169-Plat
Self-Organization of the Phosphatidylinositol Lipids Signaling System for Random Cell Migration
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Phosphatidylinositol (PtdIns) lipids have been identified as key signaling mediators for random cell migration as well as chemoattractant-induced directional migration. However, how the PtdIns lipids are organized spatiotemporally to regulate cellular motility and polarity remains to be clarified. Here, we found that self-organized waves of PtdIns 3,4,5-trisphosphate [PtdIns(3,4,5)P3] are generated spontaneously on the membrane of Dictyostelium cells in the absence of a chemoattractant. Characteristic oscillatory dynamics within the PtdIns lipids signaling system were determined experimentally by observing the phenotypic variability of PtdIns lipid waves in single cells, which exhibited characteristics of a relaxation oscillator. The enzymes phosphatase and tensin homolog (PTEN) and phosphoinositide-3-kinase (PI3K), which are regulators for PtdIns lipid concentrations along the membrane, were essential for wave generation whereas functional actin cytoskeleton was not. Defects in these enzymes inhibited wave generation as well as actin-based polarization and cell migration. On the basis of these experimental results, we developed a reaction-diffusion model that reproduced the characteristic relaxation oscillation dynamics of the PtdIns lipid system, illustrating that a self-organization mechanism provides the basis for the PtdIns lipids signaling system to generate spontaneous spatiotemporal signals for random cell migration and that molecular noise derived from stochastic fluctuations within the signaling components gives rise to the variability of these spontaneous signals. PNAS 107 12399-12404

170-Plat
The Combination of Pulsatile and Switch-Like Behaviors of p53 in Response to DNA Damage
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Upon DNA damage, p53 is stabilized and activated to induce diverse cellular outcomes including cell cycle arrest and apoptosis. The p53 dynamics can exhibit distinct modes, depending on cell and stress types. In an analog mode, high (low) levels of p53 lead to cell death (survival) in response to lethal (sublethal) DNA damage. By contrast, p53 levels exhibit a series of discrete pulses in a digital mode, wherein it is the number of p53 pulses that determines the cell fate [1]. Here, we explore whether both the modes of p53 are exploited in one cellular response. We propose a modular model for the cell fate decision between survival and death, which is governed by the p53 network. At low damage levels, p53 levels exhibit few pulses, and the cell returns to normal proliferation after DNA damage is fixed. For irreparable damage, the amount of p53 first displays four pulses and then switches to high levels, and the cell undergoes apoptosis. The negative feedback loop between p53 and Mdm2 and that between ATM and p53 via Wip1 are responsible for p53 oscillations, whereas the switching behavior occurs when the positive feedback loop between p53 and PTEN predominates over the negative feedback loops. Such a combination of pulsatile and switch-like behaviors of p53 may represent a flexible and reliable control mode, avoiding unnecessary cell death or promoting execution of apoptosis. This work also underscores that both the nature and strength of feedback loops determine p53 dynamics.
[1] X.-P. Zhang, F. Liu, Z. Cheng, and W. Wang (2009) Cell fate decision mediated by p53 pulses. Proc. Natl. Acad. Sci. USA. 106, 12245-12250.

171-Plat
Time-Keeping in the Transcriptional Cascades of the Developing C. elegans Embryo
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Cell differentiation and cell proliferation are coordinated during animal development by mechanisms that remain largely unknown. We use the fact that the timespan of development in C. elegans can be varied significantly by changes in temperature and mutations to discern the nature of the coordination mechanism at play in this organism. We have measured time profiles of gene expression in key organogenesis pathways of C. elegans embryos that have grown under conditions and genetic backgrounds that significantly change the overall rate of development. We used the single molecule RNA fluorescence in situ hybridization technique recently developed by our group to obtain absolute RNA transcript numbers with single molecule sensitivity. The data allow us to determine the extent to which RNA dynamics remain locked to the cell cycle as lifespan is varied and infer the existence of coordinator processes from changes of apparent induction thresholds in transcriptional cascades.

PLATFORM O: Actin & Actin-binding Proteins

172-Plat
Structural Polymorphism in F-Actin
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Actin plays a major role in many cellular processes including motility, cell division, endocytosis, and exocytosis. Actin has also maintained an exquisite degree of sequence conservation over large evolutionary distances for reasons that are not understood. Generating an atomic model of the actin filament (F-actin) has been driven by the desire to explain phenomena from muscle contraction to cytokinesis in mechanistic detail. To understand how key mobile elements of actin contribute to the intrinsic structural polymorphism of F-actin we carried out electron microscopic studies of the frozen-hydrated actin filaments. We show that frozen-hydrated actin filaments possess substantial structural polymorphism. We demonstrate at higher resolution (~ 10 Å) that within the actin filament subdomain 2 of actin (SD2) can undergo significant structural alterations from an ordered position to complete disorder, and that its dynamics is coupled with that of the C- and N-termini of actin molecule. Our observations reconcile the multiplicity of structural conformations of actin observed by x-ray crystallography with the multiplicity of conformations seen within F-actin. We link a number of disease-causing mutations in the human ACTA1 gene to the most structurally dynamic elements of actin. Since F-actin is structurally polymorphic it cannot be described using only one atomic model, and must be understood as an ensemble of different states.

173-Plat
Multiple Actin Structures in Monomeric and Filamentous States
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One of the most abundant proteins in eukaryotes is actin, a ubiquitous protein that plays a role in cell dynamics like cell migration. The dynamics of actin filament treadmilling is regulated by two actin structural states: globular actin (G-actin) and filamentous actin (F-actin). Although recently it was reported that the structure of the two actins differ (Oda., et al, Fujii., et al), there is still much to resolve on the matter of their dynamic structures.
Here we observed the dynamics of the actin structural states under various conditions by using single-molecule FRET in combination with TIRF microscopy. We found that bothF-actin and G-actin have at least two distinct states, and that the population distribution of these states depends on the ionic conditions. Furthermore, these states were sensitive to actin binding proteins like myosin and actinin. We are currently investigating actin structural states by performing FRET measurements to observe various positions of actin in the presence of the actin binding proteins myosins.

174-Plat
Observation of Individual Actin Filaments Reveals that ATP Hydrolysis is a Random Mechanism
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After their polymerization into a filament, actin monomers hydrolyze their ATP and release the resulting inorganic phosphate (Pi). This phenomenon is of primary importance for the filament's assembly dynamics, as well as for its interaction with various regulatory proteins. However, the long debated question of whether Pi release follows a random or a vectorial mechanism still awaits conclusive experimental data.
We have developed a novel experimental approach, combining microfluidics, optical tweezers and fluorescence microscopy, in order to manipulate and observe individual actin filaments in a controlled biochemical environment. Our data provides a direct measurement of the Pi profile in actin filaments, and clearly shows that Pi release occurs according to a random mechanism.
In addition, using the same experimental setup, we have monitored the Pi profile in filaments polymerized in presence of profilin, and we have measured the impact of profilin on the kinetics of filament disassembly. We show that profilin

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

175-Plat
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.

177-Plat
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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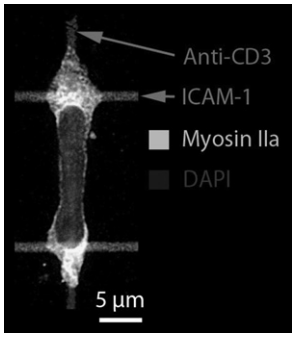

Cytoskeletal reorganization is a key element of immune synapse function. Actomyosin contractility leads to a rapid and dynamic flow of material towards the interface center, leading to complex regulation of immune receptors within this structure. Here, surfaces presenting micropatterned, spatially separated 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