Supplemental Information for

Low q Bicelles are Mixed Micelles

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Methods

Preparation of bicelle samples

For SAXS, mixtures of DMPC/DHPC were prepared from q = 0.1 to 1.0 in increments of 0.1 at 6% total amphiphile weight per volume in 10 mM phosphate buffer, pH 6.6 with 7% D2O and diluted with buffer to adjust the total amphiphile concentration as needed. A freeze-thaw cycle was performed on mixtures which were not optically transparent after vortex mixing. For SANS experiments, similar mixtures were prepared at q = 0.3 and 0.7 for 6% total amphiphile concentrations using DMPC-d54, an analog of DMPC with deuterated acyl chains, as the lipid component.

For fluorescence anisotropy experiments, 2.3% (w/w) DMPC – DHPC mixtures having q values ranging from 0 to 1.50 (0.05, 0.30, 0.50, 0.75, 1.00, 1.50) were prepared on a 3-gram scale using the following methodology. First, a DMPC stock was made by dissolving the lipid to 50 mg/mL in chloroform. To a 2 mL microcentrifuge tube, DMPC in chloroform was added (83, 370, 510, 630, 720, and 827 μL respectively). After this, DPH dissolved in methanol was added to each tube to give a final concentration of 6 μM. The samples were then dried under vacuum overnight. The samples were then hydrated by the addition of 2.78 mL of water, and 75μL buffer (400 mM HEPES, 4.0 M NaCl pH 7.4). Finally, DHPC was added as a 25% (w/w) stock to achieve clear homogeneous solutions (250, 196, 169, 146, 129, and 107 μL respectively).

Vesicles were prepared by dissolving 16 mg of DMPC in 600 μL of chloroform. To this DPH dissolved in methanol was added to give a final concentration of 30 μM (1:500 DPH to lipid molar ratio). The sample was then dried under vacuum overnight. The sample was then rehydrated using 2.78 mL of water and 75 μL buffer (400 mM HEPES, 4.0 M NaCl pH 7.4). This solution was sonicated for 5 minutes using a microtip to generate small unilamellar vesicles. The solution became clear and was then centrifuged at 20,000 x g for 5 minutes to remove titanium and large lipid aggregates. This solution was then allowed to sit 12 hours to allow fusion of small unilamellar vesicles. The solution was then centrifuged at 20,000 x g for 5 minutes. The supernatant was diluted 4 fold and was then used in fluorescence experiments.

SAXS acquisition

Small-angle scattering measurements were collected at the Advanced Photon Source (Argonne, IL) and processed as described previously.[1-2]

SANS acquisition

Solvent deuteration was modified for contrast variation experiments by changing the D_2O content in the buffer from 0% to 99% via dialysis. Neutron scattering data were obtained at the High-Flux Isotope Reactor (Oak Ridge, TN).The experimental match point for each data set was calculated from the square root of the total signal intensity as a function of the percentage of D_20 in the solvent.[3]

SANS Fitting Approach

The SasView cylinder-based core-shell bicelle model was used for all data fits. The scattering length density (SLD) of the solvent was calculated using known SLD values of $-5.5x10^{-7}$ $Å^{-2}$ and $6.3x10^{-6}$ $Å^{-2}$ for H_2O and D_2O, respectively. The chemical composition of the phosphocholineheadgroup along with reported molecular volume was used to calculate the face SLD to be $1.44\ x10^{-6}$ $Å^{-2}$.[4] This procedure was repeated to determine SLDs for whole DHPC ($6.71x10^{-7}$ $Å^{-2}$),whole d54-DMPC ($5.39x10^{-6}$ $Å^{-2}$), DHPC tail ($-7.49x10^{-8}$ $Å^{-2}$), and d54-DMPC tail ($7.04x10^{-6}$ $Å^{-2}$).[5-8] Ratios of DHPC and d54-DMPC whole lipid SLDs were calculated for the rim SLD, while ratios of DHPC and d54-DMPC tails were calculated for the core SLD. These SLD values were confirmed using the online resource MULCh: modules for the analysis of contrast variation data.[9]The face thickness range was set to 7-12 Å based on known length of the phosphocholineheadgroup while the rim thickness range was set between 10-25 Å based on the lengths of DHPC and d54-DMPC.[4, 7-8, 10]The original length range was 15-36 Å based on the lengths of two DMPC or DHPC tails as well as the SAXS data. The radius started at 20 Å but had no set range since there was no prior evidence to the radius length. The q=0.7 6% (w/w) total amphiphile concentration (C_L) and q=0.3 6% C_L Q ranges were set as 0.02-0.20 and 0.035-0.3 respectively to remove noise. The following solvent D_2O percentages were fit for each set based on available data as well as not fitting within 20% of the contrast match point (CMP): q=0.7 6% C_L: 0, 10, 20, 30, 80, 90, 100; and q=0.3 6% C_L: 0, 10, 20, 70, 80, 90, 100. All spectra in a set were fit using the above parameter ranges and SLD values corresponding to the amount of mixing observed in the MD simulations (q=0.3: 76% DHPC and 24% DMPC corresponding to a core

