soluble actin, actin oligomers, and actin regulators in the crowded filament network is little explored. We combined single molecule fluorescence microscopy with image analysis and modeling to quantify the role of diffuse actin species and their gradients in actin reorganization at the leading edge of motile cells. Actin, capping protein, and Arp 2/3 complex, were marked with fluorescent probes at low concentrations and imaged at high spatiotemporal resolution in XTC fibroblasts. Particle tracking was used to mark the appearance and disappearance of bright spots that correspond to proteins becoming associated to, or dissociated from,the actin network. Image correlation analysis was used to quantify the motion of proteins in the cytoplasm. We developed conditional image correlation methods to study local dynamics prior to assembly and immediately following disassembly. From these data we create a map of the lamellipodium showing the dynamics of lamellipodium proteins and their turnover. Bounds on the fraction of actin that leaves the filament network as oligomers was determined by measuring the distribution of diffusion coefficients which correspond to different oligomer lengths. We used numerical simulations to model these turnover dynamics and to simulate FRAP experiments. These results help resolve apparenty disparities in measurements found through FRAP and single molecule speckle microscopy.

101-Plat
Depolymerization of F-Actin Produces a Pulling Force At the Plasma Membrane *in vivo*
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We report that depolymerization of F-actin filaments produces a pulling force on the plasma membrane as predicted by calculations based upon energetics. We do this by monitoring the axial membrane force produced upon forming a long (> 15 μm) membrane tube filled with an actin bundle formed from a mammalian cell. The filopodium is formed with an optical trap which is also used to measure the force. We observe a dynamic sawtooth force riding atop the equilibrium force which increases slowly (10 s of seconds), stalls and decays rapidly back (ms) to equilibrium. Examination of the magnitude and time course of the force shows that the rise and decay of the axial membrane force is due to depolymerization and polymerization of F-actin at the barbed end of the bundle. From the magnitude of the force we determine the number of filaments (< 20) within the bundle, and establish that the on and off rate decays exponentially with the axial membrane load exhibiting a length constant of ≈ 3 nm. We determine the on and off rates of G-actin at the barbed end and calculate that a filament produces a pushing and pulling force of 4 to 5 pN upon polymerization and de-polymerization. Cooperativity within the filaments of the bundle is observed; the load is borne by > 1 filament.
Supported by R01DC00354 and R01DC02775.

102-Plat
Vinculin and Fak Facilite Cell Invasion in Dense 3D-Extracellualr Matrix Networks
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The cytoskeletal adaptor protein vinculin and the non-receptor tyrosine kinase focal adhesion kinase (FAK) modulate the dynamics of integrin-based cell adhesions via different mechanisms. Vinculin contributes to the mechanical link of actin filaments to ligand-bound integrin receptors, connecting the contractile actomyosin cytoskeleton to the extracellular matrix (ECM). Vinculin̄s incorporation into adhesion sites is associated with decreased cell motility on 2D-ECM substrates. FAK associates with integrins in adhesion sites directly and indirectly. Activity regulation of the kinase is involved in stress sensing and the control of adhesion site turnover. To date, the effects of vinculin and FAK on cell invasion and migration through dense 3D-ECM gels have not been addressed. Here, we investigated vinculin knock-out and vinculin expressing wild-type mouse embryonic fibroblasts. Vinculin knock-out cells were 4-fold more motile on 2D-collagen-coated substrates compared to wild-type cells, but 3-fold less invasive in dense 3D-ECMs. Similarly, FAK knock-out cells were 3-fold less invasive in dense 3D-ECMs. Using magnetic tweezer microrheology measurements, vinculin and FAK knock-out cells were shown to be softer, remodel their cytoskeleton more dynamically and adhere less firmly to collagen, all of which is consistent with their enhanced 2D motility but does not explain the reduced 3D invasiveness. Traction microscopy revealed that vinculin- and FAK-expressing cells were both able to generate at least 3-fold higher traction forces. These findings suggest that vinculin and FAK facilitate 3D-ECM invasion through upregulation and enhanced transmission of traction forces, as needed to overcome the steric hindrance of dense matrix gels.

103-Plat
Probing the Response of Structural Proteins To Mechanical Stimulation in Neuroblasts
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Mechanotransduction is an essential component in neural processes as many sensory neurons respond to pain and touch as well as neurites experience mechanical stimulation during the process of growth. Although the mechanistic details of these responses have yet to be elucidated, since neural behavior is related to mechanical stimulation and affects the functioning and outgrowth of neurons, this field has the potential for directly affecting multiple areas including regeneration. These responses are related to the structural organization of the neurons and one protein in this area that is of interest is advillin. Advillin is a member of the gelsolin/villin family of actin binding proteins. To understand the mechanical affects related to cell structure in neural outgrowth, we used a custom fabricated device to investigate the effects of static mechanical stretching while examining molecular connections including advillin and actin. Neuro-2A cells were first seeded on a polydimethylsiloxane (PDMS) membrane and a uniform 1% strain was applied to the membrane for 1 hour. This allowed us to investigate neuroblast response to static strain. Our results suggest that actin and advillin are relevant in the mechanotransduction pathway of Neuro-2A neuroblasts through the sensing of the matrix stiffness as well as static mechanical stretching. We believe that this area will provide greater understanding of mechanotransduction in neuroblasts, as well as being important in areas such as biophysics, cell-matrix interactions, and mechanobiology.

Platform J: Interfacial Protein-Lipid Interactions

104-Plat
Thermodynamics of Membrane-Mediated β-Amyloid Formation: A Free Energy Description Based on X-Ray, CD, and GUV Experiments
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Jarrett and Lansbury's (1993) nucleation-dependent polymerization model describes the generic process of β-amyloid formation for a large number of diverse proteins and peptides. Here we discuss a membrane-mediated version of the JL model. From our recent experiments of X-ray diffraction, CD and GUV, we found correlations between the membrane bound conformation of penetratin and its effect on the bilayer thickness, in four different lipids with various degrees of chain unsaturation. We found that the interface of a lipid bilayer provided energetically favorable binding sites for penetratin in the α-helical form. Such bindings are characterized by a membrane thinning in proportion to the amount of bound molecules per lipid (P/L). Therefore increasing P/L elevates the energy level of the bound states E_α, until it becomes equal to that of a second binding phase at P/L=P/L*. In the case of antimicrobial peptides, all peptides above P/L* would bind to the second phase which forms pores. In contrast, penetratin forms β-aggregates in the second phase. Further binding of monomers to the aggregate is energetically favorable because the monomers contact the growing aggregate at multiple sites. This means that the binding energy for the monomers to the β-aggregate E_β decreases with the growth of the aggregate, because in average larger aggregates would present more available contact sites. Thus membrane binding facilitates nucleation-dependent β-aggregation. This free energy description could be the prototype for membrane-mediated β-amyloid formation.

105-Plat
Interactions of Lipidated Ras Proteins With Raft Membranes Studied By Time-Lapse Atomic Force Microscopy
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The existence of membrane subdomains with different lipid composition and the relationship between lipid-domain formation and the conformation and functional properties of membrane-associated proteins is one of the central