Temperature-responsive mixed core nanoparticle properties determined by composition of statistical and block copolymers in the core

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Temperature-responsive mixed core nanoparticle properties determined by composition of statistical and block copolymers in the core

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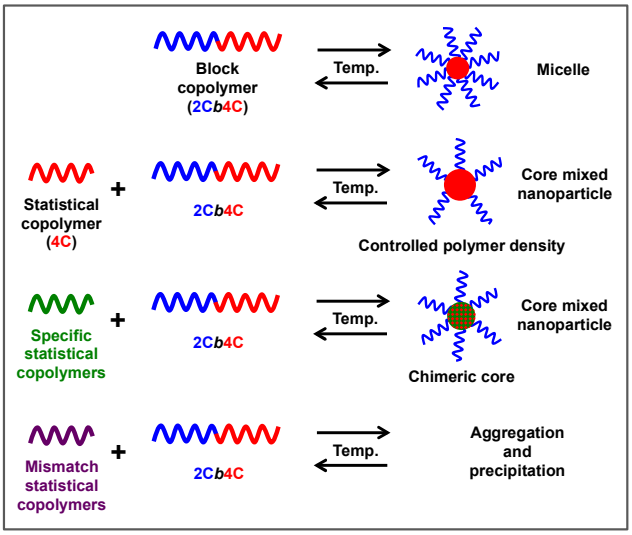
Mixed core nanoparticles were prepared from self-assembled statistical and block copolymers by controlling solution temperature. An equal mass of statistical copolymers was loaded into block copolymer micelle cores. A chimeric core was also constructed by adjusting the physicochemical properties of the mixed statistical copolymers.

Various types of multi-stimuli responsive block copolymer nanoparticles have been applied in a wide range of fields, such as drug delivery, diagnosis, tissue engineering, sensors, and devices.[1] These block copolymers are designed for each application and the nanoparticles take on the stimuli responsive properties of the copolymers. Therefore, there is a limitation on the number and location of stimuli responses. *ABC* type tri-block copolymers (poly*A*-*b*-poly*B*-*b*-poly*C*: where *A* is a hydrophobic segment), for example, form nanoparticles in aqueous solution with only two kinds of stimuli responses (*i.e. B* and *C* segments) and the *C* segment is always located on the surface of each nanoparticle.

Recently, self-assembly of heterogeneous block copolymer mixtures has been proposed for the preparation of multi-stimuli-responsive nanoparticles.[2] Using this system, the numbers, locations, and compositions of the multi-stimuli responsive nanoparticles are easily controlled by the mixture ratios. The self-assembly process typically requires toxic organic solvents and disintegration of the nanoparticles is difficult after the preparation. To overcome these challengers, temperature has been selected in order to trigger the self-assembly process. Temperature responsive polymers are the most studied stimuli-responsive polymers and have been applied in protein conjugates, drug delivery, cell engineering, and devices.[3] By increasing solution temperature, these temperature responsive polymers can reversibly change their physicochemical properties, which is known as lower critical solution temperature (LCST).

In a previous study, we demonstrated a simple approach for the preparation of mixed shell (multi-stimuli responsive shell) micelles using two or three types of temperature responsive block copolymers.[4] These block copolymers had a common LCST and formed mixed shell nanoparticles above the LCST. The properties of the shell were precisely adjusted by tuning the mixture ratios of the block copolymers. However, the temperature responsive core was difficult to load with hydrophobic materials directly. When dissolved in aqueous solution, this difficulty arose due to weak hydrophobic-hydrophobic interactions in the core. Moreover, the polymer density of the shell could not be controlled using this approach.



Scheme 1 Schematic representation of mixed core nanoparticles consisting of self-assembled compatible statistical and block copolymers.

