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.

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[1] R. Beck, J. Deek, J.B. Jones, C.R. Safinya. Nature Materials, In Press
[2] J.B. Jones, C.R. Safinya, Biophys. J. 95, 823 (2008)

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

plate-plate rheometer by applying a cyclic sinusoidal strain of a given amplitude, and analyze the non-linear stress-strain relationship. With each cycle, the maximum stress and the linear modulus of the material decrease, and the transition from a linear to a strain-stiffening response occurs at higher strains. If the strain amplitude is increased and a new set of cycles is taken, this 'working' of the material repeats for the now higher strain amplitude. However, for each strain step, the first oscillation of the set of cycles is qualitatively different from the following ones. First, when compared to the stress-strain response of a gel that has not been previously worked at smaller strains, the two match closely. Secondly, upon unloading, this first oscillation shows a significantly increased dissipation.
Upon addition of covalent crosslinks by incubating the collagen gels with 2% glutaraldehyde solution after polymerisation, the stress-strain relationship becomes independent of the loading history. We hypothesize that the microscopic mechanism responsible for the history dependence is due to intra-fibrillar slip of adjacent collagen monomers. We present evidence from direct observation using confocal microscopy to image collagen gels under shear that supports this hypothesis.

2881-Pos
Direct Detection of Tension Recovery After Local Stretching of Cell Surface
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Cellular response to the externally applied force has a vital effect on cell proliferation, propagation and finally on its ultimate fate. Details of the molecular basis of the mechanical response are , however, little known. Here we measured mechanical and structural responses of a cell under locally applied force on an atomic force microscope (AFM) equipped with a fluorescence microscope for live cell imaging. When a colloidal AFM probe was first pressed on the cell surface and then pulled up, the tensile force sensed by the cantilever was recovered after initial relaxation. This recovery of the tensional activity was inhibited when cells were treated with cytochalasin D, the inhibitor of actin polymerization, or blebbistatin, the inhibitor of ATPase activity in myosin II, suggesting that the tension-recovering activity was driven by actin-myosin contractility. Our method allows us to investigate the dynamic processes of the mechanical maintenance of subcellular structures in a single cell.

2882-Pos
Probing the Micrheology of Mesenchymal Stem Cell Migration to Tumors
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Mesenchymal stem cells (MSCs) are excellent candidates for the development of cell-based gene delivery systems; however, extended cell culture, required for therapeutic development, alters MSC morphology, reducing MSC migration upon reinfusion. Spontaneous migration of MSCs to tumors is mediated by tumor secretion of proangiogenic chemokines. Multiple particle tracking microrheology was used to investigate the effect of tumor-secreted molecules on MSC viscoelasticity, which was correlated with MSC migration and morphology. Within 24 hours after MSC treatment with tumor-conditioned media (TCM), MSCs were elongated, with more than 5-fold difference in the length of lamellipodia. Within 24 hours, the migration of MSCs, measured using a Boyden chamber assay, toward TCM was increased 10-fold over control media. The mean squared displacements (MSDs) of 100-nm carboxylated polystyrene particles, injected into the cytoplasm of human MSCs using the Biolistic Particle Injection System, were determined with 33 ms temporal and 5 nm spatial resolution using multiple particle tracking. The frequency dependent elastic and viscous moduli were calculated from the complex shear moduli, which were determined from the Fourier transform of the time-dependent MSDs, using the frequency-dependent Stokes-Einstein equation. Pretreatment of MSCs with TCM resulted in rapid changes in cytoplasmic viscoelasticity with a 9.8-fold increase in the average elastic moduli, which increased from 35 to 344 dyn/cm2, and a 3.5-fold decrease in the average viscous moduli, which was reduced from 99 to 28 dyn/cm2, within 1 hour ($n = 6$-8 cells per group). We hypothesize that tumor-secreted molecules increase MSC mobility by altering cytoskeletal organization. Changes in MSC viscosity may be in part to reduced actin cross-linking during cytoskeletal reorganization. Increased MSC rigidity may be due to MSC elongation, which leads to the formation of polymer entanglements as the ratio of cell length to width is greatly increased.

