motors can reverse direction and exhibit random-walk like behavior when transitioning between microtubules within in the bundle at long times.

3951-Pos Board B679
Kinesin Motion in the Presence of Obstacles on Microtubules
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Kinesin molecules walk by successively moving their heads to binding site on microtubules. This walk involves 16 nm advances of each kinesin head, realized by a conformational change in the structure of the molecule and by diffusion. Most previous studies of kinesin focus on movement on microtubules which have almost every binding site accessible. However, obstacles such as other molecular motors or different proteins can occupy binding sites in front of a kinesin. This study focuses on predicting the effects of obstacles on the dynamics of kinesin. First, a novel quantitative model is developed to capture the diffusion of kinesin heads in the absence of obstacles. To obtain probabilities of a head to bind at different neighboring sites, this model considers the combined effects of the head geometry on the extension of neck linkers, the deformation of the head, the interaction of the head and microtubules, and the dynamic behavior of the coiled-coil structure of kinesin neck. The model reveals that the unwinding of the neck and the binding of the head with tilted configuration are required to obtain the comparable probability of side walk of kinesin as was measured in previous experiments. Then, the motion of kinesin in the presence of obstacles is studied by using this model. The results of previous experiments with obstacles suggest that the unbinding rate of kinesin increases in the presence of interference with obstacles. Thus, this effect is also incorporated in the model. The effects of the size and number of obstacles on the velocity and run length of single kinesins are predicted. The new model can also be exploited to other unsolved problems regarding the motion of kinesin heads such as mechanical interferences between kinesin heads during collective transport.

3952-Pos Board B680
Experimental and Computational Investigations into Cooperative Cargo Transport by Mixtures of Kinesins from Different Families
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Intracellular cargo transport often involves multiple motor types, either having opposite directionality or having the same directionality but different speeds. Although a significant progress has been made in characterizing them at the single-molecule level, predicting ensemble behavior of motors is challenging. To understand how diverse kinesins attached to the same cargo coordinate their movement and uncover the force-dependent properties of them, we carried out microtubule gliding assays using pairwise motor mixtures 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 fast motor ratio varied from 0 to 1. Coarse-grained computational model of gliding assays recapitulated these experimental findings for the ensemble behavior. The simulations incorporate force-dependent velocities from the literature along with mechanical interactions between motors bound to the same microtubule. The force dependence of unbinding appears to be the key parameter that determines behavior in the multi-motor assays and motor compliance plays a minimal role in the observed gliding speed. Simulations also make predictions for the force dependent dissociation rates for single molecule experiments. 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.

3953-Pos Board B681
The Rice Kinesin Oskch1 is a Dynamic Cross-Linker of Actin Filaments and Microtubules
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The interaction between microtubules and actin filaments impacts many processes during plant growth and development including cell division and expansion. Despite numerous reports of co-localization between the two filament types in the past, only recently plant kinesins with a calponin-homology domain (KCH) were identified as putative dynamic cross-linkers. OsKCH1, a KCH from rice, has been described to interact with cortical microtubules and actin filaments both in vivo and in vitro. However, it remained unsolved whether this interaction is static or dynamic. Here, we show that OsKCH1 drives the active sliding of actin filaments along microtubules in vitro. We found that the sliding velocities corresponded to the velocities of microtubules propelled along OsKCH1 immobilized on glass substrates. This suggests that the motor interacts statically with actin filaments and dynamically with microtubules. We propose that kinesins with a calponin-homology domain play an essential role in the dynamic interaction between microtubules and actin filaments in plant cells.

3954-Pos Board B682
Measurements of Single Fluorescent Motor Proteins: The Right Way
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Cytoskeletal motor proteins are required in many cellular processes, such as intracellular transport and mitosis. Therefore, the biophysical characterization of motor protein movement along their filamentous tracks is essential. Commonly, stepping motility assays are used to determine the stepping and detachment rates of various molecular motor proteins by measuring their speed, run length and interaction time. However, comparison of these results proved to be difficult because the experimental setup (e.g. bead assay vs. single-molecule fluorescence assay), the experimental conditions (e.g. temperature, buffer or filament preparation) and data analysis (e.g. normal vs. exponential distribution) can influence the results. Here, we describe a method to evaluate traces of fluorescent motor proteins and propose an algorithm to correct the measurements for photobleaching and the limited length of the filaments. Additionally, bootstrapping is used to estimate statistical errors of the evaluation method. The method was tested with numerical simulations as well as with experimental data from kinesin-1 stepping experiments to show that the run length of kinesin-1 is independent of the microtubule length distribution. Our work will not only improve the evaluation of experimental data, but will also allow for better statistical comparison of two or more populations of motor proteins (e.g. motors with distinct mutations or motors linked to different cargos).

