late-stage ovarian cancer patients is attributed to chemoresistance against drugs like cisplatin and paclitaxel. Though alterations in physical properties of the tumor microenvironment in a variety of different epithelial cancers, including ovarian cancer, is well appreciated, how such alterations influence normal versus drug-resistant cells remains obscure. In this study, we have compared the behaviour of normal and cisplatin-resistant ovarian cancer cells in response to changes in extracellular matrix (ECM) density. In comparison to control cells, cisplatin-resistant cells were less spread, possessed less number of cell matrix adhesions, and lower degradation potential. Further, cisplatin-resistant cells were found to possess higher baseline contractility compared to normal ovarian cancer cells, as assessed by trypsin de-adhesion assay. However, the enhanced contractility of cisplatin-resistant cells remained insensitive to changes in ECM density. Western blots revealed lower expression levels of the focal adhesion protein vinculin and higher expression levels of the actin crosslinking protein α-actinin in cisplatin-resistant cells compared to control cells. Together, our results point to a potential mesenchymal to amoeboidal transition in ovarian cancer cells that attain drug resistance.

Key Words: chemoresistance, tumor microenvironment, extracellular matrix, contractility, focal adhesions, actin bundling proteins.

**830-Pos Board B585**

**A Single-Chain Model to Predict Buckling in Active Gels**

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We present a single-chain theory to describe the dynamics of active actin gels, driven by motor proteins. Molecular motors create active cross-links between the semiflexible filaments. We model the semiflexible filaments as bead-spring chains; the active interactions between filaments are accounted for using a mean-field approach in which filaments have prescribed probabilities to undergo a transition from one motor attachment state into the other depending on the state of the probe filament. The level of description of the model includes the change in the end-to-end distance of the filaments, the attachment state of the filaments, and the motor-generated forces, as stochastic state variables which evolve according to a proposed differential Chapman-Kolmogorov equation. The motor-generated forces are drawn from a stationary distribution of motor stall forces that can be measured experimentally. The general formulation of the model allows accounting for physics that is not possible, or not practical, to include in available models that have been postulated on coarser levels of description. To obtain analytical results that provide insight into the microscopic mechanisms underlying the dynamics of active gels we first treat the special case of filaments as one-dimensional dumbbells, approximate the elasticity of the semiflexible filaments with a Hookean spring law, and assume that the transition rates are independent of the tension in the filaments. We show that even in this simplified form, the model predicts the buckling of individual filaments that is thought to be the underlying mechanism in the self-contraction of non-sarcomeric actin-mysoin bundles [Lenz et. al., PRL 108, 238107 (2012)]. The active dumbbell model also explains the violation of the fluctuation-dissipation theorem observed in microrheology experiments on active gels [Mizuno et. al., Science 315, 370-373 (2007)].

**831-Pos Board B586**

**Metavinculin Induced Changes at the Actin Interprotomer Contacts and the Mechanism of Resulting Severing**

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Vinculin is a ubiquitously expressed adhesion protein of cell-matrix and cell-cell junctions. A larger splice-isoform of vinculin, metavinculin, is specifically expressed in cardiac and smooth muscles where it co-localizes with vinculin. Metavinculin has a 68 amino acid insert at its C-terminal actin binding tail (metavinculin tail: MVT). Mutations in the MVT insert are associated with disrupted intercalated disc organization and dilated cardiomyopathies, suggesting a distinct and important role for this isoform in cardiac cells. In vitro, metavinculin was shown to sever and organize actin filaments into fine meshworks while vinculin bundles them. Therefore, we focused on characterizing the structural changes MVT induces in F-actin and the severing activity of MVT. By using mutagenesis, cross-linking and site-directed labeling, we determined that MVT causes changes in the actin interprotomer contacts and dynamics. Specifically, the spectra of acrylodan attached to cysteine residues engineered in dynamic loops of actin -D-loop, W-loop, and C-terminus- are changed in the presence of MVT indicating a rearrangement of actin-actin contacts. Enhanced excimers fluorescence due to pyrenes attached to cysteines 265 and 374 shows that MVT binding affects mainly lateral actin contacts. By using a seeded actin elongation assay as a reporter of severing by MVT, we confirmed that MVT has a biphasic mode of actin severing. At partial decoration with MVT -as defined by co-pelleting of MVT-actin complexes- we observed increasing amounts of filament severing. At higher decoration, above 60%, MVT had a stabilizing effect on filaments. Our work enhances our understanding of MVT induced severing and changes in actin filaments structure, which appears to be important for the physiological role of this tissue-specific vinculin isoform.

**832-Pos Board B587**

**Mechanical Properties of Branched Actin Networks Assembled from Yeast Extracts**

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The actin cytoskeleton is essential for cell mechanics and motility. Its dynamic nature and the host of actin binding protein (ABP) that modifies its architecture and growth make its description challenging to achieve. Here we propose to study actin branched networks through their mechanical properties. We previously developed a powerful experimental method to measure the mechanics of in vitro dense branched actin networks assembled from a minimal number of regulatory proteins (Pujol et al. PNAS 2012). This method is based on magnetic beads and allows for the rapid acquisition of orders of magnitude more experiments than previous techniques. We now focus on a recent in vitro system that reconstitutes actin branched gels in a near-physiological environment, from yeast cell extracts. These reconstituted actin networks are analogous to endocytic actin patches assembled in vivo, with more than 80 regulatory proteins present (Michelot et al., Curr. Biol, 2010). Compared to gels assembled from purified proteins, these gels were notably stiffer and more prone to plastic deformations, exhibiting failure and long-time relaxation. Because of the large number of experiments that can be carried out with the magnetic technique and the ease of yeast mutant generation, we are able to test the effects of the absence of numerous ABPs on the actin network mechanics. The lack of two cross-linkers Sac6 (fimbrin) and Scp1 (calponin) softens the gels and modifies their long-term evolution. The absence of Aip1, a protein involved in actin filament severing and network disassembly, changes the plastic reorganization properties of the actin networks. In the future, the combination of the mechanical magnetic probing technique and the yeast actin reconstituted system will provide an avenue to precise the role of many other actin protein partners implicated in the mechanics of the cytoskeleton.

**833-Pos Board B588**

**Response of Actin Networks at Intermediate Distances**

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We report the observation of a large-distance intermediate response in an experimental system of entangled F-actin gels. The tools of 1-point and 2-point microrheology were used to characterize the local and distance-dependent responses of the actin networks, respectively. The 2-point response at intermediate distances, arising from the effect of mass displacement rather than momentum diffusion, is enhanced by the much softer local microenvironment of the tracers compared to the bulk properties of the gel. Consequently, the intermediate behavior sets in at particle separations much larger than the mesh size, ξ, of the actin gel. Results from several networks with different mesh sizes will be presented, emphasizing this inherent property of complex fluids and its relation to ξ.

**834-Pos Board B589**

**Actin Filament Severing by Vertebrate Cofilin is Driven by Linked Cation Release**

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The dynamic remodeling of actin cytoskeleton drives cell movement. The essential actin regulatory protein cofilin accelerates actin remodeling by

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.

**836-Pos Board B591**

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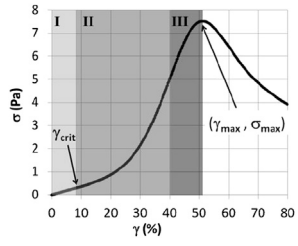
The cellular actin cytoskeleton plays a central role in the ability of cells to properly sense, propagate, and respond to external stresses and other mechanical stimuli. The actin binding protein calponin has been previously implicated in actin cytoskeletal regulation and is thought to act as an actin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