Morphological Characterization of DMPC/CHAPSO Bicellar Mixtures: A Combined SANS and NMR Study

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1. Models used to fit the SANS data

1.1 Disk with polydisperse radius

Lipid nanodiscs (bicelles) can be modeled by discs with a radius, R and a thickness, b [see schematic in Fig. S1 (a)]. A Schultz distribution is used to describe the polydispersity of their radii. The scattering form factor can then be written as follows [S1].

\[ P(q) = \frac{\phi}{V_{\text{poly}}} \int_0^\infty \int_0^{\pi/2} f(r) dr \sin \alpha \, d\alpha \, , \]

where \( \phi \) and \( V_{\text{poly}} \) are the volume fraction and the individual volume of a disc, respectively. \( f(r) \) is the normalized Schultz distribution of radius. \( F(q, \alpha) \) is the scattering amplitude and is defined as

\[ F(q, \alpha) = 2\pi R^2 b (\rho_{\text{lip}} - \rho_D) \frac{\sin \left( \frac{ab\cos\alpha}{2} \right)}{\frac{ab\cos\alpha}{2}} \frac{J_1(qR\sin\alpha)}{qR\sin\alpha} , \]

where \( \rho_{\text{lip}} \) and \( \rho_D \) are the neutron scattering length densities (SLDs) of the discs and D\(_2\)O, respectively. \( J_1(x) \) is the 1\(^st\) order Bessel function, while \( \alpha \) is the angle between the disc normal and the scattering vector. Throughout the fitting procedure, \( \rho_D \) and \( \rho_{\text{lip}} \) were set to 6.4x10\(^{-6}\) and 2.7x10\(^{-7}\) Å\(^{-2}\), respectively, representing the SLDs of D\(_2\)O and lipid. The bilayer thickness was constrained between 30 and 65 Å.

1.2 Elliptical cylinder

An elliptical cylinder model is used to describe the ribbon morphology [Figure S1 (b)], with the minor semi-axis, b and the major semi-axis, a (= \( \varepsilon b \)) describing the elliptical cross-section and \( l \), the length of the cylinder. The following equation is used to describe the form factor of the elliptical cylinder [S3].
The minor axis is constrained between 15 and 35 Å, representing half of the lipid bilayer thickness.

1.3 Polydisperse spherical shell

The polydisperse spherical shell model is used to describe vesicles [Figure S1(c)] with an inner radius, \( R_i \), and a thickness, \( t \), where \( R_i \) is assumed to follow the Schulz distribution. SLDs inside and outside the shell are set to \( \rho_D \) (6.4x10^{-6} Å^{-2}). The following expression describes the polydisperse spherical shell model [S4-5].

\[
I(q) = \phi \left( \rho_{up} - \rho_D \right)^2 \int_0^{\pi/2} \int_0^{\pi/2} \Lambda_1^2 \left[ \frac{K^2 \cos \beta \cos \alpha}{q^2 \cos \alpha} \right] d\alpha d\beta
\]

and \( \Lambda_1(x) = \frac{2J_1(x)}{x} \).

Figure S1. (a) Schematic of a bicellar nanodisc with a radius, \( R \) and a bilayer thickness, \( b \). A Schultz function is used to account for the polydispersity of \( R \) when fitting the data. (b) Schematic of an elliptical cylinder used to model the ribbon structure, where \( a \), \( b \) and \( l \) are the minor and major semi-axes of the elliptical cross-section and the length of the cylinder, respectively. (c) The polydisperse spherical shell model used to describe ULV structure. \( R_i \) is the inner radius of the vesicle, and \( t \) is the lipid bilayer thickness. During the fitting procedure, the SLDs inside and outside of the shell are held to the same value, which is that of \( D_2O \). A Schultz function is included to account for the polydispersity \( R_i \). [S2]
Supporting Information

\[ A_{ves}(q, R_i) = \frac{4\pi(\rho_{lip} - \rho_D)}{q^3} \left\{ \sin[q(R_i + t)] - q(R_i + t) \cdot \cos[q(R_i + t)] \right\} - \sin(qR_i) + qR_i \cdot \cos(qR_i) \]

\[ F(R_i) = \frac{p^2 \left( \frac{R_i}{\langle R_i \rangle} \right)^{(1-p^2)/p^2} e^{-\frac{R_i}{\langle R_i \rangle}}}{\langle R_i \rangle^{1/p^2}} \]

where \( \Gamma(x) \) is Gamma function.

