Langmuir

Article

pubs.acs.org/Langmuir

Tuning Micelle Dimensions and Properties with Binary Surfactant Mixtures

Ryan C. Oliver,[†] Jan Lipfert,[‡] Daniel A. Fox,[†] Ryan H. Lo,[†] Justin J. Kim,[†] Sebastian Doniach,[§] and Linda Columbus*,[†]

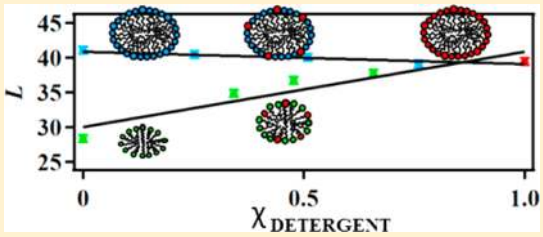
[†]Department of Chemistry, University of Virginia, Charlottesville, Virginia 22904, United States

[‡]Department of Physics and Center for Nanoscience (CeNS), University of Munich, Munich, Germany

[§]Departments of Physics and Applied Physics, Biophysics Program, and Stanford Synchrotron Radiation Laboratory, Stanford University, Stanford, California 94305, United States

Supporting Information

ABSTRACT: Detergent micelles are used in many areas of research and technology, in particular, as mimics of the cellular membranes in the purification and biochemical and structural characterization of membrane proteins. Applications of detergent micelles are often hindered by the limited set of properties of commercially available detergents. Mixtures of micelle-forming detergents provide a means to systematically obtain additional micellar properties and expand the repertoire of micelle features available; however, our understanding of the properties of detergent mixtures is still limited. In this study, the shape and size of binary mixtures of seven different detergents commonly used in molecular host−guest systems and membrane protein research were investigated. The data suggests that the detergents form ideally mixed micelles with sizes and shapes different from those of pure individual micelles. For most measurements of size, the mixtures varied linearly with detergent mole fraction and therefore can be calculated from the values of the pure detergents. We propose that properties such as the geometry, size, and surface charge can be systematically and predictably tuned for specific applications.



INTRODUCTION

Micelles are used extensively in a broad range of applications such as remediation,[1,2] pharmaceuticals,[3,4] nanodevices,[5−7] and membrane mimics in membrane protein structural and functional studies.[8−11] However, the full potential of micelles for these uses is limited by a lack of understanding of micelle physical and geometrical properties. The size and shape of detergent micelles is dependent on the detergent monomer properties, and relative qualitative predictions based on the chain length and headgroup size are well established.[12,13] However, the ability to manipulate the shapes and sizes for specific applications rationally and systematically has not been realized. In this study, the physical properties of micelles composed of binary mixtures of detergents (Figure 1) are investigated with small-angle X-ray scattering (SAXS) and isothermal titration calorimetry (ITC).

Because of their use in membrane protein investigations, phosphocholine and maltoside detergents with different chain lengths were investigated (Table 1 and Figure S1). In addition, mixtures with anionic lyso-PG detergents were selected to probe the effect of charged detergents on the observed trends and to investigate the ability to modify the micelle surface charge. The extent of detergent mixing was evaluated by measuring the critical micelle concentrations (CMC) of detergent mixtures with ITC and the measured CMC values were as predicted by ideal mixing. Using SAXS, the shapes and sizes of mixed micelles were determined as a function of the micellar mole fraction. The SAXS results show that mixed micelles have properties different from those of individual commercially available components, thereby expanding the available micelle sizes and shapes. In addition, the geometrical properties of the micelle mixtures are predictable from the pure detergent micelle properties based on a linear dependence on mole fraction. With this increased understanding, a rational detergent selection approach may be implemented for applications such as membrane protein studies[8] and the design of supramolecular micelle/receptor systems.[14]

Currently, the selection of micelles for most applications is empirical and historical, and rational approaches have been limited by the lack of understanding of physical properties of the micelles and the interaction that the micelles have with biomolecules. This study embarks on increasing our understanding of the physical properties of micelles and expanding the toolbox with micelle mixtures. Our results demonstrate that mixed micelles will provide a platform for systematically investigating micelle−biomolecule interactions that are important in stabilizing function.

Received: August 28, 2014
Revised: October 10, 2014

   dx.doi.org/10.1021/la503458n | *Langmuir* XXXX, XXX, XXX−XXX

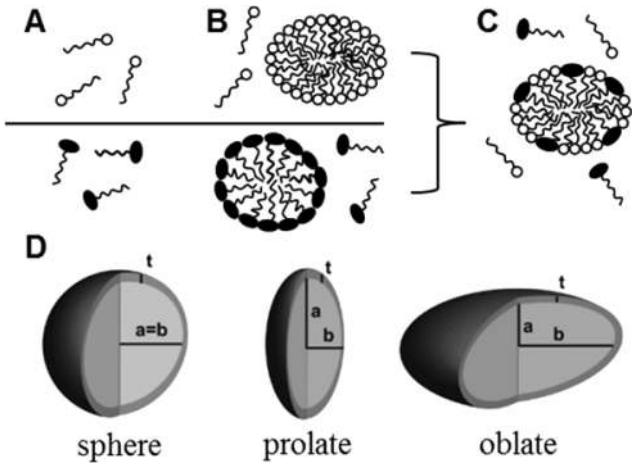


Figure 1. Schematic of detergent mixing and mixed micelle formation. (A) Two micelle-forming detergents with similar alkyl chain lengths and different headgroups (black and white) at concentrations below their CMC values. (B) At concentrations above the CMC, monomers self-assemble to form micelles, which remain in equilibrium with a CMC concentration of free monomers. (C) The combination of two different detergents results in mixed micelles with new physical properties and CMC values. (D) Ellipsoid core−shell models represent the overall detergent micelle structure having a core composed of detergent alkyl chains and shell formed by detergent headgroups. The core radii are labeled *a* and *b*, and the shell thickness is labeled *t*.

MATERIALS AND METHODS

Sample Preparation. Detergents *n*-octyl-*β*-D-maltopyranoside (OM), *n*-nonyl-*β*-D-maltopyranoside (NM), *n*-decyl-*β*-D-maltopyranoside (DM), *n*-undecyl-*β*-D-maltopyranoside (UM), *n*-dodecyl-*β*-D-maltopyranoside (DDM), *n*-tridecyl-*β*-D-maltopyranoside (13M), *n*-tetradecyl-*β*-D-maltopyranoside (14M), 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC), *n*-decyl-phosphocholine (FC10), *n*-dodecyl-phosphocholine (FC12), and *n*-tetradecyl-phosphocholine (FC14) were purchased from Anatrace (Affymetrix). 1-Myristoyl-2-hydroxy-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) (14:0 lyso PG, LMPG) and 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) (16:0 lyso PG, LPPG) were purchased from Avanti Polar Lipids. Detergent structures are shown in Figure S1, detergent properties are given in Table 1, and micelle size and shapes (determined from SAXS data, see below) for the pure detergents are provided in Table 2. Deuterium oxide (D_2O) was purchased from Cambridge Isotope Laboratories, and all other chemicals were obtained from Fisher unless otherwise noted.

