Supporting Information

Structure of Phospholipid Mixed Micelles (Bicelles) Studied by Small-Angle X-ray Scattering

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Models

The main function fitted for all the models is the intensity expression:

$$I_{mod}(q) = nP(q)S(q) \quad \text{(Eq. S1)}$$

where n is the number density of particles, $P(q)$ is the form factor describing the structure of the particles, and $S(q)$ is an effective structure factor, which accounts for interparticle interference effects. The parameter q is the scattering vector modulus $q = 4\pi\sin(\theta)/\lambda$, where 2θ is the scattering angle, and λ is the wavelength of the X-rays. The form factors on absolute scale use the excess scattering length density differences ($\Delta\rho_i$) for the head and tail of the molecule with respect to the solvent:

$$\Delta\rho_{head} = \left(\frac{Z_{head}}{V_{head}} - \frac{Z_w}{V_w}\right) r_T \quad \text{(Eq. S2)}$$

$$\Delta\rho_{tail} = \left(\frac{Z_{tail}}{V_{tail}} - \frac{Z_w}{V_w}\right) r_T \quad \text{(Eq. S3)}$$

where Z_i is the number of electrons in the lipid head or tail, V_i is the volume of the head or tail, Z_w is the number of electrons of a water molecule, V_w is the volume of a water molecule, r_T is the Thomson radius of an electron (2.8179×10^{-15} m). Volumes of the entire molecules and the hydrocarbon tails are taken from the literature (see Table S 1 for references). To find the volume of the head groups we subtracted the tail volume from the total volume for both the molecules and used the average of those values, since the head group is the same for DHPC and DMPC.

Table S 1 Volumes used in the models

Molecule (or part of)	Temperature (˚C)	Volume (Å^3)	Reference
DHPC (total)	25	677.2	[1]
DHPC tails	37	329.8*	[2]
DHPC head	25/37	347.4	[1-2]
DMPC (total)	37	1107.8	[3]
DMPC tails	37	766.6	[2]
DMPC head	37	341.2	[2-3]
Head group average	25/37	344.3	

*Value is extrapolated from a series of temperature dependent volumes

Model 1 - Core-shell ellipsoid based on the form factor of an ellipsoidal of revolution[4]

Form factor of core-shell ellipsoid:

$$P_{sc-ell}(q) = \int_0^{\frac{\pi}{2}} \left(\Delta\rho_{shell} V_{tot} A_{sph}(qr_{tot}) e^{-q^2\sigma_{outer}{}^2/2} + (\Delta\rho_{core} - \Delta\rho_{shell}) V_{core} A_{sph}(qr_{core}) e^{-q^2\sigma_{inner}{}^2/2}\right)^2 \sin(\alpha)\, d\alpha \quad \text{(Eq. S4)}$$

$$A_{sph}(qr) = \frac{3[\sin(qr) - qr\cos(qr)]}{(qr)^3} \quad \text{(Eq. S5)}$$

$$r_{tot} = R_{tot}(\sin^2\alpha + \varepsilon_{tot}{}^2\cos^2\alpha)^{½} \quad \text{(Eq. S6)}$$

$$r_{core} = R_C(\sin^2\alpha + \varepsilon_{core}{}^2\cos^2\alpha)^{½} \quad \text{(Eq. S7)}$$

where R_{tot} is the outer polar radius of the ellipsoid, R_C is the core radius and the relations between them are $R_{tot} - R_C = \varepsilon_{tot}R_{tot} - \varepsilon_{core}R_{Core} = D_{shell}$, where D_{shell} is the shell thickness. The parameter ε_{core} is the eccentricity of the ellipsoidal core and ε_{tot} is the eccentricity of the total ellipsoid. The

term $e^{-q^2\sigma^2/2}$ is the interface smearing function that accounts for the interface between core and shell, and shell and solvent, respectively, not being sharp. The parameter σ is the width of the Gaussian smearing function that the core and the total ellipsoid, respectively, are convoluted by to introduce the smearing. The model also includes a fitted flat scattering background. Model 1 is used for the pure DHPC sample and the calculation of the aggregation number is straight forward

