1623-Pos Board B533
Stress Accumulation Originating from Mechanical Asymmetry Promotes Actin Filament Severing at Boundaries of Bare and Cofilin-Decorated Segments
Jérémy Roland, Cristian Suarez, Laurent Blanchoin, Jean-Louis Martiel, **Enrique M. De La Cruz**.
The regulatory protein, cofilin, severs actin filaments and increases the number of ends from which subunits add and dissociate. Structural and biochemical analyses demonstrate that cofilin binding alters the conformation and mechanics of actin filaments such that cofilin-decorated filaments are ~20-fold more compliant in bend and twist than native actin filaments. Equilibrium and kinetic binding models as well as direct visualization of cofilin binding to filaments favor a mechanism in which severing occurs at or near boundaries of bare and cofilin-decorated segments. It is hypothesized that shear stress associated with conformational fluctuations accumulates locally at boundaries of mechanical asymmetry, thereby leading to preferential severing at junctions of bare and cofilin-decorated segments. In this work, we evaluate if mechanical and conformational periodicity in filaments promotes stress accumulation at junctions of asymmetry (i.e. boundaries). We have derived mathematical expressions of the actin filament elastic free energy, accounting for contributions from bending, twisting and twist-bend coupling, and used a computational modeling approach to evaluate the distribution of energy and stress of model filaments strained by external mechanical (buckling or torque) loads applied to filament ends. Our results indicate that mechanical asymmetry introduced by cofilin binding promotes the accumulation of shear stress at boundaries between bare and cofilin-decorated segments that likely increases the probability of failure (i.e. severing) under active or passive, thermal deformation, analogous to the fracture of some non-protein materials. Elastic coupling between twisting and bending is critical for stress accumulation at boundaries.

1624-Pos Board B534
Fast Magic Angle Sample Spinning NMR Yields a View of the F-actin - Cofilin Complex with Atomic Resolution
Andrew H. Butterworth, Si Yan, Dmitri Kudryashov, Emil Reisler, Tatyana Polenova.
Proteins of the ADF/cofilin family are vital regulators of the actin cytoskeleton in eukaryotes. Binding of cofilin results in dramatic reorganization of the F-actin structure and potentiates filament severing, and its accelerated depolymerization from the pointed end. Although the X-ray structure of monomeric actin with cofilin-homology domain of twinfilin has been recently solved, F-actin evades crystallization and therefore the analysis of cofilin interaction with F-actin at the atomic level calls for alternative approaches. While considerable insight into the cofilin F-actin complex has been gained through chemical cross-linking and radiolytic footprinting, these approaches were unable to generate high resolution information on this interaction While considerable insight into the cofilin F-actin complex has been gained through chemical cross-linking and radiolytic footprinting, novel approaches are still desirable. Here we demonstrate that Fast-MAS Solid State NMR is a high sensitivity approach to studying this system. Isotopically labeled S. cerevisieae yeast cofilin, in complex with polymerized yeast actin, allows for an atomic resolution view of cofilin within the complex. Intramolecular conformational changes occurring in cofilin upon binding to actin can be deduced from dipolar and scalar coupling based spectra. Additional studies of the free cofilin have been performed in the solution and solid states for comprehensive comparisons. Therefore, our data demonstrates the general feasibility of the SS-NMR approach for studying the interaction between F-actin and actin binding proteins.

1625-Pos Board B535
Observations of Twist and Disorder in F-actin from Cofilin Binding
Diana Y. Wong, David Sept.
The regulation of actin polymerization is vital for cellular function. Cofilin is one important regulatory protein and has increasingly been credited as being a player in a cell's homeostasis. Cofilin binds and severs actin filaments, thereby leading to depolymerization and creation of new barbed ends for elongation. Mutagenesis experiments and cryo-EM work have provided critical information about the interaction of cofilin with the actin filament, however the molecular details of cofilin binding and the mechanism of twisting and severing have not been elucidated. We have performed a series of molecular docking and molecular dynamics studies using muscle actin and human cofilin I. After determining a model for bound cofilin, we performed both all-atom and coarse-grained molecular dynamics simulations on bare actin filaments, fully decorated filaments, and filaments with cofilin bound at isolated sites. We find that the binding of cofilin as domains or in isolated sites affects the average twist angles as well as the twist fluctuations. Decorated filaments not only have a greater average twist, in agreement with cryo-EM studies, but also a lower local fluctuation of twist angle. Additionally, we show how cofilin introduces local disorder in a filament. These results shine light on the cofilin's effects on F-actin twisting and bending and provide some clues about cooperative binding kinetics and filament severing.

