Shell-crosslinked nanostructures from amphiphilic AB and ABA block copolymers of styrene-*alt*-(maleic anhydride) and styrene: polymerization, assembly and stabilization in one pot†

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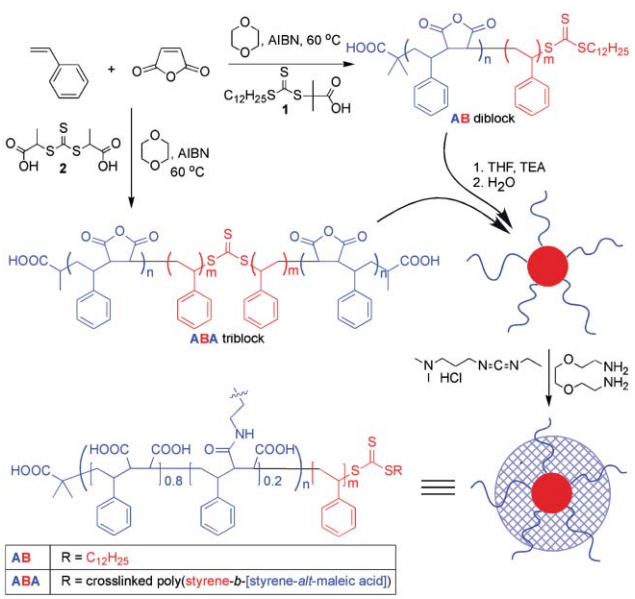
Shell-crosslinked nanostructures having unusual rosette morphologies have been produced by a simple process from styrene and maleic anhydride.

Nanoparticles formed by the regioselective crosslinking of the outer regions of amphiphilic block copolymer micelles (by covalent coupling of chain segments constituting the shell[1–5] or an intermediary layer[6,7]) have attracted great interest in recent years as well-defined nanoreactors,[7] agents for encapsulation,[3–5] transduction,[3] and drug delivery,[4] among other potential applications. The block copolymer precursors are programmed for multimolecular assembly into complex nanostructures based upon their compositions and structures,[8] which are controlled during synthesis using living polymerization techniques and *via* post-polymerization modifications. For physical and chemical compatibility purposes, protecting groups are often utilized during polymerization to establish the polymer structure and then are removed to reveal polar, reactive functionalities to promote supramolecular assembly and provide for covalent crosslinking.[9]

Armes and coworkers have developed an approach towards reducing the synthetic complexity of copolymer synthesis and micelle formation,[2,6,7] whereby the direct production of micelles from block copolymers was accomplished by aqueous atom transfer radical polymerization of monomer units having pH- and thermally-tunable degrees of hydrophilicity. In another approach, the assembly of polymer mixtures into non-covalently crosslinked micelles has been followed by shell-crosslinking to establish robust nanomaterials by a block copolymer-free strategy.[10] In this report, we present an alternative synthetic simplification strategy, which maintains the elegance of block copolymer assembly and utilizes a one-pot formation of well-defined, regioselectively-crosslinked nanostructures from commercially-available monomers.

To demonstrate this one-pot synthetic strategy, poly(maleic anhydride-*alt*-styrene)-*block*-styrene AB and ABA block copolymers were prepared from maleic anhydride (MA) and styrene (STY) *via* radical addition fragmentation chain transfer (RAFT) polymerization,[11] hydrolyzed and organized into micellar assemblies upon the addition of water, and crosslinked by addition of a diamine in the presence of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC), yielding shell-crosslinked nanostructures (Scheme 1). Each step within the overall one-pot methodology was performed independently to confirm the structure and composition of the products and to study its influence on the resulting assemblies and crosslinked nanostructures.



Scheme 1 Synthesis of di- and tri-block poly(styrene-*alt*-maleic anhydride)-*block*-polystyrene copolymers and formation of micelles and shell-crosslinked nanoparticles.

