1522-Pos Board B292
Microfluidic-Enabled Real-Time Imaging of Lipid Domains in Bilayer Membranes
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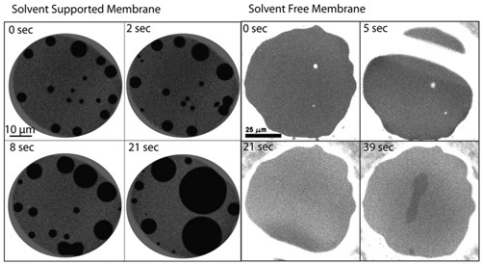
A microfluidic bilayer lipid membrane system supporting correlated opto-electrical membrane characterization has been developed and applied to the real time imaging of lipid domains by confocal microscopy. Annulus supported bilayers are formed by a novel ''kiss and retreat'' method and the device supports rapid buffer exchange in both chambers. Additionally, the system has been extended for the creation of solvent-free asymmetric bilayers by a microfluidic adaptation of the monolayer folding method. Because the dynamic folding process itself may be imaged, the very early stages of phase separation and domain growth may be observed. By enabling the simultaneous measurement of transmembrane current and optical observation of lipid microdomains in real-time, the microfluidic platforms open the door to new multi-domain measurements of dynamic lipid-lipid and lipid-ligand interactions that are not feasible using established tools.



(left) Confocal image sequences of liquid ordered domains in a bilayer formed by kiss and retreat method. Under ~5 Pa transmembrane pressure, l_o domains are observed to emerge, grow and merge. (right) Confocal image sequence of bilayer formation by monolayer folding, sequentially revealing a single monolayer, the folding process, and the initial emergence of liquid ordered domains.

1523-Pos Board B293
High-Speed Quantum Dot Tracking in Plasma Membranes Reveals Short-Lived Small-Size Corralled Diffusion
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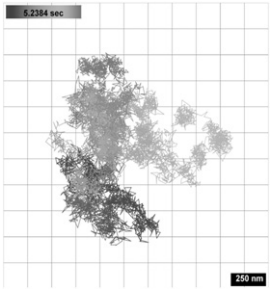
In this work, we show that tracking of single quantum dots (QDs) is possible at image acquisition rates up to ~2,000 Hz. We image with a spatial precision of ~30 nm for several seconds using a standard wide-field fluorescence microscope and an EMCCD camera.
Using this system, we show that membrane proteins and lipids, exogenously labeled with functionalized QDs, undergo corralled diffusion in the plasma membrane of live cells where the molecules move between corrals of ~100 nm in diameter within a lifetime of ~50 ms (figure). We further investigate the cholesterol and actin dependence of corralled diffusion.
Observation of these dynamic features is dependent on three parameters; the diffusion rate of the investigated molecule, the corral size, and the image acquisition rate. If not imaged at sufficient speed, corralled diffusion appears as slow free diffusion. Therefore, we compare our experimental result with computer simulations to find the relation between these three parameters.
The QDs used in this imaging system has a signal brightness and stability advantage compared to conventional fluorophores allowing for long-term fast imaging, and a size advantage to gold particles used for high-speed single particle tracking.



