Kinesins, Dyneins, and Other Microtubule-based Motors

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Decrypting the Structural, Dynamic and Energetic Basis of Kinesin Interacting with Tubulin Dimer in Three ATPase States by All-Atom Molecular Dynamics Simulation
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We have employed molecular dynamics (MD) simulation to investigate, with atomic details, the structural dynamics and energetics of three major ATPase states (ADP, APO and ATP state) of a human kinesin 1 complexed with a tubulin dimer. Starting from a recently solved crystal structure of ATP-like kinesin-tubulin complex by Knossow lab, we have performed flexible fitting of cryo-electron-microscopy maps of kinesin-decorated microtubules, followed with extensive MD simulation (400ns for each state), which is to our knowledge the longest MD simulation of kinesin-tubulin complex published to date. Our modeling and simulation have revealed extensive conformational changes at the tubulin-binding site and the nucleotide-binding pocket of kinesin between ADP, APO and ATP state, featuring a more twisted central β-sheet and a highly flexible and open switch I in APO state. We have found kinesin undergoing large structural fluctuations in APO state toward the direction of ATP state, which allosterically couple nucleotide-binding pocket with tubulin-binding site. We have identified a dynamic network of hydrogen bonds spanning the nucleotide-binding pocket and the kinesin-tubulin binding interface, which are predicted to control and couple nucleotide and tubulin binding during kinesin's ATPase cycle. In addition, we have employed binding free energy analysis to identify a set of key residues involved in kinesin-tubulin binding. Our simulation has also shed light on several outstanding issues in kinesin literature (such as the role of neck linker docking in regulating nucleotide binding, and possible extension/shortening of α4 helix during the ATPase cycle). This study has provided a most comprehensive structural and dynamic picture of kinesin's major ATPase states, and offered promising targets for future mutational and functional studies to investigate the molecular mechanism of kinesin motors.

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A New Mechanism of Kinesin Molility: Conversion of Random Motion to Directional Motion with Ratchet Structure
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A dynamic model for the motility of kinesin along microtubule (MT), including stochastic-force generation and step formation, is proposed. In our model, force is generated by the impulse from the collision between the randomly moving long-chain stalk and the ratchet-shaped outer surface of microtubule. The force produces greater tension on the trailing head than on the leading head, and stochastically triggers the trailing head to flip over the leading head. Kinesin progresses along the protofilament in a non-conventional hand-over-hand fashion. We demonstrate the applicability of the dynamic model in a real vibrating simulation system including (a) ratchet structures similar to the outer surface of MT, (b) a bead chain connected to two heads, similarly to the stalk of the real kinesin motor, and (c) the attractive interaction between the heads of the simulated kinesin and microtubule. Most of the dynamical and statistical features of the motility of kinesin such as the step formation, the distribution of the dwell time, and the functional dependence of the velocity on the loading force (V-F relation) are reproduced in our simulating system. It is also found that the dependence of the velocity on the amplitude of vibration changes with the length of the stalk domain. Based on this finding, we propose an experiment to measure the dependence of the velocity of the motion of kinesin along MT as a function of the ATP concentration for the mutants of kinesin with various lengths of the stalk domain. Because the stalk domain has no effect on the motility of kinesin in the hand-over-hand model, such an experiment can discriminate between the hand-over-hand model and the dynamic model.

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Quantification of IFT-Dynein Dynamics in C.elegans
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Cytoplasmic dyneins are the main drivers of microtubule-based retrograde transport in eukaryotic cells. Cytoplasmic dynein 1 plays a role in retrograde intracellular transport and cell division, whereas cytoplasmic dynein 2, also known as IFT-dynein, co-operates with kinesin motors to assemble and maintain cilia in a process called intraflagellar transport (IFT). While cytoplasmic dynein 1 has been the subject of many recent studies, relatively little is known about IFT-dynein. Here, we focus on the mechanism and dynamics of IFT-dynein: how does it behave in vivo at the ensemble and single-molecule level? To this end, we use fluorescence microscopy to visualize labeled IFT-dynein motors in the chemosensory cilia of living C. elegans. Transgene worms were generated using the Mos1-mediated single copy insertion (MosSCI) method to ensure endogenous motor expression levels. Time-lapse fluorescence movies showed that IFT-dynein moves in trains consisting of tens of motor proteins. The movies were processed to kymographs, from which location-dependent velocities and motor numbers were obtained using in-house developed kymograph-analysis software. This analysis revealed that IFT-dynein train velocities and motor numbers are dynamic, changing along the cilium. Double-labeled constructs allowed us to look more closely into motor co-operation, determining the dynein:kinesin ratio at different positions in the cilium.
To obtain insight into the behavior of individual motors, we employed photoactivation of PA-GFP-labeled IFT-dynein, which allowed, for the first time, the tracking of individual IFT-dynein motors in vivo. Single-motor trajectories revealed distinct features of IFT-dynein motility: diffusive behavior at the ciliary base, pauses, turns, directed motion and switches between these behaviors. This combined ensemble and single-molecule approach has provided novel quantitative insight into IFT-dynein dynamics in living organisms, shedding light on the complex functioning of dynein motors in general.

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3D Real-Time Orbital Tracking in Zebrafish Embryos: High Spatiotemporal Analysis of Mitochondrial Dynamics in Neurons
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The main function of mitochondria is to provide cells with adenosintriphosphate (ATP) in regions with high-energy demand. A complex machinery of motor proteins (kinesin, dynein, myosin, etc.) and signaling molecules like calcium ions are responsible for the distribution and recycling of mitochondria in cells. A malfunction in the dynamics of these complexes is one possible reason for neurodegenerative diseases. However, the exact mechanism behind this process is not yet fully understood.
To follow the trajectory of individual mitochondria in rohon-beard sensory neurons, we use a home-built, three-dimensional, real-time orbital-tracking microscope with a spatial resolution of a few nm in three dimensions and an acquisition speed of up to 500 Hz. Environmental information is recorded simultaneously with a built-in widefield microscope.
Photoactivatable GFP (Tracking microscope) and TAG-RFP (Widefield microscope) are coexpressed in neuronal mitochondria by coinjecting neuron specific driver constructs with separate UAS responder constructs into fertilized single-cell zebrafish eggs. Zebrafish expressing the markers are measured individually three days past fertilization.
By photoactivating mitochondria, we are able to obtain single-mitochondria trajectories with a traveled distance of more than 100μm with nm precision. Due to our high spatial and temporal resolution, we can identify several different dynamic populations involved in mitochondrial transport. The environmental information gives insight into the colocalization behavior between stationary and moving mitochondria. By combining the results from the fast and precise tracking microscope with the widefield data, we obtain an in vivo overview over the dynamic processes in rohon-beard sensory neurons which can be used for further disease related experiments.

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Motor Coordination in Long-Distance Transport in Axons
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Retrograde transport of nerve growth factor signaling endosomes by microtubular motors, from the axon terminals to cell bodies, is vital for the survival of neurons. The robustness of this fast long-distance axonal transport and biased directionality could be attributed to the cooperative mechanics of multiple motors and/or intracellular regulation mechanisms. Here, we present a comprehensive motion analysis of retrograde nerve growth factor (NGF)-endosome trajectories in axons to show that cooperative motor mechanics and intracellular motor regulation are both important factors determining the endosome directionality. We used quantum dot (QD) to fluorescently label NGF and acquired trajectories of retrograde QD-NGF-endosomes with < 20 nm accuracy at 32 Hz, using pseudo-total internal reflection fluorescence imaging. Using a combination of transient motion analysis and Bayesian parsing, we segregated the trajectories into sustained periods of retrograde (dynein-driven) motion,