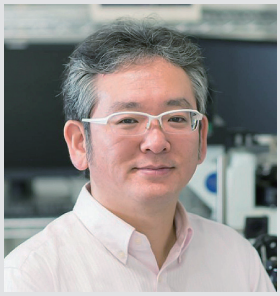
Operation and Design Principles of Biological Molecular Machines

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 mechanical forces and torques that drive their unidirectional motions from the energy of chemical reaction or the electrochemical potential across the cell membrane.

We unveil operation 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.

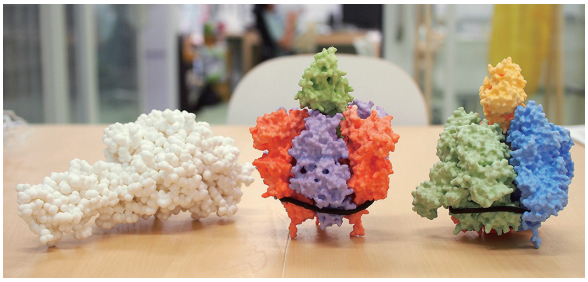

Figure 1. Protein molecular machines. (Left) A linear molecular motor chitinase A. (Center and Right) Rotary molecular motors F_1-ATPase and V_1-ATPase, respectively.

Selected Publications

- 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).
- A. Nakamura, T. Tasaki, Y. Okuni, C. Song, K. Murata, T. Kozai, M. Hara, H. Sugimoto, K. Suzuki, T. Watanabe, T. Uchihashi, H. Noji and R. Iino, "Rate Constants, Processivity, and Productive Binding Ratio of Chitinase A Revealed by Single-Molecule Analysis," *Phys. Chem. Chem. Phys.* **20**, 3010–3018 (2018).
- T. Uchihashi, Y. H. Watanabe, Y. Nakazaki, Y. Yamasaki, T. Watanabe, T. Maruno, S. Uchiyama, S. Song, K. Murata, R. Iino and T. Ando, "Dynamic Structural States of ClpB Involved in Its Disaggregation Function," *Nat. Commun.* **9**, 2147 (2018).

1. Small Stepping Motion of Processive Dynein Revealed by Load-Free High-Speed Single-Particle Tracking[1)]

Cytoplasmic dynein is a dimeric motor protein which processively moves along microtubule. Its motor domain (head) hydrolyzes ATP and induces conformational changes of linker, stalk, and microtubule binding domain (MTBD) to trigger stepping motion. Here we applied scattering imaging of gold nanoparticle (AuNP) to visualize load-free stepping motion of processive dynein (Figure 2). We observed artificially-dimerized chimeric dynein, which has the head, linker, and stalk from Dictyostelium discoideum cytoplasmic dynein and the MTBD from human axonemal dynein, whose structure has been well-studied by cryo-electron microscopy. One head of a dimer was labeled with 30 nm AuNP, and stepping motions were observed with 100 μs time resolution and sub-nanometer localization precision at physiologically-relevant 1 mM ATP. We found 8 nm forward and backward steps and 5 nm side steps, consistent with on- and off-axes pitches of binding cleft between αβ-tubulin dimers on the microtubule. Probability of the forward step was 1.8 times higher than that of the backward step, and similar to those of the side steps. One-head bound states were not clearly observed, and the steps were limited by a single rate constant. Our results indicate dynein mainly moves with biased small stepping motion in which only backward steps are slightly suppressed.

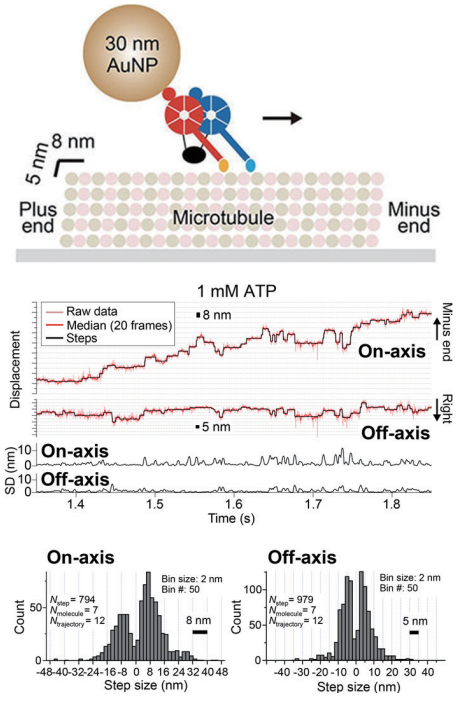

Figure 2. (Top) Schematic of single-molecule imaging of dynein motion. (Middle) Typical trajectory of motion. (Bottom) Distribution of step size in on- and off-axis.

2. Single-Molecule Imaging Analysis Reveals the Mechanism of a High-Catalytic-Activity Mutant of Chitinase A from *Serratia marcescens*[2)]

Chitin degradation is important for biomass conversion and has potential applications for agriculture, biotechnology, and the pharmaceutical industry. Chitinase A from the Gram-negative bacterium *Serratia marcescens* (*Sm*ChiA, Figure 3) is a processive enzyme that hydrolyzes crystalline chitin as it moves linearly along the substrate surface. In a previous study, the catalytic activity of *Sm*ChiA against crystalline chitin was found to increase after the tryptophan substitution of two phenylalanine residues (F232W and F396W), located at the entrance and exit of the substrate binding cleft of the catalytic domain, respectively. However, the mechanism underlying this high catalytic activity remains elusive. In this study, single-molecule fluorescence imaging and high-speed atomic force microscopy were applied to understand the mechanism of this high-catalytic-activity mutant. A reaction scheme including processive catalysis was used to reproduce the properties of *Sm*ChiA WT and F232W/F396W, in which all of the kinetic parameters were experimentally determined. High activity of F232W/F396W mutant was caused by a high processivity and a low dissociation rate constant after productive binding. The turnover numbers for both WT and F232W/F396W, determined by the biochemical analysis, were well-replicated using the kinetic parameters obtained from single-molecule imaging analysis, indicating the validity of the reaction scheme. Furthermore, alignment of amino acid sequences of 258 *Sm*ChiA-like proteins revealed that tryptophan, not phenylalanine, is the predominant amino acid at the corresponding positions (Phe-232 and Phe-396 for *Sm*ChiA). Our study will be helpful for understanding the kinetic mechanisms and further improvement of crystalline chitin hydrolytic activity of *Sm*ChiA mutants.

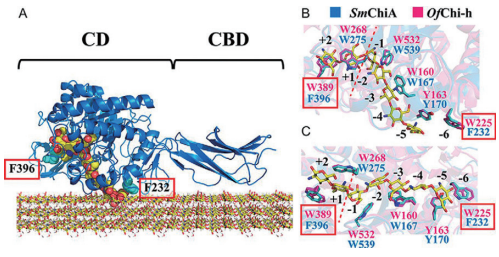

Figure 3. (A) Structural model of *Sm*ChiA bound to crystalline chitin. (B and C) Side and bottom views of aromatic amino acid residues (cyan and pink) and bound chitin (yellow) in the catalytic cleft of *Sm*ChiA and OfChi-h.

References

1) J. Ando *et al.*, *Sci. Rep.* **10**, 1080 (2020).
2) A, Visootsat *et al.*, *J. Biol. Chem.* **295**, 1915–1925 (2020).

Awards

NAKAMURA, Akihiko; Early Career Award in Biophysics, 2019 Annual Meeting of the Biophysical Society of Japan (2019).
IIDA, Tatsuya; Student Presentation Award, 2019 Annual Meeting of the Biophysical Society of Japan (2019).

* IMS International Internship Program