Supporting information

for

Complementary light scattering and synchrotron small angle Xray scattering studies of the micelle-to-unimer transition of polysulfobetaines

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Polymer **2** was analysed by DLS at 1 mg mL⁻¹ in 0.5 M NaCl solution, to ensure the polymer was molecularly dissolved, and a D_h of 18 ± 1 nm was obtained. This is significantly bigger than that seen for **1** at 1 mg mL⁻¹ (5 ± 1 nm) (see ESI), indicating that the increase in D_h is a result of the increased size of the polymer chain. Analysis of a solution of **2** in water gave an assembly with D_h of 74 ± 2 nm, showing that self-assembly occurs in a salt-free environment.



Figure S1: Number average DLS traces of POEGMA homopolymer 1 ($D_h = 5 \pm 1 \text{ nm}$), diblock copolymer 2 in 0.5 M NaCl solution ($D_h = 18 \pm 1 \text{ nm}$), and diblock copolymer 2' in pure water ($D_h = 74 \pm 2 \text{ nm}$), determined at 25 °C, all at 1 mg mL⁻¹.

Calculating the absolute molecular weight of copolymers 2 - 5 in salt solution

Calculation of dn/dc for the di- and triblock copolymers

The refractive index (RI) increment (dn/dc) was determined using a Shodex RI-101 deflection refractometer. A range of concentrations of polymer in 0.5 M NaCl solution from 0.5 mg mL⁻¹ to 2 mg mL⁻¹ were measured. The RI response for each concentration was plotted against the concentration and the dn/dc calculated using the following equation.

$$\frac{dn}{dc} = \frac{slope \times n^o}{K}$$

Where slope is the gradient of the linear fit of the refractive index response vs the concentration n° is the RI of the solvent and K is the instrument constant. The dn/dc was calculated for diblock copolymer **2** in 0.5 M NaCl as 0.130 mL g⁻¹.



Figure S2: Plot of concentration *vs* RI response for **2** in salt solution. The dn/dc was calculated as 0.130 mL g⁻¹ using the slope of the linear fit.

The dn/dc was also calculated for triblock copolymers **3**, **4** and **5** in 0.5M NaCl solution. The calculated dn/dc values for the triblock copolymers are all very similar and are displayed in Table S1.

Table S1: Calculated *dn/dc* values for triblock copolymers 3, 4 and 5 in 0.5 M NaCl.

Triblock polymer	$dn/dc (mL g^{-1})$
3	0.126
4	0.124
5	0.125

Plotting the inverse of the relaxation time for the fast mode divided by $q^2 (\tau_{fast}^{-1}/q^2)$ against the scattering vector squared (q^2) showed that there was no angular dependence of the dissolved polymer chains (see Figure S3).



Figure S3: Plots of $\tau_{\text{fast}^{-1}/q^2}$ vs q^2 for A) **2** at 1 mg mL⁻¹ in 0.5 M NaCl, B) **3** at 1 mg mL⁻¹ in 0.5 M NaCl, C) **4** at 1 mg mL⁻¹ in 0.5 M NaCl, D) **5** at 1 mg mL⁻¹ in 0.5 M NaCl, showing there to be no significant angular dependence of the scattering of the dissolved polymer chains.

Analysis of the self-assembled structures of copolymers 2' - 5' in water

The dn/dc was calculated for the self-assembled solutions 2'-5' in water with the results shown in Table S2.

Table S2: Calculated *dn/dc* values for self-assembled solutions 2'-5' in water.

Polymer	$dn/dc (mL g^{-1})$		
2'	0.127		
3'	0.125		
4'	0.125		
5'	0.128		

Plotting the inverse of the relaxation time for the fast mode divided by $q^2 (\tau_{fast}^{-1}/q^2)$ against the scattering vector squared (q²) showed that there was no angular dependence of the self-assembled particles (see Figure S4).



Figure S4: Plots of $\tau_{fast}^{-1}/q^2 vs q^2$ for A) **2'** at 1 mg mL⁻¹, B) **3'** at 1 mg mL⁻¹, C) **4'** at 1 mg mL⁻¹, D) **5'** at 1 mg mL⁻¹, showing there to be no significant angular dependence of the scattering of the self-assembled solutions.

The absolute M_w of the self-assembled structures, **2'-5'**, in water was determined by SLS analysis. The results can be seen in Table S3. The aggregation number was calculated by comparison of the M_w for the micelles with the M_w calculated for a single polymer chain.



Table S3: Molecular weights and aggregation numbers for the self-assembled solutions 2'-5' as determined by SLS.

Figure S5: Plot of $Kc/R_{\theta, fast}$ vs concentration for self-assembled solutions 2', 3', 4' and 5' in water at 20°C. The M_w was calculated using the intercept of the linear fit to the SLS data.

Second virial coefficients, A₂, can be obtained from the slope of the graph shown in Figure S5. For 2' the gradient of the $Kc/R_{\theta, fast}$ vs concentration is essentially flat, meaning that the polymer-solvent interaction strength and polymer-polymer interaction strength are equal. For assemblies 3'-5' the gradient is positive meaning that the polymer-solvent interaction strength is greater than polymer-polymer interactions and hence the self-assembled structures will remain in solution and not tend to aggregate.