In this study, in order to construct nanoparticles suited for drug delivery applications, we focused on a mixed core design approach using statistical and block copolymers as shown in Scheme 1. Hydrophobic statistical polymers were effectively loaded in the micelle cores (the loading capacity 100 %) while slightly increasing in size (1.1 times larger than the original micelle, ~ 5 nm of diameter increase). The block copolymers are inclined to encapsulate the shorter statistical polymer chains. The polymer density of the shell was also controlled by the mixture ratios of statistical and block copolymers. Moreover, a chimeric core (consisting of copolymer segments having different compositions) was constructed by mixing statistical copolymers with unique structures, along with the block copolymers. Nanoparticle-formation was strongly affected by the

properties of the statistical copolymers, including molecular weight, compositions, and lower critical solution temperature (LCST). In other words, the block copolymer could recognize the specific physicochemical properties of the statistical copolymers, when incorporated in the nanoparticle's chimeric core. Using this system, nanoparticle properties were easily customized by selecting the specific combination of statistical and block copolymers. The nanoparticle preparation method was also straightforward. The copolymers were simply dissolved in aqueous solution at a temperature below the LCST and the desired nanoparticles were formed rapidly above the LCST. To the best of our knowledge, this is the first report that temperature responsive block copolymer can recognize its optimum statistical copolymers when they form mixed core nanoparticles.

Fig. 1 shows structures of the statistical and block copolymers. Two-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA)/oligo(ethylene glycol) methacrylate (OEGMA), 6-acetylthiohexyl acrylate (ATA), and 2-(diisopropylamino)ethyl methacrylate (DP) monomers were selected to control the temperature responsive properties, biodegradability, and pH responsive properties, respectively. In the field of biomaterials, functionalization of nanoparticles is very important for drug delivery. The desirable nanoparticle core can be prepared using our system. The LCSTs of P(MEO_2MA-*co*-OEGMA) and P(MEO_2MA-*co*-OEGMA-*co*-ATA-*co*-DP) were adjusted to be at 60 and 35 °C, respectively. It was possible to predict the LCSTs even in the complex copolymer composition (*e.g.* four-components copolymer) using a combination of the relationships between the LCSTs and contents of X in two-component copolymers P(MEO_2MA-*co*-X)s. The LCSTs of the statistical P(MEO_2MA-*co*-X) copolymers (X= OEGMA, ATA, or DP) were measured (in milliQ, pH 7.4 PBS, and pH 5.5 buffer) and used to design the LCSTs of the P(MEO_2MA-*co*-OEGMA-*co*-ATA-*co*-DP) (4C) and P(MEO_2MA-*co*-OEGMA)-*b*-P(MEO_2MA-*co*-OEGMA-*co*-ATA-*co*-DP) (2C*b*4C) (Table S1 and Fig. S1~8). Thiol (SH) groups have been used in drug delivery systems as a promising reversible cross-linker (-S-S-), due to their stability under physiological conditions and for their capability to be reductively cleaved once internalized in cells.[5] However, it is

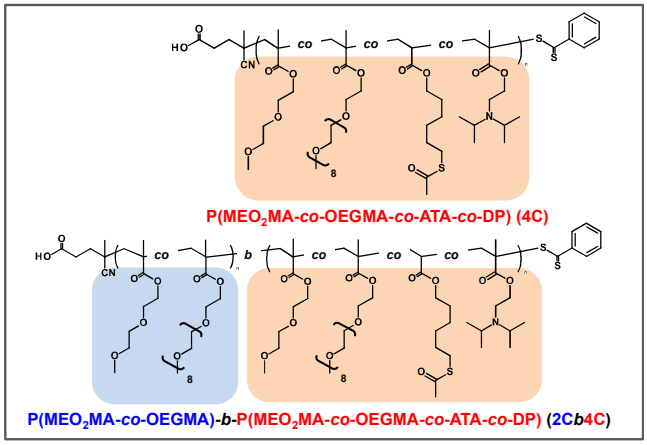


Fig. 1 Structures of statistical copolymer: P(MEO_2MA-*co*-OEGMA-*co*-ATA-*co*-DP) (4C) and block copolymer: P(MEO_2MA-*co*-OEGMA)-*b*-P(MEO_2MA-*co*-OEGMA-*co*-ATA-*co*-DP) (2C*b*4C).

