Actin plays a major role in many cellular processes including cell motility, cell division, endocytosis, and exocytosis. External and internal mechanical forces lead to cytoskeletal rearrangements in the cell. Recently, we proposed that actin filaments may be directly involved in mechanosensing. Single molecule experiments suggest that F-actin is stiff with respect to extension. We used cryo-electron microscopy and image analysis to examine variation in the axial rise (the distance between two adjacent actin protomers along the one-start left handed helix) within frozen-hydrated actin filaments. We show that F-actin can be found in both stretched and compressed structural states. The magnitude of such mechanical deformation is far beyond what has been suggested for F-actin, and the axial rise can vary from 25 to 30 Angstroms. We demonstrate that the structural state of the filament strongly correlates with the variability of the axial rise, and actin's stretching stiffness depends upon the structural state of the subdomain 2, just as we had previously shown for the bending stiffness. We show that actin binding proteins that cross-link adjacent actin protomers, such as cofilin and the actin binding domain 2 of fimbrin, dramatically reduce the variability of the axial rise within the decorated filaments. Our results demonstrate the coupling between the mechanical properties of F-actin and its structural state and provide a structural basis for actin's role in mechanosensing.

3312-Pos Board B467
Cyclic Mechanical Reinforcement of Actin Interactions
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The fundamental mechanism underlying force regulation in actin dynamics is not understood. To investigate actin depolymerization under dynamic force environments, single molecule experiments were conducted using atomic force microscopy (AFM).
A custom-made AFM and force-clamped experimental procedures were used. To measure G-actin/G-actin (GG) interaction, G-actin was immobilized on the AFM cantilever tip and the polystyrene petri-dish was functionalized with G-actin. For G-actin/F-actin (GF) interaction, F-actin was prepared and immobilized on the petri-dish. To mimic dynamic force, once binding was detected during the tip retraction, force was applied to it via one of the programming paths; (1) loading the bond to 10, 15, 20 and 25 pN forces, and then reducing the force to 5 pN; (2) loading 1.5-, 2.5- or 3.5-cycle with a 10 pN peak force and holding at 10 pN to measure bond lifetime.
GG and GF dissociations were qualitatively similar to each other. When loaded by a linear ramp, actin dissociations exhibited a biphasic transition from a catch bond to a slip bond. In the catch bond region, bond lifetimes increased as force increased to a maximum at 12 pN in GG and at 20 pN in GF interactions. Interestingly, after applying cyclic forces, the post-priming bond lifetimes of GG and GF were significantly prolonged at low force range (5-10 pN), instead of reverting instantly to a low affinity state. This shows that cyclic force is more effective in strengthening actin-actin bonds than a linear ramp.
Our study demonstrated that kinetics of actin depolymerization is force dependent and the mechanical priming process by cyclic force application significantly enhanced bond lifetime of actin/actin. We hypothesize that the mechanical reinforcement of actin/actin interaction is an important regulatory mechanism underlying cytoskeletal dynamic rearrangement by affecting the actin depolymerization kinetics.

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Toxoplasma Gondii Actin Assembles via Isodesmic Polymerization
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The protozoan parasite Toxoplasma gondii relies on actin polymerization for motility and host cell invasion. Despite this strict requirement for actin filaments, T. gondii actin (TgACTI) remains largely unpolymerized in vivo and recombinant TgACTI only forms short filaments in vitro. TgACTI has several amino acid substitutions when compared with muscle actin, and our previous work showed that these changes significantly diminish the lateral contacts and interactions within the actin filament. The net result is that the two strands of the filament assemble with less cooperativity and not surprisingly this has significant effects on the polymerization kinetics. Here we show that the highly unusual characteristics of TgACTI result from isodesmic polymerization rather than the nucleation-elongation kinetics of conventional eukaryotic actins. TgACTI polymerization kinetics lack a lag phase and critical concentration, and all of the results from dynamic light scattering, dilution induced depolymerization, and sedimentation can be fit using a single pair of kinetic rate constants. These findings expand the repertoire of how actin functions in cell motility and offer clues about the evolution of self-assembling, stabilized protein polymers.

3314-Pos Board B469
Interplay of Stochastic Processes during Actin Depolymerization
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The dynamic polymerization (assembly) and depolymerization (disassembly) of actin filaments are pivotal for cell motility, cell adhesion, and cell division. The hydrolysis of actin-bound ATP destabilizes the filaments over time by enhancing the depolymerization velocity. However, abrupt changes of depolymerization were observed in single filament experiments, which were interpreted in terms of global structural transitions leading to increased filament stability with aging [Kueh et al. (2009), Science 325: 960]. In contrast to this proposal, we have demonstrated that these interruptions are caused by local transitions which turned out to be the photo-induced dimerization of neighboring filament subunits [Niedermayer et al. (2012), PNAS 109: 10769]. Here, we discuss and generalize the idea that led to this crucial notion of local transitions within the filaments. The time from the initiation of depolymerization until the occurrence of the first interruption represents a stochastic variable and the distribution function of this variable is a fingerprint of the unknown transition mechanism. By modeling the underlying stochastic processes - association and dissociation of actin subunits as well as hypothetical transitions -, we compute the distribution functions for many possible transition mechanisms and compare these functions to experimental data. We also generalize our theoretical description to accommodate for instance finite size effects or the influence of ATP hydrolysis. Furthermore, we outline how stochastic modeling may help deciphering other fundamental issues in actin dynamics.

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Actin Filament Nucleation by Smooth Muscle Leiomodin-1
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The formation of new filaments is an essential, but kinetically unfavorable, step in actin cytoskeleton remodeling. In cells nucleation has to be catalyzed by proteins called nucleators that bring together at least two actin monomers and stabilize a polymerization nucleus. Among known filament nucleators Leiomodin (Lmod) is the only one specifically expressed in muscle cells. Lmod is related to the F-actin pointed-end capping protein tropomodulin (Tmod), with which it shares two actin-binding sites: a flexible N-terminal region and a leucine-rich repeat (LRR) domain. In addition, Lmod contains a unique C-terminal extension that features a WH2 domain, another actin-binding site.
There are three muscle-type specific isoforms of Lmod: smooth muscle Lmod1, cardiac and striated muscle Lmod2, and the fetal isoform Lmod3. To date, only Lmod2 has been shown to nucleate actin filaments. Lmod1 is quite different in sequence from Lmod2 (37% identity, most of which is concentrated in the LRR). Here we demonstrate that despite these differences smooth muscle Lmod1 is also an actin filament nucleator. However its activity and mechanism of nucleation vary significantly from that of Lmod2.

3316-Pos Board B471
A Novel Mechanism of Actin Nucleation by Rickettsia Sca2
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Pathogens destabilize the host cytoskeleton for their survival and for motility. Pathogen interaction with host gives an advantage of illustrating the key cytoskeleton functions like phagocytosis, micropinocytosis, autophagy, motility, membrane trafficking and signal transduction. Sca2 is a Rickettsial outer membrane protein is known to nucleate host actin and forms actin comet tails for the pathogen motility. Yet, the mechanism remains a mystery. Sca2 passenger domain is 1482 amino acid length protein and it has three WH2 domains flanked with two proline rich domains. Here, we have used a battery of biophysical approaches such as, Fluorescence, TIRF microscopy, ITC, X-ray crystallography and SAXS to determine the structural basis for nucleation and elongation of actin by Sca2. using biochemical and TIRF assays we have found that Sca2 consists of two domains, where one domain nucleates actin and the other is needed for processivity. We have determined the