

Dynamics of Biomolecular Machines in Function Revealed by Theoretical Methods

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Biomolecular machines, such as molecular motors and transporters in the cell, are known to change their structure when they function. For example, ATP synthase, which synthesizes ATP in mitochondria, is a molecular motor that uses chemical energy to rotate unidirectionally. Transporters, which transport substrate molecules across the cell membrane, perform substrate transport by changing their structure between an inwardly and outwardly open structure relative to the membrane. Our goal is to elucidate the mechanism of these elaborate and dynamic nanomachines created by nature at the atomic and molecular level, and to control their functions based on our findings.

We would like to understand the mechanism of biomolecular machines by “seeing” the motion of biomolecular machines at the moment they function at the molecular level, on a computer. However, this is not an easy task, because biomolecular machines are huge molecules, and their functioning time scale is slow (for a molecular scale) at milliseconds or longer. Conventional atomistic molecular dynamics (MD) simulations cannot cover millisecond-long functional dynamics, especially for a large system like typical biomolecular machines. Therefore, we have developed and applied methods such as coarse-grained modeling, enhanced

sampling and importance sampling to capture the motion at the moment of function.

We have been working on biomolecular motors such as ATP synthase. ATP synthase is a rotary motor that produces most of ATP required in the cell. It is composed of two rotary motors: F_0 and F_1 . F_0 motor is embedded in the membrane and driven by proton gradient, while F_1 motor is driven by ATP hydrolysis reaction. We clarified how the rotation of F_1 motor is driven by a key chemical step, P_i release after ATP hydrolysis reaction, by accelerating atomistic MD simulations with external forces.

Transporters are membrane proteins that transport their substrates across the membrane. We have studied Na^+/H^+ antiporter, which exchanges sodium ions and protons inside and outside the cell. The ion transport process by the Na^+/H^+ antiporter was simulated in atomic detail with transition path sampling technique to capture the moment of the ion transports. The simulations predicted the mutation that can speed up the ion transport. The mutation was tested in experiments and shown to speed up the ion transport twice faster than the wild type. Therefore, we succeeded in controlling the function of the transporter based on mechanism obtained from simulations.

Selected Publications

- K. Okazaki and G. Hummer, “Phosphate Release Coupled to Rotary Motion of F_1 -ATPase,” *Proc. Natl. Acad. Sci. U.S.A.* **110**, 16468–16473 (2013).
- K. Okazaki and G. Hummer, “Elasticity, Friction, and Pathway of γ -Subunit Rotation in F_0F_1 -ATP Synthase,” *Proc. Natl. Acad. Sci. U.S.A.* **112**, 10720–10725 (2015).
- K. Okazaki, D. Wöhlert, J. Warnau, H. Jung, Ö. Yildiz, W. Kühlbrandt and G. Hummer, “Mechanism of the Electroneutral Sodium/Proton Antiporter PaNhaP from Transition-Path Shooting,” *Nat. Commun.* **10**, 1742 (2019).

1. Mechanism of Na⁺/H⁺ Antiporter and Engineering of a Faster Transporter

Na⁺/H⁺ antiporters control pH and Na⁺ concentration in the cell by exchanging sodium ions and protons across lipid membranes. They belong to the cation/proton antiporter (CPA) superfamily, and prevail in all domains of life. The archaeal Na⁺/H⁺ antiporters PaNhaP and MjNhaP1 as well as human NHE1, which is linked to a wide spectrum of diseases from heart failure to autism and has no structure solved yet, are electroneutral antiporters of the CPA1 family, exchanging one proton against one sodium ion. As a model system in mechanistic studies of electroneutral Na⁺/H⁺ exchange, we studied the transport mechanism of PaNhaP.¹⁾

Na⁺/H⁺ antiporters use the gradient of either sodium ion or proton to drive the uphill transport of the other ion (Figure 1A). The conformational transition of the transporter makes the ion-binding site accessible from either side of the membrane in the alternating manner. For PaNhaP, the inward-open conformation was obtained by X-ray crystallography, while the outward-open conformation is not known experimentally. We modelled the outward-open conformation by MDFF flexible fitting to the low-resolution outward-open structure of the homologous MjNhaP1 from cryo-EM, followed by the long equilibrium MD simulations. It was shown that the transporter domain moves ~3.5 Å in the direction normal to the membrane to take the outward-open state.

