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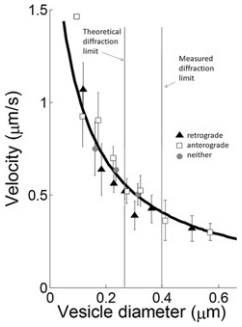
Extending the Range of In Vivo Multimotor Force-Velocity Curves by Sizing Vesicles Below the Diffraction Limit

Jed C. Macosko[1], Yuri Shtridelman[1], Clayton T. Bauer[1], David DeWitt[2].
[1]Wake Forest, Winston-Salem, NC, USA, [2]Liberty University, Lynchburg, VA, USA.

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

Brian R. Long, Tania Q. Vu.
Oregon Health and Science University, Portland, OR, USA.

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

Zhuo Gan[1], **Sripad Ram**[1], Carlos Vaccaro[1], Raimund J. Ober[1,2], E. Sally Ward[1].
[1]UT Southwestern Medical Center, Dallas, TX, USA, [2]University of Texas at Dallas, Richardson, TX, USA.

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

Sylvain Gabriele[1], Anne-Marie Benoliel[2], Pierre Bongrand[2], Olivier Theodoly[2].
[1]University of Mons-Hainaut, Mons, Belgium, [2]Universite de la Méditerranée, INSERM-CNRS, Marseille, France.

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

Brian D. Adair[1], Mehmet Altintas[2], Clemens C. Möller[1], Jan Flesche[1], Changli Wei[2], Christian Faul[2], Kirk Campbell[1], Changkyu Gu[1], Sanja Sever[1], Andrey Shaw[3], Peter Mundel[2], Jochen Reiser[2].
[1]Massachusetts General Hospital and Harvard Medical School, Charlestown, MA, USA, [2]Leonard Miller School of Medicine, University of Miami, Miami, FL, USA, [3]Washington University, St. Louis, MO, USA.

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