Mixed micelle solutions were prepared by combining and diluting two detergent micelle stock solutions in a final buffer consisting of 20 mM phosphate buffer, pH 6.2, 150 mM NaCl, and 10% D_2O (necessary for the NMR deuterium lock). Detergent concentrations were determined with ^{1}H 1D NMR by comparing peak amplitudes to those of standards with known concentrations.[8] Mixed micelle solutions were prepared from stock solutions of the pure detergents with a total micelle concentration of approximately 1 mM.

Hen egg white lysozyme (Fisher, BP535) in 40 mM acetate buffer, pH 3.8, with 150 mM NaCl, horse heart cytochrome c (Sigma, C7150) in 100 mM acetate buffer, pH 4.6, with 0.5 M guanidinium hydrochloride, and bovine serum albumin (Sigma, A8531) in 20 mM HEPES buffer, pH 7.8, with 50 mM NaCl were used as SAXS molecular weight standards. Five concentrations were measured for each protein standard (up to 10.6 mg/mL lysozyme, 4.2 mg/mL cytochrome c, and 8.6 mg/mL albumin) to determine any concentration-dependent effects on scattering.

Measuring the Critical Micelle Concentration of Detergent Mixtures Using Isothermal Titration Calorimetry. The critical micelle concentration (CMC) denotes the concentration of detergent monomers in equilibrium with detergent micelles, below which micelles do not form. When two detergent species are mixed, rather than remaining as discrete micelles, a mixed micelle is formed. Studies of the detergent association and mixed micelle formation[15−18] have yielded the following generalized relationship for the critical micelle concentrations of a detergent mixture

$$\frac{1}{\mathrm{CMC}_{\mathrm{mix}}} = \frac{\chi_{\mathrm{A}}}{\mathrm{CMC}_{\mathrm{A}}} + \frac{\chi_{\mathrm{B}}}{\mathrm{CMC}_{\mathrm{B}}} \quad (1)$$

where $\mathrm{CMC}_{\mathrm{mix}}$ is the CMC of the mixture, $\mathrm{CMC}_{\mathrm{A}}$ and $\mathrm{CMC}_{\mathrm{B}}$ are the CMCs of the two pure components, and χ_{A} and χ_{B} are the respective mole fractions of each detergent.

Isothermal titration calorimetry (ITC) experiments were conducted to examine this relationship between CMC and mixed micelle fraction in selected mixed micelles using a VP-ITC microcalorimeter (MicroCal) at 30 °C with stirring at 300 rpm. The 1.5 mL sample

Table 1. Physical Properties of Selected Pure Detergents

detergent	abbr.	FW (Da)	CMC (mM)[a]	V_{mon} (Å^3)[b]	ρ_{det} (e/Å^3)[c]	N_{lit}
1,2-dihexanoyl-*sn*-glycero-3-phosphocholine	DHPC	454	15	677	0.363	27, 35
n-decylphosphocholine	FC10	323	11	494	0.360	24,[a] 45-53
n-dodecylphosphocholine	FC12	351	1.5	548	0.354	54,[a] 60−80
n-tetradecylphosphocholine	FC14	380	0.12	602	0.348	108[a]
n-octyl-*β*-D-maltopyranoside	OM	454	19.5	590	0.416	6,[a] 26
n-nonyl-*β*-D-maltopyranoside	NM	469	6	617	0.412	25[a]
n-decyl-*β*-D-maltopyranoside	DM	483	1.8	644	0.407	69,[a] 82−90
n-undecyl-*β*-D-maltopyranoside	UM	497	0.59	671	0.402	71[a]
n-dodecyl-*β*-D-maltopyranoside	DDM	511	0.17	698	0.398	78−149,[a] 135−145
n-tridecyl-*β*-D-maltopyranoside	13M	525	0.024	725	0.394	186[a]
n-tetradecyl-*β*-D-maltopyranoside	14M	539	0.01	752	0.388	ND
1-myristoyl-2-hydroxy-*sn*-glycero-3-phosphor-(1′-*rac*-glycerol)	LMPG	478	0.16	639	0.404	90[d]
1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphor-(1′-*rac*-glycerol)	LPPG	507	0.018	693	0.395	125, 160−170

[a]Reported by Anatrace (Affymetrix, Inc.). CMCs are reported for conditions of detergent in H_2O, except for the CMC of OM, which is reported in 20 mM HEPES, pH 7.5 with 100 mM NaCl. [b]Monomer volumes were calculated from published specific densities using the Tanford formula ($V_{\mathrm{tail}} = N(24.7 + 26.9n_c)$) for alkyl chain volumes to adjust for different chain lengths. [c]The detergent electron density values were computed by summing the number of electrons from the chemical composition and dividing by the molecular volume. [d]A measured value was not found in the literature, although many studies report an aggregation number. However, a rough estimate can be made from the PDC molecular weight reported by Tian et al. of ∼60 kDa (detergent contribution of 44 kDa), which yields an aggregation number estimate of ∼90. All other aggregation numbers are from refs 13 and 20 and are reported in the same buffer used in this study.

dx.doi.org/10.1021/la503458n | *Langmuir* XXXX, XXX, XXX−XXX

Table 2. Geometrical Parameters of Single Detergent Micelles[a]

	shape	ρ_1 (e/Å^3)	ρ_2 (e/Å^3)	a (Å)	b (Å)	t (Å)	a/b	$R_{g_{expt}}$ (Å)	L_{expt} (Å)	rN_{expt}
DHPC	oblate	0.253	0.464	8.3−8.7	16.6−17.1	4.5−5.0	0.49−0.52	17.0 ± 1.5	22.0−24.0	38−40
FC10	prolate	0.273	0.490	20.4−20.9	13.3−13.6	2.7−3.0	1.50−1.57	25.0 ± 1.5	29.3−30.2	32−35
FC12	prolate	0.277	0.490	24.3−24.8	16.1−16.4	2.7−3.0	1.48−1.54	32.0 ± 2.0	34.9−35.8	46−49
FC14	prolate	0.280	0.490	29.6−30.1	18.8−19.1	2.7−3.0	1.55−1.60	46.5 ± 4.0[b]	40.3−41.2	75−81
OM	oblate	0.268	0.520	11.0−11.4	18.4−18.8	5.4−5.8	0.59−0.62	21.5 ± 1.0	27.4−28.6	42−45
NM	oblate	0.270	0.520	11.7−12.1	21.4−21.8	5.4−5.8	0.54−0.57	24.8 ± 1.0	30.3−31.5	23−25[c]
DM	oblate	0.273	0.520	13.4−13.8	22.7−23.1	5.4−5.8	0.59−0.61	26.7 ± 1.0	32.2−33.4	71−77
DDM	oblate	0.277	0.520	15.3−15.7	27.9−28.3	5.4−5.8	0.54−0.56	32.0 ± 1.0	36.8−38.0	114−123
13M	oblate	0.278	0.520	15.9−16.3	31.6−32.0	5.4−5.8	0.50−0.52	43.5 ± 4.0[b]	41.8−43.0	74−80[c]
14M	oblate	0.280	0.520	17.4−17.8	33.3−33.7	5.4−5.8	0.52−0.53	49.4 ± 4.0[b]	44.4−45.6	124−134[c]
LMPG	oblate	0.280	0.470	16.6−17.6	23.5−24.5	5.3−6.1	0.68−0.75	27.0 ± 1.5	38.5−41.3	90−100
LPPG	oblate	0.281	0.470	18.0−19.0	27.0−29.0	5.6−6.4	0.62−0.70	30.0 ± 1.5	43.5−46.3	160[d]

[a]Ellipsoidal shapes and corresponding parameters from fits to experimental SAXS scattering profiles. [b]For FC14, 13M, and 14M, the Guinier range (qR_g <1.3) deviated from linear resulting in an unreliable R_g estimate. An average $R_{g_{expt}}$ from the lower-concentration data (≤150 mM) was used to minimize these influences. [c]The aggregation number for these detergents was determined at a concentration of 40 mM compared to 60 mM for the other detergents. The aggregation numbers for maltosides have previously been reported to be concentration-dependent.[20] [d]The aggregation number was previously determined and reported in ref 20.

