with this, when the Kinesin-2 neck linker was matched to the effective length of Kinesin-1 by deleting three residues and substituting an alanine for a proline, the Kinesin-2 run length nearly matched that of Kinesin-1. These results demonstrate that run length scales with neck linker length for both Kinesin-1 and Kinesin-2 and is sufficient to account for differences in processivity. In addition, we find that adding positive charge to neck linker inserts enhances processivity, providing a possible explanation for the lack of dependence of run length on neck-linker length observed by others. Our data is consistent with the hypothesis that increasing neck linker compliance reduces processivity by disrupting front head gating and potentially provides a unifying principle across kinesin families - longer neck-linkers lead to less processive motors.

1912-Pos
Optimal Size of the Neck Linker is Important for the Coordinated Processive Movement of Kinesin-1
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Kinesin-1 is a dimeric motor protein that walks along microtubules by alternately moving two motor 'heads'. Several recently published papers including ours provided evidences that kinesin dimer takes one-head-bound state while waiting for ATP and ATP-binding triggers the tethered head to bind to the forward tubulin-binding site. However, it is still not clear why rebinding of the tethered head, which is freely diffusing, to microtubule is prohibited during the ATP-waiting state. To explain this mechanism, we proposed a model based on the crystal structural analysis (Makino et al, this meeting) that ADP release of the tethered head is prohibited because the neck linker would be stretched out if both heads become nucleotide-free due to a steric hindrance posed on the neck linker. This model predicts that if the neck linker is artificially extended, the tethered head can easily rebind to the microtubule. To test this prediction, we engineered neck linker extended mutants by inserting poly-Gly residues and observed their conformational states using single-molecule FRET technique. We found that 5 amino acid extension of the neck linker allows the tethered head to rapidly rebind to the microtubule even in the absence of ATP, and that in this state both neck linkers adopt backward-pointing conformation. The neck linker extended mutants showed processive motility with reduced velocities compare to the wild-type, although the microtubule-activated ATPase rate was not changed, which are consistent with our previous results using poly-Pro insertion (Yildiz et al 2008). There results suggest that optimal size of the neck linker is important to prevent rebinding of the tethered head while waiting for ATP and to efficiently couple ATP hydrolysis energy with forward step.

1913-Pos
The Neck Linker of Kinesin-1 Functions as a Regulator of ATP Hydrolysis Reaction
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Kinesin-1 is a highly processive motor that moves along microtubule in a hand-over-hand manner. The neck linker that connects two motor domains has pivotal role in the head-head coordination but its exact role is still controversial. It have been widely believed that the neck linker acts as a mechanical element to propel the tethered head forward, however, we recently proposed an alternative model (biased-capturing model) based on crystallographic and cryo-EM analyses, in which the neck linker docking is not required for the forward stepping. We hypothesized that the neck linker docking rather functions to activate rate-limiting ATP hydrolysis reaction.
To test this hypothesis, we engineered a series of monomeric kinesin mutants whose neck linker was truncated and carried out biochemical and structural analyses. As the neck linker was deleted further from the C-terminus, microtubule-activated ATPase rate of the mutant kinesin decreased and it becomes almost undetectable when whole neck linker was removed. Single molecule fluorescent imaging showed that the neck linker-less monomer stably bound to the microtubule even in the presence of 1 mM ATP. Cryo-EM observation of the neck linker-less mutant on the microtubule in the presence of saturating AMP-PNP displayed a structure similar to that of nucleotide-free wild-type kinesin.
These results indicate that kinesin without the neck linker can bind to the microtubule but is incapable of proceeding ATP hydrolysis reaction, which is consistent with the idea that the neck linker acts as an activator of ATP hydrolysis reaction. This mechanism can explain the front head gating mechanism for head-head coordination: the neck linker of the leading head is pulled backward and the head cannot proceed ATP hydrolysis so that the head cannot detach until the trailing head detaches from microtubule.

