

1994-Plat**Force Dependent Unbinding Kinetics of Actin Crosslinking Proteins Using a Four-Bead Optical Tweezers Assay**

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While the interplay between actin filaments and actin crosslinking proteins has been extensively characterized in bulk studies, only very few experiments address this interaction on the level of single molecules. Hence, the longstanding question whether unfolding of crosslinking proteins is relevant to the mechanical response of the actin cytoskeleton remains to be elucidated.

To this end, we developed a four-bead optical tweezers assay which allows probing individual actin-crosslinker-actin bonds. Two actin filaments decorated with crosslinking proteins are suspended crosswise between two trapped beads each, forming a double dumbbell geometry. A feedback control keeps formed bonds under constant force thus enabling us to measure the force dependence of lifetime distributions. As the probed filaments are freely suspended in solution, the presented experimental approach mimics the situation in actin networks very well. We used this assay to characterize the well-known actin crosslinking proteins α -Actinin und Filamin. Our results indicate that unfolding of these proteins in a physiological context seems very unlikely as the bond between filament and crosslinking protein ruptures first.

1995-Plat**The Actin-binding Site of Adducin Is Regulated by Intramolecular Interactions that Occur Within a Natively Unfolded Domain**

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Adducin is an actin-binding protein with convergent functions in cell motility, including binding of calmodulin, capping of the barbed end of actin filaments, and recruitment of spectrin to the fast growing end of actin filaments. This protein consists of an unfolded C-terminal tail domain containing a positively charged phosphorylation site domain (PSD) that binds to actin, an alpha helical neck domain responsible for oligomerization, and an N-terminal globular head domain with unknown function. We recently established that MARCKS, a protein with a homologous PSD, forms intramolecular interactions that regulate the accessibility of this domain to its binding partners. Our data establish that the PSD of adducin is similarly regulated. Salt bridge formation between the PSD and a negatively charged region within the tail domain stabilize this interaction. The result is a unique configuration in which the tail is looped over upon itself without canonical structural elements so as to sterically regulate binding events at the PSD. Moreover, our data show that oligomerization of adducin results in activation of the PSD. Oligomerization is shown to be mediated either by self-association (occurring in the alpha helical neck domain) or by association with spectrin. Thus, these results explain at a structural level earlier observations showing that spectrin-adducin interactions affect actin-binding function. Finally, a polymorphism of adducin that has been associated with adverse cardiovascular outcomes in humans is shown to affect the actin-binding function of adducin by altering adducin oligomerization, and, therefore, by changing the availability of the PSD.

1996-Plat**Towards a Molecular Understanding of Actin Bundle Stability and Mechanics**

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Fascin and fimbrin are highly conserved actin-binding proteins used by cells to crosslink filamentous actin into compact ordered bundles. Despite the importance of these ubiquitous actin-binding proteins to cytoskeletal function, the molecular basis for their crosslinking and mechanical properties remains unknown. Here, we present a multi-scale approach aimed at reconciling the conserved sequence and structure of fimbrin and fascin with the unique properties that they endow to actin bundles. Equilibrium and nonequilibrium measurements indicate differential mediation of bundle mechanical properties, as well as the capacity for remodeling under sustained load. Atomistic simulations of fimbrin and fascin reveal distinct mechanisms of conformational flexibility that may have consequences on their cooperative actin-binding and mechanical properties in situ. Integrated structure-based modeling combined with experimental assays of bundle mechanics provides a promising approach to reconciling the conserved structure and sequence of actin crosslinking proteins with emergent actin bundle stability and mechanical properties.

