



Cite this: *Polym. Chem.*, 2021, **12**, 1768

In situ cross-linking polymerization-induced self-assembly not only generates cross-linked structures but also promotes morphology transition by the cross-linker†

Jamshid Kadirhanov,[‡] Cheng-Lin Yang,[‡] Zi-Xuan Chang, Ren-Man Zhu, Cai-Yuan Pan, Ye-Zi You,[‡] Wen-Jian Zhang[‡] * and Chun-Yan Hong[‡] *

In comparison with the post-polymerization cross-linking strategy, *in situ* cross-linking by divinyl comonomers in polymerization-induced self-assembly (PISA) is a more straightforward and convenient approach to produce structurally stabilized nano-objects. However, cross-linking usually lowers the chain mobility and hence prevents morphology transition, so formation of higher order morphologies (worm-like micelles or vesicles) by the *in situ* cross-linking strategy in PISA has always been a great challenge. In this work, not only cross-linked structures but also a promoting effect on morphology transition has been observed during the *in situ* cross-linking PISA. Worm-like micelles, lamella and vesicles with stabilized (cross-linked) structures are produced by RAFT dispersion copolymerization of 2-(diisopropylamino)ethyl methacrylate (DIPEMA) and the divinyl comonomer cystaminebismethacrylamide (CBMA). The morphology transition is not prevented due to the slower consumption of CBMA than that of the DIPEMA, and the cross-linking process is mostly delayed to the late stage of polymerization. What is surprising, compared with RAFT dispersion polymerization of DIPEMA in the absence of CBMA, is that polymeric nano-objects with higher order morphologies are generated in most cases of the RAFT dispersion copolymerization of DIPEMA and CBMA. The slower consumption of CBMA generates branched structures of the solvophobic blocks, which significantly promote the morphology transition. Cleavage of the cross-linkers (disulfide linkage) by incubating with glutathione (GSH) leads to the morphology transition of the nano-objects to the lower order ones, which further demonstrates the promoting effect on morphology transition in *in situ* cross-linking PISA.

Received 13th January 2021,
Accepted 24th February 2021

DOI: 10.1039/d1py00046b

rscl.li/polymers

Introduction

Polymeric nano-objects have aroused great attention in nano-reactors, Pickering emulsifiers, drug delivery systems, *etc.*^{1–6} Polymerization-induced self-assembly (PISA), which efficiently simplifies the procedure by combining polymerization and self-assembly in one-pot (multiple steps including synthesis of the block copolymer, tedious purification and sophisticated post-polymerization assembly are usually needed in the traditional self-assembly method), has been established as a

powerful strategy to produce various block copolymer nano-objects.^{7–32} Moreover, polymeric nano-objects can be produced at much higher concentrations (up to 50% solid) by PISA, while typically less than 1% solid is produced by the traditional self-assembly method.³³ The above superiorities make PISA a promising technique for industrial-scale production of polymeric nano-objects.^{7–33}

The structural stability of polymeric nano-objects is essential to maintain the morphology- and size-dependent performance.^{13,34–37} Chemical cross-linking has been demonstrated to be a robust strategy to enhance the structural stability of polymeric nano-objects. Both the post-polymerization cross-linking strategy and *in situ* cross-linking strategy have been exploited to enhance the structural stability of PISA-based nano-objects.^{38–51} There are two steps in post-polymerization cross-linking: introducing reactive functionalities into the polymeric nano-objects by (co)polymerization of functional (co)monomers in PISA and then triggering the cross-linking reactions of the functional handles with complementary com-

CAS Key Laboratory of Soft Matter Chemistry, Department of Polymer Science and Engineering, University of Science and Technology of China, Hefei, 230026 Anhui, P.R. China. E-mail: zwj85@ustc.edu.cn, hongcy@ustc.edu.cn

†Electronic supplementary information (ESI) available: ¹H NMR spectra of CBMA, polymers, and the reaction mixture, GPC traces of the polymers, TEM images of the nano-objects, *etc.* See DOI: 10.1039/d1py00046b

‡These authors (J. Kadirhanov and C. L. Yang) contributed equally to this work.

pounds (or stimulus).^{38–45} Compared with the post-polymerization cross-linking strategy, *in situ* cross-linking by copolymerization of divinyl comonomers (cross-linkers) in PISA eliminates the additional step of post-polymerization reaction, which seems to be a more convenient approach.^{46–53} However, cross-linking usually limits the chain mobility of the solvophobic blocks, and the morphology transition of spherical micelles to higher order morphologies (worm-like micelles or vesicles) will be prevented accordingly. In this regard, preparation of stabilized worm-like micelles or vesicles by copolymerization of divinyl comonomers (crosslinkers) in a single-step is usually problematic. Recently, An and co-workers reported the fabrication of stabilized vesicles by *in situ* cross-linking of PISA via reversible addition–fragmentation chain transfer (RAFT) dispersion copolymerization of diacetone acrylamide (DAAM) and an asymmetric cross-linker allyl acrylamide (ALAM).⁴⁹ ALAM bears two vinyl groups with different reactivities, an acrylamido moiety with a similar reactivity to the monomer DAAM and an allyl moiety with lower reactivity. Therefore, DAAM copolymerizes with the acrylamido moieties in ALAM at the early stage of PISA, and the reaction of the allyl moiety which induces cross-linking is mainly delayed to the late stage of PISA (after the formation of vesicles). The same group also demonstrated that cross-linked worm-like micelles could be produced by the *in situ* cross-linking strategy using asymmetric cross-linkers.⁵⁰ We fabricated dual stimulus responsive and cross-linked vesicles by the *in situ* cross-linking strategy via RAFT dispersion copolymerization of the monomer and cross-linker with disparate reactivities, 2-(diisopropylamino)ethyl methacrylate (DIPEMA) and cross-linker cystaminebismethacrylamide (CBMA).⁵¹ The RAFT polymerization activity of the cross-linker CBMA is lower than that of the monomer DIPEMA, so the cross-linking reaction is mainly delayed to the late stage of PISA (after the formation of vesicles). *In situ* cross-

linking by divinyl comonomers during PISA in a single step has been demonstrated to be a feasible and convenient approach in fabrication of cross-linked nano-objects with higher order morphologies (worm-like micelles or vesicles), and is worth devoting much more effort for further research.

