activity is not coupled to ATP cleavage, but that it accelerates Pi release at the barbed end of actin filaments.

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Differential Effects of Crowding Reagents on the Interaction of Profilin and Thymosin Beta 4 with Actin
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Actin polymerization *in vivo* is spatially organized, with dynamic actin polymerization occurring at the leading edge of a motile cell, and occurs in the context of molecular crowding effects, including osmolyte concentration, that also vary with location. The current study tests the hypothesis that molecular crowding could influence interactions of actin with actin-regulatory proteins so as to shift the pool of actin monomers to a polymerization competent state within the active polymerization zone. In support of this hypothesis, we found that a naturally occurring osmolyte trimethylamine *N*-oxide (TMAO) dramatically increases affinity, K_T, of the actin sequestering protein thymosin b4 (TB4) to monomeric actin and significantly decreases barbed end actin critical concentration Ac in presence of ATP. The N- and C-terminal alpha helices in TB4 helices are known to be unstructured in aqueous solution and to adopt helical conformation in organic solvents or upon binding to actin. The effects of TMAO are consistent with a decrease in free energy of the actin-TB4 interaction through facilitating alpha helix formation. The effect of TMAO on the affinity, K_P, of ATP-G-actin to a globular actin regulating protein, profilin, is much weaker, as might be expected based on structural considerations. Unlike TMAO, polyethylene glycol (PEG) affects A_c, K_T, and K_P by similar extents. We also found that the TMAO facilitates ternary complex formation between actin, profilin, and TB4 which may very significantly increase the amount of unpolymerized actin in presence of profilin and TB4, and therefore inhibit actin polymerization. We conclude that spatial variation in osmolytes could, in principle, explain how actin sheds TB4 prior to polymerization.

176-Plat
Structural Reorganization of Parallel Actin Bundles by Crosslinking Proteins: Incommensurate States of Twist
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We construct a coarse-grained model of parallel actin bundles crosslinked by compact, globular bundling proteins, such as fascin and espin, necessary components of filapodial and mechanosensory bundles. Consistent with structural observations of bundles, we find that the optimal geometry for crosslinking is overtwisted, requiring a coherent structural change of the helical geometry of the filaments. We study the linker-dependent thermodynamic transition of bundled actin filaments from their native state to the overtwisted state and map out the ''twist-state'' phase diagram in terms of the availability as well as the flexibility of crosslinker proteins. We predict that the transition from the uncrosslinked to fully-crosslinked state is highly sensitive to linker flexibility: flexible crosslinking smoothly distorts the twist-state of bundled filaments, while rigidly crosslinked bundles undergo a phase transition, rapidly overtwisting filaments over a narrow range of free crosslinker concentrations. The critical stiffness which divides these two regimes is determined by the stiffness of the actin filament to torsional deformation. Additionally, we predict a rich spectrum of intermediate structures, composed of alternating domains of sparsely-bound (untwisted) and strongly-bound (overtwisted) filaments. This model reveals that subtle differences in crosslinking agents themselves modify not only the detailed structure of parallel actin bundles, but also the thermodynamic pathway by which they form, thereby allowing different cell types to modulate the sensitivity of bundle formation to crosslinker availability by altering properties of the crosslinking proteins alone.

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Structure, Conformational Dynamics, and Evolutionary Conservation of Human Fascin-1
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Cells express a range of actin-binding proteins to crosslink filamentous actin (F-actin) into compact ordered bundles ranging from stereocilia and microvilli to filopodia. Human fascin-1 is found nearly exclusively in filopodia, which protrude from the leading edge of migrating cells in normal and cancerous cells. Previously, we and others have found that fascin-1 confers unusual mechanical integrity to these actin bundles, which play a role in sensing the extracellular environment. Here, we examine the packing, conformational flexibility, and evolutionary sequence conservation of full length Homo sapiens fascin-1. Unlike the ubiquitous crosslinking proteins fimbrin and alpha-actinin that consist of dual calponin homology domain pairs, fascin-1 consists of four β-trefoil domains organized into a double-lobe-like structure. Sequence analysis suggests that these β-trefoil domains are stabilized by bulky hydrophobic residues in the core of each domain, and that interfacial residues between the two lobes of fascin play an important role in stabilizing the molecule. Conformational dynamics analysis reveals an allosteric coupling between highly conserved surface patches near the putative actin-binding sites of the molecule, with potential implications on its ability to crosslink F-actin tightly and confer unusual mechanical properties to F-actin bundles. Future mutational and structural studies motivated by this work will further elucidate the molecular basis for the unique function of fascin in human cells.

178-Plat
A coarse-Grained Monte Carlo Model of Cytoskeletal Actin Filament Alignment under Cyclic Stretch
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The cytoskeleton is a dynamic system linked to the cell'S environment through sites of potential mechanical interaction such as focal adhesions, integrins, cellular junctions, and the extracellular matrix. The physiologic mechanical stimulation experienced by cells such as endothelium is comprised of multiple mechanical modes (e.g., stretching and shear), thus presenting a challenge to characterize their influence on cell structure. Furthermore, physiologically, both endothelial cells and fibroblasts align themselves perpendicular to the direction of cyclic stress. Here, we simulate this behavior using a minimalistic coarse-grained Monte Carlo model of the actin filament network undergoing uniaxial cyclic stretch. A filament network is prescribed within a two-dimensional circular space through filaments connecting nodes. Perimeter nodes represent focal adhesion complexes and interior nodes represent actin binding proteins. Filaments representing actin filaments are randomly generated between nodes. During a stretch cycle, the perimeter nodes are stretched and a Gauss-Seidel relaxation iteration is applied to adjust the position of the interior nodes until the system reaches equilibrium. This equilibrium is defined to occur when the cumulative stress on the nodes from filaments falls below a prescribed tolerance. This repositioning results in a gradual alignment of the filaments in the direction perpendicular to stretch with increasing cycle count. In addition, we corroborate our model with experimental data showing gradual alignment of NIH 3T3 fibroblasts perpendicular to 1 Hz cyclic stretch. With this work, we test the hypothesis that a first-principles mechanical model of filament assembly in a confined space is by itself capable of yielding the remodeling behavior observed experimentally. We believe that this work is of interest to a wide variety of fields including physics, biology, mechanics, and computer science.

179-Plat
Regulation of Immune Synapse Cytoskeleton Mechanics by CD3 and LFA1
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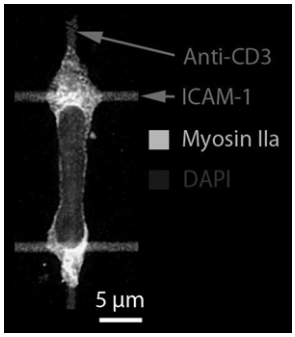

Cytoskeletal reorganization is a key element of immune synapse function. Actomyosin contractility leads to a rapid and dynamic flow of material towards the interface center, leading to complex regulation of immune receptors within this structure. Here, surfaces presenting micropatterned, spatially separated ligands are used to determine the viscoelastic properties of the immune synapse cytoskeleton. Anti-CD3 and ICAM-1, which activate the T Cell Receptor complex and LFA-1 integrin, respectively, are patterned as orthogonal lines forming a grid. Human CD4+ T cells spread anisotropically along these lines in response to the different adhesion signals. Measurement of cell spreading along each direction and the free cell-edge curvature radius allowed analysis of how these two receptor systems differentially influence cytoskeletal mechanics. Activation of CD3 alone induced a high degree of cytoskeleton fluidity and low accumulation of elastic tension. Concurrent engagement of LFA-1 lead to higher accumulation of elastic potential energy but higher