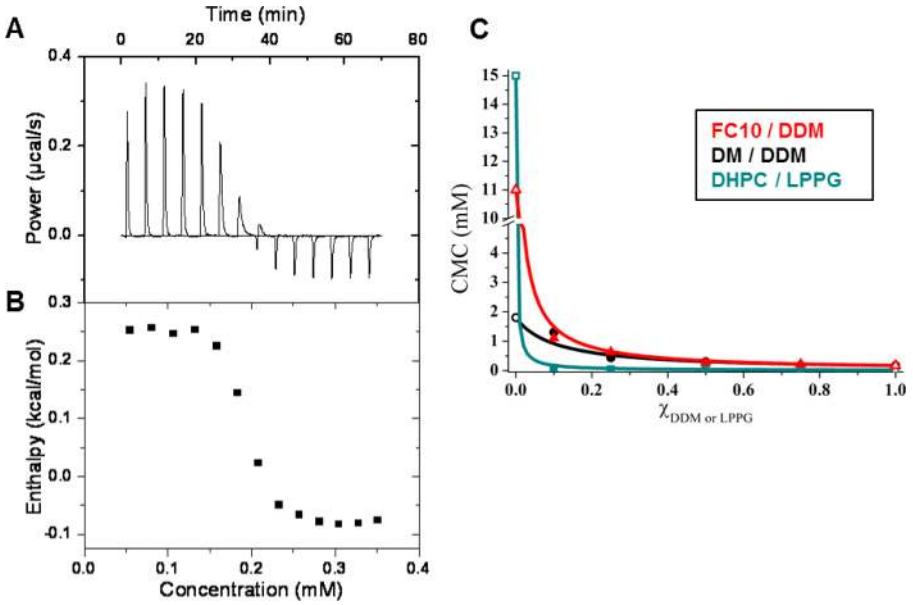


Figure 2. Critical micelle concentrations (CMCs) of detergent mixtures determined by ITC. (A) Measured power as a function of time, which is then used to generate a plot of the change in enthalpy versus concentration. (B) The inflection point corresponds to the CMC. Representative data for the DM/DDM mixture with a molar ratio of 1:3 is shown. (C) CMC values are plotted versus the mole fraction of either DDM or LPPG in the mixed micelle. Mixtures of two nonionic maltosides with different alkyl chain lengths (DM and DDM, black circles), a zwitterionic phosphocholine and nonionic maltoside (FC10 and DDM, red triangles) with similar hydrophobic radii but different alkyl chain lengths, and a zwitterionic phosphocholine and anionic phosphatidyl glycerol (DHPC and LPPG, teal squares) with different lengths and numbers of alkyl chains. The lines represent that expected using eq 1, and unfilled symbols indicate the pure detergent CMC values (Table 1). The error in the CMC values is estimated by the concentration difference between the two points that flank the inflection point of the isotherm (e.g., panel B) and has an upper limit of 15%.

cell contained 20 mM phosphate buffer at pH 7. The 1.5 mL reference cell contained water. Binary detergent stock solutions were degassed and loaded into the calorimeter syringe. Binary detergent stock solutions containing DM and DDM, FC10 and DDM, and DHPC and LPPG each in ratios of 1:3, 1:1, 3:1, and 9:1 were prepared in 20 mM phosphate buffer at pH 7. To observe sufficient baselines before and after micelle formation, stock concentrations needed to be in excess of 20 times the CMC as predicted by eq 1. The titrant was injected into the sample cell in 10 μL aliquots for 20 s per injection, with an equilibration time of 300 s between injections. The change in total volume after each injection was accounted for in the calculation of total detergent concentration in the cell. A binding isotherm was generated by plotting the integration of the resulting power versus time for each injection versus the total detergent concentration (Figure 2A), and the local maximum of the first derivative was used to determine the CMC. The CMCs of different detergent mole ratios were plotted and compared to the ideal mixing predicted by eq 1.

SAXS Data Collection and Core−Shell Model Fits. SAXS data were measured at XOR/BESSRC undulator beamline 12-ID of the Advanced Photon Source (Argonne, IL), with a sample-to-detector distance of 2 m and a Pilatus 2 M detector. The data were collected at 25 °C using a custom-made sample holder[19] and an X-ray energy of 12 keV (corresponding to a X-ray wavelength of λ = 1 Å). The usable range of momentum transfer q was 0.01 < q < 0.28 Å^{-1} ($q = 4\pi \sin(\theta)/\lambda$, where 2θ is the scattering angle). Buffer-only scattering profiles recorded under otherwise identical conditions were subtracted

dx.doi.org/10.1021/la503458n | Langmuir XXXX, XXX, XXX−XXX

for background correction. Additional details of the beamline setup and measurements are as previously described.[13,20−22]

Micelle sizes and shapes were determined from ellipsoid core−shell model fits to the SAXS scattering profiles using the NCNR (NIST Center for Neutron Research) analysis toolkit,[23] adapted for X-ray scattering as previously described for a comprehensive series of pure detergents relevant to membrane protein investigations.[13,20] To fit the scattering profile, the previously published procedure was used.[13,20] Briefly, electron densities were calculated for the core and shell by summing the number of electrons from the chemical composition and dividing by the molecular volume; these values were held constant during fitting for identical headgroups and chain lengths. Additionally, the shell thickness t was held constant for identical headgroups during fitting. The axial dimensions (a and b) of the ellipsoid were allowed to vary; however, only models with a short dimension that was similar to or shorter than the extended alkyl chain length were considered as physically reasonable models. For binary mixed micelles, core and shell electron densities were calculated as the mole fraction weighted linear combination of the electron densities of the individual detergents (which assumes ideal mixing as discussed in the Binary Detergent Mixtures Exhibit Ideal Mixing section). Scattering profiles, Guinier plots of the low-q scattering data, and close-up views of regions containing the second maxima in the scattering profiles are shown in Figures S2−S6.

