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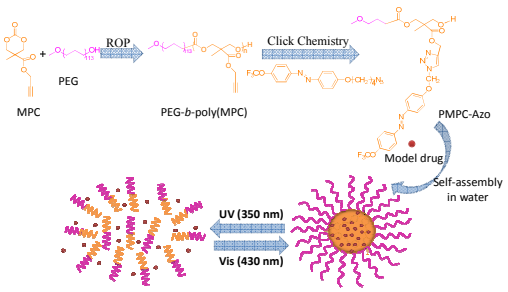
Graphic Abstract

Photo-responsive reversible micelles based on azobenzene-modified poly(carbonate)s via Azide-Alkyne click chemistry

Ding Hu, Yefei Li, Yile Niu, Ling Li, Jingwen He, Xiangyu Liu, Xinnian Xia, Yanbing Lu*, Yuanqin Xiong and Weijian Xu



We provide convenient method to construct photo-responsive poly(carbonate)s via ring-opening polymerization of cyclic carbonate followed by Azide-Alkyne click chemistry.

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Cite this: DOI: 10.1039/xoxxooooox

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxooooox

www.rsc.org/

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Photo-induced reversible amphipathic copolymer PMPC-Azo was click conjugated by connecting the amphiphile poly(ethylene glycol)-modified poly(carbonate)s (PEG-*b*-poly(MPC)) and azide-functional trifluoromethoxy-azobenzene (Azo-N_3). The resulting copolymer self-assembled into spherical micelles with a hydrophobic Azo core stabilized by a hydrophilic PEG corona in aqueous solution. As characterized by time-resolved UV-vis spectroscopy, dynamic light scattering (DLS) and transmission electron microscopy (TEM), these micelles showed reversible self-assembly and disassembly in aqueous solution under the alternative UV and visible light irradiation. Model drug Nile Red (NR) was then encapsulated into the micelles successfully. Light-controlled release and re-encapsulation behaviors were demonstrated by fluorescence spectroscopy. The cell cytotoxicity of PMPC-Azo micelles was also evidenced by MTT assay. This study provides a convenient way to construct smart nanocarriers for controlled release and re-encapsulation of hydrophobic drugs.

Introduction

During the last decades, the stimuli-responsive block copolymers (BCP) have been widely investigated for "on-off" drug delivery and "on-demand" nanomedicines because of their switch properties.[1-5] The most frequently used stimuli are pH, temperature, light, redox potential, ultrasound, charge, gases, biomolecules and enzymes.[6-14] Compared with other stimuli-responsive systems, light-responsive BCP don't need any changes in the surroundings. In addition, the wavelength and intensity of illumination can be adjusted accurately and the time, direction and area of illumination are easy to be controlled.[15-18] Light-responsiveness is usually provided by photochromic molecules attached to the polymers. Azobenzene (Azo) is a well-known and well-used compound because it can undergo *trans-cis* photo isomerization in response to UV and visible light. Azo isomerization is easy to change the hydrophobicity of the BCP micelles since the *cis* form of azobenzene is more polar than the *trans* form.[15] There are several reports on amphipathic BCP decorated with azobenzenes.[19-23] Chen et al. reported the construction of photo-responsive micelles from azobenzene-modified hyperbranched polyphosphates, which showed reversible self-assembly and disassembly behaviors via irradiating with UV and visible light.[24] Recently, Blasco et al. reported a series of light- responsive vesicles based on linear-dendritic amphiphile, which could be tailored the photo-responsive properties of the vesicles and consequently the release rate via adjusting the percentages of Azo and hydrocarbon chains.[25]

Amphiphilic copolymers have been widely studied for their potential applications in biomedical and pharmaceutical fields. Among them, block copolymers of aliphatic poly(carbonate)s combined with poly(ethylene glycol) (PEG) have been widely investigated for drug delivery due to their low toxicity, biocompatibility, and biodegradability.[26-30] PEG is a hydrophilic and nonionic polymer that has been widely used as a biocompatible polymer in both academia and industry for chemical and biological applications.[31-33] Polymeric micelles containing PEG as the hydrophilic shell are able to form a palisade preventing the adsorption of proteins and enzymes and subsequent non-specific uptake by the re-ticuloendothelial system (RES) after intravenous injection.[34,35] Several groups have grafted PEG onto polyesters using different synthetic pathways. Yang et al. reported the ring opening copolymerisation of acryloyl carbonate (AC) and ε-caprolactone (CL) using methoxy PEG as an initiator.[36] Hydrophilic and amphiphilic PEG-poly(trimethylene carbonate)

hydrogels were prepared using PEG-bisazides via "Click" chemistry by Truong et al.[37]

However, there have been limited reports of the synthesis stimuli-responsive poly(carbonate)s. Zhong's group prepared pH-responsive biodegradable polymers, which were comprised of a novel acid-labile polycarbonate hydrophobe and PEG.[38] And then, they developed reduction-responsive poly-(carbonate)s via ring-opening copolymerization of ε-CL and pyridyl disulfide-functionalized cyclic carbonate monomer.[39,40] However, there is no report on the light-responsive biodegradable biomaterials based on poly(carbonate)s.

In this study, we developed a facile method to prepare amphiphilic block copolymer with poly(carbonate)s as hydrophobic chain using ring opening polymerization of cyclic carbonate. Then, copper-catalyzed Huisgen's 1,3-dipolar cycloaddition of azides with alkynes as the coupling reaction was employed to form reversible light-responsive biodegradable micelles based on poly(carbonate)s hydrophobe. This amphiphilic copolymer can self-assemble into micelles consisting of a hydrophobic core surrounded by a hydrophilic shell. Such core-shell polymeric micelles allow the properties of reversible transition via irradiating with UV and visible light. The micelle formation, reversible light-responsive self-assembly and disassembly of the micelles, cytotoxicity of the copolymer micelles as well as light-responsive model drug release were investigated.

Experimental Section

Materials

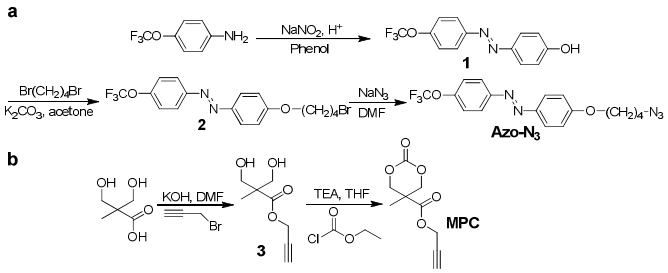
Monomethoxy poly(ethylene glycol) (PEG_{5k}, Mn=5000), 4-(Trifluoromethoxy)-aniline, ethyl chloroformate, 1,4-dibromobutane and 2,2-bis(hydroxyl methyl) propionic acid were obtained from Sigma-Aldrich and used as received. Dichloromethane (DCM), dimethylformamide (DMF), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), triethylamine were dried over calcium hydride for 24 hours at room temperature and distilled under reduced pressure. The thiourea catalyst (TU) was synthesized as reported previously and recrystallized from dry methylene chloride.[26,41] Tetrahydrofuran (THF) was dried by refluxing over a benzophenone-sodium mixture until a deep blue color appeared and distilled. All other reagents and solvents were purchased from Sinopharm Chemical Reagent Co. Ltd, China and used as received.

