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Supplementary Information

Normal and Shear Forces between Boundary Sphingomyelin Layers under Aqueous

Conditions

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Figure S1. Differential scanning calorimetry (DSC) traces of egg SM-SUVs. DSC tests were performed on a TA Q200 differential scanning calorimeter. For the test, 35 mg of 6 mM egg SM-SUVs prepared in water was amounted into a Tezo pan and sealed. Conductivity water was used as a reference. Heating and cooling scans were controlled at $0.5 \,^{\circ}$ C min⁻¹, in a temperature range of 20 - 65 $^{\circ}$ C.



Figure S2. Size distribution (a) and zeta potential (b) at different ionic concentrations in 0.3 mM egg SM-SUV dispersion. In Figure (a), magenta and blue curves indicate liposomes prepared in water and 150 mM NaNO₃; pink, green, black, red curves represent dispersions in 0.03, 0.3, 1, and 5 mM Ca(NO₃)₂, respectively. No aggregation of SUVs was observed in the presence of 0.03 to 3 mM Ca²⁺. In Figure (b), the filled symbols were zeta potential values measured immediately after mixing liposomes with Ca²⁺. The zeta potential of egg SM-SUVs increases from -13.6 mM, without Ca²⁺, increases to positive values as Ca²⁺ concentration increases to 0.3 mM, indicating that the Ca²⁺ ions adsorb to the vesicle surface and cause charge reversal.



Figure S3. Morphologies of egg SM-SUVs on mica across 0.3 mM egg SM-SUV dispersion in water (a-b) and after replacing 0.3 mM SUV dispersion in 150 mM NaNO₃ with water (c-d) by AFM. Under 0.3 mM egg SM-SUV dispersion in water, although SFB results show few egg SM-SUVs adsorbed on mica, AFM image (a) shows egg SM patches on mica. This is possibly because the AFM tip brings liposomes to mica surface when imaging with tapping mode, which overcomes the relatively weak electrostatic repulsion and facilitates adsorption. The gap between egg SM patches indicates repulsive forces. In c-d, defects and phase separation appear on the supported egg SM bilayer, implying detachment of egg SM-bilayer from mica in water.