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.
To further validate our method we extended TOCCSL by utilizing two-color co-localization and photo-activation. While two-color TOCCSL allows for direct imaging of mobile nanoplatforms containing different probe molecules and thus supporting the lipid raft concept, photo-activation based TOCCSL addresses an additional population of observed probes. Since TOCCSL is suitable for characterizing the mobile fraction of marker-proteins/lipids, the information about slowly diffusing or immobile nanoplatforms is not accessible. This can be circumvented by substituting the fluorescent marker by a photo-activatable protein linked to the molecule of interest. By irreversibly switching a small fraction of markers from a dark into the fluorescent state single molecule brightness and diffusion analysis after activation will add to the characterization of nanodomains.
Key Publication: Brameshuber et al., JBC 2010, 285(53): 411765-71

1262-Pos Board B154
The Immunomodulator Enterotoxin Influences BCR Signaling by Stabilizing Lipid Domains
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Adjuvants may potentiate immune response at the cell level by either enhancing receptor activation at the membrane or by increasing uptake of antigen. To investigate the mechanism behind the adjuvancy of LTIIb, a toxin that binds GM3, we studied its effects on the membrane structure, the mobility of BCR and B cell membrane signaling. bimFCS, a novel technique for probing interaction between membrane molecules and membrane domains, confirms that LTIIb pre-clusters cholesterol-stabilized domains. LTIIb, or its binding subunit alone, is found to reduce the mobility of BCRs, as measured by FRAP measurements and induce membrane signaling, as confirmed by calcium imaging. The calcium signaling pathway, however, seems to compete with that of BCR activation through IgM crosslinking. The B subunit of Choleratoxin (CTB), commonly used for labeling GM1, though also modulates cholesterol-stabilized domains, does not affect BCR mobility or trigger calcium signaling. These results show that GM1 and GM3, though both enriched in cholesterol-stabilized domains, behave differently, at least in CH27 B cells, upon crosslinking.

1263-Pos Board B155
Quantifying the Effect of BCR Clustering on Plasma Membrane Organization
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The B cell antigen receptor (BCR) is an integral part of the adaptive immune system that communicates binding of antigen in the extracellular environment through the plasma membrane. Antigen binding to the BCR results in phosphorylation of intracellular tyrosine activating motifs (ITAMs), which subsequently bind to and activate numerous proteins involved in BCR regulation. Interestingly, many of the proteins regulating the early stages of the BCR signaling pathway are linked to the inner leaflet by saturated lipid anchors which tend to be associated with liquid-ordered membrane phase in model membranes. Also, the BCR becomes transiently detergent resistant following antigen-induced BCR clustering, suggesting that BCR clusters become coupled to membrane order following stimulation. In this work, we aim to characterize how BCR clustering could reorganize plasma membrane lipids by quantifying co-localization of BCR with fluorescent markers of liquid-ordered and liquid-disordered phases. We utilize two-color super-resolution fluorescence localization microscopy (STORM and PALM) in live and chemically fixed CH27 B cells to simultaneously image BCR and membrane anchored proteins, and we quantify their co-clustering using correlation functions. Our results from chemically fixed cells show that proteins anchored to the plasma membrane inner leaflet through saturated acyl-chain lipid modifications exhibit increased co-localization with BCR upon antigen stimulation, whereas those without lipid modifications or those anchored through branched acyl-chain modifications are not significantly co-localized with BCR before or after stimulation. These results are contributing to our long term goal of elucidating the role of lipid mediated interactions in the regulation of BCR signaling.

1264-Pos Board B156
Eisosomes and Plasma Membrane Organization
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A vast body of evidence coming from different microscopy techniques has been instrumental in concluding the 10-year-long debate on whether biological membranes presented lateral segregation of proteins and lipids. Currently, the existence of membrane domains in both eukaryotes and prokaryotes has been common ground. However, the mechanisms that sustain membrane domain formation and maintenance remain largely unknown. Our work is focused on the study of Eisosomes, recently discovered plasma membrane domains in S. cerevisiae. In a first piece of work, we identified new eisosomal components and also showed that eisosomes are involved in sphingolipid metabolism (1). Thereafter, we showed that Pil1 and Lsp1, the major proteinaceous components of eisosomes, are able to form self-assemblies that bind and curve membranes both in vivo and in vitro. We also showed that Lsp1 and Pil1 membrane-sculpting abilities are associated with the generation and organization of membrane domains (2). Thus, our currently published work support the hypothesis that a mechanism for membrane eisosome domain formation is membrane curvature generation directed by Pil1-Lsp1 assemblies.
1. Aguilar PS., et al. (2010) Nature Structural and Molecular Biology 17, 901-908.
2. Olivera-Couto A., et al. (2011) Molecular Biology of the Cell 22, 2360-2372.

1265-Pos Board B157
Nano-Domains of Cell Membrane Stiffness, Proteins Diffusion and Concentration Characterized by Thermal Noise Imaging
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The cell membrane is thought to contain transient spatial domains: cholesterol-stabilized lipid nano-domains and corrals formed by interactions with the membrane cytoskeleton. Due to their small size and transient nature, these cannot be visualized directly and are challenging to characterize in intact cells. One possibility is to measure the diffusion of membrane proteins interacting with these domains. However, the diffusion should be measured with nanometer spatial and microsecond temporal resolution to correctly plot the protein's path in the membrane. We show here that not only is the spatio-temporal resolution of thermal noise imaging (TNI) in an optical trap sufficient to plot the membrane protein's path, but the trapping also allows gathering sufficient data within one small membrane area to "image" the membrane. We create for high resolutions maps of the local diffusion, local attraction potentials and membrane stiffness by using TNI to confine a single membrane protein to diffuse for seconds in an area of 300nm x 300nm. Using a GPI-anchored green fluorescent protein (GFP), which is often used a marker for cholesterol-stabilized nano-domains, to probe the membrane of PtK2 cells we detect domains that are at the same time stiffer, concentrate the protein and show slower diffusion. These align along linear feature and show convex polygons shape. These domains are further stabilized by addition of Ganglioside cross-linking toxins and disappear after removal of the cholesterol. Another marker, GFP-labeled transferrin receptor molecule, detects linear features and linearly demarcated areas of increased protein concentration.

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Temperature-Dependent Phase Behavior of the Synaptosomal Membranes from Mammalian and Marine Invertebrate Synaptosomes
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Given reports of raft domains in rodent brain synaptosomes, we probed for their conservation in a poikilotherm, the Woods Hole squid Loligo. Because rafts are described as liquid-ordered phase-separated domains, we expected phase transition temperatures above body temperature if raft function is important to neuronal activity. We tested this hypothesis by comparing synaptosomes, intact nerve endings, from animals that live at two very different temperatures: mouse (body temperature-37°C) and squid (body temperature-20°C). We measured the temperature-dependence of the lipid phase of intact synaptosomes in the absence of exogenous probes by using line-width and spinning sideband intensities of lipid hydrocarbon chain resonances, using proton magic angle spinning NMR spectra as a function of temperature between 0 and 40°C. We also