difficult to directly polymerize monomers having thiol groups by radical polymerization as the thiol groups can act as radical scavengers. The ATA monomer was selected due to the presence of a protected thiol group and its easy deprotection after polymerization.[6] In fact, the deprotection efficiency of the ATA in P(MEO_2MA-*co*-ATA) was controlled from 36.7 to 67.7 % by

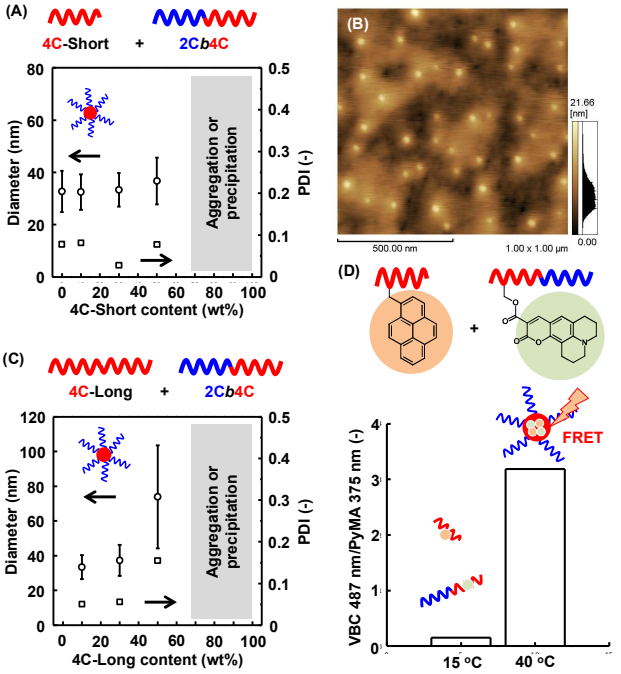


Fig. 2 Mixed core nanoparticles with high loading capacity. (A) Diameter and polydispersity index (PDI: size distribution) of 0.5 wt% mixture solution of 2C*b*4C and 4C-Short in pH 7.4 PBS at 40 °C. The temperature of solution was increased from 15 to 40 °C (increase of 5 °C for every 10 min.). (B) AFM image of nanoparticles consisting of block copolymers of 2C*b*4C. (C) Diameter/PDI of 0.5 wt% mixture solution of 2C*b*4C and 4C-Long in pH 7.4 PBS at 40 °C. (D) FRET Intensity of VBC (487 nm)/PyMA (375 nm) of of mixture solution of P(MEO_2MA_{48}-*co*-$OEGMA_{20}$)-*b*-P(MEO_2MA_{23}-*co*-$OEGMA_2$-*co*-ATA_3-*co*-DP_2-*co*-$VBC_{0.3}$) and P(MEO_2MA_{34}-*co*-$OEGMA_4$-*co*-ATA_5-*co*-DP_5-*co*-$PyMA_{0.8}$) (total 0.01 wt%: mixture ratio = 1/1) at 15 and 40 °C.

varying the concentration of the reducing agent and the reaction time (Fig. S9). DP monomer (pK_a = 6.3) has been applied as a pH responsive polymer in biomaterials. Protonated DP polymers can help in endosomal release due to the proton sponge effect.[7] Statistical copolymers of 4C (4C-Short: M_n = 5800, M_w/M_n = 1.35, 4C-Long: M_n = 10900, M_w/M_n = 1.36) and block copolymer of 2C*b*4C (M_n = 27600, M_w/M_n = 1.31) were prepared as the model multi-stimuli responsive copolymers in this study (Scheme S1~3, Table S2). The 4C segment of block copolymer 2C*b*4C had the same temperature responsive block as statistical copolymer 4C (LCST: 35 °C in pH 7.4 PBS) (Scheme 1, and Fig. S10~12). 2C*b*4C also had a carboxylic acid group at the distal end of the polymer chain. After self-assembly these groups were located on the surface of each nanoparticle. The location of the reactive functional group can strongly affect the solution properties of the nanoassemblies and can also be exploited for further modification.[8] In order to synthesize the block copolymers, it was necessary to synthesize the 2C segment first as a macro-CTA. Fine structure of block copolymers were not observed when they were polymerized form 4C segment (*i.e.* 4C*b*2C) (Table S3).