Analysis of the micelle to unimer transition by synchrotron SAXS

Variable temperature synchrotron SAXS studies, between 5 and 50 °C, were performed in order to determine the morphology of the self-assembled diblock copolymer, **2'** in water. A solution of **2'** (at 1 mg mL⁻¹ in 18.2 M Ω cm water) was placed in a 1.5 mm diameter quartz capillary. The capillary was held in a temperature controlled sample holder and temperatures of 5, 10, 19, 24, 28, 36, 40, and 50 °C were targeted. The sample was allowed to equilibrate for 10 minutes at each temperature before the measurement was taken. The measurements were collected at a sample to detector distance of

3.252 m to enable access to a *q* range of 0.0015 to 0.07 Å⁻¹, where *q* is the scattering vector and is related to the scattering angle (2 θ) and the photon wavelength (λ) by the following equation:

$$q = \frac{4\pi\sin(\theta)}{\lambda}$$

All patterns were normalised to fixed transmitted flux using a quantitative beamstop detector. The scattering from a blank (H₂O) was subtracted for each measurement. The two-dimensional SAXS images were converted in one-dimensional SAXS profile (I(q) versus q) by circular averaging, where I(q) is the scattering intensity. The functions used for the fitting from the NIST SANS analysis package were "Debye"¹ and "Core-Shell with Constant Core/Shell Ratio" models.²⁻⁴ ScatterBrain and Igor software were used to plot and analyse the data. The scattering length density of the solvent and the monomers were calculated using the "Scattering Length Density Calculator" provided by NIST Center for Neutron Research.⁵ Limits for q range were applied for the fitting from 0.002 to 0.05 Å⁻¹.



Figure S6: SAXS profiles for 2' at varying temperatures between 5 °C and 50 °C in water with a core-shell fit shown as a solid line.

Temperature	Morphology (Ratio	Volume fraction (Ratio	Number of micelles
(°C)	micelle:unimer)	micelle:unimer $\times 10^4$)	(/Vtot ×10 ¹⁰)
5	Micelles	27:0	344
10	Micelles	20:0	261
19	Micelles and unimers	1:324	129
24	Micelles and unimers	1:410	86
28	Micelles and unimers	1:431	56
36	Micelles and unimers	1:3831	3
40	Micelles and unimers	1:4460	1
50	Unimers	0:2341	0

Table S4: Showing the morphologies present at each temperature and the ratio of micelles to unimers, of 2' as calculated by

 SAXS analysis.

Count rate data from DLS analysis of the self-assembled structures



Figure S7: Plots of count rate (\blacktriangle) and size (\blacksquare , D_h) with increasing temperature for A) **2**' in water, B) **3**' in water, C) **4**' in water, D) **5**' in water.

Studying the morphology transition by variable temperature ¹H NMR spectroscopy

In order to investigate the solution properties of the diblock copolymer, **2'**, in water, variable temperature ¹H NMR spectroscopy was performed. A higher concentration of polymer solution was used, compared to the SAXS or LS measurements to ensure reliable spectroscopic results were obtained. An NMR sample of **2'** at 5mg mL⁻¹ was made in D₂O with an internal standard of DMF. This NMR sample was initially analysed by variable temperature DLS to confirm that the micelles still underwent the micelle-to-unimer transition at this higher concentration (see Figure S8).



Figure S8: DLS analysis with temperature of **2**^{\circ} at 5 mg mL⁻¹. Error bars are one standard deviation calculated from at least three measurements at each temperature.

¹H NMR spectroscopy was performed at temperatures ranging from 5 to 65 °C with 10 °C increments. DMF was used as an internal standard to help calculate the percentage hydrophilicity of the polymer. The COH peak of the DMF at δ 8.0 ppm was set at an integration of 1 and three separate peaks relating to the DMAPS block at δ 2.7, 3.7 and 4.1 ppm were integrated relative to this DMF peak (Figure S9). The integration of each peak at the highest temperature was assumed to be 100% hydrophilic, *i.e.* all the DMAPS side chains are hydrated. The integrations of the same peaks at different temperatures were compared to these "100%" peaks to calculate the percentage remaining hydrophilicity present in the polymer at that temperature.



Figure S9: A) ¹H NMR spectrum showing the three DMAPS peaks used for calculating remaining hydrophilicity in **2**', and B) – E) plots showing how the % relative hydrophilicity of the DMAPS segment of diblock copolymers **2'-5'**, respectively, change with increasing temperature.

Figure S9 shows how the integration of the 3 characteristic DMAPS signals changes with variable temperature for polymer samples **2'-5'**. This figure indicates that the betaine block never becomes fully hydrophobic for any of the betaine copolymers, even at temperatures of 5 °C, indeed at this temperature approximately 25% of the block remains hydrophilic, for all samples. Therefore the amphiphilic balance of the polymer is not directly proportional to the block lengths of the DMAPS and hence the amphiphilic balance is not as expected. We propose that this higher hydrophilicity in the diblock explains why micelles are formed instead of vesicles.

Encapsulation and release experiments



Figure S10: Fluorescence spectra showing the effect of filtering the micelle solution of **2'** multiple times has on the fluorescence response ($\lambda_{ex} = 500 \text{ nm}$) following incubation with Nile Red.

The same encapsulation and release experiments were performed (as described in the manuscript) on the self-assembled micelle **3'** and similar results to that observed for the assemblies formed from **2'** were seen.



Figure S11: Fluorescence spectra ($\lambda_{ex} = 500$ nm) showing the decrease in fluorescence after heating the micelle solution of 3'.



Figure S12: Dry-state TEM image of micelles 3', at 0.1mg mL-1, imaged on a graphene oxide support, scale bar 200nm.



Figure S13: Dry-state TEM image 4', at 0.1mg mL-1, imaged on a graphene oxide support, scale bar 100nm, showing the dissociation of the micelles on the graphene oxide support.



Figure S14: Dry-state TEM image of 5', at 0.1mg mL-1, imaged on a graphene oxide support, scale bar 100nm showing the dissociation of the micelles on the graphene oxide support.

References

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