SUPPORTING MATERIAL
Interactions between adsorbed hydrogenated soy phosphatidylcholine (HSPC) vesicles at physiologically-high pressures and salt concentrations

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Liposomes Preparation Hydrogenated Soy PC (HSPC, M_w = 762.10 g/mol, >99% purity) was purchased from Lipoid (Ludwigshafen, Germany). Multilamellar vesicles (MLVs) were prepared by hydrating HSPC in 150 mM $NaNO_3$ (Fluka, >99.999% purity) at 62º C (above the gel-to-liquid crystalline phase transition temperature, T_m, of HSPC, 53ºC (1)). MLVs were downsized to form SUVs, ~75 nm in diameter (via dynamic light scattering), at a concentration of 30 mM HSPC (by phospholipid concentration), by stepwise extrusion through polycarbonate membranes starting with a 400-nm and ending with 50-nm-pore-size membrane, using a Lipex 100 mL extruder system (Northern Lipids, Vancouver, Canada)(2). Water was treated with either a Milli-Q® purification system (comprised of a reverse osmosis stage (RiOs™), a UV treated reservoir and a Milli-Q® Gradient A10 purification system) or a Barnstead Nanopure system. The resistance of water was 18.2 MΩ cm with total organic compound (TOC) of 3 - 4 ppb (Milli-Q®) or < ca.1 ppb (Barnstead®). The pH of the water was in the range 5.5 - 6, likely due to ions leached from glassware and dissolved atmospheric CO_2. 150 mM NaNO3 salt solutions were used for immersing the liposome-coated surfaces for the subsequent force measurements. Sodium ions were used to emulate physiological salt concentrations, due to the high natural abundance of Na^+ in biological fluids. The nitrate group was chosen as the counterion because of its compatibility to our experimental system, as the mica surfaces in the SFB are back-silvered and this coating was found to be sensitive to some ions (notably Cl^-), which cause degradation of the silver layer.

Dynamic Light Scattering: Liposomes were characterized for size distribution by dynamic light scattering (DLS) using, for the final stage liposomes, an ALV-NIBS High Performance Particle Sizer (Langen, Germany) equipped with an ALV-5000/EPP multiple digital correlator at a scattering angle of 173°. The DLS data indicated that over 95% of the liposomes in the bulk solution were 75±3 nm in diameter.

Zeta Potential measurement: ζ-potential was measured using a Malvern Zetasizer Nano ZS instrument (Worcestershire, UK). Controls showed that HSPC liposomes in low (5mM) salt concentrations had a zeta potential of 6.20±0.26 mV. At the high salt concentrations used in our study (0.15M) the ζ-potential values, measured with somewhat lower accuracy, were in the range 1.3±0.6 mV, demonstrating the neutral nature of the zwitterionic lipids.

Determination of phospholipid concentration: Phospholipid (PL) concentration was determined using the modified Bartlett assay as described elsewhere (3).

Surface Preparation: HSPC-SUV were adsorbed on atomically smooth mica surface by placing freshly cleaved mica in 10 ml 150 mM $NaNO_3$ and then adding 360±10 μL of the SUV dispersion (of 30 mM HSPC phospholipid concentration) for 1.5-2 hours of incubation at room temperature. Then mica surfaces were washed to remove excess, non-adsorbed liposomes by placing the adsorbed surfaces in a beaker filled with 150mM $NaNO_3$ for few minutes along with a delicate shake motion. All preparations were done in a laminar flow hood to prevent contamination.

Cryo-Scanning Electron Microscopy (Cryo-SEM) Cryo-SEM samples (mica surfaces covered with HSPC liposomes) were prepared as was described above. Samples were frozen by plunging into liquid ethane and transferred to a BAF 60 freeze fracture device (BAl-Tec AG, Liechtenstein). Water was sublimed at -80°C for 2 hrs. Samples were rotary-shadowed with 3 nm Pt at an angle of 45°. Samples were transferred to an Ultra 55 SEM (Zeiss, Germany) using a VCT 100 vacuum-cryo transfer system (Bal-Tec AG, Liechtenstein) and observed at voltages of 2.5 - 5 kV.

Surface Force Balance (SFB) Normal and lateral force profiles were measured using the SFB between the two atomically smooth mica surfaces, as shown schematically in the inset of Fig. 2 and described in detail elsewhere(4). All experiments were carried out according to the following procedure: after mounting back-silvered mica sheets in the SFB, the equipment was placed in the measurements room and the air-contact positions (zero-position) of the interference fringes of

equal chromatic order (FECO) were measured. Then, the SFB was replaced in the laminar hood and the lenses were taken out, each to a 20 ml beaker filled with 10 ml of 150 mM $NaNO_3$ solution. 360 μL of the HSPC suspension was added to each of the beakers and the surfaces were incubated 1.5-2 hours in the beakers to enable adsorbance of the HSPC liposomes onto the mica surfaces. At the end of the adsorbance procedures the lenses were washed by immersing for ca. 5 mins in a 400 ml beaker filled with 150 mM $NaNO_3$ in order to remove liposome excess. Then, the bottom lens was withdrawn, with the liposome-coated mica surface wetted, from the salt solution, mounted in the apparatus and immersed in the 0.15M $NaNO_3$ solution added to the cell. This procedure lasted under one minute, and the liposome layer remained wetted throughout. Following this, the upper lens was mounted in the SFB and immersed in the 0.15M salt solution, likewise remaining wetted throughout the less-than-one minute required for this. The apparatus was then positioned in the temperature-controlled (T = 23.7 ± 0.3° C) surface-forces measurement room. The procedure of mica surface preparation and addition of salt solution to the SFB was preformed in a laminar hood to avoid contamination, thus reducing the accuracy of the absolute separation measurements from ± (0.2 - 0.3) nm to ± ca.0.5 nm, due to shifts in the optical fringe positions upon dismounting and remounting the lenses, as well as due to relocation of the apparatus. Results are shown from 3 different experiments and different contact points within each experiment (ca. 9 in all).

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