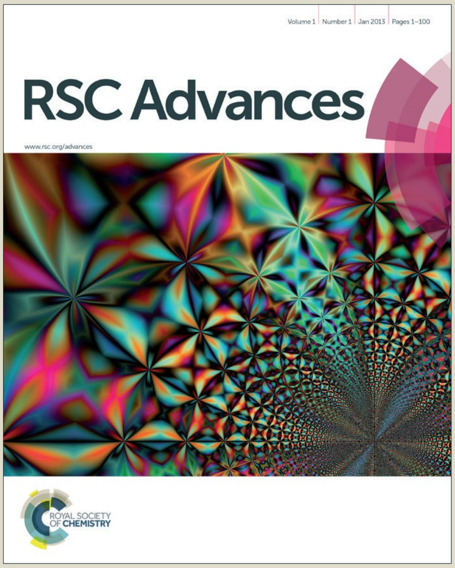
RSC Advances

This article can be cited before page numbers have been issued, to do this please use: T. Yuan, J. Dong, G. Han and G. Wang, *RSC Adv.*, 2016, DOI: 10.1039/C5RA26894J.



View Article Online
DOI: 10.1039/C5RA26894J

RSC Advances

PAPER

Polymer nanoparticles self-assembled from photo-, pH- and thermo-responsive azobenzene-functionalized PDMAEMA

Tingting Yuan, Jie Dong, Guoxiang Han, Guojie Wang*

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

A series of amphiphilic azobenzene-functionalized poly (dimethylaminoethyl methacrylates) have been synthesized by quaternization between [3-(4-(4-bromo-butyloxy)phenyl)-trifluoromethyl]-diazene and the dimethylaminoethyl units of poly(dimethylaminoethyl methacrylate) (PDMAEMA). The azobenzene-functionalized polymers could self-assemble into micellar nanoparticles in water. Upon UV light irradiation, the spherical nanoparticles could only swell a little. Although the loaded Nile Red could not be released from the nanoparticles under UV light irradiation, the fluorescence intensity of Nile Red changed reversibly when altering UV and visible light irradiation. At pH 3, the nanoparticles could be swelled greatly and the loaded Nile Red could be released completely. At pH 10, the nanoparticles could be shrunk and the loaded Nile Red could be released a little. At high temperature, the nanoparticles could be shrunk, where little of Nile Red could be released from the nanoparticles, while the fluorescence intensity of Nile Red could change reversibly during the heating and cooling process.

1. Introduction

In the past few decades, stimuli-responsive polymers that respond to external stimuli such as light,[1] temperature,[2] pH value[3] and enzyme[4] have attracted significant attention in material science and technology due to their applications in developing smart materials.[5] Among them, stimuli-responsive amphiphilic polymers which can self-assemble into nanoparticles in aqueous media draw a lot of attention because of their potential applications in catalysis,[6] nanoreactors,[7] logic gates,[8] and drug delivery.[9] Zhao's group designed a micellar system that could be dissociated upon UV or near-infrared irradiation based on a diblock copolymer comprising hydrophilic PEO and hydrophobic PNBM.[10] Ren et al. reported a pH-responsive dendritic star-block copolymer PCL-b-PDMAEMA which could self-assemble into complex micelles with bimodal size distribution in aqueous solution.[11] Shi et al. prepared temperature-responsive multilayered micelles formed from the complexation of PNIPAM-b-P4VP block-copolymer and PS-b-PAA core-shell micelles.[12] Most of the stimuli-responsive nanoparticles reported so far are focused on being responsive to a single stimulus. Multiple stimuli-responsive systems, which can achieve more functionalities and be modulated through more parameters, may present many intriguing possibilities.[13,14] We have ever reported multiple responsive micellar nanoparticles self-assembled from polymers comprised of photo-responsive pyrene-, perylene- and nitrobenzyl-containing polymethacrylate and temperature/pH-responsive poly(dimethylaminoethyl methacrylate) (PDMAEMA).[15-19] Photo and pH responsive polymer nanoparticles self-assembled from azobenzene and pyrene functionalized polymers based on poly(acrylic acid) (PAA) have also been explored.[20,21] Here we report a photo-, pH- and thermo-responsive polymer nanoparticle self-assembled from azobenzene-functionalized PDMAEMA.

