Low q Bicelles are Mixed Micelles

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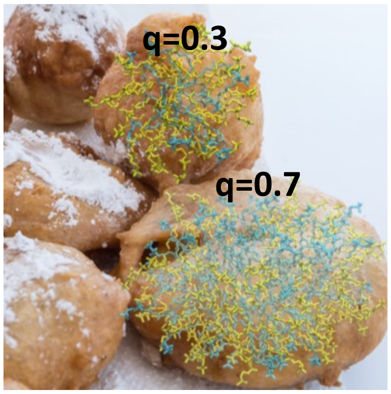
ABSTRACT. Bicelles are used in many membrane protein studies as they are thought to be more bilayer-like than micelles. We investigated the properties of “isotropic” bicelles by SANS, SAXS, fluorescence anisotropy, and MD. All data suggest that bicelles with a q-value below 1 deviate from the classic bicelle which contains lipids in the core and detergent in the rim. Thus, not all isotropic bicelles are bilayer-like.

TOC GRAPHICS



For nearly two decades, bicelles have had a wide variety of applications, most commonly as bilayer mimics for structural[1-14] and functional[15-20] investigations of membrane-associated proteins. A bicelle is a bilayer micelle; a disc-shape aggregate typically formed by a mixture of detergents (Figure 1A) and lipids (Figure 1B). Bicelle self-assembly was first determined in 1984[21-22] and since, bicelles have been characterized using many methods such as small angle neutron scattering (SANS),[23-24] and NMR.[25-31] The classically described ("ideal") bicelle contains a central disk-shaped lipid bilayer encircled by a rim of detergents which screen the hydrophobic lipid tails from water (Figure 1C).[32-33] Thus, in the “ideal” bicelle the lipid and detergent molecules are segregated spatially. Bicelles vary in size and shape depending on the ratio of lipid to detergent (known as the q-value)[34], the structure of the lipid and detergent monomers[25], total concentration of amphiphiles[35-36], and temperature[29-30]. For solution NMR structural studies, bicelles with low q-values (< 0.7; also known as fast-tumbling “isotropic” bicelles) have demonstrated some utility for polytopic integral membrane proteins.[24, 31, 37-38] Several of these studies suggest that the stabilization of membrane protein fold is due to the more “bilayer” nature of bicelles compared to micelles. That is, the segregated lipid core in bicelles is more similar in structure to the native membrane.

However, recent studies of binary mixtures of detergents of different alkyl chain lengths and head groups indicated that these compositions are fully mixed (Figure 1D).[39-40] This observation led to a hypothesis that bicelles with q-values below 1, for which the detergent concentration is higher than the lipid concentration, may not have segregated lipid cores, as previously suggested.[36] Here, we investigate the structure and segregation of bicelles with q-values less than 1 formed by dihexanoylphosphatidylcholine (DHPC; Figure 1A) and dimyristoylphosphatidylcholine (DMPC; Figure 1B), which have been studied for almost 30 years.[41-44] Several measurable structural and physical properties allow the mixing of lipids and detergents to be tested. As with mixed micelles, the average head group – head group distance (L) is expected to vary with concentration in mixed bicelles and can be determined via small angle X-ray scattering (SAXS), a model-free measurement.[39-40] Since the two components of the bicelle have different scattering length densities, SANS can determine their degree of mixing. The gel to liquid phase transition temperature (T_m), measured using the fluorescence anisotropy of diphenylhexatriene (DPH), is an independent measurement of the extent of bilayer formation. Finally, the shape, size, and lipid-detergent mixing can be quantified directly using molecular dynamics (MD) simulations.

