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

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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

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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

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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

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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

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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

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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

demonstrated that this mechanosensory response is tunable by varying the lever arm length of myosin-II heavy chain, showing that myosin-II is an active force sensor in this mechanosensory system. We now focus on how mechanical inputs mediated through myosin-II lead to changes in biochemical signaling pathways, specifically the cortexillin-I regulatory and spindle signaling pathways. Rac1A (a small GTPase), IQGAP1, and IQGAPA (GTPase effectors) can form complexes with cortexillin-I. In the absence of both IQGAP1 and IQGAPA, cortexillin-I does not localize normally to the cleavage furrow during cell division. However, IQGAPA, but not IQGAP1, is required for myosin-II mechanosensing. Kif12, a mitotic-kinesin-like protein in Dictyostelium cells, is part of the chromosomal passenger complex, including INCENP and Aurora kinase. Kif12 is also recruited to the cell cortex inside the micropipette in a myosin-II-dependent and/or IQGAPA-dependent manner during cell division. Thus, mitotic spindle signaling proteins are responsive to mechanical stress sensed by myosin-II. Overall, myosin-II is a key mechanical stress sensor and these mechanical inputs are fed back to the mitotic spindle signaling system.

836-Pos

Mechano-Chemical Feedbacks Play a Major Role in Regulating Actin Mesh Growth in Lamellipodial Protrusions

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During cell motion on a substratum, eukaryotic cells project sheet-like lamellipodia which contain a dynamically remodeling three-dimensional actin mesh. A number of regulatory proteins and subtle mechano-chemical couplings determine the lamellipodial protrusion dynamics. To study these processes, we constructed a microscopic physico-chemical computational model, which incorporates a number of fundamental reaction and diffusion processes, treated in a fully stochastic manner. Our work sheds light on the way lamellipodial protrusion dynamics is affected by the concentrations of actin and actin-binding proteins. In particular, we found that protrusion speed saturates at very high actin concentrations, where filament nucleation does not keep up with protrusion, resulting in sparse filamentous networks, and, consequently, high resistance forces on individual filaments. We also observed maxima in lamellipodial growth rates as a function of Arp2/3, a nucleating protein, and capping proteins. We provide detailed physical explanations behind these effects. In particular, our work supports the actin funneling hypothesis explanation of protrusion speed enhancement at low capping protein concentrations. Our computational results are in agreement with a number of related experiments. Overall, our work emphasizes that elongation and nucleation processes work highly cooperatively in determining the optimal protrusion speed for the actin mesh in lamellipodia.

837-Pos

In Silico Study of Formation and Collapse of T-Killer Cell Synapse Mediated by Receptor Recycling and Actin Network

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T-killer cells of the immune system eliminate virus-infected and tumorous cells through direct cell to cell interactions. Reorientation of the killing apparatus inside the T-killer cell to the interface with the target cell ensures specificity of the immune response. Several research works were reported to explain the mechanism of reorientation but the most adversary situation, when the cell's initial orientation is complete opposite to the desirable direction, always left skepticism toward the suggested mechanism. We have constructed a computational model that incorporate T-killer cell receptor dynamics and all the possible mechanical properties which involve not only intrinsic physiology of T-killer cell but also the synapse formation with target cell. The model studies show that the actin network nucleation and degradation is a crucial part in the T-killer cell synapse formation. Furthermore, the role of actin network provides a safety feature for the T-cell reorientation mechanisms by allowing T-cells to detach from the target cell when they are stranded in situations in which reorientation is not available. Our computational model also provides insights into the actin network near the T-cell synapse including retrograde flow development.

