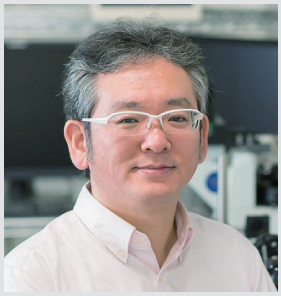
Operation and Design Principles of Biological Molecular Machines

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



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Activity of life is supported by protein molecular machines, which show higher performance than 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 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. In addition, with the help of structure-based rational design and site-saturation mutagenesis, we engineer non-natural protein molecular motors to understand their design principles. Furthermore, we challenge to engineer synthetic molecular motors.

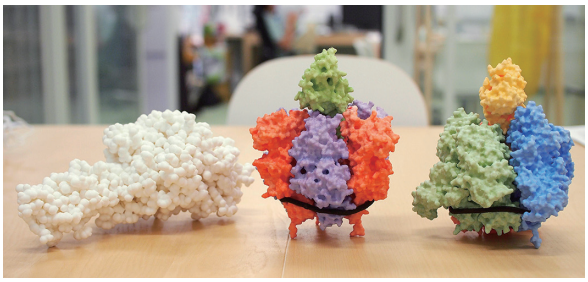

Figure 1. Protein molecular machines. (Left) A linear molecular motor chitinase A. (Center and Right) Rotary molecular motors F_1-ATPase and V_1-ATPase, respectively.

Selected Publications

- R. N. Burton-Smith, C. Song, H. Ueno, T. Murata, R. Iino and K. Murata, "Six States of *Enterococcus hirae* V-Type ATPase Reveals Non-Uniform Rotor Rotation during Turnover," *Commun. Biol.* **6**, 755 (2023).
- 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.* **15**, 1591–1598 (2023).
- 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).
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- 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).
- 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. Mechanism-Based Design of DNA-Nanoparticle Motor with High Speed and Processivity Comparable to Motor Proteins[1)]

DNA-nanoparticle motor is a burnt-bridge Brownian ratchet moving on RNA-modified surface driven by Ribonuclease H (RNase H), and one of the fastest nanoscale artificial motors. However, its speed is still much lower than those of motor proteins. Here we resolve elementary processes of motion and reveal long pauses caused by slow RNase H binding are the bottleneck (Figure 2). As RNase H concentration ([RNase H]) increases, pause lengths shorten from ~100 s to ~0.1 s, while step sizes are constant (~20 nm). At high [RNase H], speed reaches ~100 nm s^{-1}, however, processivity, run-length, and unidirectionality largely decrease. A geometry-based kinetic simulation reveals switching of bottleneck from RNase H binding to DNA/RNA hybridization at high [RNase H], and trade-off mechanism between speed and other performances. A mechanism-based newly-designed motor with 3.8-times larger DNA/RNA hybridization rate simultaneously achieves 30 nm s^{-1} speed, 200 processivity, and 3 μm run-length comparable to motor proteins.

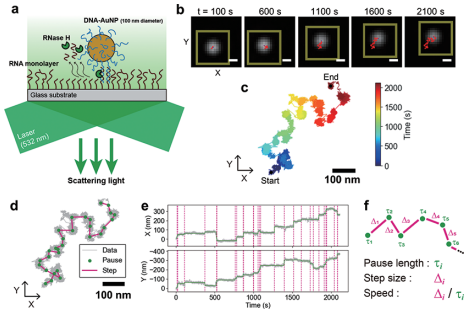

Figure 2. (a) Schematic illustration of the experimental system for high-speed/high-precision single particle tracking of DNA-AuNP motor. DNAs on the AuNP surface are hybridized with RNAs on the glass surface. RNAs that hybridized with DNAs are selectively hydrolyzed by RNase H. The motion of DNA-AuNP motor is visualized using total-internal reflection dark-field microscopy. (b) Motion of DNA-AuNP motor on RNA surface at 36 nM RNase H. Recording rate: 20 fps. Red lines indicate trajectories of the particle centroid. Dark yellow squares show the regions of interest to calculate the centroid for each frame. Scale bar: 200 nm. (c) Whole trajectory of the centroid of DNA-AuNP motor shown in (b). (d) Pauses (green dots) and steps (magenta lines) superimposed to raw trajectory shown in (c). (e) Time-course of XY-coordinates. Green solid lines are fittings to raw trajectories, and magenta dashed lines indicate positions of detected steps. (f) Schematic illustration and definitions of pause length, step size, and speed.