SLD of $1.64x10^{-6}$ $Å^{-2}$and a rim SLD of $1.80x10^{-6}$ $Å^{-2}$; q=0.7: 49% DHPC and 51% DMPC corresponding to a core SLD of $3.56x10^{-6}$ $Å^{-2}$and a rim SLD of $3.08x10^{-6}$ $Å^{-2}$). The SLD values were then allowed to vary and values for radius, rim thickness, face thickness, and length as well as SLDs for core and rim were converged upon after several rounds of fitting. The percent of DHPC and DMPC in the core and rim (Table 1, Table S1, Table S2, Table S5) were calculated by the ratios of pure DHPC and DMPC SLDs (Table S3) [SLD=(%DMPC)(DMPCSLD)+(%DHPC)(DHPC SLD) where the percentages of DMPC and DHPC add to 100%. The radii (Table 1) were calculated as radius+rim and ½length+face thickness (Figure S4).

Anisotropy Measurements and Curve Fitting

Fluorescence emission spectra were acquired with magnetic stirring using a 1 × 1 cm quartz cuvette on an Agilent Eclipse fluorometer (Santa Clara, CA). The excitation and emission slit widths were both set to 5 nm. The fluorescence emission intensity was measured (excitation 355 nm, emission 430 nm) with polarizers parallel to each other (both oriented at 0° from vertical) and repeated in the perpendicular configuration (excitation 0° and emission 90°). The correction factor for emission monochromator transmission efficiency was obtained from the ratio of emission intensity at 0° and 90° with the excitation polarizer oriented at 90°. Melting curves were generated for both pure DMPC vesicles and DMPC-DHPC bicelles by examining the change in DPH anisotropy as a function of temperature over the range of 2 - 36 °C. Each melting temperature was determined from the inflection point of the melting curve using a sigmoidal fit in Igor Pro 6.22A (WaveMetrics, Inc., Lake Oswego, OR). Melting temperatures obtained represent the average of two experiments.

Molecular dynamics (MD) simulations

We performed MD simulations for mixtures of DMPC (lipid)and DHPC (detergent) in water. The Gromacs v. 4.6.5 software [11]with the Stockholm lipid(Slipid) force field [12-14]and TIP3p water model [15]were used. The starting configuration constituted a loose spherical aggregate of DMPC and DHPC in a water cube. The ratio of DMPC/DHPC was varied to produce a range of q-values (0.3 and 0.7), resulting in 6 different system setups with varying numbers of lipids and detergents in the box. The summary of simulations is given in Table S6. The hydration level was selected at 1/400 lipid (and detergent) to water

ratio, which corresponds to ~100 mM and ~9%w/v. Aggregation of lipids and detergents in the course of simulation resulted in one or more bicelles/micelles in the simulation box. We considered the largest and most stable aggregate for each setup; the actual number of molecules in the considered aggregate was therefore lower than the total number of molecules in the box. Note that the actual q-value of the aggregate differed from the total value in the box (see Table S6).

The standard simulation parameters for Slipids force field were used. The temperature was maintained at 303 K with the v-rescale thermostat[16]with a relaxation time of 1 ps. The pressure was maintained at 1 bar with the isotropic coupling scheme and Parinello-Rahman barostat[17]with the time constant of3 ps. A cut-off of 1 nm was used for van der Waals and electrostatic interactions; the energy-pressure dispersion correction and the PME method[18-19]were used for long-range interactions. The integration time step was 2 fs, the neighbor list was updated every 10 steps. The simulation time was 1 microsecond for each setup.

