2P163 人工基質 RTP を用いた F_1 -ATPase の回転触媒機構の解明
Base moiety of ATP is dispensable for driving the rotation of F_1 -ATPase

Ayako Yukawa¹, Ryu Iwatate², Rikiya Watanabe¹, Mako Kamiya², Yasuteru Urano², Hiroyuki Noji¹ (¹Grad. Sch. Eng., Univ. Tokyo, ²Grad. Sch. Med., Univ. Tokyo.)

F_1 -ATPase (F_1) is a rotary motor protein driven by ATP hydrolysis. We have previously examined the role of base moiety of ATP on the rotation of F_1 by using base-substituted analogs; GTP and ITP. These analogues supported the rotation of F_1 . Based on these findings, it was hypothesized that the base moiety is dispensable for the rotation of F_1 . To verify this, we synthesized an artificial substrate “ribose-triphosphate” (RTP), lacking the base moiety, and examined its competency to drive the rotation of F_1 . As a result, we found that RTP can also drive F_1 rotation, although its binding affinity was 10^6 times lower than ATP. Thus, we confirmed that the base moiety of ATP is important for its binding to F_1 , but is dispensable for driving the rotation.

2P164 DNA を回転子を持つ新規回転分子モーターの創製
Creation of a hybrid F_1 motor with DNA as the rotor

Kosuke Iwamoto¹, Ryota Iino¹, Risa Yamauchi¹, Takayuki Uchihashi², Hiroyuki Noji¹ (¹Grad. Sch. Eng., Univ. Tokyo, ²Col. Sci. and Eng., Univ. Kanazawa)

F_1 -ATPase (F_1) is a molecular motor that rotates the rotor γ -subunit against the stator $\alpha_3\beta_3$ ring by hydrolyzing ATP. The three β -subunits generate a torque for the unidirectional rotation. They show the well-coordinated power-stroke motion in the isolated $\alpha_3\beta_3$ ring (Uchihashi, Iino et al Science 2011). This result raised a hypothesis that the $\alpha_3\beta_3$ can rotate the objects other than the γ -subunit. To verify this hypothesis, we have created a hybrid motor that consists of the $\alpha_3\beta_3$ as the stator and DNA as the rotor. Double-stranded DNA was successfully incorporated into the $\alpha_3\beta_3$ ring. When observed using gold nanorod as a probe, some molecules showed three pausing positions, although unidirectional rotation has not been observed yet.

2P165 F_1 -ATPase 内の DELSEED-loop のトルク伝達機構の解明
Elucidation of torque-transmission mechanism of DELSEED-loop in F_1 -ATPase

Kazuma Koyasu¹, Mizue Tanigawara², Rikiya Watanabe¹, Hiroyuki Noji¹ (¹Dept. Applied Chem., Grad. Sch. Eng., Univ. Tokyo, ²Grad. Sch. Frontier Biosci., Osaka Univ.)

F_1 -ATPase (F_1) is an ATP-driven rotary motor whose torque is generated at the interface between the catalytic β subunit and the rotary γ subunit. Previously, we reported that the mutant F_1 of which residues in DELSEED-loop (D-loop) of β C-terminus domain were substituted with glycine to disrupt the interaction with γ (Gh^{loop}) halved the torque, suggesting the crucial role of D-loop in torque-transmission. Here, we investigated how the glycine substitution of D-loop affects the angular dependencies of the equilibrium constants of ATP binding and ATP hydrolysis. Gh^{loop} showed evidently lower angular dependency of ATP binding step while that of ATP hydrolysis step was not affected by the mutation, suggesting that D-loop transmits the torque in ATP binding process.

2P166 高速配向イメージングによる F_1 -ATPase の触媒サブユニットの構造変化計測

Direct observation of domain motion of the catalytic β subunit of F_1 -ATPase using high-speed orientational imaging

Sawako Enoki, Ryota Iino, Hiroyuki Noji (Grad. Eng., Univ. Tokyo)

F_1 -ATPase is an ATP-driven rotary molecular motor. Rotation of the rotor subunit γ is driven by coordinated conformational change of three catalytic subunits β in the stator ring. To elucidate the mechanism how each catalytic subunit β rotates the γ subunit, direct observation of the conformational change of individual subunit β at high temporal and spatial resolutions is requisite. Here, we attached a gold nanorod to the subunit β , and observed defocused scattering images of gold nanorod with time resolution of 3 μ s. As results, we could resolve rapid and reversible changes of the catalytic subunit β between open and closed states driven by ATP hydrolysis reaction. We will discuss the observed conformational transition process and the rotation mechanism of F_1 -ATPase.

2P167 サポートド膜を用いた F_0F_1 の一分子回転計測
Single molecule observation of F_0F_1 -ATP synthase in the supported lipid membrane

Yoshiki Moriizumi, Rikiya Watanabe, Kazuhito V. Tabata, Hiroyuki Noji (Dep. Appl. Chem., Grad. Sch. Eng., Univ. Tokyo.)

F_0F_1 -ATP synthase is the rotary motor, in which the mechanical rotation mediates the energy conversion between ATP hydrolysis and the proton translocation. F_0F_1 from Thermophilic *Bacillus* PS3 (TF_0F_1) is the best-characterized F_0F_1 in single-molecular studies. While 3-step rotation of F_1 -part, reflecting the 3-fold symmetry of TF_1 structure, has been well studied, 10-step rotation of TF_0F_1 corresponding to the structural 10-fold symmetry of F_0 has not been observed. In this study, we developed the novel experimental setup in which TF_0F_1 was reconstituted to the supported lipid membrane. Using this setup, we observed the ATPase rotation of TF_0F_1 , some of which showed 10-step rotations and we will report the kinetic analysis of the stepping behavior of TF_0F_1 .

2P168 Basic properties of rotary dynamics of *Enterococcus hirae* V1-ATPase motor protein

Yoshihiro Minagawa¹, Hiroshi Ueno², Yoshiko Ishizuka-Katsura⁴, Noboru Ohsawa⁴, Takaho Terada⁴, Mikako Shirouzu⁴, Shigeyuki Yokoyama⁴, Hiroyuki Noji¹, Takeshi Murata³, Ryota Iino¹ (¹Dept. of App. Chem., Grad. Sch. of Eng., Univ. Tokyo, ²Dept. of Phys., Fac. of Sci. and Eng., Univ. Cyuo, ³Dept. of Chem., Grad. Sch. of Sci., Univ. Chiba, ⁴RIKEN, SSBC)

we observed rotation of *Enterococcus hirae* V₁-ATPase (EhV₁) by using a 40-nm gold colloid as a load-free probe. EhV₁ rotated unidirectionally with maximum rotation speed ($V_{\max}$) of 107 rps and the Michaelis constant (K_m) of 154 μ M. Second-order binding rate constant for ATP ($k_{\text{on}}^{\text{ATP}}$) determined by $3 \times V_{\max}/K_m$ was $2.1 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$. At saturating ATP concentration, EhV₁ showed three catalytic pauses separated by 120° each other. The distribution of duration times of the catalytic pause showed convex-shape and was reproduced by consecutive reaction with two time constants of 2.5 ms and 0.5 ms. No substeps were resolved in the rotation observed around K_m . The $k_{\text{on}}^{\text{ATP}}$ determined from duration times at 10 μ M ATP was $2.3 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$, consistent with the value of $3 \times V_{\max}/K_m$.