Characterization

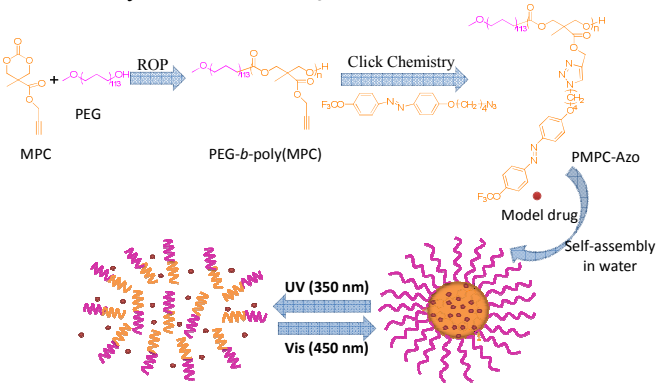
^{1}H and ^{13}C NMR spectra were recorded on an INOVA-400 NMR spectrometer in chloroform-d ($CDCl_3$). Mass spectroscopy analyses were conducted with an LCQ-Advantage (Thermo Finnigan, United States). The number-average molecular weight (Mn) and molecular distribution (polydispersity index, PDI = M_w/M_n) of the polymers were determined at room temperature using a Waters GPC (Waters, USA) equipped with four TSK HXL series polystyrene divinylbenzene gel columns (300 × 7.8 mm). Calibration was established with polystyrene standards from Polymer Laboratories. THF was used as solvent with a flow rate of 1 mL min^{-1}. Transmission electron microscopy (TEM) images were obtained on an H-7000 NAR transmission electron microscope (Hitachi) with a working voltage of 100 kV. A drop of the micelle aqueous solution (0.5 mg mL^{-1}) was deposited onto a 230 mesh copper grid coated with carbon and allowed to dry at room temperature before measurement. The mean size of the micelles was determined by dynamic light scattering (DLS) using a Malvern Nano S instrument (Malvern, UK). Measurements of the solution of micelles (0.5 mg mL^{-1}) were performed at a scattering angle of 90° and at room temperature (25 °C). UV-vis spectra were carried out with a UV-vis Hitachi UV-4100 spectrophotometer and the spectra were collected within the range of 200-800 nm.

Synthesis of 4-Hydroxy-4'-trifluoromethoxyazobenzene (1)

4-(Trifluoromethoxy)aniline (4.5 g, 25.4 mmol) was dissolved in 1.5M aqueous H_2SO_4 (70 mL) and kept in an ice bath at 0 °C. Sodium nitrite (2.35 g, 34.0 mmol) in water (15 mL) was added dropwise to the former solution and stirred for 3 h. Then, the mixture was added dropwise to a solution of sodium carbonate (15.7 g, 147.9 mmol), sodium hydroxide (1.0 g, 25.0 mmol) and phenol (2.5 g, 26.6 mmol) in water (100 mL) at 0 °C and stirred for 3 hours. The resultant mixture was poured into deionized water, and then neutralized with 2M aqueous HCl. The crude product was filtered and washed with a large amount of deionized water, then dried in vacuum oven at 60 °C. Yield: 5.7 g (79.6%).



Scheme 1 Synthesis of Azo-N_3 and MPC



Scheme 2 Synthesis of amphiphilic block copolymer PMPC-Azo and facile access to form reversible light-responsive micelles for the drug package and release.

^{1}H NMR ($CDCl_3$): δ = 6.95-7.94 (m, 8**H**, ArH), 5.30 (s, 1H, ArO**H**);
^{13}C NMR ($CDCl_3$): δ = 158.50, 150.93, 150.45, 146.99, 125.15, 123.99, 121.39, 115.87.

Synthesis of 4-(4-bromobutyloxy)-4'-trifluoromethoxy-azobenzene (2)

To a 100 mL three-neck round bottomed flask was added 1,4-dibromobutane (12.9 g, 60 mmol), K_2CO_3 (2.76 g, 20 mmol), KI (0.12 g, 0.7 mmol) and acetone (30 ml). To a 50 mL pressure equalising dropping funnel, compound **1**(5.6 g, 20 mmol) in 40 mL acetone were added. The solution was added dropwise to the flask. The reaction mixture was magnetically stirred at 60 ^{o}C for 12 hours. The salts formed were filtered and washed with acetone. The solvent of the filtrate was evaporated off by a rotary evaporator. The crude product was washed with very small amount of ethanol and chloroform, then recrystallized from methanol and dried in vacuum oven at 50 ^{o}C. Yield: 5.8 g (69.5%).
^{1}H NMR ($CDCl_3$): δ = 6.99-7.94 (m, 8H, Ar**H**), 4.08-4.11 (t, 2H, C**H$_2$**CH$_2$CH$_2$CH$_2$Br), 3.50-3.53 (t, 2H, CH$_2$CH$_2$CH$_2$C**H$_2$**-Br), 1.96-2.14 (m, 4H, CH$_2$C**H$_2$**C**H$_2$**CH$_2$Br);
^{13}C NMR ($CDCl_3$): δ = 161.74, 151.11, 150.38, 146.99, 125.07, 124.08, 121.27, 114.72, 67.29, 33.41, 29.62, 27.81.

Synthesis of 4-(4-azidebutyloxy)-4'-trifluoromethoxy-azobenzene (Azo-N_3)

Compound **2** (5 g, 12.0 mmol) and sodium azide (1.56 g, 24.0 mmol) were dissolved in DMF (15 mL), and the mixture was stirred at room temperature for 24 hours. The solution was added DCM (100 mL) and reversed extraction with water three times (100 mL × 3). The organic phase was dried with anhydrous magnesium sulfate, filtered and evaporated to dryness. The product was collected as a yellow solid powder. Yield: 4.1 g (90.1%).
^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.01-7.96 (m, 8H, Ar**H**), 4.09-4.12 (t, 2H, C**H$_2$**CH$_2$CH$_2$CH$_2$N$_3$), 3.40-3.43 (t, 2H, CH$_2$CH$_2$CH$_2$C**H$_2$**N$_3$), 1.81-1.98 (m, 4H, CH$_2$C**H$_2$**C**H$_2$**CH$_2$N$_3$);
^{13}C NMR (400 MHz, $CDCl_3$): δ = 161.66, 151.10, 150.39, 147.00, 125.36, 124.06, 121.27, 114.72, 67.63, 51.01, 26.44, 25.57.

