disassembly in the lamelipodia of migrating cells. The model also provides insights on the critical interplay of capping protein and ADF/cofilin in the regulation of F-actin assembly. Because this model and the simulation results are ''open source'', in the sense that they are publicly available and editable through the *Virtual Cell* database (http://vcell.org), they can be accessed, analyzed, modified and extended. (Supported by NIH grants P41 RR013186, U54 RR022232 and U54 GM64346)

670-Pos Board B549
Real-time Observation Of Actin Polymerization Regulated By The Gelsolin-family Of Proteins
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Cell motility is governed by the concerted assembly and disassembly of actin filaments.[1] Actin filament length is regulated by a variety of actin-modifying proteins such as gelsolin, vilin and adseverin. To understand the mechanism of cell movement in health and disease, a detailed understanding of the self-assembly of actin monomers into filaments and its regulation by the actin-modifying proteins is required. Conventional bulk measurements yield ensemble-averaged data which do not allow an understanding of the process at the single molecule level. Total internal reflection fluorescence (TIRF) microscopy coupled with fluorescence spectroscopy measurements provide a means to follow the actin dynamics with single molecule sensitivity. In the present work, *in vitro* real-time TIRF assays of actin polymerization in the presence of full length and truncated actin-regulating proteins such as gelsolin, vilin and adseverin will be presented. Use of calcium ions as a switch to activate gelsolin[2,3] is further corroborated in the time-lapse movies.
Footnotes
[1] T.D. Pollard, Annu. Rev. Biophys. Biomol. Struct. 2007. 36:451-477.
[2] R.C. Robinson et al, Science 1999. 286:1939-1942.
[3] K. Narayan et al, Febs Lett. 2003. 552:82-85.

671-Pos Board B550
Computational Modeling of Antigen Processing and Presentation by Dendritic Cells
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The stimulation of T lymphocytes (CD4+ or CD8+) by dendritic cells (DCs) is a key event in the initiation and establishment of immune responses against pathogens. Understanding the intracellular mechanisms that govern how DCs acquire, process, and present antigens would lead to more rational vaccine design. Although individual intracellular events have been elucidated, a quantitative view of how the various networks of antigen trafficking affect T cell stimulation is lacking. In this work, we developed a stochastic model to examine the critical steps involved in antigen delivery for T cell stimulation, including antigen internalization, trafficking in endosomal/lysosomal environments, access to various antigen presentation pathways, and stimulation of either CD4+ or CD8+ T cells. Kinetic parameters for various processes were either obtained from previous reports if available, or derived from our own experimental data. In particular, we aim to identify rate-limiting steps of antigen trafficking and processing that regulate T cell stimulation. Furthermore, we examine how characteristics of the vaccine, such as size and attachment of targeting ligands, affect whether delivered antigen stimulates CD4+ or CD8+ T cell responses. The development of the computation model of antigen delivery will lead to greater insight into the intracellular processes involved in the type of T cell response elicited and to more rational design of effective vaccines.

672-Pos Board B551
Regulation and single-molecule mechanics of microtubule-based motors in living Chlamydomonas
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Whether motors of different directionality are functionally coordinated in cells or operate in a semi-random ''tug of war'' is unclear. We tested the hypothesis that the microtubule-based motion of the transmembrane protein FMG-1 in the flagella of Chlamydomonas is functionally coordinated for unidirectional transport. A laser trap was used to position microspheres on the plasma membrane of paralyzed Chlamydomonas flagella. The anterograde and retrograde movements of the microsphere were measured with nanometer resolution as microtubule-based motors moved FMG-1. Based on stall forces, we find that an average of 10 motors act to move the microsphere in either direction, with mean step sizes of 4 and 8 nm. Reverse steps were uncommon, and quiescent periods separated every transport event, suggesting the exclusive activation of motors of one direction. Temperature-sensitive mutants of kinesin-2 showed exclusively retrograde steps after jumps to the non-permissive temperature. These data suggest that molecular motors in living cells can be reciprocally coordinated to engage in large numbers and for transport in a single direction, even when motors of mixed directionality are present. The predominance of 4 nm steps suggests sub-steps or other novel behavior of these motors in the cytoplasm. This novel technique should prove beneficial for studying the mechanics, regulation and bidirectional coordination of molecular motors in vivo.

673-Pos Board B552
Multiple-Motor-Based Transport
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Intra-cellular transport via processive molecular motors plays an important role in maintaining cell structure and function. In many cases, cargoes move distances longer than expected for single motors; there is significant evidence that this increased travel is in part due to multiple motors working together to move cargoes. However, while we understand much about the function of single motors both experimentally and theoretically, our understanding of how multiple motors work together to move cargoes is less developed. We start with a Monte-Carlo model of single motor to theoretically investigate how multiple motors work together. We have investigated the effect of non-linear force-velocity curves and stochastic load sharing on multiple motor transport using stochastic model. We are particularly interested in cargo transport by a few molecular motors which is motivated by in-vivo results that only a few motors are engaged to transport cargo. Predictions for average travel distances and mean velocities obtained from stochastic model are significantly different from those predicted using steady-state model. Our theoretical study of multiple motor transport using stochastic model also shows that single-motor force-velocity curve plays an important role in determining the ensemble function when only a few motors are engaged.

674-Pos Board B553
Transport Of Micrometer-Sized Vesicles By Kinesin In Vitro
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Cytoskeletal motor proteins (e.g., kinesin) are responsible for directed transport in cells. Motor proteins can also be used in artificial bionanotechnological systems to provide a controlled cargo transport. We explore this possibility by using giant unilamellar vesicles (GUVs) as a micrometer-sized cargo model and establish an in vitro system to transport this cargo by kinesin (rK430) molecules along surface-attached microtubules (MTs). Kinesin was linked to GUVs (diameter 1–4 μm) via biotin-streptavidin interaction. MTs and moving GUVs were visualized using fluorescence wide-field imaging microscopy. We observe directed transport of GUVs along MTs with traveling distances of up to 100 μm and velocities of ~0.7 μm/s being in a good agreement with the velocity of kinesin motion along MTs (~0.8 μm/s). The long walking distances, as well as the visualization of the GFP-labeled kinesin molecules by total internal reflection fluorescence imaging, suggest that a large number (>10) of kinesin molecules is involved in the transport of a single GUV. Apart from its biotechnological importance, this system might additionally be useful to gain further understanding of vesicle transport processes in cells.

675-Pos Board B554
Intermittent Search Strategies for Delivering mRNA to Synaptic Targets
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We model the motor–driven transport of an mRNA containing granule along a dendrite in terms of a random intermittent search for a synaptic target. The granule is injected at one end of a one–dimensional track with an absorbing boundary at the other end. The particle switches between a stationary phase and a mobile phase that is biased in the anterograde direction. A single hidden target is located at a fixed but unknown location on the track. We calculate the hitting probability and conditional mean first passage time for finding the target, and determine conditions for an optimal search strategy.

676-Pos Board B555
Microtubule elasticity: Connecting all-atom simulations with continuum mechanics
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The mechanical properties of microtubules have been extensively studied using a wide range of biophysical techniques. These experiments have sought to understand how the mechanics of these cylindrical polymers is related to their