The second model treats actin contraction by myosin as the primary determinant of shape and speed. Simulations show that this model is able to produce very realistic keratocyte-like shape and also reproduces the bistability that is observed in keratocyte fragments. We next consider a simple biochemical regulation model that has been proposed for the polarization of the Rac/Rho system. Here a high concentration of Rac at the front of the cell stimulates actin polymerization, and Rho at the rear of the cell induces contraction of myosin. Interestingly, volume conservation is not required for this model to work, yet the model behaves very differently if the cell has fixed volume than when the volume is self-regulating. The final model assumes that microtubule-based transport of vesicles to the leading edge limits the rate of protrusion. As all of these models are able to produce steady crawling locomotion, it suggests that these mechanisms may serve redundant roles in driving cell motility.

830-Pos

Crowding Effects on Association Reactions at Membranes

Jun Soo Kim, **Arun Yethiraj**.
University of Wisconsin, Madison, WI, USA.

The effect of macromolecular crowding on the binding of ligands to a receptor near membranes is studied using Brownian dynamics simulations. The receptor is modeled as a reactive patch on a hard surface and the ligands and crowding agents are modeled as spheres that interact via a steep repulsive interaction potential. When a ligand collides with the patch it reacts with probability. The association rate constant can be decomposed into contributions from diffusion-limited and reaction-limited rates. The simulations show that the diffusion-limited rate is a non-monotonic function of the volume fraction of crowding agents for receptors of small sizes. This is because crowding decreases the rate of diffusion to the surface but inhibits the escape of the ligand from the vicinity of the surface. The association rate constant has a qualitatively different dependence on the macromolecular crowding, for different values of the reaction probability. The simulation results are used to predict the velocity of the membrane protrusion driven by actin filament elongation. Based on the simple model where the protrusive force on the membrane is generated by the intercalation of actin monomers between the membrane and actin filament ends, we predict that crowding increases the local concentration of actin monomers near the filament ends and hence accelerates the membrane protrusion.

831-Pos

The Equilibrium and Nonequilibrium Mechanics of Cytoskeletal Networks

Andrew R. Missel[1], Mo Bai[2], William S. Klug[2,3], Alex J. Levine[1,3].
[1]Department of Chemistry and Biochemistry, UCLA, Los Angeles, CA, USA, [2]Department of Mechanical and Aerospace Engineering, UCLA, Los Angeles, CA, USA, [3]California Nanosystems Institute, UCLA, Los Angeles, CA, USA.

The cytoskeleton is a complex, chemical heterogeneous network of semiflexible protein filaments, cross-linking proteins, and molecular motors that control the mechanics of some eukaryotic cells. Investigations of the mechanics of simplified biopolymer networks have shown that these materials differ substantially from better understood polymer gels in at least two< respects: (i) they show large deviations from the predictions of continuum elasticity in sparsely cross-linked networks but undergo a non-affine to affine cross-over in denser networks and (ii) when endogenous molecular motors are present to drive the network out of equilibrium, the elastic moduli of the nonequilibrium network increase by more than one hundred fold.
In this talk we report analytic calculations and numerical simulations of equilibrium and non-equilibrium networks. Previous theoretical studies of the non-affine to affine transition in cytoskeletal networks have been confined to statistically isotropic random networks of monodisperse filaments. Biologically relevant and experimentally realizable networks are highly polydisperse and are frequently comprised of filaments with a preferred orientation. We examine, via numerical simulation, the individual effects of uniaxial order, filament polydispersity, and motor activity on the non-affine to affine transition. Finally, we demonstrate analytically how one can use the correlated motion of pairs of tracer particles embedded in the network (i.e. two-particle microrheology) to experimentally determine the density of active motors in in vitro networks and in living cells.

832-Pos

Fiber Network Elasticity as Function of Crosslinker Density

Susan Sporer[1], Sebastian Kapfer[1], Christoph Arns[2], Klaus Mecke[1], Gerd E. Schröder-Turk[1].
[1]Friedrich-Alexander-University, Erlangen, Germany, [2]University of New South Wales, Sydney, Australia.