In the first stage, the block copolymers were synthesized from commodity monomers and without the need for sequential monomer additions and post-polymerization modification, allowing for significant cost and labor savings over current techniques for the synthesis of similar nanostructures. The propensity of MA and STY to form alternating copolymers regardless of their molar ratio is well-known. The living copolymerization of MA with STY has been reported under RAFT[11,12] or nitroxide-mediated (NMRP)[13,14] polymerization conditions. Of these, RAFT was selected, as it can be performed at temperatures lower than those required for NMRP,[13] allowing the formation of MA–STY copolymers with structures much closer to the alternating ideal.[11,12] The practical problems of synthesis, purification, and storage, commonly associated with RAFT agents, were ameliorated by the use of crystalline and virtually odorless RAFT agents, *S*-dodecyl *S*′-2-(2,2-dimethylacetic acid) trithiocarbonate, **1**, and 2,2′-bis(propionic acid) trithiocarbonate, **2**.[15] Therefore, RAFT copolymerization of MA and STY was conducted in the presence of excess STY to afford an alternating copolymer initially, which

† Electronic supplementary information (ESI) available: Full experimental details, AFM images and DLS correlation functions. See http://www.rsc.org/suppdata/cc/b5/b504313a/

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Table 1 Polymers synthesized in this study

	STY : MA : RAFT : AIBN (initial ratios)	Conv.[a]	M_n (theory)[b]	M_n (GPC)	PDI	%STY[c]	Composition[d]
3	250 : 50 : 1[e] : 0.2	93%	29 200	23 100	1.08	77.7	(STY-*alt*-MA)$_{52}$–STY$_{118}$
4	200 : 100 : 1[e] : 0.2	94%	29 100	29 800	1.09	56.3	(STY-*alt*-MA)$_{117}$–STY$_{25}$
5	300 : 100 : 1[e] : 0.2	86%	35 700	35 400	1.10	68.3	(STY-*alt*-MA)$_{109}$–STY$_{113}$
6	150 : 50 : 1[f]: 0.2	96%	20 200	20 600	1.08	69.3	(STY-*alt*-MA)$_{28}$–STY$_{63}$–(STY-*alt*-MA)$_{28}$

[a] Estimated by ^{1}H NMR analysis of final reaction mixture. [b] Calculated from conv. × ([STY] + [MA])/[RAFT]. [c] From elemental analysis. [d] Ideal structure, calculated using %STY from elemental analysis, M_n from GPC, assuming no transitional region between poly(STY-*alt*-MA) and polySTY segments. [e] RAFT = **1**. [f] RAFT = **2**.

was then extended by a homopolystyrene block segment, once the MA was exhausted. In this way, block copolymers were formed in which the length of the first STY-*alt*-MA block was determined by the ratio of MA to RAFT agent, while the length of the homo-STY block was determined by the ratio of excess STY to RAFT agent and the overall conversion.[11,13,14]

Di- and triblock copolymers with a range of compositions were conveniently prepared by dissolving appropriate amounts of maleic anhydride, styrene, RAFT agent **1** or **2**, respectively, and 2,2′-azobisisobutyronitrile (AIBN) in 20 mL dioxane and heating for 21 h (reaching 75–96% conversion) (Table 1). The resulting polymers exhibited two glass transition temperatures (T_g) at 77 and 170 °C, of varying intensities depending on the relative amounts of STY and MA, indicating the presence of microphase-separated domains in the bulk. Molecular weight distributions were unimodal and of narrow polydispersities, as observed by gel permeation chromatography (GPC). The architecture of the ABA triblock copolymer, **6**, was confirmed by pyrolysis[16] of the polymer (30 min at 250 °C), which resulted in cleavage at the central trithiocarbonate group to produce polymer chains having a number-average molecular weight half that of **6**, and a narrow molecular weight distribution (M_n = 11 100, PDI = 1.12).

In the cases of isolated block copolymers, aggregates were formed by slow addition (10 mL h^{-1}) of an equal volume of water to a solution of copolymer in THF (2 mg mL^{-1}) in the presence of a catalytic amount of triethylamine,‡ followed by dilution to 0.67 mg mL^{-1}, dialysis against deionised water and, finally, dilution to 0.5 mg mL^{-1}. The resulting solutions varied in appearance from colorless to bluish-white and were characterized by transmission electron microscopy (TEM), atomic force microscopy (AFM) and dynamic light scattering (DLS) (Table 2).† In solution, the assemblies of **3–5** were narrowly distributed in size, while the size distribution of **6** was significantly broader.

