Thermally induced micelle to vesicle morphology transition for a charged chain end diblock copolymer

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Supporting Information

Materials

AIBN (2,2’-Azobis(2-methylpropionitrile)) was recrystallised from a 9:1 mixture of hexanes/ acetone and stored in the dark at 4 °C. Tert-butyl acrylate (tBuA) was purified via vacuum distillation over CaH₂ and then stored at 4 °C. N-iso-propyl-acrylamide (NIPAM) was recrystallised from a 9:1 mixture of hexanes/ acetone and stored at 4 °C. All other materials were used as received from Sigma-Aldrich Co. Tetrahydrofuran (THF), N,N-dimethylformamide (DMF), dichloromethane (DCM), ethyl acetate and hexanes were used as received from Fisher Scientific unless otherwise stated. When used, dry solvents were collected and used directly from an in house drying and degassing solvent tower delivery system.

Instrumentation

¹H NMR and ¹³C NMR were obtained at 400 or 500 MHz with a Bruker DPX-400 or DPX-500 spectrometer respectively in CDCl₃ unless otherwise stated. Chemical shifts are reported in ppm (δ) relative to CHCl₃ (7.26 ppm for ¹H and 77.2 ppm for ¹³C) using TMS as an internal reference. The molecular weight of each polymer block was determined by ¹H NMR spectroscopy (128 scans on a 500 MHz cryo probe) to give Mₙ(NMR). This was calculated by comparing the integration of the peaks corresponding to the RAFT CTA, 3 end groups (e.g. the phenyl ring protons at 7.20 ppm) and the N-methyl peaks of the acrylamide at 4.02 ppm for the PNIPAM block and the tbutyl protons of the PₜBuA side chain at 1.38 ppm, (see Figures S2, S3 and S4). All infrared spectra were collected on a Perkin Elmer Spectrum 100 FTIR ATR unit. Molecular weights Mₙ(GPC) and molecular weight distributions for all polymers were estimated by size exclusion chromatography (SEC) with HPLC grade DMF (Fisher) containing 1 wt % LiBr as eluent at 40 °C at a flow rate of 1.0 mL/ minute. Data was analyzed with Cirrus GPC software (Polymer Laboratories) using polymethyl methacrylate standards (EasiCal PMMA, Polymer Laboratories).

Dynamic Light Scattering (DLS) Measurements:

Hydrodynamic diameters (Dₜₕ) and size distributions of the nanostructures in aqueous solutions were determined by dynamic light scattering (DLS). The DLS instrumentation consisted of a Malvern Zetasizer NanoS instrument operating at 25 °C or 65 °C with a 4 mW He-Ne 633-nm laser module. Measurements were made at a detection angle of 173° (back scattering), and Malvern DTS 5.02 software was used to analyze the data. DLS measurements during the transition were taken by transferring the cuvette to the DLS instrument (ensuring no heat loss occurs) and recording measurements in triplicate (with 15 runs recorded per measurement) at 65 °C, after equilibrating for 5 minutes. All size distributions and polydispersity data shown for micelles, 6 and vesicles, 7 are averages of at least three DLS measurements.
Transmission Electron Microscopy (TEM) Measurements:

To prepare samples for TEM analysis at room temperature, an argon plasma treated, carbon coated, copper grid was placed onto a droplet of the sample solution for 2 minutes (film side down) and excess liquid was removed by blotting onto filter paper and the sample was allowed to air dry. The sample was then stained using a 1% solution of uranyl acetate for 1.5 minutes, blotted with filter paper to remove any excess liquid and allowed to air dry. To prepare samples for TEM analysis at 65°C, an argon plasma treated, carbon coated, copper grid was placed into the solution to be analysed at 65°C for 2 minutes and excess liquid was removed rapidly via placing under vacuum (to prevent cooling of the sample upon drying). The samples were then stained using a 1% solution of uranyl acetate (unless otherwise stated) for 1.5 minutes, blotted onto filter paper to remove any excess liquid and allowed to air dry. All samples were then examined with a transmission electron microscope (JEOL TEM-1200), operating at 100 kv. Average sizes of micelles, 6 and vesicles, 7 were determined from counting the sizes of at least 100 particles.