Determination of Micelle Aggregation Numbers. For mixed micelles of two detergents (e.g., detergents A and B), the total aggregation number (N_T) is the sum of all detergent monomers in the micelle. Partial aggregation numbers (N_A, N_B) can be used to describe the populations of each component in the mixed micelle such that $N_T = N_A + N_B$. If the mole fraction of each component and the total aggregation number are known such that $N_T = \chi_A N_T + \chi_B N_T$, partial aggregation numbers can be determined according to the following relationships (this again assumes ideal mixing as discussed in the Binary Detergent Mixtures Exhibit Ideal Mixing section below):

$$N_A = \chi_A N_T, \quad N_B = \chi_B N_T \tag{2}$$

In this instance and hereafter, χ_A refers to the micellar mole fraction and is equal to [A]/([A] + [B]), where [A] and [B] are the detergent concentrations corrected for the monomeric detergent concentration. (The CMC is calculated from eq 1, and the mixing ratio is used to determine each monomeric detergent concentration.) Total aggregation numbers were estimated using two methods: first from the forward scattering intensity $I(0)$ of the SAXS measurements and comparison to well-characterized molecular weight standards and then from the best-fit geometric models by analyzing the volume of the hydrophobic core. For SAXS measurements of macromolecules, the zero-angle scattering intensity $I(0)$ (which is determined from Guinier analysis of the data, see below) is proportional to the square of the total scattering contrast $V(\rho - \rho_s)$ (eq 3), where V is the molecular volume, ρ is the average electron density of the particle, and ρ_s is the electron density of the solvent. κ is the proportionality constant determined from $I(0)$ measurements of molecular weight standards (described in the Sample Preparation section) of known concentration, volume, and electron density:

$$I(0) = \kappa c[V(\rho - \rho_s)]^2 \tag{3}$$

Consequently, a micelle of N detergent monomers scatters N-fold more strongly than N monomers, and the micellar aggregation number can be determined from the measured $I(0)$ and that expected for a monomer, as described by the following equation:

$$N = \frac{I(0)_{micelle}}{I(0)_{monomer}} = \frac{I(0)_{micelle}}{\kappa(C - CMC_{mix})(\rho_{mix} - \rho_s)^2 (V_{mix})^2} \tag{4}$$

For mixed micelles, C is the total concentration of both detergent monomer components, and CMC_{mix} is the CMC adjusted for the detergents used and the ratio of mixing (eq 1). The solvent electron density (ρ_s) was 0.34 e/Å^3 in all calculations. For mixed micelle values such as electron densities (ρ_{mix}) and monomer volumes (V_{mix}), a mole-fraction-weighted linear combination of the values from each component was used, as demonstrated for the mixed micelle volume:

$$V_{mix} = \chi_A V_A + \chi_B V_B \tag{5}$$

After calculating a total aggregation number, partial aggregation numbers ($N_{A,expt}$ and $N_{B,expt}$) were determined using mixed micelle mole fractions.

Alternatively, aggregation numbers were determined from the best-fit micelle models fit to the experimental data. The total volume of the hydrophobic core was divided by the volume per monomer

$$N_{core} = \frac{\frac{4}{3}\pi ab^2}{V_{tail}} \tag{6}$$

where V_{tail} is the volume occupied by a hydrocarbon chain, based on Tanford's formula for the alkyl chain volume ($V_{tail} = 27.4 + 26.9n_c$, where n_c is the number of alkyl chain carbons)[12] and the elliptical core axes (a and b). Again, the mole-fraction-weighted linear combination of tail volumes V_{tail} for the individual components was used in the evaluation of eq 6 for mixed micelles. This approach implies maximum detergent packing to fill the mixed micelle volume. The individual detergent aggregation numbers ($N_{A,model}$ and $N_{B,model}$) are extracted from the total aggregation number (N_{core}) using a weighted linear combination for multiple component mixed micelles.

Determination of the Short Dimension across the Micelle Core (L_{expt}). A local maximum in the scattering intensity for $0.1 < q < 0.3$ Å^{-1}, corresponding to a length scale of $20 < d < 60$ Å, is characteristic of X-ray scattering profiles from detergent micelles. The position of this maximum (q_{max}) indicates the most frequently occurring distance of separation among the detergent headgroups across the micelle core (L_{expt}) and correlates with the micelle's hydrophobic thickness along the minor elliptical axis.[13,20] L_{expt} is calculated directly from the position of the second maximum in the experimental SAXS profile for each micelle as

$$L_{expt} = 2\pi/q_{max} \tag{7}$$

L_{model} is calculated from the geometrical model that was fit to the scattering data

$$L_{model} = 2r + t \tag{8}$$

where r is the minor elliptical core axis (a for oblate, b for prolate) and t is the shell thickness.[13] The length of the shorter core axis is constrained by the maximum extended alkyl chain length because exceeding this limit results in unoccupied volume in the center of the micelle.[12,13,20] The dependence of L_{expt} on the mixed micelle composition for two detergents A and B was fit by the linear relationship $L(\chi_A) = L_B + (L_A - L_B)\chi_A$. Theoretical L values calculated on the basis of the number of carbons in the alkyl chain (n_c) and the headgroup length ($2(1.265n_c + 1.5) + t$) are used to evaluate the ability to predict this parameter of detergent mixtures.

Guinier Analysis and Determination of Micelle Radius of Gyration R_g. In the limit in which interparticle correlations are negligible, the scattering intensity for very low momentum transfer q is given by the Guinier approximation[24,25]

$$I(q) \approx I(0)e^{-R_g^2 q^2/3} \tag{9}$$

Thus, the forward scattering intensity $I(0)$ and radius of gyration $R_{g,expt}$ can be determined by linearly fitting the low-q data in a plot of $\ln(I)$ as a function of q^2. Linear Guinier fits were evaluated using the program PRIMUS,[26] where we limited the upper bound of the fitting range such that $qR_g \leq 1.3$. A Guinier analysis of the experimental SAXS profiles at several detergent concentrations was performed to probe the effects of increasing concentration and to obtain reliable estimates of micellar $R_{g,expt}$ and $I(0)$. In addition, model-dependent $R_{g,model}$ can be calculated from the core−shell model according to the relationship

dx.doi.org/10.1021/la503458n | Langmuir XXXX, XXX, XXX−XXX

$$R_{g_{model}}^{\ 2} = \frac{1}{5}\frac{(\rho_2 - \rho_s)(a+t)(b+t)^2[(a+t)^2 + 2(b+t)^2] + (\rho_1 - \rho_2)ab^2(a^2 + 2b^2)}{(\rho_2 - \rho_s)(a+t)(b+t)^2 + (\rho_1 - \rho_2)ab^2} \quad (10)$$

where ρ_1, ρ_2, ρ_s, a, b, and t are electron densities for the inner and outer cores, solvent electron density, elliptical core axes, and shell thickness, respectively.[20]

RESULTS AND DISCUSSION

Binary Detergent Mixtures Exhibit Ideal Mixing. When two detergents are mixed, the extent of mixing and the resulting micelle structure and properties are unknown a priori. In the extreme scenario, a mixture of detergents A and B could give rise to pure A and pure B micelles in solution. At the other extreme, ideal mixing between the two detergents is achieved, and the resulting micelle composition is equal to the bulk detergent concentration ratio (Figure 1). The formation of ideal mixed micelles containing nonionic headgroups with different alkyl chain lengths was previously reported.[16,27,28] However, interactions between different detergent headgroups, such as electrostatic interactions, are proposed to hinder the ideal mixing of detergents in micelles.[12] Deviations from ideal mixing were observed in mixtures of dodecyl sulfate and octyl(oxyethylene) dodecyl ether in which CMC values were lower than those predicted for ideal mixing (eq 1) because of repulsive forces between headgroups in the mixed micelle as compared to that formed by the pure component.[29]

We investigated the extent of mixing for a set of commercially available detergents that are commonly used in membrane protein research by ITC and SAXS. Mixtures that tested the hypotheses that headgroup charge and chain length influenced ideal mixing were investigated: (i) identical nonionic headgroups differing in alkyl chain length (DM and DDM), (ii) a zwitterionic (FC10) and a nonionic headgroup (DDM) with different alkyl chain lengths, and (iii) a zwitterionic (DHPC) and an ionic headgroup (LPPG) differing in the alkyl chain length and the number of alkyl chains.

