Electronic Supplementary Material (ESI) for Chemical Communications.

Measurements of the effect of membrane asymmetry on the mechanical properties of lipid bilayers

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Supplementary Information

DOPC and POPC lipids were purchased from Sigma Aldrich. Fluorescent lipids were purchased from Avanti Polar Lipids. All other reagents were purchased from Sigma Aldrich unless otherwise specified.

SI 1 –Phase Transfer

The phase transfer method of GUV generation requires lipid to first be dissolved in oil. To prepare lipid-in-oil solutions, lipid was dissolved in chloroform which was then removed under a stream of nitrogen followed by lyophilisation for two hours. Oil was then added to give a 2 mg ml^{-1} solution, and lipids were dissolved by sonication at 50°C for 60 minutes. Hexadecane was used for the emulsion oil (containing the inner leaflet lipid), and mineral oil was used for the column oil (containing the outer leaflet lipid).

A water-oil column was then set-up. To do this, polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning) sheets with 1 cm diameter holes were bonded to glass slide by a 20 second exposure in a plasma oven (Fig. S1). Plasma exposure also modified the PDMS so it was hydrophilic, leading to a favourable concave meniscus of the aqueous solution.[1]

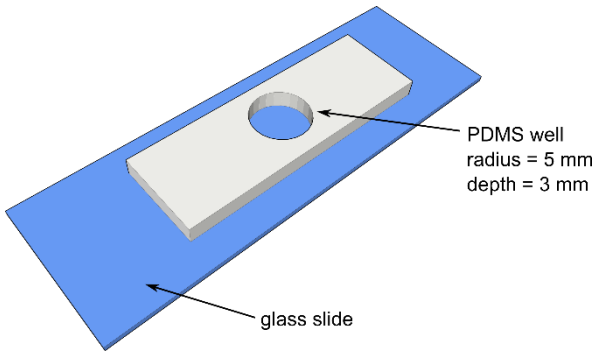

Fig. S1. Schematic of the well used to hold the water/oil column used to generate vesicles.

Immediately after plasma treatment, 150 µL of 500 mM glucose in deionised (DI) water solution was added to the well, followed by the addition of 150 µL of lipid in mineral oil solution (2 mg ml^{-1}). The column was left to stabilise for two hours before the addition of the emulsion, leading to the assembly of a well-packed interfacial lipid monolayer.

Then, we prepared a water-in-oil emulsion where droplets were composed of 450 mM sucrose in DI water, with lipid dissolved in hexadecane oil (2 mg ml^{-1}). A lipid monolayer thus self-assembled around the droplets. The emulsion was generated by pipetting up and down a mixture of 10 µL sucrose solution and 100 µL hexadecane five times. This emulsion solution was then pipetted into the pre-prepared water/oil column. Due to the density difference between the sucrose-containing emulsion droplets and the glucose-containing column aqueous phase (1.15 vs. 1.09 g cm^{-3}) the lipid-coated droplets descended through the column and were encased by a second monolayer. Emulsion droplets were thus transformed into GUVs (diameter distribution: 5 – 80 µm). By having different lipid compositions in the oil phases used in the two steps an asymmetric distribution of lipids in the bilayer was achieved. The monolayer coating the droplets formed the internal monolayer, whilst the monolayer originally lying at the water/oil interface of the column formed the external monolayer.

We used two different oils to assemble the two leaflets in order to minimise intermixing of the two lipid types before vesicles are generated. This is because (i) mineral oil is slightly denser than hexadecane allowing two distinct oil layers to assemble and (ii) the different chemical potentials of the oils slows intermixing. In addition, the formation of a well-packed lipid monolayers around the droplet is rapid, and there is minimal energetic driving force for the addition of further lipid from the surrounding bulk oil. We would also like to note that if any exchange does occur, it is not significant enough to compromise vesicle asymmetry. This is supported by our fluorescence validation of asymmetry experiments, by the different bending rigidities seen with asymmetric vesicles, and by proof-of-asymmetry experiments conducted by others.