Atomic Force Microscopy (AFM) Measurements:

Measurements and distributions for the vesicles, 7 were determined by tapping-mode atomic force microscopy (AFM) under ambient conditions in air. The sample solutions were prepared for AFM analysis by deposition of a drop (2 μL) onto freshly cleaved mica and allowed to dry in oven at 65°C. The number-average particle diameter (D_{avg}) value was generated from the sectional analysis of at least 30 particles.

Static Light Scattering (SLS) Measurements:

SLS measurements were performed on a Malvern Instruments Autosizer 4800, equipped with an APD detector and a Malvern 7132 50 ns 16-bit digital auto-correlator, using a 50mW green laser beam. SLS measurements were carried out over an angle range of 30-170° in 7° stepwise increments. Toluene was used as a calibration standard. SLS data was collected for 4 or more different concentrations of the vesicles, 7 at 20 different angles for each concentration and was analysed using the Zimm plot method on Malvern PSW0078 – Advanced software to determine z-average radius of gyration (R_g).
**Figure S1.** Synthesis of functionalised RAFT CTA, 3.

**RAFT CTA, 1.**

Octadecanethiol (2.00 g, 6.98 mmol) was added to a stirred suspension of potassium phosphate (1.56 g, 7.33 mmol) in acetone (20 mL) and the reaction mixture was stirred for ten minutes. Carbon disulfide (1.59 g, 20.94 mmol) was added and the solution turned bright yellow. After stirring for thirty minutes, 4-(chloromethyl)benzyl alcohol (1.09 g, 6.98 mmol) was added and the reaction mixture was stirred at room temperature for a further 18 h. The reaction mixture was then filtered and all volatiles were removed under reduced pressure. The crude product was purified directly *via* flash column chromatography on silica gel eluting with 1:1.5 ethyl acetate:hexanes (Rf of 1 = 0.5) to give RAFT CTA, 1 as a yellow solid (2.27 g, 67 %). IR (ν<sub>max</sub>/cm<sup>-1</sup>): 684, 718, 814, 864, 945, 970, 1015, 1027, 1057, 1077, 1156, 1196, 1214, 1240, 1388, 1418, 1470, 1515, 2850, 2916, 2956, 3330. 1H NMR (CDCl<sub>3</sub>, 400 MHz): δ 0.85 (t, 3H, C<sub>H</sub><sub>3</sub>(CH<sub>2</sub>)<sub>15</sub>), 1.08-1.40 (br m, 30H, (C<sub>H</sub><sub>2</sub>)<sub>15</sub>), 1.60 (m, 2H, S=CSCH<sub>2</sub>C<sub>H</sub><sub>2</sub>), 3.30 (t, 2H, S=CSC<sub>H</sub><sub>2</sub>), 4.52 (s, 2H, S=CSC<sub>H</sub><sub>2</sub>Ph), 4.60 (s, 2H, Ph CH<sub>2</sub>OH) 7.25 (m, 4H, ArH). 13C NMR (CDCl<sub>3</sub>, 400 MHz): δ 14.5-37.5 (CH<sub>3</sub>(CH<sub>2</sub>)<sub>17</sub>), 41.4 (S=CSC<sub>H</sub><sub>2</sub>Ph), 65.4 (PhCH<sub>2</sub>OH), 127.7-140.8 (ArC), 224.1 (SC=S).