In this work, we demonstrate that not only the enhanced stability of the nano-objects but also the promoting effect on morphology transition is observed by *in situ* cross-linking PISA using PEG₉₀-CPADB-mediated RAFT dispersion copolymerization of DIPEMA and CBMA (Fig. 1). For comparison, PEG₉₀-CPADB-mediated RAFT dispersion polymerization of DIPEMA, which generates linear PEG₉₀-*b*-PDIPEMA_x nano-objects, was also carried out. For *in situ* cross-linking PISA by RAFT dispersion copolymerization of DIPEMA and CBMA, the slower consumption of CBMA results in branched structures of the solvophobic P(DIPEMA-*co*-CBMA) blocks at the early stage of PISA, which greatly promotes morphology transition, leading to the formation of higher order morphologies accordingly. Continuous consumption of the cross-linker results in the formation of cross-linked nano-objects. The cross-linkers (disulfide linkages) in the PEG₉₀-*b*-P(DIPEMA-*co*-CBMA)_x nano-objects can be cleaved by incubating with glutathione (GSH), leading to morphology transition of the nano-objects to the lower order ones (Fig. 1), which further demonstrates the promoting effect on the morphology transition in *in situ* cross-linking PISA.

Experimental

Materials

Polyethylene glycol monomethyl ether 4000 (mPEG₉₀-OH) purchased from TCI was used as received. 4-(4-Cyanopentanoic acid) dithiobenzoate (CPADB) was synthesized according to

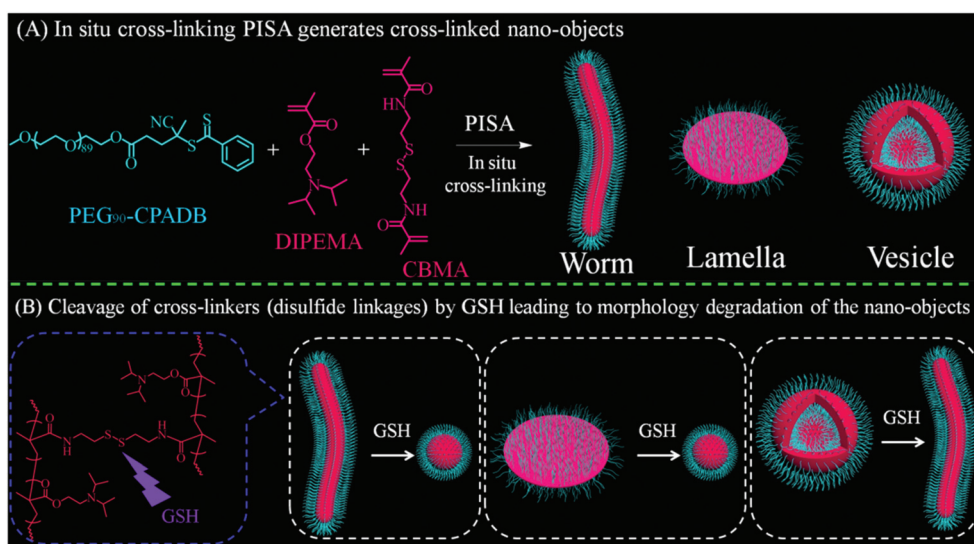


Fig. 1 (A) *In situ* cross-linking polymerization-induced self-assembly (PISA) generates cross-linked nano-objects. (B) Morphology degradation of the nano-objects by incubating with glutathione (GSH).

the method reported in a previous article.⁵⁴ *N,N'*-Dicyclohexylcarbodiimide (DCC, >99%, MACKLIN), 4-dimethylaminopyridine (DMAP, >99.0%, TCI), cystamine dihydrochloride (99%, Energy Chemical), methacryloyl chloride (95%, Aladdin), 2,2'-bipyridine (>99.0%, TCI) and glutathione (reduced form) (GSH, ≥98%, Sigma-Aldrich) were used as received. 2-(Diisopropylamino) ethyl methacrylate (DIPEMA, Sigma-Aldrich, 97%) was purified by passing through an alkaline alumina column. Other solvents are of analytical grade and used directly without purification.

Synthesis of the macro-RAFT agent PEG₉₀-CPADB

mPEG₉₀-OH (8 g, 2.0 mmol), CPADB (2.23 g, 8 mmol) and DMAP (98 mg, 0.8 mmol) were dissolved in 50 mL of dichloromethane (CH₂Cl₂), and then stirred at 0 °C using a low-temperature equipment (DHJF-8002) for 30 min. DCC (1.65 g, 8 mmol) was dissolved in 10 mL of CH₂Cl₂, and then the solution of DCC was added dropwise to the above mixture for 30 min. The reaction mixture was stirred at 0 °C for three days. The insoluble byproduct was removed by filtration, and then most of the solvent was removed using a rotary evaporator. The concentrated solution was added dropwise into a large amount of glacial diethyl ether to form some pink precipitate. The product was obtained by filtration, and then dissolved in CH₂Cl₂ again. The above mentioned precipitation procedure was repeated three times. The product was dried under vacuum for 2 days to obtain some pink powder.

Synthesis of the crosslinker *N,N'*-cystaminebismethacrylamide (CBMA)

Cystamine dihydrochloride (9 g, 40 mmol) and 100 mL of deionized water were added into a 250 mL round-bottomed flask. 10 mL of sodium hydroxide (6.4 g, 160 mmol) aqueous solution was added into the flask, and then the flask was put into an ice water bath. After stirring at 0 °C for 30 min, a solution of methacryloyl chloride (8.32 g, 80 mmol) in 20 mL of CH₂Cl₂ was added dropwise to the above reaction mixture, during which some white precipitate was formed. The reaction was carried out for another three hours at room temperature. The precipitate was obtained by filtration and washed three times with deionized water. The crude product was recrystallized twice in ethyl acetate to obtain some white powder.

RAFT dispersion polymerization of DIPEMA using PEG₉₀-CPADB as the macro RAFT agent

A typical formula of DIPEMA/PEG-CPADB = 150 at 20 wt% solid in the ethanol/water mixture (70/30, w/w) was introduced here. PEG-CPADB (43 mg, 0.01 mmol), DIPEMA (320 mg, 1.5 mmol), AIBN (0.55 mg, 3.33×10^{-3} mmol) and ethanol/water (70/30, w/w, 1.45 g) were added into a glass tube. After three freeze-pump-thaw cycles, the tube was sealed under vacuum. Then the polymerization was carried out in an oil bath at 70 °C for 24 h under stirring. Finally, the polymerization was quenched by rapid cooling to room temperature and exposing to air. Other RAFT dispersion polymerizations with

various feed molar ratios of DIPEMA/PEG₉₀-CPADB were performed using the same procedure.