Crosslinkers determine the architecture of polymer networks and thus are of great importance for the resulting mechanical properties. A simple morphological model is proposed for the investigation of the linear elastic response of 3D fiber networks to randomly disconnecting network nodes. Isotropic ordered and disordered, 4-coordinated networks are modeled as homogeneous bodies in the shape of a network with a given volume fraction with locally isotropic elastic moduli. The 4-coordinated nodes are randomly split into two locally unconnected fibers representing a morphology change of the network at constant volume fraction. The effective shear modulus is studied using a voxel-based finite element method. Our results show a strong, continuous decrease of the shear modulus with decreasing number of nodes in the network without a percolation transition. The morphology of the networks is characterized by the Euler number that linearly depends on the fraction of split nodes, and that is easily extracted from 3D confocal microscopy data. Associating all network nodes with fiber junctions connected by a crosslinking molecule, this approach is a first order model for elasticity of biological networks with varying crosslinker density.

833-Pos

Heterogeneity and Flow in Biological Networks and Implications for Cargo Transport

Eliza Morris, David Nelson, David Weitz.
Harvard University, Cambridge, MA, USA.

Single molecule experiments have measured stall forces and procession rates of molecular motors on isolated cytoskeletal fibers in Newtonian fluids. But in the cell, these motors are transporting cargo through a highly complex cytoskeletal network. To compare these single molecule results to the forces exerted by motors within the cell, an evaluation of the response of the cytoskeletal network is needed. Using magnetic tweezers, we quantify force-velocity curves for magnetic beads moving through entangled F-actin networks [12 uM]. Below a certain critical force, we see an elastic response with a plateau indicating a shear modulus of 0.1 Pa, comparable to bulk rheological measurements. Above this critical force we find a viscous response with a viscosity of approximately 0.3 Pa.s. The exact value of the critical force ranges from roughly 6-14 pN, reflecting the spatial heterogeneity of the network. This non-Newtonian force-velocity relationship, as well as the considerable heterogeneity in the network response, suggests the local cytoskeletal environment is an important factor when considering cargo transport inside the cell.

834-Pos

Regulation of Nonmuscle Myosin IIA Assembly

K. Ilker Sen, Wendy Zencheck, Michael D. Brenowitz, Steven C. Almo, Anne R. Bresnick.
Albert Einstein College of Medicine, Bronx, NY, USA.

In eukaryotic cells, nonmuscle myosin II filament assembly is critical for essential motile processes such as cytokinesis, cell motility, and the maintenance of cell morphology. Although myosin II filament dynamics are believed to be under strict spatial and temporal control, the mechanisms modulating filament assembly and disassembly are poorly understood. In this work, we examined the molecular mechanisms regulating myosin IIA filament assembly that rely on the intrinsic dynamics of the C-terminal coiled-coil of the myosin IIA heavy chain and on non-covalent interactions with S100A4 protein, a major metastasis factor. Sedimentation equilibrium, hydrogen-deuterium exchange, and thermal melt CD spectroscopy showed that the C-terminal coiled-coil of the nonmuscle myosin IIA heavy chain exhibits significant conformational plasticity. Based on these observations, we propose that the plasticity of the C-terminal coiled-coil is an inherent regulatory mechanism for modulating myosin IIA filament assembly. We are testing this hypothesis by introducing stabilizing mutations into the C-terminal coiled-coil and examining how the loss of plasticity modulates salt-dependent oligomerization of myosin IIA and filament assembly as assayed by turbidity and critical concentration measurements.

835-Pos

Myosin II is an Active Stress Sensor at the Core of a Cell Division Control System

Yee Seir Kee[1,2], Richard Firtel[3], Pablo Iglesias[1], Douglas Robinson[2].
[1]Johns Hopkins University, Baltimore, MD, USA, [2]Johns Hopkins School of Medicine, Baltimore, MD, USA, [3]University of California San Diego, San Diego, CA, USA.

Mechanosensing is important in many cellular processes such as cell motility and cell division. Cells experience mechanical stress from the surrounding environment and also its internal cytoskeleton structure. Previous studies from our lab showed that cellular mechanosensing is crucial for regulating cytokinesis shape change. Using micropipette aspiration (MPA) to generate stress on the cell cortex, we discovered that mechanical stress stimulates the accumulation of myosin-II (a contractile force generating protein) and cortexillin-I (an actin bundling protein) at the deformation site. These proteins then contract the cortex, correcting the shape of the cell. Both myosin-II and cortexillin-I are required for this mechanosensory system during cytokinesis. Recently, we