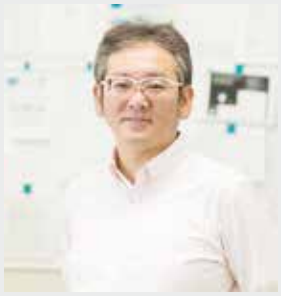
Operation and Design Principles of Biological Molecular Machines

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 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

- 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).
- Y. Shibafuji, A. Nakamura, T. Uchihashi, N. Sugimoto, S. Fukuda, H. Watanabe, M. Samejima, T. Ando, H. Noji, A. Koivula, K. Igarashi and R. Iino, "Single-Molecule Imaging Analysis of Elementary Reaction Steps of *Trichoderma reesei* Cellobiohydrolase I (Cel7A) Hydrolyzing Crystalline Cellulose I_α and III_I," *J. Biol. Chem.* **289**, 14056–14065 (2014).
- Y. Minagawa, H. Ueno, M. Hara, Y. Ishizuka-Katsura, N. Ohsawa, T. Terada, M. Shirouzu, S. Yokoyama, I. Yamato, E. Muneyuki, H. Noji, T. Murata and R. Iino, "Basic Properties of Rotary Dynamics of the Molecular Motor *Enterococcus hirae* V_1-ATPase," *J. Biol. Chem.* **288**, 32700–32707 (2013).
- 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. One Nanometer Steps and Rate-Limiting Step of *Serratia marcescens* Chitinase A Resolved by Gold Nanoprobe[1)]

Serratia marcescens chitinase A (*Sm*ChiA) is a monomeric linear molecular motor moving on and hydrolyzing crystalline chitin processively. We have directly resolved steps and pauses in the motion of *Sm*ChiA with high-resolution single-molecule imaging analysis with gold nanoparticle. By using total internal reflection dark-field microscopy and 40-nm gold nanoparticle as a low-load probe, movement of *Sm*ChiA was observed at 1,000–2,000 frames/s with 0.3 nm localization precision (Figure 2). The step sizes were 1.1 nm and –1.2 nm for forward and backward steps (Figure 3), respectively, consistent with the length of the product, chitobiose (~1 nm). The ratio of forward to backward steps was 5.5, corresponding to the energy difference of 1.7 k_BT. Frequent backward steps and low energy difference indicate that *Sm*ChiA operates as the Brownian ratchet. Furthermore, detailed analysis of the distribution of pause duration revealed that the rate-limiting step of chemo-mechanical coupling of *Sm*ChiA is the decrystallization of single polymer chain from the crystalline chitin, not bond cleavage and product release. These results give us important insights to engineer non-natural chitinases which show better performances than the natural ones.

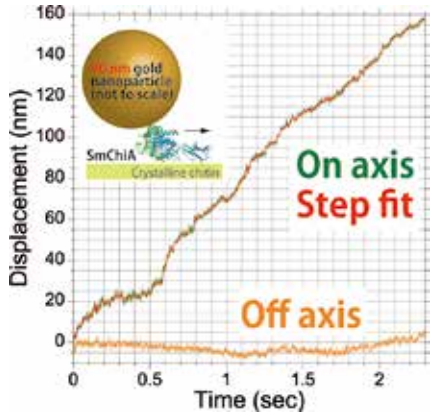

Figure 2. Example of stepping movement of *Sm*ChiA.

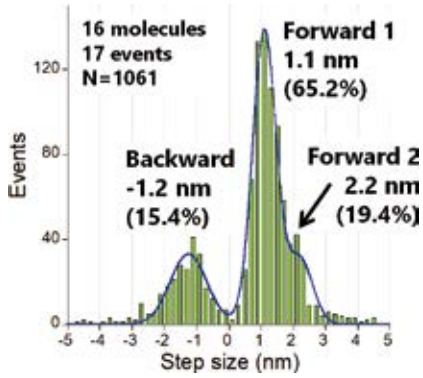

Figure 3. Distribution of step size of *Sm*ChiA.

2. Chemo-Mechanical Coupling Scheme of Rotary Molecular Motor *Enterococcus hirae* V_1-ATPase[2)]

A rotary molecular motor V-ATPase (Figure 4, left) is an ion pump driven by ATP hydrolysis. To understand the chemo-mechanical energy conversion mechanism, we conducted single-molecule analysis of V_1 moiety of *Enterococcus hirae* V-ATPase (Figure 4, right). We found that 120° steps (3 pausing positions per turn) reflecting the coordinations among three catalytic sites of V_1 were further divided into 40° and 80° substeps. At low ATP concentration ([ATP]), pause duration before 40° substep was dependent on [ATP], indicating that ATP binding triggers 40° substeps. On the other hand, at high [ATP], two time constants (both ~1 ms) independent on [ATP] were obtained. When slowly hydrolyzing ATPγS was used as a substrate, the pause before 40° step became longer (140 ms), indicating that cleavage of phosphate bond of ATP occurs during this pause. Time constant (2.5 ms) of pause duration before 80° step was also [ATP] independent. In the presence of ATPγS and high concentration of ADP, 80° backward steps were frequently observed, indicating that ADP binding triggers 80° forward step. From these results and rotation behavior of an arginine finger mutant, we proposed a model of chemo-mechanical coupling scheme of V_1 (Figure 5).

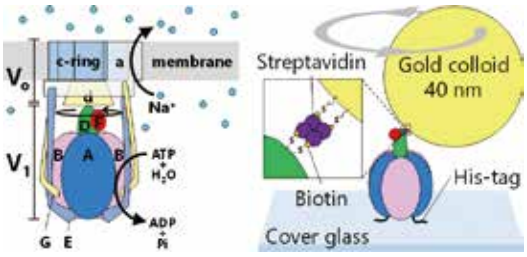

Figure 4. V-ATPase (left) and single-molecule rotation assay of V_1 (right).

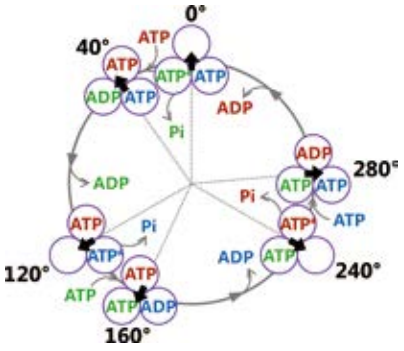

Figure 5. Chemo-mechanical coupling scheme of V_1.

References

1) A. Nakamura and R. Iino, in preparation.
2) T. Iida, Y. Minagawa, H. Ueno, F. Kawai, T. Murata and R. Iino, in preparation.