To characterize the mixing of lipids and detergents, we calculated the enrichment values of DHPC around DMPC. The enrichment value is given by the ratio of the local concentration of DHPC around DMPC to the bulk concentration (defined as the molar ratio of DHPC and corresponding to 1/(q+1) at the given q-value). The calculations were performed using custom python scripts employing MDAnalysis library [20]. To characterize the bicelle shape, the bicelle headgroup layer was fitted to a 3D ellipsoid using custom Matlab scripts, and the principal radii of the ellipsoid were calculated. We also calculated the SAXS scattering profiles of the bicelle structures obtained in MD using FoXS software.[21]

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Supplemental Tables

Table S1: Fit parameters for the SANS profiles for q=0.7 bicelles using the core-shell bicelle model						
D_2O	Radius	Rim	Face	Length	Core DMPC	Rim DHPC
%	(Å)				%	
0	17	12	7	19	63	59
10	15	11	7	23	52	49
20	14	11	7	18	52	49
30	11	11	7	25	51	49
80	27	19	12	36	52	49
90	19	19	12	36	55	52
100	16	13	7	32	59	51
AVG	17	15	8	27	55	51
Range	10-27	11-23	7-12	18-36	51-63	49-59

Table S2: Fit parameters for the SANS profiles for q=0.3 bicelles using the core-shell bicelle model						
D_2O	Radius	Rim	Face	Length	Core DMPC	Rim DHPC
%	(Å)				%	
0	6	12	7	16	24	77
10	4	13	7	15	23	76
20	5	10	7	15	32	77
30	21	10	12	23	24	78
80	15	11	7	21	60	100
90	14	11	7	19	62	100
100	15	11	7	18	56	100
AVG	11	11	8	18	40	87
Range	4-21	10-13	7-12	15-23	23-62	76-100

Table S3: Scattering length density for different bicelle components	
Component	**Scattering length density (x 10^{-6} $Å^{-2}$)**
DMPC with deuterated alkyl chains	5.39
DHPC (rim in ideal bicelle)	0.671
PC head group (face)	1.44
Deuterated dimyristoyl chains (core in ideal bicelle)	7.04
Dihexanoyl chains	-0.075

Table S4: Match point comparison for bicelles				
Bulk q value	**Effective q value***	**Match point (% D_2O)**		
		Theoretical for bulk q value	**Theoretical for effective q value**	**Experimental**
0.70	0.78	54	56	57
0.30	0.32	40	41	41

*Effective q-value is calculated by subtracting the critical bicelle concentration (cbc) of DHPC monomer from the overall concentration of amphiphiles (q_{eff}=[DMPC]/{[DHPC]-CBC} CBC_{DHPC}=7mM)

Table S5: Comparing q=0.3 and q=0.7 bicelles				
	q=0.7		q=0.3	
	Average	Range	Average	Range
Radius	17	10-27	11	4-21
Rim Thickness	15	11-23	11	10-13
Face Thickness	8	7 - 12	8	7 - 12
Length	27	18-36	18	15-23
Core % DMPC	55	51-63	40	23-62
Rim % DHPC	51	49-59	87	76-100

Table S6: Bicelle properties from MD simulations								
#	n (n*)	q (q*)	Rg,nm	L,nm	a,nm	b,nm	c,nm	comment
1	34 (33)	0.3 (0.3)	1.6	2.6	2.4	2	1.8	mixed micelle
2	75 (41)	0.3(0.32)	1.7	2.8	2.4	2.1	1.8	mixed micelle
3	75 (74)	0.5(0.51)	2.1	3.5	3.0	2.5	2.3	ellipsoidal bicelle
4	150(110)	0.5(0.72)	2.5	4.2	4.2	2.8	2.3	de-mixed bicelle
5	120(116)	0.7(0.76)	2.6	4.2	4.0	3.1	2.2	de-mixed bicelle
6	150 (96)	0.7(0.92)	2.4	4.2	3.5	3.1	2.2	de-mixed bicelle

Here n is total number of lipids and detergents in the simulation box, n* is the actual number of lipids and detergents in the aggregate, q is q-value (lipid-to-detergent ratio) in the simulation box, q* is the actual q-value in the bicelle (or micelle), Rg is radius of gyration, L parameter is determined from the second peak of the SAXS profile using FoXS software, a, b, and c are the principal radii of an ellipsoid, fitted to the bicelle surface (headgroups layer).

