Experimental report

16/09/2019

Proposal: 8-05-446 **Council:** 10/2018

Title: Localisation of membrane proteins in curved lipid bilayers using oriented lipid cubic phases

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: Monoolein

Instrument	Requested days	Allocated days	From	To
D22	4	2	24/07/2019	26/07/2019

Abstract:

This proposal will test hypothesized curvature – driven localization of membrane proteins within lipid cubic phases, using our recent advances in preparing cubic phase films with high out-of-plane orientation, combined with 2D Grazing Incidence-SANS.

Preliminary experimental report (**8-05-446**) D22 (July 2019)

We have recently (July 2019) carried out GI-SANS experiments on beamline D22 at ILL using the solid-liquid cell (Figure 1(c-e)) with lipid films spin-coated onto silicon substrates. In these experiments we made three significant breakthroughs:

- First GI-SAS data showing highly oriented lipid cubic phase films at the solid-water interface (Fig 1 c, top) demonstrating success of the approach. The films show the same orientation as our previously published GI-SAXS data from films containing glycerol, in humidified air; the GI-SANS data under water are much more representative of Q_{II} films in most applications. Furthermore, the lipid could be contrast matched dynamically by replacing the H_2O with D_2O.
- First GI-SANS data confirming localization of the peptide gramicidin (gD); by contrast matching out the lipid, the remaining signal from the (unlabeled) gD showed reduced signals from the √3 reflections (Fig 1(d)), consistent with localization into more curved regions (Fig 1(b)).
- Addition of membrane protein (OmpF) AFTER formation of Q_{II} phase, by flowing OmpF solution into the cell containing the Q_{II}^D phase film, and allowing to equilibrate (Fig 1(e)). This greatly widens the potential of our approach: unlike the gD, which was co-dissolved in organic solvent and spin-coated with the lipid at the start of the experiment, most proteins (like OmpF) would be denatured under such conditions, and so require a different approach. The addition of OmpF induced a phase transformation to another Q_{II} phase. Data from the phase including the OmpF, with or without contrast matching the lipid (Fig 1(e) top and bottom) again show a change in relative intensity of spots. We are currently analyzing the data to determine predicted localization of OmpF in curved vs flat regions of the new phase.

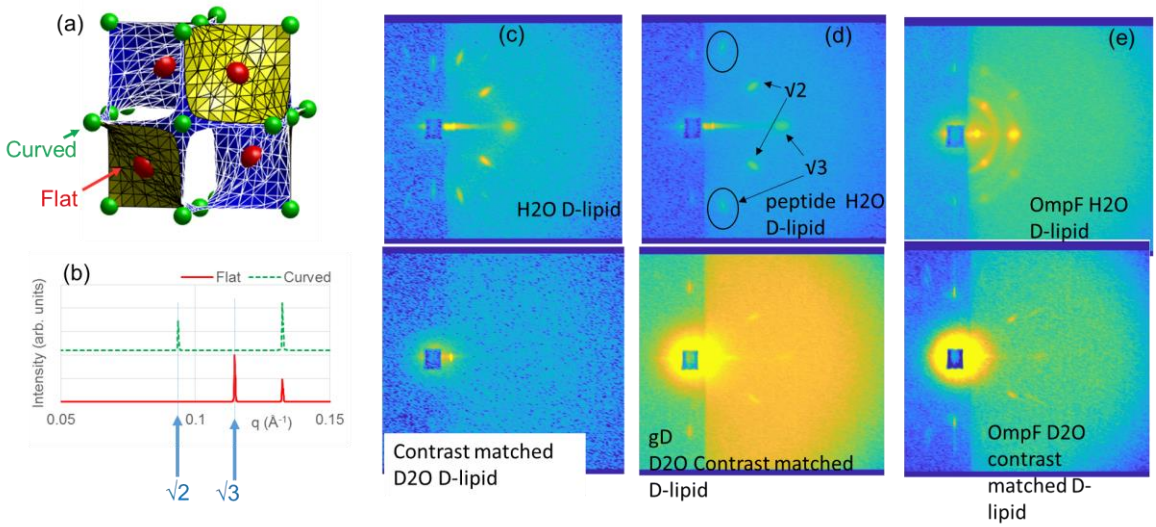

Figure 1 (a) schematic showing the lipid bilayer midplane surface of the D phase, highlighting locations of local high (green) or low (red) gaussian curvature, where different membrane components might be expected to be localized; (b) simulated diffraction patterns from "dummy atoms" located only at the flat (below) and curved (above) regions. (c-e) GI-SANS patterns from spin-coated cubic films of deuterated lipid in the liquid/solid cell on D22, with (bottom) and without (top) contrast matching the majority lipid component (d-MO). (c): d-MO adopts Q_{II}^D phase with (111) direction normal to surface (top) and can be contrast matched in D2O (bottom). (d): with 10% gramicidin (gD) the lipid still adopts the aligned Q_{II}^D phase. However, contrast matching the lipid signal reduces the intensity of the √3 reflections, especially noticeable with the off-axis reflections (circled in the top figure, absent in the bottom). (e) addition of the protein OmpF induces a phase change to the Q_{II}^P phase. Contrast matching the lipid causes disappearance of certain reflections (further analysis ongoing).