RAFT dispersion copolymerization of DIPEMA and CBMA using PEG₉₀-CPADB as the macro RAFT agent

A typical formula of DIPEMA/PEG₉₀-CPADB = 60 and CBMA/DIPEMA = 5% at 20 wt% solid in the ethanol/water mixture (70/30, w/w) was introduced here. PEG-CPADB (43 mg, 0.01 mmol), DIPEMA (128 mg, 0.6 mmol), CBMA (22 mg, $0.6 \times 5\%$ mmol), AIBN (0.55 mg, 3.33×10^{-3} mmol) and ethanol/water (70/30, w/w, 0.77 g) were added into a glass tube. After three freeze-pump-thaw cycles, the tube was sealed under vacuum. Then the polymerization was carried out in an oil bath at 70 °C for 24 h under stirring. Finally, the polymerization was quenched by rapid cooling to room temperature and exposing to air. Other RAFT dispersion copolymerizations with various feed molar ratios of DIPEMA/PEG₉₀-CPADB but a constant feed molar ratio of CBMA/DIPEMA = 5% or 10% were performed using the same procedure.

Incubation of the resultant PEG₉₀-*b*-P(DIPEMA-*co*-CBMA) nano-objects with glutathione (GSH)

The disulfide bond in the cross-linkers can be cleaved by glutathione (GSH), and the cross-linked structures of the PEG₉₀-*b*-P(DIPEMA-*co*-CBMA) nano-objects will be destroyed accordingly. A typical operation is as follows: 10 μL of the sample was taken and diluted to 0.1 wt% with 2 mL of GSH solution (10 mM, ethanol/water 70/30, w/w). After 24 hours of incubation, these samples were characterized by TEM.

Characterization

The ¹H NMR spectra of the polymers were recorded in CDCl₃ or DMSO-*d*₆ using a Bruker DMX300 spectrometer. The molecular weight and polydispersity of the polymers were assessed by gel permeation chromatography (GPC) using a Waters 1515C equipment with a RI 2414 detector, two Waters styragel columns (HR1 and HR4), and THF as the mobile phase (flow rate: 1.0 mL min⁻¹). Commercial polystyrene standards with molecular weights of 500–600 000 g mol⁻¹ were used to calibrate the apparatus. Transmission electron microscopy (TEM) measurements were performed using a HT7700 electron microscope at an accelerating voltage of 100 kV. The PISA-generated dispersions of the nano-objects were diluted with ethanol/water (70/30, w/w) to 0.1–0.5 wt% and deposited on copper grids. In order to obtain clear TEM images, phosphotungstic acid was used for staining all the samples.

Results and discussion

Esterification reaction of polyethylene glycol monomethyl ether (PEG₉₀-OH) and 4-(4-cyanopentanoic acid) dithiobenzoate (CPADB) was carried out at 0 °C using a low-temperature equipment (DHJF-8002) for 3 days to synthesize the macro RAFT agent PEG₉₀-CPADB. Fig. S1A† shows the ¹H NMR spectrum of the resultant PEG₉₀-CPADB. The degree of CPADB

functionalization of the PEG₉₀-CPADB (F_{CPADB}) was calculated to be 98% based on the integral values of the aromatic protons in the CPADB unit (g, h, and f) and the methyl end group of the PEG chain (a). Fig. S1B† shows the GPC trace of the resultant PEG₉₀-CPADB ($M_n = 5900 \text{ g mol}^{-1}$ and $M_w/M_n = 1.05$). The PEG₉₀-CPADB was used as the macro RAFT agent in the subsequent RAFT dispersion polymerization.

CBMA was successfully synthesized as shown by the ¹H NMR spectrum (Fig. S2†), and used as the cross-linker in the following experiments. Using PEG₉₀-CPADB as the macro chain transfer agent, RAFT dispersion copolymerization of DIPEMA and CBMA with target $\text{DP}_{\text{DIPEMA}} = 150$ and feed molar ratio of CBMA/DIPEMA = 5% was carried out. During the RAFT dispersion copolymerization of DIPEMA and CBMA, the consumption of the cross-linker CBMA is obviously slower than that of the monomer DIPEMA (Fig. 2, Fig. S3, and Table S1†), which can be attributed to the lower transfer coefficient of methacrylamide group- $\text{C}(\text{Me})_2\text{C}(=\text{O})\text{NH}(\text{alkyl})$ than that of methacrylate group- $\text{C}(\text{Me})_2\text{C}(=\text{O})\text{O}(\text{alkyl})$ in RAFT polymerization.^{51,55} For the samples of PEG₉₀-*b*-P(DIPEMA_{*x*}-*co*-CBMA_{*y*}) generated at the early stage of RAFT dispersion copolymerization of DIPEMA and CBMA, multimodal GPC traces gradually appeared due to the branched structures of the solvophobic blocks (Fig. 2A). GPC characterization of PEG₉₀-*b*-P(DIPEMA_{*x*}-*co*-CBMA_{*y*}) generated at the late stage of polymerization was not performed due to the cross-linked (or partially cross-linked) structures. For comparison, PEG₉₀-CPADB-mediated RAFT dispersion polymerization of DIPEMA with target $\text{DP}_{\text{DIPEMA}} = 150$ in the absence of CBMA was also carried out (Fig. S4–S6 and Table S2†). The GPC traces of the

PEG₉₀-*b*-PDIPEMA_{*x*} block copolymers formed by RAFT dispersion polymerization of DIPEMA at different polymerization times are unimodal ($M_w/M_n = 1.08$ – 1.17), and they gradually shifted to the higher molecular weight area with increasing polymerization time (Fig. 3B and Table S2†). After 1 h of polymerization, PEG₉₀-*b*-PDIPEMA₂₂ spherical micelles (Fig. 4A₁) and PEG₉₀-*b*-(PDIPEMA₂₅-*co*-CBMA_{0.57}) spherical micelles (Fig. 4A₂) were formed in the RAFT dispersion polymerization of DIPEMA and RAFT dispersion copolymerization of DIPEMA and CBMA, respectively. After 2 h of polymerization, spherical micelles of PEG₉₀-*b*-PDIPEMA₅₃ were formed in the RAFT dispersion polymerization of DIPEMA (Fig. 4B₁), and worm-like micelles of PEG₉₀-*b*-P(DIPEMA₇₀-*co*-CBMA_{1.13}) were formed in the RAFT dispersion copolymerization of DIPEMA and CBMA (Fig. 4B₂). The higher order morphology of PEG₉₀-*b*-P(DIPEMA₇₀-*co*-CBMA_{1.13}) when compared to that of PEG₉₀-*b*-PDIPEMA₅₃ might be partially attributed to the higher $\text{DP}_{\text{DIPEMA}}$ of the former polymer. However, only a mixture of spherical micelles and short worm-like micelles was formed even at $\text{DP}_{\text{DIPEMA}} = 93$ after 4 h of RAFT dispersion polymerization of DIPEMA (Fig. 4C₁). The above results indicate that the branched structures of the solvophobic blocks also contribute to the promoting effect on morphology transition in the RAFT dispersion copolymerization of DIPEMA and CBMA. After 4 h of RAFT dispersion copolymerization of DIPEMA and CBMA, PEG₉₀-*b*-P(DIPEMA₁₀₉-*co*-CBMA_{1.98}) vesicles were formed (Fig. 4C₂). Continuous

