MOLECULAR SIMULATIONS ON BLUE WATERS AID THE UNDERSTANDING OF HOW PLANTS TRANSPORT SUGARS BETWEEN CELLS

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EXECUTIVE SUMMARY

SWEETs are a new family of sugar transporter proteins in plants that play a crucial role in various fundamental processes such as nectar production, pollen development, and plant–microbe interaction. SWEETs function is based on a rocker-switch mechanism; that is, an outward-facing (OF) to inward-facing (IF) transition to transport substrate molecules across the cell membrane. The OsSWEET2b crystal structure in rice was recently obtained in the IF state and provided the first glimpse of structural information on this class of proteins. However, these transport proteins are very flexible in nature and it is difficult to understand the structural changes based on a single, static X-ray crystal structure. In this study, we performed all-atom unbiased molecular dynamics (MD) simulations to investigate the conformational dynamics of the OsSWEET2b transporter. For the first time, we characterized the complete sugar-transport cycle of a plant transporter, and determined the critical residues that mediate the transport of sugar.

RESEARCH CHALLENGE

Global climate change and increasing world population pose a great threat to the current agricultural economy. Although genetic engineering has emerged as a successful approach that enhances crop productivity under optimum environmental conditions, it still fails to meet the current food demand. An alternative solution to increase crop yield may be to change the phenotype of plants. (The phenotype is the set of observable characteristics of an individual plant resulting from the interaction of its genotype with the environment.) Two years ago, researchers produced the largest pumpkin, setting a world record of 1,190 kg [1]. This experimental study suggested that these plants store sugar in the phloem (the vascular tissue of plants) and transport it from the leaves to various organs [2]. The sugars (glucose) are produced in the leaf during photosynthesis and transported to the phloem via the SWEETs' sugar transporters [3].

In our research study, we performed hundreds of microsecond-long simulations to understand the functional dynamics of SWEETs and characterized how the sugars are exported and transported to the cell. Our study provides novel insights into the molecular mechanism of sugar transport.

METHODS & CODES

MD simulations rely on numerical integration of Newton's equation of motion for the interacting atoms. This results in time-dependent trajectories for all atoms of the system, which together provide a simulation of the biomolecule's dynamical motion. Our simulations were performed in AMBERv14 [4]. AMBER is highly parallelized to massively accelerate complex molecular simulations and enhance sampling efficiency. Our simulations generated several terabytes of data that cannot be analyzed visually. CPPTRAJ [5] is the processing and analytical tool written in C++ that is available with the AMBER suite for trajectory processing. In addition, we used Python modules such as Pytraj [6] and MDTraj [7] for data analysis and processing. The MD data we obtained were featurized to biologically relevant reaction coordinates and converted to Python array numPy files for efficient processing and analysis. We used Markov State Models (MSM) to cluster the data based on relevant kinetics [8]. From the clusters, MSM constructed a transition probability matrix to find the rate of transition from one state to another. Using transition path theory

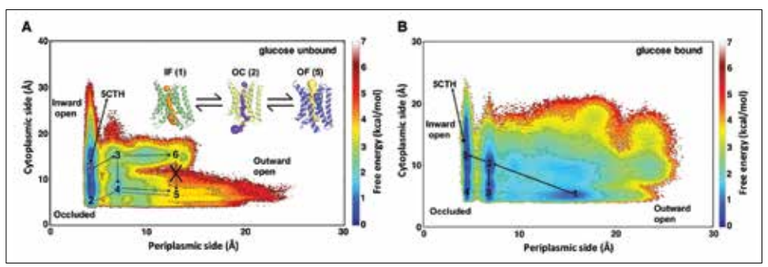

Figure 1: The numbers 1–6 in Fig. 1A and 1B represent the inward-open (1A, 5B); occluded (2A, 4B); intermediate states (3A, 4A, 6A, 2B, and 3B); and outward-open (5A, 1B). The pore channel opening and closure at the periplasmic and cytoplasmic side are obtained by measuring the distances between the gating residues. The crystal structure is shown as a black star. The dominant paths 1A (grey line), 2A (dotted black line), 3A (thin black line), 1B (dotted grey line), and 2B (black line) are shown as arrows.