Fig. 2 shows the diameters and PDI of mixed nanoparticles consisting of 2C*b*4C and 4C-Short (Fig. 2(A)) or -Long (Fig. 2(C)) at 40 °C in PBS. The solution temperature was adjusted from 15 to 40 °C with increasing temperature at 5 °C for every 10 min. The

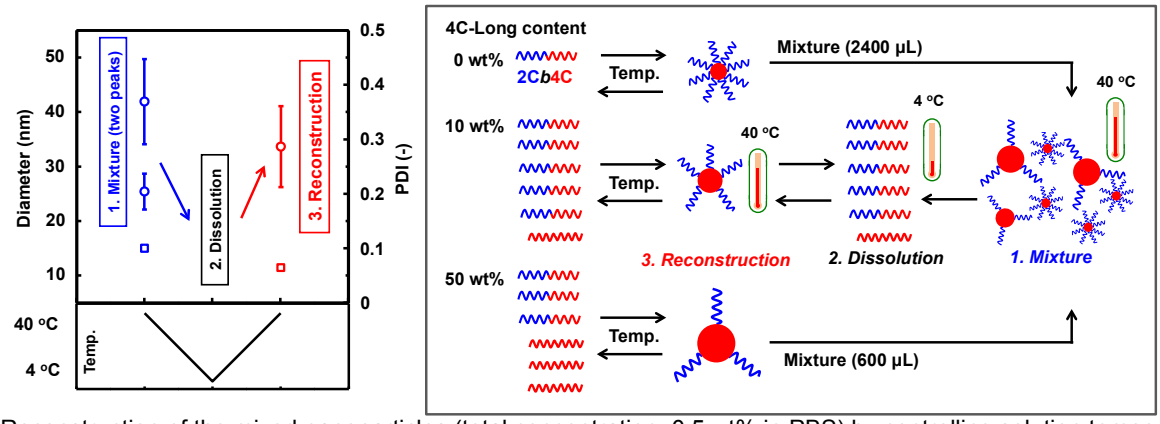
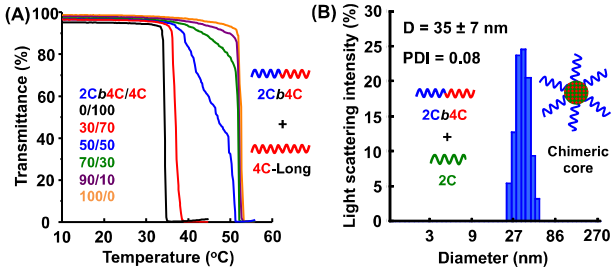


Fig. 3 Reconstruction of the mixed nanoparticles (total concentration: 0.5 wt% in PBS) by controlling solution temperature. The nanoparticles having different sizes were mixed at 40 °C (Mixture of 2400 μL of 2C*b*4C and 600 μL of 2C*b*4C/4C-Long (1/1 wt)). The total 4C-Long content was 10 wt%.