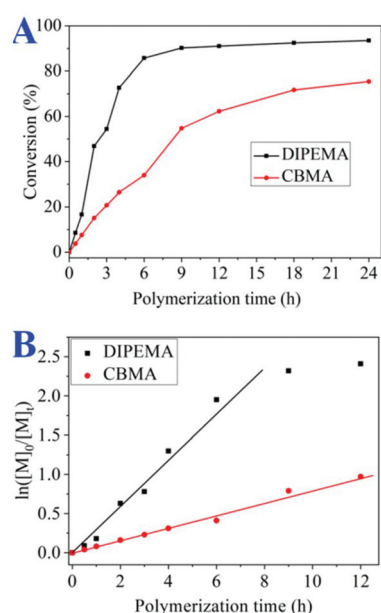


Fig. 2 (A) Polymerization kinetics for PEG₉₀-CPADB-mediated RAFT dispersion copolymerization of DIPEMA and CBMA with DIPEMA/PEG₉₀-CPADB = 150 and CBMA/DIPEMA = 5%. (B) Plots of $\ln([M]_0/[M]_t)$ versus polymerization time according to the data in (A).

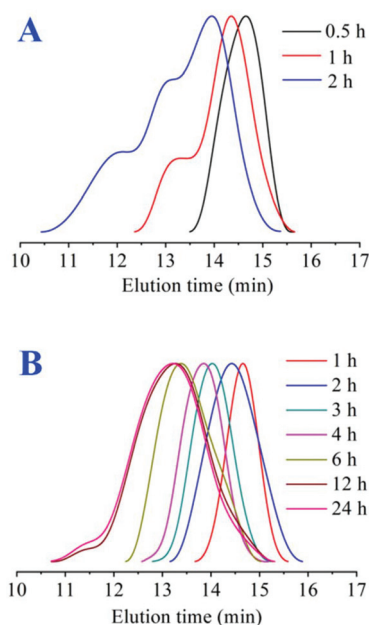


Fig. 3 (A) GPC traces of the PEG₉₀-*b*-P(DIPEMA_{*x*}-*co*-CBMA_{*y*}) block copolymers obtained at different polymerization times of the PEG₉₀-CPADB-mediated RAFT dispersion copolymerization of DIPEMA and CBMA (DIPEMA/PEG₉₀-CPADB = 150, CBMA/DIPEMA = 5%). (B) GPC traces of the PEG₉₀-*b*-PDIPEMA_{*x*} block copolymers obtained at different polymerization times of the PEG₉₀-CPADB-mediated RAFT dispersion polymerization of DIPEMA (DIPEMA/PEG₉₀-CPADB = 150).

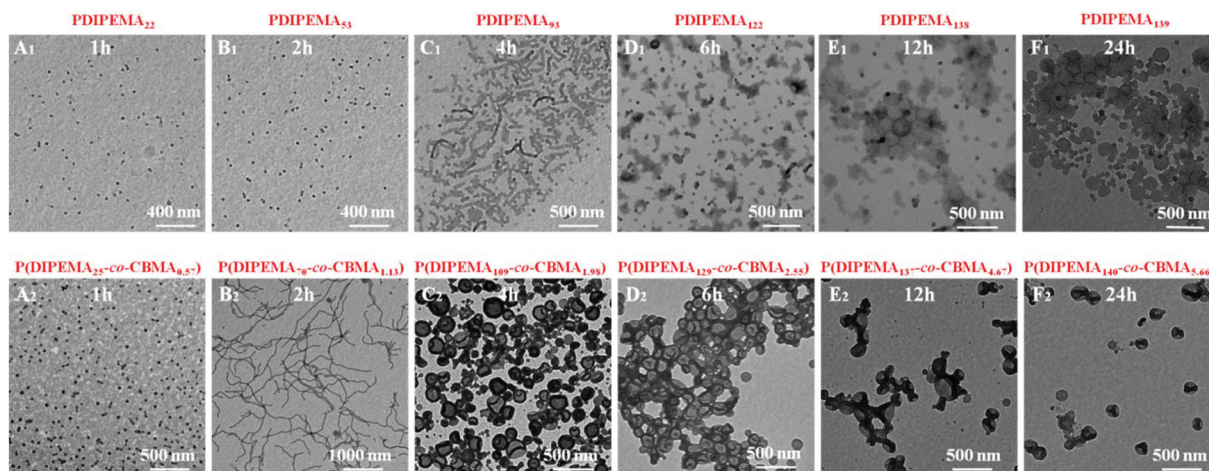


Fig. 4 TEM images of the PEG₉₀-PDIPEMA_x nano-objects obtained at different polymerization times by RAFT dispersion polymerization of DIPeMA with target DP_{PDIPEMA} = 150 (A₁–F₁), and the PEG₉₀-P(DIPEMA_x-co-CBMA_y) nano-objects obtained at different polymerization times by RAFT dispersion copolymerization of DIPeMA and CBMA with target DP_{PDIPEMA} = 150 and feed molar ratio of CBMA/DIPEMA = 5% (A₂–F₂). The red note on the top of each image is the composition of the solvophobic blocks in the corresponding nano-objects. The subscript number of DIPeMA is the actual DP_{PDIPEMA}, and the subscript number of CBMA is the averaged CBMA units in the formed P(DIPEMA-co-CBMA) blocks, which were calculated according to the ¹H NMR spectra as shown in Fig. S3 and S4.†

polymerization leads to gradual growth of the solvophobic P(DIPEMA-co-CBMA) blocks and the averaged CBMA units in the formed P(DIPEMA-co-CBMA) blocks (Fig. 2, Fig. S3 and Table S1†), leading to the formation of cross-linked vesicles (Fig. S7†). In comparison, pure vesicles are not formed in the RAFT dispersion polymerization of DIPeMA even at DP_{PDIPEMA} = 139 (Fig. 4). The above results demonstrate that *in situ* cross-linking PISA by RAFT dispersion copolymerization of DIPeMA and CBMA not only generates stabilized vesicles but also promotes morphology transition, which has been usually ignored by researchers in previous work.^{49–51}

