sequences, we explore the rich dynamics and emergent properties of actin with an emphasis on assembling networks of actin bundles under appropriate stimuli, mimicking these processes in living cells. Due to the sharing of filaments within different bundles, long actin filaments form networks by percolation, when induced by specific bundling agents in micro-confinements. Using this bottom-up approach, we observe the dynamics of hierarchical assembly and disassembly processes and find exceptional attributes such as spindle-like structure formation and internal stress generation in networks. Furthermore, our research suggests that such percolated networks are very much likely to exist within living cells in a dynamic fashion with a long-range connectivity resulting in enhanced responsiveness to internal and external signals.

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F-Actin has Slow Dynamics and Concerted Movement as Indicated by H/D Exchange Rate Mapping

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We are investigating local dynamics within F- and G-actin by the method of Hydrogen-Deuterium Exchange-MS rate mapping. G-actin studies are underway, and F-actin results are near completion. Using extended time courses, we find HDX transitions that reveal new and unexpected insight into the properties of F-actin. Virtually all actin residues assessed so far are near one or the other extreme - very fast dynamics for ~ 25% of actin, or very slow dynamics for the remainder. Thus, the majority of the actin monomer exhibits very high protection from exchange, indicating a highly stabile character. The extent of HDX was flat and unchanging between 30 and 600 s of D2O exposure, consistent with published work of Stokasimov and Rubenstein. The new results, examining longer D2O exposures for the first time, show that HDX rose very notably between 600 s and 3600 s. Interestingly, the kinetics are very similar in the many peptides, and a single rate constant can fit transitions in almost the entire set of peptides. The observed rate of 3 x 10-4 s-1 implies high local folding stability, with a protection factor of ~ 100,000-fold. This suggests that part of the actin monomer is very tightly folded except for a low probability global movement that facilitates exchange in many areas of actin. The current work shows that F-actin is amenable to study by examination of HDX rates, shows that F-actin dynamic mapping can reveal new insight into actin's properties, and provides a basis for further study in the presence of other proteins such as tropomyosin.

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Characterization and Stabilization of Fascin-Bundled Actin Filaments Transported by Heavy Meromyosin

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Maintenance and reconstruction of cell shape and migration rely on dynamic formation of actin-bundles from actin-filaments (F-actin) and the interaction of these bundles with myosins in cytoskeletal networks. Of particular interest are protruding finger-like and sub-micrometer filopodia at the leading edge of cell. These contain unipolar actin-bundles with parallel filaments cross-linked via protein fascin. Here, we reconstruct fascin-actin-bundles in vitro from purified proteins and allow them to interact with myosin II motor-fragments, heavy meromyosin (HMM). We investigate the size distributions of fascin-actin-bundles transported by HMM on the trimethylchlorosilane-derivatized surfaces in the absence and presence of quantum dots (Qdots) in the in vitro motility assay. In the absence of Qdots, the number of filaments in a bundle (12.8 ± 3.3; n=50) was decreased by HMM-driven transport within 5 min after addition of adenosine-triphosphate (ATP) as compared with that of bundles without ATP (40.2 ± 12.7; n=35). Thus, the number of bundles of intermediate to large size (2-4 and >5 filaments in a bundle) significantly decreased during this time. This effect was prevented if fascin-actin-bundles were biotinylated and transported by HMM in the presence of streptavidin-Qdots (3 nM). Interestingly we also found that some of large fascin-actin-Qdot-bundles were further assembled into huge bundles 10-30 min after ATP-incubation. In this case, tracking of Qdots showed that there was no rotation around the long axis of bundle during transportation as has sometimes been observed for F-actin. We discuss the usefulness of actin-bundles for reconstruction of the in vitro cytoskeletal transport systems in engineered environments (e.g. biocomputation) but also consider issues related to the in vivo functions, e.g. the possible role of other actin-binding proteins (here-mimicked by streptavidin-Qdots) in the production of higher-order structures composed of fascin-actin-bundles. (Support from EU-FP7-FET-ABACUS grant number 613044).