$$N_{agg} = \frac{V_{core}}{V_{DHPC\ tail}} \quad \text{(Eq. S8)}$$

where the volume of the core, V_{core} for an ellipsoid of revolution is

$$V_{core} = \frac{4}{3}\pi R_C^3 \varepsilon_{core} \quad \text{(Eq. S9)}$$

The shell thickness *D* is a fit parameter, which allows the shell to accommodate water as the volume of the shell can be larger than the 'dry' volume of the headgroups. The total scattering of the shell is $N_{agg}\Delta\rho_{head}V_{head}$ and the shell volume is $V_{shell} = \frac{4}{3}\pi R_{tot}^3 \epsilon_{tot} - V_{core}$, so that the excess scattering length density of the shell is $\Delta\rho_{shell} = N_{agg}\Delta\rho_{head}V_{head}/V_{shell}$.

Model 2 - Short core-shell cylinder with an ellipsoidal cross section based on the form factor of an ellipsoidal cylinder[5]

The form factor of a cylinder with an elliptical cross section is:

$$P(q) = \frac{2}{\pi}\int_0^{\frac{\pi}{2}}\int_0^{\frac{\pi}{2}}\left[\Delta\rho V \frac{2J_1(qr(R_a,R_b,\phi,\alpha)}{qr(R_a,R_b,\phi,\alpha)}\frac{\sin(qW_c\cos\alpha)}{qW_c\cos\alpha}\right]^2 d\phi\sin\alpha\ d\alpha \quad \text{(Eq. S10)}$$

where $J_1(x)$ is the first order Bessel function of the first kind, *Wc* is the half length of the cylinder and *r* is:

$$r(R_a,R_b,\phi,\alpha) = \left[{R_a}^2\sin^2\phi + {R_b}^2\cos^2\phi\right]^{1/2}\sin\alpha \quad \text{(Eq. S11)}$$

where R_a and R_b are the cross-section semi axes, φ and α are the orientational angles of the cylinder with respect to the scattering vector **q**. To obtain the expression for a core-shell cylinder, the same approach is used as for model 1. Form factor amplitudes ($A_i(q)$) for the core and total particle, respectively, weighted by the related volumes and scattering length density differences, are added to build the core-shell structure amplitude and then squared to obtain the form factor amplitude The final expression contains integrals over the orientation angles. The model also includes a fitted flat scattering background. Model 2 is used for the micelles with the composition $Q = 0.5$. To calculate the aggregation number both the volume of the tails of DHPC and DMPC must be included

$$N_{agg} = \frac{V_{core}}{V_{DHPC\ tail}/(1+Q)+V_{DMPC\ tail}\,Q/V_{DHPC\ tail}} \qquad \text{(Eq. S12)}$$

Similar to Model 1, the total excess scattering of the head groups and the volume of the head group shell are used for calculating the excess scattering length density of the shell.

Model 3 - Core-shell rectangular cuboid form factor[6].

In order to fit the data for Q = 1, 2, 3.2 and 4, it was necessary to associate part of the DMPC hydrocarbon tails with the shell of the aggregate. The core is composed of the DHPC tails and the end of the DMPC tail (5 carbons from each hydrocarbon tail, i.e., 10 carbons per molecule), and a fraction F of the rest of the DMPC tails. The shell is composed of all head groups and the remaining fraction (1 – F) of the DMPC tails. When fitting the model to the data, this "rest" (see Figure S1) of the DMPC tails can then be shifted into and out of the core by the fit parameter F. The form factor of a rectangular cuboid is:

$$P(q, W_C, b, c) = \frac{2}{\pi}\int_0^{\pi/2}\int_0^{\pi/2}\left[\Delta\rho V \frac{\sin(q\,W_c\,\sin\alpha\,\cos\beta)}{q\,W_c\,\sin\alpha\,\cos\beta}\frac{\sin(q\,b\,\sin\alpha\,\sin\beta)}{q\,b\,\sin\alpha\,\sin\beta}\frac{\sin(q\,c\,\cos\alpha)}{q\,c\,\cos\alpha}\right]^2 \sin\alpha\, d\alpha\, d\beta$$

(Eq. S13)

where $2W_C$, $2b$ and $2c$ are the dimensions of the cuboid, α and β are the orientational angles with respect to the scattering vector **q**. The aggregation number is

$$N_{agg} = \frac{V_{core}}{V_{DHPC\,tail}/(1+Q)+(V_{DMPC\,tail-end}+F\,V_{DMPC\,tail-rest})Q/(1+Q)} \quad \text{(Eq. S14)}$$

Similar to Model 1, the total excess scattering of the head groups and the volume of the head group shell are used for calculating the excess scattering length density of the shell.