Table 2 Characterization of micellar assemblies formed by polymers **3–6** before and after crosslinking to form shell-crosslinked nanoparticles

	Before crosslinking				After crosslinking			
	D_h/nm[a]	Poly[a]	H/nm[b]	D/nm[c]	D_h/nm[a]	Poly[a]	H/nm)[b]	D/nm[c]
3	41.2 (3)	0.06 (2)	50 (8)	49 (7)	84 (2)	0.19 (5)	48 (11)	38 (8)
4	45.0 (4)	0.06 (2)	39 (6)	38 (7)	100 (1)	0.125 (2)	49 (11)	36 (8)
5	58 (3)	0.05 (4)	56 (11)	53 (11)	102 (2)	0.10 (2)	62 (17)	47 (11)
6	30 (4)	0.19 (1)	23 (4)	27 (6)	44 (1)	0.14 (2)	32 (9)	22 (6)

[a] Hydrodynamic volume (D_h) and polydispersity (Poly) measured by DLS. Numbers in parentheses represent standard error in the final digits. [b] Average height (and standard deviation) measured by AFM. [c] Average diameter (and standard deviation) measured by TEM.

When observed by TEM, assemblies of **3–5** frequently appeared to be composed of multiple subunits, arranged in a rosette pattern (Fig. 1A–C). This ordering was evident particularly for the structures formed from **5** (Fig. 1C) which were typically composed of 4, 5 or 6 subunits arranged circularly. The diameters by TEM (Table 2) were measured for the entire assemblies, rather than individual subunits. The agreement between diameters measured by DLS and by TEM suggests that these assemblies were present in solution and not an artefact of substrate adsorption. Higher-order assembly was not exhibited by **6**, which formed much smaller aggregates without visible microstructure (Fig. 1D).

AFM of the micelles confirmed the dimensions observed by TEM and DLS. Higher-order structure was not observed by AFM, suggesting that the images obtained by TEM reveal the internal arrangement of polystyrene core domains in a continuous poly(styrene-*alt*-maleic acid) matrix. Intricate, internal segregation patterns have been observed for microscopic assemblies[17] and are well known for block copolymers in the bulk, however, capture of such uniform structures within assemblies that are tens of nanometres in dimension is of significant interest.

Crosslinking was obtained *via* carbodiimide-mediated amidation,§ resulting in robust nanostructures. Dilution of the aqueous solutions with 9 volumes of THF caused disassembly of the noncrosslinked diblock copolymer assemblies, with the effect that no light scattering was observed from these solutions by DLS. In contrast, the crosslinked nanomaterials remained intact on dilution with THF. While the micelles of the triblock copolymer **6** did not dissociate completely on addition of THF, a clear difference was observed between crosslinked and noncrosslinked samples, with

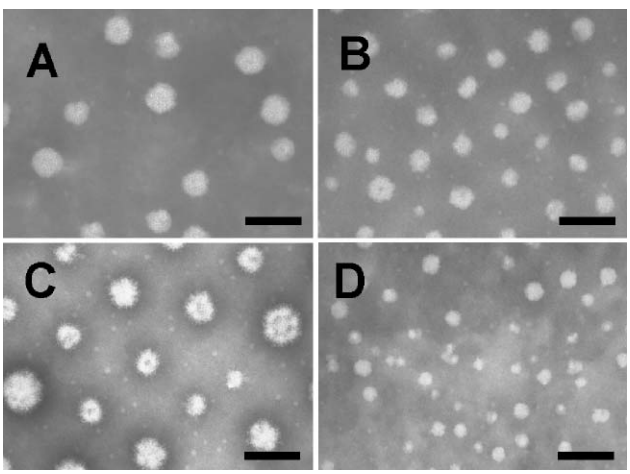

Fig. 1 TEM images of **3** (A), **4** (B), **5** (C) and **6** (D), showing rosette-like arrangements of subunits in assemblies of **5** (C). Scale bars are 100 nm.