diameter of 2C*b*4C homo-particles was 33 ± 8 nm (PDI 0.079) (with spherical structures measured by AFM in Fig. 2(B)). On the other hand, the diameters of mixed, 4C-Short and 2C*b*4C nanoparticles were slightly increased while maintaining a narrow PDI < 0.1 as shown in Fig. 2(A). The diameters of the mixed nanoparticles were controlled within 5 nm, depending on the mixture ratios of 4C-Short, up to 50 wt% of the 4C-Short. When the mixture content was over 70 wt%, aggregation/precipitation was observed. The diameters of the mixed nanoparticles were not affected by the rate of solution temperature increase (*i.e.* directly increasing from 15 to 40 °C (Fig. S13)). Using fluorescence resonance energy transfer (FRET) between pyrene modified statistical copolymer and courmarin 343 modified block copolymer, we also demonstrated that the nanoparticle core consisted of both polymeric species.[9] Strong FRET fluorescence was observed after assembling the nanoparticles due to the close proximity of the pyrene and courmarin 343 in the mixed core (Fig. 2(D), Scheme S4~5, Table S4, and Fig. S14~16). Fig. 2 (C) shows diameters of mixed 4C-Long and 2C*b*4C nanoparticles. The diameters were slightly increased as compared to that of 4C-Short with same weight percentage. Interestingly, the diameter was greatly increased with a high PDI > 0.1 at 50 wt% 4C-Long. These results suggest that the copolymer with low molecular weight is favorable for producing nanoparticles with narrow PDI.

Mixed nanoparticles can be easily reconstructed by controlling only the solution temperature without the use of organic solvents and/or the addition of other chemicals. Fig. 3 shows the reconstruction process for a mixture of nanoparticles having different sizes (Mixture of 2400 μL of 2C*b*4C and 600 μL of 2C*b*4C/4C-Long (1/1 wt)). The total 4C-Long content was 10 wt%. Immediately after mixing at 40 °C, two peaks (25 ± 3 and 42 ± 8 nm) were observed in the diameter histogram (Fig. S17). The solution temperature of the nanoparticles was decreased to 4 °C and no DLS peaks were observed due to the disintegration of the nanoparticles into unimers. By increasing the solution temperature to 40 °C again, we found that the nanoparticles were reconstructed with narrow PDI (34 ± 7 nm, PDI 0.07). The size and PDI was in agreement with the mixed nanoparticle consisting of 10 wt% of 4C-Long in Fig. 2(C).

The nanoparticles described above were composed of 4C and 2C*b*4C with identical monomer compositions (*i.e.* MEO_2MA, OEGMA, ATA, and DP). These results lead us to one simple question: Is it possible to construct mixed core nanoparticles consisting of statistical and block copolymers having different compositions and LCSTs? Harada *et al.*, have shown that electrostatically assembled polyion block copolymers can recognize the chain length of the mixed polymer blocks and assemble fine structured micelles.[10] In our case, it is important to form nanoparticles not only by changing the chain lengths but also the LCSTs as shown in Fig. 2. Therefore, several copolymers with different chain lengths, compositions, and LCSTs were prepared to



(C)

		Units	LCST (°C)	Nanoparticle
Block copolymer 2C*b*4C	∿∿∿	68 / 36	60 / 35	O
Mixed copolymers[a]				
4C-Short	∿∿	24	35	O
4C-Long	∿∿∿∿	47	35	Δ (PDI > 0.1)
$P(MEO_2MA_{65}$-*co*-$OEGMA_5)$		70	35	×
$P(MEO_2MA_{34}$-*co*-$OEGMA_2)$		36	33	×
$P(MEO_2MA_{33}$-*co*-$OEGMA_2)$		35	38	×
$P(MEO_2MA_{62}$-*co*-$OEGMA_3)$		65	31	×
$PMEO_2MA_{84}$[b]		84	27	×
$P(MEO_2MA_{19}$-*co*-$OEGMA_2)$	∿∿	21	42	O
$P(MEO_2MA_{77}$-*co*-$OEGMA_9)$[b]		86	43	×