Poly(2-(N, N-dimethylamino)ethyl methacrylate) (PDMAEMA) is a pH- and thermo-responsive polymer.[22] PDMAEMA exhibits pH-responsive behavior because of the protonation/deprotonation of amine groups in the polymer, which is partially charged at neutral pH. In addition, PDMAEMA also exhibits temperature-responsive behavior because of the H-bonding interactions between the polymer and water at low temperature (hydrophilic) and the hydrophobic interactions between N,N-dimethylaminoethyl groups at high temperature (hydrophobic).[23] It is well known that azobenzene undergoes trans to cis isomerization under UV irradiation resulting in a shape change accompanied by an increase in the dipole moment of the molecule.[24-26] It is noted that light is a clean and highly efficient stimulation source which can be triggered from outside of the system and controlled both spatially and temporally with great ease and convenience.[27] To achieve the triple-stimuli sensitive polymer, light-sensitive azobenzene chromophore was incorporated into temperature/pH sensitive polymer (PDMAEMA) through quaternization with the dimethylaminoethyl unit. The

---

*School of Materials Science and Engineering, University of Science and Technology Beijing, Beijing 100083, China. *E-mail: guojie.wang@mater.ustb.edu.cn; phone: 86-10-62333619*

† Electronic Supplementary Information (ESI) available: ^{1}H NMR, GPC, UV/vis absorption spectra and fluorescence spectra See DOI:10.1039/c000000x

View Article Online
DOI: 10.1039/C5RA26894J

synthesized azobenzene-functionalized PDMAEMA could self-assemble into nanoparticles in water, the morphological changes of which under the stimulation were revealed by DLS and TEM. Moreover, the properties of the loaded Nile Red, a hydrophobic dye, in the polymer nanoparticles were also explored under the stimulation of photo, pH and temperature by absorption and fluorescence spectroscopy.

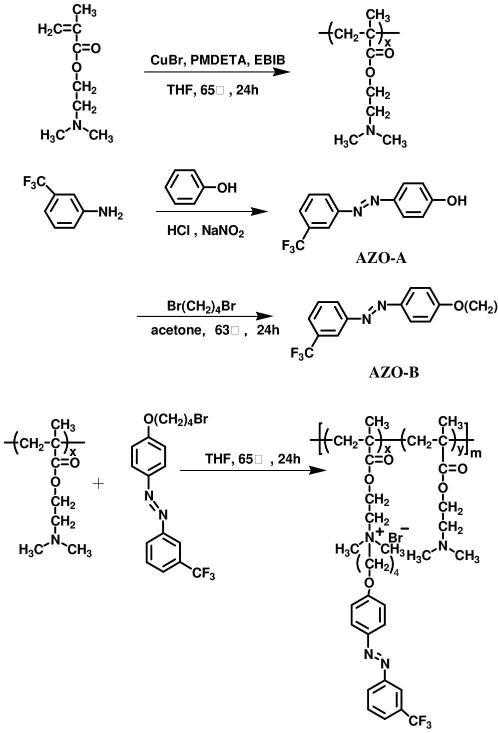
2. Experimental

2.1 Materials

2-(Dimethylamino) ethy methacrylate (99%), 3-(Trifluoromethyl) aniline, ethyl α-bromoisobutyrate (98%), copper bromide (98%), 1,1,4,7,10,10-hexamethyltriethylenetetramine (97%), Phenol from Aldrich and the conventional reagents were used as received. All atmosphere sensitive reactions were done under nitrogen. Deionized water with a conductance below 10^{-6} S cm^{-1} was used in the experiments.

2.2 Polymer Synthesis

Azobenzene-functionalized PDMAEMA was prepared according to the reported procedures.[15,20] The synthetic route is shown in Scheme 1.



Scheme 1. Synthetic route of the azobenzene-functionalized polymer PDMAEMA-AZO

PDMAEMA was synthesized via atom transfer radical polymerization (ATRP). In a typical experiment, a 10 ml Schlenk flask dried under vacuum were charged with CuBr (0.076 g, 5.3×10^{-4} mol), 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA, 0.144 g, 5.3×10^{-4} mol), anhydrous tetrahydrofuran (THF, 8 mL), ethy 2-bromoisobutyrate (EBIB, 0.065 mL, 5.3×10^{-4} mol) and dimethylaminoethyl methacrylate (DMAEMA, 8.334 g, 5.3×10^{-2} mol) under a N_2 atmosphere with a stirrer. The reaction mixture was deoxygenated more than three times using freeze-pump-thaw cycles and sealed. Then the flask was placed in an oil bath at 70 °C for 12 h. The solution was diluted with dichloromethane and passed through an alumina column to remove the copper salts. The polymer was precipitated twice from an excess of hexane, filtered and dried at 40 °C under vacuum for 48 h. Yield : 72% ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.1 (t, 2H, $OCH_2CH_2N(CH_3)_2$), 2.6 (t, 2H, $OCH_2CH_2N(CH_3)_2$), 2.3 (s, 6H, $OCH_2CH_2N(CH_3)_2$), 2.1-1.6 (broad, 2H, aliphatic main chain), 1.3-0.7 (broad, 3H CH_3 main chain). GPC: Mn= 2.0×10^4 g mol^{-1}, PDI = 1.08.