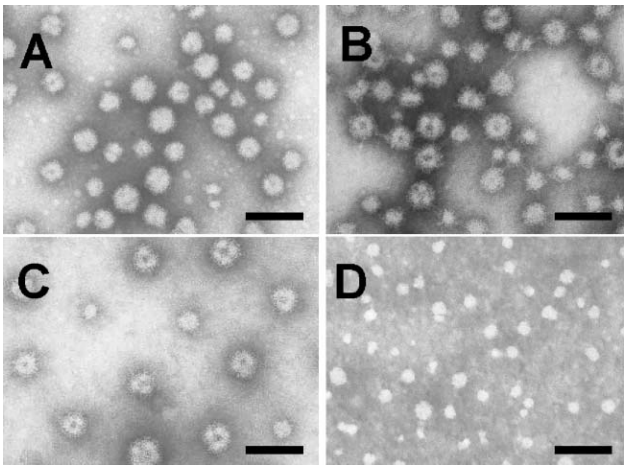

Fig. 2 Transmission electron micrographs of **3** (A), **4** (B), **5** (C), and **6** (D), crosslinked to a nominal crosslink density of 20%. Scale bars represent 100 nm.

the crosslinked nanostructures showing greater resistance to THF-induced disassembly. Further confirmation of crosslinking was provided by IR analysis of the lyophilized crosslinked structures, which showed an amide carbonyl absorption band at 1636 cm^{-1}. There was a significant increase in the hydrodynamic diameter and polydispersity in solution, as measured by DLS, suggesting increased hydrophilicity and swelling in aqueous solution upon incorporation of the hydrophilic crosslinker.

The structures formed from **3–5**, as observed by TEM (Fig. 2), indicate that crosslinking was accompanied by an increase in the complexity of the microstructure (*cf.* Fig. 1). The mechanism of formation of these unusual structures is currently under investigation. The crosslinked morphologies from **6** were similar in appearance to their noncrosslinked precursors.

Finally, crosslinked nanoparticles were formed directly from the monomers without intervening purification steps.¶ The resulting mixture contained nanoparticles with D_h 164 $\pm$ 2 nm, and a polydispersity of 0.08 (measured by DLS). The persistence of the particles in 9 : 1 THF : H_2O indicated that crosslinking had occurred.

The production of regioselectively crosslinked nanoparticles having well-defined, amphiphilic characteristics and rosette-like morphologies *via* a one-pot route from commodity monomers and involving intermediate block copolymer assemblies is a significant synthetic advance. Further studies are expected to lead to increased understanding of the structure of the internal sub-assemblies and the mechanism for their formation, ultimately, to allow for manipulation of those domains and enhanced complexity.

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Notes and references

‡ In the absence of triethylamine, precipitation occurred on water addition.
§ In a typical procedure, 1,2-ethylenedioxy bis(ethylamine) (10 mol% relative to maleic anhydride units) was added to a micellar solution of polymer. After stirring for 20 min, EDC was added (20 mol% relative to maleic anhydride units). The solution was stirred overnight at RT, then dialyzed for 3 days against deionised H_2O to remove the urea byproduct.
¶ MA (0.5 g) and STY (1.5 g) were heated at 60 °C in the presence of 2.0 g dioxane, 18 mg **1**, and a trace of AIBN for a period of 16 h under N_2. The resulting mixture (conversion = 89% by NMR) was dissolved in 1 L of THF. 1,2-Ethylenedioxy bis(2-ethylamine) (18.9 mg, 10 mol% relative to maleic anhydride) was added with vigorous stirring to a 250 mL aliquot of this solution, followed immediately by 250 mL nanopure water. The THF was removed by evaporation *in vacuo* (at RT) and the resulting micellar solution was diluted to 1 L with nanopure water.