Fig. 4 Mixed nanoparticles having a chimeric core. (A) Transmittance change of 0.5 wt% solution of 2C*b*4C and 4C-Long as a function of temperature in pH 7.4 PBS. (B) Size distribution histogram for the mixed nanoparticles consisting of 2C*b*4C and $P(MEO_2MA_{19}$-*co*-$OEGMA_2)$ at 45 °C (Total concentration: 0.5 wt%, ratio of mixture: 1/1 wt). (C) Summary of the match (**O**: fine nanoparticles) or mismatch (✖: aggregation or precipitation) for preparation of fine nanoparticles consisting of 2C*b*4C and copolymers with different compositions, chain lengths, and LCSTs ([a]Ratio of mixture: 4C*b*2C/statistical copolymers = 1/1 wt in pH 7.4 PBS). [b]Copolymers of Table S1.

investigate their ability (*match*) or inability (*mismatch*) to form stable nanoparticles. Fig. 4(A) shows the temperature dependent transmittance change of mixed 2C*b*4C and 4C-Long at different mixture ratios. The decrease in transmittance caused by the dehydration of the 4C-Long was gradually weakened with increasing content of the 2C*b*4C. Another transmittance decrease around 52 °C was noted due to the dehydration of the 2C segment of the 2C*b*4C. This slightly lower transmittance (lower light scattering) may suggest the formation of nanoparticles as shown Fig. 2. The total monomer units and the LCSTs of the 2C*b*4C segments were: 2C segment; 68 units / 60 °C and 4C segment; 36 units / 35 °C, respectively. The copolymers having long chain length and similar LCST (70 units / 35 °C) showed aggregation and precipitation upon mixing with 2C*b*4C (ratios of mixture: 1/1 wt) in Fig. S18(A). Interestingly, the statistical copolymer with low chain length (21 units) and a higher LCST of 42 °C formed mixed nanoparticles with 2C*b*4C (35 ± 7 nm, PDI 0.078), as shown in Fig. 4(B). There was a difference of 7 °C between the LCSTs of the copolymer and 4C segment in 2C*b*4C. Dehydrations of temperature responsive polymers are known to show nano-scale dehydration even below the LCST.[11] The weak interaction of the copolymer and its short chain length may lead toward the formation of fine structures. The mixture of 2C*b*4C and another copolymer having LCST around 43 °C with long chain length (86 units) showed aggregation/precipitation with increasing solution temperature (Fig. S18(F)). These results suggested the block copolymer could recognize the chain length and LCST of mixed copolymers in the formation of fine nanoparticles for the thermodynamically stability (Fig. 4(C)). Moreover, we expect that the hydrophobicity is also playing an important role in the formation of the fine nanoparticles. Copolymers having the same LCST possess different hydrophobicity due to the chemical structure of the monomer units.[12] The block copolymer may encapsulate several types of temperature responsive polymers including P(*N*-isopropylacrylamide) (PNIPAAm).

Conclusions

In conclusion, we successfully prepared mixed-core nanoparticles consisting of temperature responsive statistical and block copolymers. The formation of the nanoparticles was also strongly affected by the chain lengths, compositions, and LCSTs of the mixed copolymers. The block copolymers could incorporate the same weight of the copolymer (50 wt%, the loading capacity 100 %) in the core. Additionally, the diameters of the mixed nanoparticles were controlled within 5 nm (1.1 times larger than the original micelle) by controlling the ratios of the mixed copolymers. The assembled structures can be reconstructed simply by controlling the solution temperature. Moreover, a chimeric core was constructed by adjusting the physicochemical properties of the mixed statistical copolymers. Customization of the copolymers leads to the formation of nanoparticles with new properties such as new size, different stimuli-responsiveness, cross-linking (on-off/amount), and encapsulation.