Synthesis of 4-(3-trifluoromethylphenylazo) phenol (AZO-A)： 3-(Trifluoromethyl) aniline (3.22 g, 20 mmol) was dissolved in an aqueous solution of sodium nitrite (1.39 g in 7mL of deionized water). The obtained solution was cooled to 0-5 °C and then hydrochloric acid (6 mL) in 12 mL deionized water was added slowly with stirring in ice bath. After a clear solution of the diazonium salt was formed, the solution was added dropwise into a solution of phenol (1.88 g, 20 mmol), sodium hydroxide (1.2 g, 30 mmol) and deionized water (10 mL) at 0-5 °C. The mixture was vigorously stirred for 30 min in ice bath and hydrochloric acid aqueous solution was added to adjust the pH to 5-7. The solution was kept for 30 min, and then the solid was filtered and dried under vacuum at room temperature. After recrystallized from petroleum ether, the final yellow powder was obtained. Yield: 70%. m.p. 112 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.120 (s, 1H, Ar*H*), 8.028-8.047 (d, 1H, Ar*H*), 7.883-7.905 (d, 2H, Ar*H*), 7.665-7.684 (d, 1H, Ar*H*), 7.587-7.626 (t, 1H, Ar*H*), 6.936-6.958 (d, 2H, Ar*H*), 5.218(s, 1H, O*H*).

Synthesis of [3-(4-(4-bromo-butyloxy)phenyl)-trifluoromethyl]-diazene (AZO-B): 4-(3-Trifluoromethylphenylazo) phenol (0.532 g, 2 mmol) was dissolved in acetone (50 mL), in which 1, 4-dibromobutane (2.16 g, 10 mmol), K2CO3 (2.73 g, 20 mmol) and a catalytic amount of KI (0.28 g) were added. The reaction mixture was stirred at 65 °C for 24 h. Then the reaction mixture was cooled to room temperature and poured into water (1.5 L). The precipitate was collected by filtration. Purification of the crude material was achieved by twice recrystallization from n-hexane and then the orange crystal was obtained. Yield: 59%. m.p. 38 °C. 1H NMR (400 MHz, CDCl3) δ (ppm): 8.122 (s, 1H, ArH), 8.029-8.048 (d, 1H, ArH), 7.919-7.939 (d, 2H, Ar*H*), 7.659-7.678 (d, 1H, Ar*H*), 7.584-7.623 (t, 1H, Ar*H*), 7.023-7.109 (d, 2H, Ar*H*), 4.100-4.146 (t, 2H, $OCH_2CH_2CH_2CH_2Br$), 3.725-3.963 (t, 2H, $OCH_2CH_2CH_2CH_2Br$), 1.978-2.051 (m, 2H, $OCH_2CH_2CH_2CH_2Br$), 1.830-1.905 (m, 2H, $OCH_2CH_2CH_2CH_2Br$).

Synthesis of azobenzene-functionalized PDMAEMA (PDMAEMA-AZO) with DF of 4%: The synthesized PDMAEMA (0.314 g, 2 mmol) and AZO-B (0.16 g, 0.4 mmol) were dissolved in THF (20 ml) and then stirred for 24 h at 65 °C. Then the polymer PDMAEMA-AZO was precipitated from an excess of hexane, filtered and dried at 40°C under vacuum for 48 h. Yield: 73%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.017-8.113

(Ar*H*), 4.385-4.415 ($\mathrm{O}\mathit{CH_2}\mathrm{CH_2CH_2CH_2}$), 4.023 ($\mathit{CH_2}\mathrm{CH_2N(CH_3)_2}$), 3.453 ($\mathrm{OCH_2CH_2CH_2}\mathit{CH_2}$), 2.529 ($\mathrm{CH_2}\mathit{CH_2}\mathrm{N(CH_3)_2}$), 2.250 ($\mathrm{CH_2CH_2N(}\mathit{CH_3}\mathrm{)_2}$), 1.969-2.101 ($\mathrm{OCH_2}\mathit{CH_2CH_2}\mathrm{CH_2}$), 1.790-1.880 (broad, 2H, aliphatic main chain), 0.868-1.395 (broad, 3H, C*H*$_3$ main chain).

Synthesis of PDMAEMA-AZO with DF of 9%: PDMAEMA (0.317 g, 2 mmol) and AZO-B (0.45 g, 1.11 mmol) were dissolved in THF (20 ml) and then stirred for 24 h at 65 °C. The polymer was precipitated twice from an excess of hexane, filtered and dried at 40°C under vacuum for 48 h. Yield: 78%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.240-8.081 (Ar*H*), 4.381-4.415 ($\mathrm{O}\mathit{CH_2}\mathrm{CH_2CH_2CH_2}$), 4.022 ($\mathit{CH_2}\mathrm{CH_2N(CH_3)_2}$), 3.470 ($\mathrm{OCH_2CH_2CH_2}\mathit{CH_2}$), 2.540 ($\mathrm{CH_2}\mathit{CH_2}\mathrm{N(CH_3)_2}$), 2.254 ($\mathrm{CH_2CH_2N(}\mathit{CH_3}\mathrm{)_2}$), 1.977-2.103 ($\mathrm{OCH_2}\mathit{CH_2CH_2}\mathrm{CH_2}$), 1.792-1.883 (broad, 2H, aliphatic main chain), 0.868-1.395 (broad, 3H, C*H*$_3$ main chain).

