investigated via the construction of a Markov chain including all states assumed by the coupled kinesin-microtubule system, and mechanical rate constants optimized to fit experimental data. Further analysis with kinetic Monte Carlo yields sidestepping probabilities for each kinesin species. We also report preliminary results on the use of motion planning methods for simulating the geometry and dynamics of the protein/tubulin/obstacle system, using coarse-graining of PDB protein structures. The results of these simulations are compared to the Markov chain results, and used to reconcile and refine both methods.

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The Kinesin-8 Kif18B uses a Non-Canonical Form of Directed Motility to Target the Extreme Microtubule Plus-End

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A defining feature of kinesins is that they are motile, using ATP-hydrolyzed energy to translocate along the microtubule (MT) lattice. The motile properties of kinesins can define their subcellular distributions. For example, the mitotic kinesin-8 Kif18A uses high processivity to enriches at the plus-end of long stable MTs. In contrast, the localization of Kif18B, a second kinesin-8, is governed by its binding to EB1, a MT plus-end tracking protein. It is therefore unclear whether Kif18B requires plus-end directed motility to accumulate at MT plus-ends. Using structured illumination microscopy (SIM), we show that a sub-population of Kif18B occupies the extreme tip of MT plus-ends ahead of EB1. This observation raises the possibility that Kif18B uses plus-end directed motility to ''sample'' protofilaments corresponding to the GTP cap. Using single molecule assays, we show that Kif18B is not highly processive, and that the motor switches frequently between plus-end directed and diffusive modes of motility. Diffusion is promoted by the tail of Kif18B. The tail of Kif18B is therefore multi-functional: it allows the motor to interact with EB1, and we show here that it also contains a second MT binding site, a property that increases the MT on-rate of the motor. Our mean squared displacement analysis shows that the speed of ATP-driven plus-end motility of Kif18B is well below the velocity of growing MT plus-ends. However, computer simulations suggest that a combination of directed motility and diffusion allows Kif18B to outpace a growing MT plus-end. Collectively, our work demonstrates that the motile properties of Kif18B deviate significantly from conventional transport motors. Instead, Kif18B is designed to efficiently explore the GTP cap, enabling it to promote catastrophes and thereby regulate MT length.

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Motility of Kinetochore Kinesin CENP-E is Enhanced by Tubulin Detyrosination

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Targeted transport by intracellular motors can be regulated by posttranslational modifications of polymerized tubulins in the microtubule tracks, but little is known about such effects for motors that drive chromosome motions during mitosis. Microtubules that form mitotic spindle are differentially modified at the C-terminal residue of α-tubulin: polymers that point to the spindle equator, but not the astral microtubules, are preferentially detyrosinated. Here we examine the influence of tubulin detyrosination on CENP-E, the kinetochore-localized kinesin-7 that transports pole-proximal chromosomes to the spindle equator. We polymerized purified human tubulins that were fully tyrosinated or detyrosinated, and examined the suitability of these tracks for motility of recombinant GFP-tagged CENP-E motor. Using fluorescence microscopy we show that single molecules of CENP-E walk faster and more processively on detyrosinated microtubules. Moreover, on these tracks the CENP-E motor can generate larger force than on the tyrosinated microtubules, as determined using stationary optical trap. On both types of microtubules CENP-E took 8-nm steps, exhibited similar dwell times and frequencies of backward stepping. However, motor's detachment increased with resisting force faster when CENP-E was walking on tyrosinated microtubules, leading to the detachment from these polymers at on average smaller load, 4.5 pN vs. 6.4 pN for detyrosinated microtubules. The enhanced motility of CENP-E motor on detyrosinated microtubules, most notably its ability to carry a larger load, could potentially explain the targeted transport of mitotic chromosomes toward the spindle equator.

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Concentration Profiles of Actin-Binding Molecules in Lamellipodia with Retrograde Flow

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Motile cells form flat protrusions in the direction of motion called lamellipodia. The actin filament network inside lamellipodia flows opposite to the direction of motion (retrograde flow) due to actin polymerization at the front. Hence, actin binding molecules are subject to transport to the rear in the bound state and diffuse freely in the unbound state. We analyse this non-linear reaction-diffusion-advection process with respect to the concentration profiles of these species and provide analytic approximations for the profiles. Retrograde flow may cause a depletion zone of actin-binding molecules close to the leading edge. The existence of such zone depends on the free molecule concentration in the cell body, on the ratio of the diffusion length to the distance bound molecules travel rearward with the retrograde flow before dissociating, and the ratio of the diffusion length to the width of the region with retrograde flow and actin binding. Our calculations suggest the existence of depletion zones for the F-actin cross-linkers filamin and α-actinin in 3T3-fibroblasts, which is in line with the small elastic moduli of the F-actin network close to the leading edge found in measurements of the force motile cells are able to exert.

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Helical Buckling in Filopodia

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Filopodia are thin actin rich membrane protrusion that allow cells to interactively probe their environment by periodic protrusion and shrinkage interrupted by occasional kinks. Filopodial actin is thought to play a pivotal role in filopodial force transduction, bending, and rotation. We investigated whether, and how, actin within filopodia is responsible for filopodia dynamics by conducting simultaneous force spectroscopy and confocal fluorescent imaging of F-actin in membrane protrusions. The actin shafts frequently undergo buckling and rotational motion which was correlated with retrograde movement of actin inside the filopodium. Pulling on an object attached to the filopodium tip strongly correlated with the presence of actin near the tip region and pulling forces were found to be correlated with movement of buckles along the actin shaft. We propose a mechanism that is based on accumulation of torsional twist in the rotating actin shaft and consequently leads to torsional induced buckling and shortening of the actin shaft.

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Characteristics of Cell Shape in Two Dimensions

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The shape of a cell is closely related with its properties, and in certain conditions it has been shown that change in cell shape can change cell behavior and outcome. However in two dimensions cells have complicated outlines, and it is not yet clear how best to characterize cell shape. Here we study the characteristics of cell shape of the 10T1/2 cell line in two-dimensional culture, and a few osteosarcoma cancer cell lines. The two dimensional surfaces are treated to make them either hydrophobic or hydrophilic, and the cells imaged after fixing, using membrane and actin labeling. The cells are also treated with pharmacological modulators of the cytoskeleton, yielding a large number of different shape types, and shape perturbations. Shape parameters are calculated by first using image processing to obtain binary outlines of the cells, and then calculating a number of geometric parameters, such as area, perimeter