**3P151 Giant Acceleration of diffusion in F₁-ATPase II**

Ryunosuke Hayashi¹, Shuichi Nakamura¹, Seishi Kudo¹, Kazuo Sasaki², Hiroyuki Noji³, Kuniho Hayashi¹ (¹Dept. Appl. Phys., Sch. Eng., Tohoku Univ., ²Dept. Appl. Chem., Sch. Eng., Univ. Tokyo)

Diffusion coefficients are often measured in small biological systems to characterize their fluctuation. Giant acceleration of diffusion is one of the theories on diffusion in the field of non-equilibrium statistical mechanics. We apply this theory to F₁-ATPase which is an ATP-driven rotary motor protein. According to the theory, when we apply a constant torque to F₁ by using an electric rotating field, the diffusion coefficient of a rotary probe attached to F₁, as a function of the applied torque exhibits a resonance peak. The resonance peak corresponds to the torque value near the critical tilt of the rotary potential of F₁.

**3P152 Nucleotide binding to TF₁β subunit in relation to the effect of Pi**

Riku Nagano, Kiyoshi Obara, Hiroshi Ueno, Eiro Muneyuki (Dept. of Physics, Chuo Univ.)

Nucleotide binding is important for the rotation of F₁-ATPase. Here, we examined nucleotide binding to the isolated β subunit and αβγ subcomplex containing βγY341W mutation in relation to the effect of Pi. We found that $k_{\text{on}}^{\text{ATP}}$ and $k_{\text{off}}^{\text{ADP}}$ for the isolated β subunit were insensitive to 100 mM Pi. Next we examined the effect of Pi on nucleotide binding to αβγ subcomplex. In order to prevent ATP hydrolysis during experiment, we first used β(E190Q/Y341W) mutant for nucleotide binding to subcomplex. One hundred mM Pi partially released the bound nucleotide irrespective of ATP or ADP was pre-loaded.

**3P153 Key factors of arginine finger of F₁-ATPase clarified by an unnatural amino acid mutation**

Ayako Yukawa¹, Ryota Inou², Rikiya Watanabe¹, Shigehiko Hayashi³, Hiroyuki Noji (¹Grad. Sch. Eng., Univ. Tokyo, ²Okazaki Inst. Integr. Biosci., NINS, ³Grad. Sch. Sci., Univ. Kyoto)

A catalytically important arginine, called Arg finger (Arg), is employed in many enzymes to regulate their functions. F₁-ATPase (F₁), a rotary motor protein, possesses Arg, which contributes to catalyze ATP efficiently. In this study, to identify chemical determinants of the Arg catalysis, we mutated Arg into an unnatural amino acid Lyk, a lysine analogue with an alkyl chain elongated by one CH₂ unit. Single molecule observations showed that the terminal guanidium group in Arg is indispensable for catalyzing ATP and that geometric chain length matching prevented the inhibited-state of F₁. We showed that utilization of unnatural amino acids extends biochemical approaches for elucidation of molecular mechanism of protein functions at a high chemical resolution.

**3P154 Rotor-Stator Interactions in V₁ and V₄ from Enterococcus hirae V-ATPase**


We developed an E. coli expression system for E. hirae V-ATPase (EhV₁,V₄) which functions as a Na⁺ pump and observed the rotation of EhV₁,V₄. Even at high Na⁺ concentration, with a load-free probe, EhV₁,V₄ rotated slower than EhV, without clear three pauses separated by 120°, suggesting that EhV₁,V₄ limits the rotation of EhV. The torque of EhV₁,V₄ estimated from the continuous rotation was nearly half of that of EhV, although the stepping torque of EhV₄ was comparable to the torque of EhV₁,V₄, suggesting that EhV₄ has unstable state where EhV₄ generates low torque whereas rotor-stator interactions in EhV₁,V₄ are stabilized by two peripheral stalks. We found that the torque of EhV₁,V₄ was lower than those of other V₁ and F₁, indicating low energy conversion efficiency in EhV₁,V₄.

**3P155 Photo-control of mitotic kinesin Eg5 using novel SH reactive photochromic molecules**

Tamura Yuki¹, Mutoh Hiroyuki², Tohyma Kanako³, Kondo Kazunori³, Maruta Shinshiku¹ (¹Div. Bioinfo., Grad. sch. Eng., Univ. Soka, ²Dep. Bioinfo., Fac. Eng., Univ. Soka)

Kinesin Eg5 is essential for bipolar spindle formation during eukaryotic cell division. Previously, we prepared mutants of mitotic kinesin Eg5, which have a single cysteine in the functional region and modified with photochromic molecules, iodoacetyl-spiropyran and 4-phenylazomaleinim. ATPase activities of the modified Eg5 mutants W127C and D130C were photo-reversibly regulated by light irradiations. In this study, we synthesized novel SH group crosslinkable photochromic molecules, iodo-fulgide (IAFG) and iodo-trityl azobenzene (IATAB) and tried to control function of Eg5. IAFG and IATAB were incorporated into the Eg5 mutants, E116C, E118C, Y125C, W127C and D130C. Photo-control of ATPase and motility activities of the modified Eg5 were investigated.

**3P156 Determination of Power Stroke Distance Driven by Human Cytoplasmic Dynein**

Yoshimi Kinoshita¹, Taketoshi Kambara², Satoshi Ikeda¹, Motoshi Kaya³, Hideo Higuchi² (¹Graduate School of Science, The University of Tokyo, ²RIKEN QBIC)

Cytoplasmic dynein is a motor protein moving along microtubules toward the minus-end dominantly with 8.2nm step, and plays an important role in cellular processes. Dynein’s conformational change, called “power stroke”, is assumed to generate driving forces moving along the microtubule. However, it has not been clarified the mechanism of how the power stroke contributes to individual steps. Thus, we measured the power stroke distance of single-headed dynein using optical tweezers. Results showed that the power stroke distance is less than 8.2nm, implying the following scenario; the attached head goes on power stroke, while the other head detaches, undergoes diffusive search and rebinds to the next site on microtubule.