Cell Mechanics and Motility IV

3955-Pos Board B683
Bleb Formation in a Hela Cell Induced by Temperature Gradient
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The ability to sense and respond to temperature changes is necessary for biological systems. However, the response of individual cells to local temperature gradient is poorly understood. We have observed actomyosin dynamics within a cell which responds to local thermo-stimulation produced by focusing infrared (IR) laser. We found that the thermo-stimulation provokes spherical expansions of the membrane, termed "blebs", in mitotic HeLa cells along the temperature gradient. Similar blebs are observed during cell migration, cell spreading, cytokinesis, and apoptosis. It has been proposed that the blebs form due to an increase in intracellular hydrostatic pressure, which results in the breakage of actomyosin cortex. Blebs can be induced artificially by laser ablation or by local delivery of actin-depolymerizing drugs (Sedzinski et al., 2011; Paluch et al., 2005). Cortical tension is also important for bleb expansion (Tinevez et al., 2009). However, the specific mechanism of bleb formation remains unclear. In this study, we demonstrate that blebs are induced by temperature gradient. Different types of blebs were observed depending on the steepness of temperature gradient at the ambient temperature between 40 and 50°C. Mitotic cells which responded to the thermo-gradient formed asymmetrical actomyosin network. A similar, but uniform increase in the ambient temperature did not induce the directional bleb formation, suggesting that the temperature gradient within a cell is essential for the bleb formation. When the motor function of myosin II or the polymerization/depolymerization dynamics of actin had been suppressed, smaller blebs were produced. These results hint to a new perspective that individual cells respond to local thermo-stimulation through shifting the protein activity or concentration distribution.

3956-Pos Board B684
Actin Bundle Stabilization During Cell Spreading on Micropatterned Substrates
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Biophysical interactions between cells and their local environment drive key processes such as motility, differentiation and division. Both chemical and mechanical factors regulate these cellular responses. The substrate topology

highly influences cell spreading, morphology and cell fate. Moreover, when cells spread on constraining adhesive micropatterned substrates, reproducible cell cytoskeleton and adhesion site organizations are observed. This suggests that a simple physical energy minimizing process drives cell adhesion when considering two cellular tensions at the interface, one along and the other normal to the cell interface. These active tensions are due to myosin II activity that maintains cell shape and which relies on the stabilization of actomyosin bundles.
To characterize cellular active tensions, we investigate how peripheral contractile bundles are formed, stabilized and rearranged during cell spreading. Experimental quantitative analyses are carried out on living or fixed cells spreading on adhesive patterns, where protrusions and adhesions are spatially restricted. Using fluorescently labelled F-actin on living cells, we observe that curvature radius of peripheral bundles increase during the spreading phase concomitantly with the increase of the bundle spanning distance. This supports the idea that bundle tension may depends on its length. Once cells have covered the whole adhesive pattern, further increase of the curvature radius often happens, suggesting that cells relax to a tensile steady state on longer period.
On fixed cell populations, similar correlations between curvature radius and spanning distance are observed. Close analysis of bundle strength and myosin II distribution suggest the existence of these dynamical processes. Analysis of the assembly of extracellular matrix proteins into fibrils revealed that tangential tension (i.e. along peripheral bundles) may be prominent compared to inward assemblies, mainly observed on spreading cells on homogeneous substrate.

3957-Pos Board B685
Evaluating Tension in Actomyosin Bundles at the Cell Periphery
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During cell spreading, interactions between cells and their local environment drive key processes such as motility, differentiation and division. Both chemical and mechanical factors regulate these cellular responses including the substrate topology that highly influences cell spreading, cell morphology and cell fate. Cell shape and cytoskeletal tension are maintained by the activity of myosin II. When cells spread on micropatterned adhesive substrates, reproducible cell cytoskeleton organizations into curved actomyosin bundles at the cell periphery are observed. To characterize cellular active tensions and their dependencies on geometries and on myosin II activity, the tension of peripheral contractile bundles is determined using different techniques of cellular force measurements.
Traction force microscopy (TFM) allows measurement of cellular forces exerted on the adhesive substrate by registering fluorescent beads displacements embedded in soft gels, and by then computing the corresponding cellular stress applied on the substrate. We combine micropatterning technique and TFM to investigate the tension of the peripheral contractile bundles depending on the pattern and cell geometries.
By using ultra-soft cantilevers and three-dimensional patterned substrates, we perform mechanical probing on peripheral contractile bundles enabling, at small deformations, to obtain direct tension measurements. By adjusting indentation magnitude and deformation speed, we aim to determine viscoelastic properties of actomyosin bundles on living cells.
Both the passive (TFM) and active (cantilever-based) experiments suggest a range of bundle tensions from 10 to 100 nanonewtons. Using drugs to vary the contractility level of the cell, we can reconstruct the interdependencies between cell contractility (i.e. bundle tension and cortical tension), cell shape (i.e. bundle curvature) and substrate properties (i.e. adhesive geometry and substrate stiffness).

