dependence of the binding affinity was calculated. For the catalytic site that waits for ATP at 0° during free rotation, the affinities for both Cy3-ATP and Cy3-ADP were lowest at -40°(320°). The affinity for Cy3-ATP around 0° was 5-10 times greater than that for Cy3-ADP in the presence or absence of P_i, ensuring spontaneous rotation in the direction of ATP hydrolysis. Around -120° (240°) where ADP is to bind in synthesis rotation, Cy3-ATP and Cy3-ADP showed affinities similar to each other, but P_i selectively and greatly diminished the affinity for Cy3-ATP, warranting efficient ATP synthesis.

1L1424 化学エネルギーを可逆的かつ効率的に機械的な仕事へと変換する F₁ P10 -ATPase の作動原理を 1 分子操作技術で解明する
1YE1115 Key mechanism for high efficiency and reversibility of chemomechanical coupling in F₁-ATPase revealed by single-molecule manipulation

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F₁-ATPase (F₁) is a rotary motor protein which couples ATP hydrolysis/synthesis to rotary motion in a reversible fashion. Compared to other motor proteins, F₁ is unique for its high efficiency and reversibility in converting chemical energy into mechanical work. To elucidate its prominent chemomechanical coupling mechanism, we developed the novel single-molecule manipulation technique with a magnetic tweezers and determined the rate and equilibrium constants of ATP binding, hydrolysis of bound ATP, and P_i release at various angles. Then, we found that post ATP binding, hydrolysis, and P_i release states are progressively stabilized with counterclockwise rotation, which suggests that chemical energy derived from each reaction step is gradually converted into mechanical work to avoid energy dissipation. We also found that equilibrium constants of all reaction steps increased exponentially with rotation; however, the variations were different from each step; those for P_i release and ATP binding were much larger than that of hydrolysis. This result directly proved that P_i release and ATP binding generate the larger torque than hydrolysis. Furthermore, from the analysis of P_i release kinetics against a rotary angle, we eventually determined the timing of P_i release in free rotation, which was the last unknown piece on the puzzle of chemomechanical coupling scheme of F₁, and this finding gave us a clue for high reversibility between catalysis and mechanical work.

1L1436 F₁-ATPase の触媒部位に対するヌクレオチド結合の温度依存性
Thermodynamic parameters of nucleotide binding to the catalytic sites of F₁-ATPase

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F₁-ATPase is a molecular motor in which the central γ subunit rotates against $\alpha_3\beta_3$ ring while hydrolyzing ATP. It has been established that the γ -subunit makes a 120° step when F₁ hydrolyzes one ATP molecule. This 120° step is further divided into 80° substep and 40° substep. The 80° substep is triggered by ATP binding to a catalytic site and upon binding of the ATP, it is believed that an ADP is immediately released from another catalytic site. Thus, nucleotide binding and release at catalytic sites are very important for the mechanism of the rotation. In the present study, we focused on the nucleotide binding to F₁-ATPase and deduced the thermodynamic parameters. And we will compare the parameters for F₁-ATPase and isolated β subunit obtained by our group. In order to selectively monitor the binding to catalytic sites, we used β Y341W mutant in which fluorescence of the introduced Trp quenches upon nucleotide binding the catalytic sites. We measured the fluorescence quenching as a function of ATP concentration at a temperature range from 4 to 45 degrees and constructed a van't Hoff plot. The van't Hoff plot gave a straight line and at present, ΔH^0 and ΔS^0 of approximately -18 kJ·mol⁻¹ and 35 J·mol⁻¹·K⁻¹ have been obtained. In addition, we examined the thermodynamically parameters of the various nucleotide binding to F₁-ATPase. At the ATP, GTP, ITP and UTP, we used the mutant which has a low activation of hydrolyze nucleotide. The thermodynamically parameters show the difference value between F₁-ATPase and isolated β subunit.

1L1448 ねじれた γ 軸を持つ F₁-ATPase 変異体の高時間分解能回転観察
Observation of rotation of mutant F₁-ATPase with twisted γ at high temporal resolution

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F₁-ATPase is the sector of F₀F₁-ATP synthase that has catalytic sites responsible for the ATP synthesis reaction. An isolated $\alpha_3\beta_3\gamma$ subcomplex of F₁ is the world's smallest rotary molecular motor. The central γ -subunit rotates in the $\alpha_3\beta_3$ cylinder by repeating four stages of rotation: ATP-binding dwell (0° dwell); 80° substep rotation; catalytic dwell (80° dwell); 40° substep rotation. Previous reports indicated that 0° dwell is terminated by ATP binding to one β subunit and subsequent ADP release from another β subunit, and 80° dwell is terminated by hydrolysis and P_i release. Although the reaction scheme of F₁ has mostly been revealed, there still remains a question why these chemical events are assigned to specific angles, 0° and 80°. To address this question, we have observed rotation of the $\alpha_3\beta_3\gamma$ ($\Delta A275$) mutant, in which the γ subunit was twisted and the size of substep driven by ATP binding was unexpectedly altered to ~35° (Iai *et al.*, 41th annual meeting, 2003; Hirakawa *et al.*, 46th annual meeting, 2008). Notably, remaining ~85° was further resolved into 45° and 40° substeps, suggesting that the chemical events may be unlinked to the specific angles by mutation. In our previous contribution, rotation of this mutant was perturbed by the viscous resistance due to the large size of a marker, 200 nm duplex beads. Here we use 40 nm gold nanoparticle to quantify 2 ms dwell time with high-speed imaging. Validation of the above suggestion awaits further analysis.

