1PS039 The role of electrostatic interaction in binding-site selectivity of KIF1A on microtubule

Yukinobu Mizuhara, Kyohei Yamamoto, Jun Narita, Mitsunori Takano (Grad. Sch. of Adv. Sci. & Eng., Waseda Univ.)

KIF1A, a well-studied kinesin superfamily motor protein, functions as a monomer, where the biased binding to MT, which leads to force-generation, has been observed both *in vitro* and *in silico* experiments. Cryo-electron microscopy indicated that KIF1A binds to the α - β contact region within the dimer (intra-dimer binding site), although there is another almost equivalent potential binding site between the adjacent dimers (inter-dimer binding site). It has been indicated that the binding-site selectivity is achieved by the difference in the KIF1A-MT electrostatic interaction between the intra- and inter-dimer binding sites. On the other hands, binding of KIF1A to the inter-dimer binding site was also observed in our previous simulation study using a residue-based coarse-grained. However, it is possible that the binding-site selectivity was smeared in our simulation due to the coarse-graining of the protein model. Therefore, in this study, we examined this possibility by using the all-atom model, and calculated the binding energy between KIF1A and the $\alpha\beta$ tubulin dimer. We found that the binding energy for KIF1A binding to the intra-dimer binding site is lower than that to the inter-dimer binding site. We then analyzed the binding energy at the residue level, and examined whether the observed binding-energy difference is originated from the electrostatic interactions (including the critical role of loop 7 of KIF1A as has been suggested).

1PS040 Regulation of motor activity of kinesin-6 by phosphorylation of the N-terminal extension domainAkihiko Sato¹, Tim Davis², Shin Yamaguchi¹, Masanori Mishima², Junichiro Yajima¹ (¹Graduate School of Arts and Sciences, Univ. Tokyo, ²Centre for Mechanochemical Cell Biology Warwick Medical School University of Warwick)

Kinesin-6 is a microtubule-based motor protein localized at centralspindle in anaphase. It has been demonstrated that phosphorylation of the motor domain of kinesin-6 on a conserved site within a N-terminal extension destabilised the interaction between microtubules and kinesin-6. Furthermore, deletion of the N-terminal extension also reduced its affinity for microtubules. The molecular mechanism in which the N-terminal extension domain controls the interplay between microtubule and kinesin-6 is unknown. Here, we investigate the influence of the N-terminal extension domain on motor activity. To do this, we have engineered *C.elegans* kinesin-6 motor domain construct with the mutation of one residue to aspartic acid (T9D) in its N-terminal region predicted to mimic phosphorylation of the conserved threonine. Using a microtubule sliding assay in which a quantum dot is attached to the microtubule (Yajima J. *et al.* Nat. Struct. Mol. Biol. 2008), we found that microtubules sliding over wildtype kinesin-6 underwent short-pitch rotation around their longitudinal axes, as revealed by the tracking of the quantum dot in 3D, which has not been previously seen for kinesin-6. In the case of T9D, sliding velocity decreased to 10% of wildtype kinesin-6 and rotations of sliding microtubules could not be observed. This result suggests that phosphorylation in the N-terminal extension domain will regulate torsional force as well as axial sliding force in kinesin-6.

1PS041 カーボンナノチューブを用いたキネシン運動の局所加熱**Local heating of kinesin motors on carbon nanotubes**

Yuichi Inoue, Mitsunori Nagata, Akihiko Ishijima (IMRAM, Tohoku Univ.)

We demonstrated that single carbon nanotubes (CNTs) can be used as a heat conductor to induce local temperature change for skeletal muscle myosin (Annual meeting, 2011). However, adsorption of myosin on CNTs required very high concentration of myosin as 30 mg/ml to observe continuous sliding of actin filaments. To reduce number of motors on CNTs toward single molecular level, here we used kinesin as a typical processive motor.

Multiwalled CNTs (~170 nm diameter, ~10 μ m length) were coated with kinesin (~0.1 mg/ml) purified from a bovine brain to observe sliding of fluorescently-labeled microtubules in the presence of 1 mM ATP. During the sliding, a local point of the CNT was temporarily heated with a laser irradiation (645 nm, ϕ ~2 μ m, 2.7 mW) to supply external heat.

Microtubules moved on the kinesin-coated CNTs at a speed of ~0.80 μ m/s. By the local heating with laser, the speed increased to ~18.0 μ m/s, indicating temperature jump over 10°C as observed for the myosin-coated CNTs.

Our results suggest that the local heating using CNTs can be applied to kinesin

motors and processivity of kinesin provides a good reference for the CNTs as a nano heater at single molecular level for future applications of various proteins *in vitro* and *in vivo*.