RAFT dispersion copolymerization of DIPeMA and CBMA with target DP_{PDIPEMA} = 150 and feed molar ratio of CBMA/DIPEMA = 10% was also carried out. ¹H NMR, GPC and TEM were used to trace the polymerization (Fig. S8–S10 and Table S3†). After 1 h of polymerization, spherical micelles were formed in the composition of PEG₉₀-b-P(DIPEMA₂₂-co-CBMA_{0.89}) (Fig. S10A†). After 2 h of polymerization, a mixture of worm-like micelles and lamella was formed in the composition of PEG₉₀-b-P(DIPEMA₆₀-co-CBMA_{1.93}) (Fig. S10B†). In comparison with the PEG₉₀-b-P(DIPEMA₇₀-co-CBMA_{1.13}) worm-like micelles shown in Fig. 4B₂, PEG₉₀-b-P(DIPEMA₆₀-co-CBMA_{1.93}) nano-objects (Fig. S10B†) have lower DP_{PDIPEMA} but a slightly higher order morphology (a mixture of worm-like micelles and lamella). The slightly higher content of CBMA units in PEG₉₀-b-P(DIPEMA₆₀-co-CBMA_{1.93}) than that in PEG₉₀-b-P(DIPEMA₇₀-co-CBMA_{1.13}) results in higher branching degrees of the solvophobic P(DIPEMA-co-CBMA) blocks, which exhibit a more pronounced promoting effect on morphology transition. After 3 h of polymerization, a mixture of lamella and vesicles was formed in the composition of PEG₉₀-b-P(DIPEMA₇₂-co-CBMA_{2.82}), but further morphology transition to pure vesicles was limited to some extent in the subsequent

polymerization (Fig. S10†). Compared with the RAFT dispersion copolymerization of DIPeMA and CBMA with a feed molar ratio of CBMA/DIPEMA = 5% (Fig. 4A₂–4F₂), the RAFT dispersion copolymerization of DIPeMA and CBMA with CBMA/DIPEMA = 10% exhibits a more pronounced effect on promoting morphology transition at the early stage of polymerization, but a slightly limited effect on morphology transition at the late stage of polymerization (Fig. S10†).

RAFT dispersion copolymerization of DIPeMA and CBMA with a fixed feed ratio of CBMA/DIPEMA = 5% (molar ratio) but different DIPeMA/PEG₉₀-CPADB molar ratios was carried out to fabricate the PEG₉₀-b-P(DIPEMA_x-co-CBMA_{5%}) nano-objects. For comparison, RAFT dispersion polymerization of DIPeMA with varying target DP_{PDIPEMA} in the absence of CBMA was also carried out to fabricate the PEG₉₀-b-PDIPeMA_x nano-objects. ¹H NMR, GPC and TEM were used to assess the monomer conversion in polymerization, the molecular weight and polydispersity of the block copolymer, and the morphologies of the resultant nano-objects (Fig. 5, Fig. S11 and Table S4†). In order to obtain high conversion of DIPeMA and CBMA, all polymerizations were carried out for 24 h. Table S4† shows that the conversion of DIPeMA in each case was above 90%, and the conversion of CBMA reached 70–80%. At the similar DP of the solvophobic blocks, the PEG₉₀-b-P(DIPEMA_x-co-CBMA_{5%}) nano-objects (5% of CBMA in feed) usually exhibit a higher order morphology compared to the PEG₉₀-b-PDIPeMA_x nano-objects (Fig. 5). For the RAFT dispersion polymerization of DIPeMA, spherical micelles were obtained at DIPeMA/PEG₉₀-CPADB = 60 and 80, a mixture of spherical micelles and worm-like micelles was obtained at DIPeMA/PEG₉₀-CPADB = 100 and 120, and a mixture of spherical micelles and vesicles was produced at DIPeMA/PEG₉₀-CPADB = 150 (Fig. 5A₁–E₁). For the RAFT dispersion copolymerization of