838-Pos

Intimacy Between Actin Network Flow and Turnover in the Lamella of Crawling Fragments

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Elucidating the dynamics of the actin cytoskeleton that generates the driving force for cell migration is fundamental to understanding the mechanisms of actin-based cell migration, which is important to various physiologically relevant processes including metastasis and angiogenesis.
In this study, based on the hypothesis that actin cytoskeleton in migrating cells is a spatiotemporally self-regulating structure, we aimed at elucidating the dynamic coupling between actin network flow and turnover by focusing on flow dynamics in the lamella of one of the simplest but elegant motility systems; crawling fragments derived from fish keratocytes. Using a combination of fluorescent speckle microscopy and particle imaging velocimetry, we have succeeded in quantitatively mapping the flow in the lamella of these simple motility systems where it was previously reported to be stationary. Moreover, by correlating network flow with turnover, we have demonstrated that whereas polymerization mediates network assembly at the front, surprisingly, network flow convergence modulates network disassembly toward the rear of the lamella, suggesting that flow and turnover are coupled during migration. Furthermore, we found that polymerization is not just limited to the usually reported narrow rim along the leading edge, but occurs over an extended ~8 ∈ 1/4m wide region at the posterior of the lamella. We suggest that this is necessary to maintain the structural integrity of the lamella for rapid cell migration, as in fish keratocytes. These results obtained using simple but remarkable motility systems present new interesting concepts about actin network dynamics during cell migration that will definitely have profound impact on cell migration research. We believe that this study will make a major contribution toward biophysical understanding of cell migration, and aid in the development of quantitative models for exploring the yet unknown mechanisms of the process.

839-Pos

Quantitative Analysis of Cell Edge Dynamics and Cell Shape in Non-Polarized Fish Epidermal Keratocytes

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Cell migration requires the coordination of several processes such as leading edge protrusion, adhesion formation and disassembly, and trailing edge retraction. The quantitative analysis concerning the correlation between these processes will be useful to understand the coordination mechanism. A major difficulty in the quantitative analysis stems from the complex morphology of migrating cells. To cope with this difficulty, we selected the non-polarized fish epidermal keratocytes as a simplified experimental system that includes basal migratory mechanisms. We acquired a mixture of non-polarized stationary keratocytes and polarized highly motile keratocytes by disaggregating the large epidermal sheets. The time-lapse micrographs of non-polarized keratocytes were used to analyze cell edge dynamics and cell peripheral shape. We adopted the protrusion and retraction rate and cell peripheral curvature as quantitative parameters. Non-polarized keratocytes did not exhibit net translocation, however, active protrusion and retraction were observed around the cell periphery. Protrusion rate was negatively correlated with the cell peripheral curvature. In contrast, retraction rate was positively correlated with the cell peripheral curvature. The plot of protrusion and retraction rates over the entire cell periphery showed that protrusion and retraction waves were traveling laterally in both directions along the cell periphery. The lateral traveling velocity of each wave was constant. The wave persistence varied from 10 s to 100 s. These results indicate that the cell has the positive feedback mechanism maintaining stable protrusion and retraction and that the rate of protrusion and retraction is related to the cell peripheral shape. Quantitative analysis together with theoretical and molecular biological studies will shed light on the mechanism of cell migration.

840-Pos

Mechanisms Underlying Protrusion-Retraction Waves at the Leading Edge of Migrating and Spreading Cells

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Motility is fundamental to many cell types and plays key roles in immune response, tissue development, and cancer metastasis. Recent studies of migrating mouse embryonic fibroblast cells revealed protrusion-retraction cycles and lateral waves at the leading edge [Giannone G, et al, Cell, (2004); Giannone G, et al, Cell (2007)]. Each cycle entails membrane protrusion powered by actin polymerization, interrupted every ~24 s by ~5 s partial retraction episodes attributed to myosin II proteins which pull back the growing lamellipodial actin network until the latter separates from the leading edge membrane. We developed a mathematical model and extended our experimental observations in order to quantitatively describe the mechanisms underlying this motility behavior. We find the retraction waves are caused by propagation of myosin powered tears between the lamellipodium and cell membrane. Once a tear is nucleated its