Synthesis of 2, 2-Bis(hydroxyl methyl)propionate (3)

In a 250 mL round-bottom flask, 2, 2-bis(hydroxyl methyl)-propionic acid (19.21 g 143.3 mmol), KOH (8.6 g, 153.6 mmol) and DMF (100 mL) was added. The mixture was stirred at 100 ^{o}C for 2 h, then propargyl bromide (18.28 g, 153.6 mmol) was added dropwise over 30 min. After 72 hours of reaction, the reaction mixture was filtered, the solvent was evaporated under reduced pressure, and the residues were dissolved in 200 mL of DCM and extracted three times with saturated salt water (100 mL × 3). The organic phase was concentrated to yield crude product, which was purified by column chromatography (eluent: ethyl acetate/petroleum ether = 1/5, v/v). Yield: 10.68 g (43.4%).
^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.76-4.77 (d, 2H, CHCC**H$_2$**CO), 3.91-3.94 (d, 2H, C**H$_2$**OH), 3.72-3.75 (d, 2H, C**H$_2$**OH), 2.51-2.52 (t, 1H, C**H**CCH$_2$CO), 1.11 (s, 3H, C**H$_3$**CC).
^{13}C NMR (400 MHz, $CDCl_3$): δ = 175.01, 75.20, 67.82, 52.43, 49.29, 16.95.

Synthesis of 5-Methyl-5-propargylxycarbonyl-1,3-dioxane-2-one (MPC)

Compound 3 (8.60 g, 50 mmol) was mixed with ethyl chloroformate (10.86 g, 100 mmol) and THF (100 mL) in a sealed vessel that was purged with nitrogen and cooled in an ice bath. After stirring an hour, triethylamine (10.14 g, 100 mmol) was added dropwise over 30 min under a nitrogen atmosphere. The reaction was allowed to stir for 3 hours, after which it was allowed to warm to 25 ^{o}C and left to stir overnight. The solution was then filtered, evaporated to dryness, and the product was precipitated in a mixture of ethyl acetate and diethyl ether (1:1) as white crystals. Yield: 9.24 g (93.3%).
^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.79-4.80 (d, 2H, CHCC**H$_2$**CO), 4.71-4.74 (d, 2H, **CH$_2$**OCO), 4.22-4.25 (d, 2H, C**H$_2$**OCO), 2.54-2.55 (t, 1H, C**H**CCH$_2$CO), 1.37 (s, 3H, C**H$_3$**CC).
^{13}C NMR (400 MHz, $CDCl_3$): δ = 170.33, 147.22, 76.36, 75.95, 72.71, 53.47, 40.18, 17.39.

Typical procedure for the synthesis of PEG-*b*-poly(MPC)

The ring-opening polymerization of MPC was carried out under an inert atmosphere of nitrogen using standard Schlenk-line techniques. In a typical experiment, MPC (0.594 g, 3 mmol), PEG_{5k}(0.601 g, 0.12 mmol), TU (0.055 g, 0.15 mmol), DBU (0.005 g, 0.03 mmol) and dried DCM (10 mL) were placed in a dried Schlenk tube, fitted with a rubber septum. The solution was further degassed by three freeze-pump-thaw cycles. The resulting mixture was stirred at room temperature for 7 hours, followed by precipitation in ice-cold diethyl ether and centrifugation. The resulting product was collected by filtration and dried in vacuum to yield a white powder. Yield: 1.087 g (91.0%).
^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.71 (m, OC**H$_2$**CCH), 4.25-4.33 (m, OC(O)OC**H$_2$**), 3.62 (m, OC**H$_2$**C**H$_2$**O), 3.36 (s, C**H$_3$**O), 2.53 (s, CH$_2$CC**H**), 2.30 (br s, O**H**), 1.27 (s, C**H$_3$**).
GPC (THF, RI): *M*n (PDI) = 10544 g mol^{-1} (1.11).

Synthesis of PMPC-Azo via ''click'' chemistry

In a Schlenk tube, PEG-*b*-poly(MPC) (302.9 mg, propargyl group, 0.74 mmol), Azo-N_3 (289.2 mg, 0.74 mmol), sodium ascorbate (17.1 mg, 0.074 mmol), and DMF (4 mL) were introduced. The tube was fitted with a rubber septum. The solution was further degassed using three freeze-pump-thaw cycles. A DMF solution of copper sulfate (10.55 mg, 0.037 mmol) was then added in the Schlenk tube. The solution was stirred at room temperature for 24 hours. The crude material

Table 1 Molecular characteristics of amphiphiles PEG-*b*-poly(MPC) and the PMPC-Azo

Entry	M_w/M_n [a]	$M_{n,GPC}$ [a]	$M_{n,NMR}$ [b]	cmc [c] (mg mL^{-1})
PEG-*b*-poly(MPC)	1.11	10544	9752	0.0145
PMPC-Azo	1.12	16285	15005	0.0157

[a] Both molecular weight ($M_{n,GPC}$) and the polydispersity (M_w/M_n) of the amphiphiles were determined by GPC. [b] $M_{n,NMR}$ was determined by ^{1}H NMR. [c] cmc: the critical micellar concentration of the amphiphiles was determined by fluorescence spectroscopy (Fig. 3).

was purified by dialysis (dialysis tubing 3500 MWCO) against deionised water which was renewed regularly. After 3 days, the final product, PMPC-Azo, was obtained by lyophilization. Yield: 450.0 mg (76.0%).
^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.84-7.87 (m, Ar**H**), 7.69 (br s, N_3C**H**C), 7.28-7.30 (d, Ar**H**), 6.92-6.94 (d, Ar**H**), 5.24 (s, C(O)OC$\mathbf{H_2}$), 4.43 (m, NC$\mathbf{H_2}$), 4.24 (m, OC(O)OC$\mathbf{H_2}$), 4.02 (m，$CH_2C\mathbf{H_2}OAr$), 3.64 (m, OC$\mathbf{H_2}C\mathbf{H_2}$O), 3.38 (s, C$\mathbf{H_3}$O), 2.11 (m, $NCH_2C\mathbf{H_2}CH_2CH_2$), 1.82 (m, $NCH_2CH_2C\mathbf{H_2}CH_2$), 1.20 (s, C$\mathbf{H_3}$).
GPC (THF, RI): *M*n (PDI) = 16285 g mol^{-1} (1.12).

