Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

Separation of liquid domains in model membranes induced with high hydrostatic pressure

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Phase separation videos

Two videos of giant unilamellar vesicles (GUVs) during pressure ramps accompany this document.

Materials

1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC), 1,2-diphytanoyl-sn-glycero-3-phosphocholine, (DPPC) and cholestanol and 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt) Rh-DPPE were purchased from Avanti Polar Lipids (Alabaster, AL) and were used without further purification. Lipid mixtures were prepared by co-dissolving the required molar ratios of lipids in chloroform.

GUV formation

A lipid mixture of 25:50:25 mol % of DPhPC /DPPC /cholestanol were dissolved in chloroform to a concentration of 1 mg/ml. To this mixture 0.8 mol % Rh-DPPE was added. ITO-coated microscope slides (Sigma Aldrich, Gillingham, UK) were cleaned by sonication in mild detergent, rinsed in distilled water and dried under a nitrogen stream. The sides and lipid solution were heated to 60 °C and 20 µl of solution was spread evenly on the conducting side of the slide. The lipid film was dried under vacuum for 30 mins to remove all solvent. A 5 mm thick PDMS spacer with a central cut-out was used to separate the slides with the conductive sides facing the each other, and the resulting chamber was filled with 197 mM sucrose solution which had been preheated to 60 °C. The chamber was incubated at 60 °C and an alternating electric field (1 V, 10 Hz) was applied across the ITO plates using a function generator (Aim-TTi, TG315). After two hours, the electric field was changed to 1 V, 2 Hz for a further hour. The chamber was allowed to cool to room temperature and the resulting solution was used for fluorescent imaging within 48 hours.

High Pressure Fluorescence Microscopy

A custom built high pressure microscopy cell mounted on a Nikon Eclipse TE2000-E inverted microscope was used to image the GUV sample under high pressure. The cell comprises a high tensile strength stainless steel body with 1 mm thick, 5 mm diameter sapphire optical windows, which can withstand pressures of up to approximately 2500 bar. Hydrostatic pressure was applied to the sample via a water filled pressure generator (4000 bar, Si-Tec) and hydraulic network similar to that described previously.[1] The pressure was measured using a strain gauge pressure transducer (4000 bar full scale, 0.25 % precision, Top Industrie, France). The pressure cell temperature was controlled via a circulating water bath and recorded using a PT-100 sensor. Pressure and temperature data were recorded using a National Instruments compactDAQ system. An extra-long working distance objective lens (Nikon CFI Super Plan Fluor ELWD ADM 20X, N.A. 0.45, W.D. 8.2 - 6.9 mm) was used to image the sample in either phase contrast or fluorescence mode. Images were acquired using an Andor Zyla sCMOS based camera (Andor Technology, Belfast, UK) and recorded using custom built software with temperature and pressure logging.

A 10 µl sample of the GUV suspension was diluted in 90 µl of 200 mM glucose solution. A sample holder comprising a 1.5 mm thick 6 mm diameter PTFE disc with a 3 mm internal aperture was fixed to the lens side pressure cell window and 10 µl of the diluted GUV solution loaded into the sample holder. The disk was sealed with a 6 mm coverglass to isolate the sample solution from the pressurizing water.