1PS042 多分子キネシン間の協調性は運搬物を効率的に長距離輸送するのに重要である**Multiple kinesin molecules coordinate to ensure the long-distance walking: a DNA-kinesin hybrid nanomachine study**Yuya Miyazono¹, Masayuki Endo², Takuya Ueda³, Hiroshi Sugiyama², Yoshie Harada², Hisashi Tadakuma³ (¹Grad. Sch. Appl. Phys., Univ. Tokyo, ²iCeMS, Univ. Kyoto, ³Grad. sch. of Frontier Sci., Univ. Tokyo)

Kinesin drives cellular transport using energy derived from ATP. However, how multiple kinesin affect the vesicle transport remains elusive. Here we created a hybrid nanomachine (DNA-tile-kinesin) using DNA-origami-tile as the skeletal structure and kinesin as the functional module. Single molecule imaging of DNA-tile-kinesin hybrid, on which 1 to 9 kinesin molecules were attached, allowed us to evaluate the effects of number of kinesins participating in vesicle transport. Our results show that the number of kinesin involving movement affects run length but not velocity of the DNA-tile-kinesin. Furthermore, disturbing the coordination of multiple kinesins, by replacing 1 molecule of 9 molecules to defect kinesin, revealed that coordination is crucial for efficient transport. Thus, the intermolecular coordination facilitates high processive movement of transport complex.

**1PS043 フォトクロミック阻害剤を用いた有糸分裂キネシン Eg5 の光制御
Photo-regulation of mitotic kinesin Eg5 using photochromic inhibitors**Kumiko Ishikawa¹, Hideo Seo¹, Kanako Touyama², Shinsaku Matuta¹ (¹Div. Bioinfo., Grad. Eng., Soka Univ., ²Dept. Bioinfo., Fac. Eng., Soka Univ.)

Kinesin Eg5, a member of the kinesin-5 family, is essential for formation of bipolar spindle during cell division. Eg5 is overexpressed in tumor cell and induce significant cell multiplication. Monastrol and Strityl-L-cysteine (STLC), potent inhibitor specific for kinesin Eg5 ATPase activity shut off mitotic division and results in apoptosis. Therefore, these inhibitors are attracting attention as an anti-cancer drug. In this study, we have tried to design and synthesize Monastrol and STLC analogues composed of photochromic molecules (azobenzen and spiropyran derivatives) as kinesin Eg5 inhibitors, and their photo-reversible inhibitory effects for Eg5 were examined upon ultra-violet (UV) and visible (VIS) light irradiations. STLC analogue, PAM-Cys was synthesized by coupling reaction of 4-phenylazophenyl maleimide with SH-group of cysteine. PAM-Cys showed reversible absorption spectral changes upon UV-VIS light irradiations suggesting the cis-trans isomerization of azobenzen moiety of PAM-cys. Preliminary experiments revealed that the Microtubules dependent ATPase activity of EG5 was inhibited by PAM-Cys reversibly upon UV-VIS light irradiation. We also examined inhibitory effect of spiropyran derivative, 1', 3', 3'-Trimethylspiro [1 (2H) -benzopyran-2, 2'-indoline] (TMSBI) for Eg5. TMSBI inhibited ATPase activity of Eg5 upon UV light irradiation more effectively than VIS light irradiation.

**1PS044 フォトクロミック阻害剤を利用したキネシン Kif18A の光制御
Photo-regulation of kinesin Kif18A using photochromic inhibitor**Hideo Seo¹, Kumiko Ishikawa¹, Kanako Touyama² (¹Div. Bioinfo., Grad., Eng., Soka Univ., ²Dept. Bioinfo., Fac. Eng., Soka Univ.)

The shape and function of the mitotic spindle depends on the cooperation action of various kinesins. Recently, it was demonstrated that the kinesin Kif18A is a central component for the correct alignment of chromosomes at the spindle equator. BTB-1 is a small molecule potent inhibitor for Kif18A. BTB-1 inhibits ATPase activity of Kif18A but not of other mitotic kinesins. BTB-1 also inhibits the motility of Kif18A in a reversible manner. BTB-1 inhibits Kif18A in an ATP competitive but microtubule-uncompetitive manner and slows down the progression of cells through mitosis. Azobenzen is one of the photochromic molecules that change their shapes and properties drastically upon ultra-violet (UV) and visible (VIS) light irradiation. Previously, we have incorporated azobenzen derivative into functional region of ATP driven motor protein and succeeded to control microtubule dependent ATPase activity reversibly by UV-VIS light irradiation. Interestingly, BTP-1 shows structural similarity to azobenzen derivatives. In this study, we examined photo-reversible inhibitory effects of various azobenzen derivatives for ATPase activity of Kif18A. Cis