**RAFT CTA, 2.**

1 (1.00 g, 2.07 mmol) was dissolved in dry dichloromethane (60 mL) under nitrogen and thionyl chloride (0.985 g, 0.604 mL, 8.28 mmol) was then added slowly at 0 ℃. The reaction mixture was stirred for 18 h at room temperature under nitrogen. The reaction was then carefully quenched at 0 ℃ *via* the slow drop wise addition of brine. The crude product was washed with 1M sodium hydroxide solution (3 x 100 mL), water (3 x 100 mL) and saturated brine (3 x 100 mL), dried over magnesium sulphate, filtered and concentrated under reduced pressure. The crude product was purified *via* flash column chromatography on silica gel eluting with 1:9 dichloromethane:hexanes (Rf of 2 = 0.5) giving RAFT CTA, 2 as a yellow solid (0.405 g, 39 %). IR (ν<sub>max</sub>/cm<sup>-1</sup>): 689, 715, 813, 868, 972, 1019, 1061, 1081, 1159, 1201, 1218, 1243, 1395, 1420, 1471, 1515, 2851, 2919, 2957. 1H NMR (CDCl<sub>3</sub>, 400 MHz): δ 0.85 (t, 3H, CH<sub>3</sub>(CH<sub>2</sub>)<sub>15</sub>), 1.08-1.40 (br m, 30H, (CH<sub>2</sub>)<sub>15</sub>), 1.60 (m, 2H, S=CSC<sub>H</sub><sub>2</sub>CH<sub>2</sub>), 3.30 (t, 2H, S=CSC<sub>H</sub><sub>2</sub>), 4.52 (s, 2H, S=CSC<sub>H</sub><sub>2</sub>Ph), 4.60 (s, 2H, Ph CH<sub>2</sub>Cl) 7.25 (m, 4H, ArH). 13C NMR (CDCl<sub>3</sub>, 400 MHz): δ 14.5-37.5 (CH<sub>3</sub>(CH<sub>2</sub>)<sub>17</sub>), 41.2 (S=CSC<sub>H</sub><sub>2</sub>Ph), 46.2 (PhCH<sub>2</sub>Cl), 129.3-137.4 (ArC), 223.8 (SC=S).
RAFT CTA, 3.

2 (0.175 g, 0.350 mmol) was dissolved in chloroform (5 mL) and N, N-dimethyl-ethyl-amine (0.153 g, 0.230 mL, 2.09 mmol) was added to the solution. The reaction mixture was then stirred for 18 h at room temperature. Upon reaction completion as monitored by thin layer chromatography (R_f of 2 = 0.5, R_f of 3 = 0) ca. 20 mL of hexane was added to the solution and upon cooling in an ice bath to ca. 0 °C, the resultant yellow precipitate was collected via suction filtration to give RAFT CTA, 3 as a yellow solid (0.178 g, 95 %). IR (ν_{max}/cm^{-1}): 720, 810, 870, 908, 1037, 1056, 1121, 1185, 1399, 1421, 1467, 1515, 1614, 2850, 2916, 2955. 1H NMR (CDCl3, 400 MHz): δ 0.80 (t, 3H, CH(CH2)15), 1.16-1.48 (br m, 30H, (CH2)15), 1.32 (t, 3H, NCH2CH3), 1.63 (m, 2H, S=CSCH2CH2), 3.20 (s, 6H, N(CH3)2), 3.62 (t, 2H, S=CSCH2), 3.30 (t, 2H, S=CSCH2), 4.58 (s, 2H, S=CSCH2Ph), 4.96 (s, 2H, Ph CH2N(CH3)2) 7.33 (d, 2H, ArH), 7.55 (d, 2H, ArH). 13C NMR (CDCl3, 400 MHz): δ 14.5-37.7 (CH3(CH2)17), 40.8, 59.5, 66.9, 127.6-138.4 (ArC), 223.4 (SC=S).

Supplementary Material (ESI) for Chemical Communications

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Figure S2. Assigned 1H NMR spectrum (400 MHz, CDCl3) of functionalised RAFT CTA, 3.
Chain end functionalised PNIPAM, 4.

N-isopropylacrylamide (0.5 g, 4.42 mmol), 3 (0.079 g, 0.147 mmol) and AIBN (0.0073 g, 0.044 mmol) were dissolved in dry DMF (1.6 mL) and placed in an oven-dried ampoule with a stirrer bar, under the flow of nitrogen. The ampoule was degassed at least three times via freeze/pump/thawing cycles and released to and sealed under nitrogen. The polymerisation mixture was then heated and stirred at 65 °C for 5 h. Upon completion, the polymerisation mixture was cooled to stop the polymerisation and precipitated into 1:9 diethylether:hexanes (ca. 200 mL) to afford chain end functionalised homopolymer, PNIPAM, 4 (0.480 g, 96 %). $M_n^{\text{NMR}} = 3,400$ g/mol, $M_n^{\text{GPC}} = 3,900$ g/mol, (using PMMA standards), $M_n/M_n = 1.12$. IR ($\nu_{\text{max}}$/cm$^{-1}$): 1131, 1171, 1366, 1386, 1458, 1538, 1640, 2927, 2970, 3282. $^1$H NMR (CDCl$_3$, 500 MHz): δ 0.85 (t, 3H, $\text{C}_3\text{H}_3$($\text{CH}_2$)$_{17}$ in CTA end group), 1.05-1.20 (br, 6H, ($\text{C}_3\text{H}_3$)$_2\text{CH}$ in polymer side chain), 1.29-2.38 (br, 3H, $\text{C}_2\text{H}$ and $\text{C}_2\text{H}$ in polymer backbone), 3.19 (t, 2H, $\text{SC}=$SS$\text{C}_2\text{H}$ in CTA end group), 3.29 (q, 2H, $\text{NCH}_2\text{CH}_3$ in charged end group), 4.75 (s, 4H, $\text{CH}_2\text{PhCH}_2$ in CTA end group), 6.2-7.2 (br, 1H, (CH$_3$)$_2$CH) 7.45 (m, 4H, ArH in end group).

