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