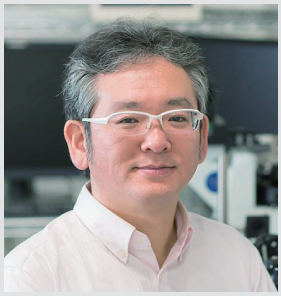
Operation and Design Principles of Biological Molecular Machines

Department of Life and Coordination-Complex Molecular Science
Division of Biomolecular Functions



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Activity of life is supported by various molecular machines made of proteins. Protein molecular machines are tiny, but show very high performance, and are superior to man-made machines in many aspects. One of the representatives of protein molecular machines is linear and rotary molecular motors (Figure 1). Molecular motors generate the mechanical forces that drive their unidirectional motions from the energy of chemical reaction or the electrochemical potential across the cell membrane. We unveil operational principles of molecular motors with advanced single-molecule functional analysis. With the help of site-saturation mutagenesis and robot-based automation, we also engineer non-natural molecular motors to understand their design principles.

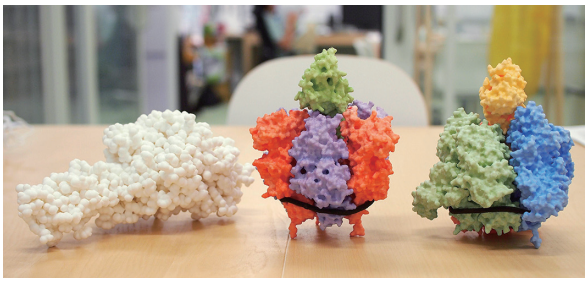

Figure 1. Protein molecular machines. (Left) A linear molecular motor chitinase A. (Center and Right) Rotary molecular motors F_1-ATPase and V_1-ATPase, respectively.

Selected Publications

- T. Kosugi, T. Iida, M. Tanabe, R. Iino and N. Koga, "Design of Allosteric Sites into Rotary Motor V_1-ATPase by Restoring Lost Function of Pseudo-Active Sites," *Nat. Chem.* (2023). DOI: 10.1038/s41557-023-01256-4
- A. Otomo, T. Iida, Y. Okuni, H. Ueno, T. Murata and R. Iino, "Direct Observation of Stepping Rotation of V-ATPase Reveals Rigid Component in Coupling between V_o and V_1 Motors," *Proc. Natl. Acad. Sci. U. S. A.* **119**, e2210204119 (2022).
- A. Nakamura, N. Kobayashi, N. Koga and R. Iino, "Positive Charge Introduction on the Surface of Thermostabilized PET Hydrolase Facilitates PET Binding and Degradation," *ACS Catal.* **11**, 8550–8564 (2021).
- A. Visootsat, A. Nakamura, P. Vignon, H. Watanabe, T. Uchihashi and R. Iino, "Single-Molecule Imaging Analysis Reveals the Mechanism of a High-Catalytic-Activity Mutant of Chitinase A from *Serratia marcescens*," *J. Biol. Chem.* **295**, 1915–1925 (2020).
- J. Ando, A. Nakamura, M. Yamamoto, C. Song, K. Murata and R. Iino, "Multicolor High-Speed Tracking of Single Biomolecules with Silver, Gold, Silver-Gold Alloy Nanoparticles," *ACS Photonics* **6**, 2870–2883 (2019).
- T. Iida, Y. Minagawa, H. Ueno, F. Kawai, T. Murata and R. Iino, "Single-Molecule Analysis Reveals Rotational Substeps and Chemo-Mechanical Coupling Scheme of *Enterococcus hirae* V_1-ATPase," *J. Biol. Chem.* **294**, 17017–17030 (2019).
- J. Ando, A. Nakamura, A. Visootsat, M. Yamamoto, C. Song, K. Murata and R. Iino, "Single-Nanoparticle Tracking with Angstrom Localization Precision and Microsecond Time Resolution," *Biophys. J.* **115**, 2413–2427 (2018).
- A. Nakamura, K. Okazaki, T. Furuta, M. Sakurai and R. Iino, "Processive Chitinase is Brownian Monorail Operated by Fast Catalysis after Peeling Rail from Crystalline Chitin," *Nat. Commun.* **9**, 3814 (2018).

1. Six States of *Enterococcus hirae* V-Type ATPase Reveals Non-Uniform Rotor Rotation during Turnover[1)]

The vacuolar-type ATPase from *Enterococcus hirae* (EhV-ATPase) is a thus-far unique adaptation of V-ATPases, as it performs Na^+ transport and demonstrates an off-axis rotor assembly (Figure 2). Recent single molecule studies of the isolated V_1 domain have indicated that there are subpauses within the three major states of the pseudo three-fold symmetric rotary enzyme. However, there was no structural evidence for these. Herein we activate the EhV-ATPase complex with ATP and identified multiple structures consisting of a total of six states of this complex by using cryo-electron microscopy. The orientations of the rotor complex during turnover, especially in the intermediates, are not as perfectly uniform as expected (Figure 3 and 4). The densities in the nucleotide binding pockets in the V_1 domain indicate the different catalytic conditions for the six conformations. The off-axis rotor and its' interactions with the stator a-subunit during rotation suggests that this non-uniform rotor rotation is performed through the entire complex.

