Experimental report for ESRF Experiment SC 3664

Domain formation and patterning in bicontinuous cubic lipid phases

One of the key functions of biomembranes is to provide a dynamic lipid matrix within which reactions can take place and this function is thought to be facilitated by lateral inhomogeneity and patterning within these membranes. While there has been significant research into lateral phase separation in flat model membranes both in lamellar liquid crystals and unilamellar vesicles, there has been very little work done to investigate phase separation and patterning in curved lipid phases. Non-lamellar lipid structures are known to play highly important roles *in-vivo* and in this experiment we aim to observe liquid ordered (L_o) – liquid disordered (L_d) phase separation within bicontinuous cubic lyotropic lipid phases (structures consisting of a bilayer draped over an infinite periodic minimal surface (IPMS) with Im3m, Pn3m or Ia3d symmetry). We expect that the higher bending modulus L_o phase will preferentially partition to the flat-points of the bicontinuous cubic structure with the L_d phase lying at the saddle points. These structures may prove extremely useful in stabilising membrane proteins for *in-cubo* crystallisation.

Mixtures of monoolien (MO) (which is known to form a Pn3m bicontinuous cubic structure in excess water), monopalmitin (MP) and cholesterol (Chol) were studied in the range 50 – 100 mol% MO, 0 – 50 mol% MP and 0 – 50 mol% Chol in excess water from 5 to 80 °C and 0 to 2000 bar to explore the structural landscape of these mixtures and identify the conditions at which single phase and phase separated bicontinuous cubic structures are formed.
Chol was found to incorporate into the MO Pn3m phase up to 15 mol%, causing a small swelling (increase of approximately 10 Å in the lattice parameter). Above this concentration, Chol drives a phase transition to an inverse hexagonal phase, however no excess Chol crystals were observed during these experiments.

Many of the mixtures displayed formation of two distinct structures which we believe indicates large scale phase separation. However, MO:MP:Chol 80:10:10 at 24.5 °C shows the formation of a single fluid bicontinuoius cubic (Im3m) structure with a lattice parameter of approximately 190 Å which may indicate the formation of a phase separated cubic phase (Figure 1).

We are currently carrying out electron density reconstructions for all the fluid structures formed and hope that this will confirm phase separation in this sample.