Effects of Fluorescent Probes on Lipid Membrane Physical Properties

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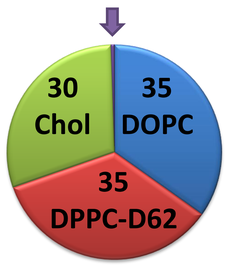
Motivation: Trace Impurities in Lipid Membranes

Many biological molecules are present in cells in trace amounts, and yet they play important functional roles. Examples include caveolin fragments, PIP2 and GM1 [2, 3, 5]. Other studies have shown that minor impurities can alter lipid membrane phase diagrams [4, 6, 8, 9].

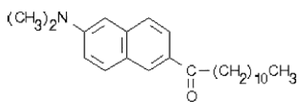
The Big Question

Do minor components present in *trace* amounts alter liquid disordered-liquid ordered (ld-lo) membrane phase behavior?

Membrane Composition

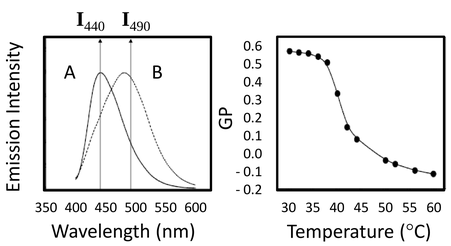


Laurdan Emission Spectrum and General Polarization



When Laurdan intercalates into a more disordered part of the membrane, its emission spectrum is red shifted, decreasing the general polarization (GP) [1].

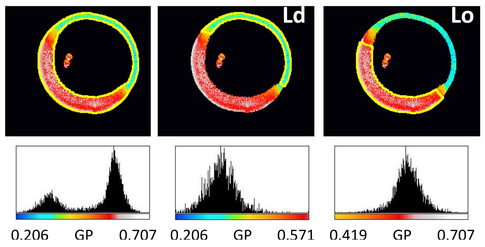
$$\mathrm{GP} = \frac{\mathrm{I}_{440} - \mathrm{I}_{490}}{\mathrm{I}_{440} + \mathrm{I}_{490}}$$



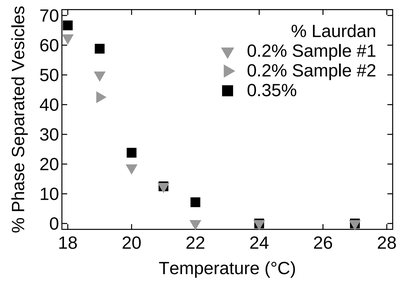
A: Gel DPPC at 35°C
B: Liquid crystalline DPPC at 50°C
Figure from [7].

Two Photon Excitation Fluorescence Microscopy of Giant Unilamellar Vesicles

Ld-lo phase separation at the equatorial plane observed using the fluorescent probe Laurdan.

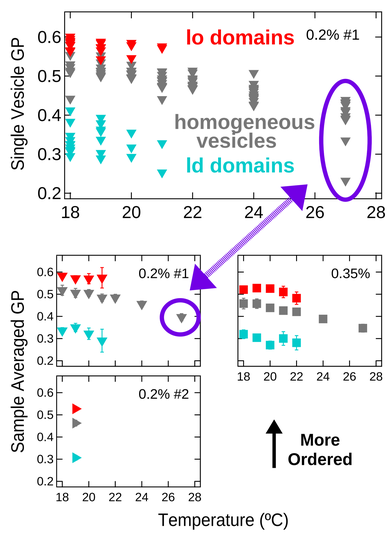


Microscopy: Counting Phase Separated Vesicles

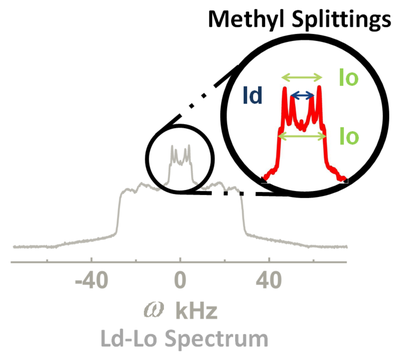


Microscopy: General Polarization

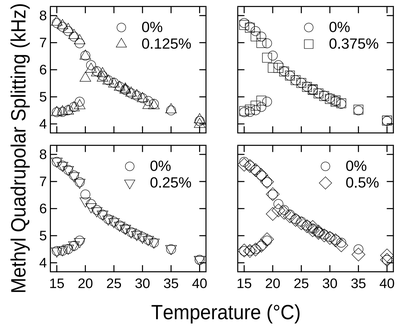
For phase separated vesicles, the GP of ld and lo regions are measured separately. For homogenous vesicles, the GP for the entire vesicle is measured. The GP for multiple vesicles in a given sample are then averaged.



^{2}H NMR Spectroscopy of Multilamellar Vesicles

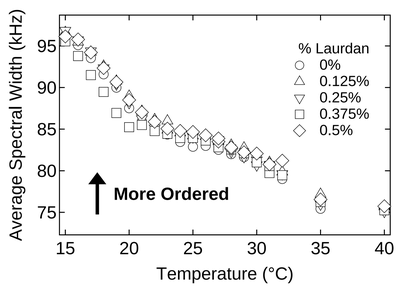


^{2}H NMR: Flexibility of Membrane Interior



^{2}H NMR: Overall Membrane Fluidity

$$\mathrm{M}_1 = \frac{\sum_{\omega=-75\mathrm{kHz}}^{75\mathrm{kHz}} |\omega|\mathrm{I}(\omega)}{\sum_{\omega=-75\mathrm{kHz}}^{75\mathrm{kHz}} 2\mathrm{I}(\omega)}.$$



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Abstract

It is currently accepted that nano-scale lateral compositional heterogeneity plays functional roles in cell membranes. Ordered and disordered lipid phases can coexist at appropriately chosen compositions, temperatures and pressures. Fluorescence is a popular family of techniques used to study membranes, however recent systematic studies show that fluorescent probe behaviour can be altered by membrane composition, probe concentration, and the presence of other probes. Using deuterium nuclear magnetic resonance spectroscopy (^{2}H NMR), we found that trace amounts of the carbocyanine probe DiIC12 are enough to alter phase coexistence behaviour of 35:35:30 DPPC-D62:DOPC:cholesterol membranes, while other probes like Laurdan, Naphthopyrene, and another carbocyanine probe DiOC18, did not affect the membrane appreciably. Laurdan is particularly well suited to the study of phase separation in lipid membranes. It partitions equally well into ordered and disordered lipid phases, and displays a phase-dependent emission spectral shift. Laurdan general polarization (GP) parameter, which characterizes said emission spectral shift, has been used to characterize membrane fluidity. We examine the relationship between Laurdan GP and ^{2}H NMR order parameters.

Summary

Both fluorescence microscopy and ^{2}H NMR show that trace amounts of the fluorescent probe Laurdan does not affect membrane phase behaviour. Laurdan can be used to measure the phase behaviour of 35:35:30 DPPC:DOPC:Chol without modifying it. This is in contrast to several other recently-studied probes. We hypothesize that Laurdan's ability to partition nearly equally among different lipid phases is responsible for this difference.

Outstanding Questions

- Is it generally true that trace components that partition equally between ld and lo phases do not alter phase behaviour?
- Which biological molecules present in trace amounts in membranes partition unequally between ld and lo phases?

References

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