A microfluidic bilayer lipid membrane system supporting correlated opto-electromagnetic characterization has been developed and applied to the real-time imaging of lipid domains by confocal microscopy. Annexin 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 multidomain 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, 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.

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 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 erg6Δ), and animal (CHO) cells, modelled 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 thatzymosterol, 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-ANEPPES increased as: wild type < erg6Δ < CHO. Concomitantly, in model systems, cholesterol and zymosterol had much larger effects than ergosterol in di-4-ANEPPES 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-ANEPPES 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.
