micro- and nano-meter scales, which organization critically depended on i) the lateral pressure, ii) the presence of either SP-B or SP-C and iii) the presence of cholesterol. In films mimicking surfactant lipid composition, SP-B promoted formation of a continuous network of interconnected ordered-like phase, highly intermingled with more disordered nano-regions. In contrast, SP-C kept less polymorphic condensed micro- and nano-domains, with a high degree of heterogeneity. The films made of the lipid mixture containing high proportion of DPPC and palmitate showed a dominant solid-like/ordered phase irrespective of their protein content. The implication of the micro- and nano-structure of the different lipid and lipid-protein systems on the mechanical stability of the interfacial films will be discussed.

1847-Pos Board B757
Imaging of Mobile Stable Nanoplatforms in the Live Cell Plasma Membrane
Mario O. Brameshuber, Julian Weghuber, Verena Ruprecht, Imre Gombos, Ibolya Horváth, László Vigh, Paul Eckerstorfer, Endre Kiss, Hannes Stockinger, Gerhard J. Schuetz.
The plasma membrane has been hypothesized to contain nanoscopic lipid platforms, which are discussed in the context of ''lipid rafts'' 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 direct imaging of nanoscopic stable platforms with raft-like properties diffusing in the live cell plasma membrane. Our method senses these platforms by their property to assemble a characteristic set of fluorescent marker-proteins or lipids on a time-scale of seconds. A special photobleaching protocol was used to reduce the surface density of labeled mobile platforms down to the level of well-isolated diffraction-limited spots, without altering the single spot brightness. The statistical distribution of probe molecules per platform was determined by single molecule brightness analysis. For demonstration, we used the consensus raft marker glycosylphosphatidylinositol-anchored monomeric GFP and the fluorescent lipid analogue Bodipy-GM1 which preferentially partitions into liquid ordered phases. For both markers we found cholesterol-dependent homo-association in the plasma membrane of living CHO and Jurkat T cells in the resting state, thereby demonstrating the existence of small, mobile, stable platforms containing these probes. We further applied the technology to address structural changes in the plasma membrane during fever-type heat shock: at elevated temperatures the mGFP-GPI homo-association disappeared, accompanied by an increase in the expression of the small heat shock protein Hsp27.

1848-Pos Board B758
Plasma Membrane Organization
Rolf Harkes, Anna Pezzarossa, Franziska Zosel, Thomas Schmidt.
Plasma membrane organization
The composition of the plasma membrane has long been modeled as a mosaic fluid (Singer and Nicolson, 1972). Studies in the last few years have identified microdomains, lipid rafts and caveolae, that constrain membrane proteins within a small region of the cellular plasmamembrane. These domains facilitate anchoring of different signaling proteins for example H-Ras that has been shown to co-localize with lipid rafts upon activation (Lommerse et al. 2005, Rotblat et al. 2004). The signaling cascade on the plasma membrane of cells is dependent on the location of different membrane proteins. Therefore it is of interest to further investigate these domains.
Photo Activated Localization Microscopy (PALM) achieves sub-diffraction limited resolution by imaging single fluorophores and determining their position at high precision. By using photoactivatable fluorescent proteins the amount of fluorescing molecules can be altered by variation of the intensity of the activation beam. This way single molecule fluorescence microscopy is achieved. Dendra2 is a fluorescent protein thatwas photoconverted from a green to a red fluorescent state using a 405nm light-puls. For this research 3T3-cells have been transfected to express H-Ras-Dendra2. Using Photo Activated Localization Microscopy (PALM) the position of H-Ras was determined with a precision of 30 nm. By analyzing the trajectories of single molecules of H-Ras we showed that a significant percentage of H-Ras molecules was confined within small (~200 nm) domains.
Choleratoxin B (CtxB) is a ligand to the ganglioside GM1 which has been used as a marker for lipid rafts at the outside of the lipid membrane. Since it binds to five GM1 molecules it was suggested that CtxB enlarges the size of lipid rafts. Here we have treated cells with CtxB that indeed enlarged both the outside and inside lipid raft and therefore the confinement of H-Ras.