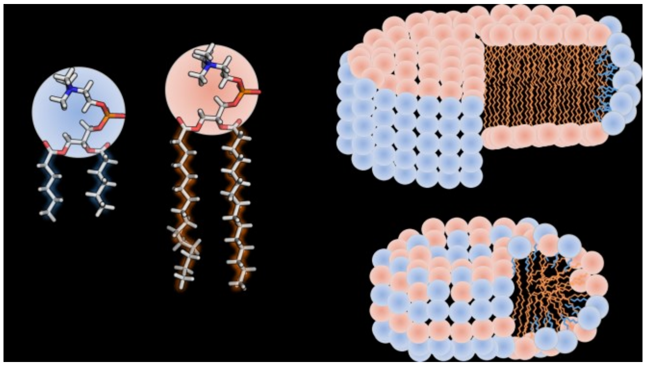


Figure 1. Structures of detergent (A), lipid (B), and cartoons of idealized bicelles (C) and mixed micelles (D).

For an ideal bicelle with a fully segregated core, the average head group to head group distance (L) equals to twice the length of DMPC tails plus a head group (one half on each side) (~43 Å; Figure 1C). But, if the detergent and lipid components mix, then the parameter L will be less and decrease linearly with the concentration of DHPC in the core[39-40]. This structural feature can be determined with SAXS, SANS, and MD simulations.

In the SAXS scattering profile, the second maximum (Q_{max}) corresponds to the distance between opposing electron rich head groups, L.[45] Our data show that at q-values from 0.5 to 1, the model-free dimension L remains constant at 42 Å (Figures 2 and S1), suggesting a segregated bicelle (core). However, below q=0.5 L varies linearly with q-values (Figure 2), indicative of mixing of lipid and detergent in the core. A linear fit produces a y-intercept of 22 Å, the approximate L of pure DHPC.[40, 46]

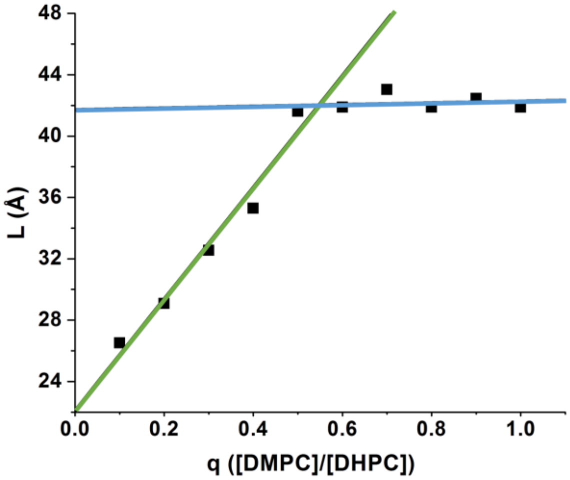


Figure 2. Bicelle dimensions vary with q-values between 0 and 0.5. The L values are measured directly from the SAXS profiles (L; $L=2\pi/Q_{max} \approx 2\,(1.5 + 1.265n_c) + t$, where n_c is the number of carbons in the alkyl chain and t is the head group thickness) for bicelles with varying q (6% (w/w) amphiphile). Linear fits to the data points for $q \leq 0.5$ (green) and data points for $q \geq 0.5$ (blue) are shown.

To further investigate the structure of bicelles, SANS experiments were conducted on bicelles with q-values of 0.3 and 0.7 (see SI for methods) with different solvent scattering length densities (varied percentages of D_2O in H_2O). Each scattering profile (Figure 3, S2, and S3) was fit to the core-shell bicelle model (Figure S4). The obtained dimensions (Table S1, S2, and S5) agree with the SAXS data (Table 1) and provide additional information about the shape. Higher detergent concentrations reduce the size and result in a more spherical shaped bicelle.