Synthesis of PDMAEMA-AZO with DF of 17%: PDMAEMA (0.264 g, 1.68 mmol) and AZO-B (0.3 g, 1.11 mmol) were dissolved in THF (20 ml) and then stirred for 24 h at 65 °C. The polymer was precipitated twice from an excess of hexane, filtered and dried at 40°C under vacuum for 48 h. Yield: 65%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.001-8.072 (Ar*H*), 4.381-4.415 ($\mathrm{O}\mathit{CH_2}\mathrm{CH_2CH_2CH_2}$), 4.018 ($\mathit{CH_2}\mathrm{CH_2N(CH_3)_2}$), 3.506 ($\mathrm{OCH_2CH_2CH_2}\mathit{CH_2}$), 2.521 ($\mathrm{CH_2}\mathit{CH_2}\mathrm{N(CH_3)_2}$), 2.241 ($\mathrm{CH_2CH_2N(}\mathit{CH_3}\mathrm{)_2}$), 1.956-2.101 ($\mathrm{OCH_2}\mathit{CH_2CH_2}\mathrm{CH_2}$), 1.794-1.881 (broad, 2H, aliphatic main chain), 0.861-1.224 (broad, 3H, C*H*$_3$ main chain).

2.3 Preparation of Polymer Nanoparticles and Encapsulation of Nile Red[10,19]

Preparation of polymer nanoparticles: The azobenzene-functionalized polymer PDMAEMA-AZO (2 mg) was first dissolved in THF (1 mL). Then suitable amount of water (1 mL) was added dropwise into the solution at a rate about 5-6 drop min^{-1} with stirring to induce the formation of the nanoparticles. After 3 h of stirring, more water (9 mL) was added to quench the nanoparticles. THF was removed by evaporation for 7 days at room temperature.

Preparation of polymer nanoparticles loaded with Nile Red: The azobenzene-functionalized polymer PDMAEMA-AZO (2 mg) and Nile Red (0.2 mg) were dissolved in THF (1 mL); 1 mL of water was then added dropwise into the solution at a rate about 5-6 drop min^{-1} with stirring to induce the formation of nanoparticles and the simultaneous encapsulation of Nile Red. The driving force for encapsulation of Nile red is the hydrophobic interaction between the dye and hydrophobic segments of the azobenzene-functionalized polymer. After 3 h of stirring, more water (9 mL) was added to quench the nanoparticles. THF was removed by evaporation and precipitated NR was filtered by microfiltration (450 nm pore filter). The polymer nanoparticles with different pH were obtained by adding aqueous HCl or NaOH solutions to the above solutions.

2.4 Instrumental Analysis

^{1}H NMR spectra were recorded from $CDCl_3$ solution on a Bruker AM 400 spectrometer. The average molecular weights and molecular weight distributions of the obtained polymers were determined by a gel permeation chromatography (GPC) (Waters 1515) with styragel columns relative to polystyrene standards using tetrahydrofuran (THF) as eluent. Fourier transform infrared (FTIR) spectra were recorded on PerkinElmer spectrophotometer at room temperature. Polymer nanoparticles were examined using a JEM 2010 TEM operating at 100 kV. The samples for TEM observation were prepared by placing 5 μL of nanoparticle solution onto carbon-coated copper TEM grid. Dynamic light scattering (DLS) experiments and zeta-potentials were carried out on the ALV/SP-150 spectrometer equipped with an ALV-5000 multi-digital time correlator and a solid-state laser (ADLS DPY 425II, output power ca. 400 MW at λ = 632.8 nm) as the light source. The fluorescence spectra were recorded on a F-280 fluorescence spectrophotometer. The UV-vis spectra of the samples were recorded by a JASCO V-570 spectrometer. A UV-LED irradiator (LAMPLIC UVEC-4□, λ= 365 nm) and a VIS-LED irradiator (LAMPLIC UVEC-4□, λ= 450 nm) were used to induce the photo-isomerization of azobenzene.

2.5 Degree of Functionalization

The degree of functionalization (DF) of PDMAEMA-AZO was determined by UV-vis spectroscopy. As the molar absorptivity ε (λ_{max}) in the range of 300 to 400 nm of the azobenzene chromophores in the polymer was assumed to be almost the same as that of the corresponding molecule [3-(4-(4-bromo-butyloxy)phenyl)-trifluoromethyl]-diazene (AZO-B), the degree of azobenzene-functionalization was calculated from the absorption intensities of the azobenzene-functionalized polymer and the compound AZO-B in THF.