1626-Pos Board B536
Actin Filaments Stabilize Locally at Random Sites
Thomas Niedermayer, Antoine Jégou, Guillaume Romet-Lemonne, Marie-France Carlier, Reinhard Lipowsky.
After the polymerization of actin monomers into filaments, the actin-bound ATP is hydrolyzed into ADP, a process that was believed to decrease the filament stability. Recent experiments suggest the opposite behavior, however, namely that actin filaments become increasingly stable with time. Several mechanisms for this stabilization have been proposed, ranging from structural transitions of the whole filament helix to pure artifacts arising, e.g., from the capping or surface attachment of the filament ends. We performed novel fluorescence microscopy experiments on single filaments to clarify this controversial issue. We find that filaments do indeed cease to depolymerize in an abrupt manner, and that this transition happens on relatively long time scales that exceed those of both ATP cleavage and phosphate release. We also developed a theory that allows us to distinguish the different possible stabilization mechanisms. A detailed comparison of theory and experiment implies that the sudden truncation of the shrinkage process does neither arise from artifacts nor from a collective transition of the whole filament. Instead, our results provide strong evidence for a local stabilization process occurring at random sites within the filament.

1627-Pos Board B537
Mechanism of Actin Nucleation by Arp2/3 Complex Visualized by Single Molecule Fluorescence
Benjamin A. Smith, Karen Daugherty-Clarke, Bruce Goode, **Jeff Gelles**.
Nucleation of actin filaments by the Arp2/3 complex is a critical process in cell motility and endocytosis. It is well established that activity of Arp2/3 complex is stimulated by interactions with the VCA domain of WASP family proteins and pre-existing (mother) filaments. However the kinetic mechanism by which these proteins, together with monomeric actin, cooperate to rapidly assemble a critical nucleus for new (daughter) filament growth is not fully understood. We used multi-wavelength single molecule fluorescence microscopy to directly visualize in vitro the dynamic interactions of individual fluorescently labeled S. cerevisiae Arp2/3 complexes with actin filaments and to follow the pathway of new filament nucleation. We observed that Arp2/3 complex associated with mother filament sides, not ends. The majority of Arp2/3 complexes rapidly dissociated from filament sides after a characteristic distribution of lifetimes, while the remaining sub-population nucleated daughter filaments. Pointed ends of daughter filaments remained stably associated with the mother filament for durations estimated to be orders of magnitude longer than the dissociation times of Arp2/3 complexes that did not generate daughter filaments. Arp2/3 complexes nucleated some daughter filaments even in the absence of VCA, consistent with a mechanism in which VCA shifts an equilibrium between nucleation-inactive and nucleation-primed states of filament-bound Arp2/3 complexes. Taken together, these novel measurements reveal key features of the kinetic mechanism of actin nucleation by Arp2/3 complex and will help to more clearly define the VCA-regulated steps in the pathway.

1628-Pos Board B538
Actin Polymerization Dynamics - Insights from *In vitro* TIRF Microscopy
Balakrishnan Kannan, Mårten Larsson, Wei Lin Lee, Maria Hernandez-Valladares, Robert C. Robinson.
Actin elongation is a bi-molecular reaction between monomeric actin (G-actin) and filamentous actin (F-actin), in the first approximation. It can be controlled by changing the ability of either G-actin or F-actin to participate in the reaction. Either of the two mechanisms alone is not sufficient to maintain a large pool of G-actin ready to polymerize in a signal-controlled fashion [1]. Mammalian cells have hundreds of actin-binding proteins (ABP) which bind either or both the forms of actin. Profilin sequesters G-actin and makes them predisposed towards F-actin barbed-end addition, cofilin severs F-actin and depolymerizes it into G-actin. On the other hand, capping protein (CP) caps the barbed-end and stops further elongation of F-actin [2]. Gelsolin-family of proteins [3] sever F-actin as well as cap filaments. *In vitro* TIRF microscopy [4] has been used to monitor real-time actin dynamics in the presence of ABPs [5], [6]. Representative results on some ABPs which alter actin assembly will be presented.
[1] Pantaloni et al, Science (2001) 292: 1502-06
[2] J.A. Cooper et al, Int. Rev. Cell Mol. Biol. (2008) 267: 183-206
[3] P. Silacci et al, Cell Mol Life Sci (2004) 61: 2614-2623