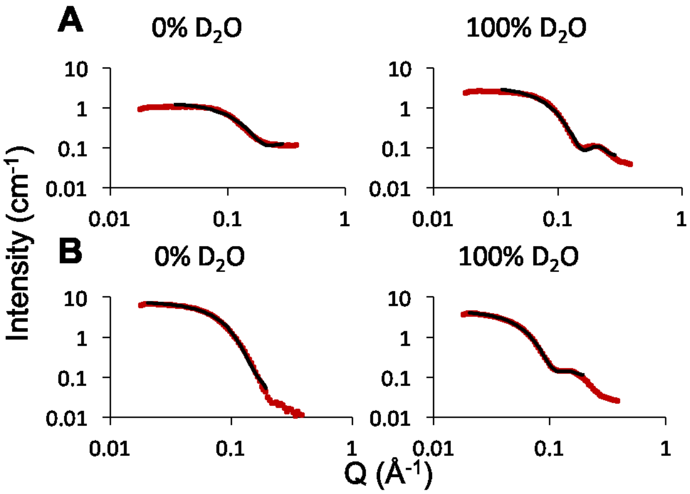


Figure 3. Examples of model fits to SANS scattering profiles of bicelles with q=0.3 (A) and q=0.7 (B) (6% (w/w) amphiphile). The scattering profiles of bicelles with varying percentages of D2O (red) and the fits using the core-shell bicelle model (black; parameters listed in Table S1, S2, and S5, all fits shown in Figure S3 and S4) are shown. Scattering profiles with D2O concentrations ± 20% the match points were not included in the fits.

We also investigated the bicelle properties using all-atom MD simulations (see SI for details) for q<1 bicelles. The simulation results support the SANS and SAXS analysis. At higher q, bicelles become less spherical compared to lower q, as evident from the principal radii of an ellipsoid fitted to the aggregate shape (Table 1, Table S6). This trend is observed in the SANS models; however, the average radii from the SANS models are slightly smaller than the MD models. Some discrepancies are expected due to the differences in the methods related to ensemble properties (multiple bicelles in experiments with a certain degree of polydispersity vs. a single bicelle in the simulation box). However, the MD dimensions are within the ranges obtained from the SANS fits (Table S1, S2, and S6). The small radius is comparable to half the

SAXS-derived L dimension (the SAXS value is smaller by half of a head group since L is the distance measured from the middle of each head group). Furthermore, L values derived from the simulated SAXS data (from the MD obtained bicelle structures) are equal to the SAXS values for q=0.7 (42 Å), but are somewhat larger than those for q=0.3 bicelles (Figure 2, Table S6). Altogether, the difference in the radii between q=0.3 and q=0.7 bicelles is indicative of a structural change in isotropic bicelles above and below q≈0.5. The linear changes in L observed in SAXS experiments, and the overall geometry determined by all three methods suggest that the bicelles with q<0.5 do not have fully segregated lipid cores. We therefore investigated bicelle detergent-lipid mixing.

The classical bicelle model predicts that the concentration of lipids and detergents in the core and rim will deviate from their bulk concentration: the bilayer forming lipid DMPC is expected to preferentially partition to the core, while the detergent DHPC to the rim. We used SANS, MD, and fluorescence anisotropy to investigate the extent of mixing in bicelles with q-values of 0.3 and 0.7.

Table 1. Comparison of experimental and MD derived radii and DHPC concentration for q=0.7 and q=0.3 bicelles.

		Radius (Å)		DHPC		
q		1	2	Expected[#] (%)	Observed (%)	Ratio[^]
0.7	SAXS	-	21	56	-	-
	SANS	32	22		45/51*	0.8/0.9
	MD	40	27		49	0.9
0.3	SAXS	-	16	76	-	-
	SANS	22	17		60/87*	0.8/1.1
	MD	24	19		76	1

Dash indicates the parameter is not determined. See supplemental methods for calculations. [#]If fully mixed, *Average values for the core/rim are given, [^]Ratio of expected to observed DHPC.

In SANS experiments, bicelles formed by DHPC with protonated alkyl chains and DMPC with deuterated alkyl chains were used to distinguish a segregated versus a mixed bicelle.[47] Deviations from the DHPC or DMPC alkyl chain scattering length density (SLD; Table S3) in the "rim" and "core", respectively, indicates lipid/detergent mixing because of the SLD contrast between DHPC and DMPC (Table S3, see supplemental methods for calculation). To verify the SLD values and the effective q-values of the bicelle the theoretical match points and the experimental match points were compared and are in good agreement (Table S4). The SLD values from the core-shell bicelle fits to the SANS data (Table S1 and S2 and Figure S2 and S3) indicate that the core composition is 38 – 77% DHPC and 23 – 62% DMPC in q=0.3 bicelles and the core composition is 37 – 49% DHPC and 51 – 63% in q=0.7 bicelles. Although a broad range of DHPC is observed for the q=0.3 bicelles, fully mixed values (76%) are observed (Table 1).