Preparation of micelles

Micelles of PEG-*b*-poly(MPC) and PMPC-Azo were prepared by a dialysis method. 10.0 mg copolymer was dissolved in DMF (2 mL), then DI water (20 mL) was slowly added under vigorous stirring. After vigorous stirring for another 2 h at room temperature, the micelles were obtained and further dialyzed against DI water for 24 h to remove DMF (MWCO of 1000 Da). The final polymer concentration was adjusted by adding DI water to 0.5 mg/mL.

Fluorescence measurement of the critical micellar concentration

The critical micellae concentrations (CMC) of PEG-*b*-poly(MPC) and PMPC-Azo amphiphiles was determined by a dye solubilization method using Nile Red (NR) as a probe molecule. NR in THF (0.1 mg/mL, 30 μL) was added to a glass vial via a microsyringe. After THF was evaporated, a micellar solution (2 mL) was added. The concentration of the micellar solution was varied from 0.1 to 5 × 10^{-4} mg/mL. Then the solution was stirred for 5 h. The fluorescence measurements were taken at an excitation wavelength of 550 nm and the emission was monitored from 570 nm to 750 nm.

Light-responsive release and re-encapsulation of NR

NR was selected as hydrophobic model drug to be encapsulated into the PMPC-AZO micelles and fluorescence spectroscopy was used to investigate the release and re-encapsulation behaviours of the micelles. NR in THF (0.1 mg/mL, 30 μL) was added to a glass vial via a microsyringe. After THF was evaporated, a 0.5 mg/mL micellar solution (4 mL) was added. Then the solution was stirred for 5 h. After the encapsulation process, the fluorescence spectrum of the micelles was recorded immediately. The micelles were then exposed to 365 nm UV light for 15 min and subsequently irradiated with 450 nm visible light for 80 min. Fluorescence measurements were taken at an excitation wavelength of 550 nm and the emission monitored from 570 to 750 nm.

In vitro cytotoxicity assay

The HeLa cells were used for studying the cytotoxicity of the micelles. They were seeded in a 96-well plate at the density of 9.6 × 10^3 cells/well and incubated in DMEM at 37 °C in 5% CO_2 for 24 h. Then, the medium was removed and replaced with 200 μL polymer micelles. The aggregate concentrations of each formulation were prepared by serial dilution with DMEM medium. After treatment for 24 h, 20 μL of fresh medium containing 10% of MTT of 5 mg/mL stock was replaced to each well. The plates were incubate d for 4 h, and then 230 μL of DMSO was added to each well to dissolve intracellular MTT formazan crystals, followed by absorbance at 490 nm using a microplate reader (Varioskan Flash, Thermo Scientific). Experiments were done in triplicate.

Results and discussion

Synthesis and characterization of amphiphile PMPC-Azo

The monomers of Azo-N_3 and MPC were synthesized according the design route, which was showed in Scheme 1. Amphiphiles PMPC-Azo was prepared via a two-step method (Scheme 2). Firstly, the PEG-*b*-poly(MPC) block copolymer was synthesized through the ROP of MPC with PEG_{5k} as a macroinitiator. The ^{1}H NMR spectrum of PEG-*b*-poly(MPC) in $CDCl_3$ is shown in Fig. 1C. The degree of polymerization (DP) of the polycarbonate backbone was determined to be 24 by comparing the integrals of peaks at δ = 3.36 (CH_3O–, methyl protons of poly(ethylene glycol) end group) with δ = 4.28 (–$C(O)OCH_2CCH_2O$–, the methylene protons of the carbonate units), which closed to the theoretical value (Table 1). GPC analysis showed that the obtained copolymer had a narrow polydispersity index (PDI) of 1.11 and a *M*n of 10544 g moL^{-1}, which was in agreement with that determined by ^{1}H NMR end group analysis.
Secondly, the modification of PEG-*b*-poly(MPC) to prepare amphiphiles PMPC-Azo was performed in DMF with $CuSO_4$/sodium ascorbate as catalytic system at room temperature for 24 hours via click reaction (Scheme 2). The

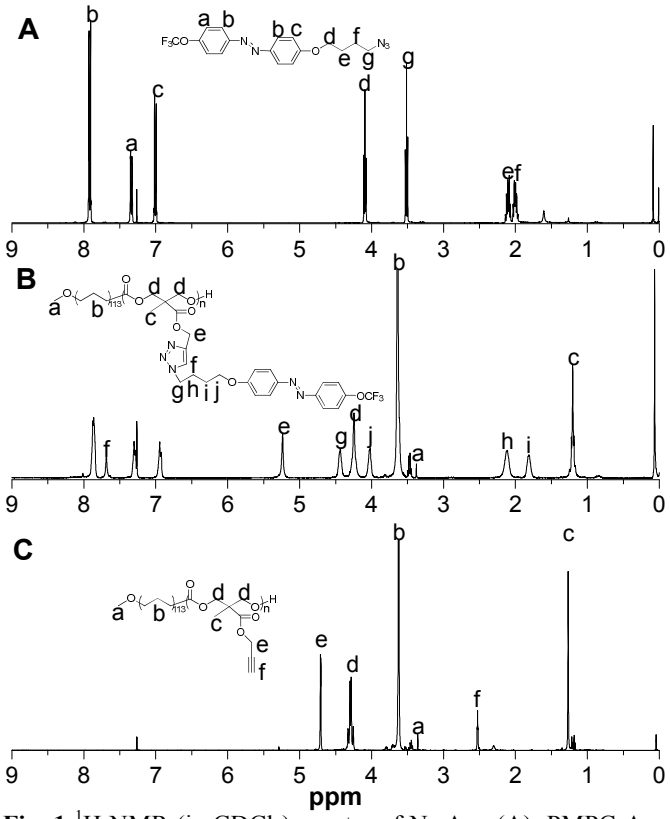


Fig. 1 ^{1}H NMR (in $CDCl_3$) spectra of N_3-Azo (A), PMPC-Azo (B) and PEG-*b*-poly(MPC) (C)

crude copolymer was purified by dialysis in order to remove the copper salt. Fig. 1 compares the ^{1}H NMR spectra of Azo-N_3, PMPC-Azo and PEG-*b*-poly(MPC). The signal at δ=2.54 in Fig. 1C was assigned to the protons of the alkynyl of PEG-*b*-poly(MPC), but it disappeared in the Fig. 1B after click chemistry modification, indicating that the propargyl group of PEG-*b*-poly(MPC) have been completely grafted with Azo-N_3. Compared to Fig. 1A and Fig. 1C, Fig. 1B revealed that signals assignable to Azo-N_3 were detected at δ 1.75–2.15, 6.90–7.34 and 7.87, while signals at δ 1.22, 3.37, 3.65 and 4.26 owing to PEG-*b*-poly(MPC) were also in the amphiphiles PMPC-Azo. Moreover, the weak signal at δ 7.69, assigned to the proton of the triazole ring, evidenced the attachment of Azo-N_3 branches to the polyester backbone. GPC result revealed that PMPC-Azo copolymer had a narrow PDI of 1.12 and an *M*n of 16285 g mol^{-1}, close to that calculated by end group analysis from ^{1}H NMR (Table 1). GPC trace of the graft copolymer showed a slight shift to lower retention time while maintaining narrow distributions with dispersities similar to that of the unmodified copolymer PEG-*b*-poly(MPC) (Fig. S6). It is evident that PMPC-Azo graft copolymer can be readily prepared from propargyl–functionalized polycarbonate via the copper catalyzed azide-alkyne cycloaddition (CuAAC) click reaction.