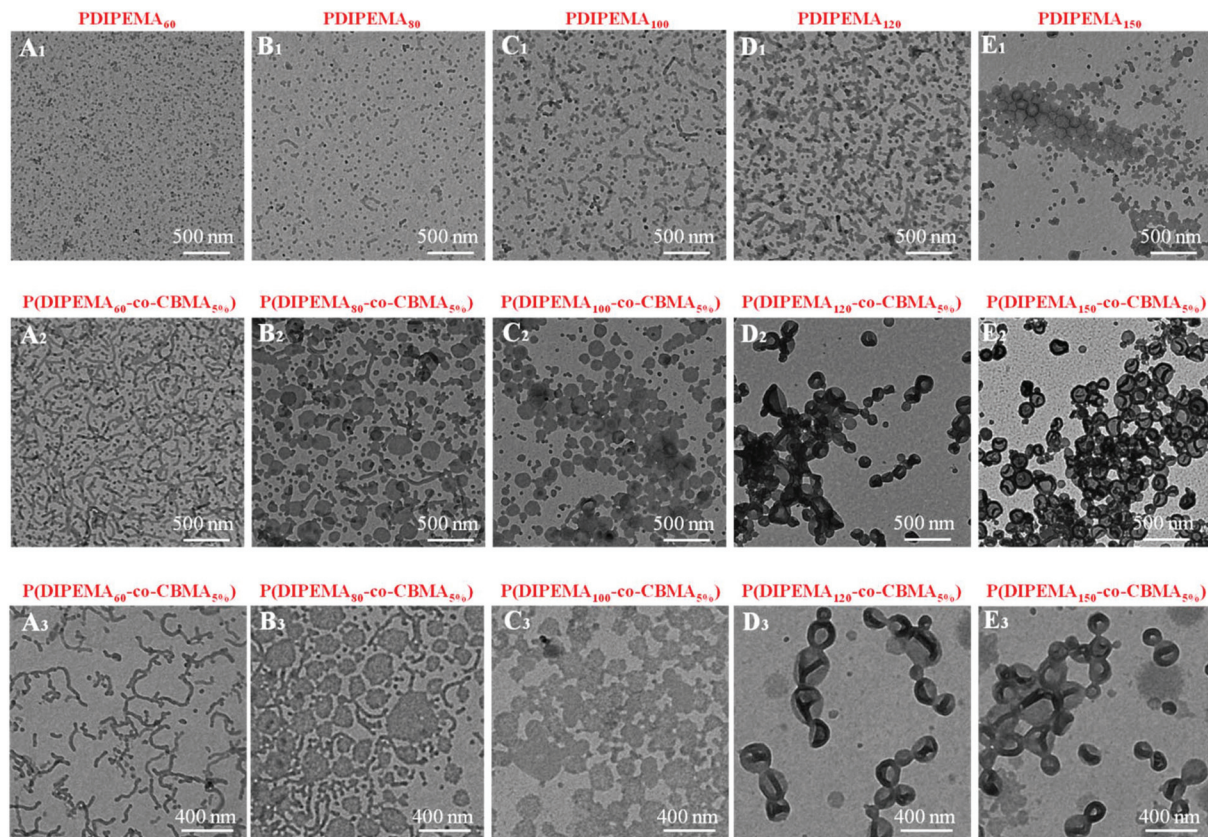


Fig. 5 TEM images of the PEG₉₀-*b*-PDIPEMA_x nano-objects obtained by RAFT dispersion polymerization of DIPEMA with varying feed molar ratios of DIPEMA/PEG₉₀-CPADB, (A₁) 60, (B₁) 80, (C₁) 100, (D₁) 120 and (E₁) 150. TEM images of the PEG₉₀-*b*-P(DIPEMA_x-*co*-CBMA_{5%}) nano-objects obtained by RAFT dispersion copolymerization of DIPEMA and CBMA with a constant feed molar ratio of CBMA/DIPEMA = 5% but different feed molar ratios of DIPEMA/PEG₉₀-CPADB, and (A₂) 60, (B₂) 80, (C₂) 100, (D₂) 120 and (E₂) 150. (A₃–E₃) are the TEM images of the nano-objects (A₂–E₂) after being dispersed in a good solvent (THF). All the nano-objects shown in (A₃–E₃) were processed using the following steps: (1) the nano-objects were dispersed in THF, (2) the dispersions in THF were diluted with ethanol/water (7/3) to a specific concentration (0.1 wt%–0.5 wt%) for TEM characterization.

DIPEMA and CBMA, worm-like micelles were formed at DIPEMA/PEG₉₀-CPADB = 60, a mixture of lamella and a small amount of spherical micelles and worm-like micelles were produced at DIPEMA/PEG₉₀-CPADB = 80 and 100, and vesicles were formed at DIPEMA/PEG₉₀-CPADB = 120 and 150 (Fig. 5A₂–E₂). Tetrahydrofuran (THF) is a good solvent for both PEG and PDIPEMA blocks. In order to demonstrate the structural stability of the cross-linked nano-objects, the PEG₉₀-*b*-P(DIPEMA_x-*co*-CBMA_{5%}) nano-objects were dispersed in THF. TEM images show that all the nano-objects well maintained their original morphologies after being treated with THF (Fig. 5A₃–E₃). The above results indicate that *in situ* cross-linking PISA by RAFT dispersion copolymerization of DIPEMA and CBMA results in not only superior structural stability but also promoting effect on morphology transition.

In acidic solution (pH = 5), the tertiary amine in the DIPEMA units was protonated, which induced hydrophobic-to-hydrophilic transition of the P(DIPEMA-*co*-CBMA) blocks.⁵¹ Such hydrophobic-to-hydrophilic transition resulted in size increasing rather than disaggregation of the nano-objects, which further demonstrated the structural stability of the

cross-linked nano-objects (Fig. S12 and Table S5†). However, after incubation of the cross-linked nano-objects in acidic solution (pH = 5) with 10 mM GSH for 24 h, all the nano-objects fully dissociated (the volume-averaged diameter was less than 10 nm as obtained by DLS) due to the simultaneous cleavage of the cross-linker (disulfide linkage of the CBMA units) and hydrophobic-to-hydrophilic transition of the P(DIPEMA-*co*-CBMA) blocks (Table S5†). In order to further demonstrate that branched structures of the solvophobic blocks promote morphology transition, the PEG₉₀-*b*-P(DIPEMA_x-*co*-CBMA_{5%}) nano-objects were dispersed into neutral solution of GSH (10 mM) to destroy the cross-linked/branched linkage of the nano-objects (the solvophobicity of the P(DIPEMA-*co*-CBMA) blocks was unchanged). After incubating with GSH for 24 h, the worm-like micelles of PEG₉₀-*b*-P(DIPEMA₆₀-*co*-CBMA_{5%}) (as shown in Fig. 5A₂) and lamella of PEG₉₀-*b*-P(DIPEMA₈₀-*co*-CBMA_{5%}) (as shown in Fig. 5B₂) were both degraded to spherical micelles (Fig. 6A and B), and the vesicles of PEG₉₀-*b*-P(DIPEMA₁₂₀-*co*-CBMA_{5%}) (as shown in Fig. 5D₂) were degraded to worm-like micelles and spherical micelles (Fig. 6C). The vesicles of PEG₉₀-*b*-P(DIPEMA₁₅₀-*co*-CBMA_{5%}) (as shown in

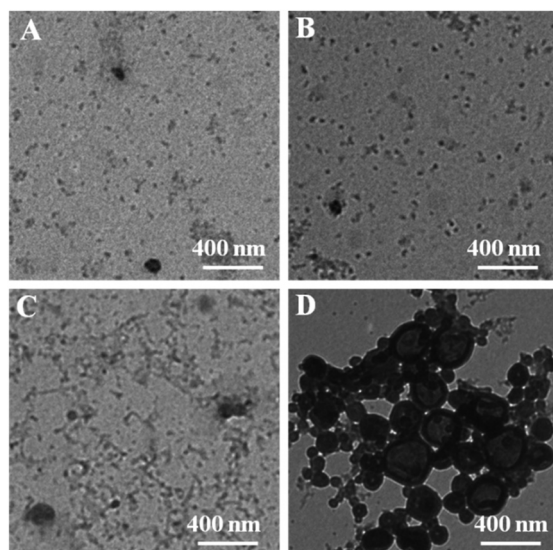


Fig. 6 TEM images of the PEG₉₀-*b*-P(DIPEMA_x-*co*-CBMA_{5%}) nano-objects (as shown in Fig. 5) after incubation in an aqueous solution of GSH (10 mM) for 24 h, (A) PEG₉₀-*b*-P(DIPEMA₆₀-*co*-CBMA_{5%}), (B) PEG₉₀-*b*-P(DIPEMA₈₀-*co*-CBMA_{5%}), (C) PEG₉₀-*b*-P(DIPEMA₁₂₀-*co*-CBMA_{5%}) and (D) PEG₉₀-*b*-P(DIPEMA₁₅₀-*co*-CBMA_{5%}).

