and motility. When artificially dimerized, myosin VI moves processively along an actin filament towards its pointed end, that is, in the direction opposite to all other characterized myosins. Similarly to myosin V, it walks in a "hand-over-hand" fashion; however, as we have shown experimentally, the asymmetry between the ADP binding affinities to the trailing and the leading heads, which supports the unidirectional motility by ensuring that ADP dissociates more readily from the trailing head, is less pronounced in myosin VI than in the myosin V-6IQ construct. The distribution of the step sizes is significantly broader in the case of myosin VI compared to myosin V, which suggests that during the processive stepping the azimuthal angle of myosin's VI lever arm is quite variable, implying the existence of the off-axis load during the double-headed binding of myosin VI to actin. To reveal whether the off-axis load may be affecting the efficiency of the processive stepping, we directly measured the ADP affinity of individual single-headed myosin VI molecules bound to actin under the external loads applied at different angles to the long axis of actin filament. The obtained results indicate that the off-axis load significantly affects ADP affinity of the myosin's VI motor domain and is an important factor regulating the efficient processive movement of myosin VI.

3P160 Kinesin neck linker の stiffness 計測 3B1150 Measurement of the stiffness of kinesin neck linker

Takuya Yamada, Takuya Kobayashi, Tomohiro Shima, Motoshi Kaya, Hideo Higuchi, (Department of Physics, The University of Tokyo)

Conventional kinesin is a dimeric motor protein that hydrolyzes ATP and moves processively along microtubules in a hand-over-hand manner. The neck linkers, which connect the two motor domains together, have been generally thought to play an important role in the unidirectional stepping of the protein. A conformational change of the neck linker of the front head, which occurs upon binding of ATP, is called "neck linker docking" and is thought to bias the position of the rear head towards the forward direction. Recently, however, the question has aroused whether the neck linker docking could solely produce 7 pN of the stall force. To address this question, we designed the novel experimental system using optical tweezers and fluorescence imaging techniques. In order to investigate how much the force and displacement can be generated by the "docking" of neck linkers, those of single kinesin heads are measured. The forces exerted on the neck linker were measured by the position of two trapped beads attached to both ends of the microtubule, and the displacements of the neck linker were estimated as the displacement between quantum dots (QDs) attached to the neck linker of truncated kinesin monomer and QDs attached to the microtubule. The preliminary result showed that the stiffness changes at different force levels, implying the different conformational states of kinesin heads at different loads.

3P161 ミオシンのアクチン結合クレフト閉口とレバーアーム・スイングは熱力学的に有利な構造変化か？ Are the actin-binding cleft closure and the lever-arm swing of myosin thermodynamically-favored structural changes?

Jun Ohnuki, Takato Sato, Kei-ichi Okazaki, Mitsunori Takano, (Graduate School of Advanced Science and Engineering, Waseda University)

Force generation in actomyosin is widely believed to be caused by the two large-scale structural changes of myosin: the closure of the actin-binding cleft that enables myosin to firmly bind to the actin filament and the swinging of the lever-arm that exerts mechanical force. However, the atomic-level picture of the structural change (if any) and the coupling between the chemical and structural state remains elusive. Computational approach possesses a potential capability to develop the picture. Actually, our present molecular dynamics study captured the sign of allosteric response in the actin-binding cleft that is consistent with the previous experiments. On the other hand, the swinging of the lever arm (power stroke) was found to be significantly disfavored from the viewpoint of the potential energy of the whole system including solvents, which is apparently at odds with the lever-arm model and makes the estimation of the entropic contribution indispensable. Therefore, we are calculating the free energies along the two relevant reaction paths, the width of the actin-binding cleft and the lever-arm position along the actin filament, by using the umbrella sampling technique, and investigate the possibility of the nucleotide-induced structural change from the viewpoint of thermodynamics, with the hope to connect the thermodynamics with the atomic-level picture and to clarify the contribution of solvents.