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Notes and references

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†Electronic supplementary information (ESI) available: Characterization of copolymers, block copolymers and their assemble behaviors. See DOI: 10.1039/c000000x/

1 (a) M. A. Cohen Stuart, W. T. S. Huck, J. Genzer, M. Müller, C. Ober, M. Stamm, G. B. Sukhorukov, I. Szleifer, V. V. Tsukruk, M. Urban, F. Winnik, S. Zauscher, I. Luzinov and S. Minko, *Nat. Mater.*, 2010, **9**, 101; (b) R. Haag, *Angew. Chem. Int. Ed.*, 2014, **43**, 278; (c) P. Schattling, F. D. Jochum and P. Theato, *Polym. Chem.*, 2014, **5**, 25; (d) A. S. Hoffman, *Adv. Drug Deliver. Rev.*, 2013, **65**, 10.

2 (a) V. V. Palyulin and I. I. Potemkin, *Macromolecules*, 2008, **41**, 4459; (b) S. H. Kim, J. P. K. Tan, F. Nederberg, K. Fukushima, Y. Y. Yang, R. M. Waymouth and J. L. Hedrick, *Macromolecules*, 2009, **42**, 25.

3 (a) H. Wei, S.-X. Cheng, X.-Z. Zhang and R.-X. Zhuo, *Prog. Polym. Sci.*, 2009, **34**, 893; (b) C. Weber, R. Hoogenboom and U. S. Schubert, *Prog. Polym. Sci.,* 2012, **37**, 686; (c) P. S. Stayton, T. Shimoboji, C. Long, A. Chilkoti, G. Chen, J. M. Harris and A. S. Hoffman, *Nature*, 1995, **30**, 472; (d) K. J. Mackenzie and M. B. Francis, *J. Am. Chem. Soc.*, 2013, **135**, 293; (e) H. Sekine, T. Shimizu, K. Sakaguchi, I. Dobashi, M. Wada, M. Yamato, E. Kobayashi, M. Umezu and T. Okano, *Nat. Commun.*, 2013, **4**, 1399; (f) K. Okabe, N. Inada, C. Gota, Y. Harada, T. Funatsu and S. Uchiyama, *Nat. Commun.*, 2012, **3**, 705.

4 Y. Kotsuchibashi, M. Ebara, N. Idota, R. Narain and T. Aoyagi, *Polym. Chem.*, 2012, **3**, 1150.

5 (a) K. Miyata, Y. Kakizawa, N. Nishiyama, A. Harada, Y. Yamasaki, H. Koyama and K. Kataoka, *J. Am. Chem. Soc.*, 2004, **126**, 2355; (b) Q. Zhang, N. R. Ko and J. K. Oh, *Chem. Commun.*, 2012, **48**, 7542.

6 E. Hrsic, I. Zografou, B. Schulte, A. Pich, H. Keul and M. Möller, *Polymer*, 2013, **54**, 495.

7 H. Yu, Y. Zou, Y. Wang, X. Huang, G. Huang, B. D. Sumer, D. A. Boothman and J. Gao, *ACS Nano*, 2011, **5**, 9246.

8 (a) Y. Kotsuchibashi and R. Narain, *Polym. Chem.*, 2014, **5**, 3061; (b) D. J. Keddie, G. Moad, E. Rizzardo and S. H. Thang, *Macromolecules*, 2012, **45**, 5321; (c) H. Willcock and R. K. O'Reilly, *Polym. Chem.*, 2010, **1**, 149.

9 (a) S. W. Hong, K. H. Kim, J. Huh, C.-H. Ahn, and W. H. Jo, *Chem. Mater.*, 2005, **17**, 6213; (b) C. Li and S. Liu, *Chem. Commun.*, 2012, **48**, 3262.

10 A. Harada and K. Kataoka, *Science*, 1999, **283**, 65.

11 R. Motokawa, K. Morishita, S. Koizumi, T. Nakahira and M. Annaka, *Macromolecules*, 2005, **38**, 5748.

12 (a) J.-F. Lutz, Ö. Akdemir and A. Hoth, *J. Am. Chem. Soc.*, 2006, **128**, 13046; (b) S. Sugihara, K. Yamashita, K. Matsuzuka, I. Ikeda and Y. Maeda, *Macromolecules*, 2012, **45**, 794.