectric boundary of the membrane.

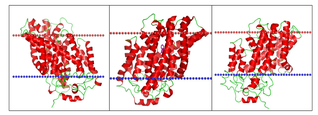
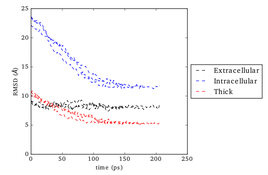


Figure 3. Membrane-oriented structures of Mhp1[7]: Outward-facing (left): Occluded (center); Inward-facing (right). All transitions were generated using these structures.

Figure 4. The acceptance probability of a timestep as a function of RMSD-to-target for three values of the soft-ratcheting parameter



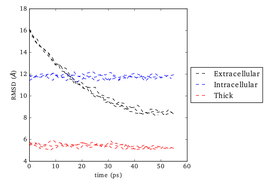


Figure 5. Gate order parameter time-series for inward-to-occluded (top) and outward-to-occluded transitions generated using DIMS

Maxwell Demon discrete Molecular Dynamics (MDdMD)[2]

Flexible enough to function with any available resolution, MDdMD biases a basic discrete molecular dynamics algorithm using a Maxwell-Demon engine, which appropriates information from the deformation modes of a protein to generate physiologically plausible transitions.

Figure 6. Gate order parameter time-series for inward-to-occluded (top) and outward-to-occluded transitions generated using MDdMD

GOdMD[3]

This method – designed by the same researchers (Sfriso et. al) who released MDdMD – can be thought of as a leaner, less-detailed version of its predecessor. It is, however, expanded in the sense that it contains what the creators of the algorithm refer to as, “a novel multiple-well Go-like scheme.”

Figure 7. Gate order parameter time-series for inward-to-occluded (top) and outward-to-occluded transitions generated using GOdMD

PATH-ENM[4]

PATH-ENM is a mixed elastic network model (MENM) whose network energy surface is produced by the combination of the partition functions of the potential energy landscapes for both initial and target configurations.

Figure 8. Gate order parameter time-series for inward-to-occluded (top) and outward-to-occluded transitions generated using PATH-ENM

Sampling and simulation methods (continued)

Interpolated-ENM (iENM)[5]

This MENM was designed to find an exact solution for the saddle points of the double-well potential energy landscape constructed from the ENM potentials of the initial and target configurations of a protein.

Figure 9. Gate order parameter time-series for inward-to-occluded (top) and outward-to-occluded transitions generated using iENM

ANMPathway[6]

ANMPathway, like the previously mentioned ENMs, produces an energy surface by combining the potentials of initial and target configurations. However, ANMPathway further seeks out a minimum energy configuration on the 3n-dimensional cusp, where the edges of the two basins meet. Transitions are then generated via steepest descent minimization on either side of the cusp.

Figure 10. Gate order parameter time-series for inward-to-occluded (top) and outward-to-occluded transitions generated using ANM-Pathway

Path Similarity Analysis

Summary

Acknowledgements & References