Self-assembling behavior of amphiphilic copolymers

The amphiphilic copolymers PEG-*b*-poly(MPC) and PMPC-Azo self-assembled into hydrophobic cored micelles stabilized with hydrophilic PEG coronae (Scheme 2), and their self-assembly behaviors were investigated in detail by means of fluorescence spectroscopy, DLS and TEM. Using hydrophobic Nile Red as a probe, fluorescence spectroscopy can conveniently monitor the micellar self-assembly and determine the critical micellar concentration (CMC) of the amphiphiles.[24,41] As shown in Fig. 2, the emission fluorescence intensity gradually increased with increasing amphiphile concentration, suggesting the spontaneous self-assembly of micelles. PMPC-Azo showed a similar CMC value to PEG-*b*-poly(MPC) (0.0157 mg mL^{-1} vs. 0.0145 mg mL^{-1}), suggesting that the self-assembled micelles are thermodynamically stable in aqueous solution. Those values are consistent with literature data reported for graft copolymers.[24,35,41] The size and morphology of the self-assembled micelles were then measured by DLS (Fig. 3A and B) and TEM (Fig. 4A and B), respectively. From TEM images, spherical morphology was observed with an average size of 30 nm for PEG-*b*-poly(MPC)

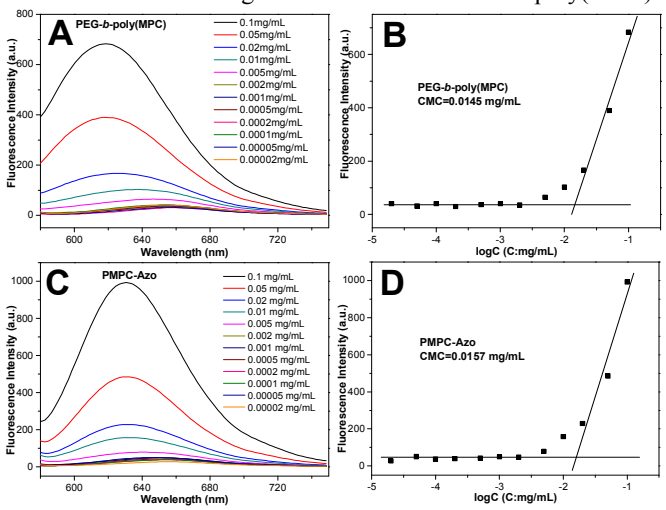


Fig. 2 Fluorescence emission spectra of Nile Red in PEG-*b*-poly(MPC) or PMPC-Azo of varying concentrations and their relevant emission intensity at 630 nm versus the log of concentration

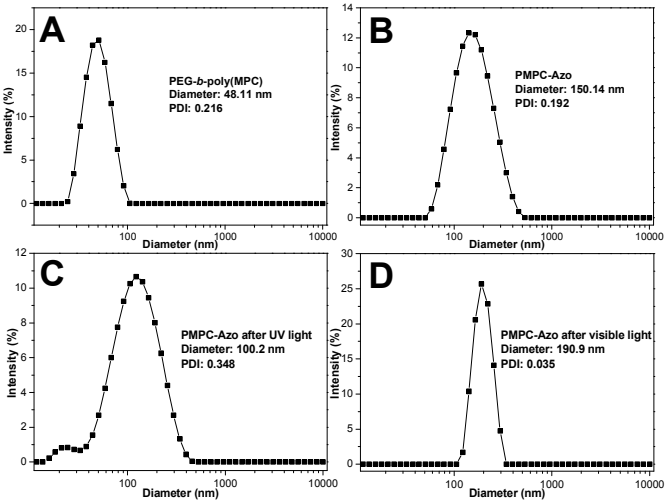


Fig. 3 Mean size distributions of PEG-*b*-poly(MPC) micelles (A) and PMPC-Azo micelles (B) determined by DLS

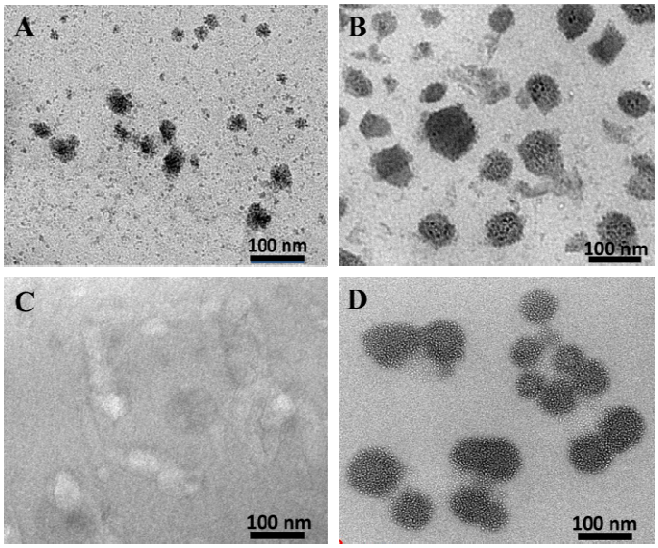


Fig. 4 TEM photographs of the micelles: PEG-*b*-poly(MPC) (A), PMPC-Azo (B), PMPC-Azo after 15 min of 365 nm irradiation (C), subsequent 80 min of 450 nm irradiation (D)

micelles and an average size of 80 nm for PMPC-Azo micelles. Despite having a common spherical morphology, PMPC-Azo micelles have a bigger DLS-determined diameter than PEG-*b*-poly(MPC) micelles, which can be attributed to expanded hydrophobic nucleus. These results demonstrate that the amphiphiles PMPC-Azo self-assembled in aqueous solution into spherical micelles with about 150 nm in size, which had an Azo core stabilized by a PEG corona.

