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_o and F_1. F_o 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.[1)]

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.[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 oxalate forms 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), which exists in the membrane of the bacterium, is responsible for oxalate uptake into and formate efflux out of the bacterium. 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 structure is in the occluded conformation with the bound oxalate.

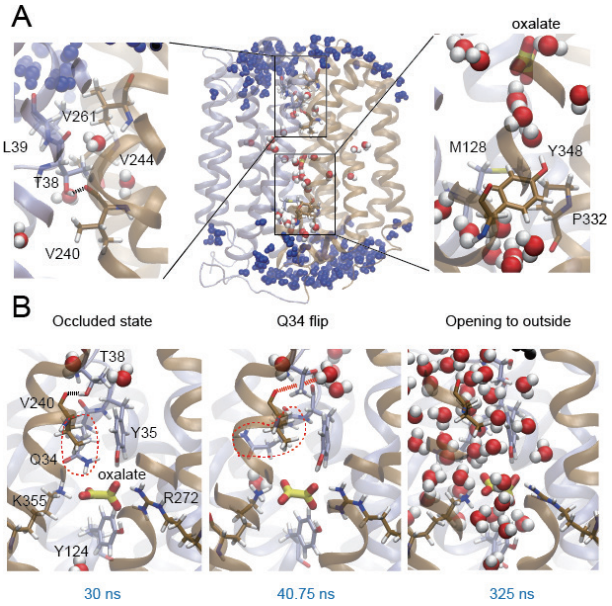

Figure 1. (A) The determined periplasmic and cytoplasmic gates. (B) The conformational transition from the occluded to the outward-open state.[3]

The atomistic MD simulation from the occluded conformation of OxlT and analysis of the water molecule density revealed the presence of gates above and below the substrate binding pocket that control the influx of water and substrate molecules.[3] The periplasmic gate consists of a hydrogen bond between Thr38-Val240 and a hydrophobic structure around it (Figure 1A left). The cytoplasmic gate consists of a hydrophobic structure composed of Met128, Pro332, and Tyr348 (Figure 1A right). Furthermore, in microsecond-scale simulations, OxlT undergoes a conformational change from the occluded conformation to the outward-open conformation.[3] The overall conformational change was preceded by a localized change in the flip of the Gln34 side chain at the oxalate binding site and the dissociation of the Thr38-Val240 hydrogen bond mentioned above, followed a few hundred nanoseconds later by the opening of the periplasmic gate to the open conformation (Figure 2B). Thus, the Gln34 side chain and the Thr38-Val240 hydrogen bond are considered to be "latches" for the periplasmic gate.

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.[4] 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.[5]

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.[6] By matching structural fluctuations between AA and CG simulations, a CG protein model called "Gō-MARTINI" was developed and optimized.[7] This model should prove useful for describing protein dynamics that are involved in membrane remodeling processes.

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