In MD simulations, the segregation of lipids and detergents can be quantified by comparing the local concentration of DHPC around DMPC (see SI for details). There is on average 76% DHPC around DMPC in q=0.3 bicelles and 49% in 0.7 bicelles. It is interesting to note that full segregation was not observed in either case indicating a certain degree of mixing even in isotropic bicelles with q>0.5. Thus, mixed nearly-spherical micelles were observed for q~0.3 and partially segregated ellipsoid bicelles for q~0.7 (representative structures are shown in Figure 4; the observed characteristics of q=0.3 bicelles are similar to previously reported simulations.[36]

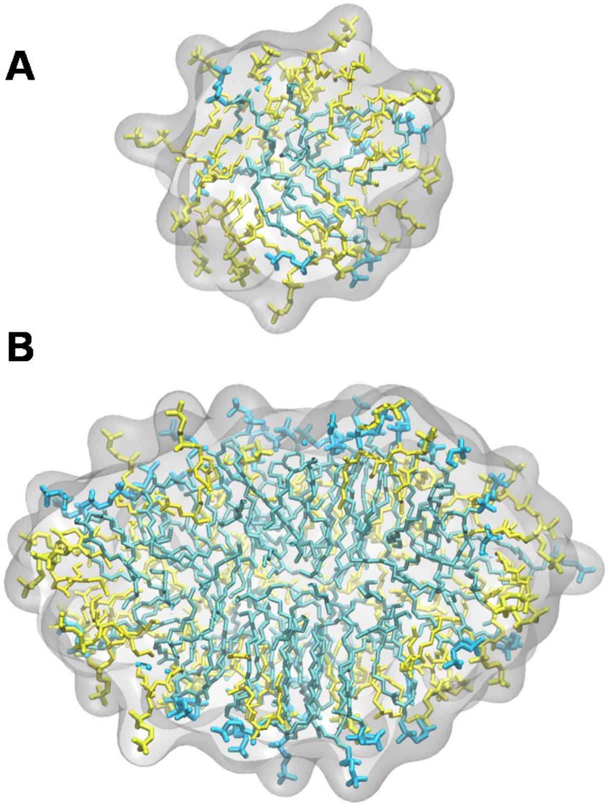


Figure 4. Snapshots from MD simulations of A) q=0.3 and B) q=0.7 bicelles (9% (w/w) amphiphile). DHPC and DMPC are rendered as sticks and colored yellow and blue, respectively.[48] The surface is shown as transparent gray and a portion of the bicelle is removed to view the interior distribution of the DMPC and DHPC tails.

The fluorescence anisotropy of diphenylhexatriene (DPH) detects changes in the fluidity of lipid bilayers as a function of temperature, from which the main phase transition temperature (T_m) of a lipid bilayer can be determined.[49-51] To benchmark this technique, the T_m of pure DMPC vesicles was measured to be 23.1 ± 0.4 °C, consistent with other methods (Figure 5A).[36] The T_m of bicelles is expected to be identical to that of DMPC vesicles if DPH partitions into a region comprised purely of DMPC. However, if significant mixing between DMPC and DHPC occurs, a decrease of T_m compared to DMPC vesicles will be observed, as DHPC disrupts acyl chain packing between DMPC molecules.