Photo-induced reversible self-assembly and disassembly

In order to investigate photo-responsive self-assembly and disassembly behaviors of the micelles, the PMPC-Azo micelles were evidenced by time-resolved UV-vis spectroscopy (Fig. 5). DLS and TEM measurements of the samples were then conducted and the images are shown in Fig.3 and Fig. 4. Without irradiation, the amphiphile PMPC-Azo could form spherical micelles with a diameter of about 150 nm in solution (Fig. 3B) and about 80 nm in dry state (Fig. 4B). The diameter determined by TEM were smaller than those determined by DLS analysis, which was probably due to the shrinkage of the PEG shell upon drying.[38,42] Upon irradiation with 365 nm UV light for 20 min, the diameter of the micelles became smaller and the polydispersity became bigger (Fig. 3C). At the same time, the spherical micelles were disassembled as shown in Fig. 4C. This phenomenon was due to change the hydrophilic-hydrophobic balance of PMPC-Azo since the isomerization of *trans*-azobenzene into cis-azobenzene under 365 nm UV irradiation.[15,20,23] Meanwhile, UV-vis spectroscopy was also employed to monitor this irradiation process (Fig. 5A). The intensity of the characteristic absorption peak of π–π^* transition of *trans*-azobenzene at around 340 nm gradually decreased with the increased irradiation time and then did not change. In addition, the absorption band at 440 nm which is ascribed to n-π^* transition of *cis*-azobenzene became gradually stronger. These results suggested the completion of isomerization of *trans*-azobenzene into *cis*-azobenzene within 15 minutes. As mentioned in the Introduction, the *cis* form of azobenzene is more polar than the *trans* form, resulting the destroy of the hydrophilic–hydrophobic balance of PMPC-Azo. As a result, the micelles disassembled.

After further irradiating the sample with visible light of 450 nm for 80 min, the copolymer formed micelles with an diameter of about 190 nm and a polydispersity of 0.035 (Fig. 3D and Fig. 4D). This phenomenon occurred because of the isomerization of *cis*-azobenzene into *trans*-azobenzene. Fig. 5B showed the changes of the corresponding UV-vis spectrum for the sample in the visible light irradiation process. It can be seen that the absorption band at around 340 nm which corresponds to *trans*-azobenzene showed regularly decrease in the n–π* absorption of *cis*-azobenzene, suggesting the isomerization of *cis*-azobenzene into *trans*-azobenzene.[17,19,21] Due to recover the hydrophilic–hydrophobic balance of PMPC-Azo, the micelles reassembled after 80 min of 450 nm irradiation. Compared the Fig. 5A and Fig. 5B, after irradiating UV light and visible light, the intensity of absorption at 340 nm and 450 nm was almost as

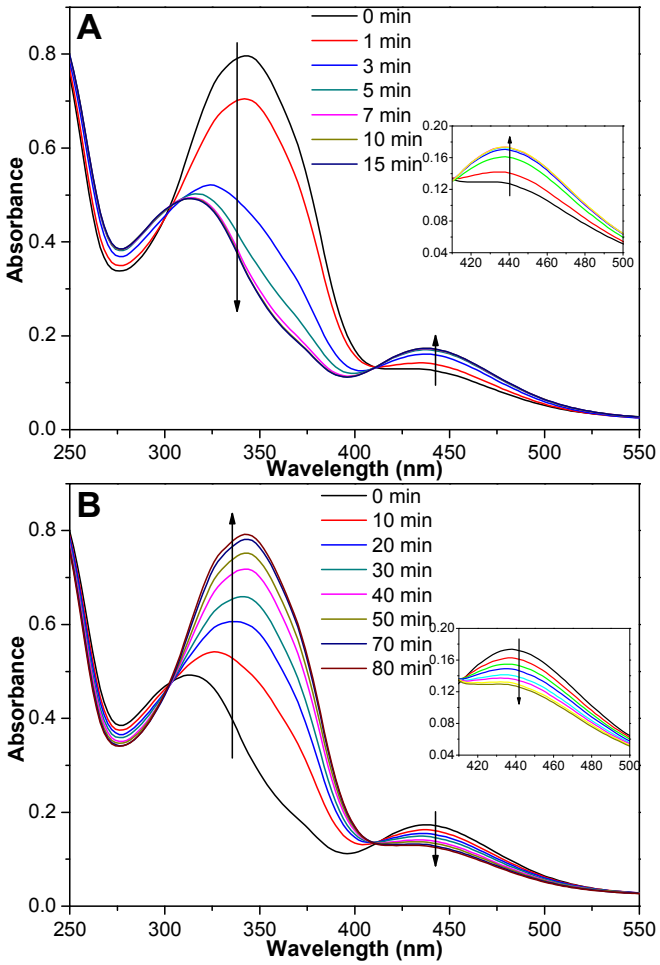


Fig. 5 UV-vis spectra of the PMPC-Azo micelles: (A) 365 nm UV light irradiation induced *trans*-to-*cis* transition, and (B) 450 nm visible light irradiation induced *cis*-to-*trans* transition

same as the original sample. These results indicated that the PMPC micelles could undergo photo-induced reversible self-assembly and disassembly. In order to further confirm completely reversible process, the PMPC-Azo micelles were irradiated with alternative UV and visible light (Fig. S6). It can be seen that the photo-isomerization of azobenzene groups in the PMPC-Azo could take place reversibly for many times. After three cycles, the characteristic absorption peak of π–π* transition of *trans*-azobenzene at 340 nm was still the same as the original micelles.

Model drug release and reload of NR-loaded micelles

Nile Red (NR), as a hydrophobic model drug, was used to demonstrate the concept of photo-triggered drug release, because the fluorescence intensity of NR is known to increase substantially in hydrophobic environments such as the interior of micelles.[42] After loading of NR, the fluorescence spectrometry of the samples was carried out immediately. As shown in Fig 6, the NR-loaded micelles of PMPC-Azo exhibited a photo-triggered drug release profile. During the 15 minutes of 365 nm irradiation, the emission fluorescence intensity of NR gradually decreased with the increase of irradiation time, indicating that the model drug NR was released into water from the disintegration of micelles (Fig.6A). By comparing fluorescence emission intensities at 613 nm, we find that 20% and 82% of drug molecules were released into water after irradiation with UV light for 1 min and 15 min, respectively. This phenomenon is attributed to the isomerization of Azobenzene under UV irradiation resulted in the micelle collapsed. On the contrary, the characteristic peak of NR became much stronger in Fig. 6B when the solution was irradiated by 450 nm visible light. Obviously, some fluorescence dye molecules were re-encapsulated into hydrophobic cores of the micelles. It is noted that the emission peak did not increase anymore after 80 min of irradiation, suggesting not all NR molecules were encapsulated in the process. The reason for the incomplete re-encapsulation might be the rate of micelle formation is higher than that of drug loading.[43] These results proved that the drug release and re-encapsulation behaviors of the PMPC-Azo micelles can be controlled by irradiating with UV or visible light.

