Functional Dynamics of Biomolecular Machines Revealed by Theoretical Methods

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Functional dynamics plays an important role when biomolecular machines fulfill their functions. For example, motor proteins walk on the rail or rotate relative to the stator by using ATP hydrolysis energy. Transporter proteins transport their substrates across the membrane by changing their conformation between inward-open and outward-open conformations. We aim to understand design principles of these precise, yet dynamic nano-machines developed by nature.

Functional dynamics of biomolecular machines involve wide spectrum of intricate motions and reactions. In order to understand such dynamics, we need a multiscale approach to cover full range of these motions and reactions. Conventional atomistic molecular dynamics simulations alone cannot cover millisecond-long (or even longer) functional dynamics, especially for a large system like typical biomolecular machines with more than hundreds of thousand atoms including water molecules. Thus, we use both atomistic and coarse-grained molecular simulations, as well as kinetic models based on statistical mechanics, to tackle this problem.

We have been working on ATP synthase that produces most of ATP required for living activities. The ATP synthase is composed of two rotary motors, F_o and F_1. The F_o motor is embedded in membrane and its rotation is driven by proton gradient. The F_1 motor is a catalytic part that produces ATP from ADP and P_i. However, the F_1 motor by itself (F_1-ATPase) rotates the central stalk, γ-subunit, in the opposite direction by hydrolyzing ATP. Thus, the two motors are driven by different energy sources and rotate in the opposite directions. In order to understand how ATP synthase works, we have to look into both individual motors and ATP synthase as a whole.

We are also working on other types of biomolecular machines like chitinase that shows a unidirectional motion by hydrolyzing chitin, Na^+/H^+ antiporter that exchanges sodium ions and protons inside and outside the cell. Methodological development and application of sampling rare events are our interests too.

Selected Publications

- K. Okazaki and G. Hummer, "Phosphate Release Coupled to Rotary Motion of F_1-ATPase," *Proc. Natl. Acad. Sci. U.S.A.* **110**, 16468–16473 (2013).
- K. Okazaki and G. Hummer, "Elasticity, Friction, and Pathway of γ-Subunit Rotation in F_oF_1-ATP Synthase," *Proc. Natl. Acad. Sci. U.S.A.* **112**, 10720–10725 (2015).
- M. Sugawa, K. Okazaki, M. Kobayashi, T. Matsui, G. Hummer, T. Masaike and T. Nishizaka, "F_1-ATPase Conformational Cycle from Simultaneous Single-Molecule FRET and Rotation Measurements," *Proc. Natl. Acad. Sci. U.S.A.* **113**, E2916–E2924 (2016).
- H. Jung, K. Okazaki and G. Hummer, "Transition Path Sampling of Rare Events by Shooting from the Top," *J. Chem. Phys.* **147**, 152716 (2017).
- 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). doi:10.1038/s41467-018-06362-3

1. Mechanochemical Coupling Mechanism of F_1-ATPase

Many single-molecule studies as well as crystallographic studies have clarified how the γ-subunit rotation is coupled to ATP hydrolysis reactions at three catalytic sites of F_1. As summarized in Figure 1B, main points are, 1) 120° step inferred from three-fold symmetry is further divided into 80° and 40° substeps, 2) the 80° substep is driven by ATP binding and ADP release, 3) the 40° substep is driven by P_i release and ATP hydrolysis reaction, 4) typical crystal structures correspond to catalytic dwell (before 40° substep). There are still some remaining questions, though. What is the timing of P_i release: Just after the hydrolysis reaction or after ADP release? What conformation does it take in ATP-binding dwell (before 80° substep)?

We resolved the timing of P_i release by using atomistic molecular dynamics simulations.[1] The question is, essentially, from which catalytic site, DP-site or E-site, P_i is released. Since the P_i release takes ~millisecond, a biasing method called metadynamics was employed to facilitate the functional dynamics. Different pathways were observed depending on the site P_i was released. From the E-site it went through P-loop toward outside of the ring structure, while from the DP-site it went through switch II toward inside of the ring structure (Figure 1C). We estimated mean first-passage time from free energy profile (Figure 1D) and diffusion coefficient and concluded that P_i is release from the E-site. That is, P_i is released after ADP release, which is unique among other members of ATPases.