SI 2 - Generation of electroformed vesicles

Electroformed vesicles were made by depositing 5 µL of lipid in chloroform (1 mg ml^{-1}) between a PDMS spacer on an indium tin oxide (ITO) microscope slide. The chloroform was left to evaporate and was placed in a lyophiliser for two hours to before a second ITO slide was placed above the spacer. The slides were clamped together, and the central volume was filled with filtered 450 mM sucrose in de-ionised (DI) water. Crocodile clips were attached to the two ITO slides, and an alternating voltage was applied for one hour (1V, 10 Hz) leading to GUV formation. 10 µL of this solution was then added to 90 µL of 500 mM glucose on a glass slide, and vesicles were observed using a phase contrast microscope.

SI 3 – Vesicle Imaging

Vesicle were imaged under phase contrast microscopy on a Nikon Eclipse TE-2000-E inverted microscope with a high speed CMOS camera (Luminera Infinity-1, exposure of 1ms, average frames per second). For fluorescent experiments an illuminating mercury arc lamp was used together with standard TRITC and FITC filters to image the Rh-PE and NBD-PE lipids respectively (200ms exposure time).

SI 4 -Contour Extraction Using Radial Integration method

A key step in measuring the bending rigidity is accurately finding the membrane contours from the captured images. To do this we developed an image analysis program using the LabVIEW software package (National Instruments, Austin, Texas), shown in Fig. S2. The vesicle edges from the phase contrast images were detected as maximum intensity points. Initially, an approximate vesicle centre, maximum and minimum radii are specified by the user. The image pixel intensity is then radially integrated between the minimum and maximum radii in 1 degree arcs. The maximum intensity is found to sub pixel resolution for each integrated arc using cubic interpolation and taken as the membrane edge position. The collection of points is fit to a circle using the least square algorithm to extract the centre of the vesicle. The membrane edge position is then converted to polar coordinates relative to the vesicle centre for the Fourier transform and for further analysis.

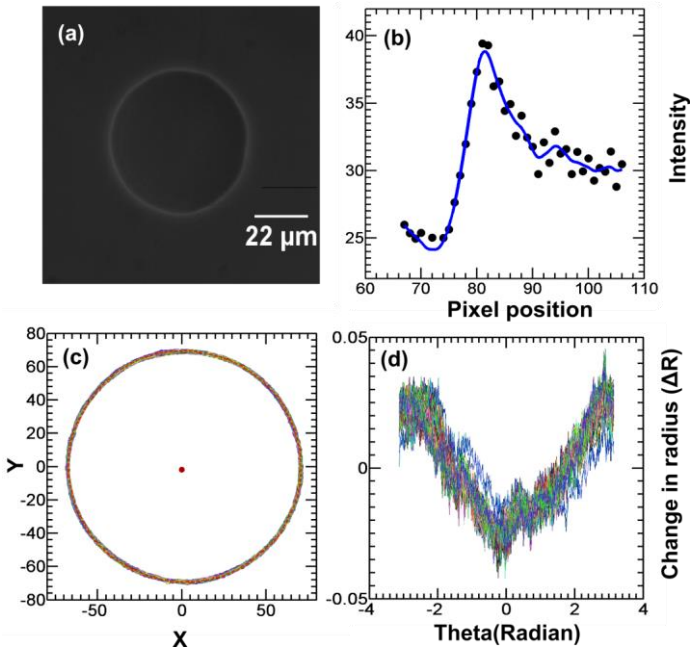

Fig. S2. Contour extraction routine. (a) Typical image of a vesicle under phase contrast. (b) The intensities after radially integrating slices of the edge (black points) and interpolation to obtain the co-ordinates of the edge.(c) Contours of 20 frames after centring to the found centre. (d) Converting x-y co-ordinates to r-theta for Fourier transform to obtain the power spectrum.

SI 5 - Fluctuation Analysis

To extract vesicle bending rigidities we used the following fluctuation analysis method, explained in more detail by Yoon et al.[2] Since the edges of the vesicles are computed in two dimensions (x-z plane), setting y=0, the bending energy of deformation is then given by:

$$\langle h(q_x, y=0)^2 \rangle = \frac{1}{L}\frac{k_B T}{2\sigma}\left[\frac{1}{q_x} - \frac{1}{\sqrt{\frac{\sigma}{\kappa}+q_x^2}}\right] \qquad \text{(S1)}$$

Where and h is the amplitude of mode q_x, L is the vesicle circumference, K_B is the Boltzmann constant, T is the temperature, σ is the membrane tension, and κ is the bending rigidity. For bending dominated fluctuations, $\sigma/(q_x 2\kappa) \rightarrow 0$, to give:

$$\langle h(q_x, y=0)^2 \rangle = \frac{1}{4L}\frac{k_B T}{\kappa q_x^3} \qquad \text{(S2)}$$

Where $L = 2\pi\langle r\rangle$, is the average circumference of the contours from all frames.