**Figure S3.** Assigned $^1$H NMR spectrum (400 MHz, CDCl$_3$) of end functionalised homopolymer, PNIPAM, 4.
Chain end functionalised PNIPAM-b-PrBuA, 5.

$t\text{BuA}$ (0.195 g, 1.53 mmol), 4 (0.200 g, 0.051 mmol) and AIBN (0.0025 g, 0.0152 mmol) were dissolved in dry DMF (0.4 mL) and placed in a oven-dried ampoule with a stirrer bar, under the flow of nitrogen. The ampoule was degassed at least three times via freeze/ pump/ thawing cycles and released to and sealed under nitrogen. The polymerisation mixture was then heated and stirred at 85 ºC for 16 h. Upon completion, the polymerisation mixture was cooled and precipitated into hexanes (ca. 200 mL). The resulting polymer was collected via decanting off the solution and dissolving the remaining polymer in THF and then removing the THF under reduced pressure to afford chain end functionalised diblock copolymer, PrBuA-b-PNIPAM, 5 (0.350 g, 89 %) $M_n^{\text{NMR}} = 6,600$ g/mol, $M_n^{\text{GPC}} = 5,800$ g/mol, $M_w/M_n = 1.29$ (using PMMA standards). IR ($\nu_{\text{max}}$/cm$^{-1}$): 754, 822, 845, 1007, 1026, 1051, 1146, 1256, 1367, 1391, 1457, 1547, 1644, 1726, 2931, 2974, 3277. $^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ 0.83 (t, 3H, $CH_3(CH_2)_{17}$ in CTA end group), 1.05-1.20 (br, 6H, $(CH_3)_2CH$ in polymer side chain), 1.29-2.38 (br, 3H, $CH_2$ and $CH$ in polymer backbone), 1.30-1.62 (br, 9H, $(CH_3)_3C$ in polymer side chain), 6.20-7.10 (br, 1H, $(CH_3)_2CH$), 7.11 (d, 2H, ArH in end group), 7.19 (d, 2H, ArH in end group).

Figure S4. Assigned $^1$H NMR spectrum (400 MHz, CDCl$_3$) of end functionalised diblock, PrBuA-b-PNIPAM, 5.
**PNIPAM-b-P^t^BuA Micelles, 6**

The diblock copolymer PNIPAM-b-P^t^BuA, 5 was dissolved in THF at a concentration of 2 mg/ mL. Deionised nanopure water was added slowly to the stirred THF solution via a metering pump at a rate of 10 mL/ h. Once all of the water had been added, the opaque micelle solution was transferred to dialysis tubing (MWCO = 3.5 kDa) and dialysed against deionized nanopure water exhaustively (incorporating 6 changes) to remove all of the THF, affording a clear solution of PNIPAM-b-P^t^BuA micelles, 6 at a concentration of 1 mg/ mL. $D_h$ (DLS by number) = 13.2 nm (100 %), $D_h$ (DLS by volume) = 22.3 nm (100 %), $D_h$ (DLS by intensity) = 21.1 nm (51.6 %), 105 nm (48.4 %), PDI (DLS) = 0.45; $D_{avg}$ (TEM): 28 ± 4 nm.

**PNIPAM-b-P^t^BuA Vesicles, 7**

A 2 mL solution of micelles, 6 was placed in a sealed glass cuvette equipped with a small stirrer bar and placed in an oil bath at 65 °C. The sample was then heated with stirring at 500 rpm for the desired time, to achieve the micelle to vesicle transition. $D_h$ (DLS by number) = 145 nm (100 %), $D_h$ (DLS by volume) = 147 nm (100 %), $D_h$ (DLS by intensity) = 154 nm (100 %), PDI (DLS) = 0.47; $D_{avg}$ (TEM): 105 ± 15 nm.