2.6 Critical Aggregation Concentration

The critical aggregation concentration (CAC) of the azobenzene-functionalized polymer PDMAEMA-AZO was determined by the fluorescent method with Nile Red as a probe. Polymer nanoparticles loaded with Nile Red in aqueous solution were prepared with concentrations ranging from 0.5 mg mL^{-1} to 0.0001 mg mL^{-1}. Then fluorescence measurements were taken at an excitation wavelength of 550 nm and the emission monitored from 560 to 750 nm. The maximum fluorescence intensity emission was plotted against the logarithm of polymer concentration to measure the critical aggregation concentration.

3. Results and Discussion

3.1 Synthesis and Characterization.

The azobenzene-functionalized polymer PDMAEMA-AZO has been successfully synthesized as described in the following and the procedure is shown in Scheme 1. The pH- and thermo-responsive polymer PDMAEMA was first synthesized through atom transfer radical polymerization (ATRP) of the monomer DMAEMA, the number-average molecular weight (M_n) and polydispersity index (PDI) of which were determined to be 2.0 × 10^4 g mol^{-1} and 1.08, respectively, by gel permeation chromatography (GPC), shown in Figure S1. Then [3-(4-(4-bromo-butyloxy)phenyl) -trifluoromethyl]-diazene was synthesized and incorporated into PDMAEMA through

View Article Online
DOI: 10.1039/C5RA26894J

quaternization with the dimethylaminoethyl unit to achieve the azobenzene-functionalized PDMAEMA with DF of 4%, 9% and 17%. The incorporation of trifluoromethyl group into azobenzene was expected to obtain a large polarity change during the process of photo-isomerization from trans to cis.[20]

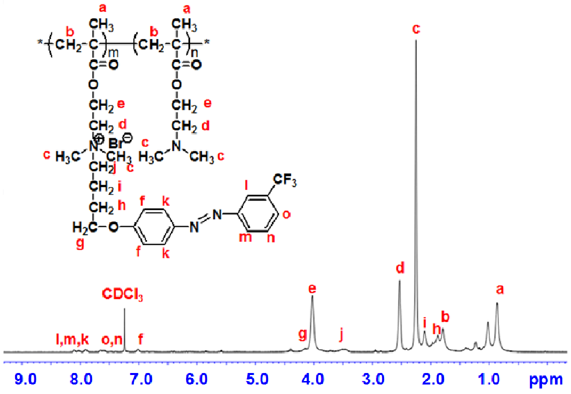


Figure 1. ^{1}H NMR spectrum of PDMAEMA-AZO with DF of 4% in CDCl$_3$

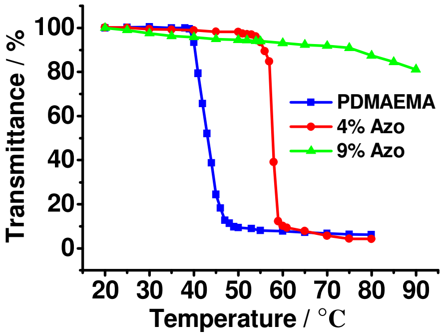


Figure 2. Temperature dependence of optical transmittance at 500 nm for the aqueous solutions of PDMAEMA and PDMAEMA-AZO with DF of 4% and 9%.

Figure 1 shows the typical ^{1}H NMR spectrum of the polymer PDMAEMA-AZO with DF of 4%. The FTIR spectra of PDMAEMA, PDMAEMA-AZO with DF of 4%, 9% and 17% are shown in Figure S3, from which it can be seen that a carbon-nitrogen vibration peak from the ammonium groups at 3420 cm^{-1} increased with the increase of DF. The LCST curves of PDMAEMA and PDMAEMA-AZO with DF of 4% and 9% are shown in Figure 2. The LCST of the synthesized PDMAEMA was measured to be 43°C, while the LCST of the copolymer PDMAEMA-AZO with DF of 4% increased to 57°C and the LCST of the copolymer with DF of 9% was too high to be detectable. The LCST increased with the increase of the degree of functionalization, since the functionalization endowed the polymer with hydrophilic quaternary ammonium groups and the increase of hydrophilicity of the polymer induced the increase of LCST.[28]

3.2 Photo-responsive Properties of PDMAEMA-AZO in THF.

The UV-vis measurements were performed to examine the photoisomerization behavior of the azobenzene-functionalized polymer in THF. As shown in Figure 3, the absorption bands at 352 and 442 nm were ascribed to the π-π* transition of the trans form and n-π* transition of the cis form of the azobenzene group, respectively. Upon UV light irradiation, the absorption band at 352 nm became progressively weaker with the increase of irradiation time, while the absorption band at 442 nm slightly increased, shown in Figure 3a. The changes of the absorption bands induced by UV irradiation were indicative of the photo-isomerization of the trans-azobenzene to the cis-azobenzene. Then the polymeric solution was exposed to visible light of 450 nm, and the opposite change of UV-vis spectra could be observed, shown in Figure 3b, which implied the process of back-conversion from cis to trans form.

