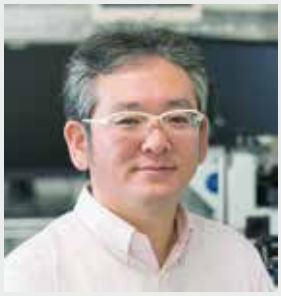
Operation and Design Principles of Biological Molecular Machines

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



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Professional Employment
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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 will unveil operation principles of molecular motors with advanced single-molecule functional analysis and structural analysis. With the help of computer science, we will also engineer new, non-natural molecular motors to understand their design principles. Our ultimate goal is controlling living organisms with created molecular machines.

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

- 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).
- F. Kawai, A. Nakamura, A. Visootsat and R. Iino, "Plasmid-Based One-Pot Saturation Mutagenesis and Robot-Based Automated Screening for Protein Engineering,"*ACS Omega* **3**, 7715–7726 (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).
- H. Isojima, R. Iino, Y. Niitani, H. Noji and M. Tomishige, "Direct Observation of Intermediate States during the Stepping Motion of Kinesin-1," *Nat. Chem. Biol.* **12**, 290–297 (2016).
- A. Nakamura, T. Tasaki, D. Ishiwata, M. Yamamoto, Y. Okuni, A. Visootsat, M. Maximilien, H. Noji, T. Uchiyama, M. Samejima, K. Igarashi and R. Iino, "Direct Imaging of Binding, Dissociation, and Processive Movement of *Trichoderma reesei* Cel6A and Its Domains on Crystalline Cellulose," *J. Biol. Chem.* **291**, 22404–22413 (2016).
- H. Ueno, Y. Minagawa, M. Hara, S. Rahman, I. Yamato, E. Muneyuki, H. Noji, T. Murata and R. Iino, "Torque Generation of *Enterococcus hirae* V-ATPase," *J. Biol. Chem.* **289**, 31212–31223 (2014).
- T. Uchihashi, R. Iino, T. Ando and H. Noji, "High-Speed Atomic Force Microscopy Reveals Rotary Catalysis of Rotorless F_1-ATPase," *Science* **333**, 755–758 (2011).

1. Single-Nanoparticle Tracking with Angstrom Localization Precision and Microsecond Time Resolution[1)]

Gold nanoparticle (AuNP) has been used as a probe of single-molecule imaging of molecular motors. We investigated lower limit of the localization precision in dark-field imaging of AuNP. We confirmed that the localization precision is inversely proportional to square root of photon number, and the lower limit is determined by detector saturation. To overcome the limit, we developed an axicon lens-based annular illumination total internal reflection dark-field microscopy, which illuminates AuNP with high laser intensity to obtain high signal intensity and observes AuNP with small image pixel size to avoid detector saturation. As results, with 40 nm AuNP, 1.3 Å and 5.4 Å localization precisions have been achieved at 1 ms and 33 μs time resolutions, respectively (Figure 2). We then observed transition pathways from bound to unbound states of the kinesin-1 head (Figure 3). During transitions, large leftward trails were not observed along the microtubule short axis indicating that the rear head passes the microtubule-bound leading head from the right, which results in unidirectional rotation of kinesin-1.

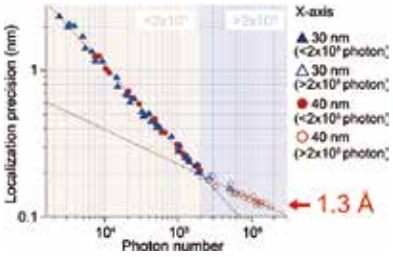

Figure 2. Photon number dependence of the localization precision for the 40 nm and 30 nm AuNPs.

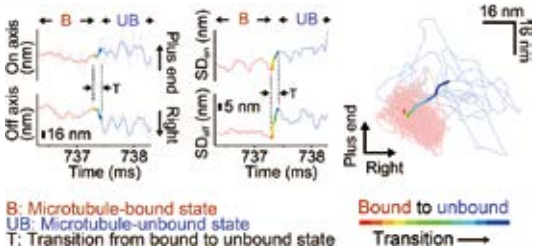

Figure 3. Transition from bound to unbound state of the kinesin-1 head labeled with 40 nm AuNP at 10 μs time resolution.

2. Processive Chitinase Is Brownian Monorail Operated by Fast Catalysis after Peeling Rail from Crystalline Chitin[2)]

Processive chitinase is a linear molecular motor which moves on the surface of crystalline chitin driven by processive hydrolysis of single chitin chain (Figure 4). Here, we analysed the mechanism underlying unidirectional movement of *Serratia marcescens* chitinase A (*Sm*ChiA) using high-precision single-molecule imaging, X-ray crystallography, and all-atom molecular dynamics simulation. *Sm*ChiA showed fast unidirectional movement of ~50 nm s^{-1} with 1-nm forward and backward steps, consistent with the length of reaction product chitobiose. Analysis of the kinetic isotope effect revealed fast substrate-assisted catalysis with time constant of ~3 ms. Decrystallisation of the single chitin chain from crystal surface is the rate-limiting step of movement with time constant of ~17 ms, achieved by binding free energy at the product binding site of *Sm*ChiA (Figure 5). Our results demonstrate that *Sm*ChiA operates as a burnt-bridge Brownian ratchet wherein the Brownian motion along the single chitin chain is rectified forward by substrate-assisted catalysis.

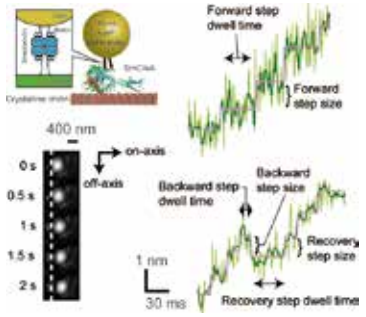

Figure 4. Examples of image and trajectory of *Sm*ChiA probed by 40 nm AuNP.

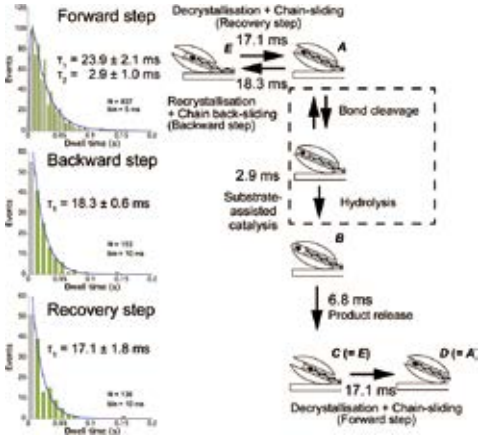

Figure 5. Distribution of dwell times and summary of time constants of elementally steps.

References

1) J. Ando, A. Nakamura, A. Visootsat, M. Yamamoto, C. Song, K. Murata and R. Iino, in press.
2) A. Nakamura, K. Okazaki, T. Furuta, M. Sakurai and R. Iino, *Nat. Commun.* **9**, 3814 (2018).

Award

ANDO, Jun; Young Scientist Presentation Award of the Spectroscopical Society of Japan 2017 (2017).

* IMS International Internship Program