Supplemental Figures

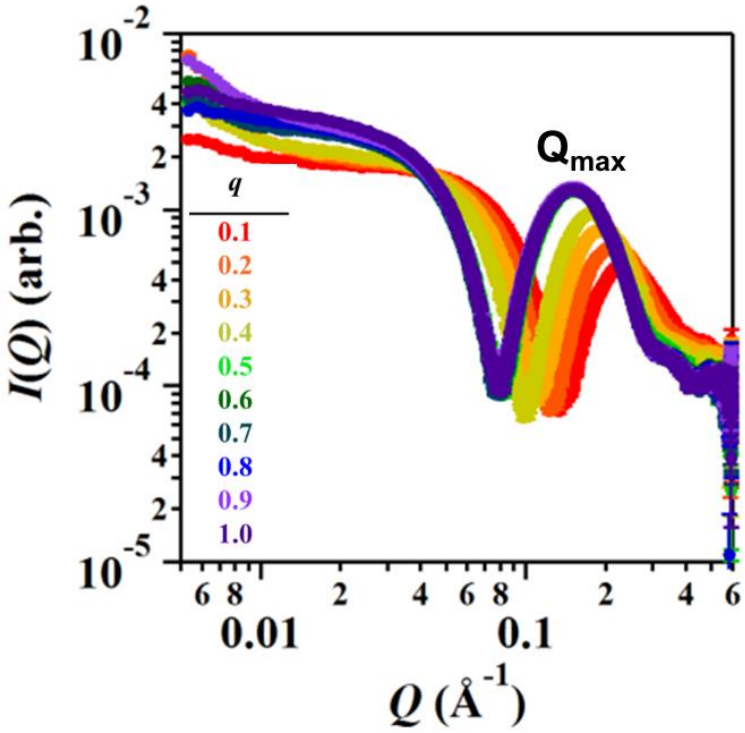

Figure S1. SAXS scattering profiles for bicelles with q-values ranging from 0.1 to 1.

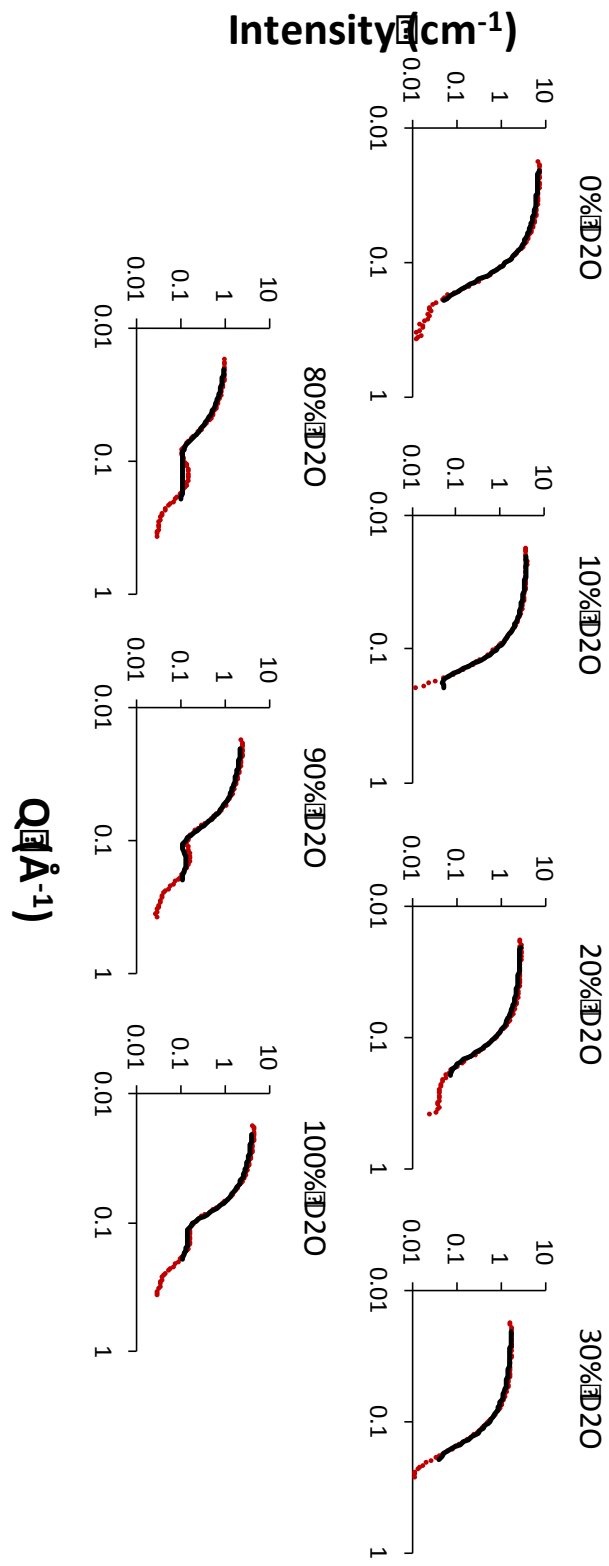

Figure S2. Core-shell bicelle model fits (black) to the experimental SANS scattering profiles (red) for q = 0.7 bicelles in different solvent D_2O concentrations. The parameters are given in Table S1 and a schematic of parameters is provided as Figure S4.