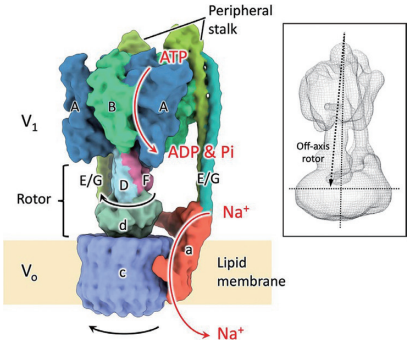

Figure 2. Schematic drawing of the EhV-ATPase. A-, B-, D-, E-, F-, G- and d-subunits form the V_1 domain, while a- and c-subunits form the V_o domain. The a-subunit and c-ring are embedded in lipid membrane. The rotation of the D/F/d rotor shaft and c-ring proceeds clockwise when viewed from V_1 to V_o domains, as indicated. Turnover is driven by the entry of ATP into a binding pocket at the interface of each A/B dimer, the hydrolysis reaction drives conformational changes which cause the rotation of the rotor. Inset shows the off-axis rotor.

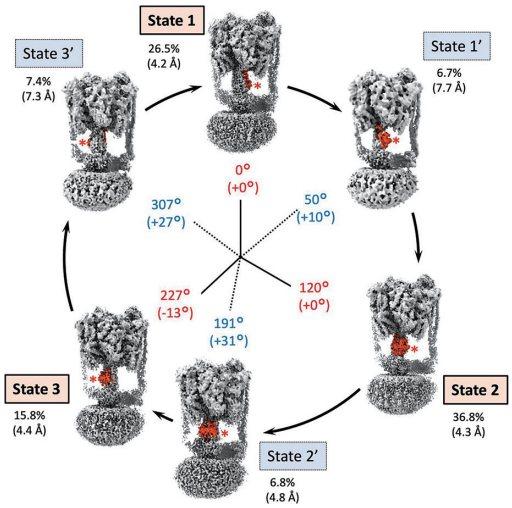

Figure 3 (top right). The six state structures of EhV-ATPase isolated. The F-subunit position is highlighted in red for easier identification of orientation of the rotor. Starting in State 1 at "12 o'clock" on the circle and proceeding clockwise when turnover is viewed from the V_1 to V_o domains. The six state structures are defined as State 1, State 1', State 2, State 2', State 3, and State 3' with comparisons to the other V-ATPases. Total rotation of the rotor at F subunit is labelled in red for the major states and blue for the intermediate states internally of the circle. The gaps from the orientations based on the single-molecule imaging studies (120° major pauses, and 40/80° subpauses in the major pauses) are in brackets. Relative percentages of the total final particles used and their resolutions (brackets) for each reconstruction are indicated externally of the circle. The cryo-EM maps of EhV-ATPase are aligned according to the orientation of the F-subunit.

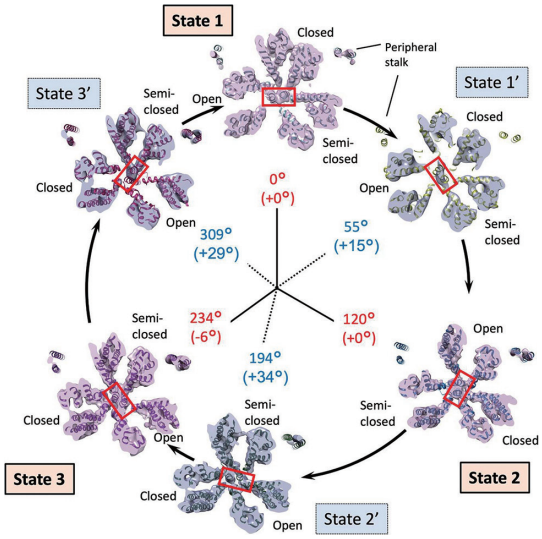

Figure 4. V_1 domain cross-section view of the six states. The figure is laid out as shown in Figure 3 viewed from the V_1 to V_o domains. The rotor D subunit is boxed in red, demonstrating the positions of a pair of the longest helices. The catalytic conformations of the A/B subunit in V_1 domain are indicated with "Open," "Closed," and "Semi-closed." The positions of peripheral stalk are labelled.

Reference

1) R. N. Burton-Smith, C. Song, H. Ueno, T. Murata, R. Iino and K. Murata, *Commun. Biol.* **6**, 755 (2023).

Award

HARASHIMA, Takanori; Best Presentation Award, 2022 Annual Meeting of the Biophysical Society of Japan Chubu Branch (2023).

* IMS International Internship Program