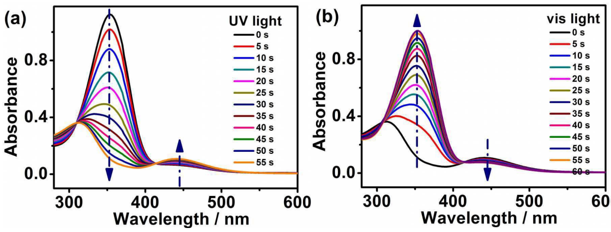


Figure 3. UV-vis spectra of the azobenzene-functionalized polymer (DF, 4%) in THF under irradiation of UV light (365 nm, 5 mW cm^{-2}) (a) and visible light (450 nm, 7 mW cm^{-2}) (b), respectively.

3.3 Self-assembly of PDMAEMA-AZO in Water.

The synthesized azobenzene-functionalized polymers are amphiphilic polymers composed of hydrophobic azobenzene units and hydrophilic unquaternized PDMAEMA and quaternized amine groups. When the azobenzene-functionalized polymers dissolve in water at room temperature and neutral pH, the random copolymers may self-assemble into supramolecular multimolecular micelles,[29,30] where the hydrophobic azobenzene units form the core and the hydrophilic unquaternized PDMAEMA and quaternized amine groups form the shell. As a strong evidence for self-assembly behavior of amphiphilic polymers into nanoparticles, CAC value of PDMAEMA-AZO with DF of 4% in aqueous solution was measured to be 0.09 mg mL^{-1}, shown in Figure 4a. The CAC values of the copolymers with DF of 9% and 17% were measured to be 0.05 and 0.04 mg mL^{-1}, respectively, shown in Figure S4. The CAC values decreased with the increase of the functionalization degree, since the increased hydrophilicity could enhance the thermodynamical stability of the self-assembled nanoaggregates.[31,32] Transmission electron microscopy (TEM) and Dynamic light scattering (DLS) were used to investigate the self-assembled nanostructures of the polymer PDMAEMA-AZO. Figure 4b shows the TEM image of the polymer nanoparticles, from which it can be seen that the core-shell spherical structure with a diameter of approximately 120 nm was formed. Figure 4c shows the size distribution

View Article Online
DOI: 10.1039/C5RA26894J

curve of the polymer nanoparticles obtained from DLS experiment, where the hydrodynamic diameter (D_h) of the polymer nanoparticle was about 125 nm. The TEM images of the polymer assemblies from PDMAEMA-AZO with DF of 9% and 17% are shown in Figure S5 and S6, from which it can be seen that the diameters of the nanoparticles from the two polymers were approximately 120 nm and 130 nm, respectively.

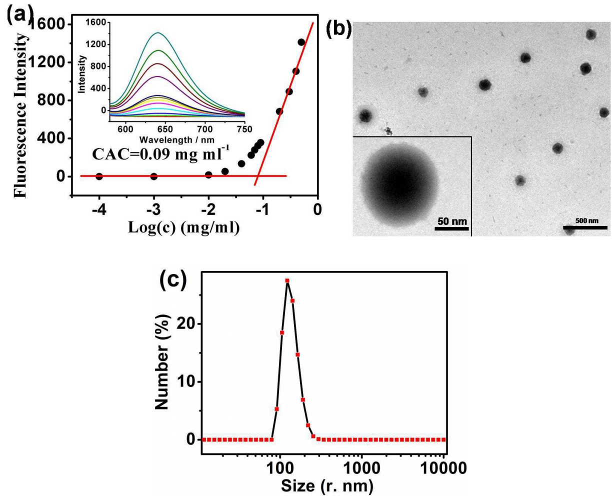


Figure 4. (a) Plot of the maximum fluorescence emission intensity of Nile Red (λ_{ex}=550 nm) vs the log of concentration of the polymer PDMAEMA-AZO (DF, 4%). Inset: Fluorescence emission spectra of Nile Red in aqueous polymer solution with different concentrations of the polymer. (b) and (c) TEM image and DLS curve of the nanoparticles self-assembled from the polymer PDMAEMA-AZO (DF, 4%).

