ESRF	**Experiment title:** Surface diffraction at single supported membranes: Lipid phase separation at solid-liquid interfaces.	**Experiment number:** SC-1798
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Report:

Solid supported membranes were investigated in aqueous environment by means of x-ray reflectivity and fluorescence microscopy. Simultaneous studies with both methods were conducted at ESRF using a microfluidic setup [1].

Motivation: Lipid bilayers which are composed of lipids with high and low phase transition temperatures (T_m) show phase separation over a wide range of temperature and composition. Free lipid vesicles may have separate solid (gel) and fluid phases, together with a third intermediate state (the liquid-ordered phase) which is induced by the presence of cholesterol [2]. Our main goal was to find evidence for liquid-liquid coexistence in lipid bilayers on solid supports and to characterize these systems on a nanoscopic and microscopic scale.

We have studied mixtures of cholesterol, saturated sphingolipids (high T_m) and unsaturated phospholipids (low T_m) in various stoichiometric ratios on smooth silicon wafers (average roughness 2 Å). Lateral phase separation, which was previously observed by our group on less smooth wafers (Fig. 1(a)), could not be observed on these substrates at first. However, after insertion of GM_1-ganglioside into the bilayer, a change in membrane structure was detected (Fig. 1(b+c)) together with the appearance of small domains (Fig. 1(d)). GM_1 is a marker lipid that is known to partition into liquid-ordered phases.

Our results are manifold: The preparation of fluid, homogeneous membranes and their phase behavior depends critically on the substrate morphology. Lateral ordering is inhibited on ultra-smooth substrates, probably due to restricted possibilities for membrane fluctuations. We have shown that insertion of glycolipids such as GM_1 may overcome these inhibitions by inducing conformational changes in the membrane.

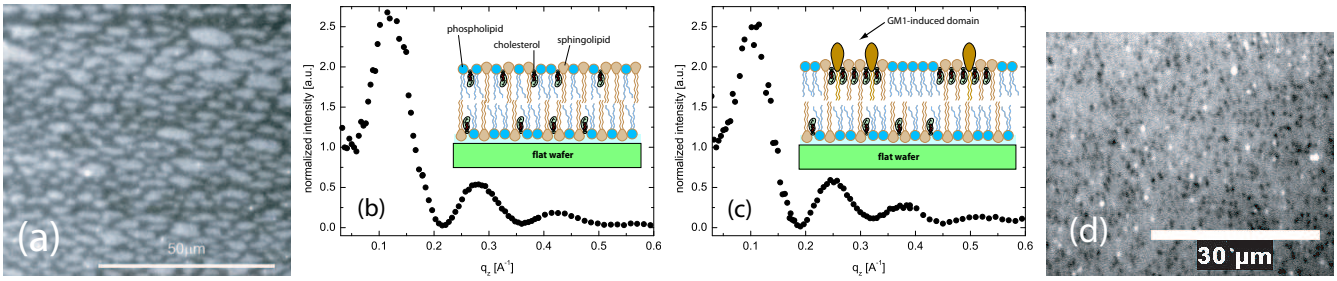

Fig. 1(a): Phase separation of a 1:2:2 cholesterol:sphingomyelin:phospholipid mixture on a wafer with 5-6 Å roughness. (b) Fresnel Reflectivity of the same mixture on a wafer with 2 Å roughness. (c) Fresnel Reflectivity after insertion of GM_1. (d) Fluorescence microscopy reveals small domains (image corresponds to sample of (c)).

At lower cholesterol content, such lipid domains are expected to be of sub-microscopic scale. We have carried out several attempts to resolve nanoscopic structures by grazing-incidence diffraction. Unfortunately, the off-specular signal gain from single supported bilayers was insufficient for a quantitative evaluation. For future experiments, we suggest the application of strong marker lipids (e.g. gold conjugated phospholipids) which may yield sufficient contrast to resolve separate phases in single bilayers.

During this experiment we could optically investigate beam damage effects due to excess illumination. Membrane homogeneity and defects prior and after illumination were studied using fluorescence microscopy. Fig. 2(a) shows the reflectivity of a single bilayer of 1-Stearoyl-2-Oleoyl-sn-Glycero-3-Phosphocholine (SOPC). After exposure of the sample for several minutes to the full beam intensity (a typical situation for grazing-incidence diffraction), the reflectivity became clearly degenerated (Fig. 2(b)). Thus, in order to avoid misinterpretation of synchrotron data, a microscopical pre- and post-characterization is of great value for quantifying the implications of the beam damage (Fig. 2(c)). Such considerations are mandatory if proteins are incorporated in the bilayer which are particularly sensitive to radiation.

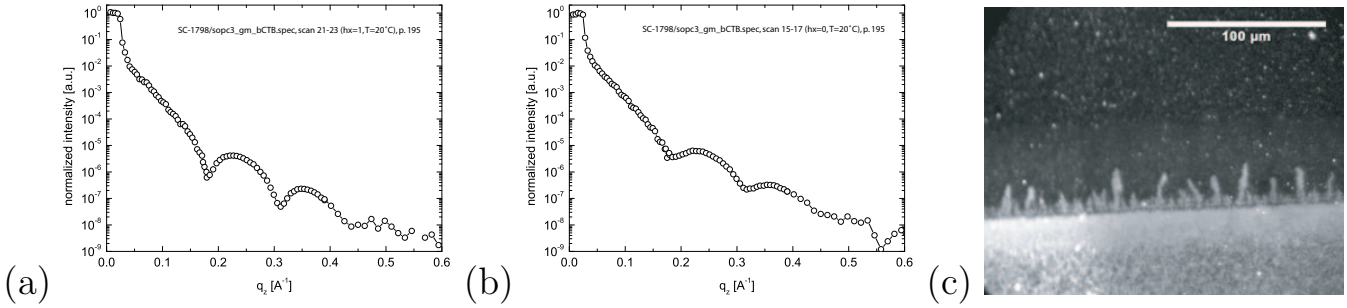

Fig. 2(a): Reflectivity of a SOPC-membrane (first scan). (b) Scan of the same spot, but after an exposure for several minutes with the full beam intensity. (c) Microscopy image of the illuminated surface area (black stripe). Intact lipids (bright tubes) are re-diffusing into the damaged region.

[1] C. Reich et al., Rev. Sci. Instr. **76**, 095103 (2005). M. Hochrein et al., Langmuir **22**, 538-545 (2006). ESRF experimental reports no. IH-SI-194 and SC-1635.

[2] S. L. Veatch, S. L. Keller, Phys. Rev. Lett. **94**, 148101 (2005)