Platform: Microtubular Motors

187-Plat
Kinesin Backstepping Under 3D Load
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During the dwells between steps kinesin is bound via one head to the microtubule. ATP binding to the microtubule-attached head triggers escape from this waiting state, which we call the parked state. Kinesin then takes either a backwards (minus-end-directed) or a forwards (plus end directed) 8nm step according to the external load that it experiences. The dwell time before forwards steps increases exponentially with hindering load until it equals the dwell time for backsteps, which is ~1s. The 1s backstep dwell time appears to depend very little on the load, suggesting that backstepping is a leakage pathway that reports the failure of the biasing mechanism. Several groups have reported that mutating the the neck linkers, which tether the two heads together, alters stepping behaviour. At least one of the gating mechanisms that coordinate molecular walking appears disrupted, possibly because the connection between the heads loses tension, or possibly for other reasons. Stall force might be reduced because backsteps are more probable, or because forward steps are less probable, or indeed both. To explore the influence of directional load on the stepping probabilities, we have done experiments in which the upwards component of force is varied. Using single bead optical trapping, but with smaller (360nm) beads, alters the attachment angle of the motor, and the balance between the horizontal and vertical vectors of force. At this stage we find no change in the stall force. We are exploring the same problem using a custom AFM with a cantilever that is very stiff in the lateral and vertical directions, but compliant in the axial direction. Stall forces in this framework are lower, and we are working to discover why.

188-Plat
Kinesin-8 is a Weak Motor Protein with a Weakly Bound Slip State
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Kinesin-8 is a highly processive plus-end directed motor protein that is conserved in eukaryotes from yeast to human. Different members of the Kinesin-8 family have been shown in vivo to control the length of microtubules by interacting with their plus ends. High processivity is crucial for these motors to reach the microtubule plus ends, where they induce catastrophes or otherwise interfere with microtubule dynamics. In this study, we characterized yeast (Kip3) and human (Kif18A) kinesin-8s using optical tweezers. Both kinesin-8 motors stalled under load forces of only ~1 pN, much less than the stall forces measured for other kinesins. Furthermore, we found that higher load forces caused the motor to slip backwards on the microtubule towards the minus end; the motor could recover from this state and resume plus-end motility. The low forces are consistent with kinesin-8s being regulators of microtubule dynamics rather than cargo transporters; the slip state, in which the motor remains in a weakly bound state, may be an adaptation for high processivity.

189-Plat
Engineered Dynein Mutants Reveal Minimal Structural and Catalytic Requirements for Processive Motility
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Cytoplasmic dynein is a molecular motor responsible for minus-end directed transport along microtubules. In contrast to kinesins and myosins, the detailed mechanism of dynein processivity and force generation remains unclear. Dynein's structure and evolutionary origin are different from these motors, suggesting unique mechanistic features. In this work, we engineered novel dynein constructs with altered mechanical, chemical and geometric properties to test the roles of rigid linkage between monomers, interaction between the ATPase rings and the proposed linker swing mechanism in maintaining dynein processivity. We found that a rigid linkage between monomers and dimerization through the N-terminal tail domains are not essential for dynein processivity. Instead, processivity minimally requires the linker domain of one monomer with an active ATPase ring to be attached to a partner monomer, which can be replaced by an inert protein retaining the microtubule-binding domain. To understand how one active head could provide motility to an inactive partner, we quantified the force-dependent microtubule release rates of dynein monomers. A clear directional asymmetry for detachment was observed, with significantly faster release towards the minus-end, providing insight into dynein's directionality. These results led us to a detailed mechanistic model of dynein processivity, directionality and force generation.

190-Plat
Coordination of Individual and Ensemble Dynein Motors Studied using Tools from DNA Nanotechnology
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The microtubule-based motor cytoplasmic dynein powers the transport of a diverse array of cargo, allowing cells to organize their contents, move, divide and respond to signals. Dynein is a ~1 MDa homodimer that can take many consecutive (processive) steps along its microtubule track by converting ATP hydrolysis into mechanical motion. It remains largely unknown how a dynein dimer coordinates the respective mechano-chemical cycles of its two motor domains to produce processive motion and how ensembles of motors work together to move cargo in concert inside cells. We have developed novel tools using DNA nanotechnology for dissecting the coordination of dynein both at the single molecule and ensemble levels.
To determine how dynein coordinates its two motor domains to achieve processive motion, we have developed a novel method to create stable dynein heterodimers by utilizing the hybridization of complementary DNA oligonucleotides. Two-color, high-precision, single molecule experiments reveal that dynein, unlike kinesin and myosin, uses both a stochastic and a coordinated stepping mechanism dependent upon the distance between the motor domains within the dimer.
To determine the mechanism of cargo motility by ensembles of motors, we have used DNA origami to build a synthetic cargo scaffold to which precise numbers and arrangements of motors can be attached. Our preliminary results in an unloaded system suggest that increasing the number of dynein motors enhances cargo processivity but does not affect velocity. Studies of opposite polarity motors are underway to determine whether cargos driven by both dynein and kinesin exhibit stochastic bidirectional movements.

191-Plat
Forces in Intracellular Transport: Calibrated Optical trap Recordings in Living Cells
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Many cellular cargos move bidirectionally along microtubules and actin, driven by teams of plus- and minus-end directed motor proteins. To probe the forces exerted on cargos in intracellular transport, we examined latex beads phagocytosed into living mammalian macrophages. These latex bead compartments (LBCs) are encased in membrane and transported along the cytoskeleton by a complement of endogenous kinesin, dynein, and myosin motors. The size and refractive index of LBCs makes them well-suited to manipulation with an optical trap. We developed novel methods to calibrate the optical trap in the complex, viscoelastic environment of the cytoplasm using spectra of thermal motions and forced sinusoidal positioning of the trap laser and the microscope stage. This method provides an accurate, in situ characterization of the fluctuating motions and forces on LBCs inside living cells without reliance on calibrations performed outside of the cell or on different beads, as in previous studies. Correlation analysis of quadrant photodiode signals is used to minimize spurious signals from scattering particles out of the LBC's plane. For both LBCs in live cells and purified LBCs in vitro, we find that centrally and peripherally directed forces exerted on LBCs in live cells, or plus- and minus-end directed forces on microtubules in vitro, are of similar magnitude. In vitro and in live cells, the force traces are indicative of transport by multiple motors, with maximum forces of 15-20 pN and detachment occurring in multiple steps. These observations suggest bidirectional transport of LBCs is driven by opposing teams of stably-bound motors that operate near force balance. Supported by NIH grant GM087253 to ELFH and YEG and GM089077 to AGH.

192-Plat
Deciphering Orientation and Rotational Information of Cargoes at Pauses During Axonal Transport
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The study of axonal transport is essential in learning motor protein working mechanisms and neuronal functions. Direct visualization of the transport events in living neurons has led to the current understanding of the mechanism of axonal transport. However, the mechanism for generating the pauses in the transit of cargoes is still largely unknown, mainly because the molecular or nanoparticle probes used in the previous studies could not reveal the