2883-Pos
Toward Magnetic Control of Cell Polarity
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Cell polarity is involved in many aspects of cell and developmental biology. It is of fundamental importance for processes as diverse as cell motility, division, or differentiation. Cell polarization manifests itself through complex signaling and transport mechanisms by which molecules are asymmetrically localized within the cell. Whereas usual genetic and biochemical approaches are adapted to identify the elements in a transduction pathway which are necessary for the emergence of a cell polarity, they are not sufficient to know if the sole localized activity of a given effector is sufficient for the cell to acquire a polarity or to determine the kinetics of polarity formation. To address these important issues, we present a novel approach based on functionalized magnetic nanoparticles which are used to induce a localized signaling event of polarization. By doing so, we are able to monitor the cellular dynamic response to a local perturbation while preserving the complexity of the interaction feedbacks needed for the emergence of a global polarity. In our experiments, fluorescent magnetic nanoparticles (100-500 nm in size) are coated with purified constitutively active Cdc42 proteins, a key regulator of cell polarity. Once injected in the cytoplasm of live cells, these nanoparticles are manipulated using a customized magnetic setup able to exert forces on the order of 1-100 pN. We monitor at the single cell level the dynamics of nanoparticles and analyze the role of diverse factors (cytoskeleton, ER, substrate rigidity) on their intracellular mobility. Finally, we measure in different cell lines (Hela, 3T3) the effect of the local signalization on downstream effectors such as actin dynamics.

Bacteria & Motile Cells: Signal Transduction

2884-Pos
Generating Alternating Bidirectional Gradient Fields for Dynamic Measurements of Chemotactic Response
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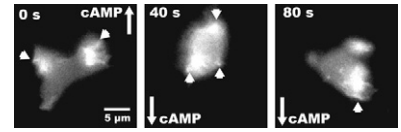
Prerequisite to a quantitative analysis of biochemical signalling networks is a well defined stimulus in space and time and a defined marker of biochemical response at the single cell level. We combine time-lapse fluorescence microscopy with a microfluidic chamber, which allows applying a defined temporal sequence of a spatially homogeneous chemical gradient over an ensemble of cells. The distribution of a chemoattractant in the flow chamber is characterized and the performance of the device found in good agreement with finite element calculation. To elucidate the dynamics of cellular response we investigate the velocity distribution of the amoeba Dictyostelium discoideum as a response to alternating cAMP gradients in opposing directions. We find pronounced directional migration at low switching frequencies, while at switching frequencies above 0.01 Hz stochastic cellular motility exhibiting seemingly non-responsive cells is observed. We demonstrate that the dynamics of intracellular polarization as displayed by the distribution of Lim-GFP is delayed with respect to the external change in gradient. We expect the microfluidic set-up to be useful for comparison of experimental data and computational systems modelling of cellular responses.



2885-Pos
The Response of Single E. Coli Cells to Changes in External Osmolarity
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The extreme concentrations of chemicals in a bacterium's cytoplasm generate a large osmotic pressure that inflates the cell. Using a number of interconnected systems, bacteria actively regulate their turgor pressure to resist changes in their local environment. In response to osmotic shock and changes in internal ion concentration, the osmosensory transporters ProP/U, BetT/U, TrkAH and KdpFABC transport external chemicals such as proline, choline and potassium into the cell, whereas the mechano-sensitive channels MscS and MscL export solutes from the cell in response to increased membrane strain. Although each has been shown to play a role in the regulation of turgor, details of how the different systems are coordinated by a cell is poorly understood. Previous measurements of osmoregulation in bacteria have been unable to directly probe the adaptation of turgor pressure, focusing instead on the activity of various transporters, or the change in cellular survival rates. Here we move beyond these limited measurements using AFM and fluorescence imaging to monitor turgor pressure and cell volume adaptation on a single cell level with a time