and we aim to utilize such structures to synthesize artificial cytoskeletons with various levels of complexity. DNA nanotube polymerization was monitored using total internal reflection microscopy at the single molecule level. We observed that DNA nanotubes exhibit asymmetric polymerization, similar to actin and microtubule polymerizations. We measured the association and dissociation rates of DNA nanotube polymerization at different monomer concentrations and temperatures and compared our measurements with the polymerization theory developed for the cytoskeleton and the kinetic DNA tile assembly model. Finally, the coupling between DNA nanotube polymerization and a DNA nanotechnology analog of nucleotide hydrolysis could potentially recapitulate cytoskeleton-based dynamics, such as treadmilling and dynamic instability, where the polymerization and depolymerization co-exist at steady state without ever reaching equilibrium.

2870-Pos
Stiff-Filament Microrheology
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Active and passive microrheology techniques for probing viscoelastic properties of biological samples require the embedding of micron-sized particles. This can give rise to local perturbations and surface interactions. These effects have to be taken into account during data evaluation and form an obstacle for the investigation of living cells.
A way of circumventing these influences is the use of parts of the system itself, such as the microtubules in cells, as local probes by observing their thermal bending fluctuations in the surrounding medium. A detailed analysis of the spatial and temporal bending fluctuations can give information about local shear moduli and stress fluctuations in biopolymer networks in the absence of probe artifacts.
We have investigated a network of filamentous actin by attaching nanometer-sized gold particles to embedded microtubules and have measured thermal motions of the gold particles with an optical trap by laser interferometry with high bandwidth. The results agree well with the expectations, providing a proof of principle of the new approach.

2871-Pos
Quantitative Investigation of Individual Contractile Actin Bundles *in vitro*
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Much is known regarding the structure and function of contractile actomyosin networks in cellular physiology, however, details of their biophysical properties remain far from clear. For example, we lack a clear understanding of how the transmission of forces from myosin motor filaments influences structural changes in dynamic actin networks. We attempt to address these unknowns by measuring the dynamic structure and biophysical properties of *in vitro* 2-D actomyosin bundles. By working with a small number of purified components, this enables us to create a simple assay to study the effects of small changes in concentrations of one component has on the emergent biophysical properties of the resultant contractile bundle.
Here we have created a reconstituted 2-D network of actin that is suspended from, and anchored to, a surface using polystyrene beads. Smooth muscle myosin (ADP) is added, resulting in bundling of actin within the network. Interestingly, the resultant structure after myosin addition allows inter-actin bundling to occur, creating a web-like structure. The addition of ATP initiates contraction and results in large scale restructuring of the actin bundles. During network contraction, the intensity of individual actin bundles increases as the individual filament arc length decreases. In addition, the web-like structure of the network diminishes during contraction. Under some conditions, presumably where the myosin/actin ratio is above a critical threshold, the filaments break due to excessive contraction. These tethered filaments, no longer under tension, contract at a greater rate than those still anchored by both ends.
In conclusion, actomyosin filaments can be assembled *in vitro* without a passive crosslinker; ADP/ATP myosin can bundle actin, even during contraction. Large-scale restructuring of 2-D actomyosin networks occur when tension is applied through myosin motor activity. These observations are consistent with sliding filament theory of actomyosin contraction.

2872-Pos
Mechanical Perturbation of Immunological Synapse and Cortical Actin Flow in T Cells
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Reorganization of membrane components plays an important role in signal transduction. Patterned hybrid live cell-supported membrane junctions provide spatial controls over the lateral transport of signaling molecules inside the cell. Here, we introduce a new technique which allows us to mechanically manipulate the membrane curvatures at hybrid membrane junctions. We demonstrate that large scale of protein patterns in the T cell immunological synapse can be altered merely by imposing a defined membrane curvature from supporting substrates. Our observation suggests that mechanical perturbations of membrane junctions via geometrical modulations result in decreasing actin velocity as well as remodeling actin retrograde flow. We also explore the effects of membrane diffusion barriers on cytoskeletal regulations and receptor transport processes. Flow-based particle tracking algorithms reveal that actin centripetal retrograde flow directs the inward transport of T cell receptor (TCR) clusters. We find that slower actin flow over confined TCR clusters whereas it stays the same level elsewhere. Actin flow regains its velocity after passing through confined TCR clusters. We demonstrate that the dissipated coupling of TCR clusters and actin network can feedback into a frictional coupling model.