3P162 *in silico* 変異実験で探るキネシン-微小管間の静電相互作用の役割 Role of the electrostatic interactions between kinesin and microtubule studied by *in silico* mutagenesis

Kyohei Yamamoto, Jun Narita, Mitsunori Takano, (Graduate School of Advanced Science and Engineering, Waseda University)

Kinesin moves toward the plus-end of microtubule (MT) using ATP hydrolysis energy. In the case of KIF1A, a single molecule experiment (SME) has demonstrated that binding of KIF1A to MT is biased and accompanied by a net movement toward the plus-end, suggesting that a Brownian-ratchet-like mechanism is inherent in KIF1A and that interaction between kinesin and MT plays a key role. The important role of electrostatic interaction has been suggested; mutagenesis studies indicated that basic residues in kinesin and acidic residues in α - β -tubulins are involved in the interaction, and the KIF1A-MT complex structure suggested that two almost-identical MT binding sites for KIF1A may be discriminated by electrostatic interaction. In this study, to clarify the role of the electrostatic interaction, we conducted an *in silico* SME in combination with mutagenesis. We constructed a system composed of KIF1A (or conventional kinesin) and MT using a coarse-grained model and KIF1A-MT complex structure (KIF1A was moved away from MT by ~ 5 nm), and then observed the binding process of KIF1A to MT. We found that the net plus-end-directed movement of KIF1A, which was found in our previous study, disappears when the electrostatic interactions are all turned off. We then introduce charge-neutralizing mutations to key residues, including the acidic residues in MT as identified in the recent study by Uchimura et al., and study the effect of the mutations on the binding affinity and the unidirectional movement.

3P163 F_1 -ATPase の構造比較：酵素構造・反応サイト・回転の共役 Structural comparison of F_1 -ATPase: Coupling among enzyme structures, reaction sites, and rotations

Kei-ichi Okazaki¹, Shoji Takada², (¹ School of Advanced Science and Technology, Waseda University; ² Graduate School of Science, Kyoto University)

F_1 -ATPase is a rotary motor that converts ATP hydrolysis energy into mechanical work, and has been studied both by x-ray crystallography and single molecule experiments. The single molecule experiments have established the coupling scheme between the γ rotation and the nucleotide states in the three catalytic sites. On the other hand, the x-ray structures have shown that $\alpha\beta$ -subunit in F_1 alternatively take the three different structures ($\alpha\beta_E$, $\alpha\beta_{DP}$ and $\alpha\beta_{TP}$) depending on the nucleotide states, which leads to the rotation of γ . But, as recently suggested [Okuno et al. (2008)], the x-ray structures are said to represent only the catalytic dwell conformation, and the other conformation, the ATP-binding dwell conformation, is not known so far. Although x-ray structure itself is a static snap shot, extensive structural comparison can provide insights into the working mechanism of this motor.

Here, we performed an exhaustive structural comparison using all available high resolution and asymmetric F_1 -complex structures (29 in total). We conducted principal component analysis of all 87 $\alpha\beta$ -subunits and their nucleotide binding sites to clarify the coupling between nucleotide binding and $\alpha\beta$ structure. We also calculated the rotational angles of γ to see the coupling between γ -angle and $\alpha\beta$ structure. Furthermore, we modeled and calculated angles of the C-terminal helix of β to identify the partially closed conformation of β that was observed in the single molecule experiment [Masaie et al. (2008)].

3P164 生体分子モーターのステップ運動解析のためのアルゴリズム開発 New algorithm for analyzing stepping motion of biological molecular motors

Takeshi Nakagawa, Kazuo Sasaki, (Dept. of Applied Physics, Tohoku Univ.)

Certain biological molecular motors such as kinesin, dynein and myosin move stochastically along a track (a microtubule or an actin filament) in a stepwise manner. The distributions of step size and dwell time (duration between successive steps) provide important information on the mechanism of how these molecular machines work. One needs to employ a certain algorithm to extract the step sizes and dwell times from the noisy experimental data. Recently the step finding algorithm proposed by Kerssemakers [J.W.J. Kerssemakers et al. Nature 442, 709 (2006)] has been used frequently. However as the standard deviation of the noise increases, the performance of the method becomes considerably worse; it misses steps buried in the noise. We propose a new step finding algorithm which improves the performance against noise. Our method is briefly explained as follows: (i) adopting the first stage of the Kerssemakers algorithm, a series of step functions are fitted to the data with unrealistically large number of steps ("overfitting"); (ii) the two dwell adjoining each step are tested by the two sample Student's t-test, and the step is removed and the two dwells are combined to a single dwell if the mean displacements of the dwells are considered to be identical. Our algorithm detects more steps than the Kerssemakers algorithm for the data with large noise. We will explain the detail of our method and compare its performance with the Kerssemakers