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

geometry, and likewise how microtubule bending and stiffness are influenced by the addition of drugs such as Taxol. Here we present a novel method for connecting all-atom molecular dynamics simulations with continuum mechanics and show how this can be applied to the microtubule system. Our coarse-graining technique applied to the microscopic simulation system gives us the correct macroscopic predictions for Young's modulus and persistence length and clearly demonstrates how Taxol binding decreases the stiffness of microtubules. The techniques we develop should be widely applicable to other macromolecular systems.

677-Pos Board B556

Extending the Range of In Vivo Multimotor Force-Velocity Curves by Sizing Vesicles Below the Diffraction Limit

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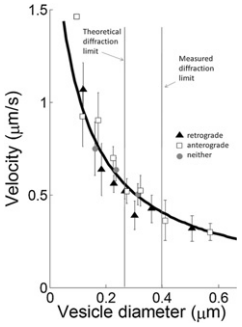
In our previous work (Shtridelman et al. 2008, Cell Bioch. & Biophys), we presented three force-velocity curves corresponding to 1, 2, and 3 motors/vesicle. We constructed these curves via velocities obtained directly from vesicles (NT2 cells, 37°C) and via forces obtained indirectly from Stokes' Law using measured intracellular viscosity, and vesicle diameters and velocities as inputs. The range of these earlier curves was restricted by the diffraction limit. In our current work, we use the image intensity–obtained with a differential interference contrast (DIC) microscope–as a proxy for vesicle diameters smaller than the diffraction limit. This novel sizing method is surprisingly robust, allowing us to extend the range of our in vivo multimotor force velocity curves. As with our previous curves, our newly extended 1-motor in vivo curve is similar to in vitro single kinesin force-velocity curves obtained at 35°C and, qualitatively, to single dynein curves obtained at 25°C. However, the 2- and 3- motor curves have proportionally higher extrapolated stall forces and overall velocities. The figure accompanying this abstract shows the primary size-velocity data we used to generate our extended in vivo multimotor force-velocity curves.



678-Pos Board B557

Characterizing Intracellular Structure and Dynamics Through Trajectory Analysis and Single-particle Tracking of QDs in Live PC12 Cells

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Quantum dots (QDs) are brightly fluorescent nanoparticles being investigated for potential use as probes of intracellular structure and function. Previously, we reported dynamic tracking of individual, activated QD-receptor complexes functionalized with nerve growth factor (NGF) in live PC12 cells, revealing a variety of complex trajectories using fluorescence microscopy. Here, we apply detailed numerical analysis of QD trajectories because commonly-used mean-squared displacement (MSD) analysis yields average quantities that fail to capture the complex information and interesting features of trajectories traced by ligand-activated receptor complexes in live cells.

Furthering our research on trafficking of QD-receptor complexes, we focus on trajectories likely to display active transport because of their high aspect ratio. We measure the distribution of particle locations and movements relative to the overall curvilinear shape of each QD trajectory by fitting a spline curve to the set of positions traced out by the QD complex. Distribution of measured particle distances from the spline curve is sharply limited in some trajectories, showing clear spatial confinement within curvilinear regions that are well-characterized by the spline curve and range from 100–300 nm in width.

We also measure non-equilibrium fluctuations associated with molecular motor motion along spline curve fits by calculating excess kurtosis of the displacement distribution parallel to the fitted spline curve. However, in some trajectories, the displacements along the spline curve follow a Gaussian distribution, consistent with diffusive processes and illustrating the importance of careful analysis to distinguish between superficially similar trajectories.

Our ongoing work will incorporate these features into trajectory analysis software that can discriminate between diffusive trajectories that happen to have a high aspect ratio, diffusion confined to linear structures and trajectories that are linear because of active motor transport.

679-Pos Board B558

High Resolution Live-Cell Imaging Reveals Novel Pathways for Lysosomal Delivery

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Lysosomes play a central role in the degradation of proteins and other macromolecules. The mechanisms by which receptors are transferred to lysosomes for constitutive degradation are poorly understood. We have analyzed the processes that lead to the lysosomal delivery of the neonatal Fc receptor, FcRn. FcRn is a specific receptor for IgG and is ubiquitously expressed in many cell types. Structurally and functionally, FcRn is distinct from the other classical Fc receptors and is responsible for regulating and transporting IgG in the body. Our studies provide support for a novel pathway for receptor delivery. Specifically, unlike other receptors that enter intralumenal vesicles in late endosomes, FcRn is transferred from the limiting membrane of such endosomes to lysosomes, and is rapidly internalized into the lysosomal lumen. By contrast, LAMP-1 persists on the limiting membrane. Receptor transfer is mediated by tubular extensions from late endosomes to lysosomes or by interactions of the two participating organelles in kiss-and-linger like processes, whereas full fusion is rarely observed. The persistence of FcRn on the late endosomal limiting membrane, together with selective transfer to lysosomes, allows this receptor to undergo recycling or degradation. Consequently, late endosomes have functional plasticity, consistent with the presence of the Rab5 GTPase in discrete domains on these compartments.

680-Pos Board B559

Microfluidic Investigation Reveals Distinct Roles for Actin Cytoskeleton and Myosin II Activity in Capillary Leukocyte Trafficking

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Circulating leukocyte stiffness is considered as the initiating event of lung injury in many pathological situations such as Acute Respiratory Distress Syndrome (ARDS). Recent studies on ARDS patient leukocytes suggest a role of the actin cytoskeleton organization to explain that leukocytes from sepsis shock and ARDS patients are significantly more rigid than normal leukocytes. These recent works highlight our lack of knowledge of the precise role of actin organization and call into question the importance of myosin II activity on the control of circulating leukocyte mechanical properties. To address these issues, we present an efficient microfluidic approach to measure relevant parameters of leukocyte trafficking in narrow capillaries. We have evaluated the mechanical deformation of single circulating leukocytes in a 4-microns-wide constriction during their entry, transit and shape relaxation stages. We intend to precise the specific role that actin cytoskeleton and myosin II play in the passage of circulating leukocytes through narrow capillaries by specifically enhancing (latrunculin A) or inhibiting (jasplakinoide) microfilaments and myosin II activity (blebbistatin). Our findings confirm the major role of actin filaments organization on the deformation rate during the entry stage. Interestingly, our results bring new insights into the specific role of actin cytoskeleton and especially myosin II activity during the transit stage of the leukocytes in a constriction. We demonstrate that the cell velocity in the constriction is lower as the actin network is more organized and that the membrane unfolding of sequestred leukocytes is controlled by myosin II activity. In the last stage, we show that the relaxation process appears to be largely independent of actin organization and actin-myosin activity, whereas a deformed state of the cell is required for normal trafficking of leukocytes through physiological capillary segments.

681-Pos Board B560

CD2AP Structure And Progression Of Renal Disease

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CD2AP is a scaffolding molecule that was originally cloned as an interaction partner of CD2 in T lymphocytes. In the kidney, CD2AP is strongly expressed in podocytes, a cell type that regulates the filtration barrier. The protein directly interacts with filamentous actin and a variety of cell membrane proteins