Weighted Ensemble Simulation of Alternating Access in Sugar Symporter vSGLT

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I. Introduction

In the field of computational biology, homology modeling provides a simple approach to the determination of tertiary protein structure. This method is based on research suggesting that if the primary sequences of two proteins share an approximately forty percent or higher sequence affinity, then the structure of one can be used to predict that of the other. Sequence affinities under forty percent exist in what is commonly called the "twilight zone (*4*)." Although protein models dependent on low sequence affinities are typically considered less reliable, new research suggests that in protein families that are related by function, structure is more conserved than we might predict even when primary sequence is not (*1*).

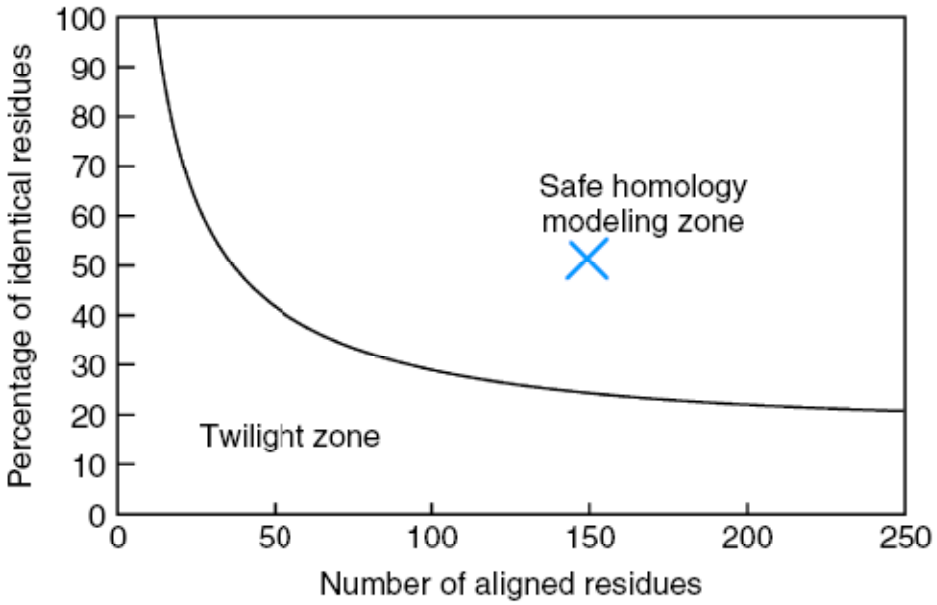

Figure 1. Two zones of sequence alignments. Blue X indicates an example of two proteins with 150 aligned residues that share a 50% sequence affinity (from *4*).

Faham et al. determined the crystal structure of the *Vibrio parahaemolyticus* sodium/galactose symporter (vSGLT), a member of the solute sodium symporter (SSS) family of membrane transporters.

Members of this family use energy generated from the flow of Na^+ down its concentration gradient, coupling solute flow with ion flow to allow the solute passage into the intracellular matrix (*1*, *3*). The ~3.0 angstrom structure of vSGLT crystallizes as a parallel dimer but functions as a monomer (Figure 2) and contains fourteen transmembrane helices, ten of which form a central core. This core is formed by the inverted repeat of five helices, transmembrane (TM) helices TM 2-6 and TM 7-11. The N and C terminus both contact the periplasm. Seven helices (TM 2-4, TM 7-9, and TM 11) provide residues for ligand selectivity, while the other helices provide stability. Galactose binds at the center of the protomer and is held in place by the hydrophobic residues and protein mass above it. When in complex with galactose, the protomer contains a large cavity that extends from just below the binding site and into the intracellular matrix. Since galactose is held above this cavity by the hydrophobic residue Tyr 263 (*3*), Faham et al. declared this the inward-facing structure of vSGLT. Kinks in TM 2 and TM 7 suggest their importance in transport (*1*).

