The Liquid Ordered Cholesterol Phase in Lipid Membranes Contains Highly Ordered Lipid Rafts

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Changing the incoming wavelength of the neutrons in a triple axis spectrometer will have an effect on the energy resolution, Q resolution and coherence length of the beam. The standard triple axis setup typically strives for high energy and Q resolutions, while maximizing the coherence length of the probe. We have found that by operating in a low energy resolution setup with a shorter neutron coherence length we are able to observe small, highly ordered structures in systems which lack long range order. This technique was first used to detect coexisting fluid and gel nanodomains in a single component lipid bilayer system [1].

In this experiment we used the same technique to examine a high-cholesterol, lipid bilayer sample. From this, we are able to present experimental evidence that the liquid ordered, Io, phase in saturated DPPC membranes contains highly ordered lipid domains and that the cholesterol does not uniformly disperse throughout the bilayer.

The sample examined consisted of highly oriented multi-lamellar stacks of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) containing 32.5% cholesterol prepared on polished Si wafers. Sixteen such Si wafers were stacked with aluminum spacers to allow for full hydration of the membranes. The “sandwich” sample was kept in a temperature and humidity controlled chamber.

With this sample a series of in-plane scans were conducted in both the standard high energy resolution setup and the modified low energy resolution setup of the spectrometer. Using the standard triple axis spectrometer settings we observed a spectra similar to that of a single component, fluid lipid bilayer (shown in Figure 1a), suggesting that the cholesterol are uniformly dispersed. However, when the spectrometer was settings were altered, such that it was operating with a lower energy resolution, we observed the same broad fluid peaks, but also additional peaks that are believed to be associated with highly ordered lipid domains caused by the non-uniform distribution of cholesterol in the membrane (Figure 1b).

![Figure 1 A comparison between the in-plane scans of DPPC62 and 32.5 mol% cholesterol bilayers using (a) the conventional high energy resolution setup and (b) the low energy resolution setup. A disordered structure was observed in (a) while the pronounced signals in (b) are indicative of the formation of highly ordered lipid domains. The corresponding molecular structures are shown in the lattice cartoons; the reflections are indexed by their Miller indices, [hkl].](image)

Reference