To obtain the expression for a core-shell rectangular cuboid, the same approach is used as for models 1 and 2. Form factor amplitudes for the core and total particle, respectively, weighted by the related volumes and scattering length density differences, are added to build the core-shell structure amplitude and then squared to obtain the form factor amplitude. The model also includes a fitted flat scattering background.

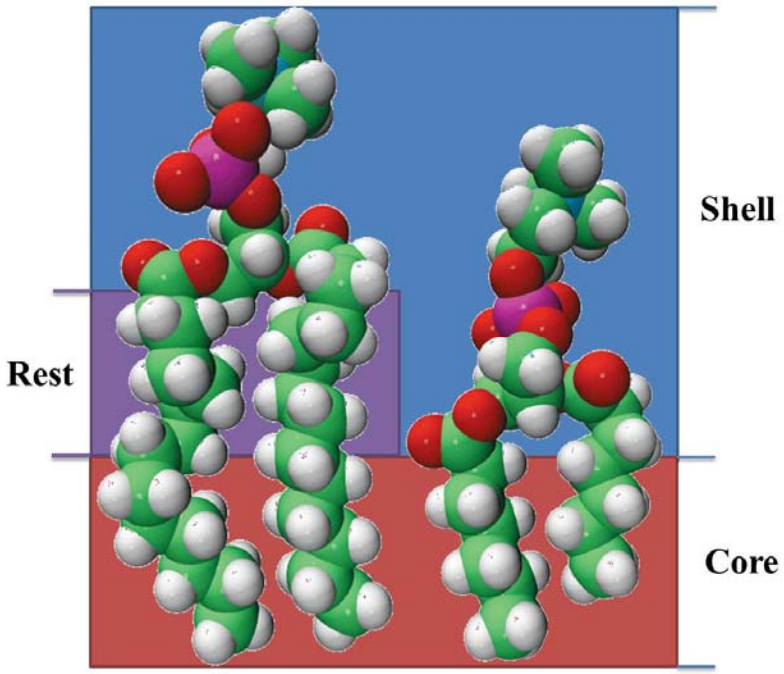

Figure S 1 Schematic explanation of the division of molecules into core and shell.

Model 4 - Combination of a multilamellar structure made up of bilayers and free bilayers[7]

The data from samples containing only DMPC were fitted using a model containing contributions from both a multilamellar structure and single bilayers[7]

$$I_{model}(q) \propto \frac{S_{mul}\, F(q)^2\, S(q) + S_{bi}\, F(q)^2}{q^2} \qquad \text{(Eq. S15)}$$

where S_{mul} is the scale for the multilayer contribution, $F(q)$ is the form factor amplitude, $S(q)$ is the structure factor, S_{bi} is the scale for the single bilayer and q is the scattering vector modulus. The model also includes a fitted flat scattering background.

The form factor amplitude of the cross section uses box functions smeared by Gaussians and it includes contributions from the head group (shell) and the core of the bilayer.

$$A(q) = \Delta\rho_H(2D_W\ + 2W_c)\exp\left(\frac{-\sigma_H{}^2q^2}{2}\right)\frac{\sin(q(D_W+W_c))}{q(D_W+W_c)} - (\Delta\rho_C - \Delta\rho_H)2\mathrm{W}_c\exp\left(\frac{-\sigma_C{}^2q^2}{2}\right)\frac{\sin(qW_c)}{qW_c}$$

(Eq. S16)

where $A(q)$ is the form factor amplitude, σ_i is the standard deviation of the Gaussian defining the electron density profile of the head or core, $\Delta\rho_i$ is the scattering length density difference of the component, q is the scattering vector, D_W is the thickness of the head group shell, and W_c is the half width of the core. The index H is for head group and index C for core.

The structure factor is from the modified Caillé theory[8 8b] and given as

$$S(q) = N + 2\sum_{k=1}^{N-1}(N-k)\cos(k\ q\ d)\exp\left(-\left(\frac{d}{2\pi}\right)^2 q^2\eta\ \gamma\right)(\pi k)^{-\left(\frac{d}{2\pi}\right)^2 q^2\eta} \qquad \text{(Eq. S17)}$$

where N is the number of bilayers in the stack, q is the scattering vector modulus, d is the repeat distance of a bilayer, η is the Caillé parameter and γ is Euler's constant. Polydispersity in the number of layers were included using a Gaussian of width σ_N.