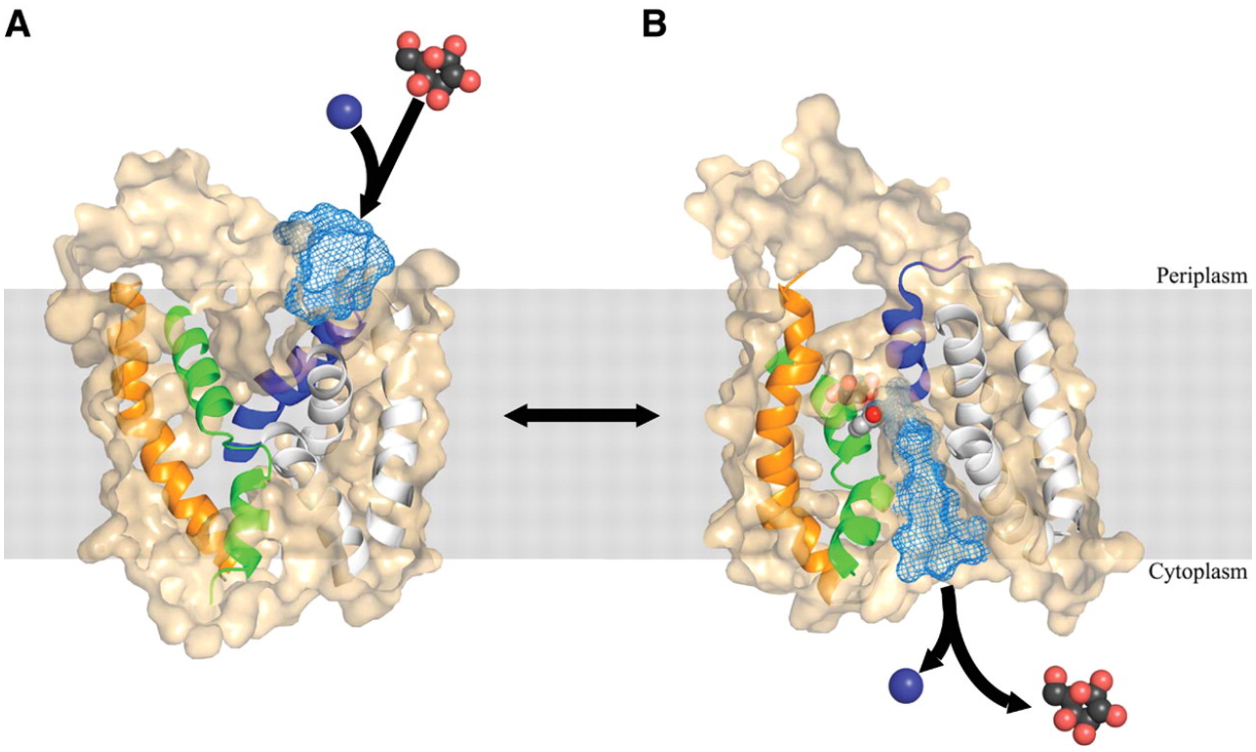

Figure 1. Structure of vSGLT. A) Outward-facing model based on LeuT. B) Inward-facing model based on X-ray crystallography. Helices shown are those that likely play an important role in alternating access. (From *1*)

Faham et al. noted similarities in the core structures (TM 2-11 in vSGLT, TM 1-10 in LeuT) and Na^+ binding sites of vSGLT and LeuT, a leucine transporter from the neurotransmitter sodium symporters (NSS) family. However, at an 11.5% sequence affinity, the two fall into the "twilight zone" seen in Figure 1. It is amazing, therefore, that such a structural affinity has been observed between inward-facing vSGLT and outward-facing LeuT. This conservation of tertiary structure facilitates the reliable modeling of outward-facing vSGLT (*1*, *3*).

The recent unveiling of both inward and outward facing structures for vSGLT (*1*) marks a turning point in the study of alternating access, the process by which a protein alters its conformation to expose a binding site on either side of the membrane and allow passage of solutes and ions into or out of the cell (*3*). Although alternating access has been studied indirectly, the research currently underway at the University of Pittsburgh in collaboration with the University of California, Los Angeles is providing the first direct evidence for alternating access via molecular dynamics simulations.

Although traditional molecular simulations can accurately describe <100 ns events, they are incapable of describing the rare conformational changes of large-scale protomers, which occur on the microsecond or millisecond time scales (*5*). Fortunately, in 1996, Huber and Kim outlined a path sampling method known as the weighted ensemble (WE) approach which is not so restricted (*2*). The WE approach employs models of any level of detail and saves computational time by focusing on rare transition events rather than random equilibrium motions. This approach generates a collection of unbiased trajectories. It is easily implemented, well-suited for sampling multiple distinct pathways, and yields a reaction rate along with each trajectory (*5*). The weighted ensemble approach begins with the initiation of multiple trajectories and uses statistical weighting to generate results, replicating trajectories that advance along a progress coordinate and pruning those trajectories which do not (*2*, *5*).

Previously, collaborators at the University of Pittsburgh and the University of California, Los Angeles have created models of the outward-facing structure of vSGLT based on LeuT and used the

weighted ensemble approach to model likely pathways for alternating access. We will analyze these trajectories and generate new models and trajectories based on Mhp1, a member of the nucleobase-cation-symport-1 (NCS1) family from *Microbacterium liquefaciens* that shares greater (16%) sequence affinity with vSGLT than LeuT (*6*).

II. Research Objectives

Our objectives are threefold, as listed:

- **Objective 1:** Analyze weighted ensemble trajectories generated from an outward-facing model of vSGLT based on the structure of LeuT
- **Objective 2:** Use homology modeling based on Mhp1 to propose a more accurate outward-facing structure for vSGLT
- **Objective 3:** Create more accurate alternating access trajectories for vSGLT, again using the WE approach

Our overall research objective is to provide an accurate description of the alternating access pathway for the galactose transporter vSGLT.

III. Methodology

The following will be used in the course of this research:

- **ClustalW** – for the determination of sequence affinity between vSGLT, Mhp1, and LeuT
- **Modeller** – for the creation of models for the outward-facing structure of vSGLT based on Mhp1
- **VMD** – for molecular visualization and analysis of trajectories generated using the WE approach
- **MatLab** – for graphical and statistical analysis of trajectories generated using the WE approach

IV. Conclusion

Following modeling techniques that have already been applied successfully laboratory, we intend to utilize structural similarity rather than sequence affinity as a basis for accurate homology modeling of vSGLT. We will compare WE trajectories generated from models that are based upon LeuT or Mhp1, with which vSGLT shares a higher sequence affinity. With our results, we not only hope to generate accurate models of alternating access for vSGLT but to confirm successful and computationally feasible means of capturing rare transition states in other sizeable protomers. We further hope that our research will have important implications by contributing to a more complete understanding of the critically important processes of membrane transport.

V. References

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