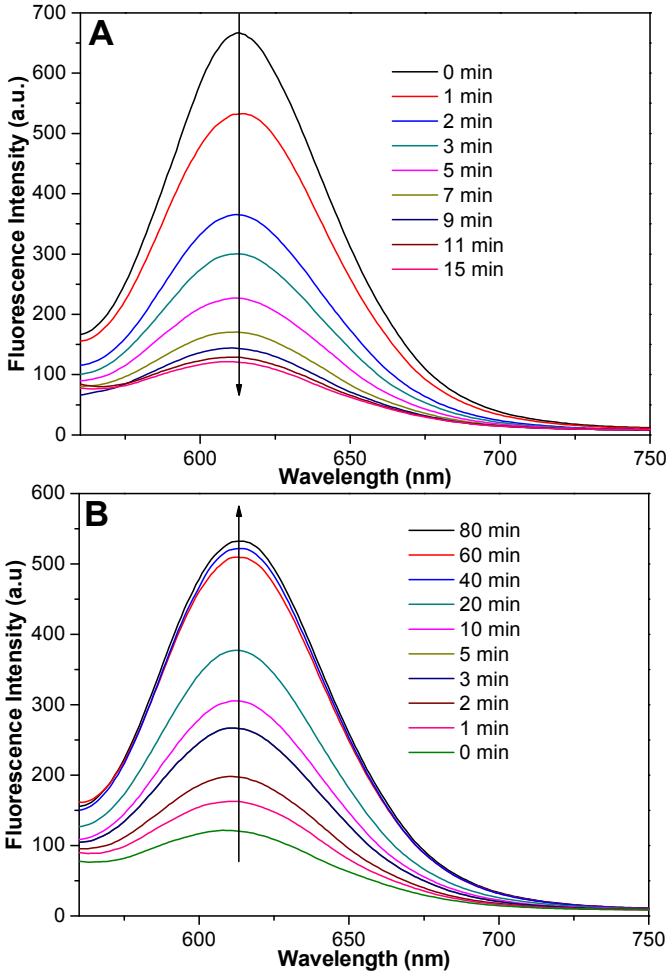


Fig. 6 Fluorescence emission spectra of PMPC-Azo micelles with encapsulated NR after irradiation with: (A) 365 nm UV light for 15 min and (B) subsequent 450 nm visible light for 80 min

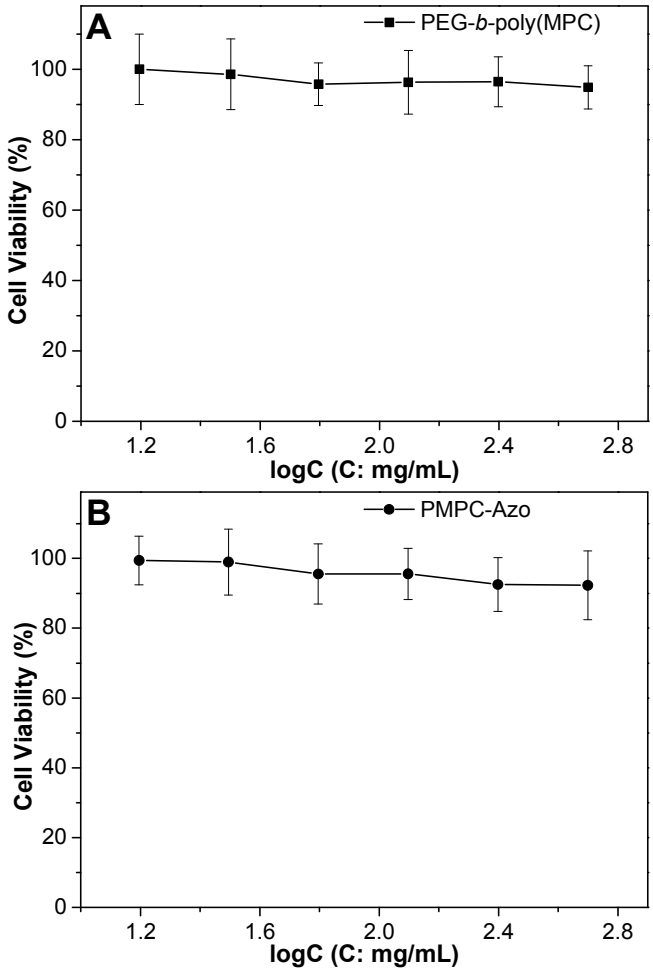


Fig. 7 Cell viability of the HeLa cell line against different concentrations of micelles after being cultured for 48 hours: (A) PEG-*b*-poly(MPC); (B) PMPC-Azo.

Cell cytotoxicity of PMPC-Azo micelles

In order to test cell cytotoxicity of PMPC-Azo micelles, MTT assay was used to evaluate the cytotoxicity of the micelles against HeLa cells after 48 hours of culture. As shown in Fig. 7, when the micelles concentration of PMPC-Azo was 500 mg mL^{-1}, the cell viability still remained about 95%, demonstrating the low cytotoxicity. This result can be mainly attributed to the excellent biocompatibility of the PEG and PC. It has been reported that biocompatible nanocarriers with diameter less than 200 nm may avoid the reticuloendothelial system (RES) recognition.[1,2,4] Therefore, the micelles fabricated in this study have potential to be an ideal drug carrier system.

Conclusions

In this paper, we present an Azo-decorated block poly(carbonate) copolymer PMPC-Azo via a convenient click conjugation of photo-responsive azobenzene molecules to propargyl-functionalized poly(carbonate)s. DLS and TEM measurements revealed that the polymer can self-assemble into spherical micelles with an average diameter of 150 nm in aqueous solution. The CMC of the micelles was determined as 0.0157 mg mL^{-1} by fluorescence spectroscopy using Nile Red as a fluorescence probe. Under alternative UV and visible light irradiation, the amphiphile PMPC-Azo carried on a reversible micelle transition in water. Controlled release of model drug NR under 365 nm UV light and re-encapsulation under 450 nm visible light were confirmed by fluorescence spectroscopy. The reversibly light-responsive micelles with excellent biocompatibility, biodegradability and an appropriate size are highly promising as smart carriers for controlled delivery of hydrophobic molecules for biomedical applications.

Acknowledgement

Financial support from Planned Science and Technology Project of Hunan Province (2012WK4017) and the Fundamental Research Funds for Central Universities are gratefully acknowledged.