2873-Pos
A Comparison of Polymer Blocking Agents in the *in vitro* Motility Assay
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Blocking agents are used in *in vitro* motility assays to stabilize the motor proteins myosin or heavy meromyosin (HMM) and to prevent non-specific binding of actin to regions of microscope coverslip that are devoid of motors. Bovine serum albumin (BSA) or casein is typically used for blocking, but there is occasional need for non-protein blocking agents. We compared skeletal myosin and HMM function in motility assays as a function of the blocking agent that was used; these blocking agents included polysorbate (Tween) 20 and six different molecular weights of polyvinyl pyrrolidone (PVP) ranging from 10 kDa to 1.3 MDa, as well as BSA and β-casein as controls. *In vitro* motility assays were preformed and actin filament movement was quantified using automated particle tracking algorithms. PVPs of all molecular weights supported the motility of both HMM and myosin, though there was a slight downward trend in mobility at the highest molecular weights. When HMM was used in the motility assay, Tween showed poor mobility (1.7 um/s) compared to BSA (9.4 um/s). In contrast, full length myosin showed high mobility when blocked with Tween (8.3 um/s) compared to BSA (6.6 um/s). To determine whether Tween is a direct inhibitor of HMM function, NH_4-activated ATPase assays were performed in solution with either BSA or Tween. There was no significant difference in ATPase rates between these two conditions. However, when the NH_4-activated ATPase assay was repeated with HMM bound to a flow cell, Tween inhibited the ATPase activity. Thus while Tween does not directly inhibit the motor domains of HMM, it may adversely alter its binding to hydrophobic surfaces. In conclusion, low- to mid-molecular weight PVPs are excellent polymer blocking agents for actin-myosin or actin-HMM motility assays.

2874-Pos
Observation of Microinjected Fluorescent Myosin in Contractile Smooth Muscle Cells
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It is not known if myosin filaments in smooth muscle (SM) are fixed or dynamic structures. Currently models of the ultra-structure of SM myosin filaments suggest that the filaments are indeed labile structures. This would explain the ability of SM cells to generate maximum force over a wide range of lengths, also called plasticity. However, time-lapse in vivo studies have not been carried out in SM cells. The aim of this study is to analyze SM myosin filament assembly in live SM cells through the development of a microinjection technique using fluorescently labeled myosin.
Methods: Monomeric smooth muscle myosin purified from chicken gizzards was fluorescently labeled with Alexa Fluor 555 and microinjected into cultured SM3 cells. Images of microinjected cells were then gathered using a Leica Deconvolution microscope. Cells were then stimulated using electric field stimulation (EFS) to induce contraction. Images were collected and analyzed for filament dynamics.
Results: An in vitro motility assay showed that the purified myosin is functional. The myosin maintains its ability to assemble into filaments after labeling as determined by sedimentation assays. The microinjection technique was successful, resulting in live cultured cells containing exogenous myosin that could be imaged. Within 25 minutes of microinjection, monomeric myosin could be seen dispersed throughout the cells (excluding the nucleus). After EFS stimulation high-resolution images of the labeled myosin were obtained.
Conclusion: Using cultured SM cells that retain their ability to contract provides an effective tool in the analysis of myosin filament assembly in live cells.

Further analysis of exogenous myosin dynamics will be performed under different stimuli and will be used to address the question of myosin dynamics.

2875-Pos

Dynamics and Rheology of Transiently Crosslinked Cytoskeletal Networks

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Cells derive their mechanical properties largely from the cytoskeleton: a network of stiff biopolymers and associated proteins, capable of cross-linking and force generation. The short-timescale mechanical response of biopolymer gels has been studied extensively, while little is known about the biologically more relevant long-time behavior. It is on these longer timescales that the cytoskeleton remodels in response to internal and external cues. Here we present predictions for the viscoelastic behaviour of semiflexible polymer networks cross-linked with physiological transient linkers. Our model allows us to elucidate the mechanisms by which the network can relax as a result of the constant breakage and formation of links in the network.

2876-Pos

To Determine the Structure of Vimentin Head Domain Using SDSL-EPR Approach

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Cysteines were placed in vimentin head spanning 1-108 positions using basic genetic engineering approaches. The mutants were expressed in bacteria, purified by FPLC, and spin-labeled using O-87500 [MTSL-d15]. Labeled proteins were assembled into filaments by dialysis from 8M urea and filament assembly was checked by electron microscopy. EPR measurements were carried out in JEOL X-band spectrometer fitted with a loop-gap resonator. The SDSL EPR spectral data indicated that 48/50 positions tested displayed spectral broadening indicating the close proximity of these positions (<2.1 nm). Mixing protein spin labeled at a given residue, with protein labeled at sites slightly ''up''- or "downstream", decreased the level of dipolar interaction in all cases. This data provide strong evidence that the polypeptide backbones of head domains remain in close proximity, forming a symmetric structure which folds back on the rod domain, bringing residues 17 of the head and 137 of the rod into close proximity. By monitoring in vitro assembly process, we show that head-head interactions occur early in the assembly process. We show that spin labels in the region of residues 50 and 103 display high level of mobility at protofilament stage of assembly but becoming more compacted and motionally constrained upon assembly into intact filaments, suggesting that head domain structure is dynamic and changes during assembly. Finally, because the vimentin head domain is the major site of phosphorylation, we compared specific spin-spin interactions in the phosphorylated state, and have identified distinct structural changes resulting from phosphorylation of head domain. This report presents the first evidence-based structural model for the head domain of any IF protein. We provide data which shows that head domain structure is dynamic, changing with both assembly into filaments, but also with phosphorylation, a physiologic regulator of vimentin assembly/disassembly.