ITC was used to determine the CMC of the three mixtures at different mole fraction ratios (Figure 2; Materials and Methods). Most of the experimentally determined CMC values for the binary detergent mixtures investigated in this study agreed well with the prediction of eq 1 for ideal mixing (Figure 2). The agreement of the experimentally determined CMC values with the ideal mixing predictions suggests that the panel of detergents investigated here exhibits ideal (or close to ideal) mixing.

In addition to the ITC measurement, we investigated the degree of mixing using SAXS. In general, the scattering profile for a dilute solution of a mixture of different scattering species is simply the sum of the scattering profiles from the individual species.[30] As a consequence, a mixture of pure A and pure B micelles would give a scattering profile simply equal to the sum of the scattering profiles from a pure A and pure B sample. In contrast, if a mixed micelle is formed that has a size and shape different from those of pure micelles, then the scattering profile of the binary mixture would be different from the sum of the scattering profiles of the two pure detergent samples. We systematically compared the scattering profiles for the binary mixtures investigated in this work with the sum of the scattering profiles from the pure components, measured at the same concentration. In all cases, the sum of the scattering profiles from the pure detergent samples was different from the scattering profile of the mixture. Representative examples are shown in Figure 3. These differences strongly suggest that the mixtures do not partition into micelles that are similar to the pure micelles of the individual components but that instead mixed micelles are formed that exhibit shapes significantly different from that of pure micelles. In summary, the evidence from both ITC and SAXS data suggests that the detergent mixtures investigated in this study form (nearly) ideal mixed micelles, justifying the use of mole fraction weighted averages for many of the micelle properties used to fit the micelle scattering profiles (e.g., the electron density or tail group

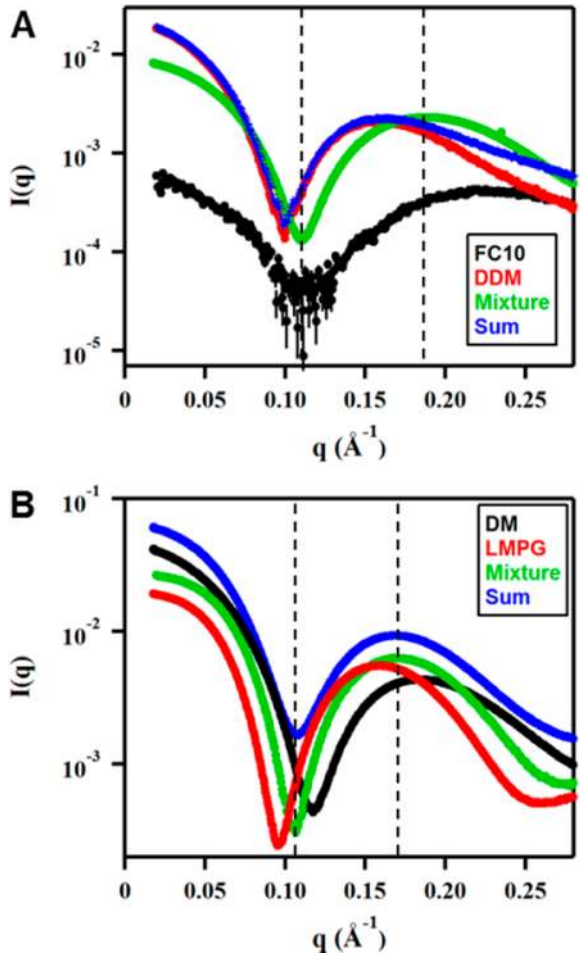


Figure 3. Detergent mixture scattering profiles are different from the sum of the pure detergent scattering profiles. (A) Scattering profiles are shown for pure detergents FC10 (36 mM, black) and DDM (17 mM, red). The scattering profile from the binary detergent mixture (39 mM FC10 and 19 mM DDM, green) is different from the sum of the two pure detergent scattering profiles (blue), indicating that the micelle is a mixture of both detergents. (B) Scattering profiles are shown for pure detergents DM (58 mM, black) and LMPG (54 mM, red). The scattering profile from the binary detergent mixture (46 mM DM and 39 mM LMPG, green) is again different from the sum of the two pure detergent scattering profiles (blue), indicating that the micelle is a mixture of both detergents. Dashed lines are added to guide the eye.

dx.doi.org/10.1021/la503458n | Langmuir XXXX, XXX, XXX−XXX

volumes; Materials and Methods)). In addition, the linear dependence on the detergent mole fraction of many of the model-independent and model-dependent parameters (described in the following sections) strongly supports the observation that these detergents from mixed micelles.

Modulating the Micelle Size and Shape in Binary Detergent Mixtures. The size of a micelle can be assessed with different parameters such as the aggregation number (N) and micelle geometrical dimensions, which can be determined using SAXS with model-dependent and model-independent approaches. With respect to geometrical dimensions, the ellipsoidal radii (model-dependent) and L_{expt} (model-independent) are two properties of particular interest because they correlate with the micelle hydrophobic thickness, which may be of importance for various applications such as stabilizing membrane proteins.[8] L_{expt} was determined from the position of the second scattering maximum for several binary detergent mixtures with the same headgroup (maltosides; Figure 4) and

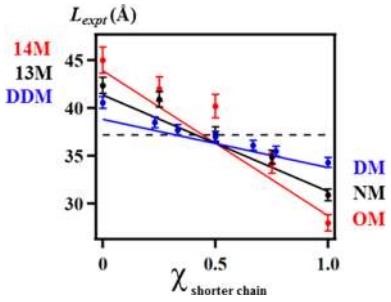


Figure 4. Characteristic micelle thickness L_{expt} varying linearly with micellar mole fraction. L_{expt} of micelle mixtures composed of maltosides with different alkyl chains: 8 and 14 carbons (OM and 14M, red), 9 and 13 carbons (NM and 13M, black), and 10 and 12 carbons (DM and DDM, blue). The dashed line indicates the L_{expt} value for 11M. Solid lines correspond to the predicted micelle thicknesses. R^2 values for each fit are 0.875 (OM and 14M), 0.913 (NM and 13M), and 0.795 (DM and DDM).