Fig. 5E₂) can maintain their morphologies after incubation with GSH for 24 h (Fig. 6D), which may be due to the fact that vesicles were also formed at DP_{DIPEMA} = 150 without CBMA (Fig. 5E₁). The above results further demonstrate that the branched structures of the solvophobic blocks generated by *in situ* cross-linking PISA can promote morphology transition. The incorporation of the CBMA units (5%) into the solvophobic blocks also results in a slight change in the chemical composition and the corresponding solvophobicity, which may also have influence on morphology transition. However, upon close inspection of Fig. 5A₁–E₁ and 6, it can be seen that the degraded nano-objects of PEG₉₀-*b*-P(DIPEMA-*co*-CBMA) usually exhibit a similar morphology to the PEG₉₀-*b*-PDIPEMA nano-objects at a similar DP_{DIPEMA}, which demonstrates that such slight changes of chemical composition (or solvophobicity) have negligible influence on morphology transition and the promoting effect on morphology transition is mainly attributed to the changes in the topological structures of the solvophobic blocks.

For another comparison, RAFT dispersion copolymerization of DIPEMA and CBMA with a fixed feed molar ratio of CBMA/DIPEMA = 10% but varying DIPEMA/PEG₉₀-CPADB ratios was also carried out. Compared with the PEG₉₀-*b*-P(DIPEMA_x-*co*-CBMA_{5%}) nano-objects obtained at 5% of CBMA (Fig. 5A₂–E₂), most of the PEG₉₀-*b*-P(DIPEMA_x-*co*-CBMA_{10%}) nano-objects obtained at 10% of CBMA have lower order morphologies (Fig. S13†), which may be due to the fact that more CBMA feed results in the faster formation of cross-linked structures to limit morphology transition. However, compared with the PEG₉₀-*b*-PDIPEMA_x nano-objects (Fig. 5A₁–E₁), most of the PEG₉₀-*b*-P(DIPEMA_x-*co*-CBMA_{10%}) nano-objects also exhibit

higher order morphologies (Fig. S13†), which further illustrates the promoting effect on morphology transition in *in situ* cross-linking PISA by RAFT dispersion copolymerization of DIPEMA and CBMA. Moreover, the PEG₉₀-*b*-P(DIPEMA_x-*co*-CBMA_{10%}) nano-objects also have robust cross-linked structures, which can maintain their original morphologies after being treated with a good solvent (THF) (Fig. S14†).

Conclusions

In summary, not only cross-linked structures of the nano-objects but also the promoting effect on morphology transition has been observed in *in situ* cross-linking PISA by RAFT dispersion copolymerization of DIPEMA and CBMA. Compared with the RAFT dispersion polymerization of DIPEMA, RAFT dispersion copolymerization of DIPEMA and CBMA usually generates higher order morphologies. The slower consumption of CBMA at the early stage of polymerization generates branched structures of the solvophobic blocks, leading to the promoting effect on morphology transition accordingly. The reaction of CBMA mainly occurs in the late stage of polymerization (after most of the monomer DIPEMA is reacted), which generates the cross-linked structures. Cleavage of the disulfide linkage at the branched/cross-linked points by GSH leads to morphology transition of the nano-objects to the lower order ones, which further demonstrates the promoting effect on morphology transition in *in situ* cross-linking PISA.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

We acknowledge the financial support from the National Natural Science Foundation of China (21774113, 52021002 and 21704095).

Notes and references

- 1 L. D. Blackman, Z. Y. Oo, Y. Qu, P. A. Gunatillake, P. Cass and K. E. S. Locock, *ACS Appl. Mater. Interfaces*, 2020, **12**, 11353–11362.
- 2 L. D. Blackman, S. Varlas, M. C. Arno, Z. H. Houston, N. L. Fletcher, K. J. Thurecht, M. Hasan, M. I. Gibson and R. K. O'Reilly, *ACS Cent. Sci.*, 2018, **4**, 718–723.
- 3 X. L. Hu, Y. G. Zhang, Z. G. Xie, X. B. Jing, A. Bellotti and Z. Gu, *Biomacromolecules*, 2017, **18**, 649–673.
- 4 K. L. Thompson, C. J. Mable, A. Cockram, N. J. Warren, V. J. Cunningham, E. R. Jones, R. Verber and S. P. Armes, *Soft Matter*, 2014, **10**, 8615–8626.

