MOLECULAR BASIS OF THE NITRATE TRANSPORT MECHANISM IN PLANTS

Allocation: Illinois/ 400 Knh
PI: Diwakar Shukla[1]

[1]University of Illinois at Urbana–Champaign

EXECUTIVE SUMMARY

Growing evidence suggests that efficient use of nitrogen is required to improve crop productivity. The gene NRT1.1 has been identified as a nitrate transporter that enables nitrogen uptake by plants. NRT acts as a nitrate sensor and transports substrate molecules across the membrane. It belongs to the major facilitator family whose function relies on the alternate access mechanism where the substrate binding site of the transporter protein alternatively opens and closes at either side of the membrane. The crystal structure of NRT1.1 was determined in the inward-facing (IF) state as a homodimer and provided the first glimpse of structure information on this class of proteins. These proteins undergo intrinsic conformational changes; the dynamics among the functionally important intermediate states remains elusive. In this project, the research team investigated the conformational dynamics and substrate transport mechanism of NRT1.1 using molecular dynamics simulations.

RESEARCH CHALLENGE

Nitrogen is an essential nutrient required for plant growth and development. Plants uptake nitrogen as nitrate ions through the nitrate transporter (NRT1.1), which actively mediates the transport of nitrate ions from the soil into cells. NRT1.1 exhibits a dual affinity mode [1]. At high nitrate concentration, NRT1.1 acts as a low-affinity transporter (LAT) and at high concentration, it is phosphorylated at Thr101, resulting in a high-affinity transporter [1,2]. Recently, the crystal structure of NRT1.1 bound to a nitrate ion (NO_3^-) was obtained as a homodimer in the IF state [3,4]. NRT1.1 belongs to the major facilitator superfamily that contains 12 transmembrane helices and functions based on an alternate access mechanism to transport signaling molecules. NO_3^- was bound to the protonated His356 and Thr360 on TM7 in the binding site, and biochemical studies show that the mutation of these residues results in a loss of ion transport [3,4]. The phosphorylation of Thr101, the phosphorylation site residue, increases the affinity of the nitrate ions. However, the conformational dynamics and mechanistic basis of NO_3^- uptake remain elusive. In this project, the research team performed long microsecond simulations to understand the functional dynamics of NRT1.1 and characterize the NO_3^- transport mechanism. The results will provide molecular-level understanding of the phosphorylation-mediated increased affinity switch mechanism of NRT1.1.

METHODS & CODES

The simulations were performed with AMBER14 [5]. The simulations produced massive amounts of data (several TB); the Python package MDTraj was used for data processing and analysis [6,7]. The MD data were featurized to biologically relevant reaction coordinates and Markov state models (MSM) were constructed using MSMBuilder [8]. MSM is a statistical model constructed to explore the kinetics of the biological events. MD simulation data will be clustered based on kinetically relevant microstates and the transition matrix that was constructed. Using the transition matrix, the rate of probability of transition to the different states were obtained. The state population are easily converted to free energies using Boltzmann distribution. The obtained free energies of individual states are called the "MSM weighted free energy" of populations.

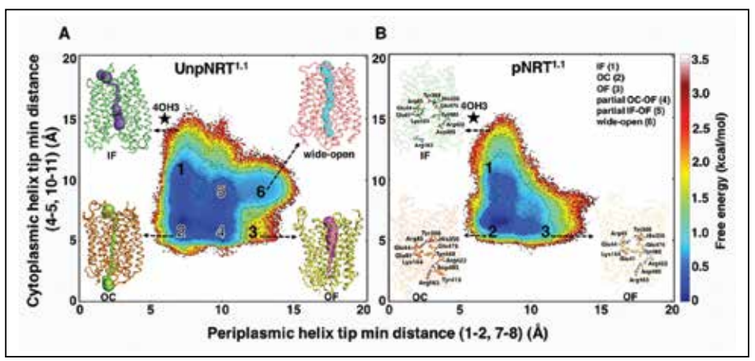

Figure 1: Conformational dynamics of NRT1.1. The conformational free energy landscape of unphosphorylated (A) and phosphorylated NRT1.1 (B). The pore channel radius for various intermediate states is shown in 1A. The polar interactions that stabilize the key states are shown in 1B. The starting inward-facing structure is shown by the black star