We also identified conformational state of the ATP-binding dwell by combining single-molecule FRET measurements and systematic structural analysis.[2] We found that an ε-inhibited *E. Coli* structure that has half-closed β_{DP} and loose $\alpha\beta_E$ interface is consistent with the conformation taken in the ATP-binding dwell.

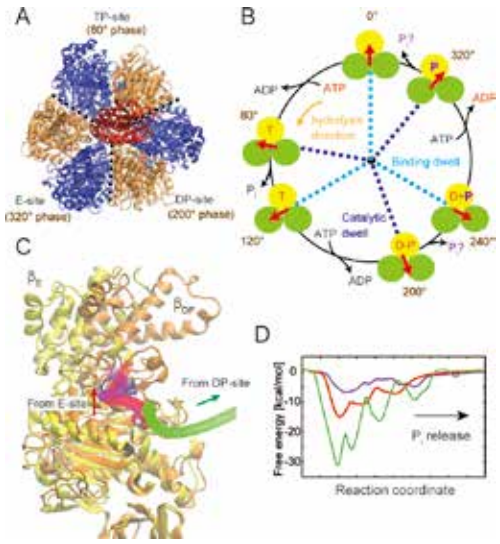

Figure 1. Mechanochemical coupling scheme and P_i release in F_1-ATPase.

2. Torsional Elasticity and Friction of Rotor in F_oF_1-ATP Synthase

It has been known that there is a symmetry mismatch between F_o c-ring and F_1 $\alpha_3\beta_3$ ring. The F_1 $\alpha_3\beta_3$ ring has (pseudo) three-fold symmetry, while the F_o c-ring in animal mitochondria has 8-fold symmetry. Thus, the common rotor, γ-subunit, has to rotate by 120° steps (or 80°+40° substeps) in F_1 part, while it has to rotate by 45° steps in F_o part. Therefore, it has to have torsional elasticity to solve the mismatch. In order to estimate torsional elasticity as well as viscosity of the γ-rotation, we built a simple viscoelastic model (Figure 2B) and fitted it against atomistic simulation trajectories in which external torque was applied on γ.[3]

The estimated torsional elasticity is consistent with values from single-molecule experiments. By using this elasticity, we identified pathways and associated free energies of the coupled F_oF_1 rotation (Figure 2C). It turned out that with the two-substep F_1 the pathway is blocked by high-energy states. To solve this situation, F_1 needs three substeps as was measured for human mitochondrial F_1 recently. From the estimated torsional friction, we predict that γ-rotation can rotate as fast as 1 MHz and this fast rotation can be observed with an attached bead as small as 20 nanometer diameter.

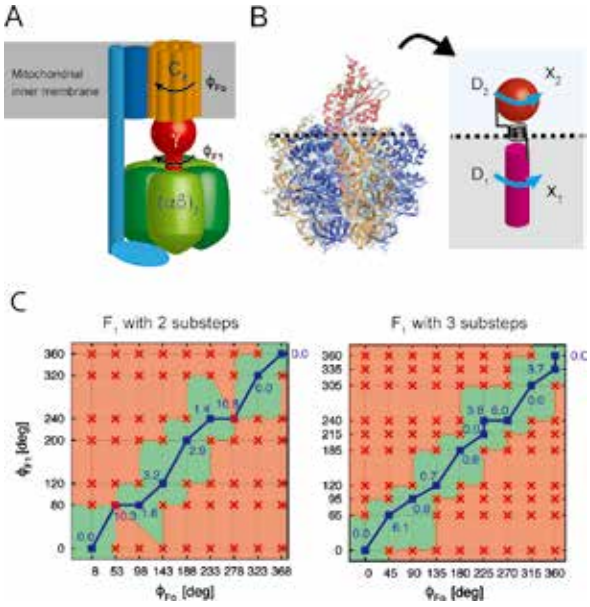

Figure 2. Viscoelastic model of F_oF_1-ATP synthase.

References

1) K. Okazaki and G. Hummer, *Proc. Natl. Acad. Sci. U.S.A.* **110**, 16468–16473 (2013).

2) M. Sugawa, K. Okazaki, M. Kobayashi, T. Matsui, G. Hummer, T. Masaike and T. Nishizaka, *Proc. Natl. Acad. Sci. U.S.A.* **113**, E2916–E2924 (2016).

3) K. Okazaki and G. Hummer, *Proc. Natl. Acad. Sci. U.S.A.* **112**, 10720–10725 (2015).