Dynamics of Biomolecular Machines in Function Revealed by Theoretical Methods

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Keywords Theoretical Biophysics, Biomolecular Machines, Molecular Simulation

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 inward-open and outward-open states relative to the membrane. We aim to elucidate the mechanisms of these elaborate and dynamic nanomachines created by nature at the atomic and molecular levels and to control their functions based on our findings.

We would like to understand the mechanisms 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 dynamics, especially for a large system like typical biomolecular machines. Therefore, we have developed and applied methods such as coarse-grained modeling and enhanced 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 the ATP required in the cell. It is composed of two rotary motors: F_o and F_1. The F_o motor is embedded in the membrane and driven by a proton gradient, while the F_1 motor is driven by the ATP hydrolysis reaction. We clarified how the rotation of the F_1 motor is driven by a key chemical step, Pi release after the ATP hydrolysis reaction, by accelerating atomistic MD simulations with external forces.[1)]

Transporters are membrane proteins that transport their substrates across the membrane. We have studied a 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 a transition path sampling technique to capture the moment of the ion transport. The simulations predicted the mutation that could speed up 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 the mechanism obtained from simulations by creating the faster transporter.[2)]

Selected Publications

- K. Okazaki and G. Hummer, "Elasticity, Friction, and Pathway of γ-Subunit Rotation in F_oF_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).
- R. Kobayashi, H. Ueno, K. Okazaki and H. Noji, "Molecular Mechanism on Forcible Ejection of ATPase Inhibitory Factor 1 from Mitochondrial ATP Synthase," *Nat. Commun.* **14**, 1682 (2023).

1. Mechanism of Oxalate Transporter

Oxalate is contained in our daily food, such as spinach and nuts. Excess amounts of oxalate form insoluble salts with calcium ions, causing kidney stone disease. *Oxalobacter formigenes*, an oxalate-degrading bacterium that lives in the intestine, absorbs oxalate as its sole carbon source and excretes formate, a metabolic degradation product. As a result, *Oxalobacter formigenes* contributes to reducing the risk of kidney stone disease by lowering the oxalate level. The oxalate transporter (OxlT) in the bacterium's membrane is responsible for oxalate uptake and formate efflux. The crystal structures of the two different conformations taken by OxlT during its transport cycle have been determined by our collaborators.[3] One structure is in the outward-open conformation, while the other is in the occluded conformation with the bound oxalate.

We have identified the inward and outward gates of OxlT using MD simulations from the experimental structures.[3] An unresolved inward-open conformation was obtained by performing accelerated MD simulations, in which boost potential was applied to residues around the inward gate (Figure 1A). The obtained inward-open conformation was validated by an additional simulation observing the substrate formate binding from the inside of the membrane (Figure 1B). The contact analysis was performed to identify key interactions that change during the conformational change (Figure 1C). The residues that break contacts include D280 at the cytoplasmic side. The S162 and T258 formed a contact at the periplasmic side.

Then, we used the state-of-the-art structural prediction AI AlphaFold2 to see how mutations of the identified residues affect AlphaFold prediction (Figure 1D). While AlphaFold predicted only the outward-open conformations with the wild-type sequence, the mutation D280L or S162C/T258C made AlphaFold also predict the inward-open conformation. These mutations likely stabilize the inward-open conformation.

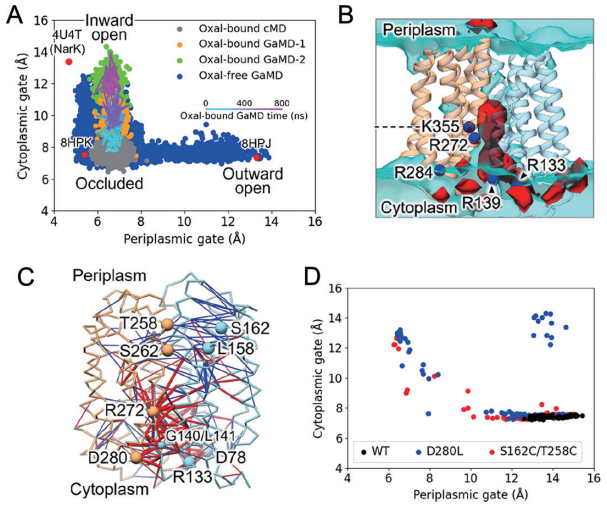

Figure 1. (A) Accelerated MD simulations discover an inward-open conformation. (B) Formate density in the inward-open state. (C) Contact analysis to identify important residue pairs. (D) AlphaFold structures for wild type and mutants.[4]

2. Integration of AlphaFold with MD Simulation

The computational cost of all-atom MD simulations for biomolecular machines is so high that direct simulation of the functional motions is impossible. We introduce a method that integrates AlphaFold with MD simulation to overcome this difficulty.[5] This method first generates broad structures by AlphaFold with reduced MSA depth, including multiple stable conformations and intermediates. Then, MD simulations are conducted from these structures to cover the broad conformational space that is involved with the function. The method was tested with the transporter protein NarK. It successfully uncovers a missing conformational state and transition dynamics between stable states.

3. 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 the progress of a reaction between two metastable states. We have developed a machine learning method to identify reaction coordinates based on the committor function.[2,6] We have applied a deep neural network and Explainable Artificial Intelligence (XAI) for this problem.[6]

References

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Awards

OHNUKI, Jun; Young Scientist Excellence Award of the Protein Science Society of Japan (2024).
KOBAYASHI, Ryohei; Early Career Award, The Biophysical Society of Japan (2023).