Operation and Design Principles of Biological Molecular Machines

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Keywords Molecular Machines, Protein Engineering, Single-Molecule Analysis

Activity of life is supported by various molecular machines made of proteins. These biological molecular machines are tiny, but show high performance, and are superior to man-made machines in many aspects.

One of the representatives of the 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.

We will unveil operation principles of molecular motors with single-molecule functional analysis and advanced structural analysis. With the help of computer science, we will also engineer new, non-natural molecular machines 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

- S. Enoki, R. Iino, Y. Niitani, Y. Minagawa, M. Tomishige and H. Noji, "High-Speed Angle-Resolved Imaging of Single Gold Nanorod with Microsecond Temporal Resolution and One-Degree Angle Precision," *Anal. Chem.* **87**, 2079–2086 (2015).
- A. Yukawa, R. Iino, R. Watanabe, S. Hayashi and H. Noji, "Key Chemical Factors of Arginine Finger Catalysis of F_1-ATPase Clarified by an Unnatural Amino Acid Mutation," *Biochemistry* **54**, 472–480 (2015).
- R. Iino, H. Ueno, Y. Minagawa, K. Suzuki and T. Murata, "Rotational Mechanism of *Enterococcus hirae* V_1-ATPase by Crystal-Structure and Single-Molecule Analyses," *Curr. Opin. Struct. Biol.* **31**, 49–56 (2015).
- 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. Direct Observation of Intermediate States during the Stepping Motion of Kinesin-1[1)]

The dimeric motor protein kinesin-1 walks along microtubules by alternatingly hydrolyzing ATP and moving two motor domains ('heads'). Nanometer-precision single-molecule studies demonstrated that kinesin takes regular 8-nm steps upon hydrolysis of each ATP; however, the intermediate states between steps have not been directly visualized. Here, we employed high-temporal resolution dark-field microscopy to directly visualize the binding and unbinding of kinesin heads to or from microtubules during processive movement (Figure 2). Our observations revealed that upon unbinding from microtubules, the labeled heads were displaced rightward and underwent tethered diffusive movement. Structural and kinetic analyses of wild-type and mutant kinesins with altered neck linker lengths provided evidence that rebinding of the unbound head to the rear-binding site is prohibited by a tension increase in the neck linker and that ATP hydrolysis by the leading head is suppressed when both heads are bound to the microtubule, thereby explaining how the two heads coordinate to move in a hand-over-hand manner.

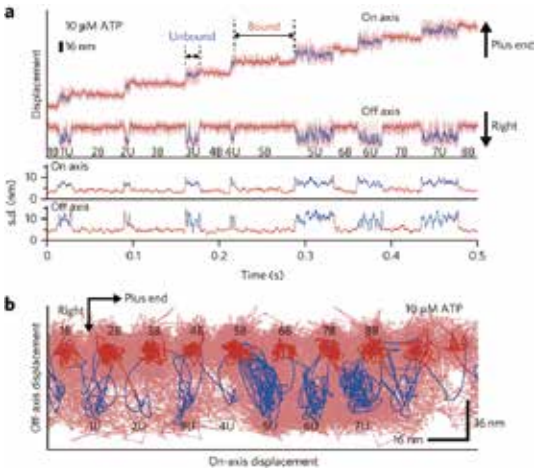

Figure 2. (a) Typical trace for the centroid position of the gold probe attached to a kinesin head (light red lines), toward the microtubule long axis (on axis) and perpendicular to the microtubule axis (off axis). Red and blue lines depict the median-filtered traces (window size of 51 frames) for the bound and unbound states, respectively. Lower panel shows the s.d. of on- and off-axis positions for each time frame t (calculated as [t–20, t+20]). (b) Two-dimensional plot of the gold probe shown in a. Numbers denote the temporal order of the bound (B) and unbound (U) states.

2. Direct Imaging of Binding, Dissociation, and Processive Movement of *Trichoderma reesei* Cel6A and Its Domains on Crystalline Cellulose[2)]

Trichoderma reesei Cel6A (*Tr*Cel6A) is a cellobiohydrolase that hydrolyzes crystalline cellulose into cellobiose. Here, we observed the binding, dissociation, and movement of single-molecule intact *Tr*Cel6A on a crystalline cellulose, in addition to isolated catalytic domain (CD), cellulose-binding module and linker (CBM-Linker), and CBM (Figure 3). The CBM-Linker had a binding rate constant almost half that of intact *Tr*Cel6A, whereas those of the CD and CBM were only one-tenth of intact *Tr*Cel6A. These results indicate that the linker region largely contributes to initial binding on crystalline cellulose. After binding, all samples showed slow and fast dissociations, likely caused by the two different bound states due to the heterogeneity of cellulose surface. The CBM showed much higher (12-times) specificity to the high-affinity site than to the low-affinity site, whereas the CD did not, suggesting that the CBM leads the CD to the hydrophobic surface of crystalline cellulose. The intact molecules showed slow, processive movements (8.8 ± 5.5 nm/s) in addition to fast diffusional movements (30–40 nm/s), whereas the CBM-Linker, the CD, and a full-length but catalytically inactive mutant showed only fast diffusional movements. These results suggest that in addition to direct binding, surface diffusion also contributes to the searching of the hydrolysable point of the cellulose chains. The duration time constant for the processive movement was 7.7 s. Our results reveal the role of each domain in the elementary steps of the reaction cycle and provide the first direct evidence of the processive movement of *Tr*Cel6A on crystalline cellulose.

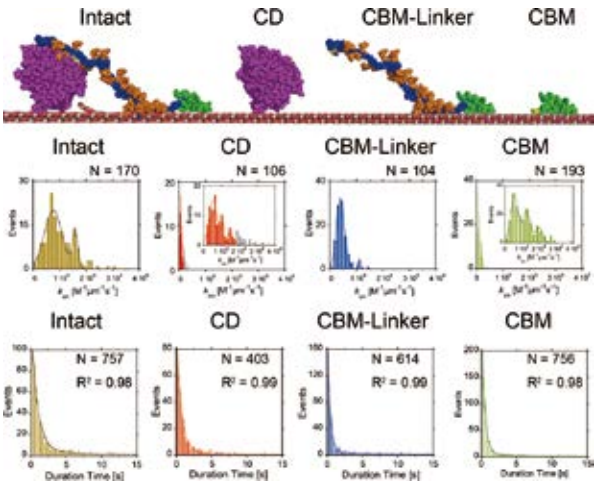

Figure 3. (Top) Domain structures of Intact, CD, CBM-Linker, and CBM of *Tr*Cel6A. (Middle) Distributions of the binding rate constant. (Bottom) Distributions of the duration time on cellulose.

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