## Thermally induced micelle to vesicle morphology transition for a charged chain end diblock copolymer<sup>†</sup>

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A quaternary amine end functionalised diblock copolymer (PtBuA-b-PNIPAM) has been synthesised using RAFT polymerisation and shown to undergo a thermally induced morphology transition from micelles to vesicles, as evidenced by TEM, AFM, SLS and DLS analyses.

Precise morphological control over self-assembled polymers is currently the subject of intense research interest with various nanosized architectures being studied, including spherical micelles, vesicles, cylinders, worm-like micelles and rods.<sup>1–3</sup> Polymeric nanostructures are particularly attractive materials since their size, structure and function can be readily tailored to suit many desirable applications in nanoscience including drug delivery and catalysis through careful selection of the polymer structure and properties prior to self-assembly.<sup>4–8</sup> Furthermore, research groups have been able to tune the physical properties of the block copolymer building blocks such that the morphology of the resultant self-assembled system is responsive towards stimuli such as temperature and pH.<sup>9–19</sup>

Of particular interest is the ability for polymeric assemblies to exhibit transitions between different morphologies (and hence give rise to a change in physical properties) as a result of an alteration in the packing parameter<sup>20</sup> (p) of the constituent polymers in response to applied external stimuli.<sup>21-27</sup> Recently, Grubbs and co-workers have investigated thermoresponsive morphology transitions for triblock copolymers which incorporate a thermoresponsive central block. Initial studies on these systems (and those by Zhuo et al.) report that the time taken for the triblock polymer assemblies to exhibit this thermally induced transition can be in the order of weeks.<sup>25,26</sup> Herein, we report a thermally induced morphology transition from micelles to vesicles for a diblock copolymer, end functionalised with a permanently hydrophilic charged group. We propose that this charged end group is a crucial component in ensuring the stability of the diblock copolymer in solution when self-assembled into a nanostructure.

We synthesised an end functionalised diblock copolymer *via* reversible addition fragmentation chain transfer (RAFT) polymerisation<sup>28</sup> utilising a novel quaternary amine functionalised chain transfer agent (CTA), see Fig. S1 and S2 (ESI†).<sup>29</sup>

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**Fig. 1** Schematic of the stimuli induced change in the hydrophilic to hydrophobic ratio of the target copolymer.

The diblock copolymer used in this study contains a thermoresponsive polymer block (poly(*N*-isopropyl acrylamide) or PNIPAM) located between a permanently hydrophobic block and a permanently hydrophilic charged quaternary amine 'headgroup', Fig. 1.

The structure and properties of the diblock copolymer were carefully chosen to enable a significant modification of p for the diblock copolymer, upon changing the temperature of the surrounding solution. It was proposed that the copolymer would have a low p below the LCST of PNIPAM and form smaller aggregates with higher interfacial curvature (*i.e.* spherical micelles) and above the LCST the p value would increase, thus forming larger aggregates with lower interfacial curvature<sup>26</sup> (*i.e.* vesicles), see Fig. 2.

The diblock copolymer used in this study was poly(*tert*butylacrylate-*b*-*N*-isopropylacrylamide), synthesised using a quaternary amine functionalised RAFT CTA, **3**, giving rise to a well-defined permanently charged, end functionalised diblock copolymer, PtBuA-b-PNIPAM, **5** ( $M_n$  (NMR) = 6600 Da,  $M_n$  (GPC) = 5800 Da,  $M_w/M_n = 1.29$ ), see Scheme 1.

The self-assembly of copolymer, **5**, into micelles, **6**, proceeded *via* dissolution of the copolymer in tetrahydrofuran (THF) at a concentration of 2 mg mL<sup>-1</sup>. Deionised nanopure water was then added slowly to the stirred THF solution followed by exhaustive dialysis into nanopure water to remove the THF giving a final concentration of *ca*. 1 mg mL<sup>-1</sup>. Dynamic light scattering (DLS) of the resultant aggregates at 25 °C showed the presence of exclusively micelles by volume



**Fig. 2** Schematic representation of the thermally induced micelle to vesicle morphology transition.

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Scheme 1 Synthesis of diblock copolymer, PtBuA-b-PNIPAM, 5, utilising RAFT CTA, 3.

and number distributions with an average hydrodynamic diameter ( $D_{\rm h}$ ) of 22 nm (PDI = 0.45). A small number of larger aggregates could be observed in the DLS intensity data ( $D_{\rm h}$  = 105 nm). Analysis of a dried sample of the micelle solution, **6**, at 25 °C, by transmission electron microscopy (TEM) stained with uranyl acetate, showed the presence of spherical micelles  $D_{\rm avg} = 28 \pm 4$  nm (see Fig. 4 and S13 (ESI†)), with a very small population of larger aggregates, which could be the larger structures observable by DLS.

Upon heating and stirring a 1 mg mL<sup>-1</sup> solution of the micelles, 6, at 65 °C (above the LCST of PNIPAM at ca. 32 °C, and the glass transition temperature  $(T_g)$  of PtBuA at 40 °C), no significant change in the  $D_{\rm h}$  (by DLS) of the aggregates occurred over a period of 6 days. However, around day 7, a dramatic change in the  $D_{\rm h}$  of the aggregates was observed, increasing from 22 nm to 147 nm (PDI = 0.47), suggesting a possible morphology change, see Fig. 3. Longer heating and stirring times (for a further 8 days) showed no further increase in the size of the aggregates by DLS, although the PDI by DLS decreased to 0.22. No precipitation or discolouration of the clear solution occurred, indicating the lack of an observable cloud point of the polymer in solution (see Fig. S12, ESI<sup>†</sup>), which is consistent with other literature reports for similar thermoresponsive systems.<sup>26</sup> In a similar method reported by Grubbs et al., we observed a broad LCST at around 43 °C in the VT <sup>1</sup>H NMR spectra of 5 in  $D_2O$ .<sup>26</sup>

To confirm the existence of vesicles after heating for 7 days at 65 °C, the solution was analysed by TEM. Upon deposition of the sample (at 65 °C) and positive staining with ammonium molybdate (selective for the positively charged outer shell), much larger aggregates with  $D_{avg} = 105$  nm were observed exclusively, which had a clearly definable bilayer structure and showed fusion-like behaviour with each other, indicative of a vesicle type morphology (see Fig. 4).