3.4 Photo-responsive Behaviors of Nanoparticles .

To evaluate the photo-responsive properties of the polymer nanoparticles, UV-vis spectrophotometer was employed to monitor the absorption spectra upon irradiation of UV light (365 nm, 5 mW cm^{-2}) and visible light (450 nm, 7 mW cm^{-2}). Figure 5a shows the UV-vis absorption spectra of the polymer nanoparticles self-assembled from the polymer with DF of 4% upon UV light irradiation, from which it can be seen that the intensity of the π-π* transition band at 350 nm decreased while the intensity of the n-π* transition band at 440 nm slightly increased with the increase of UV irradiation time, indicating the isomerization from trans-azobenzene to cis-azobenzene. The photostationary state for the copolymer with DF of 4% was obtained after irradiating for 30 seconds. It is noted that it took more time to reach the balance between cis and trans when the degree of azobenzene functionalization increased. For the copolymers with DF of 9% and 17%, the photosationary time increased to 60s and 80s, respectively (see ESI†). When the polymer solution was subsequently exposed to visible light irradiation, the absorption band of π-π* transition increased and the n-π* transition band decreased, shown in Figure 5b, indicating the reversible isomerization from cis-azobenzene to trans-azobenzene. It should be noted that the absorption bands blue-shifted 2 nm compared with those in THF (shown in Figure 3), which confirmed that the azobenzene units formed H-aggregates in the nanoparticles.[33] Figure 5c shows the TEM image of the nanoparticles after UV light irradiation, where the spherical morphology of polymer nanoparticles could be still observed and the size of the nanoparticles increased to 125 nm. Figure 5d shows the DLS curves of the nanoparticles before and after UV light irradiation and then after visible light irradiation, from which it can be seen that the D_h of nanoparticles increased from 125 nm to 138 nm under UV light irradiation, subsequently, when visible light was applied, the D_h of nanoparticles could be recovered (125 nm). The photo-responsive morphological changes of nanoparticles self-assembled from the polymers with DF of 9% and 17% are shown in Figure S5 and S6, from which it can be seen that the spherical nanoparticles could be also swollen a little after UV light irradiation. Under UV light irradiation, the trans-azobenzene can be isomerized to the cis-azobenzene, resulting in the increase of the polarity of azobenzene. In this study, although the trifluoromethyl-azobenzene with a large polarity change upon UV irradiation was introduced,[20] this polarity change is still insufficient, which can not result in dissociation of the micellar aggregates,[34] where the cis-azobenzene units still exist in the core.

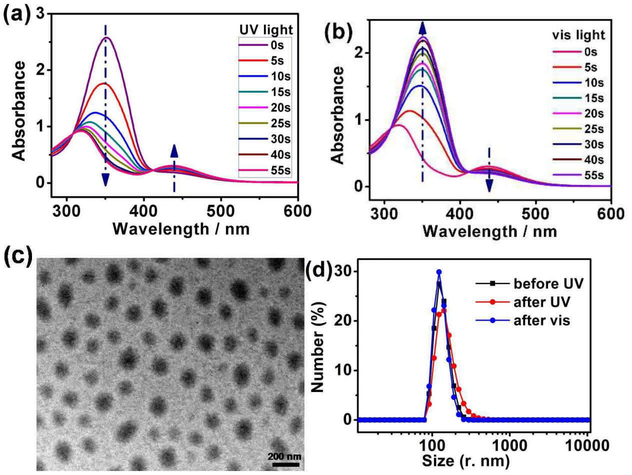


Figure 5. (a) and (b) UV-vis spectra of the nanoparticles from the polymer PDMAEMA-AZO (DF, 4%) under irradiation of UV light (365 nm, 5 mW cm^{-2}) and visible light (450 nm, 7mW cm^{-2}), respectively. (c) TEM image of the nanoparticles after UV irradiation. (d) DLS curves of the nanoparticles before and after UV light irradiation and then after visible light irradiation.

Nile Red, a model hydrophobic dye, is well known for monitoring the formation and disruption of the nanoparticles under stimulation. Figure 6a shows the UV-vis absorption spectral change of the nanoparticle self-assembled from the polymer with DF of 4% loaded with Nile Red exposed to UV