different headgroups (Figure 5; properties of all micelle mixtures are listed in Supporting Information Tables S1−S4). The maltoside alkyl chain length mixtures investigated were 10 and 12, 9 and 13, and 8 and 14 carbons. A linear decrease in L_{expt} is observed as the mole fraction of the shorter chain detergent is increased. As expected from the linear dependence on mole fraction, micelles with equimolar ratios have a similar L_{expt} to that of 11M (dashed line in Figure 4), which corresponds to the average number of alkyl carbons in the equimolar mixture. The linear trends followed that predicted from ideal mixing. L_{expt} also varies linearly with mole fraction for mixtures of detergent with different headgroup properties such as nonionic maltoside with zwitterionic phosphocholine (Figure 5A−C) and anionic phosphatidyl glycerol headgroups (Figure 5D,E). As expected, L_{expt} remains approximately constant for mixtures of detergents with similar L_{expt} for the pure components (e.g., OM, FC10, DM, and FC12, Figure 5B). For most mixtures, the linear trend in L_{expt} was followed as predicted; however, for LMPG and LPPG mixtures, the trend was not followed. The L_{expt} observed for pure LMPG is smaller than that predicted; if the observed L_{expt} is used for the calculation of the L value of the mixtures, then the R^2 values are 0.821 (FC10 and LMPG), 0.712 (FC12 and LMPG), and 0.837 (DM and LMPG). The LPPG and DHPC mixture did not follow the predicted trend and could be due to the two alkyl chains in the more lipidlike detergent. This deviation emphasizes the importance of studying lipid-detergent mixtures to investigate the effects of lipids on micelle shapes and sizes. A linear trend was also observed for L_{model} ($= t + 2a$) as a function of the micelle mole fraction (example in Figure 6A). Significant deviations from that predicted and larger deviations from the linear trend were observed compared to the L_{expt} measurements and are likely due to the approximation of the shell thickness and electron density of the headgroup mixture. In most cases, the major axis of the ellipsoid was also linearly dependent on the detergent mole fraction, indicating that additional properties that reflect the micelle size such as micelle volume (V_{model}), aggregation numbers (N, N_A, and N_B; model-dependent and independent measurements), and R_g (model-dependent and model-independent measurements) may be linearly dependent on the detergent mole fraction.

For mixtures of maltosides (OM, DM, and DDM) and phosphocholines, V_{model} varies approximately linearly with mole fraction (examples for mixtures with FC12 are shown in Figure 6B). Because V_{model} is dependent on the radii, each of which vary linearly with the mole fraction, the linear dependence of the volume is not surprising; however, the plot highlights the volume similarities between prolates and oblates with different dimensions. For instances, OM and FC12 have similar volumes but differ in minor radius length and L_{expt} because they are different shapes (prolate vs oblate). V_{model} may be an important parameter to consider in terms of the membrane protein hydrophobic surface area or maximum load capacity of pharmaceuticals. Aggregation numbers are another measurement of the micelle size and are extracted from the volumes using the mole fractions and detergent monomer volumes. In most cases, the aggregation number (N_{model}) varied linearly with the micelle mole fraction (N_{total}: Figures 6D and S7). Deviations from linearity were observed for OM and FC12, FC14 with OM and DDM, and LMPG with FC10, FC12, and DM. In each case, the micelles change shape and are mixtures of relatively small and large micelles. Another assessment of micelle size is the radius of gyration R_g. The dependence of the model-independent $R_{g,expt}$ with respect to the mixed micelle composition is linear for many mixtures (Figure S8) but not all. In the cases of longer-chain prolate phosphocholine and oblate maltoside micelle mixtures, there appears to be a transition at the higher mole fractions of phosphocholine that deviates from the linear dependence (Figure S8B,C). More data is required in this transition region to be able to discuss molecular explanations of this trend.

These trends in micelle size allow for the rational design of micelles of specific sizes using binary mixtures, which will be useful for many basic science and industrial applications. In each application, if a particular micelle property is hypothesized to be important, then mixtures with similar properties can be explored to test the hypothesis systematically. This approach was previously used to investigate the influence of micelle hydrophobic dimensions on membrane protein folding and NMR spectral quality.[8]

Modulating Micelle Shape. The micelles in this study are ellipsoids and can vary in the type of ellipsoid (prolate or oblate) and the ellipticity (the extent of deviation from spherical). Although in some cases the oblate and prolate ellipsoid models fit almost equally well to the scattering profiles, the models can be distinguished. A comparison of the core minor axis dimension b to the expected maximal alkyl chain length and a comparison of the experimental and model-

dx.doi.org/10.1021/la503458n | Langmuir XXXX, XXX, XXX−XXX

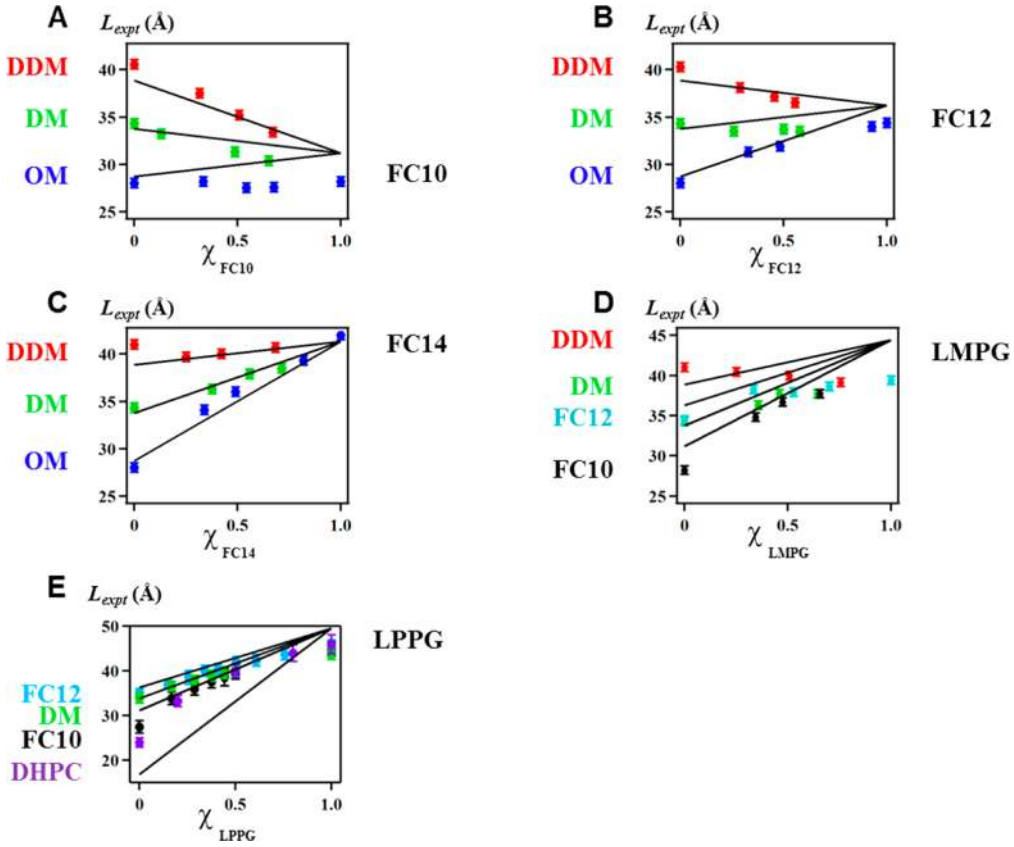


Figure 5. Linear dependence of the characteristic micelle thickness L_{expt} on micellar mole fraction for binary detergent mixtures. Binary combinations of maltosides, phosphocholines, and LMPG are plotted as a function of mole fraction of phosphocholine detergent (A–C), LMPG (D), and LPPG (E). Solid lines are the predicted micelle thicknesses. R^2 values for each series are 0.398 (DM and FC10), 0.846 (DDM and FC10), 0.727 (OM and FC12), 0.646 (DDM and FC12), 0.970 (OM and FC14), 0.961 (DM and FC14), 0.462 (FC10 and LMPG), 0.352 (DHPC and LPPG), 0.781 (FC10 and LPPG), 0.361 (DM and LPPG), and 0.598 (FC12 and LPPG). R^2 values are not reported for mixtures in which L_{expt} remains relatively constant.

derived R_g and L values typically distinguishes the appropriate model.[13] The best-fit geometric models indicate that maltosides are oblate, whereas phosphocholines are prolate.[13,20] The ellipticity of mixed micelles of maltoside (OM, DM, and DDM) and FC12 detergents varies approximately linearly with mole fraction (Figure 6C). However, above a mole fraction of ~0.55, the shape of the micelle changes from oblate to prolate. Thus, the shape and ellipticity are tunable properties using binary mixtures. Two micelles of nearly equal volumes but having different shapes (high and low aspect ratios) can be mixed to obtain a similar volume mixed micelle with a modulated ellipticity.