To obtain the amplitudes associated with each mode, it is thus required to calculate the Fourier transformation of the fluctuations about the mean radius of the vesicle. After finding the contour coordinates and representing them in polar coordinates, the mean radius of the vesicle $\langle r\rangle$ is calculated for all $r(\theta_n)$ where n is the number of points on the circumference per frame, over all frames. The amplitude of fluctuation about the mean radius is given by $h_m = r(\theta_n) - \langle r\rangle$.

The fast Fourier transform (FFT) of these fluctuations is performed by using an inbuilt LabVIEW function which calculates a set of Fourier coefficients, c_l:

$$c_l = \sum_{m=1}^{N} h_m e^{-i2\pi\frac{(l-1)(m-1)}{N}} \qquad \text{(S3)}$$

where N is the total number of points on the circumference per frame. The modes are given

$$q_x^l = \frac{l}{\langle r\rangle} \quad \text{with } l = 1, 2, \ldots, N/2 \qquad \text{(S4)}$$

Finally the relation between the continuous Fourier transform and discrete Fourier transform is:

$$h_q^2 = c_l^2 \times \left(\frac{1}{N}\right)^2 \qquad \text{(S5)}$$

The bending rigidity is then given by:

$$\kappa = \frac{k_b T}{4Lq_x^3}\frac{c_l^2}{N^2} \qquad \text{(S6)}$$

The bending rigidity is calculated by fitting (S3) to the power spectrum

$$\left(\frac{c_l}{N}\right) \text{ vs } q_x$$

The above equations describe the data well above mode 6, as these are not dominated by the membrane tension. Additionally, modes above 20 cannot be detected reliably due to their fast relaxation rate compared to the exposure time and the limited optical resolution. We therefore only apply modes 6 – 20 to the analysis.

An image showing a typical power spectrum and subsequent fit for a fluctuating vesicles is shown in Fig. S3.

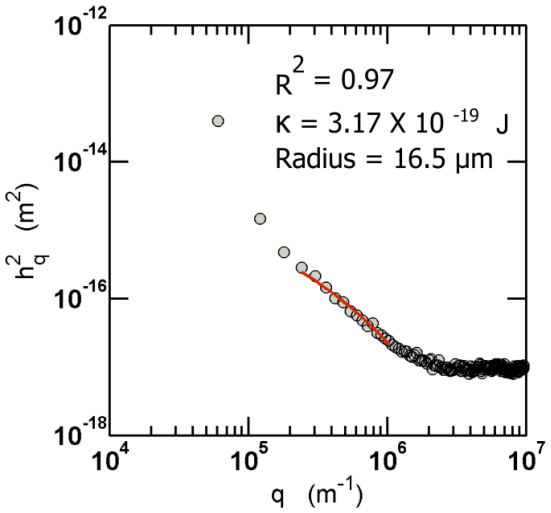

Fig. S3. Typical power spectrum of a fluctuating vesicle. Around 4000 frames were analysed following the contour extraction. The spectrum if fit to equation (S1) to obtain the bending rigidity (κ) value. Modes from 1-5 and 20 onwards are discarded for fitting as they are not bending dominated regions.

SI 6 – Discussion of maintenance of lipid asymmetry

When working with asymmetric vesicles it is important to consider the role of flip-flop, and whether asymmetry is lost over the course of the experiment. The fact that there are significant difference in bending rigidities between asymmetric and symmetric vesicles, and that the errors associated with these values are similar, indicates that asymmetry is maintained throughout the course of the experiments — if not, the errors in the asymmetric experiments would be considerably larger that the symmetric ones, as we would be measuring vesicles with a range of different asymmetries depending on when recordings were taken. In addition, others have shown that flip flop half-life for DOPC and POPC lipids are in the order of hours/days, further supporting our observation that asymmetry is maintained over the course of the experiment. [3-6]

SI 7 – Bending Rigidities of Asymmetric Membranes

A model for the bilayer curvature elastic stress can be constructed by considering the two monolayers separately.[7] The outer and inner monolayers can be assumed to have independent spontaneous curvature values (H_{0out} and H_{0in} respectively) and independent bending rigidities (κ_{out} and κ_{in}) which are dependent on lipid composition.

