mutant microtubules were modified with photochromic molecules and the effect upon UV-VIS light irradiation for gliding speed and kinesin ATPase activity were studied.

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Photocontrol of Motor Proteins using Photo-Responsive Calmodulin Dimer
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Calmodulin (CaM) is a physiologically important Ca^{2+}-binding protein that participates in numerous cellular regulatory processes. CaM undergoes a conformational change upon binding to Ca^{2+}, which enables it to bind to target proteins for specific responses. For example, Ca^{2+}/CaM regulates function of myosin V, myosin light chain kinase (MLCK) etc. Previously, we succeeded to photocontrol CaM function using photochromic molecule N- (4-phenylazophenyl) maleimide (PAM), which reversibly undergoes *cis-trans* isomerization upon ultraviolet (UV) and visible (VIS) light irradiation. The CaM mutants, which have a single cysteine in the functional region of CaM, were prepared and modified with PAM. The binding of PAM-CaM to target peptide M13 was controlled reversibly upon UV and VIS light irradiation. In this study, we prepared the photo responsive dimer CaM by cross-linking of CaM mutants with bifunctional photochromic compound, 4,4'-azobenzene-dimaleimide (ABDM) in order to apply to photo control of motor proteins. For the CaM dimer, it is expected that the relative special configuration of each of CaM cross-linked with ABDM changes by UV-VIS light irradiation. Subsequently, we prepared the fusion protein, K355-M13 composed of kinesin motor domain and M13 peptide. The monomeric K355-M13 formed dimer configuration by CaM dimer. In the presence of Ca^{2+}, two K355-M13 bound to the each site of CaM dimer resulted in formation of kinesin dimer linked by CaM-ABDM-CaM. The phtocontrol of the motor activity and microtubule dependent ATPase activity of the photochromic kinesin dimer was studied. Application of CaM-ABDM-CaM to MLCK and myosin was also performed.

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Molecular Shuttle with Calcium Dependent Cargo-Loading System using Kinesin-Cam Fusion Protein and Liposome
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Recently, attention is being focused on the application of molecular shuttle based on kinesin and microtubules. Essential point regarding molecular shuttles is the cargo-loading system. Since the intrinsic physiological mechanism underlying the selective binding of cargo to the tail domain of kinesin has not been clarified thus far, artificial binding systems have been utilized for cargo binding. For instance, in a previous study, biotin-avidin and antigen-antibody systems were used for cargo loading. These systems are known to be highly specific with extremely strong binding. However, they have a defect that the shuttle cannot release the cargoes. Therefore, it is required that the molecular shuttle should have the reversible cargo-loading function. In this study, we developed a new molecular shuttle system in which the cargoes of liposome labeled with M13 peptide reversibly binds to the shuttle of kinesin-CaM fusion protein in a Ca2+ concentration dependent manner. We prepared K560-CaM that consists of the N-terminal motor domain, neck region, α-helical coiled-coil region and CaM at the C-terminus. K560-CaM showed Ca2+ dependent reversible binding to M13-GFP. Liposome labeled with fluorescent probe was prepared and its stability as a cargo was examined. Also maleimide- conjugated liposome was prepared. We also designed and synthesized the M13 peptide that has a single cysteine at N-terminus. It is known that the peptide is combined with a maleimide on the surface of the liposome. Currently we are studying the Ca2+ dependent interaction between M13-liposime and K560-CaM.

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The Neck Linker of Kinesin-1 Functions as a Regulator of ATP Hydrolysis Reaction
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Kinesin1 is a highly processive motor that moves along microtubule in a hand-over-hand manner. The neck linker that connects two motor domains has been thought to act as a mechanical amplifier that propels the tethered head forward, however, we recently showed that the neck linker docking is not essential for the forward stepping. We hypothesized that the neck linker docking rather functions to activate ATP hydrolysis reaction. To test this hydrolysis, we engineered monomeric kinesin mutants whose neck linker was truncated and carried out biochemical and structural analysis. As the neck linker was deleted further from the C-terminus, microtubule-activated ATPase rate decreased and became almost undetectable when the whole neck linker was removed. Single molecule imaging showed that the neck linker-less monomer stably bound to the microtubule even in the presence of saturating ATP. Cryo-EM observation of the neck linker-less mutant on the microtubule in the presence of saturating AMPPNP displayed a structure similar to that of nucleotide-free wild-type kinesin. These results are consistent with the idea that the neck linker acts as an activator of ATP hydrolysis reaction, and also explains the front head gating mechanism for dimeric kinesin: the neck linker of the leading head is pulled backward so that the head cannot proceed ATP hydrolysis cycle until the trailing head detaches from microtubule.

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Exploring the Origin of Directionality of Ncd Motor using Structure-Based Model
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Ncd motor proteins are the member of kinesin superfamily which serve important purpose in terms of cargo transport upon microtubule track. While the Ncd proteins are structurally very similar to other proteins of kinesin family, they move towards the negative end of microtubule as opposed to others. Here, we explore the origin of such unique directionalily of Ncd motor proteins using structure-based model and identify the important structural elements responsible for the motion. We find that the flexibity of the junction region between coiled-coil stalk and motor head acts as ATP dependent switch to create specific directionality. We also identify the nature of domain motion in free Ncd using covariance analysis which describes the symmetry breaking character of conformtional fluctuation. The novelty of our present study is that simple structure-based model can describe the essential feature of complex functionality of Ncd motors.

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The Mechanism of Cytoplasmic Dynein Processivity
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Cytoplasmic dynein is a homodimeric AAA+ motor that transports a multitude of cargos towards the microtubule minus end. It is currently unknown how the two catalytic head domains interact and move relative to each other during processive movement. We have tracked the relative positions of both heads with nanometer precision and directly observed that the heads move independently along the microtubule. The heads remain widely separated and the stepping behavior of the heads varies as a function of interhead separation. Consistent with a lack of tight coordination, only one active head is sufficient for processive movement and the active head drags its inactive partner head forward. Only a single active ATPase ring is sufficient for processivity, and the linker swing provides required force to drive minus end directed motion. These results show that dynein is the first dimeric motor that moves processively without interhead coordination, a mechanism fundamentally distinct from hand-over-hand motion of kinesin and myosin.

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In Vitro Gliding Assays Indicate that Chlamydomonas Dynein Moves Microtubules Polymerized from Chlamydomonas Axonemal Tubulin Faster than those Polymerized from Porcine Brain Tubulin
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The axonemal dyneins drive the motility of cilia and flagella. Dynein activity must be coordinated spatially and temporally within the axoneme to generate the regular, repeating waveforms characteristic of cilial motion. Recent studies have suggested that the mechanochemical properties of dynein, in particular the dependance of velocity and dissociation constants on force, may be sufficient to achieve coordination. Because the processes involving these properties require interactions between the dyneins and the microtubules, it is likely that they are dependent on the specific dynein-tubulin interactions. We investigated this possibility using microtubule gliding assays with outer arm dynein from Chlamydomonas. We found that that the translocation speed of microtubules polymerized from Chlamydomonas tubulin is 1.6 times faster than the speed of porcine brain microtubules. Additionally, we found that the source of the tubulin also influenced how translocation speed depended on microtubule length. Both of these results suggest that dynein from Chlamydomonas prefers