Modulating Micelle Surface Properties. Physical properties of the micelle surface are dependent upon the detergent headgroup composition and ratio of detergents in the mixed micelles. For example, detergent headgroups can be anionic, cationic, zwitterionic, or neutral, thus proper mixing ratios can produce micelles with a predictable net surface charge. Provided that detergents are well-mixed, the surface charge density of mixed micelles can be calculated from the partial aggregation number of the charged species and the micelle geometry. The incorporation of such charged headgroups modulates the surface potential of micelles.[31] In this study, the potential for spherical ionic LPG micelles using a geometric mean of the elliptical axes (Table S4) for the spherical axis is calculated to be between −60 and −140 mV. Although the actual value is sensitive to the micelle geometry and degree of counterion binding, properties of the detergent monomer even at low concentration can have a significant impact on the micelle surface potential. The micelle surface charge may modulate interactions between micelles; repulsive surfaces are likely to be better for NMR studies, and attractive forces will likely facilitate particle interaction conducive to crystallization. The micelle surface potential may also be important in stabilizing soluble domains of membrane proteins or provide necessary electrostatic interactions at the membrane–headgroup interface that occur in the native membrane environment. In addition, the micelle surface potential may also enhance molecular host–guest interactions or better recapitulate a biological membrane for molecular host investigations.[5]

Similar to the incorporation of charged species, amphiphiles with unique headgroup properties can also be added to micelles. Evidence suggests that certain lipids or lipid headgroup types may be required to produce functional membrane protein complexes. On the basis of our observations of (near) ideal and complete mixing between components, we expect very small quantities (approximately less than 1%) of such amphiphiles to be incorporated without significant changes in micelle size and shape. However, in cases such as cholesterol or cholesterol analogs where the concentrations are typically high, the micelle size and shape will be significantly altered. This rationalization also applies to the incorporation of modifications along the acyl chain, such as spin-labeled lipids and fluorinated surfactants. A probe can be present and equally

dx.doi.org/10.1021/la503458n | Langmuir XXXX, XXX, XXX–XXX

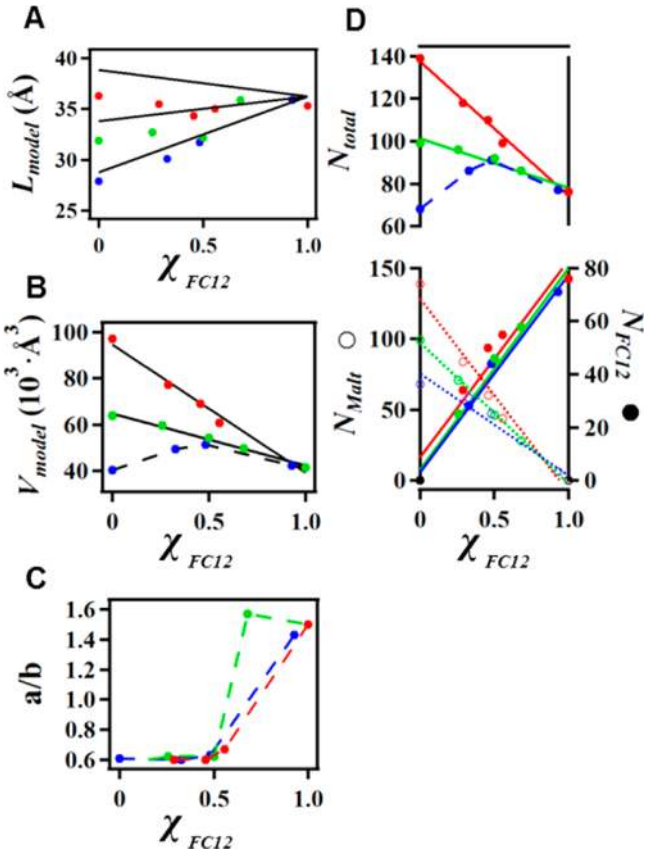


Figure 6. Core−shell model parameters vary linearly with the micellar mole fraction for binary mixtures. L_{model} (A), micelle volume (B), axial ratio (a/b) (C), and aggregation numbers (N_{model}) (D) of binary mixtures containing FC12 with OM (blue), DM (green), or DDM (red) are plotted versus the detergent mole fraction of FC12. (D) The total micelle aggregation number (N_{total}, top) and the aggregation number of each component are plotted versus the mole faction of FC12 (bottom; open circles and filled circles indicate maltoside and FC12 aggregation numbers, respectively). Note that the y-axis scales are different for N_{Malt} and N_{FC12}. Dashed lines are added to guide the eye, and solid and dotted lines represent linear fits except in panel A, where the solid lines correspond to predicted L values based on ideal mixing (R^2 for the FC12 and OM (blue) mixture is 0.928).

dispersed at very low concentrations (~1 per micelle) with a minimal disruption to other micelle properties, but as the detergent mole fraction increases, changes to the micelle should be expected.

CONCLUSIONS

We have investigated binary detergent mixtures by SAXS and ITC. Our results suggest that the available detergent micelle properties (such as the characteristic thickness, aggregation number, or shape) can be expanded through binary mixtures, and the data indicate that these properties are predictable on the basis of a linear combination of the pure detergent properties. This finding allows investigators to explore and design properties systematically that are important for specific applications. For instance, as a minimal requirement, one dimension of a detergent micelle should match the hydrophobic thickness of a membrane protein. With pure detergents, there would only be a very limited set of detergents that may satisfy this requirement. However, with binary mixtures more micelles varying in other properties can be explored to stabilize membrane protein folds. The predictability of the shape, size, and surface properties of binary mixtures expands the molecular toolbox for applications that utilize detergents and provide a means to systematically test the influence these properties on the systems investigated.

ASSOCIATED CONTENT

Supporting Information

Chemical structures of detergents and SAXS characterization of the pure detergents previously unreported (OM, FC14, and LMPG). Physical parameters determined for the detergent mixtures. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: columbus@virginia.edu. Phone: +1 434-243-2123. Fax: +1 434-924-3567.

Author Contributions

The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding

This research was funded by a National Science Foundation CAREER award (MCB 0845668) and is supported by The Netherlands Organisation for Scientific Research (NWO).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Sönke Seifert for help with data collection at the Advanced Photon Source of Argonne National Laboratories.

