

Dynamics of Biomolecular Machines in Function Revealed by Theoretical Methods

Department of Theoretical and Computational Molecular Science
Division of Computational Molecular Science



OKAZAKI, Kei-ichi
Associate Professor
[keokazaki@ims.ac.jp]

Education

2004 B.S. Kyoto University
2006 M.S. Kobe University
2009 Ph.D. Kobe University

Professional Employment

2007 JSPS Research Fellow (DC2), Kobe University
2009 JSPS Postdoctoral Fellow (PD), Waseda University
2010 Part-time Lecturer, Waseda University
2012 JSPS Postdoctoral Fellow for Research Abroad, National Institutes of Health, U.S.A.
2014 Postdoctoral Fellow, Max Planck Institute of Biophysics, Germany
2016 Research Associate Professor, Institute for Molecular Science
2020 Associate Professor, Institute for Molecular Science
Associate Professor, The Graduate University for Advanced Studies

Award

2014 Early Career Award in Biophysics, Biophysical Society of Japan

Member

Assistant Professor
OHNUKI, Jun
Post-Doctoral Fellow
MAHMOOD, Md Iqbal
Graduate Student
SEKI, Takehito
GUYOT, Constantin
Secretary
CHIBA, Fumika

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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 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_0 and F_1 . The F_0 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_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).
- 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 the Inhibitor Protein IF₁ for Mitochondrial ATP Synthase

ATPase inhibitory factor 1 (IF₁) regulates mitochondrial F₀F₁-ATP synthase by inserting into the rotor–stator interface and blocking F₁ rotation. Single-molecule experiments showed that rotation only in the ATP-synthesis direction ejects IF₁, but the atomic mechanism is unclear. Using all-atom MD simulations with torque applied on the γ subunit, we found that rotation proceeded further in the synthesis than hydrolytic direction. At 120° synthesis rotation, IF₁ contacts were disrupted, destabilizing its helices, and by 240° rotation, the β subunit pulled IF₁ outward. These stepwise changes appear key to IF₁ ejection. Simulations also revealed how hydrolytic rotation is nullified by steric clash between IF₁ and β_{TP} . We further discuss the proton motive force needed to release IF₁ inhibition.

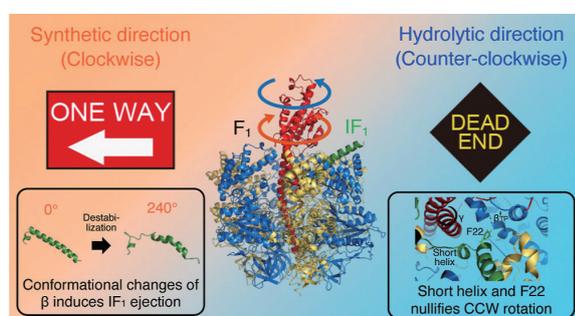


Figure 1. Rotation-direction-dependent mechanism of IF₁ inhibition for mitochondrial ATP synthase from atomistic molecular dynamics simulation.³⁾

2. Rotary Mechanism of the Prokaryotic V_o Motor Driven by Proton Motive Force

ATP synthases use the proton motive force (pmf) to rotate their c-ring and drive ATP synthesis. However, the mechanism of how pmf converts into c-ring rotation remains unclear. This study presents a 2.8 Å cryo-EM structure of the V_o domain of V/A-ATPase from *Thermus thermophilus*, revealing precise orientations of glutamate (Glu) residues in the c₁₂-ring with possible locations of water molecules that form the half channels for protons. MD simulations identified the channels and possible proton transfer pathway, and showed that protonation of specific Glu residues triggers unidirectional Brownian motion of the c₁₂-ring towards ATP synthesis.⁴⁾

Award

SEKI, Takehito; Young Presentation Award, 50th Meeting of Japan BioEnergetics Group (2024).

3. 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.

4. 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 (DNN) and Explainable Artificial Intelligence (XAI) for this problem.⁶⁾ We also developed a hyperparameter tuning approach to this problem.⁷⁾

References

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