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.

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

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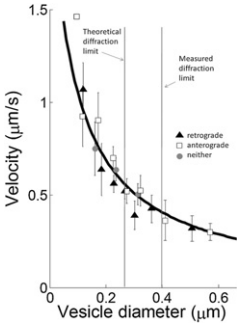
In our previous work (Shtridelman et al. 2008, Cell Bioch. & Biophys), we presented three force-velocity curves corresponding to 1, 2, and 3 motors/vesicle. We constructed these curves via velocities obtained directly from vesicles (NT2 cells, 37°C) and via forces obtained indirectly from Stokes' Law 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.



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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.

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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.

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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.

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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

including the kidney filter protein nephrin. In addition to discrete binding sites for actin and nephrin, CD2AP possesses three SH3 domains and a proline-rich region containing, in turn, binding sites for SH3 domains. CD2AP is implicated in dynamic actin remodeling and membrane trafficking that occurs during receptor endocytosis and cytokinesis. We have initiated structural studies of recombinant CD2AP protein using electron microscopy and single particle image analysis. Negative stain electron microscopy of revealed uniform particles with a size and morphology suggesting a tetrameric organization, subsequently verified with chemical crosslinking. Single particle image analysis was used to generate a three-dimensional map of the CD2AP tetramer at 21 Å resolution. The electron density map reveals an extended structure allowing the identification of specific subdomains. The tetramer is organized around a central core, including density assigned to the C-terminal coiled-coil domain, surrounded by four loosely attached arms radiating out from the center, which we have assigned to the N-terminal SH3 domains. We have further identified CD2AP as a substrate for cytoplasmic cathepsin L, a protease that is induced in early podocyte damage. Cleavage of CD2AP with cathepsin L results in a C-terminal core domain that is structurally competent but releases the CD2AP binding partner dendrin resulting in translocation of dendrin to the nucleus where it promotes apoptosis. Based on our analysis of the cathepsin L cleavage sites within CD2AP we conclude that cytosolic cathepsin L releases the N-terminal arms producing a structurally competent C-terminal core domain.

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Prestress-dependent Rheology of Semiflexible Polymers of the Cytoskeleton

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Rheological properties of living cells are essential for their physiological functions. Microrheological measurements have shown that cytoskeletal contractile stress (or prestress) and weak power-law viscoelasticity are governing principles of cell rheology, and that these two properties are closely associated in living cells for reasons that are largely unknown. In this study, we develop a stochastic model of a semiflexible polymer of the cytoskeleton that links the power-law rheology to the prestress. We describe a semiflexible polymer chain as a three-dimensional elastically-jointed chain composed of nonlinearly elastic bonds jointed by linearly elastic torsional springs. Assuming that the chain dynamics is thermally driven, we use a Monte-Carlo-based algorithm to obtain numerical simulations of the chain's creep behavior during uniaxial stretching. We obtain that the creep curves follow a power-law and that this behavior changes with prestress in a manner that is consistent with previously reported data from living cells and reconstituted crosslinked actin gels. We show that the power-law creep results from a finite-speed propagation of free energy from the chain's end points towards the center of the chain in response to externally applied stretching force. We also show that the power-law dependence on the prestress results from the chain's nonlinear, stiffening behavior that originates from both entropic and enthalpic contributions. Based on qualitative similarities between model simulations and experimental data from living cells and actin gels, it is conceivable that the mechanisms embodied in our model may also be key determinants of the overall viscoelastic properties of living cells and actin gels.

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Straining the Laws of Attraction: Mechanotransduction Studied Through Changes in Intracellular Binding Energy

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Mechanical force modulates myriad cellular functions including migration, alignment, proliferation, and gene transcription. Mechanotransduction, the transmission of mechanical forces and its translation into biochemical signals, may be mediated by force-induced protein conformation changes, and subsequently result in the modulation of protein signaling cascades. For the paxillin and focal adhesion kinase interaction, we demonstrate that mechano-induced changes in protein complex conformation, dissociation constant, and Gibbs free energy of binding can be quantified by lifetime-resolved fluorescence energy transfer microscopy and fluorescence correlation spectroscopy. A comparison with in vitro data shows that this interaction is allosteric in vivo, and spatially resolved imaging indicates that this binding constant is equal in both the cytosol and focal adhesion complexes. Further, inhibitor assays show that the mechano-sensitivity of this interaction must be mediated by soluble factors not based on protein tyrosine phosphorylation.