1L1512 Two Pauses of V₁-ATPase during Catalysis

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V₁-ATPase, the hydrophilic domain of V-ATPase, hydrolyzes ATP, like F₁-ATPase. Here, at saturating [ATP], V₁-ATPase was shown to have 2 different pauses, which is not observed in F₁-ATPase: 'Short pause', occurring frequently during rotation, is a reversible pause of 3.6 sec. And the 'final pause' is the final destination of all rotating V₁ molecules, spontaneous recovery from which was never observed. In this study, dwell time and dwell angle of both pauses are determined. Dwell time of short pause was constant under high [ADP] or [P_i]. In addition, the lifetime of active rotation was also constant under different conditions, having the mean of 17 min. Dwell angle of both short and final pauses are found to be the same as ATP binding angle, implying that all rate-limiting reactions occur at the same angle. As to the recovery from final pause, V₁ resumed rotation in the presence of external magnetic field, depending on the direction, angle and manipulation time. Finally, the characteristic distance of resuming rotation event was found to be short.

1L1524 F₁-ATPase の β サブユニット C 末領域のトルク伝達における役割
Specific interaction between C terminal domain of β and γ of F₁-ATPase is dispensable for torque transmission

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In F₁-ATPase, C-terminal domain of β rotates toward γ upon ATP binding, which is then converted into the rotation of γ . Although the importance of the direct contact of C-terminal loop with γ on torque-transmission has revealed (Furuike *et al.* 2008, *Science*, Mnatsakanyan *et al.* 2009, *J. Biol. Chem.*), the mechanism of torque-transmission by β C terminus remain elusive. We have reported the mutants of which C-terminal helices were extensively substituted with glycine (poster presentations in 2010 and 2009). Though the torque value of glycine substituted mutant was half of that of wild-type, continuous and unidirectional rotation of this mutant which indicates that the cooperatively of F₁ was maintained, suggests that the torque-transmission between β and γ is not mediated by any specific electrostatic or steric interaction and it is designed to be highly functionally robust. On the other hand, mutant of which C-terminal loop was substituted with alanine had the comparable torque with that of wild-type. The obtained experimental data was discussed in the light of the molecular dynamics simulation of mutant F₁. We will put together these previous results and the new findings into this report. The ATP synthesis activity and the angle dependency of ATP binding and hydrolysis of glycine substituted mutant with

halved torque were now under analysis. The effect of torque decrease upon the energy coupling of F_0F_1 will be discussed in this presentation.

1L1536 イースト F1-ATPase の 16° 回転に伴う水のエントロピー変化の理論解析

A theoretical analysis on water-entropy change in yeast F1-ATPase during 16 degrees rotation of gamma subunit

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We have recently proposed a novel rotation mechanism of F1-ATPase [1] in which the key factor is the water entropy. As part of the demonstration of the validity of our rotation mechanism, we analyze the water-entropy change of yeast F1-ATPase during the 16 degrees rotation of the gamma subunit. The structures before and after the 16 degrees rotation are taken from the crystal structures. For each of them, we calculate the hydration entropies of three different sub-complexes comprising the gamma subunit, one of the beta subunits, and two alpha subunits adjacent to them. The beta_E, beta_TP, and beta_DP subunits are involved in the sub-complexes I, II, and III, respectively. The calculation is performed by a hybrid of the integral equation theory of molecular liquids and the morphometric approach [2]. It follows that due to an asymmetric packing of F1-ATPase, the absolute of the hydration entropy is in the order, sub-complex I > sub-complex II > sub-complex III. Namely, the packing is the tightest in the sub-complex III. When the 16 degrees rotation occurs, the packing of the sub-complex III becomes less tight, leading to a water-entropy loss. However, the loss is almost compensated by water-entropy gains of the other two sub-complexes. The compensation is required for the smooth rotation of the gamma subunit. These results are consistent with the mechanism we have proposed [1].

[1] T. Yoshidome, Y. Ito, M. Ikeguchi, and M. Kinoshita, *J. Am. Chem. Soc.* 133, 4030 (2011).

[2] M. Kinoshita, *Front. Biosci.* 14, 3419 (2009).

1L1548 分子モーターの 1 分子非平衡熱力学

P05 Single-molecule nonequilibrium thermodynamics of molecular 1YE1000 motor

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Molecular motor is a chemical engine that converts chemical potential to mechanical motion. Therefore, it is crucial to reveal the energetics for better understanding of the design principle. However, information about the energetics has remained limited due to lack of methodology. We developed a novel methodology to measure thermodynamic quantities of molecular motors by single-molecule experiments on the basis of nonequilibrium physics. We also developed a novel analytical method to extract information regarding the mechanical potentials at each chemical state and the transition positions between states only from the single-molecule trajectories.

