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.