2. Visualizing Single V-ATPase Rotation Using Janus Nanoparticles[2)]

Understanding the function of rotary molecular motors, such as the rotary ATPases, relies on our ability to visualize the single-molecule rotation. Traditional imaging methods often involve tagging those motors with nanoparticles (NPs) and inferring their rotation from translational motion of NPs. Here, we report an approach using "two-faced" Janus NPs to directly image the rotation of single V-ATPase from *Enterococcus hirae*, an ATP-driven rotary ion pump (Figure 3 and 4). By employing a 500-nm silica/gold Janus NP, we exploit its asymmetric optical contrast—silica core with a gold cap on one hemisphere—to achieve precise imaging of the unidirectional counter-clockwise rotation of single V-ATPase motors immobilized on surfaces. Despite the added viscous load from the relatively large Janus NP probe, our approach provides accurate torque measurements of single V-ATPase. This study underscores the advantages of Janus NPs over conventional probes, establishing them as powerful tools for single-molecule analysis of rotary molecular motors.

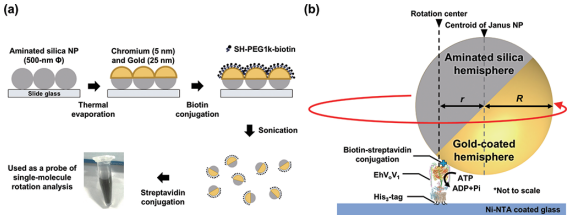

Figure 3. (a) Schematic of the Janus NP preparation process. (b) Schematic illustration of single-molecule imaging of V-ATPase rotation using a Janus NP probe. The rotor c-ring of V-ATPase is immobilized on the Ni-NTA coated coverslip via His_3-tags, while the single Janus NP is attached to the stator A-subunit of V-ATPase via biotin-streptavidin conjugation system. The black and grey dotted vertical lines indicate the rotation center and the centroid of Janus NP, respectively.

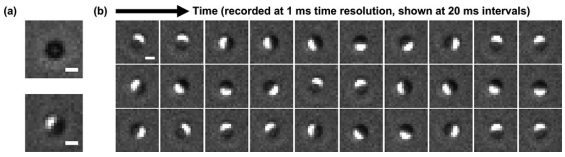

Figure 4. (a) Phase-contrast images of a single aminated silica NP (top) and a single Janus NP (bottom) non-specifically attached to the glass coverslip. (b) Time-lapse phase-contrast images demonstrating the rotational motion of a single Janus NP specifically attached to V-ATPase, driven by ATP hydrolysis. Observation was conducted at 25°C in the presence of 5 mM ATP and 300 mM NaCl. Images were recorded at 1,000 frames per second (1 ms time resolution) and are shown at 20 ms intervals. Scale bars: 500 nm.

References

1) T. Harashima, A. Otomo and R. Iino, *bioRxiv* (2024). DOI: 10.1101/2024.05.23.595615
2) A. Otomo, J. Wiemann, S. Bhattacharyya, M. Yamamoto, Y. Yu and R. Iino, *bioRxiv* (2024). DOI:10.1101/2024.08.22.609254

Awards

HARASHIMA, Takanori; Young Researcher Award at The 7th Annual Meeting of the Molecular Robotics (2024).
HARASHIMA, Takanori; Student and Early Carrier Researcher Poster Award, IUPAB2024 (2024).

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