3958-Pos Board B686
'Hum'-Corrected Comparison of Viscoelastic Properties of Normal, Tumorigenic, and Metastatic Breast Cells
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We tested the hypothesis that breast cancer cells have lower viscoelastic moduli than their normal counterparts. The moduli were evaluated by tracking peroxisomes within three human mammary epithelial cell types: normal, tumorigenic, and metastatic. Peroxisomes in these cells were labeled with GFP, and then imaged at 100 fps. From the resulting movies of peroxisome motion, mean squared displacements (MSDs) of the peroxisomes were evaluated. To assess the contribution of motor-driven 'hum' of the actomyosin network to the measured MSDs, peroxisome motion was also determined in the three cell types after they were treated with blebbistatin or sodium azide and 2-deoxy-D-glucose. Blebbistatin is a myosin II inhibitor, and sodium azide and 2-deoxy-D-glucose deplete cellular production of ATP. Our results indicate that peroxisome MSDs are larger in the presence of ATP and active myosin motors than when driven solely by thermal energy.
By inserting these MSDs into the generalized Stokes-Einstein equation, apparent viscoelastic moduli were obtained for the cytoplasms of normal, tumorigenic, and metastatic breast cells with and without the motor-driven 'hum'. The generalized Stokes-Einstein equation assumes that the only energy that drives motion of the tracked peroxisomes in the cytoplasm is thermal. Peroxisomes in all three cell types report lowered apparent cytoplasmic viscoelastic moduli in the presence of uninhibited myosin II motors than when driven solely by thermal energy. The viscoelastic moduli determined from metastatic and tumorigenic cells without hum are significantly lower than those from their normal counterparts.
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3959-Pos Board B687
Regulation for Phosphatidylinositol Lipids Signaling System by Talin
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Cellular motility is a basic function underlying various important physiological phenomena. Actomyosin system generally works as an internal force generator, and external stimulations received via receptor-mediated signaling systems and an adhesive system modulate the motility. The force-generating, signaling and adhesive systems work in cooperation to make a united anterior-posterior polarity, which ensures an effective and adaptive cell migration. The actin polymerization is induced by PI(3,4,5)P3-enriched domains on the cell membrane, which arise from a chemotactic signaling system including PI(3,4,5)P3-producing PI3-kinase and egrading PTEN in a self-organized manner. However, how the adhesive system is correlated to the PtdIns system is unknown, which should to be an essential mechanism in regulating a migration mode dependently on whether the cells move solitarily or collectively. We examined effects of loss-of-function of focal adhesion on the PtdIns dynamics in single-celled amoebae and multicellular structures of *Dictyostelium discoideum*. amoebae of a mutant lacking a component of focal adhesion, talin, by disruptions of *talA* and *talB*, did not adhere to the substratum and exhibited an enhancement of PI(3,4,5)P3 domains traveling on the cell membrane. Due to the conditional up-regulation of PI(3,4,5)P3 production, a response to chemoattractant stimulation was abrogated. Both the PI(3,4,5)P3 dynamics in resting and stimulated cells was recovered by a treatment with PI3-kinase inhibitor, indicating talin suppresses PI3-kinase activity. A single disruption of *talB*, not *talA*, reconstructed the abnormal PI(3,4,5)P3 dynamics, suggesting *talB* is more responsible. The *talB* lacking cells failed to move collectively in the multicellular structures. Therefore, talin may be coupling the adhesion and PtdIns signaling systems via PI3-kinase, and multicellular collective motion is possibly mediated through the coupling by talinB.

3960-Pos Board B688
Mechanical Properties of Vimentin Intermediate Filament Networks
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One marker of mesenchymal cells is the expression of vimentin intermediate filament (VIF) proteins, which assemble into networks. The cytoskeleton is typically comprised of these intermediate filament networks, actin, and microtubules. While some aspects of VIF networks have been characterized in bulk, their mechanical properties in situ have not been well defined. Here we use microrheological techniques to study VIF network mechanics in cells in which microtubules and actin have been removed so that VIF can be studied independently of the other cytoskeletal components. Simultaneous imaging of the network allows us to correlate network structure with its local mechanical properties.

3961-Pos Board B689
B Cell Receptor Clustering and Signaling Activation are Modulated by Physical Parameters of the Surface
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Antigen binding to the B cell receptor (BCR) induces receptor aggregation into signaling microclusters, actin dynamics and cell spreading, which trigger B cell signaling activation. Recent studies have shown that gathering of surface