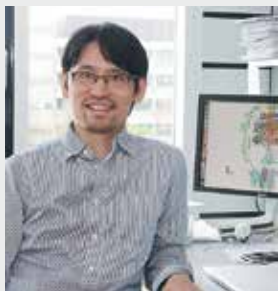


# Functional Dynamics of Biomolecular Machines Revealed by Theoretical Methods

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#### Education

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#### Professional Employment

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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 particularly focusing on ATP synthase that produces most of ATP required for living activities. The ATP synthase is composed of two rotary motors,  $F_0$  and  $F_1$ . The  $F_0$  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,  $\gamma$ -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.

#### Selected Publications

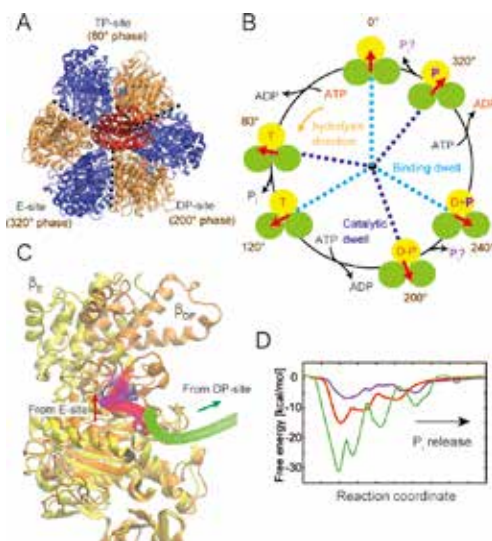
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- K. Okazaki and G. Hummer, "Phosphate Release Coupled to Rotary Motion of  $F_1$ -ATPase," *Proc. Natl. Acad. Sci. U.S.A.* **110**, 16468–16473 (2013).
- K. Okazaki and G. Hummer, "Elasticity, Friction, and Pathway of  $\gamma$ -Subunit Rotation in  $F_0F_1$ -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).

## 1. Mechanochemical Coupling Mechanism of F<sub>1</sub>-ATPase

Many single-molecule studies as well as crystallographic studies have clarified how the  $\gamma$ -subunit rotation is coupled to ATP hydrolysis reactions at three catalytic sites of F<sub>1</sub>. 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<sub>i</sub> 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<sub>i</sub> 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<sub>i</sub> release by using atomistic molecular dynamics simulations.<sup>1)</sup> The question is, essentially, from which catalytic site, DP-site or E-site, P<sub>i</sub> is released. Since the P<sub>i</sub> release takes ~millisecond, a biasing method called metadynamics was employed to facilitate the functional dynamics. Different pathways were observed depending on the site P<sub>i</sub> 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<sub>i</sub> is released from the E-site. That is, P<sub>i</sub> 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.<sup>2)</sup> We found that an  $\epsilon$ -inhibited *E. Coli* structure that has half-closed  $\beta_{DP}$  and loose  $\alpha\beta_E$  interface is consistent with the conformation taken in the ATP-binding dwell.

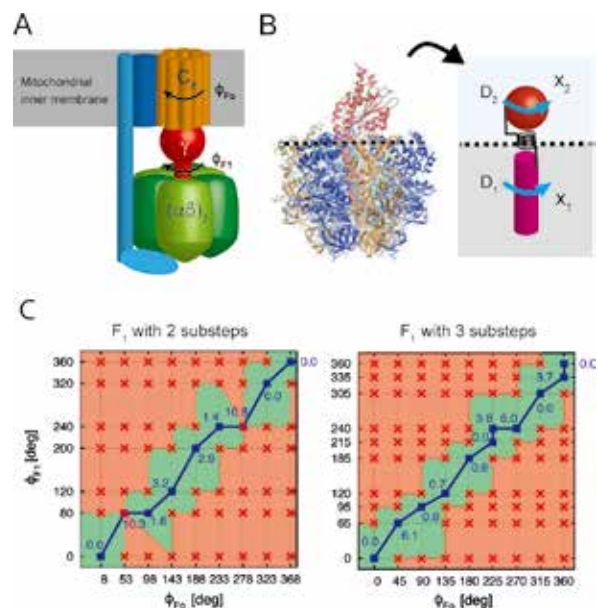


**Figure 1.** Mechanochemical coupling scheme and P<sub>i</sub> release in F<sub>1</sub>-ATPase.

## 2. Torsional Elasticity and Friction of Rotor in F<sub>o</sub>F<sub>1</sub>-ATP Synthase

It has been known that there is a symmetry mismatch between F<sub>o</sub> c-ring and F<sub>1</sub>  $\alpha_3\beta_3$  ring. The F<sub>1</sub>  $\alpha_3\beta_3$  ring has (pseudo) three-fold symmetry, while the F<sub>o</sub> c-ring in animal mitochondria has 8-fold symmetry. Thus, the common rotor,  $\gamma$ -subunit, has to rotate by 120° steps (or 80°+40° substeps) in F<sub>1</sub> part, while it has to rotate by 45° steps in F<sub>o</sub> part. Therefore, it has to have torsional elasticity to solve the mismatch. In order to estimate torsional elasticity as well as viscosity of the  $\gamma$ -rotation, we built a simple viscoelastic model (Figure 2B) and fitted it against atomistic simulation trajectories in which external torque was applied on  $\gamma$ .<sup>3)</sup>

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<sub>o</sub>F<sub>1</sub> rotation (Figure 2C). It turned out that with the two-substep F<sub>1</sub> the pathway is blocked by high-energy states. To solve this situation, F<sub>1</sub> needs three substeps as was measured for human mitochondrial F<sub>1</sub> recently. From the estimated torsional friction, we predict that  $\gamma$ -rotation can be 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<sub>o</sub>F<sub>1</sub>-ATP synthase.

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