

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

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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.

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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.

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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.

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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

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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.

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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