living cells at an unparalleled spatial and temporal resolution (50-100 nm, 4-8 min for a 30 μm scan). Furthermore, we were also able to map the elasticity of a reconstituted actin network, which has not been achieved before. In combination with optical techniques this opens up a unique simultaneous view of the mechanics of the living cell and the mechanical properties of its relevant molecular components.
Elasticity maps of a live HUVEC cell (30 μm scan)

1906-Pos
What is Measured By Passive Microbead Rheology?
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It is often claimed that the dynamic modulus G* of a viscoelastic medium can be measured by following the trajectory of a small bead subject to Brownian motion. In the pioneering manuscript that introduced the idea [T. Mason and D. Weitz, Physical Review Letters 74, 1250 (1995)], this equivalence between the autocorrelation function and G* was assumed. Later work claimed that a correspondence could be proven, but to our knowledge, the proof has never been shown. We use here an analytic solution of the forces on a sphere undergoing arbitrary displacement in an arbitrary viscoelastic medium combined with the fluctuation-dissipation theorem to derive what is actually measured in the microbead rheology experiment. We find that a convolution of G* is indeed measured in the followed autocorrelation function. Under certain restrictions the autocorrelation function is a direct measurement of the dynamic modulus as is typically used. We examine experimental data published in the literature and are unable to find any data where the restrictions do not hold. Nonetheless, the results suggest that the technique could also be used at higher frequencies, if proper analysis is made of the data.

1907-Pos
Intracellular Diffusion in Fission Yeast Cells Depends on Cell Cycle Stage
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During the cell cycle, rearrangements of the cytoskeletal network play an essential role, in particular for the success of cell division. In order to quantify the influence of cytoskeletal rearrangements on the viscoelastic properties of the intracellular space, we studied the diffusion of endogenous lipid granules within single fission yeast cells in the different stages of the cell cycle. The position of the granules was tracked with optical tweezers at nanometer and sub-millisecond resolution and the data were analyzed with a power spectral analysis. We found that the majority of the lipid granules underwent subdiffusive motion during all stages of the cell cycle, i.e. the mean squared displacement of the granule is $2Dt^{\alpha}$ with $\alpha<1$. With our experiments we have shown that α is significantly smaller during interphase than during any stage of mitotic cell division and, surprisingly, we did not find significant differences of α in the different stages of cell division. These results indicate that the cytoplasm is more elastic during interphase than during cell division and that its elasticity is relatively constant during the stages of cell division.

1908-Pos
A Comparison of Single-Cell Elasticity of Osteogenic Cells Measured with the Optical Stretcher and Holographic Microscope
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While it has been known for some time that bone mass is remodeled in response to mechanical stress, the identity of the primary mechanosensor has yet to be clearly established. To determine if cellular elasticity may play a role in the cell's ability to detect a pressure variation, we have used the optical stretcher to measure the elasticity of individual osteogenic cells. To determine cell elasticity from measurements of cellular deformation, the optical pressure on the cell surface is computed using a ray optics model which assumes a value for the index of refraction of the cell. Previously we have estimated this value from measurements of other eukaryotic cells, but the optical pressure varies significantly with small changes in refractive index. Therefore, digital holographic microscopy is used to improve estimates of this critical parameter. We consider the overall impact that a spatial variation in the index of refraction can have on the determination of the optical stress, and compare single-cell elasticity measurements of red blood cells, 2T3 osteoblast and MLO-Y4 osteocyte cells.
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Microtubule Motors-Kinesin-1

1909-Pos
An Atomic-Level Engine that Accounts for Kinesin Motility and Catalysis
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Kinesin motor proteins convert the energy of ATP hydrolysis into stepping movement along microtubules. In this process, the microtubule can be considered as kinesin's regulatory partner, responsible for activating the enzyme's functional behavior. In the absence of atomic resolution structures describing the kinesin-microtubule complex, the mechanism of this activation has remained unknown. We use cryo-electron microscopy to derive atomic models describing the complete, microtubule-attached, kinetic cycle of a kinesin motor. The resolution of our reconstructions (~8Å) enabled us to unambiguously build crystallographically-determined conformations of kinesin's key subcomponents into the density maps. The resulting models reveal novel arrangements of kinesin's nucleotide-sensing switch loops and of its microtubule binding element known as the switch II helix. Based on these models, we present a detailed molecular mechanism accounting for kinesin's force generation cycle. In this mechanism, the switch loops control a seesaw-like movement of the catalytic domain relative to the switch II helix, which remains fixed on the microtubule surface. Microtubules couple the seesaw movement to ATP binding by stabilizing the formation of extra coils at the N terminus of the switch II helix, which interact directly with the switch loops. Tilting of the seesaw to assume the ATP-bound orientation in turn elicits a power stroke by the motor domain's force-delivering element known as the neck linker. This sequence of events accounts for the essential mechanics of kinesin's force-delivery cycle, and also yields a new model for the catalytically active conformation of kinesin's ancestral relative, myosin.

1910-Pos
Free Energy Changes During Kinesin's Force-Generating Substep
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We have previously suggested that Kinesin-1 generates force by transient folding between the N-terminal cover strand and the C-terminal neck linker domains into a beta-sheet, the so-called cover-neck bundle (CNB). Once formed, the CNB has a conformational bias sufficient to move the neck linker forward. Replica exchange molecular dynamics simulations have been performed to elucidate the energetics of CNB formation with and without load. Without load, the CNB state is weakly favorable compared to non-CNB states by 0.85 kcal/mol at 300 K, which is in agreement with a previous experimental value based on electron paramagnetic resonance, 0.72 kcal/mol (Rice et al, Biophys. J. 84:1844 (2003)), although the identity of the states involved is not certain. In non-CNB states the mobile neck linker points mostly forward in the ATP-like conformation of the motor head, so there is relatively little conformational difference with the CNB-state. By contrast, when a 10-pN rearward load is applied at the end of the beta9 part of the neck linker, a new local energy minimum appears for a rearward-pointing state. Compared to the CNB state, the free energy of the rearward-pointing state is higher by 2.96 kcal/mol at 300 K. This indicates that the CNB readily forms under applied load and thus is able to move the neck linker forward. The significance of these results for the mechanism by which kinesin-1 walks on microtubules will be discussed.

1911-Pos
Neck-Linker Length is a Critical Determinant of Kinesin Processivity
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The kinesin neck-linker domain is a key mechanical element underlying processive kinesin motility. Not only is neck-linker docking thought to be the dominant conformational change in the kinesin hydrolysis cycle, chemomechanical communication between the two head domains must necessarily be transmitted through the two neck-linker domains and their shared coil-coil. Hence, the length of the neck-linker is expected to have a strong influence on kinesin run length, a quantitative measure of processivity. Across different kinesin families, motors with longer neck-linker domains, such as Kinesin-2 are generally less processive than Kinesin-1, which has the shortest neck-linker domain among N-terminal kinesins. However, there is disagreement in the literature as to whether artificially extending the Kinesin-1 neck-linker alters the motor run length. Using single molecule TIRF analysis to visualize GFP-tagged motors in 80 mM PIPES buffer, we find that lengthening the Kinesin-1 neck-linker by three amino acids results in a five-fold reduction in run length. Consistent

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