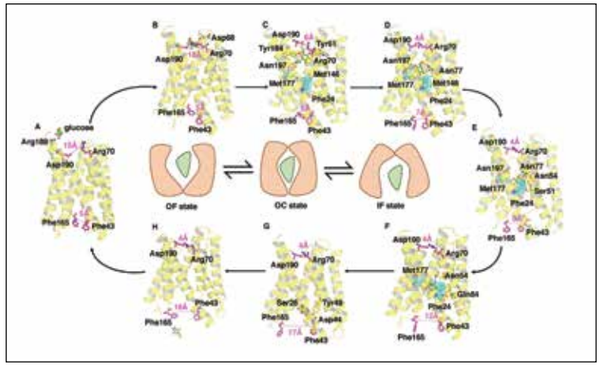

Figure 2: The glucose-binding mechanism and the glucose-driven conformational changes are shown in Fig. 2A to 2H. The distances between gating residues [Arg70 (CZ)–Asp190 (CG) and Phe165 (Cα)–Phe43 (Cα)] are shown in magenta. The intracellular hydrophobic gates Phe24–Met146–Met177 are shown in cyan. The glucose molecule is shown in green.

(TPT), the intermediate states between the source and sink states were identified [9]. The structures were extracted and visualized in VMD [10] and Pymol [11].

RESULTS & IMPACT

Conformational dynamics of OsSWEET2b: The IF state OsSWEET2b (PDB ID: 5CTH) crystal structure was used as a starting structure for MD simulations [12]. We obtained the complete transition from IF to occluded (OC) and OC to OF over a period of ~145 microseconds (μs) (Fig. 1A). The simulation data in high-dimension space were transformed to the three slowest processes and clustered to kinetically relevant states. MSM were constructed, with the final model containing 900 microstates. MSM-weighted free-energy plots were obtained by projecting the data on extracellular and intracellular gating distances (Fig. 1A and 1B).

The free-energy barrier for one complete cycle of an *apo* (ground state) transporter from IF to OC and OC to OF was estimated to be ~4 kcal/mol. Using TPT, the dominant pathways of transition were determined and Path-1A was found to be the lowest-energy pathway between IF and OF states. Path-2A was identified as an alternative high-energy pathway. The transition along Path-3A was not feasible as the transporters are wide open at both ends.

We extended the simulation from the OF state to investigate the glucose recognition, binding, and transport mechanism over a duration of ~68 μs (Fig. 1B). The conformational landscape plots show that glucose decreases the barrier among various states, and the transition between them is easily accessible compared to *apo* dynamics. We obtained two major pathways, namely Path-1B and Path-2B, using TPT. The free-energy barrier was 2–3 kcal/mol for transition of glucose from OF to IF via OC or extended OC–OF states.

Glucose translocation in OsSWEET2b: Glucose is recognized in the OF state by residues such as Arg70, Arg189, and Asp190 at the extracellular surface (Fig. 2). Glucose then diffuses to the pore channel and establishes stable contacts with binding-site residues Asn57, Asn77, and Asn197, which drive the transporter to the OC state. At this juncture, the intracellular hydrophobic gates Phe24–Met146–Met177 act as a barrier and restrict further movement of glucose. The increase in strength of the ionic contacts at the extracellular surface results in the displacement of hydrophobic gating residues; thus, it results in the entry to the intracellular side of the transporter. The downward movement of glucose leads to the IF state and forms polar contacts with Gln84, Tyr48, and Asp44.

Our study reveals atomistic-level detail of the conformational dynamics of OsSWEET2b and the free-energy barrier associated with the transport process. In this work, we have shown how the glucose regulates the SWEET function and key residues involved in the conformational switches. As a continuation of this work, we will focus on reducing the energy barrier of conformational switches between the intermediate states, thus allowing for more sugar in a plant's phloem in order to increase fruit size and crop yield.

WHY BLUE WATERS

Our work is computationally demanding and requires multiple nodes to run large numbers of simulations. Parallel computing reduces the cost and time effectively. Historically, parallel computing has been the "high end of computing," and has been used to model difficult problems in many areas of science and engineering. Blue Waters is a perfect resource that has the massive architecture to run parallel jobs. The timescales for simulation of these biological problems range from several microseconds to milliseconds. These computations are not possible to perform in a reasonable time without Blue Waters' petascale computing capability.

PUBLICATIONS & DATA SETS

Selvam, B., Y.-C. Yu, L.-Q. Chen, and D. Shukla, Molecular Basis of Glucose Transport Mechanism in Plants. In review (2018).

Cheng, K.J., B. Selvam, Y.-C. Yu, L.-Q. Chen, and D. Shukla, SWEET vs SemiSWEET: A Biophysical Investigation of their Similarities and Differences. In review (2018).