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Salt Dependence of Neurofilament Gel Phase Behavior - A Synchrotron X-ray Scattering Study

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Neurofilaments (NFs) are cytoskeletal proteins expressed in neuronal cells, and are believed to play a role in the determination and maintenance of the neuronal cell shape and mechanical integrity. NFs self-assemble as flexible cylinders from 3 protein subunits: NF-Low (NF-L), NF-Medium (NF-M), and NF-High (NF-H). The three subunits are structurally conserved with the exception of their ''tail'' domains, composed of amino acid strands of increasing length and charge respectively. Screening of tail charges is achieved by varying the salinity of the in vitro buffer. At high concentrations, the filaments interact amongst themselves through their unstructured tails that branch out from the filament core and form a viscous gel. Polarized microscopy was used to map out phase diagrams of the resultant neurofilament hydrogels, and thus characterize salt dependent phase behavior. Reassembled separate networks of NF-L + NF-M and NF-L + NF-H show phase transitions from isotropic to nematic gel phases at distinct salt concentrations, thus reflecting the differences in the governing sidearm interactions [1].
We will describe synchrotron x-ray scattering experiments that have allowed us to quantitatively study the microscopic structure of the NF gels: shifts in average interfilament spacing demonstrate the phase boundary between the isotropic and anisotropic NF-liquid crystal gel phases and how they are shifted as a result of varying the in vitro buffer salt concentrations. Funding provided by DOE DE-FG-02-06ER46314, NIH GM-59288, NSF DMR-0503347.
[1] J.B. Jones, C.R. Safinya, Biophys. J. 95, 823 (2008).

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Multi-Parameter Analysis of Spindle and Cell Cycle Dynamics in Asymmetric Cell Division

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Budding yeast is a unicellular organism which undergoes an asymmetric cell division. Successful completion of this process requires the assembly and translocation of the mitotic spindle from the mother cavity interior to the plane of cell division prior to segregation of the chromosomes. We use confocal fluorescence microscopy and automated image analysis algorithms to quantify the movement of the mitotic spindle, throughout the cell life cycle, at high spatial and temporal resolution. By selecting fluorescent proteins which specifically label the spindle poles and cell periphery, the spindle dynamics can be characterised within a coordinate system relevant to the cell division. Spindle length and orientation as well as the mother and bud cavity volumes have been extensively quantified in wild type cells. Our observations reveal fluctuations in spindle length and angle in the short pre-anaphase spindles that are distinct from longer anaphase spindles. Furthermore they indicate an apparent correlation between cell morphology and spindle dynamics. To elucidate the contributions of individual intercellular force generators to the fluctuations observed during spindle assembly and positioning, we apply the above methods to cells bearing deletions of the kinesins cin8, kip1 and kip3. It is expected that this work will ultimately enable a connection between observable single cell dynamics and biochemical signal pathways measurable by bulk assays.

Microtubular Motors I

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Synthesis Of Novel Fluorescent Atp Analogue And Interaction With Nucleotide Dependent Motor Proteins

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Previously, several kind of fluorescent ATP analogues have been synthesized for the application to the kinetic study of ATPase. However, some of the ATP analogues exist as mixture of isomers and showed small fluorescence changes during ATP hydrolysis.
For instance, 2′(3′)-O-NBD-ATP and 2′(3′)-O-Mant-ATP have isomer of 2′ and 3′ in their ribose moiety and each isomer performs differently as substrate for the ATPases. In the present study, we have tried to synthesize new fluorescence ATP analogues that have no isomer and show significant fluorescence change during ATP hydrolysis. The fluorescent ATP analogue 6-(N- (7-nitrobenz-2-oxa-1, 3-diazol-4-yl) amino) ethyl triphosphate (NBDTP) and N-methylanthraniloyl amino ethyl triphosphate (MANTTP) have been designed and synthesized, which are similar to non-nucleotide ATP analogue 2-[(4-azido-2-nitrophenyl) amino] ethyl triphosphate (NANTP). It is known that NANTP