1997-Plat**Caldesmon and Tropomyosin Synergistically Regulate Actin Dynamics**

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Our preliminary experiments suggest that actin undergoes a conformational transition during polymerization. Both caldesmon (CaD) and nonmuscle tropomyosin (Tm) arrest actin filaments at an intermediate state if added initially, but stabilize "matured" filaments when added after the transition. We have tested this hypothesis by making use of the calmodulin (CaM)-dependent dissociation of a CaD fragment, H32K, from F-actin. When CaM was added to H32K-arrested, pyrene-labeled F-actin in the presence of Ca²⁺, the pyrene-actin emission increased, reflecting the maturation of actin filaments upon dissociation of H32K. When free Ca²⁺ was removed by EGTA and H32K became re-associated with F-actin, we observed an accelerated increase in pyrene fluorescence. These results are consistent with the hypothesized conformational transition and a differential effect of CaD on the two states of actin filaments. The combined effect of CaD and Tm on the actin conformational transition was also tested during polymerization. H32K and Tm5a were first incubated with actin before polymerization was initiated. Shortly after polymerization started, CaM was added to dissociate H32K. Since Tm5a alone was sufficient to inhibit the maturation process, the transition corresponding to the conformational change was not observed, and the pyrene-actin emission jumped to the level of F-actin•Tm5a. At a later time when CaD fragment re-associated with the addition of EGTA, we saw an instant decrease, instead of a further increase, in pyrene fluorescence to the level of actin with both H32K and Tm5a, indicating that F-actin was still kept at the intermediate state by Tm5a. Since the actin cytoskeleton at the cell leading edge is extremely dynamic, it is expected that actin filaments there are kept at a less stable configuration. CaD and Tm5a may therefore function to maintain such a configuration by binding to nascent actin filaments as they are assembled.

1998-Plat**Tropomyosin Phosphorylation Has Filament-Level And Crossbridge-Level Effects On Actin-Myosin Interactions**

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The α -isoform of tropomyosin (Tm) can be phosphorylated near the C-terminus, and dephosphorylation of Tm has been shown to decrease actin-myosin ATPase rates (Heeley et al., 1989). Additionally, Tm polymerizes with adjacent Tm molecules in a head-to-tail manner. Phosphorylation of Tm might therefore influence the strength of these interactions, and thus regulate the degree of cooperative activation by myosin binding. We tested Tm phosphorylation effects by measuring the force and velocity of actin-myosin interactions at the level of single actin filaments using a combination of actin-Tm binding assays, *in vitro* motility assays, and a novel, high throughput laser trap assay to measure isometric force. In these assays we used purified heavy meromyosin (HMM) and actin filaments reconstituted with either natively phosphorylated or dephosphorylated Tm. Dephosphorylation of Tm did not result in significant changes in actin-Tm binding or unloaded sliding velocities. Isometric force measurements showed that thin filaments were cooperatively activated by myosin, but only when Tm was phosphorylated. When Tm was dephosphorylated, the reconstituted filaments behaved like bare actin filaments. These results suggest transmission of cooperative activation beyond one thin filament regulatory unit when Tm is phosphorylated. Moreover, Tm phosphorylation increased isometric force production ~50% compared to bare actin filaments at intermediate HMM surface densities. In combination with sliding velocity data, this result suggests that Tm phosphorylation may have effects at the level of a single crossbridge that cannot be explained by steric hindrance. We hypothesize that Tm phosphorylation accelerates the rate of crossbridge attachment. Single molecule kinetic and force spectroscopy experiments are underway to gain insight on this possibility.

Platform AO: Protein Folding & Stability I**1999-Plat****Solvation Free Energy of and Solvent Mediated Force on Proteins**Roland Roth^{1,2}, Yuichi Harano³, Masahiro Kinoshita⁴.

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In the theoretical study of protein folding it is important to have a fast and accurate method for predicting the free energy landscape of a protein in different geometrical configurations. One very important contribution to the free energy landscape is the solvation free energy, which originates from the interaction between the protein and the solvent.

We propose a theory for calculating the solvation free energy of a protein in a given geometrical configuration based on morphological thermodynamics. In morphological thermodynamics one separates the solvation free energy into four geometrical or morphological terms. These four terms are