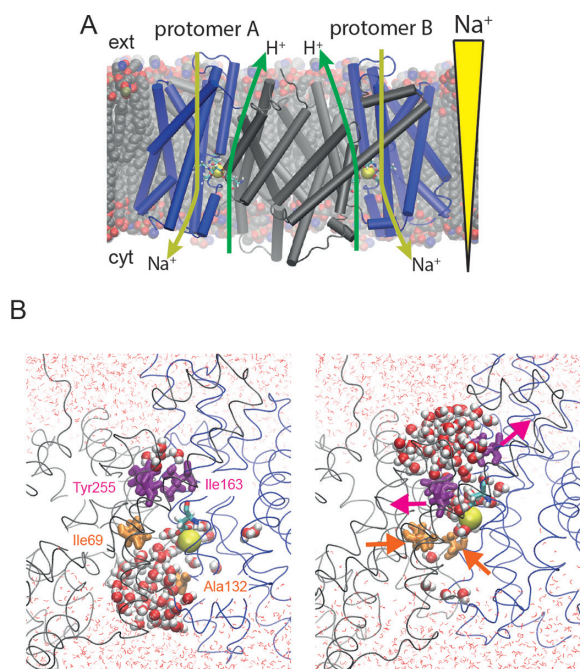


Figure 1. (A) PaNhaP dimer structure. (B) The outside (purple) and inside (orange) gates found in the transition path simulations.

By applying the transition path sampling technique, we sampled unbiased transition paths between the inward- and outward-open states. In analysis of the transition paths, we found hydrophobic gates above and below the ion-binding site, which open and close in response to the domain motions (Figure 1B). From the reaction coordinate analysis, it was

shown that open-close motion of the outside gate (Ile163-Tyr255) is a rate-limiting step of the alternating-access conformational change. Based on this result, we weakened the outside gate by mutating the residues to both alanine. It was expected that this mutation lowers the barrier and makes the ion transport faster. It was confirmed by experiments that the ion-transport speed of the mutant is indeed twice faster than the wild-type transporter.

2. Machine Learning of Reaction Coordinates

It is a challenging task to identify reaction coordinates for biomolecular systems with many degrees of freedom. Unlike order parameters or collective variables, a reaction coordinate should describe progress of a reaction between two metastable states. We have developed a machine learning method to identify reaction coordinates based on the committor function. Assuming a linear combination of many collective variables, reaction coordinates are optimized via likelihood maximization or cross-entropy minimization.²⁾ From coefficients of the optimized reaction coordinates, we can also identify rate-limiting variables, which play an important role in transition state area. We have also applied a deep neural network and Explainable Artificial Intelligence (XAI) for this problem.³⁾

3. Mechanism of Membrane Remodeling by F-BAR Protein Pacsin1

F-Bin/Amphiphysin/Rvs (F-BAR) domain proteins play essential roles in biological processes that involve membrane remodelling, such as endocytosis and exocytosis. Notably, Pacsin1 from the Pacsin/Syndapin subfamily has the ability to transform the membrane into various morphologies: striated tubes, featureless wide and thin tubes, and pearling vesicles. We clarified the membrane curvature induction and sensing characteristics of Pacsin1 by combining all-atom (AA) and coarse-grained (CG) MD simulations.⁴⁾ By matching structural fluctuations between AA and CG simulations, a CG protein model called “Gō-MARTINI” was developed and optimized.⁵⁾ The model should prove useful for describing protein dynamics that are involved in membrane remodeling processes.

References

- 1) K. Okazaki, D. Wöhlert, J. Warnau, H. Jung, Ö. Yildiz, W. Kühlbrandt and G. Hummer, *Nat. Commun.* **10**, 1742 (2019).
- 2) Y. Mori, K. Okazaki, T. Mori, K. Kim and N. Matubayasi, *J. Chem. Phys.* **153**, 054115 (2020).
- 3) T. Kikutsuji, Y. Mori, K. Okazaki, T. Mori, K. Kim, and N. Matubayasi, *J. Chem. Phys.* **156**, 154108 (2022).
- 4) M. I. Mahmood, H. Noguchi and K. Okazaki, *Sci. Rep.* **9**, 14557 (2019).
- 5) M. I. Mahmood, A. B. Poma and K. Okazaki, *Front. Mol. Biosci.* **8**, 619381 (2021).