1914-Pos
Coupling of Kinesin-1 Neck Linker Docking to the Nucleotide Binding Site
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Coupling of nucleotide binding to docking of the neck linker of kinsin-1 is important for generation of directional motility. One approach towards determining the magnitude of this coupling is to use isotopic exchange reactions to evaluate the free energy differences between states. Kinesin-1 monomer head domains catalyze the slow MT-dependent synthesis of bound ATP from bound ADP and free Pi (MT•E•ADP + Pi → MT•E•ATP + HOH) that results in oxygen isotopic exchange of $^{18}O/^{16}O$ between water and Pi. The tethered head domain of a kinesin dimer bound to MTs, however, catalyzes ATP synthesis at a 20-fold faster rate (Proc.Natl.Acad.Sci.USA 102, 18228 (2005)). This more rapid rate of ATP synthesis with a dimer suggests that the tethered head can bind to the microtubule behind the strongly attached head, because this positions the neck linker of the tethered head toward the plus end of the microtubule and would facilitate its docking on synthesis of ATP.
Isotopic exchange analysis of other constructs with alterations in the neck linker is in progress. One approach is to delete part of the neck linker and therefore prevent reversible docking. DKH335 has lost the C-terminal part of the neck linker that makes extensive contacts with the core. During net ATP hydrolysis, the full length head domain DKH346 resynthesizes ATP on average once in 40 turnovers. In contrast, DKH335 is reported here to hydrolyze ATP with no detectable ATP resynthesis (ATP resynthesis occurs only once in >500 turnovers). This is consistent with more rapid Pi release in the absence of a requirement for coupled neck linker undocking or with destabilization of bound ATP in the absence of neck linker docking.
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1915-Pos
Activity Scales and ATP Hydrolysis: Understanding the Thermodynamics of Molecular Motor Kinesin
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Kinesin is a molecular motor that transports cargo along microtubule tracks and like most other molecular motors, is powered by ATP hydrolysis. The chemical energy derived from the ATP reaction cycle is converted into mechanical work. Understanding the thermodynamics of ATP hydrolysis coupled with the motor (an enzyme), can offer insights into the mechanism and energy landscape of the system [1]. Activity scales were introduced [1] as thermodynamic parameters with this motivation.
We present a scheme to estimate activity scales for ATP hydrolysis by relating them to the free energies of formations.
Extending the concept, we show that these activity scales are well-defined for chemical species in *any* equilibrium reaction. Hence, a complex equilibrium reaction can be *decomposed* in terms of the activity scales of the respective species. The equilibrium constant for the reaction can also be calculated if the activity scales are known. A quantum mechanical simulation scheme is used to calculate activity scales. Results are presented for some gas phase equilibrium reactions involving small molecules. The accuracy of the calculated activity scales is related to the level of theory used for the quantum mechanical simulations. We discuss the implications and challenges of such simulations in solvent environments for large molecules in biochemical reactions.
[1] R. Lipowsky and S. Liepelt, J. Stat. Phys. 130, 39, 2008.
[2] Neha Awasthi, V. Knecht, and R. Lipowsky, in preparation.

1916-Pos
Single Molecule Visualization of Self-Regulated Kinesin Motility
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Kinesin-1 is an ATP-driven molecular motor that transports various cargoes in cells by binding its motor domain to microtubules. Its tail domain is thought to self regulate this binding. Here we inhibited kinesin ATPase activity and motility by interacting the heavy chain C-terminal tail region with the N-terminal motor domain. Ionic strength was found to heavily influence this self-regulation as both tail domain binding to the motor domain and ATPase activity were dependent on KCl concentration in in vitro experiments. Single molecule imaging experiments showed that the tail domain did not affect motility velocity but did lower the binding affinity of the motor domain to the microtubule. The decrease in binding was coupled to ATPase inhibition. Tail domain transfected into living cells failed to bind to microtubules, but did inhibit the interaction between the motor domain and microtubule, in agreement with the in vitro investigations. From these results, we propose a mechanism to describe this ion strength