2. Nanodisc morphology confirmed by Transmission Electron Microscopy (TEM)

TEM experiments are performed using a FET Tecnai T12 TEM with an accelerating voltage of 80 kV. 1 w/v % uranyl acetate (Structure Probe Inc., West Chester, PA, USA) is used as a negative staining agent. (Details of the experimental procedures are found in reference S6.) The round and elongated shapes observed in the TEM micrograms are presumably the top and side views of discs, respectively (Figure S2). However, it should be noted that some of the observed structures may have formed during the drying process of sample preparation. Compared with DMPC/CHAPSO [Figure S2(a)], the highly charged DMPC/DMPG/CHAPSO sample [Figure S2(b)] formed smaller (or less elongated) structures, consistent with the SANS data.

Figure S2. TEM micrographs of 0.25 wt.% (a) DMPC/CHAPSO and (b) DMPC/CHAPSO/DMPG (R=0.1) samples, which were diluted 10X. The round and rod shapes seen in the TEM images are thought to correspond to top- and edge-views of nanodiscs, respectively or nanodiscs and ribbons, respectively.
### 3. Thermal Reversibility of Morphologies in High-$C_{lp}$ DMPC/DMPG/CHAPSO Mixtures

The ability to reverse the morphological changes that took place as a result of changing temperature is a good indicator of the system’s stability. For instance, it has been shown that high-$C_{lp}$ DMPC/DMPG/DHPC mixtures can be cycled through a variety of morphologies (e.g. bicelles and lamellae) repeatedly and reversibly [S7]. To investigate whether the same behavior exists in DMPC/DMPG/CHAPSO mixtures, SANS experiments were carried out under repeated low and high temperature (low T $\rightarrow$ high T $\rightarrow$ low T $\rightarrow$ high T). SANS data (Fig S3) indicate no significant variation in morphology or $d$-spacing for the R=0.10 sample at $C_{lp}$ = 7.5, 12.5 and 25 wt%. This reversibility suggests that the morphologies corresponding to the temperature variations are either thermodynamically stable or at the same local minimum energy. It should be noted that at lower $C_{lp}$.

![Figure S3. Reversibility of SANS-observed thermotropic morphology changes undergone by DMPC/DMPG/CHAPSO, R=0.10, mixtures at different $C_{lp}$ (25, 12.5 and 7.5 wt%) upon repeated temperature cycling between 280 K and 340 K: (a) 280 K and (b) 340 K, where 1 and 2 represent, respectively, data obtained prior and subsequent to temperature cycling.](image-url)
reversibility may not be present.

4. Temperature dependence of critical micelle concentrations (CMCs) of CHAPSO and DHPC

Dynamic light scattering experiments were performed using an ALV/CGS-3MD goniometer system (ALV, Langen, Germany) with a 22mW He-Ne laser (wavelength=632 nm). An avalanche photo diode (APD)-based detector with a pseudo-cross correlator was used to improve the accuracy of the short-time measurement. All the data below were collected at a scattering angle of 90 degree. Each sample was measured for 5 min, and the scattering intensity (count rate) was averaged for the CMC plot (Figure S4). Detergent concentrations ranged from 2 to 40 mM. With increasing concentration of lipid, two distinct slopes are observed indicative of a transition from unimers to micelles. The CMC of the detergent is determined from the intersection of the two lines. The results obtained from this method are consistent with CMC values of CHAPSO and DHPC, which were determined by other techniques [S8-9]. The data also show a negligible temperature

Figure S4: Scattering intensity (count rate) as a function of detergent concentration for (a) CHAPSO and (b) DHPC. CMCs are also determined at different temperatures (i.e., 25 and 50 °C).
dependence.

References