- 5 K. L. Thompson, C. J. Mable, J. A. Lane, M. J. Derry, L. A. Fielding and S. P. Armes, *Langmuir*, 2015, **31**, 4137–4144.
- 6 W. J. Zhang, C. Y. Hong and C. Y. Pan, *Macromol. Rapid Commun.*, 2019, **40**, 1800279.
- 7 S. L. Canning, G. N. Smith and S. P. Armes, *Macromolecules*, 2016, **49**, 1985–2001.
- 8 E. E. Brotherton, F. L. Hatton, A. A. Cockram, M. J. Derry, A. Czajka, E. J. Cornel, P. D. Topham, O. O. Mykhaylyk and S. P. Armes, *J. Am. Chem. Soc.*, 2019, **141**, 13664–13675.
- 9 M. J. Derry, T. Smith, P. S. O'Hora and S. P. Armes, *ACS Appl. Mater. Interfaces*, 2019, **11**, 33364–33369.
- 10 W. J. Zhang, C. Y. Hong and C. Y. Pan, *RSC Adv.*, 2015, **5**, 42637–42644.
- 11 X. F. Xu, C. Y. Pan, W. J. Zhang and C. Y. Hong, *Macromolecules*, 2019, **52**, 1965–1975.
- 12 J. Yeow and C. Boyer, *Adv. Sci.*, 2017, **4**, 1700137.
- 13 N. J. W. Penfold, J. Yeow, C. Boyer and S. P. Armes, *ACS Macro Lett.*, 2019, **8**, 1029–1054.
- 14 S. Y. Khor, J. F. Quinn, M. R. Whittaker, N. P. Truong and T. P. Davis, *Macromol. Rapid Commun.*, 2019, **40**, 1800438.
- 15 F. Lv, Z. S. An and P. Y. Wu, *Macromolecules*, 2020, **53**, 367–373.
- 16 F. Lv, Z. S. An and P. Y. Wu, *Nat. Commun.*, 2019, **10**, 1397.
- 17 D. W. Zhou, R. P. Kuchel, S. M. Dong, F. P. Lucien, S. Perrier and P. B. Zetterlund, *Macromol. Rapid Commun.*, 2019, **40**, 1800335.
- 18 S. Varlas, R. Keogh, Y. J. Xie, S. L. Horswell, J. C. Foster and R. K. O'Reilly, *J. Am. Chem. Soc.*, 2019, **141**, 20234–20248.
- 19 S. Varlas, J. C. Foster and R. K. O'Reilly, *Chem. Commun.*, 2019, **55**, 9066–9071.
- 20 Y. X. Zhang, L. L. Yu, X. C. Dai, L. Zhang and J. B. Tan, *ACS Macro Lett.*, 2019, **8**, 1102–1109.
- 21 X. C. Dai, Y. X. Zhang, L. L. Yu, X. L. Li, L. Zhang and J. B. Tan, *ACS Macro Lett.*, 2019, **8**, 955–961.
- 22 G. Mellot, J. M. Guigner, L. Bouteiller, F. Stoffelbach and J. Rieger, *Angew. Chem., Int. Ed.*, 2019, **58**, 3173–3177.
- 23 F. D'Agosto, J. Rieger and M. Lansalot, *Angew. Chem., Int. Ed.*, 2020, **59**, 8368–8392.
- 24 Q. Z. Liu, X. Y. Wang, L. Ma, K. W. Yu, W. X. Xiong, X. H. Lu and Y. L. Cai, *ACS Macro Lett.*, 2020, **9**, 454–458.
- 25 L. Wang, Y. Ding, Q. Z. Liu, Q. Q. Zhao, X. C. Dai, X. H. Lu and Y. L. Cai, *ACS Macro Lett.*, 2019, **8**, 623–628.
- 26 D. Li, X. Chen, M. Zeng, J. Z. Ji, Y. Wang, Z. Z. Yang and J. Y. Yuan, *Chem. Sci.*, 2020, **11**, 2855–2860.
- 27 M. Zeng, X. S. Cao, H. Xu, W. P. Gan, B. D. Smith, H. F. Gao and J. Y. Yuan, *Polym. Chem.*, 2020, **11**, 936–943.
- 28 S. Y. Khor, M. N. Vu, E. H. Pilkington, A. P. R. Johnston, M. R. Whittaker, J. F. Quinn, N. P. Truong and T. P. Davis, *Small*, 2018, **14**, 1801702.
- 29 N. P. Truong, C. Zhang, T. A. H. Nguyen, A. Anastasaki, M. W. Schulze, J. F. Quinn, A. K. Whittaker, C. J. Hawker, M. R. Whittaker and T. P. Davis, *ACS Macro Lett.*, 2018, **7**, 159–165.
- 30 M. A. Touve, D. B. Wright, C. Mu, H. Sun, C. Park and N. C. Gianneschi, *Macromolecules*, 2019, **52**, 5529–5537.
- 31 S. W. Qu, R. Liu, W. F. Duan and W. Q. Zhang, *Macromolecules*, 2019, **52**, 5168–5176.
- 32 Y. Zhang, T. Y. Guan, G. Han, T. Y. Guo and W. Q. Zhang, *Macromolecules*, 2019, **52**, 718–728.
- 33 M. N. Vu, H. G. Kelly, A. K. Wheatley, S. Peng, E. H. Pilkington, N. A. Veldhuis, T. P. Davis, S. J. Kent and N. P. Truong, *Small*, 2020, **16**, 2002861.
- 34 S. Z. Li, G. Han and W. Q. Zhang, *Polym. Chem.*, 2020, **11**, 4681–4692.
- 35 W. J. Zhang, J. Kadirkhanov, C. H. Wang, S. G. Ding, C. Y. Hong, F. Wang and Y. Z. You, *Polym. Chem.*, 2020, **11**, 3654–3672.
- 36 Y. Ning, L. J. Han, M. J. Derry, F. C. Meldrum and S. P. Armes, *J. Am. Chem. Soc.*, 2019, **141**, 2557–2567.
- 37 N. J. W. Penfold, Y. Ning, P. Verstraete, J. Smets and S. P. Armes, *Chem. Sci.*, 2016, **7**, 6894–6904.
- 38 S. H. Xu, J. Yeow and C. Boyer, *ACS Macro Lett.*, 2018, **7**, 1376–1382.
- 39 W. J. Zhang, C. Y. Hong and C. Y. Pan, *ACS Appl. Mater. Interfaces*, 2017, **9**, 15086–15095.
- 40 W. J. Zhang, C. Y. Hong and C. Y. Pan, *Biomacromolecules*, 2017, **18**, 1210–1217.
- 41 S. Guan and A. H. Chen, *ACS Macro Lett.*, 2020, **9**, 14–19.
- 42 L. L. Huang, Y. Ding, Y. J. Ma, L. Wang, Q. Z. Liu, X. H. Lu and Y. L. Cai, *Macromolecules*, 2019, **52**, 4703–4712.
- 43 B. Couturaud, P. G. Georgiou, S. Varlas, J. R. Jones, M. C. Arno, J. C. Foster and R. K. O'Reilly, *Macromol. Rapid Commun.*, 2019, **40**, 1800460.
- 44 S. J. Byard, M. Williams, B. E. McKenzie, A. Blanazs and S. P. Armes, *Macromolecules*, 2017, **50**, 1482–1493.
- 45 N. Busatto, V. Stolojan, M. Shaw, J. L. Keddie and P. J. Roth, *Macromol. Rapid Commun.*, 2019, **40**, 1800346.
- 46 X. W. Zhang, A. F. Cardozo, S. Chen, W. J. Zhang, C. Julcour, M. Lansalot, J. F. Blanco, F. Gayet, H. Delmas, B. Charleux, E. Manoury, F. D'Agosto and R. Poli, *Chem. – Eur. J.*, 2014, **20**, 15505–15517.
- 47 S. Sugihara, S. P. Armes, A. Blanazs and A. L. Lewis, *Soft Matter*, 2011, **7**, 10787–10793.
- 48 W. J. Zhang, C. Y. Hong and C. Y. Pan, *Biomacromolecules*, 2016, **17**, 2992–2999.
- 49 Q. W. Qu, G. Y. Liu, X. Q. Lv, B. H. Zhang and Z. S. An, *ACS Macro Lett.*, 2016, **5**, 316–320.
- 50 B. H. Zhang, X. Q. Lv, A. Q. Zhu, J. W. Zheng, Y. Q. Yang and Z. S. An, *Macromolecules*, 2018, **51**, 2776–2784.
- 51 M. Chen, J. W. Li, W. J. Zhang, C. Y. Hong and C. Y. Pan, *Macromolecules*, 2019, **52**, 1140–1149.
- 52 J. Park, N. Y. Ahn and M. Seo, *Polym. Chem.*, 2020, **11**, 4335–4343.
- 53 J. Wang, Z. G. Wu, G. W. Wang and K. Matyjaszewski, *Macromol. Rapid Commun.*, 2019, **40**, 1800332.
- 54 Y. Mitsukami, M. S. Donovan, A. B. Lowe and C. L. McCormick, *Macromolecules*, 2001, **34**, 2248–2256.
- 55 Y. K. Chong, J. Krstina, T. P. T. Le, G. Moad, A. Postma, E. Rizzardo and S. H. Thang, *Macromolecules*, 2003, **36**, 2256–2272.