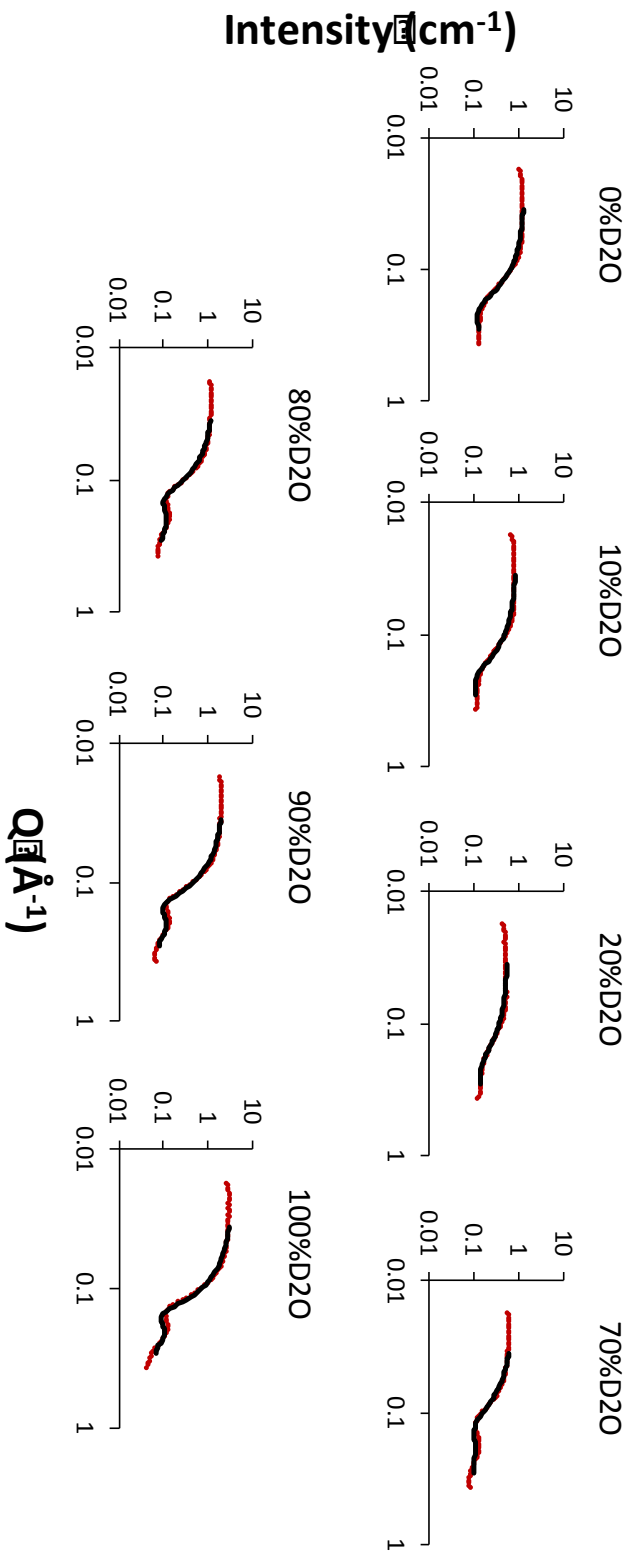

Figure S3. Core-shell bicelle model fits (black) to the experimental SANS scattering profiles (red) for q = 0.3bicelles in different solvent D_2O concentrations. Parameters are given in Table S2and a schematic is provided as Figure S4.

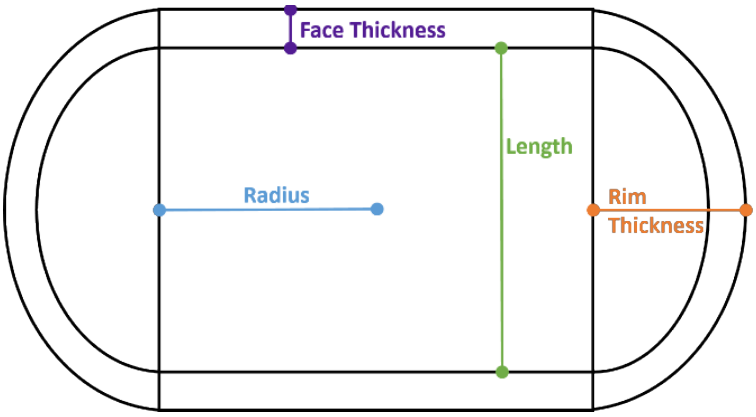

Figure S4. Core-shell bicelle model used to fit the experimental SANS scattering profiles.