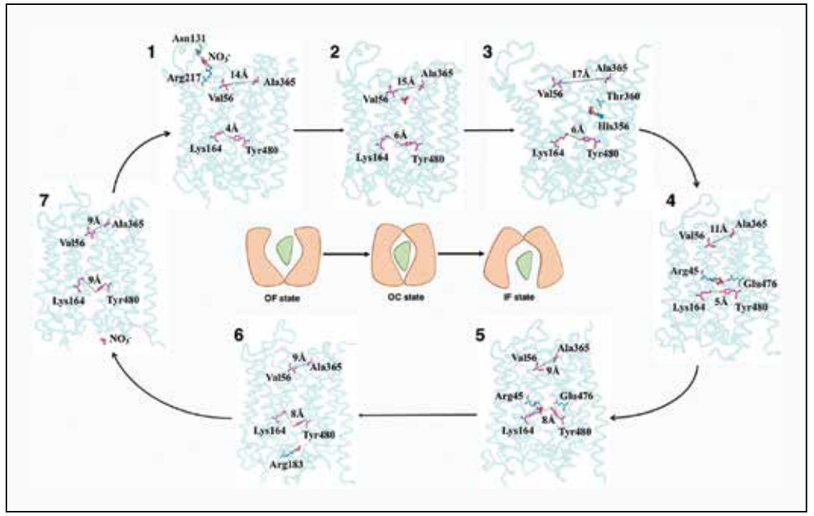

Figure 2: Nitrate transport mechanism in plants. The conformationally driven nitrate ion transport mechanism is shown in 2-1 to 2-7. The distance between the extracellular, intermediate, and intracellular gating residues is shown by black dashed lines. The nitrate ion is shown as ball and sphere.

RESULTS & IMPACT

Conformational dynamics of unphosphorylated (UnpNRT) and phosphorylated (pNRT) ensembles. The IF-state crystal structure of NRT1.1 (Protein Data Bank ID: 4OH3) [5] was the starting structure of the simulations. The research team performed extensive simulations to explore the conformational dynamics and nitrate transport mechanism of UnpNRT (~110 μs) and pNRT (~45 μs). The high-dimensional simulation data were converted to the slowest process and clustered to kinetically relevant states. MSM were constructed to gain insights into the thermodynamics and kinetics of UnpNRT and pNRT conformational ensembles. An MSM-weighted free energy landscape plot was shown by projecting the MD data obtained on the minimum helical tip distance of the pore channel radius on the extracellular and intracellular region (Fig. 1). The free energy barrier for one complete conversion cycle of IF to outward-facing (OF) structures was estimated to be approximately 2.5 kcal/mol in UnpNRT and approximately 1 kcal/mol in pNRT. The phosphorylation at Thr101 results in a conformationally driven ion-coupled transport mechanism and results in a canonical L-shaped landscape. However, UnpNRT samples more intermediate states and may result in a low-affinity transporter.

Nitrate ion transport mechanism in NRT. The nitrate ion is recognized by the group of polar and positively charged residues at the extracellular part of the transporter in the OF state (Fig. 2); the distances between the periplasmic and cytoplasmic gating residues are approximately 12 Å and 3 Å, respectively. The ion diffuses in the pore and interacts with Thr360. Nitrate ions escape from the intermediate interaction and bind to protonated His356 on TM7; in accord with the experimental finding this crucial interaction mediates the nitrate transport. The sidechain conformation of His356 is further favored by polar contacts of Glu476 and Tyr388. The tight binding of nitrate ions at the center of the transporter facilitates the conformational change from the OF to the occluded (OC) state. The breakage of polar contacts between Lys164 and Tyr480 leads to the opening of the intracellular gate and the release of nitrate to the cytoplasmic side.

This study reveals the molecular level detail of functionally important intermediate states and the nitrate ion transport mechanism using extensive simulation. In this work, the PI has also determined the key residues that drive the nitrate transport as well as the conformational switches. His future work will be focused on engineering NRT to improve the nitrate transport to increase the crop yield.

WHY BLUE WATERS

Biologically important conformational transitions are slow processes that are difficult to observe by running simulations on local hardware. Powerful resources like Blue Waters are required to study such complex biological processes in full atomistic detail and over long timescales. Blue Waters provides thousands of GPUs that are used for parallel molecular dynamics simulations to perform MSM-based adaptive sampling of the conformational energy landscape of proteins. Blue Waters increases the overall compute performance by several orders of magnitude in terms of the real time required for simulation.

PUBLICATIONS & DATA SETS

B. Selvam, J. Feng, and D. Shukla, "Atomic insights into dual affinity switch mechanism in nitrate transporter," in preparation, 2019.