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Cryo-EM of One State of F-Actin Yields a New Atomic Filament Model

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Actin is one of the most highly conserved as well as abundant eukaryotic proteins. From chickens to humans, an evolutionary separation of ~ 350 million years, there are no amino acid changes in the skeletal muscle isoform of actin. Since the functional form of actin in most instances is a polymer (F-actin), understanding the constraints on actin sequence evolution must involve an understanding of the structure and dynamics of the actin filament. The development of direct electron detectors has allowed an unprecedented advance in the ability of cryo-EM to reach near-atomic resolution for many protein polymers and protein complexes.

We have used electron cryo-microscopy and a direct electron detector and have now been able to reconstruct one state of F-actin at 4.7 Å resolution. This has allowed us to build an atomic model of this state, which differs in many details from an earlier model for F-actin derived from a substantially lower resolution reconstruction. The model explains many previous observations about F-actin, such as why the "hydrophobic plug" can be structurally polymorphic. We compare this atomic model with two other distinctly different states that we have determined at ~ 12 Å resolution, and suggest that only by understanding the multiplicity of states possible for F-actin can one understand the selective pressure on many residues and why mutations of some of these residues leads to myopathies and other human disorders.

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Mechanisms of FRNK Inhibition of FAK in Vascular Smooth Muscle Cells

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The goal of this project is to determine novel mechanisms by which Focal Adhesion Kinase related Non-Kinase (FRNK) inhibits Focal Adhesion Kinase (FAK). FAK is an important regulator of growth and migration in vascular smooth muscle cells that acts as both a kinase and a scaffolding protein. FAK is activated by numerous signaling pathways and upon activation dimerizes and phosphorylates itself. Phosphorylated FAK scaffolds additional proteins such as Src kinase which contribute to growth and migration. FRNK is endogenously expressed and inhibits FAK mediated growth and migration through an unknown mechanism. To test whether FAK directly interacts with FRNK, we co-immunoprecipitated FRNK with a FAK antibody. Significant precipitation of FRNK suggested that FAK and FRNK interact in a protein complex. We utilized fluorescence resonance energy transfer (FRET) measurements between fluorescent protein tagged FAK and FRNK to confirm direct interaction in living cells. In order to isolate FAK within focal adhesions we used total internal reflection fluorescence microscopy. In order to determine the importance of phosphorylation status on potential FAK FRNK interaction we mutated the phosphorylation site serine 910 on FRNK to aspartic acid and alanine. In co-immunoprecipitation experiments the serine 910 to alanine FRNK mutant is pulled down in greater quantity compared to wild type suggesting serine 910 phosphorylation status may play an important role in FAK FRNK binding interactions. Together the data suggest that direct FRNK and FAK interaction as regulated by serine phosphorylation plays an integral role in altering FAK signaling.

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Altered Structural State of Actin Filaments Upon MYOSIN II Binding

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The paths of actin filaments propelled over a heavy meromyosin (HMM) surface in the *in vitro* motility assay (IVMA) can statistically be described by a path persistence length (L_P^P) and has been hypothesized to be proportional to the flexural rigidity of the filaments. Here, we have studied the L_P^P at high (130 mM) ionic strength along with the persistence length of actin filaments in solution (L_P^S) to elucidate how HMM binding affects the flexural rigidity of actin filaments. Characterization and control of material properties, such as the path persistence length, is useful in engineered devices that takes advantages of the function of the muscle contractile proteins e.g. for biocomputation. It has been suggested that myosin binding reduces Lp^p for phalloidin stabilized actin filaments. This is consistent with the results presented here where the phalloidin stabilized actin filaments rigidity is reduced to the level of phalloidin free actin filaments in the IVMA. Further, reducing the MgATP concentration in the IVMA would increase the HMM head density along the actin filament hence making the effect of myosin binding more pronounced. A reduced