Analysis of the melting curves (Figure 5A) yielded the T_m for each q-value. Comparison of the T_m values obtained from the anisotropy measurements to T_m values for ideally mixed DHPC/DMPC vesicles indicates significant differences at all q-values less than 1.0, suggesting that these bicelles do not fit a fully mixed bicelle model (Figure 5B).[36] This data agrees with previously reported T_m values derived from FTIR spectroscopy of various q-value bicelles;[36] however, it does not support recent NMR data indicating similar lipid/detergent mixing in low and high q bicelles.[52] As the q-value increased, the T_m asymptotically approached the melting temperature of a pure DMPC bilayer. Only for q ≥ 1.0 a T_m close to that of a pure DMPC bilayer is obtained (± 1 °C) in agreement with FTIR measurements. (Figure 5B).[36] This suggests a variation in the lipid/detergent mixing at q-values below 1.0, in agreement with the geometrical changes determined with SAXS (Figure 2), and the geometrical and lipid-detergent mixing observed in MD and SANS studies (Table 1 and Figure 4).

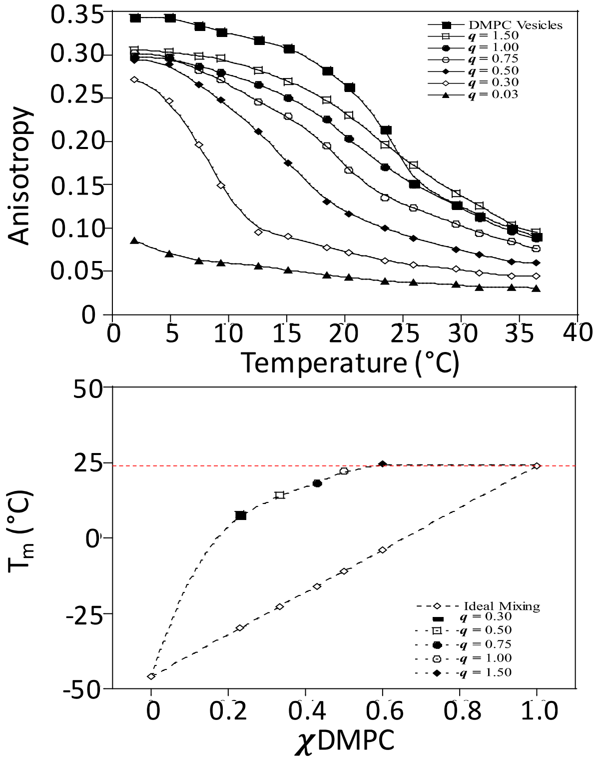


Figure 5. (A) Temperature dependence of the anisotropy value for DPH fluorescence reconstituted into bicelles with varying q values (2.3% (w/w) amphiphile). The inflection point of each melting curve was taken as the T_m. (B) Experimentally determined and calculated T_m values for bicellar solutions as a function of mole fraction DMPC. The linear black dashed line represents T_m values for ideal mixing ($T_m = \chi DHPC \times T_m(DHPC) + \chi DMPC \times T_m(DMPC)$). The red dashed line shows the T_m of pure DMPC bilayers (23.1 °C). Errors in each T_m measurement were approximately ± 0.2 – 0.4 °C.

We have shown using four independent methods – SAXS, SANS, MD, and fluorescence - that bicelle properties vary with the lipid-to-detergent ratio. The data suggest that at q-values below 1 lipid and detergent molecules partially mix, and the bicelle structure deviates from the ideal bicelle model. With increasing q-values, the lipid-detergent aggregates transition from a spherical mixed micelle through an ellipsoidal micelle to a disc-like bicelle.

These results suggest that care should be taken in interpreting membrane protein structural changes in micelles and bicelles. Isotropic bicelles with q-values less than 0.5 likely present a micellar environment, and bicelles with q-values less than 1 may not fully capture bilayer properties. A recent NMR study inferred similar bicelle differences based on protein positioning using PRE experiments.[38] Changes in protein structure in a low-q micelle/bicelle may be related to the micelle shape, size, and fluidity, or specific interactions with the lipids rather than the claimed "more bilayer-like" feature. It is interesting to note that segregation of lipids in low q bicelles may be protein mediated if the lipid interactions are pre-formed.[53-54]

ASSOCIATED CONTENT

The following files are available free of charge.

Experimental procedures and supporting figures and tables (PDF).

AUTHOR INFORMATION

Notes

[†]These authors contributed equally.

The authors declare no competing financial interests.

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