3P157 V-ATPase の中心回転軸におけるトルク伝達機構**The ingenious structure of central rotor apparatus in V_0V_1 ; torque transmission mechanism in the central rotor of V_0V_1**

Atsuko Nakanishi¹, Jun-ichi Kishikawa¹, Masatada Tamakoshi², Shou Furuike³, Ken Yokoyama¹ (¹Dept. Mol. Biosci., Kyoto Sangyo Univ., ²Dept. Mol. Biol., Tokyo University of Pharm. & Life Sci., ³Dept. Physics, Osaka Medical College)

The central rotor apparatus of V_0V_1 of *Thermus thermophilus* is composed of V_1 -DF shaft and V_0 -CL₁₂ rotor ring. In this study, we revealed that the rod domain of V_1 -DF plays important roles on both reconstitution and energy coupling in V_0V_1 by the reconstitution and fluorescence resonance energy transfer (FRET) analysis. We propose a structural model accounting for both the detachable and sticky nature of the interaction between the central rotor apparatuses.

In addition, we discuss a twist or torsion of V_1 -DF shaft in V_0V_1 during rotation.

3P158 フォトクロミック分子によって誘導されるキネシン・ADP・フルオロメタル複合体の構造変化**Conformational change of kinesin-ADP-fluorometal ternary complexes induced by photochromic molecules**

Akihisa Iwata¹, Takeshi Itaba¹, Mitsuo Ohmori², Shinya Mitsuhashi³, Shinsa Maruta^{1,2} (¹Div. Bioinfo., Grad. sch. Eng., Univ. Soka, ²Dep. Bioinfo., Fac. Eng., Univ. Soka, ³Div. Applied Bioscience, Grad. sch. Agri., Uni. Hokkaido)

In the presence of ADP, kinesin forms stable ternary complexes with phosphate analogues, BeFn, AlF₄⁻ and V_i , each of which mimic different transient state along ATPase cycle. In this study, we tried to induce transition between the different transient states of the ternary complexes in ATPase by photoisomerization incorporated into the functional region of kinesin. To monitor the conformational change of the ternary complexes by 19F-NMR, we synthesized fluorine labelled ADP analogue, 4-Fluorobenzoic-ADP (4-FB-ADP). In the presence of BeFn, 4-FB-ADP was trapped within the ATPase site of kinesin resulted in formation of Photochromic-kinesin/4-FB-ADP/BeFn ternary complexes. The conformational change of the complex induced by light irradiation was monitored by 19F-NMR.

3P159 複数のキネシンによる協調的カーゴ輸送のメカニズムの解明
Investigation of the mechanism of cooperative cargo transport by multiple kinesins

Naoto Sawairi¹, Takayuki Ariga², Mitsuhiro Iwaki^{3,4}, Michio Tomishige², Kumiko Hayashi¹ (¹Dept. Appl. Phys., Sch. Eng., Tohoku Univ., ²Dept. Appl. Phys., Grad. Sch. Eng., Univ. Tokyo, ³QBiC, RIKEN, ⁴Grad. Sch. Frontier Biosci., Osaka Univ.)

In cells, organelles are considered to be transported by multiple motor proteins such as kinesin and dynein along microtubules. It is important to elucidate the transport mechanism. In the previous study^[1], the drag force was measured using fluctuation theorem in Neuron, and the number of motors was estimated from the distribution of the drag force. Then we performed an *in vitro* single-molecule experiment on kinesin-1 with controlling the number of kinesins using DNA-origami. Multiple kinesins are connected with a 1um-sized bead via DNA-origami. We aim to reproduce the result^[1] in this *in vitro* single molecule experiments.

[1] K. Hayashi, Y. Okada, 2p145, 51th annual meeting of BSJ (2013)

3P160 MD シミュレーションによる *Enterococcus hirae*. V_1 -ATPase の回転機構の解明**Rotation mechanism of V_1 -ATPase studied by MD simulation**

Yuta Isaka¹, Takeshi Murata^{2,3}, Mitsunori Ikeguchi¹ (¹Grad. Schi. of Med. Life Sci., Yokohama City Univ., ²Fac. of Sci., Chiba Univ., ³JST, PRESTO)

V-ATPase is a molecular motor acting as a proton pump in cell membranes, which includes a proton-translocation domain (V_0) and a rotary ATP-hydrolytic domain (V_1). Recently, Arai et al. have determined a high-resolution crystal structure of V_1 , composed of the central stalk (DF) and the asymmetrically-packed hexamer ring (A_3B_3). To elucidate the relationship between rotation dynamics of DF and conformation changes of A_3B_3 , we conducted a 100-ns MD simulation using an all-atom model of V_1 with explicit solvent, and analyzed dynamical correlation among the subunits. We found that several residues exhibit highly correlated motions during MD, suggesting that the correlated motions play key roles on the rotation dynamics of V_1 .

3P161 リニアモータータンパク質キチナーゼの蛍光一分子観察**Single-Molecule Fluorescence Imaging of Linear Motor Protein Chitinase**

Tomoyuki Tasaki¹, Yusuke Shinafuji¹, Mayu Hara¹, Hiroyuki Noji¹, Ryota Iino² (¹Grad. Sch. Eng., Univ. Tokyo, ²Okazaki Inst. Integr. Biosci., NINS)

Chitinase A (ChiA) is a linear motor protein which processively hydrolyzes and moves on crystalline chitin. Turnover of ChiA measured in biochemical assay is about one-seventeenth lower than that calculated from the result of single-molecule observation with high-speed AFM (Uchiyama et al. JBC, 276 (2001), 41343-41349, Igarashi, Uchihashi et al. Nature Commun, in press.). This inconsistency can be explained if we assume the two different binding modes of ChiA to chitin, productive and non-productive bindings which accompany hydrolysis and movement or not, respectively. With single-molecule fluorescence observation, we determined fraction and velocity of productively binding ChiA. As a result, we found that most ChiA molecules bind to chitin non-productively.

3P162 ATP synthesis by elastically coupled molecular motors

Yasuhiro Imafuku¹, Nils Gustafsson², Thomas Thomas² (¹Department of Biology, Kyushu University, Japan, ²School of Physics and Astronomy, University of Birmingham, UK)

We have studied a simplified analogue of ATP synthase, consisting of two elastically coupled molecular motors pulling against each other in a molecular 'tug of war'. When one of the molecular motors is driven strongly enough by an external free-energy source, it pulls the other ATP-driven molecular motors backwards so that it synthesizes ATP.

Monte-Carlo simulations of the molecular tug of war allow us to study the dependence of the ATP synthesis rate on the free-energy drive ΔG and on the stiffness λ of the spring coupling the two motors. The model reproduces the experimentally observed dependence of ATP synthesis rate on free energy at two crucial points, the maximum ATPase velocity and the thermodynamic equilibrium where the velocity of the ATPase is zero.