1 V. Bütün, N. C. Billingham and S. P. Armes, *J. Am. Chem. Soc.*, 1998, **120**, 12135.
2 Y. Ma, Y. Tang, N. C. Billingham, S. P. Armes, A. L. Lewis, A. W. Lloyd and J. P. Salvage, *Macromolecules*, 2003, **36**, 3475.
3 K. L. Wooley, *J. Polym. Sci., Part A: Polym. Chem.*, 2000, **38**, 1397; H. M. Kao, R. D. O'Connor, A. K. Mehta, H. Huang, B. Poliks, K. L. Wooley and J. Schaefer, *Macromolecules*, 2001, **34**, 544; H. Huang, K. L. Wooley and J. Schaefer, *Macromolecules*, 2001, **34**, 547.
4 M. L. Becker, E. E. Remsen, D. Pan and K. L. Wooley, *Bioconjugate Chem.*, 2004, **15**, 699; M. L. Becker, L. O. Bailey and K. L. Wooley, *Bioconjugate Chem.*, 2004, **15**, 710.
5 M. L. Becker, J. Liu and K. L. Wooley, *Biomacromolecules*, 2005, **6**, 220; D. Pan, J. L. Turner and K. L. Wooley, *Macromolecules*, 2004, **37**, 7109.
6 V. Bütün, X.-S. Wang, M. V. d. P. Báñez, K. L. Robinson, N. C. Billingham, S. P. Armes and Z. Tuzar, *Macromolecules*, 2000, **33**, 1; S. Liu and S. P. Armes, *J. Am. Chem. Soc.*, 2001, **123**, 9910.
7 S. Liu, J. V. M. Weaver, M. Save and S. P. Armes, *Langmuir*, 2002, **18**, 8350.
8 O. Terreau, L. Luo and A. Eisenberg, *Langmuir*, 2003, **19**, 5601; S. E. Burke and A. Eisenberg, *Langmuir*, 2001, **17**, 6705; D. J. Pochan, Z. Chen, H. Cui, K. Hales, K. Qi and K. L. Wooley, *Science*, 2004, **306**, 94; Z. Li, E. Kesselman, Y. Talmon, M. A. Hillmyer and T. P. Lodge, *Science*, 2004, **306**, 98.
9 H. Huang, T. Kowalewski, E. E. Remsen, R. Gertzmann and K. L. Wooley, *J. Am. Chem. Soc.*, 1997, **119**, 11653.
10 M. Kuang, H. Duan, J. Wang, D. Chen and M. Jiang, *Chem. Commun.*, 2003, 496; X. Liu, M. Jiang, S. Yang, M. Chen, D. Chen, C. Yang and K. Wu, *Angew. Chem., Int. Ed.*, 2002, **41**, 2950; M. Wang, M. Jiang, F. Ning, D. Chen, S. Liu and H. Duan, *Macromolecules*, 2002, **35**, 5980; X. F. Yuan, M. Jiang, H. Y. Zhao, M. Wang, Y. Zhao and C. Wu, *Langmuir*, 2001, **17**, 6122; Y. Zhang, M. Jiang, J. Zhao, J. Zhou and D. Chen, *Macromolecules*, 2004, **37**, 1537.
11 M.-Q. Zhu, L.-H. Wei, M. Li, L. Jiang, F.-S. Du, Z.-C. Li and F.-M. Li, *Chem. Commun.*, 2001, 365.
12 H. de Brouwer, M. A. J. Schellekens, B. Klumperman, M. J. Monteiro and A. L. German, *J. Polym. Sci., Part A: Polym. Chem.*, 2000, **38**, 3596; X. Hao, M. H. Stenzel, C. Barner-Kowollik, T. P. Davis and E. Evans, *Polymer*, 2004, **45**, 7401; E. Chernikova, P. Terpugova, C. Bui and B. Charleux, *Polymer*, 2003, **44**, 4101; F. S. Du, M.-Q. Zhu, H. Q. Guo, Z.-C. Li, F.-M. Li, M. Kamachi and A. Kajiwara, *Macromolecules*, 2002, **35**, 6739.
13 D. Benoit, C. J. Hawker, E. E. Huang, Z. Lin and T. P. Russell, *Macromolecules*, 2000, **33**, 1505.
14 E. S. Park, M.-N. Kim, I.-M. Lee, H.-S. Lee and J.-S. Yoon, *J. Polym. Sci., Part A: Polym. Chem.*, 2000, **38**, 2239.
15 J. T. Lai, D. Filla and R. Shea, *Macromolecules*, 2002, **35**, 6754.
16 A. Postma, T. P. Davis, G. Moad and M. S. O'Shea, *Macromolecules*, 2005, **38**, in press.
17 Z. Lu, G. Liu and F. Liu, *Macromolecules*, 2001, **34**, 8814.