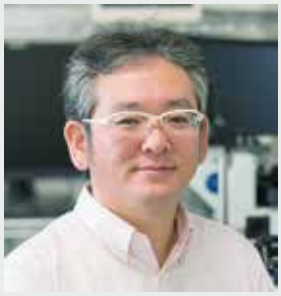
Operation and Design Principles of Biological Molecular Machines

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 and X-ray crystallographic structural analysis. With the help of computer science and robotic 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, 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).
- 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).
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1. Rotational Substeps and Chemo-Mechanical Coupling Scheme of *Enterococcus hirae* V_1-ATPase[1)]

V_1-ATPase (V_1), the catalytic domain of an ion pump V-ATPase, is a rotary molecular motor and converts chemical energy of ATP hydrolysis into mechanical rotation. To understand chemo-mechanical coupling mechanism of *Enterococcus hirae* V_1 (EhV_1), we directly observed rotation of newly-constructed EhV_1 with gold nanoparticle probe. We found that 120° steps per ATP hydrolysis were divided into 40° and 80° substeps. In the main-pause before 40° substep, time constant was inversely proportional to ATP concentration ([ATP]) at low [ATP], indicating that ATP binds during the main-pause with rate constant of 1.0×10^7 $M^{-1}s^{-1}$. At high [ATP], [ATP]-independent two time constants (0.5, 0.7 ms) were obtained. One of two time constants was prolonged (143 ms) in rotation driven by slowly-hydrolyzable ATPγS, indicating that ATP cleavage occurs during the main-pause. In another subpause before 80° substep, [ATP]-independent time constant (2.5 ms) was obtained. Furthermore, in ATP-driven rotation of an arginine-finger mutant in the presence of ADP, −80° and −40° backward steps were observed. Time constants of the pauses before −80° backward and +40° recovery steps were inversely proportional to [ADP] and [ATP], respectively, indicating that these steps are triggered by ADP and ATP bindings. Assuming that backward steps are reverse reactions, we concluded that 40° and 80° substeps are triggered by ATP binding and ADP release, respectively. The remaining time constant in the main-pause was considered to be phosphate release. Combined with previous structural information, we propose a chemo-mechanical coupling scheme of EhV_1 including substeps, largely different from those of F_1-ATPases (Figure 2).

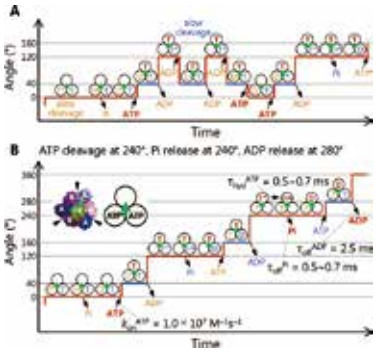

Figure 2. A model of chemo-mechanical coupling of EhV_1 including substeps and backward steps.

2. Multi-Color High-Speed Tracking of Single Biomolecules with Silver, Gold, Silver-Gold Alloy Nanoparticles[2)]

Gold nanoparticles have been used as an imaging probe to track motions of single biomolecules. Since they show high scattering signals, single-particle tracking has been performed with microsecond time resolution and nanometer localization precision. To investigate behaviors of various kinds of biomolecules simultaneously, increase of the color palette is necessary. Here we developed a multi-color, high-speed single-particle tracking system by using silver, gold, and silver-gold alloy nanoparticles. Peak wavelengths of plasmon resonance for silver and gold nanoparticles are around 400 nm and 530 nm, respectively, and those for silver-gold alloy nanoparticles can be modulated between 400 nm and 530 nm depending on their composition ratio. We constructed multi-color total internal reflection dark-field microscopy with multiple lasers at 404 nm for silver, 473 nm for silver-gold alloy, and 561 nm for gold nanoparticles. By using a spectrophotometer in the imaging optics, scattering images at each wavelength were projected onto the different portion of a two-dimensional detector (Figure 3 and 4). High contrast images of 30 nm silver, 30 nm silver-gold alloy, and 40 nm gold nanoparticles were simultaneously obtained at 404, 473, and 561 nm channels, respectively. With this system, diffusional motions of phospholipids in supported lipid membrane and stepping motions of kinesins along microtubules were observed with 2 nm localization precision and 100 μs time resolution. Furthermore, introduction of 649 nm laser enabled detection of plasmon coupling and transient dimer formation of two nanoparticles. Our method will pave the way to investigate operation mechanisms of complex biomolecular systems and multi-subunit biomolecular motors and machines.

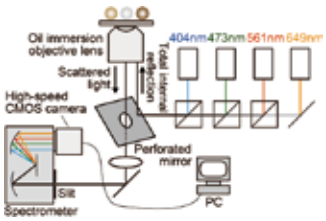

Figure 3. Spectrometer-based multi-color dark-field imaging system.

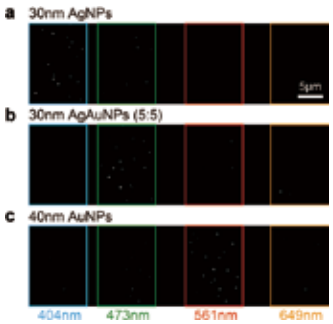

Figure 4. Spectrometer-based dark-field imaging of AgNPs, AgAuNPs (5:5), and AuNPs.

References

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2) J. Ando, A. Nakamura, M. Yamamoto, C. Song, K. Murata and R. Iino, *ACS Photonics*, published online (2019).

* IMS International Internship Program