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Direct visualization of lipid domains in free bilayers. A two photon fluorescence microscopy study

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Abstract

Combining the sectioning capability of the two-photon microscope and the partition and spectral properties of LAURDAN, PRODAN and N-Rhodamine-DPPE on the solid and fluid lipid phases, temperature-induced changes on single giant unilamellar vesicles (GUVs) were studied. We obtained images of GUVs composed of phospholipid binary mixtures labeled with the different fluorescent probes using polarized laser excitation. To characterize the lipid phase-state we used the LAURDAN Generalized Polarization function (GP). At temperatures corresponding to lipid domain coexistence we observed concurrent fluid and solid domains in GUVs composed of DPPE/DPPC, DLPC/DPPC, DLPC/DSPC, DLPC/DAPC and DMPC/DSPC. In all cases the domains grew and moved on the vesicle surface as we decreased the temperature. The size of the solid domains was up to several microns (-30 ism), displaying different shapes dependent on the lipid mixture. In the case of DPPE/DPPC (7:3 mol/mol) we observed mainly dendritic shape. Instead, in the PC mixtures (1:1 mol/mol) the solid domains showed line, circular and dendritic shape depending on the hydrophobic chain length difference of the lipid components. As judged by the LAURDAN GP values, DPPE/DPPC showed pure gel and pure fluid domain coexistence while the PC mixtures showed an increasing influence of the fluid phase on the solid phase as the hydrophobic chain length difference between the components decrease. In all cases the solid domains span the lipid bilayer showing monolayer coupling. Supported by grants from the National Institutes of Health (RR03155).