Operation and Design Principles of Biological Molecular Machines

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Activity of life is supported by various molecular machines made of proteins and nucleic acids. These biological molecular machines show high performance such as reaction specificity and energy conversion efficiency, and are superior to man-made machines in some 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 biological molecular motors and machines with single-molecule techniques based on optical microscopy. We will also try to create new biological molecular motors and machines to understand their design principles. Our ultimate goal is controlling living organisms with created molecular machines.

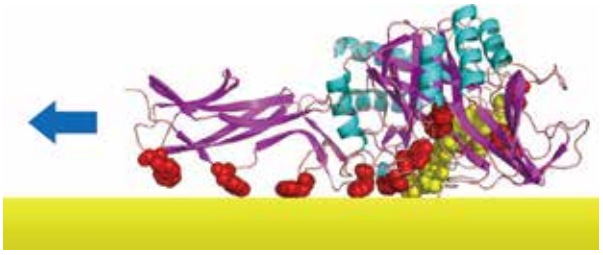

Figure 1. A linear molecular motor chitinase. Chitinase moves on the substrate crystalline chitin unidirectionally and processively, driven by the energy of hydrolysis of the chain end of the chitin.

Selected Publications

- 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).
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- 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. Key Chemical Factors of Arginine Finger Catalysis of F_1-ATPase Clarified by an Unnatural Amino Acid Mutation[1)]

A catalytically important arginine, called Arg finger, is employed in many enzymes to regulate their functions through enzymatic hydrolysis of nucleotide triphosphates. F_1-ATPase, a rotary molecular motor, possesses Arg fingers which catalyze hydrolysis of adenosine triphosphate (ATP) for efficient chemo-mechanical energy conversion. In this study, we examined the Arg finger catalysis by single-molecule measurements for a mutant of F_1-ATPase in which the Arg finger is substituted with an unnatural amino acid of a lysine analogue, 2,7-diaminoheptanoic acid (Lyk). The use of Lyk, of which the side chain is elongated by one CH_2 unit so that its chain length to the terminal nitrogen of amine is set to be equal to that of arginine, allowed us to resolve key chemical factors in the Arg finger catalysis, *i.e.*, chain length matching and chemical properties of the terminal groups. Rate measurements by single-molecule observations showed that the chain length matching of the side-chain length is not a sole requirement for the Arg finger to catalyze the ATP hydrolysis reaction step, indicating the crucial importance of chemical properties of the terminal guanidinium group in the Arg finger catalysis. On the other hand, the Lyk mutation prevented severe formation of an ADP inhibited state observed for a lysine mutant and even improved the avoidance of inhibition compared with the wild-type F_1-ATPase. The present study demonstrated that incorporation of unnatural amino acids can widely extend with its high "chemical" resolution biochemical approaches for elucidation of the molecular mechanism of protein functions and furnishing novel characteristics.

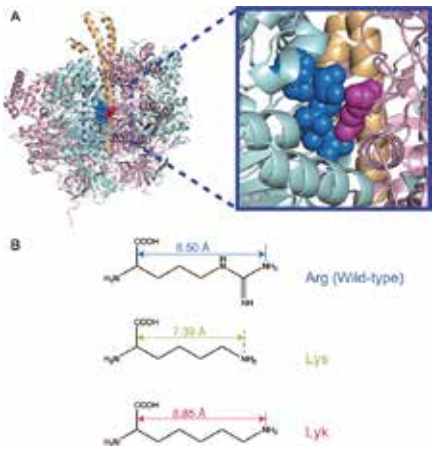

Figure 2. (A) Crystal structure of mitochondrial F_1–ATPase viewed from the side, β_{DP}/α_{DP} catalytic interface. The α, β, and γ subunits are shown in pearl pink, pearl blue, and pearl yellow, respectively. The "arginine finger" in the α subunit is shown by pink space-filling model. AMP-PNP bound to the catalytic site are shown by blue space-filling model. (B) Chemical structures and side-chain length of arginine (Arg, top), lysine (Lys, middle), and 2,7-diaminoheptanoic acid (Lyk, bottom).

2. High-Speed Angle-Resolved Imaging of Single Gold Nanorod with Microsecond Temporal Resolution and One-Degree Angle Precision[2)]

We developed two types of high-speed angle-resolved imaging methods for single gold nanorods (SAuNRs) using objective-type vertical illumination dark-field microscopy and a high-speed CMOS camera to achieve microsecond temporal and one-degree angle resolution. These methods are based on: (i) an intensity analysis of focused images of SAuNR split into two orthogonally polarized components and (ii) the analysis of defocused SAuNR images. We determined the angle precision (statistical error) and accuracy (systematic error) of the resultant SAuNR (80 nm × 40 nm) images projected onto a substrate surface (azimuthal angle) in both methods. Although both methods showed a similar precision of ~1° for the azimuthal angle at a 10 μs temporal resolution, the defocused image analysis showed a superior angle accuracy of ~5°. In addition, the polar angle was also determined from the defocused SAuNR images with a precision of ~1°, by fitting with simulated images. By taking advantage of the defocused image method's full revolution measurement range in the azimuthal angle, the rotation of the rotary molecular motor, F_1-ATPase, was measured with 3.3 μs time resolution. The time constants of the pauses waiting for the elementary steps of the ATP hydrolysis reaction and the torque generated in the mechanical steps have been successfully estimated. The high-speed angle-resolved SAuNR imaging methods will be applicable to the monitoring of the fast conformational changes of many biological molecular machines.

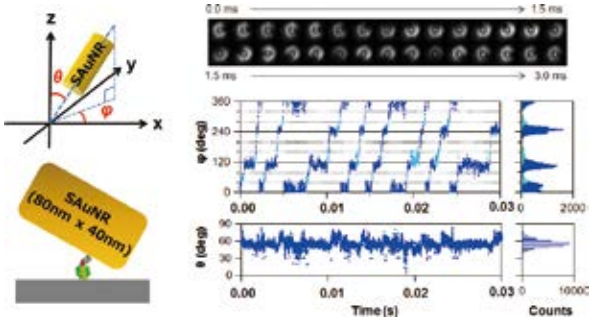

Figure 3. (Left) Schematic image of experimental system of rotation assay of F_1-ATPase using single gold nanorod (SAuNR) as a probe. (Right) Example of rotation of F_1-ATPase probed at 3.3 μs time resolution.

References

1) A. Yukawa, R. Iino, R. Watanabe, S. Hayashi and H. Noji, *Biochemistry* **54**, 472–480 (2015).
2) S. Enoki, R. Iino, Y. Niitani, Y. Minagawa, M. Tomishige and H. Noji, *Anal. Chem.* **87**, 2079–2086 (2015).