For a single rotary molecular motor F1-ATPase, we measured the "output" of the free-energy transduction, that is, the work performed by the motor and the heat through rotations for the first time under the precise control of the free energy change of ATP hydrolysis. We demonstrated that F1-ATPase has a "thermodynamic" efficiency of nearly 100%.

Furthermore, to understand the mechanism to achieve such a high efficiency, on the basis of a novel analytical method, we evaluated the mechanical potential profiles at each chemical state and the transition angular positions from only the probe's trajectory. We demonstrated that the position-dependent transition of the chemical states is the key feature for the highly efficient free-energy transduction by F1-ATPase. It is suggested that Maxwell's demon resides inside the motor. Our simple and powerful method is applicable to other molecular motors.

1L1612 F1 で見る拡散の Giant Acceleration

Giant Acceleration of Diffusion in F1-motors

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A diffusion coefficient (D), which quantitatively characterizes fluctuation of a small system (nano- or micro-sized system), has been measured frequently in single molecule observations. Applying a constant external force (f) to a small system, we investigate the force dependence of a diffusion coefficient (D(f)),

and introduce the phenomena called "the giant acceleration of diffusion" [1-2]. We show a force-dependent diffusion coefficient (D(f)) provides energetic information of the system. As an example, we first observe the giant acceleration of diffusion in F1-motors which are rotary motor proteins. We succeeded in applying a constant force (f) to F1 by using high-frequency rotating electric field [3]. According to the theories [1-2], D(f) measured in F1 should have a maximum at $f=f^*$ which corresponds to the force exerted by its rotary potential. Noting that a diffusion coefficient is one of useful measurable quantities in single molecule experiments, in future, the giant acceleration of diffusion will be widely observed in biological systems *in vitro* and *in vivo*. We hope our application will be a good example to show the availability of a diffusion coefficient measured in a non-equilibrium state achieved by an external operation.

[1] P. Reinmann et al., *Phys. Rev. Lett.* 87, 010602 (2001).

[2] K. Hayashi and S. Sasa, *Phys. Rev. E* 69, 066119 (2004).

[3] M. Washizu et al., *IEEE Trans. Industry Appl.* 29, 286 (1993).

1L1624 粗視化シミュレーションによるリボソームトランスロケーション機構の研究

Coarse-grained molecular simulation study of ribosomal translocation dynamics

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In the final step of the translation-elongation cycle, the complex of two tRNA and mRNA needs to be advanced by exactly one codon in the ribosome. To realize it, the two ribosomal subunits are thought to be largely rearranged as the so-called ratchet-like rotation. This dynamic behavior, translocation, must be essential for the sequential and accurate translation. Although many experiments including high-resolution structural study have been performed, it is still unclear how the ribosome achieves the movement dynamically.

The molecular dynamics simulation is a good tool to investigate such biological phenomena at atomic resolution. To overcome the limitation of system size and long-time scale, we have developed a coarse-grained (CG) simulation model of RNA and RNP. In the model, each nucleotide is represented as three CG beads and interactions are modeled with structure-based potential, parameters of which were determined by fluctuation matching method to achieve higher accuracy.

Then we employed the CG model to study dynamic behavior of ribosome complex. We focused on especially the relationship among the inter-subunit motion, fluctuation of the tRNAs in the bindings sites, and tRNA-mRNA movement. Some experiments have suggested that tRNAs make hybrid states in the translocation phase, and we were motivated to examine whether it is realized or not in our CG simulations.

1L1636 マイコプラズマのあしが認識するシアル酸オリゴ糖の構造

The structure of sialylated oligosaccharide recognized by mycoplasma leg

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Mycoplasmas, known as the cause of human pneumonia bind to solid surfaces and glide to one direction. Mycoplasmas catch sialylated oligosaccharides, the target of many pathogens, including influenza virus and botulinum toxin. However, there is no information on the structures of sialylated oligosaccharide recognized by mycoplasmas. In the present study, we studied the structure recognized by *Mycoplasma mobile*, using 17 sialylated compounds chemically synthesized. *M. mobile*, the fastest species, glides at 2.5-4 μm per second with the maximum force of 27 pN.

We examined the inhibitory effects of those 17 compounds by counting the number of cells bound to glass surface. The inhibitory effects showed cooperativity, where the Hill coefficient ranged 2 to 4. The most effective structure was N-acetylneuraminic acid alpha-(2,6)-galactose, whose Kd was 0.1 μM. The inhibitory effects changed significantly with the small structural modifications, suggesting that the binding is "lock and key". Next, we examined the inhibitory effects by tracing the number of cells released from glass surface after those compounds were added to gliding cells. The inhibition also showed cooperativity and similar dependency on the chemical structure. The analyses of movements before the detach from glass showed reduction of gliding speed, suggesting that the resting legs generate the drag force. The cells also showed pivoting around the subcellular position of gliding machinery, suggesting the cooperativity of leg movements.