The curvature elastic energy, g_c, for a lipid monolayer is given by equation S7 below[8] :

$$g_c = 2\kappa(H - H_0)^2 + \kappa_G K \qquad \text{(S7)}$$

Where $H = (c_1 + c_2)/2$ is the monolayer mean curvature, $K = c_1c_2$ is the Gaussian curvature (c_1 and c_2 are the principal curvatures at a given point on the surface), κ is the mean curvature modulus, H_0 is the monolayer spontaneous mean curvature and κ_G is the Gaussian modulus. If we consider a flat monolayer, H and K are both zero.

We have assumed that the curvature elastic energy of the bilayer is the sum of g_c for each monolayer and so the bilayer curvature elastic energy (g_{cB}) is given by equation S8 below.

$$g_{cB} = 2\kappa_{out}H_{0out}^2 + 2\kappa_{in}H_{0in}^2 \qquad \text{(S8)}$$

Where κ_{out} and κ_{in} are the monolayer bending modulus of the external and internal monolayers respectively.

The curvature elastic energy of the 50:50 DOPC:POPC membranes studied here can be modelled at constant overall composition but as a function of the distribution of lipids between the two monolayers. The bending modulus, κ, for pure POPC and pure DOPC monolayers can be calculated as half the bilayer bending modulus[7] values found here, and the spontaneous curvature, H_0, for POPC and DOPC have been reported previously.[9] The monolayer bending rigidity and spontaneous curvature of the mixed monolayers have been calculated as the weighted average of the pure monolayer values.

The curvature elastic energy is shown in Fig. S4 and is found to reach a minimum when the bilayer is symmetric.

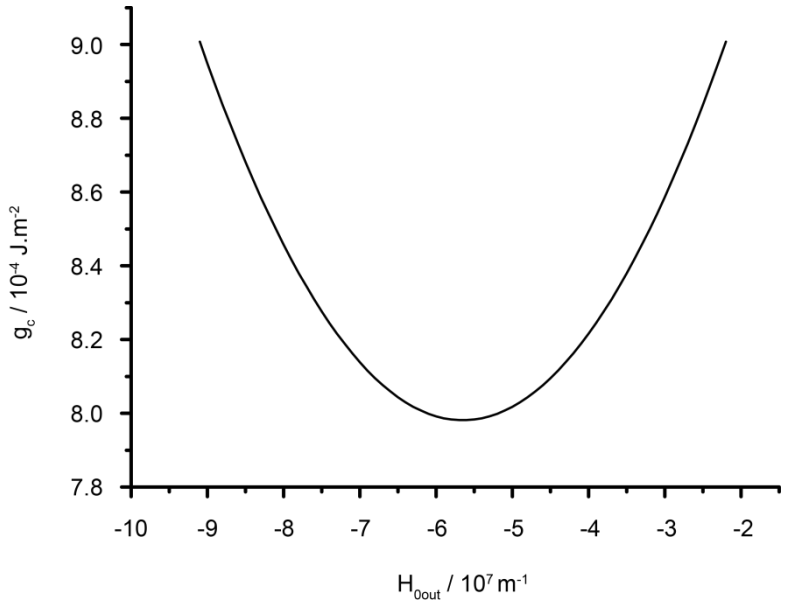

Fig. S4. The calculated curvature elastic energy associated with bilayers with an overall composition of 50:50 mol% POPC:DOPC but as a function of the distribution of lipids across the two monolayers. The left of the curve corresponds to the outer monolayer being 100% DOPC and the inner monolayer being 100% POPC, the right of the curve corresponds to outer monolayer being 100% POPC and the inner monolayer being 100% DOPC. The POPC monolayer κ and H_0 are 7.5×10^{-20} J and -2.2×10^{7} m^{-1} respectively, and the DOPC monolayer κ and H_0 are 5.0×10^{-20} J and -9.1×10^{7} m^{-1} respectively. Values for κ and H_0 of mixed lipid monolayers are calculated as the average of the values for the constituent lipids weighted to the composition.

SI References

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