2627-Pos Board B597

Investigation Of Multiparticle Motor Protein Dynamics Using Coupled Exclusion Processes

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We theoretically investigate the interaction of a microfilament and motor proteins within the context of intracellular particle transport. We model the motion of motor proteins on the microfilament as a totally asymmetric simple exclusion process (TASEP). Given that motor proteins occasionally disassociate from the microfilament into the surrounding medium where they propagate by diffusion and whence they may re-associate onto the filament, we model their behavior as a symmetric simple exclusion process (SSEP) and proceed to investigate the consequences of linking the two systems with various couplings (i.e. disassociation and re-association rates). Stationary state properties are found exactly in the limit of strong couplings between the channels. It is shown that strong symmetric couplings between TASEP and SSEP lead to an effective partially asymmetric simple exclusion process (PASEP). However, strong asymmetric couplings yield an effective TASEP with nonzero motor protein flux in the microfilament and zero diffusive flux. Treatment of intermediate couplings yields similar but not exact results. Our calculations show that in all cases there are three stationary phases determined by dynamics at entrances, at exits or in the bulk of the system, while phase boundaries depend on the strength and symmetry of couplings between SSEP and TASEP. Extensive Monte Carlo computer simulations strongly support our theoretical predictions. Theoretical calculations and computer simulations also predict that the symmetry and values of the couplings have a strong influence on motor proteins dynamics. These results suggest that by modifying interactions between motor proteins and microfilaments it is possible to control biological transport processes.

2628-Pos Board B598

EPR Spectroscopy Shows That There Are Multiple Closed Nucleotide Pocket States When Kinesin-family Motors Bind To Microtubules With A Triphosphate At The Motor Nucleotide Site

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Identifying the conformational changes in kinesin-family motors associated with the binding of nucleotide and microtubules is essential to determining an atomic-level model for the production of force and motion by these molecular motors. We have previously used EPR spectroscopy and diphosphate-state nucleotide-analog spin probes to show that the open nucleotide site closes when kinesin-family motors bind to microtubules. We have extended these studies to kinesin and ncd motors in ATP-analog states. Upon binding to microtubules, probe mobility decreases implying a closing of the nucleotide site. Comparison with the microtubule•motor•diphosphate state shows two differences: 1. In the triphosphate state, there are two spectral components implying two different closed conformations of the nucleotide pocket. The equilibrium between the conformations is independent of temperature. 2. The probe mobilities in the closed triphosphate states are less than those seen in the diphosphate state implying a further closing of the nucleotide pocket in the triphosphate state. 3. To correlate better spectroscopy and structure, molecular dynamics simulations were done on the motor-probe complex. We find the simulated probe mobility for the open x-ray structure is comparable to that observed experimentally in the absence of microtubules. Minehardt et al (2001) proposed an ncd structure with a closed phosphate tube based on myosin x-ray structures. Simulated probe mobility from this structure is comparable to the most immobile of the microtubule•kinesin-motor•triphosphate states. We conclude that the nucleotide pocket is in a closed conformation when kinesin-family motors are bound to nucleotide and microtubules, with the triphosphate states more tightly closed. The most tightly closed state has a phosphate-tube structure apparently similar to myosin.

2629-Pos Board B599

UMF: Uniform Magnetic Force; a technique used to Slow Multiple Kinesin Motors in vitro

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Can molecular motors pulling on the same load interact with each other to move faster than any one motor could? While the force generated by a single kinesin motor on a cargo in vitro has been studied extensively, the force generated by multiple kinesin motors in vitro has not been characterized as well. Using a novel Uniform Magnetic Force (UMF) apparatus, we are able to apply constant, computer controlled, uniform force over a large sample area, as opposed to a force dependent on distance from the magnetic pole. The UMF can be turned on and off rapidly, and acts upon a superparamagnetic bead bound to a microtubule through a biotin-streptavidin linkage, causing a force on the microtubule. Using Invitrogen M270 beads this force can be as high as 9pN. We employ our apparatus to investigate the velocities of microtubules being pulled by kinesin motors against a magnetic force. With a force on the order of the stall force for one kinesin we are able to see changes in the velocity of a microtubule-bead complex being pulled by kinesin motor proteins. The relative amount of velocity change is not constant for each microtubule-bead complex. This may show a dependence of velocity decrease due to the number of motors moving the microtubule.

2630-Pos Board B600

How Does The Tethered Kinesin Head Diffuse To The Next Microtubule Binding Site?

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During each step taken by a dimeric kinesin motor, one motor domain must release from its previous binding site on the microtubule and move 16 nm forward to the next binding site. This stepping process is thought to involve both concerted conformational changes and diffusive movement, but the relative role played by these two processes is not clear. We are investigating this question by computationally modeling the chemomechanical cycle of kinesin using Brownian dynamics simulations. The simulations model the diffusion of the tethered kinesin head and incorporate resistive forces from the motor's flexible neck-linker domain and a distance-dependent on-rate for binding to the next site on the microtubule. Initial simulations model the neck linker domain as an entropic spring, using parameters for a Worm-Like Chain taken from protein unfolding experiments. Because the importance of properly modeling the mechanical properties of the neck linker domain, we have refined these initial Brownian models using data from molecular dynamics simulations to predict the force-extension relationship of Kinesin-1, Kinesin-2, and mutants with engineered neck linker domains. Simulations for diffusion and binding of the tethered head are incorporated into a Monte Carlo model of the entire kinesin mechanochemical cycle to reproduce motor stepping kinetics. These simulations are being tested by comparing the predicted motor speed and processivity to results from ongoing single-molecule experiments on Kinesin-1 and Kinesin-2 motors. These simulations make specific predictions for the magnitude of the conformational changes involved in motor stepping and the kinetics of the diffusive search in relation to the overall stepping rate.

2631-Pos Board B601

Neck-linker And Neck-coil both contribute to Kinesin Processivity

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Kinesins are microtubule-based molecular motors involved in intracellular transport. These motors utilize energy from ATP hydrolysis to 'walk' along microtubules and are processive, meaning that they take multiple steps before detaching from the microtubule. The kinesin superfamily consists of 14 families of motors that have diverse structures and diverse cellular functions. A principle question in understanding motor function is: What are the structural determinants of processivity. In contrast to homodimeric motors in the canonical Kinesin-1 family, Kinesin-2 motors have two different head domains and a three amino acid extension in their neck linker. We showed previously that Kinesin-2 motors are slower than Kinesin-1 and are roughly four-fold less processive. These differences could result from biochemical differences in the head domains, reduced inter-head coordination due to differences in the neck-linker domains, or diminished electrostatic interactions between the coiled-coil domain and the microtubule track. To test the influence of the coiled-coil domain, we engineered motors containing the Kinesin-2 head and neck linker domains fused to the Kinesin-1 coiled-coil. Single-molecule fluorescence experiments of GFP-labeled motors showed enhanced processivity compared to Kinesin-2, indicating a role for the coiled-coil in motor processivity. When the Kinesin-1 neck-linker domain was extended by three amino acids, processivity fell by a factor of three, suggesting that the neck-linker domain is an important determinant of processivity. However, shortening the Kinesin-2 neck-linker significantly reduced its processivity, indicating that in a given motor, the head, neck-linker and coiled-coil domains are tuned for optimal motor function.