ABBREVIATIONS

PDC, protein-detergent complex; SAXS, small-angle X-ray scattering; ITC, isothermal titration calorimetry; CMC, critical micelle concentration; OM, octyl maltoside; NM, nonyl maltoside; DM, decyl maltoside; UM, undecyl maltoside; DDM, dodecyl maltoside; 13M, tridecyl maltoside; 14M, tetradecyl maltoside; DHPC, dihexanoylphosphatidylcholine; FC10, decyl phosphocholine; FC12, dodecyl phosphocholine; FC14, tetradecyl phosphocholine; LMPG, 14:0 lyso PG, 1-tetradecanoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol); LPPG, 16:0 lyso PG, 1-palmitoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol); D_2O, deuterium oxide

REFERENCES

(1) Chu, W.; Kwan, C. Y. Remediation of contaminated soil by a solvent/surfactant system. *Chemosphere* **2003**, *53*, 9−15.

(2) Laha, S.; Tansel, B.; Ussawarujikulchai, A. Surfactant-soil interactions during surfactant-amended remediation of contaminated soils by hydrophobic organic compounds: a review. *J. Environ. Manage.* **2009**, *90*, 95−100.

(3) Rangel-Yagui, C. O.; Pessoa, A., Jr.; Tavares, L. C. Micellar solubilization of drugs. *J. Pharm. Pharm. Sci.* **2005**, *8*, 147−165.

(4) Jhaveri, A. M.; Torchilin, V. P. Multifunctional polymeric micelles for delivery of drugs and siRNA. *Front. Pharmacol.* **2014**, *5*, 77.

(5) Javor, S.; Rebek, J., Jr. Activation of a water-soluble resorcinarene cavitand at the water-phosphocholine micelle interface. *J. Am. Chem. Soc.* **2011**, *133*, 17473−17478.

(6) Kim, Y. J.; Lek, M. T.; Schramm, M. P. pH influenced molecular switching with micelle bound cavitands. *Chem. Commun.* **2011**, *47*, 9636−9638.

(7) Su, F.; Alam, R.; Mei, Q.; Tian, Y.; Youngbull, C.; Johnson, R. H.; Meldrum, D. R. Nanostructured oxygen sensor–using micelles to incorporate a hydrophobic platinum porphyrin. *PloS one* **2012**, *7*, e33390.
(8) Columbus, L.; Lipfert, J.; Jambunathan, K.; Fox, D. A.; Sim, A. Y.; Doniach, S.; Lesley, S. A. Mixing and matching detergents for membrane protein NMR structure determination. *J. Am. Chem. Soc.* **2009**, *131*, 7320−7326.
(9) Garavito, R. M.; Ferguson-Miller, S. Detergents as tools in membrane biochemistry. *J. Biol. Chem.* **2001**, *276*, 32403−32406.
(10) Seddon, A. M.; Curnow, P.; Booth, P. J. Membrane proteins, lipids and detergents: not just a soap opera. *Biochim. Biophys. Acta, Biomembr.* **2004**, *1666*, 105−117.
(11) Vinogradova, O.; Sonnichsen, F.; Sanders, C. R., II. On choosing a detergent for solution NMR studies of membrane proteins. *J. Biomol. NMR* **1998**, *11*, 381−6.
(12) Tanford, C. *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, 2nd ed.; Wiley: New York, 1980.
(13) Oliver, R. C.; Lipfert, J.; Fox, D. A.; Lo, R. H.; Doniach, S.; Columbus, L. Dependence of micelle size and shape on detergent alkyl chain length and head group. *PloS One* **2013**, *8*, e62488.
(14) Keymeulen, F.; De Bernardin, P.; Dalla Cort, A.; Bartik, K. Paramagnetic Relaxation Enhancement Experiments: A Valuable Tool for the Characterization of Micellar Nanodevices. *J. Phys. Chem. B* **2013**, *117*, 11654−11659.
(15) Holland, P.; Rubingh, D. Nonideal multicomponent mixed micelle model. *J. Phys. Chem.* **1983**, *87*, 1984−1990.
(16) Clint, J. H. Micellization of mixed nonionic surface active agents. *J. Chem. Soc., Faraday Trans. 1* **1975**, *71*, 1327−1334.
(17) Hao, L.-S.; Deng, Y.-T.; Zhou, L.-S.; Ye, H.; Nan, Y.-Q.; Hu, P. Mixed Micellization and the Dissociated Margules Model for Cationic/Anionic Surfactant Systems. *J. Phys. Chem. B* **2012**, *116*, 5213−5225.
(18) Georgiev, G. Markov chain model of mixed surfactant systems. *Colloid Polym. Sci.* **1996**, *274*, 49−58.
(19) Lipfert, J.; Millett, I. S.; Seifert, S.; Doniach, S. Sample holder for small-angle x-ray scattering static and flow cell measurements. *Rev. Sci. Instrum.* **2006**, *77*, 046108.
(20) Lipfert, J.; Columbus, L.; Chu, V. B.; Lesley, S. A.; Doniach, S. Size and shape of detergent micelles determined by small-angle X-ray scattering. *J. Phys. Chem. B* **2007**, *111*, 12427−12438.
(21) Beno, M. A.; Jennings, G.; Engbretson, M.; Knapp, G. S.; Kurtz, C.; Zabransky, B.; Linton, J.; Seifert, S.; Wiley, C.; Montano, P. A. Basic Energy Sciences Synchrotron Radiation Center Undulator Sector at the Advanced Photon Source. *Nucl. Instrum. Methods Phys. Res., Sect. A* **2001**, *467*, 690−693.
(22) Seifert, S.; Winans, R. E.; Tiede, D. M.; Thiyagarajan, P. Design and performance of a ASAXS instrument at the Advanced Photon Source. *J. Appl. Crystallogr.* **2000**, *33*, 782−784.
(23) Kline, S. Reduction and analysis of SANS and USANS data using IGOR Pro. *J. Appl. Crystallogr.* **2006**, *39*, 895−900.
(24) Glatter, O. *Small Angle X-ray Scattering*; Academic Press: London, UK, 1982.
(25) Guinier, A. *Ann. Phys.* **1939**, *12*, 161−237.
(26) Konarev, P.; Volkov, V.; Sokolova, A.; Koch, M.; Svergun, D. PRIMUS: a Windows PC-based system for small-angle scattering data analysis. *J. Appl. Crystallogr.* **2003**, *36*, 1277−1282.
(27) Harkins, W. D.; Mattoon, R. W.; Corrin, M. L. Structure of Soap Micelles Indicated by X-Rays and the Theory of Molecular Orientation. I. Aqueous Solutions. *J. Am. Chem. Soc.* **1946**, *68*, 220−228.
(28) Puvvada, S.; Blankschtein, D. Theoretical and Experimental Investigations of Micellar Properties of Aqueous Solutions Containing Binary Mixtures of Nonionic Surfactants. *J. Phys. Chem.* **1992**, *96*, 5519−5592.
(29) Lange, v. H.; Beck, K.-H. Zur Mizellbildung in Mischlösungen homologer und nichthomologer Tenside. *Kolloid Z. Z. Polym.* **1973**, *251*, 424−431.
(30) Koch, M. H. J.; Vachette, P.; Svergun, D. I. Small-angle scattering: a view on the properties, structures and structural changes of biological macromolecules in solution. *Q. Rev. Biophys.* **2003**, *36*, 147−227.
(31) Luo, G.; Wang, H. The calculation of surface potential for spherical ionic micelles. *J. Dispersion Sci. Technol.* **2007**, *28*, 1108−1111.