DiI-C18 was characterized in phase-coexistent GUVs composed of DOPC:DPPC:Chol (2:2:1). Short chain DiI-C12 and long chain DiI-C18 partitioned to liquid-disordered and liquid-ordered membrane phases, respectively. FL of DiI-C18 stained cells (1.47 ± 0.49 ns) was higher compared to DiI-C12 stained cells (1.26 ± 0.12 ns), indicating that DiI-C18 and DiI-C12 partitioned into liquid-ordered and liquid-disordered phases respectively. In conclusion, FL of DiI is a sensitive indicator of membrane fluidity and different chain length DiI's partition to different membrane phases both in model and cell plasma membranes.

2199-Plat
Imaging of Mobile Stable Lipid Rafts in the Live Cell Plasma Membrane and their Involvement in Cellular Signaling During Heat Shock
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The plasma membrane has been hypothesized to contain nanoscopic lipid platforms, also termed lipid or membrane rafts. Based on biochemical and cell biological studies, rafts are believed to play a crucial role in many signaling processes. However, there is currently not much information on their size, shape, stability, surface density, composition and heterogeneity. We present here a method which allows for the first time the demonstration that single rafts diffuse as stable platforms in the live cell plasma membrane. Our method senses rafts by their property to assemble a characteristic set of fluorescent marker-proteins or lipids on a time-scale of seconds. The special photobleaching protocol TOCCSL (Thinning Out Clusters while Conserving Stoichiometry of Labeling1) was used to reduce the surface density of labeled mobile rafts down to the level of well-isolated diffraction-limited spots, without altering the single spot brightness. The statistical distribution of probe molecules per raft was determined by single molecule brightness analysis. For demonstration, we used the consensus markers Bodipy-GM1, a fluorescent lipid analogue, and glycosylphosphatidylinositol-anchored monomeric GFP. For both markers we found cholesterol-dependent association in the plasma membrane of living CHO and Jurkat T cells in the resting state, indicating the presence of small, mobile, stable rafts hosting these probes. We further applied the technology to address structural changes in the plasma membrane during fever-type heat shock: at elevated temperatures mGFP-GPI homo-association disappeared, accompanied by an increase in the expression of the small heat shock protein Hsp27.
1. Moertelmaier, M., Brameshuber, M., Linimeier, M., Schütz, G. J. & Stockinger, H. Thinning out clusters while conserving stoichiometry of labeling. Appl Phys Lett 87, 263903 (2005).

2200-Plat
Cholesterol and Phosphatidylinositol 4,5-Bisphosphate Synergistically Affect Endothelial Biomechanics
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Membrane cholesterol induces the formation of cell membrane microdomains that are enriched with acidic lipids such as phosphatidylinositol 4,5-bisphosphate (PIP2). PIP2 regulates a number of cellular processes by serving as cross linker between the membrane and cytoskeleton, and by association with actin-bonding proteins as cofilin, gelsolin, profilin, α-actinin, MARCKS, filamin, etc. The objective of this study is to clarify how the co-operative action of cholesterol and PIP2 impacts the biomechanics of endothelial cells. We measured the stiffness of bovine aortic endothelial cells (BAEC) by membrane indentation and tether extraction using AFM at different cholesterol level and PIP2 conditions. BAECs were transfected with Pleckstrin homology domain of phospholipase C (PH-PLC), which can sequester PIP2. Transfection with PH-PLC did not alter the force needed to elongate a tether-nanotube (i.e. tether force), but it significantly increased cell stiffness. These results imply that though PIP2 in BAEC does not act as a direct cross-linker between the plasma membrane and cytoskeleton, it is an efficient regulator of the cytoskeletal architecture. To further elucidate the role of PIP2 in endothelial cells, we enriched BAECs with exogenous PIP2 at different cholesterol levels. Enrichment with exogenous PIP2 led to the increase in membrane stiffness in cholesterol depleted BAEC. However no changes in stiffness were observed in control BAEC at normal cholesterol level. Confocal imaging showed that under normal cholesterol level most of the exogenous PIP2 delivered into BAEC was localized in discrete membrane domains and perinuclear patches. However, in cholesterol depleted BAEC the exogenous PIP2 was mostly homogeneously dispersed across the cell. On the basis of these findings we conclude that cholesterol affects BAEC's biomechanical properties by regulating the cellular localization and metabolism of PIP2, and thus by remodeling the cytoskeleton and its interaction with the plasma membrane.

2201-Plat
KcsA Redistribution Upon Lipid Domain Formation in Supported Lipid Bilayers and its Functional Implications
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In a recent study we showed that the melting behavior of Supported Lipid Bilayers (SLBs) can be influenced by ionic strength and the preparation temperature [1]. By changing these parameters we could control the coupling between the two bilayer leaflets obtaining a coupled or decoupled melting behavior. Hence, we could provide evidence that the SLB model system is also suited for the study of lipid/protein interactions which had been questioned in the past. Further, we investigated the mutual interactions between the bilayer forming lipids and the pH-gated K^+ channel KcsA. In particular, we studied the melting behavior of the SLB and the ion channel distribution by temperature controlled atomic force microscopy (AFM) in liquid. We induced the formation of solid ordered domains in SLBs made of POPE:POPG 3:1 and KcsA. We found that the KcsA proteins were excluded from the solid ordered regions. Further, the ion channels tended to accumulate at the domain boundaries or they clustered in the liquid disordered phase [2]. This behavior is in agreement with what is expected from the hydrophobic matching principle. In addition using voltage clamping with temperature control we obtained that the lateral reordering of both the proteins and lipids results in changes of KcsA functionality. Functional modifications include both the protein's opening life times and ion channel conductance, displaying a biphasic behavior.
[1] Seeger et al., Biophys.J. (2009) 97:1067
[2] Seeger et al., submitted.

Platform AL: Cell Motility & Mechanics

2202-Plat
Mesoscopic Model of Actin-Based Propulsions
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In order to study movements of actin-propelled beads, we use stochastic simulations of 'in silico' actin network, in which microscopic individual actin filaments undergoing nucleation, elongation, attachment, detachment and capping are embedded in nodes-and-springs viscoelastic network representing macroscopic actin gel. Our study shows that the combined effects of macroscopic elastic deformation and microscopic ratchet are crucial for explanation of the observed force-velocity relations and orientations of the actin-propelled ellipsoidal beads.

2203-Plat
The Effects of Filament Aging and Annealing on a Lamellipodium Undergoing Disassembly by Severing
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We construct a simplified model of a lamellipodium and use a numerical simulation to study its properties as it disassembles by filament severing. The growing lamellipodium is modeled as a 2D or 3D periodic lattice of crosslinked actin filaments. At each time step a new layer of actin filaments is added at the membrane, and existing filaments are severed stochastically. After each time step each filament is tested to determine if it remains in the connected network, defined as those filaments that are connected to the membrane by an unbroken path of filaments. Disconnected sections of the network are assumed to diffuse away rapidly and are removed after each time step. Filament aging, due to hydrolysis and other mechanisms such as association with actin binding proteins, is modeled by including several different filament chemical states, with stochastic transitions between the states. Filament annealing is included by allowing vacant sections of the network to grow new filaments off of existing filaments.
The properties of the model are studied as functions of the severing and annealing rates, as well as the number of states. We find that the network width of the multistate model is proportional to the sum of the average lifetimes of the states, and is well modeled by a simple kinetic theory. The edge of the growing network becomes sharper as either the number of states is increased or the dimensionality is increased. Annealing increases the average length of the network, and we find that the network length diverges at a critical annealing rate. These conclusions are robust to the presence of disorder and to changes in the topology of the network.