1849-Pos Board B759
Formation and Organization of Cellular Protrusions: Linking Between Actin Filaments and the Cell Membrane
Eyal Ben Isaac, Nir Gov, Arik Yochelis, Bechara Kachar.
Cells come in variety of shapes which often indicate their distinct functionality. Thus, an intimate relation between cytoskeleton and the elastic membrane must be present. Protrusions are intriguing fundamental structures in cells, serving different functions according to the cell type. We attempt to advance the understanding of protrusions, such as filopodia, by introducing a distinct reaction-diffusion-elastic model: the reaction-diffusion part is based on bistability between two uniform actin orientations (ordered and disordered) while membrane physics is derived from a Helfrich Hamiltonian. The model describes the formation, stability and interaction of protrusions. Particularly, we find that spatially localized structures can arise only via coupling between the cytoskeleton and the membrane. Experimental observations that support the model framework are also provided.

1850-Pos Board B760
Calcium Dependent Aggregation of Phosphoinositides
Adolphe Kazadi Badiambile, **Adolphe Kazadi Badiambile**, Martin Bernard Forstner.
Phosphoinositides play a crucial role in many cellular functions such as calcium signaling, endocytosis, exocytosis and the targeting of proteins to specific membrane sites. To maintain functional specificity, it has been suggested that phosphoinositides are spatially organized in ''pools'' in the cellular plasma membrane. A possible mechanism that could induce and regulate such organization of phosphoinositides is their interaction with Ca^{2+} ions. Using Langmuir monolayers, we investigated the effect of bivalent calcium cations on the surface pressure-area/lipid isotherm of monolayers of phosphatidylinositol (PI), phosphatidylinositol bisphosphate (PIP2), dioleoylphosphatidylglycerol (DOPG) and dipalmitoylphosphatidylcholine (DPPC). It is found that the decrease of area per lipid, i.e. the increase in aggregation, is temperature dependent and more pronounced at 37C than at 25C. Interestingly, despite their similar head-group net charge at physiological conditions, PI and DOPG exhibit significantly different aggregation response to Ca^{2+}. This suggests non-electrostatic contributions to the aggregation of phosphoinositides, which are discussed.

1851-Pos Board B761
Criticality in Plasma Membranes
Benjamin B. Machta, James P. Sethna, Stefanos Papanikolaou, Sarah L. Veatch.
Here we present a minimal model of plasma membrane heterogeneity that combines criticality with connectivity to cortical cytoskeleton. Our model is motivated by recent observations of micron-sized critical fluctuations in the 2d Ising Universality class in plasma membrane vesicles that are isolated from cortical cytoskeleton[1]. We incorporate criticality using a conserved order parameter Ising model coupled to a simple actin cytoskeleton interacting through point-like pinning sites. In our model small (r~20nm) and dynamic fluctuations at physiological temperatures arise from criticality. Including connectivity to cortical actin disrupts large fluctuations and macroscopic phase separation at low temperatures ($T \leq 23°C$) and provides a template for long lived fluctuations at physiological temperature ($T=37°C$). In addition, we use analytical techniques from conformal field theory and numerical simulations to investigate the form of effective forces mediated by the membrane's proximity to criticality. We show that the range of this force is maximized near a critical point and we quantify its usefulness in mediating communication using techniques from information theory. Finally we use theoretical techniques from statistical physics in conjunction with Monte-Carlo simulations to understand how criticality can be used to increase the efficiency of membrane bound receptor mediated signaling. We expect that this sort of analysis will be broadly useful in understanding and quantifying the role of lipid 'rafts' in a wide variety of membrane bound processes. Generally, we demonstrate that critical fluctuations provide a physical mechanism to organize and spatially segregate membrane components by providing channels for interaction over relatively large distances.
1. Veatch, S.L., et al., Critical fluctuations in plasma membrane vesicles. ACS Chem Biol, 2008. 3(5): p. 287-93.