

Supplementary Information

Viscoelastic changes measured in partially suspended single bilayer membranes

Imad Younus Hasan and Adam Mechler

*Department of Chemistry and Physics, School of Molecular Sciences, La Trobe University,
Bundoora, VIC, 3086 AUSTRALIA*

Differential scanning calorimetry (DSC):

Experiments were performed using a SETARAM μ DSC Evo3 instrument, in 700 μ l hastelloy pressure cells. 50 μ M liposome suspensions were prepared in 20mM phosphate buffer containing 100mM sodium chloride at pH 6.59 for all lipid mixtures. In each experiment, three zones were recorded with the same parameters. The system was equilibrated at 15 °C for 30 min; then the temperature was increased at a rate of 0.33°C min⁻¹ to 35°C; next the system was equilibrated for 30 min at 35°C, and then temperature was returned to 15°C at a rate of 0.33°C min⁻¹ followed by 30 min equilibration. In Fig. S1 the first temperature ram

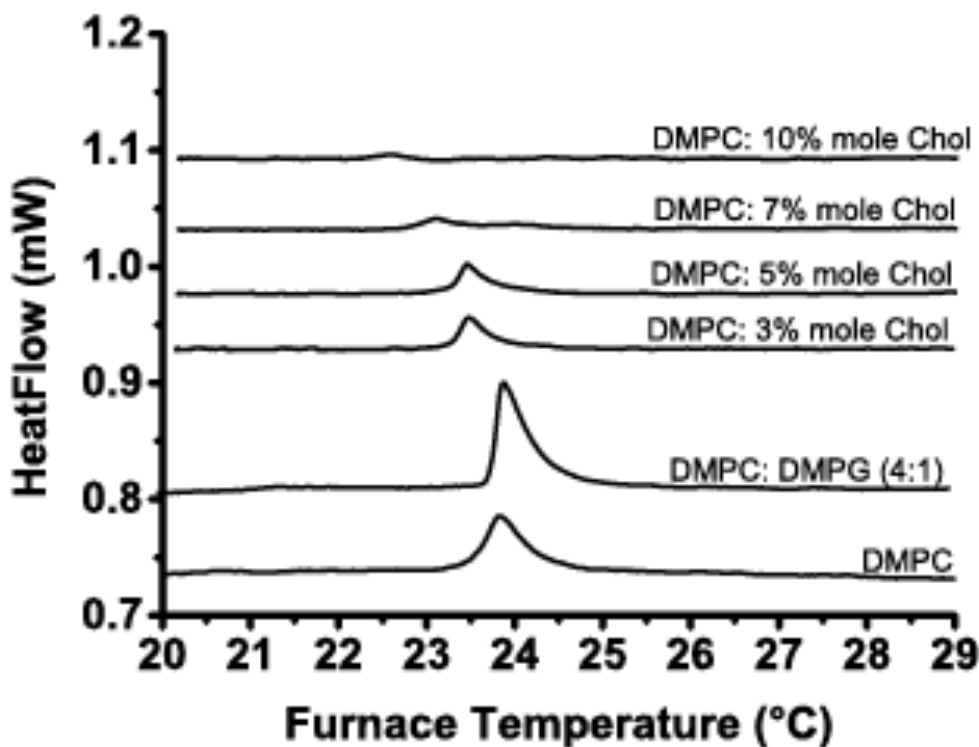


Fig. S1. DSC curves illustrating the composition-dependent changes in the main phase transition temperatures of various DMPC based membrane mixtures as indicated.

Fluorescence microscopy imaging:

Membranes were labelled with the lipophilic 0.1% DMPE-atto-594 (λ_{fl} = 627 nm) and the liposomes formed from these membranes were loaded with a 10 mM aqueous solution of 5(6)-carboxyfluorescein (λ_{fl} = 517 nm) thus the co-localization of the two dyes in unopened liposomes gives a yellow colour. Excess 5(6)-carboxyfluorescein was removed *via* dialysis. Experiments were performed with a Nikon Eclipse TM100 inverted microscope, using a UV lamp excitation source. Images were taken through a QCM-D window cell upon *in situ* membrane deposition. The setup gives a slight aberration due to the tilt of the glass cover of the QCM cell in relation to the optical axis of the microscope objective.

Fig S2 shows fluorescence images of lipid deposits (A, B) and gold chip surface as control (C) captured with the same camera settings. On the MPA surface (Fig. S2 A) the orange colour (DMPE-atto-594) is homogeneous with only a few yellow spots that reveal co-localisation of the two dyes in intact liposomes. Some 5(6)-carboxyfluorescein might be trapped underneath the membrane. Contrast enhancement did not alter the homogeneous appearance of the deposit. On oxidized gold substrate that is generally reported to support intact liposome attachment^{1, 2} the surface is covered with unopened vesicles (Fig. S2 B), in particular small unilamellar vesicles that fill the depressions of the gold morphology, as clearly visible after contrast enhancement of the same image (inset). The reference gold chip surface did not exhibit any fluorescence under the same imaging conditions. Thus fluorescence imaging confirmed that the MPA modified sensor surface is covered by a continuous membrane mostly free of intact liposomes.

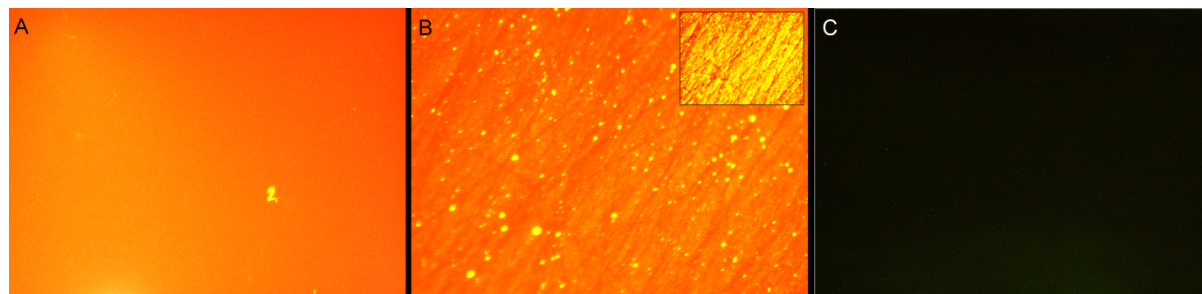


Fig. S2. Fluorescence images of A) membrane deposited on MPA (final Δf = -15 Hz and ΔD = 2.2 at 25°C); B) liposomes deposited on oxidized gold (final Δf = -7.5 Hz and ΔD = 10.5 at 25°C); inset, contrast enhanced image; C) gold chip surface in the absence of lipid.

References

1. C. A. Keller and B. Kasemo, *Biophysical Journal*, 1998, **75**, 1397-1402.
2. E. Reimhult, F. Hook and B. Kasemo, *Langmuir*, 2003, **19**, 1681-1691.