All data

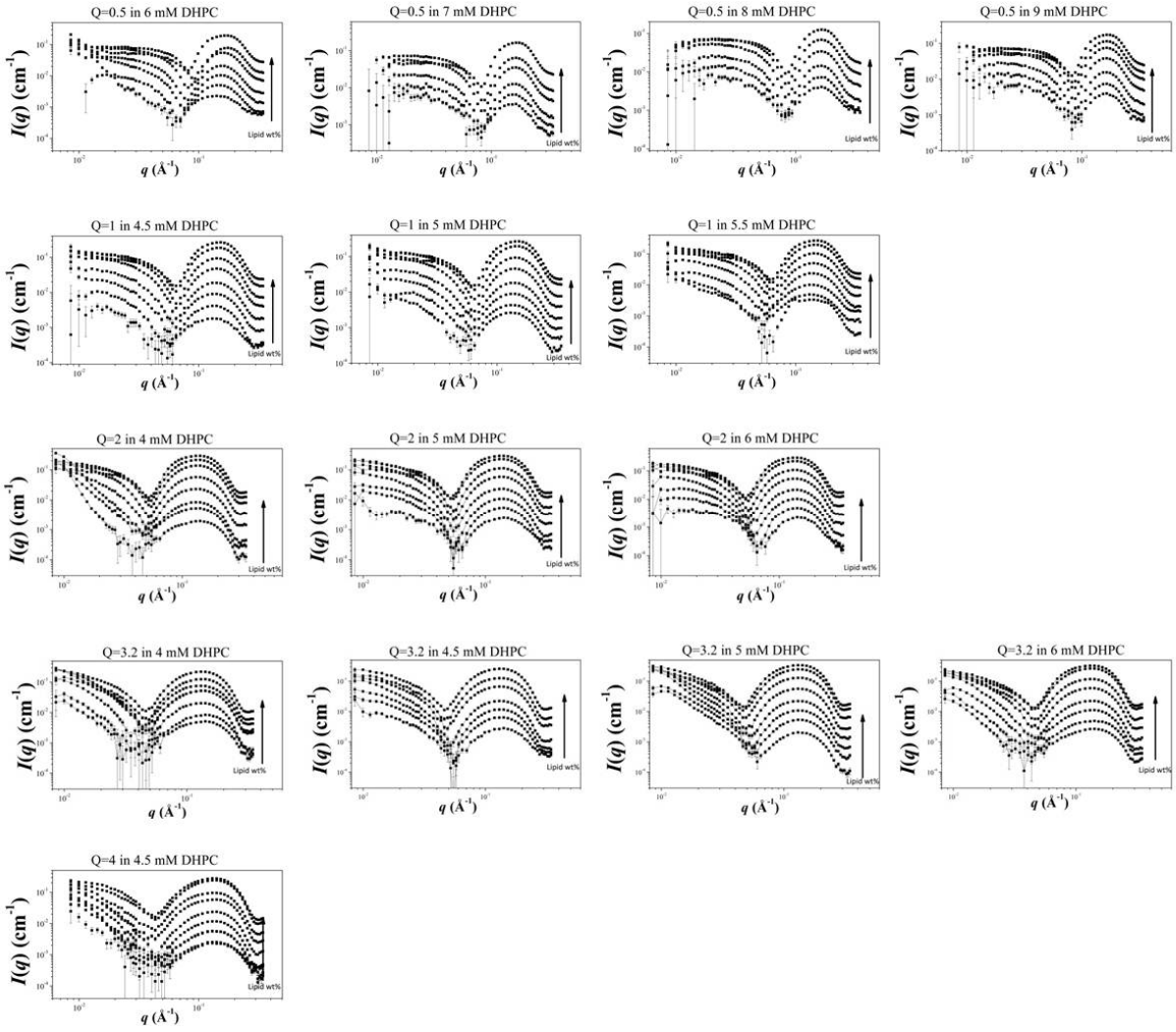

Figure S 2 Overview of scattering data used for determining appropriate DHPC concentration in the dilution media

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