



Biophysics Interdepartmental Group PhD. Thesis Defence

Thursday May 7, 2009

1:00 pm in SCIE 3317

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“PHASE BEHAVIOUR OF DOPC/DPPC/CHOLESTEROL MIXTURES REVEALED BY ^2H NMR SPECTROSCOPY AND CONFOCAL FLUORESCENCE MICROSCOPY”

ABSTRACT

The phase behaviour of lipids has long been the focus of biophysical studies, and interest in fluid-fluid phase separation in membranes has increased considerably since the “membrane raft” hypothesis was proposed. Much controversy exists in the biology and biophysics communities concerning liquid-disordered (l_d) and liquid-ordered (l_o) two-phase coexistence in natural membranes. Determination of the location of phase boundaries is an important step towards understanding the phase behaviour of different lipid systems. Biophysical techniques such as nuclear magnetic resonance (NMR) spectroscopy can be used to detect fluid-fluid immiscibility, and qualitatively determine the phase boundaries in lipid bilayer model membranes.

A partial phase diagram for the ternary lipid mixture dioleoylphosphatidylcholine (DOPC)/chain-perdeuterated dipalmitoylphosphatidyl-choline (DPPC- d_{62})/cholesterol, determined by ^2H NMR, is presented. Confocal fluorescence microscopy (CFM) using various fluorescent probes was used to confirm these results. Giant unilamellar vesicles (GUVs) composed of the same ternary lipid system showed similar phase boundaries by CFM for the l_d - l_o two phase coexistence region. An accurate method was developed for determining domain area fractions in GUVs, which can potentially be applied to any lipid system with circular domain shapes where the GUVs remain spherical under experimental conditions. Using the same ternary lipid mixture with specifically-labelled DPPC, further evidence is presented for an l_d - l_o -gel three-phase coexistence region, based on ^2H NMR measurements.

The partitioning behaviour of fluorescent probes commonly used in model membrane studies was characterized by CFM in GUVs composed of binary (DOPC/DPPC) and ternary (DOPC/DPPC/cholesterol) mixtures. In the ternary system, most of the fluorescent probes displayed partitioning preference for the l_d phase, however, two probes preferred the l_o over the l_d phase. None of the fluorescent probes showed preference for gel phase in the ternary system, however some probes partitioned into gel phases in binary mixtures of DOPC/DPPC.

Chair: Dr. Michele Oliver (Engineering)

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