Fig. 3 Change in hydrodynamic diameter,  $D_{\rm h}$  (by number), of copolymer 5 as a function of time upon heating at 65 °C at a concentration of 1 mg mL<sup>-1</sup>.



**Fig. 4** Representative TEM micrographs: (a) micelles, **6**, at 25 °C stained with uranyl acetate; (b) vesicles, **7**, at 65 °C stained with ammonium molybdate; (c) polydisperse micelles after cooling back the vesicles, **7**, to 25 °C, stained with uranyl acetate. Samples of **6** and **7** were drop cast from an aqueous solution (*ca.* 1 mg mL<sup>-1</sup>) at RT and 65 °C, respectively.

The average diameters of the vesicles by TEM were observed to be lower than in solution by DLS measurements. This is consistent with similar reports studying polymeric vesicles via TEM in the dried state.<sup>19</sup> The morphology of the vesicles, 7, was explored further in the dried state by atomic force microscopy (AFM), via drop deposition of 7 onto a mica surface at 65 °C. Individual particles could be clearly identified and they appeared to have a hollow morphology when collapsed, with an average diameter,  $D_{avg}$  of ca. 120 nm, which agrees with DLS and TEM data. Although, as with TEM, this was in the dried state and therefore showed a marked reduction from their size in solution (as measured by DLS). Fig. S15 (ESI<sup>†</sup>) shows representative AFM images for the vesicles, 7, and highlights their hollow nature and also their deformation from a uniform and spherical shape. Crosssectional AFM analysis of 7 supports this hypothesis.

To further confirm their hollow nature, static light scattering (SLS) measurements were performed on several samples of the vesicles, 7, at 65 °C. This was done to determine the average radius of gyration,  $R_{\rm g}$ , of the vesicles, 7. The ratio  $R_{\rm g}/R_{\rm h}$ (where  $R_{\rm h} = 1/2 D_{\rm h}$ ) is useful for analyzing the structure of a nanosized particle such as a vesicle, since  $R_{g}/R_{h}$  ratios close to unity indicate that a hollow particle has been formed and values close to 0.7 typically indicate a solid sphere-like particle, such as a micelle.<sup>30</sup> SLS measurements upon transformation into vesicles, 7, at lower concentrations of 0.1–0.01 mg mL<sup>-1</sup> (permitted for SLS analysis) proved successful, although the micelle to vesicle transition yielded slightly larger vesicles than at 1 mg mL<sup>-1</sup>, with  $D_{\rm h} = 211$  nm. However, morphology transformations at 1 mg mL<sup>-1</sup> also showed small amounts of size variation. SLS measurements of 7 gave  $R_g = 116.8$  for the vesicles, which gave an  $R_g/R_h$  ratio of 1.11 (see Fig. S17 for the

Zimm plot, ESI<sup>†</sup>). Furthermore, vesicles formed at lower concentrations were observable by TEM (see Fig. S14, ESI<sup>†</sup>). This supports the theory of a thermally induced transformation into hollow vesicles.

To explore the reversibility of the micelle-vesicle transition, a solution of vesicles, 7, at 1 mg mL<sup>-1</sup> was cooled to room temperature (with stirring) after being heated for 9 days. After 2 days of cooling, the mean  $D_h$  changed from *ca*. 155 nm to *ca*. 70 nm (PDI = 0.80) as observed by DLS measurements at 25 °C (see Fig. S10, ESI†). These structures were proposed to be a polydisperse mixture of micelles and were observed by TEM (see Fig. 4). Despite this morphology change back to predominantly micelles, upon cooling the sample down, the original well-defined micelles could not be solely re-isolated, but could be observed by TEM in the presence of other irregularly shaped nanostructures.

The inability for a controlled reversible restructuring of the aggregates from vesicles back to micelles is probably due to the restricted movement of PtBuA chains below their  $T_g$ , which is higher than room temperature. The PtBuA chains are not able to reorganise in solution as readily, compared to when forming the vesicles, 7 (when the copolymer is heated above both the  $T_{\rm g}$ of PtBuA and the LCST of PNIPAM). Furthermore, the relatively well-defined micelles, 6, were produced via the slow addition of water to a THF solution of the copolymer, 5, and then dialysed into water, since 5 is not easily directly solubilised in water. Thus, it is not too surprising that the original distribution of micelles could not be recovered. This problem could be overcome by synthesising a new copolymer which is more readily soluble in water at RT and utilises a lower  $T_g$  hydrophobic block. We are currently exploring the modification of the current system to incorporate such changes and therefore enable a complete and controlled thermoreversible transition, which may enable the encapsulation and release of hydrophilic substrates for drug and/or gene delivery applications. We propose that the current vesicles may show limited membrane permeability towards hydrophilic, cationically charged species which could facilitate their use in temperature responsive hydrophilic scavenger/encapsulation applications. Furthermore, it is proposed that the exterior and interior charged 'headgroup' functionality could be exploited for the binding of biologically relevant species such as proteins, DNA or RNA.

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