1524-Pos Board B294
Distribution, Orientation and Dynamics of 17β-Estradiol in Lipid Membranes Investigated by Solid-State NMR
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17β-Estradiol (E_2) is a potent estrogen, which modulates many important cellular functions by binding to specific estrogen receptors located in the cell nucleus and also on the plasma membrane. We have studied the interaction of E_2 with POPC membranes using a combination of solid-state NMR methods. Investigations were conducted on powder-type samples at lipid/E_2 ratios of 5:1 and 10:1 and at a temperature of 303K. Information about the chain dynamics and membrane packing properties was obtained using ^{2}H solid-state NMR. The spectra indicate that E_2 does not cause a condensation effect of the surrounding phospholipids, which is contrary to the effects of cholesterol, and only very modest E_2 induced alterations of the membrane structure were detected. ^{1}H magic-angle spinning NMR showed well resolved signals from E_2 as well as of POPC in the membrane-lipid layer. Two-dimensional NOESY spectra revealed intense cross-peaks between E_2 and the membrane lipids indicating that E_2 is stably inserted into the membrane. The determination of intermolecular cross-relaxation rates revealed that E_2 is broadly distributed in the membrane with a maximum of the E_2 distribution function in the upper chain region of the membrane. To further establish the orientation and dynamics of E_2 we obtained a variant in which four hydrogens were replaced by deuterium and recorded corresponding ^{2}H NMR spectra. The analysis of these spectra was performed by a model describing the orientation and dynamics of E_2 in the membrane. We determined its average orientation in the membrane and conclude that E_2 is highly dynamic in lipid membranes and may undergo rotations as it exhibits two polar hydroxyl groups on either side of the molecule.

1525-Pos Board B295
Sterol Properties Required for Microdomain Formation: From Model Systems to Living Yeast and Mammalian Cells
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This work presents new insights into the evolution of sterol-enriched domains in the plasma membrane (PM) of eukaryotes. The recent finding of sphingolipid-enriched gel domains in the PM of *Saccharomyces cerevisiae*(1) demands novel strategies to study sterol-enriched liquid ordered domains in this organism. Therefore, we employed di-4-ANEPPS fluorescence, here shown to be sensitive to *S. cerevisiae* sterols and Fluorescence Lifetime Imaging Microscopy (FLIM) for the first time in *S. cerevisiae* PM. *In vivo* studies were performed with *S. cerevisiae* (wild type and *erg6*Δ), and animal (CHO) cells, modeled by liposomes containing ergosterol, zymosterol, or cholesterol, respectively. Different fluorescent probes were used, showing that these sterols had comparable effects regarding lipid bilayer order and complete solubilization of gel domains. However, confocal fluorescence microscopy of Giant Unilamellar Vesicles showed that zymosterol, a biosynthetic precursor of ergosterol and cholesterol, has no ability to form lipid rafts.
In living cells, sterol-induced emission blue-shift and longer fluorescence lifetime of di-4-ANEPPS increased as: wild type<*erg6*Δ<CHO. Concomitantly, in model systems, cholesterol and zymosterol had much larger effects than ergosterol in di-4-ANEPPS fluorescence. However, zymosterol was unique because it yielded overlapping results for both saturated and unsaturated lipids. The two other sterols produced much stronger alterations with a saturated lipid, even though the absolute effect in di-4-ANEPPS fluorescence was often smaller than that of zymosterol. Hence, the differential regarding the interaction of sterols with saturated versus unsaturated lipids emerges as a requisite for lipid raft formation and as an important factor in sterol evolution.
(1) F. Aresta-Branco, A.M. Cordeiro, H.S. Marinho, L. Cyrne, F. Antunes, R.F.M. de Almeida (2011) ''Gel domains in the plasma membrane of *Saccharomyces cerevisiae*: highly ordered, ergosterol-free, sphingolipid-enriched lipid rafts.'' *J. Biol. Chem.* 286:5043-5054.
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1526-Pos Board B296
Microdomains Displace Laterally and Rotate in the Plasma Membrane of Cochlear Outer Hair Cells
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Guinea pig outer hair cells (OHC) are cylindrical, with a near constant diameter of ~8 μm and length ranging from 20 to 90 μm. OHC's lateral plasma membrane is literally packed with motor (prestin) and associated proteins organized in microdomains. These membrane microdomains are connected by hundreds of 25-nm long ''pillars'' to cytoskeletal microdomains of up to 10 μm2 composed by long, parallel actin filaments cross-linked by shorter spectrin tetramers. Membrane potential-dependent conformational changes in prestin molecules result in reversible changes of cell length (shortening and