![Figure S5. Raw DLS data (by Number) for micelle to vesicle transition at 25 °C, upon heating to 65 °C and cooling back to 25 °C.](image-url)
Figure S6. Raw DLS data (by number, volume and intensity) for micelles, 6 at 25 °C, showing unimodal traces by number and volume distributions (at ca. 20 nm). The presence of larger aggregates (at ca. 100 nm) can be seen in the intensity distribution data.

Figure S7. Autocorrelation function for micelles, 6 at 25 °C. The smooth, unimodal trace with a short decay time indicates one small size distribution is primarily present.
Figure S8. Raw DLS data (by number, volume and intensity) for vesicles, 7 (after stirring 6 at 65 °C for 170 hrs), showing a monodisperse size distribution of a larger size of ca. 150 nm.

Figure S9. Autocorrelation function for vesicles, 7 at 65 °C. The slower decay indicates that larger aggregates are now present in solution but also shows a slight bimodal distribution indicating the presence of other (larger) aggregates, possibly due to some sedimentation. However, this was not visible in the solution (see Figure S12).
Figure S10. Raw DLS data (by number, volume and intensity) for vesicles, upon cooling to room temperature showing two polydisperse size distributions, with one main population (by number) at ca. 70 nm.

Figure S11. Autocorrelation function for vesicles, upon cooling to room temperature with a slow decay time indicating the presence of larger aggregates and a slightly noisy baseline indicative of a polydisperse, possibly aggregating sample.
Figure S12. Photographs of a) micelles, 6 at 25 °C, b) vesicles, 7 heated at 65 °C (photo taken while still hot) and c) a sample upon cooling back to 25 °C.

Figure S13. Representative TEM micrographs of micelles, 6 at 25 °C stained with uranyl acetate.

Figure S14. Representative TEM micrographs of vesicles, 7 at 65 °C obtained at lower concentrations (ca. 0.1 mg/mL) stained with phosphotungstic acid (left) and
uranyl acetate (right). Note these images are recorded at lower concentration than those presented in the paper and this affords a larger size of particle.

Figure S15. Representative tapping mode AFM images of vesicles, 7. Solutions were drop-deposited at 65 °C onto freshly cleaved mica and allowed to dry in an oven at 65 °C. The cross sectional insert shows the hollow vesicle topography and the diameter in the dried state.

Figure S16. Representative TEM micrograph of vesicles, 7 after cooling to 25 °C stained with uranyl acetate, possibly showing the original reformed spherical micelles. However, other larger and irregular aggregates were also observed.
Figure S17. Zimm plot for vesicles. Data points marked in grey are excluded from the zimm plot analysis since these are taken at extreme angles and so deviate from the data fit.
Variable Temperature $^1$H NMR spectroscopy analysis for the diblock, 5.

**Figure S18.** A graph to show the ratio of $^1$H NMR signal intensities for PNIPAM C-H next to methyl groups (assigned as e in inset, at 3.95 ppm) w.r.t HDO as a function of temperature in D$_2$O solution (6 mg/mL) for the (P$_{tBu}$A-$b$-PNIPAM) diblock copolymer, 5.

The $^1$H NMR spectrum of the diblock copolymer, 6 in D$_2$O at 25 °C showed complete loss of P$_{tBu}$A resonances (at 1.4 ppm in CDCl$_3$) due to suppressed molecular motion of the aggregated hydrophobic chains, which is strongly indicative of a stable core-shell micelle structure with a highly viscous P$_{tBu}$A inner core, providing evidence for micelle formation.
$^1$H NMR of the diblock, 5 after heating for 2 weeks at 65 °C

Figure S19. Assigned $^1$H NMR spectrum (400 MHz, CDCl$_3$) of the end functionalised diblock copolymer, PtBuA-b-PNIPAM, 5 after heating at 65 °C for 2 weeks to undergo a transition from micelles to vesicles. Also shown is the region of the spectrum upto 12 ppm where any poly(acrylic acid) COOH peaks would appear if the PtBuA block had been hydrolysed.