Temperature and Pressure Stability of High Pressure Microscopy Cell

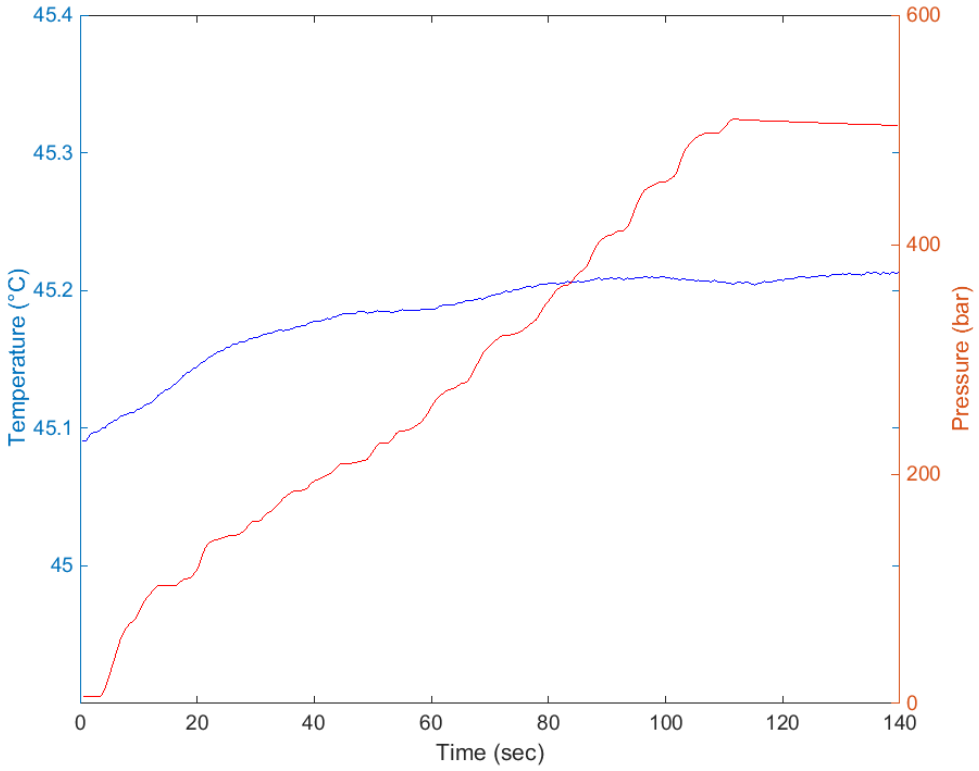

Figure S1. Temperature and pressure of the high pressure microscopy system during the pressure scan used to generate Figure 1.

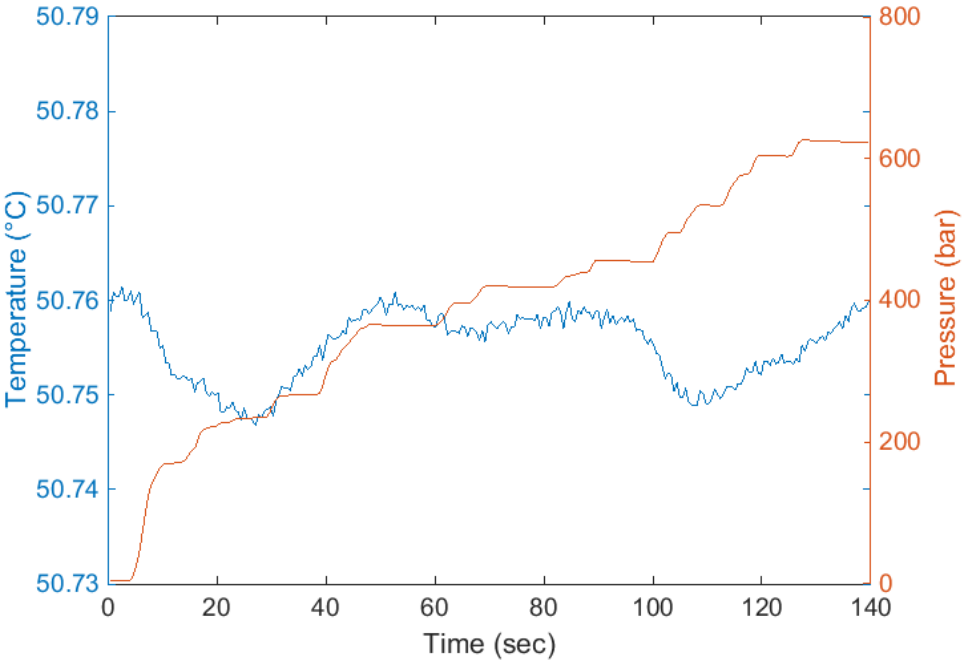

Figure S2. Temperature and pressure of the high pressure microscopy system during a pressure scan from atmospheric pressure to approximately 600 bar.

High pressure small angle X-ray diffraction (SAXS)

A dry sample of 25:50:25 mol % of the lipids DPhPC, DPPC and cholestanol was hydrated with excess water (75 wt%, MilliQ filtered). The sample was subjected to at least 10 freeze-thaw-vortex cycles and a minimum of two pressure cycles (1 to 2000 bar) before each experiment to ensure sample homogeneity. Full details of the pressure cell used for X-ray studies have been described previously.[1] Briefly, the cell consists of a high tensile strength stainless steel body with 1 mm thick, 5 mm diameter single crystal chemical-vapor deposition type IIa diamond windows. These windows provide high X-ray transmission (at 18 keV) while offering excellent pressure stability. SAXS experiments were carried out at beamlines I22 at Diamond Light Source and ID02 at the European Synchrotron Radiation Facility SAXS patterns were recorded in the range 1 to 2000 bar and 25 to 60 °C. The resulting 2D SAXS patterns were radially integrated to give scattering intensity profiles. The diffraction peaks were fitted using in-house software. Where phase separation occurs, peaks were fitted to two Voigt functions, and the center of the peaks was used to find the lamellar spacing of the individual phases.

References

(1) N. J. Brooks, B. L. Gauthe, N. J. Terrill, S. E. Rogers, R. H. Templer, O. Ces and J. M. Seddon, *Rev. Sci. Instruments*, 2010, **81**, 064103.