Notes and references

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1. N. Rapoport, *Prog. Polym. Sci.,* 2007, **32**, 962-990.
2. Y. Bae and K. Kataoka, *Adv. Drug Delivery Rev.*, 2009, **61**, 768-784.
3. J. F. Lovell, T. W. Liu, J. Chen and G. Zheng, *Chem. Rev.*, 2010, **110**, 2839-2857.
4. C. Oerlemans, W. Bult, M. Bos, G. Storm, J. F. W. Nijsen and W. E. Hennink, *Pharm. Res.*, 2010, **27**, 2569-2589.
5. N. Kamaly, Z. Xiao, P. M. Valencia, A. F. Radovic-Moreno and O. C. Farokhzad, *Chem. Soc. Rev.*, 2012, **41**, 2971-3010.
6. J. Z. Du, X. J. Du, C. Q. Mao and J. Wang, *J. Am. Chem. Soc.*, 2011, **133**, 17560-17563.
7. L. Marta, M. G. G. Jose, Q. G. Isabel, G. Alberto and P. Rodrigo, *Macromolecules*, 2011, **44**, 3739-3745.
8. K. K. Venkata, S. F. Antoni, R. Janne and M. Raffaele, *Macromolecules*, 2011, **44**, 4569-4573.
9. Y. L. Cheng, C. L He, C. S. Xiao, J. X. Ding, K. X. Ren, S. J. Yu, X. L. Zhuang and X. S. Chen, *Polym. Chem.*, 2013, **4**, 3851-3858.
10. N. Rapoport, Z. Gao and A. Kennedy, *J. Natl. Cancer Inst.*, 2007, **99**, 1095-1106.
11. Y. Wang, S. Gao, W. H. Ye, H. S. Yoon and Y. Y. Yang, *Nat. Mater.*, 2006, **5**, 791-796.
12. Q. Yan and Y. Zhao, *J. Am. Chem. Soc.*, 2013, **135**, 16300-16303.
13. G. D. G. Bruno, M. J. Alain, D. Joseph and C. D. S. Stefaan, *Langmuir*, 2006, **22**, 5070-5074.
14. C. Wang, Q. S. Chen, Z. Q. Wang and X. Zhang, *Angew. Chem. Int. Ed.*, 2010, **49**, 8612-8615.
15. J. M. Schumers, C. A. Fustin and J. F. Gohy, *Macromol. Rapid Commun.*, 2010, **31**, 1588-1607.
16. Y. Zhao, *Macromolecules*, 2012, **45**, 3647-3657.
17. A. Goulet-Hanssens and C. J. Barrett, *J. Polym. Sci. Polym. Chem.*, 2013, **51**, 3058-3070.
18. S. Swaminathan, J. Garcia-Amoros, A. Fraix, N. Kandoth, S. Sortino and F. M. Raymo, *Chem. Soc. Rev.*, 2014, **43**, 4167-4178.
19. G. Wang, X. Tong and Y. Zhao, *Macromolecules*, 2004, **37**, 8911-8917.
20. S. Kadota, K. Aoki, S. Nagano and T. Seki, *J. Am. Chem. Soc.*, 2005, **127**, 8266-8267.
21. F. D. Jochuma and P. Theato, *Chem. Commun.*, 2010, **46**, 6717-6719.
22. B. Yu, X. Jiang, R. Wang and J. Yin, *Macromolecules*, 2010, **43**, 10457-10465.
23. O. Boissière, D. Han, L. Tremblay and Y. Zhao, *Soft Matter*, 2011, 7, 9410-9415.
24. C. J. Chen, G. Y. Liu, X. S. Liu, D. D. Li and J. Ji, *New J. Chem.*, 2012, **36**, 694-701.
25. E. Blasco, J. L. Serrano, M. Piñol and L. Oriol, *Macromolecules*, 2013, **46**, 5951-5960.
26. W. Chen, H. Yang, R. Wang, R. Cheng, F. Meng, W. Wei and Z. Zhong, *Macromolecules*, 2010, **43**, 201-207.
27. W. Su, X. Luo, H. Wang, L. Li, J. Feng, X. Zhang and R. Zhuo, *Macromol. Rapid Commun.*, 2011, **32**, 390-396.
28. J. A. Edward, M. K. Kiesewetter, H. Kim, J. C. A. Flanagan, J. L. Hedrick and R. M. Waymouth, *Biomacromolecules*, 2012, **13**, 2483-2489.
29. J. Feng, R. X. Zhuo and X. Z. Zhang, *Prog. Polym. Sci.*, 2012, **37**, 211-236.
30. S. Tempelaar, L. Mespouille, O. Coulembier, P. Dubois and A. P. Dove, *Chem. Soc. Rev.*, 2013, **42**, 1312-1336.

31. Y. Yamamoto, Y. Nagasaki, Y. Kato, Y. Sugiyama and K. Kataoka, *J. Controlled Release*, 2001, **77**, 27-38.
32. R. Duncan, *Nat. Rev. Drug. Discov.*, 2003, **2**, 347-360.
33. R. B. Greenwald, Y. H. Choe, J. McGuire and C. D. Conover, *Adv. Drug. Deliv. Rev.*, 2003, **55**, 217-250.
34. P. F. Gou, W. P. Zhu and Z. Q. Shen, *Biomacromolecules*, 2010, **11**, 934-943.
35. F. Coumes, V. Darcos, D. Domurado, S. Li and J. Coudane, *Polym. Chem.*, 2013, **4**, 3705-3713.
36. R. Yang, F. Meng, S. Ma, F. Huang, H. Liu and Z. Zhong, *Biomacromolecules*, 2011, **12**, 3047-3055.
37. V. Truong, I. Blakey and A. K. Whittaker, *Biomacromolecules*, 2012, **13**, 4012-4021.
38. W. Chen, F. Meng, F. Li, S. J. Ji and Z. Zhong, *Biomacromolecules*, 2009, **10**, 1727-1735.
39. W. Chen, Y. Zou, J. Jia, F. Meng, R. Cheng, C. Deng, J. Feijen and Z. Zhong, *Macromolecules*, 2013, 46, 699-707.
40. W. Chen, Y. Zou, F. Meng, R. Cheng, C. Deng, J. Feijen and Z. Zhong, *Biomacromolecules*, 2014, **15**, 900-907.
41. R. C. Pratt, B. G. G. Lohmeijer, D. A. Long, P. N. P. Lundberg, A. P. Dove, H. Li, C. G. Wade, R. M. Waymouth and J. L. Hedrick, *Macromolecules*, 2006, **39**, 7863-7871.
42. L. Sun, B. Zhu, Y. Su and C.M. Dong, *Polym. Chem.*, 2014, **5**, 1605-1613.
43. C.J. Chen, Q. Jin, G.Y. Liu, D.D. Li, J.L. Wang and J. Ji, *Polymer*, 2012, **53**, 3695-3703.