Background: The mechanisms regulating cross-bridges (XBs) cycling during stretch and shortening are controversial. We hypothesize that XB strong to weak transition (weakening) rate increases during shortening and decreases during lengthening in an identical velocity dependent manner. Our hypothesis reproduces the muscle basic properties as the force-velocity relationship and regulation of energy consumption. The study investigates this unifying hypothesis during lengthening and shortening. **Methods:** Trabeculae were isolated from rat right ventricles (n=9). Sarcomere length was measured by laser diffraction. The number of strong XB (N_{XB}) was evaluated by measuring the dynamic stiffness. Stretches (n=42) and releases (n=48) at different velocities and instants were imposed on sarcomere isometric contractions. **Results:** Faster stretches yielded larger forces. An overt identical linear correlation between force and N_{XB} development was obtained for any stretch velocity (0-2.17μm/s), implying that the force increased due to the increase in N_{XB}, whereas the unitary force per XB (F_{XB}) was constant. The stiffness development rate linearly depended on the lengthening velocity with a proportion coefficient of 6.9 ± 0.46. Shortening yielded both a decrease in N_{XB} and F_{XB}. Interestingly, the stiffness decline rate depended linearly on the shortening velocity (0-6.27μ/s) with similar proportion coefficient of 6.08 ± 2.45. When identical perturbation (lengthening or shortening) was imposed at different instants during the twitches, similar rate of change in the stiffness and force development were observed. Thus, the phenomena are not dominated by N_{XB} but relate to an inherent property of the single strong XB. **Conclusions:** The independence of XB weakening rate on the perturbation onset time and the identical dependence on the velocity during shortening and lengthening strongly support the hypothesis that XB dynamics is dominated by a single velocity dependent kinetic.

Cytoskeletal Protein Dynamics

2865-Pos

Escherichia Coli Single FtsZ Dynamic Rings: Growing, Cyclization, Opening, Reannealing and Depolimerization

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The prokaryotic homologue of tubulin FtsZ plays a major role in cell division. *In vivo* it is localized at the center of the bacteria forming a dynamic ring that constricts during division. *In vitro* it binds and hydrolyzes GTP, and polymerises in a GTP-dependent manner. The role played by the hydrolysis in filament stability is not well understood.

In this work we have studied isolated FtsZ protofilaments by AFM obtaining information about the polymerization, steady state and depolymerization process in real-time at the single-molecule level. Previous experiments with Atomic Force Microscopy (AFM), which provide both high structural resolution and dynamics information under buffer solution, have reported complex dynamic behaviour for FtsZ filament network deposited onto a mica surface[1]. A recent model considering a labile longitudinal monomer-monomer bond with a preferential curvature and flexibility in addition to lateral attraction between monomers is enough to account for this rich dynamic behaviour[2].

In order to correlate GTPase activity with the labile nature of longitudinal bond between FtsZ monomers we used slow hydrolizable GTP analogues. We found a strong decrease on depolymerization velocity when GTPase activity is diminished allowing us to reveal cyclization, fragmentation, reannealing and release of monomers from either end or from both simultaneously as the essential features of a single FtsZ filament dynamics.

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2866-Pos

The Mechanism of E-Ring Formation During Min Oscillations

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In the bacterium Escherichia coli, the Min proteins oscillate between the cell poles to select the cell center as division site. This dynamic pattern has been proposed to arise by self-organization of these proteins, and several models have suggested a reaction-diffusion type mechanism. Recently, we have found that the proteins MinE and MinE are able to spontaneously form planar surface waves on a flat membrane in vitro. One particular feature of the patterns in vivo and in vitro is the so-called MinE ring, which defines the directionality of the traveling wave. How this accumulation of MinE at the trailing edge of the wave arises and its functional role during Min oscillation is not yet fully understood. We have applied FRET, TIRF and single molecule imaging techniques to study the behavior and interdependence of the three Min proteins in more detail. This helped us to propose a novel mechanism of Min protein wave propagation and E-ring formation.

2867-Pos

Kinetic Analysis of Actin Dendritic Nucleation from a Physicist's Standpoint

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Arp2/3-mediated polymerization of actin is an important mechanism by which cells dynamically change their shapes. Recent advances in deciphering a pathway of dendritic nucleation lay the groundwork for kinetic analyses that would estimate rate constants of the reaction steps involved in the process. Using kinetic models with various assumptions about a pathway's rate-limiting step, we have analyzed published quantitative data obtained from pyrene-labeled actin assays. All the available data are fitted well by two different models: one assumes that the nucleation is limited by the binding of the Arp2/3 complex to a mother filament whereas in the other, the rate-limiting step is the subsequent activation of the bound complex. To distinguish between these possibilities conclusively, we propose experiments with varying initial concentrations of actin monomers. The experiments need to be performed in nonequilibrium conditions, in which spontaneous formation of aster-like actin structures have been recently observed. For these conditions, the two models exhibit qualitatively different dependences of fluorescence intensity on the initial concentrations of G-actin. The work is supported by National Institutes of Health through grants 1U54 -RR022232, P41-RR13186, and 1U54-GM64346-01.

2868-Pos

Simulation Reveals Fundamental Behavior of the Actin Filament and Arp2/3 Branch Junction

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Molecular modeling and simulation now offer unprecedented opportunity to study biological events at the atomic level. Unfortunately, broad application of these approaches is elusive due to the difficulty of simulating biologically relevant length and time scales. I will use our recent investigation into key processes within the actin cytoskeleton to highlight progress we have made on these challenges.

The self-assembly of actin into a branched network of filaments is a vital process within the cytoskeleton of eukaryotic cells. This is chiefly regulated by the controlled hydrolysis of actin's bound ATP nucleotide. Although it is well established that ATP hydrolysis induces a change in actin's structure and dynamics, the exact mechanism by which this occurs is still unknown. We have used a novel computational approach to simulate various hydrolysis-induced structural transformations that have been suggested in the literature. Based on these results we are able to suggest a mechanism by which ATP hydrolysis regulates actin's structure and dynamics.

Equally important to the dynamics of the cytoskeleton is the process by which actin filaments are depolymerized in a controlled fashion. Recent experimental studies have yielded a number of hypotheses regarding the process by which actin depolymerization factor cofilin severs and depolymerizes filaments. We will present molecular and coarse-grained analyses of cofilin-bound actin filaments, and provide a molecular view of this process.

Finally, we have performed simulations of the actin branch junction - a key structural building block in the cytoskeleton. Our simulations reveal how small features of the actin protein give rise to the underlying dynamics of the entire branch junction. Strategies for coarse-graining supramolecular structures will be discussed in context of the branch junction.

2869-Pos

Toward De Novo Recapitulation of Cytoskeleton Dynamics with DNA Nanotubes

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The polymerization and depolymerization of cytoskeleton can transduce chemical energy into mechanical energy that drives cellular motility, such as chromosome segregation to cell protrusion. De novo recapitulation of the cytoskeletal phenomena with synthetic material would test our understanding of the design principles of polymerization motors. In the DNA nanotechnology tool box, DNA nanotubes are arguably the closest counterparts of cytoskeleton

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.