severing filaments and increasing the concentration of free filament ends from which subunits add and dissociate. Cofilin binding dissociates actin-associated cations and enhances filament bending and twisting compliance. The linkage between cofilin-mediated cation release, filament mechanics and severing activity has not been firmly established. Here, we demonstrate that cofilin-dependent cation release from a discrete, filament-specific cation binding site enhances the bending and twisting flexibility of actin filaments and that this local change in filament mechanics is required for severing. The work presented reveals the molecular origins of cofilin-linked changes in actin filament mechanics and severing.

835-Pos Board B590

Contractility and Dissipation in Active Actin Bundles and Networks

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Actomyosin machinery is a fundamental engine generating mechanical forces required for biological processes of non-muscle cells such as cell migration, cytokinesis, and morphogenesis. The net force generation is determined by the buildup and dissipation of forces in bundles and networks consisting of actin filaments, molecular motors, and passive cross-linkers. Although the molecular and physical properties of key elements in the actomyosin machinery have been characterized well, it still remains unclear how macroscopic force buildup and dissipation depend on the microscopic properties of individual cytoskeletal components and their local interactions.

To bridge such a gap between macroscopic and microscopic scales, we have developed a three-dimensional computational model of actomyosin bundles and networks with minimal components: actin filaments, passive cross-linkers, and active motors. Our model accounts for several key features neglected by previous studies despite their significance for force generation. Especially, the motors comprise a backbone structure with numerous heads attached as myosin thick filaments and mini-filaments, and kinetics of the individual heads is governed by mechanochemical rates for faithfully capturing behaviors of myosin II heads.

Using the model, we systematically studied how a net force in bundles and networks is determined via interplay between actin filaments, motors, and cross-linkers. We found the maximal force buildup is affected mainly by the total number and stall force of heads and how stably motors walk on actin filaments. We showed further that passive cross-linkers can help force buildup by increasing connectivity but can also act as dampers by dissipating the forces via reversible binding to actin filaments. We also investigated effects of the density, length, and dynamics of actin filaments on the net force generation.

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Direct Visualization of Tropomyosin Isoform Binding to F-Actin

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There are more than forty different tropomyosin isoforms in mammalian cells produced via alternative splicing from four separate genes. These isoforms have varying expression levels in different cell types and are targeted to specific regions of cells. In turn, the proper regulation of actin-binding protein interactions relies on the ability of different tropomyosin isoforms, each with its own unique actin-binding properties, to target a particular subset of actin filaments and define filament functions. Despite the large number of studies that have investigated the various cellular roles played by tropomyosin, the mechanism of differential tropomyosin binding to actin filaments remains uncertain.

In this work, we used TIRF microscopy to monitor Alexa-532-labeled tropomyosin binding to actin. We found that random, weak monomer binding leads to the formation of "nucleation sites" with enhanced affinity for the actin filament-a process most likely dependent on the tropomyosin end-to-end interactions. Stepwise changes in fluorescence intensity observed indicate that three tropomyosin molecules are required to form a stable nucleation site. From these sites, subsequent chain elongation is rapid and appears to depend on the strength of such end-to-end "tropomyosin linkages." This mechanism, which is strongly dependent on polymer formation rather than initial monomer binding onto the actin filament, suggests that tropomyosin isoform sorting may be intrinsic to the end-to-end linkage sites. A mixture of Tm2-Cy3 and Tm5NM1-Cy5.5 labeled tropomyosins was shown to segregate on F-actin and form distinct "patches" along actin filaments. We hypothesize that the C-terminal region of tropomyosin, which is highly variable among tropomyosin isoforms, is the primary determinant in distinguishing among different isoforms.

837-Pos Board B592

The Structure of Actin

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The protein actin exists as both a monomer (G-actin) and polymer (F-actin). There have been more than 80 different crystal structures of G-actin and related homologs over the years, and at least 4 different structures/models for F-actin have been proposed using a combination of techniques. Comparison of the G-actin crystal structure with the most recent F-actin structures suggests that there are several structural rearrangements, the most significant being a relative rotation of subdomains that results in a propeller-like twist of the protein. To investigate these structures in more detail, we performed multi-microsecond molecular dynamics simulations of the muscle ADP-actin monomer (pdb 1J6Z) and the two most recent ADP F-actin structures (2ZWH and 3MFP). We find that G-actin monomer deviates quite far from its crystal structure over the course of its simulation while the F-actin protomer shows a more modest shift from its starting point. The most interesting result is that the equilibrium structures of the monomer and F-actin protomer are much more similar than previously thought and their conformational spaces show significant overlap. We will present detailed analysis of the conformational dynamics, as well as analysis of the filament conformation, twist, and structures of the barbed and pointed ends.

838-Pos Board B593

Mechanism of Actin Network Stabilization by Changes in Polymer Flexibility by Calponin

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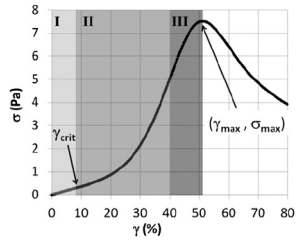
The cellular actin cytoskeleton plays a central role in the ability of cells to properly sense, propagate, and respond to external stresses and other mechanical stimuli. The actin binding protein calponin has been previously implicated in actin cytoskeletal regulation and is thought to act as an actin stabilizer, but the mechanism of its function is poorly understood.

To investigate the underlying physical mechanism, we studied an in vitro model system of crosslinked actin using bulk rheology. Networks with basic calponin exhibited a delayed onset of strain stiffening (gamma crit) and were able to withstand higher strains (gamma max) and stresses (sigma max) before failing. Using fluorescence microscopy to study the mechanics of single actin filaments, we found that calponin increased the flexibility of actin filaments. Our data are consistent with current models of affine strain behavior in semiflexible polymer networks, suggesting that calponin stabilization of actin networks can be explained by changes in single filament mechanics. Comparisons to computational models indicate that a reduction of persistence length of individual actin filaments is the primary mechanism by which calponin stabilizes actin networks against shear.



Representative stress-strain curve shown below: