measurements also revealed that, just before 16 nm step, the gold particle displaced rightward, perpendicular to the microtubule axis, accompanied with a significant increase in the fluctuation of the gold particle. The rightward asymmetry is consistent with the geometry that the proximal end of the neck linker is located right side of the head. Then the gold particles showed rapid left-forward displacement followed by decrease in the fluctuation, although we could not detect clear sub-step at this temporal resolution. The duration of the highly fluctuating period increased as the ATP concentration was decreased, indicating that the period represents ATP-waiting state. These results provide direct clue to understand how kinesin takes a step forward.

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Strain-Dependent Regulation of the Kinesin-1's Catalytic Activity as Studied by Disulfide-Crosslinking of the Neck Linker

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Kinesin is a dimeric motor protein that hydrolyzes ATP and moves along microtubules in a hand-over-hand manner. To walk by alternately moving two motor heads, the trailing head should detach from the microtubule prior to the leading head and the detached head should preferentially bind to the forward tubulin-binding site. To explain these mechanisms, we hypothesized that ATP hydrolysis reaction of kinesin motor domain can be regulated depending on the direction of the tension posed to the neck linker: backward strain posed to the neck linker suppresses ATP hydrolysis in the leading head and the forward strain posed to the neck linker suppresses ADP release at the trailing position. To test this hypothesis, we constrained the neck linker in the forward or backward extended conformation using disulfide-crosslinking between cysteine residues on the head and the neck linker, and examined these effects on the microtubule affinity and ADP release kinetics. Single molecule fluorescent observation of the GFP-fused monomeric kinesin showed that when the neck linker was constrained in a backward extended conformation, the dwell time on the microtubule in the presence of saturating ATP was increased by a factor of 15 compared to unconstrained condition. In contrast, stopped-flow measurement showed that when the neck linker was constrained in a forward extended conformation, ADP release rate after microtubule-binding was significantly decreased. These results support the idea that ATP hydrolysis cycle of kinesin's motor domain can be differently regulated depending on the direction of the neck linker extension.

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A Biosynthetic Approach to Studying Multiple Motor Complexes

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Collective motor dynamics drives important cellular processes ranging from muscle contraction to spindle organization to vesicle trafficking. Although the biomechanical and biochemical properties of individual motors have been widely studied, how motors coordinate their motility when attached to the same cargo is largely unknown. We are developing a biosynthetic approach to generate multi-motor assemblies whose biological properties can be examined in vitro and in cells. To do this, we have assembled a ''toolbox'' of protein components consisting of scaffolds and linkers. We have characterized scaffold proteins of different lengths that allow for specific separation distances between the components. We have characterized four different linker systems that enable constitutive or regulated attachment of individual motors to scaffolds. Thus, our biosynthetic approach can be used to generate multiple motor complexes with absolute control over motor type, separation, and number. The motility properties of these complexes can then be studied in vitro and in live cells to determine the structural and mechanical features that enable kinesin-1 motors to work collectively. This approach is applicable to other biological questions such as the generation of complex signaling networks as well as the assembly of artificial biological systems for engineering applications.

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Distinct Transport Regimes of Two Elastically Coupled Molecular Motors

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Intracellular transport of cargos is mainly achieved by the cooperative action of molecular motors, which pull the cargos along cytoskeletal filaments. These motors are elastically coupled, which influences the motors' velocity and/or enhances their unbinding from the filament. We show theoretically that interference between two elastically coupled motors leads, in general, to four distinct transport regimes characterized by different effects on the mean velocity and/or the binding time. To gain an intuitive insight in the emergence of these transport regimes, we compare characteristic time scales for the strain force generation. These time scale arguments allow us to predict the transport regimes for different pairs of identical motors. In addition to a weak coupling regime, pairs of kinesin motors and pairs of dynein motors are found to exhibit a strong coupling and an enhanced unbinding regime, whereas pairs myosin motors are predicted to attain a reduced velocity regime. All of the predicted regimes can be explored experimentally by varying the elastic coupling strength.

F. Berger, C. Keller, S. Klumpp, and R. Lipowsky, Phys. Rev. Lett. 108, 208101 (2012)

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Experimental and Computational Investigations into Cooperative Cargo Transport by Mixtures of Kinesins from Different Families

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Transport of intracellular cargo often involves multiple motor types, either having opposite directionality such as during bidirectional transport of vesicles, or having the same directionality but different speeds. While significant progress has been made in characterizing motors at the single-molecule level, predicting their ensemble behavior is still challenging. To uncover the force-dependent properties of diverse kinesins and to understand how diverse kinesins attached to the same cargo coordinate their movement, we carried out microtubule gliding assays using pairwise mixtures of motors from the kinesin-1, 2, 3, 5 and 7 families. To match their processivities and ensure identical binding to the glass substrate, the motors were fused to the dimerization domain and coil-1 of kinesin-1, and the neck linkers were adjusted to have a uniform length of 14 amino acids. Uniform motor densities were used and microtubule-gliding speeds were measured as the ratio of fast motors varied from 0 to 1. Depending on the motor pair, velocity versus motor fraction curves varied from convex up to nearly linear to convex down. These findings were recapitulated using a coarse-grained computational model of gliding assays. The simulations incorporate force dependent velocities and dissociation rates from the literature along with mechanical interactions between motors bound to the same microtubule. The simulations also suggest that the motor compliance plays a minimal role in the observed gliding speed compared to observations in quantum dots. The gliding assays combined with the modeling allows us to test hypotheses regarding the characteristics of diverse kinesins under predominantly axial load, avoiding the large normal forces inherent in optical tweezer experiments.

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A Spacer between the Head and Neck Coil of Kinesin-1 Relieves Inhibition Due to Crosslinking of the Heads

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Kinesin-1 is autoinhibited through crosslinking of its two motor domains (heads) by a tail domain (Kaan, et al., Science 333, 883 (2011)), in addition to crosslinking of the heads by attachment to the neck coil (NC). This 'double lockdown' would prevent undocking of the neck linker (NL) and inhibit ADP release. Inhibition by double lockdown was supported the ability of a disulfide crosslink in a S181C mutant to mimic inhibition in the absence of tails. Insertion of a flexible spacer at the junction of the NL and NC could potentially provide enough 'slack' for NL undocking and ADP release. To test this mechanism, a five amino acid spacer was introduced into the S181C mutant (a total of ten additional amino acids in the dimeric construct). At 2 μM MTs, the rate of ADP release was 0.65 s^{-1} and <0.01 s^{-1} for the crosslinked forms with or without the spacer, versus 4.5 s^{-1} when uncrosslinked. Thus ten amino acids of slack per dimer greatly accelerates ADP release, albeit not fully up to the uncrosslinked level. That this spacer is sufficient to largely relieve the inhibition suggests that the inability to undock the NL is the principal cause of the inhibition produced by double lockdown. Studies with longer and shorter spacers will better define the dependence of the ADP release rate on the amount of slack.

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Biochemical Investigations into the Kinesin-2 Chemomechanical Cycle

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Kinesin-2 and dynein motors transport intracellular cargo bidirectionally along both axonemal and cytoplasmic microtubules. These motor activities underlie intraflagellar transport, melanosome dynamics and other vital transport functions in cells, but the mechanism by which the activities of these oppositely-directed motors are coordinated is not well understood. One important question is whether the properties of kinesin-2 motors are specifically tuned for bidirectional transport rather than long-distance plus-ended transport. Consistent with this, kinesin-2 motors were found to detach much more readily than kinesin-1