2877-Pos

Tuning of Neurofilament Hydrogel Network Features - a Synchrotron X-Ray Scattering Study of Salt Dependent Network Response

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Neurofilaments (NFs) are cytoskeletal proteins expressed in neuronal cells, with a role in the maintenance and mechanical integrity of neuronal processes. NFs assemble as flexible cylinders from 3 protein subunits: NF-Low (NF-L), NF-Medium (NF-M), and NF-High (NF-H). The variable length and charge of the subunits sets the strength and range of the interactions, which are predominantly electrostatic. Reassembled (in vitro) binary system hydrogels have shown us the different contributions of individual subunits to interfilament interactions and thus to network characteristics [1,2]. We emulate cellular conditions by varying the salinity of the in vitro buffer: low salt conditions parallel higher inherent charge of the subunits, and high salt conditions parallel the lower inherent charge states of the subunits. The tunability of the network in vitro mirrors in vivo cellular control of the NF network via subunit phosphorylation, which may transition the network from a highly oriented rigid state to an isotropic gel with orientational plasticity. We describe synchrotron x-ray scattering experiments that have allowed us to quantitatively study the changes in the microscopic structure of the NF gels as a function of salt and sidearm density. At low NF-M and NF-H sidearm weight ratios, NF gels exhibit weak salt dependence. In contrast, at high weight ratios, and as a function of decreasing salt concentrations, NF gels exhibit an unexpectedly abrupt transition from highly oriented liquid crystalline gels with high filament density ($\propto 1/d$, d = interfilament spacing) to a weakly oriented (nearly isotropic) low filament density gel.

Funded by DOE DE-FG-02-06ER46314, NSF DMR-0503347.

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2878-Pos

Analysis of Single Integrin Behavior in Living Cells

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Integrins are trans-plasma membrane receptors that mediate linkages between the extracellular matrix (ECM) and the actin cytoskeleton. In migrating cells, integrins cluster into focal adhesions (FAs). Integrin binding to ECM involves induced conformational changes that increase the affinity of the extracellular domains to ECM ligands, while indirect protein-protein interactions in FAs mediate cytoskeletal linkages. How the motion of individual integrin molecules in the plasma membrane relates to integrin activation, ECM or cytoskeletal binding, and FA formation is unknown. To address these questions, we analyzed the dynamics of single integrin molecules in migrating U2OS osteosarcoma cells. Cells expressing integrin α_v or a farnesylated peptide (CAAX) fused to the photoconvertible fluorescent protein, tdEos, were analyzed by single particle tracking photoactivation localization microscopy (SPT-PALM). We imaged a high density of single photoconverted tdEos molecules by total internal reflection fluorescence microscopy (TIRFM) and tracked their trajectories using a globally-optimal SPT algorithm. Analysis of CAAX trajectories revealed that a majority of molecules (84%) exhibited Brownian diffusive behavior with 7% showing confined diffusion ($D = 2.5\text{-}3\times10^{-13}$ m^2/s). In contrast, a majority of integrin α_v trajectories exhibited confined diffusive behavior (74% confined, 21% Brownian; $D = 2\text{-}5\times10^{-14}$ m^2/s). Image segmentation allowed classification of trajectories inside and outside FAs. Surprisingly, this showed that the proportion of confined or Brownian diffusive behavior was independent of whether molecules were inside or outside FAs. Analysis of integrin α_v molecules specifically within FA showed that treatment with Mn^{2+} to induce integrin activation reduced the confinement radius of diffusion, whereas disruption of the actin cytoskeleton with latrunculin-A significantly increased the confinement radius. These results indicate that integrins can be immobilized without clustering, but can also remain mobile while clustered within FAs. In addition, both activation and cytoskeletal connection contribute to reduced integrin mobility within FAs.

2879-Pos

Direct Observation of Fiber Dynamics in Fibrin Networks Under Shear

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A blood clot stems the flow of blood in response to injury. Fibrin fibers form a network that constitutes the underlying structure of the blood clot. This network imparts its mechanical properties onto the clot itself allowing it to withstand the large range of stresses present in the body. From a materials perspective, many of the mechanical properties of fibrin networks are unusual. In particular, as they are subject to increasing strain, their shear stress increases non-linearly and their normal stress is increasingly negative. Little is known about how fiber dynamics and deformations lead to these properties in fibrin and other stiff biopolymer networks. We image fluorescently labeled fibrin networks under shear using confocal microscopy. Using this data we track individual fibers throughout the network. We report on overall and local non-affinity of fibers, strain in individual fibers and buckling as a function of shear. We do this at various protein and calcium concentrations.

2880-Pos

Non-Linear Mechanical Properties of Collagen Networks During Cyclic Loading

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Collagen is the most abundant protein in vertebrates, and its mechanical properties are responsible for the structure and function of many tissues. When subjected to large strain, collagen shows typical strain-stiffening. Here, we investigate how the strain-stiffening response of collagen changes as the material undergoes repeated large strain oscillations. We shear in vitro reconstituted collagen gels of various concentrations (0.4 - 2.4 mg/ml) repeatedly in a