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.
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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

questions in the fields of membrane biochemistry and biophysics. It has been suggested that raft domains play a role in signal transduction processes by acting as ''signaling platforms''. The three Ras isoforms are posttranslationally modified *via* lipidation on their C-termini, which is essential for correct functioning and localization at the inner leaflet of the plasma membrane.
By using semisynthetic fully functional lipidated N- and K-Ras proteins, the partitioning of Ras in liquid-disordered (l_d) and liquid-ordered (l_o, i.e., raft-like) subdomains of different artificial and natural membranes was studied by time-lapse atomic force microscopy. The results provide direct evidence that partitioning of Ras occurs preferentially into l_d domains, independent of the lipid anchor system and GDP/GTP-loading [1,2]. Whereas N-Ras proteins bearing at least one farnesyl showed a time dependent diffusion and subsequent clustering in the l_o/l_d phase boundary region, formation of new domains with accumulated protein inside a fluid-like environment was observed for the farnesylated, inactive K-Ras protein. The inserted farnesylated N-Ras proteins are expelled to the interfacial region, probably due to the lack of a particular phase preference, while for the K-Ras protein the strong electrostatic interaction between its positively charged lysines and negatively charged lipids of the membrane seems to control the partitioning behavior. Minimizing the line energy is likely to be one of the key parameters controlling not only the size and dynamic properties of rafts but also of signaling platforms.
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106-Plat
***Aggregatibacter Actinomycetemcomitans* Leukotoxin Disrupts Membranes By Inducing the Formation of An Inverted Hexagonal Lipid Phase**
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The leukotoxin (LtxA) secreted by *Aggregatibacter actinomycetemcomitans* is a member of the repeats-in-toxin (RTX) family, and like the other members of the family, is a virulence protein which destroys host cells. LtxA exhibits specificity to human white blood cells, thereby allowing *A. actinomycetemcomitans* to flourish in the human upper aerodigestive track. Scanning electron micrographs of LtxA-treated human immune cell lines showed that LtxA induces the formation of large pores, which appear to be caused by membrane bending. To understand the molecular mechanism of this interaction, we analyzed the behavior of LtxA-membrane interactions in model membranes. Freeze-fracture transmission electron microscopy revealed the presence of unique structures, such as nanotubules, in multilamellar liposomes treated with LtxA. Formation of these structures indicated that the toxin acts by bending the membrane, possibly by inducing a bilayer-to-nonbilayer transition. This phase transition was quantified using differential scanning calorimetry, and it was found that LtxA is a potent inverted hexagonal (H_{II}) phase promoter. The relationship between H_{II} phase induction and membrane disruption was determined with a calcein leakage assay. LtxA-induced leakage from calcein-encapsulating liposomes composed of lipids with negative curvature, favoring H_{II} phase formation, was significantly enhanced compared to leakage from liposomes composed of lipids with neutral curvature, favoring bilayer formation. Addition of lipids with positive curvature, inhibiters of H_{II} phase formation, or cholesterol sulfate, a bilayer stabilizer, completely eliminated calcein leakage. It appears that LtxA causes membrane disruption by inducing the formation of the H_{II} phase, and its toxic effect is therefore highly dependent on lipid curvature. Supported by NIH DE09517.

107-Plat
Lateral Pressure Profile and Curvature Frustration in Mechanosensitive Channel Gating
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Experimental evidence of a link between the function and the lipid environment of a membrane protein is increasingly available. While some of the experimental data can be explained by specific lipid-protein interactions, others can only be understood through protein induced perturbations in the membrane shape [1]. In the latter case, the free-energy of a protein state is connected to membrane thickness (hydrophobic mismatch), membrane elasticity and curvature energy [1]. Connection to membrane elasticity and curvature energy can also be formulated using the so-called lateral pressure profile. A conformational change of a membrane protein has to do work against a non-uniform pressure distribution, i.e lateral pressure profile, inside a membrane [2]. Previously it has been shown that this work might be significantly larger than thermal energy by assuming simple conformation changes [3].
Here we analyze the work done against the lateral pressure profile in mechanosensitive channel gating using a recently developed method to calculate a full 3D pressure field from molecular dynamics simulations [4]. For this purpose we have simulated closed and open states of a Mechanosensitive channel of large conductance (MscL) embedded in a DOPC bilayer using the MARTINI force field [5]. To analyze the effect of curvature frustration we have also performed simulations with symmetric and asymmetric lipid distributions. The advantage compared to previous analyzes [3] is that instead of a simple cone model, we have a more realistic model for different states of a mechanosensitive channel.
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108-Plat
Steric Confinement of Proteins in Lipid Domains Can Drive Membrane Curvature and Tubulation
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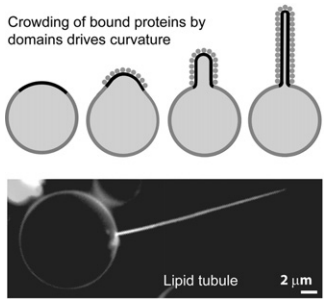
Deformation of lipid membranes into curved structures such as buds and tubules is essential to many cellular processes. Lipid micro-domains are thought to co-localize with many curved membrane structures, inspiring ongoing exploration of a variety of roles for domains in membrane bending.
We examined the role of lipid domains in spatial confinement of protein binding and discovered a new mechanism for curvature amplification that relies on global coupling. We formed giant unilamellar vesicles that contained insoluble lipid domains that strongly bound *his*-tagged proteins. We show that protein crowding on domain surfaces creates a protein layer that buckles outward, spontaneously bending the domain into stable, well-defined tubules as more proteins bind. In contrast to previously described bending mechanisms relying on local steric interactions between proteins and lipids (i.e. helix insertion into membranes), this mechanism produces tubules whose dimensions are defined by global parameters: binding energy and domain size. Our results suggest the intriguing possibility that domains can amplify membrane bending and define protrusion length scales by concentraing the steric interactions between the lipid bilayer and proteins. This mechanism may help explain the high curvatures induced by membrane bending proteins.



109-Plat
Ca^{2+}-Atpase: Lipid-Protein Interaction As Observed in Crystals and MD Simulations
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In the quest for grasping the function of complex membrane proteins, it has been realized that the surrounding bilayer may play a regulatory role. Whereas crystal structures in recent years have succeeded in providing detailed images of membrane protein structures, no structural information have appeared clearly showing how the protein is positioned in the membrane. Here we present X-ray data for the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) in the E2 and E2P states. Structural details have been extracted for the protein and the surrounding bilayer from the same crystal for each of the two configurations. Molecular dynamics (MD) simulations of SERCA incorporated in a bilayer of POPC lipids and detergent (corresponding to crystal conditions), support that the low resolution densities primarily stem from the phosphate (P) groups in the bilayer leaflets.
MD simulations of SERCA in five different single-lipid bilayers show how SERCA, regardless of its extraordinary narrow hydrophobic region, position itself in bilayers of different hydrophobic thickness. Even though membrane proteins are much less compressible than the bilayer, we see both the bilayer and the trans-membrane helix bundle adjust in concert to match their hydrophobic thickness.