View Article Online
DOI: 10.1039/C5RA26894J

light for various time. Under UV light irradiation, a remarkable decrease of the absorption band at 350 nm was observed accompanied by an increase of the absorbance at 440 nm due to the trans-to-cis photoisomerization of azobenzene, while the absorption band of Nile Red changed little, as indicated no leakage of Nile Red. Figure 6b shows the fluorescence intensity change of loaded Nile Red upon UV irradiation for different time, from which it can be seen that the fluorescence intensity of Nile Red decreased with increasing UV irradiation time since the nanoparticles became more polar after UV irradiation. Figure 6c shows the fluorescence intensity of Nile Red recovered to the initial value when the nanoparticles were illuminated with visible light. Figure S9 shows the UV-vis spectra of the nanoparticles self-assembled from the polymers with DF of 9% and 17% loaded with Nile Red under UV light irradiation, where the photo-isomerization of azobenzene could be observed and no leakage of Nile Red occurred either, whereas the fluorescence intensity of loaded Nile Red changed reversibly when altering UV and visible irradiation (Figure S11). It is known that Nile Red is an uncharged hydrophobic molecular whose fluorescence is strongly influenced by the polarity of its environment, the emission of which can be quenched when the environment becomes more polar.[35,36] The fluorescence change of Nile Red could be resulted from the polarity change of azobenzene in the nanoparticle. Upon UV light irradiation, the azobenzene could be isomerized to the cis form with a higher polarity, which would induce a decrease in emission of Nile Red,[37,38] while the polarity and emission could be recovered when the cis form isomerized to the trans form upon visible irradiation. Although the photo stimulation had little effect on the release of Nile Red loaded in the nanoparticles, the fluorescence intensity of Nile Red could change reversibly when altering UV and visible irradiation. The photo-responsive system may find applications in sensor technology which requires no leakage of the encapsulated substances for preventing environment from pollution and in catalyst system because the change of hydrophobicity might affect the activity of catalysts so as to control the chemical reactions by light.[39]

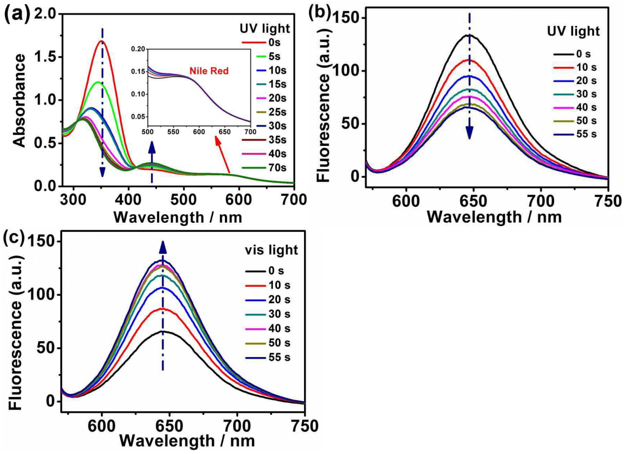


Figure 6. (a) UV-vis spectra of the nanoparticles self-assembled from PDMAEMA-AZO (DF, 4%) loaded with Nile Red under UV light irradiation (365 nm, 5 mW cm^{-2}). (b) and (c) Fluorescence spectra of the nanoparticles loaded with Nile Red under UV light irradiation (365 nm, 5 mW cm^{-2}) and visible light irradiation (450 nm, 7 mW cm^{-2}), respectively.

3.5 pH-responsive Behaviors of Nanoparticles .

It is well-known that PDMAEMA can be protonated in an acidic aqueous solution, which leads to an increase of hydrophilicity, while PDMAEMA chains can be shrunk in alkaline aqueous solution because of the deprotonation of tertiary amine groups.[40] Figure 7a shows the DLS plots of the polymer nanoparticles self-assembled from PDMAEMA-AZO with DF of 4% under acidic and alkaline conditions. Under acidic condition (pH 3), the nanoparticles could be swollen, where the D_h of the nanoparticle increased to 300 nm, much larger than that in a neutral environment, resulted from the protonation of the tertiary amine groups in PDMAEMA segments and the increase of hydrophilicity. Under alkaline condition (pH 10), the nanoparticles were shrunk and then aggregated, where the D_h of the nanoparticles increased to 400 nm. Figure 7b shows the zeta potentials of the polymer nanoparticles were measured to be 0.6, 34.3, 38.3 and 42.8 mV at pH 10, 7, 5 and 3, respectively. The potential increased with the decrease of pH value since more PDMAEMA could be protonated at lower pH. The pH-responsive morphological changes of nanoparticles self-assembled from the polymers with DF of 9% and 17% are shown in Figure S5 and S6, from which it can be seen that the nanoparticles were also swollen under acidic condition and shrunk under alkaline condition.

The controlled release of Nile Red at various pH from polymer nanoparticles were monitored through UV-vis absorption spectroscopy and fluorescence spectroscopy. Figure 7c shows the UV-vis absorption spectra of Nile Red loaded in the polymer nanoparticles self-assembled from PDMAEMA-AZO (DF, 4%) at different pH. At pH 10, the absorbance of Nile Red decreased about 10% compared with the initial value at pH 7, indicating that only some Nile Red could be released from the nanoparticles and most of Nile Red was still encapsulated in the nanoparticles. At pH 5, the absorbance of Nile Red decreased about 75%, indicating that the most Nile Red was released. At pH 3, the loaded Nile Red could be released completely, since the absorbance disappeared. Figure 7d shows the fluorescence change of Nile Red under different pH. At pH 10, the fluorescence intensity of Nile Red at 638 nm decreased about 10%, compared with the intensity at pH 7. At pH 5, the fluorescence intensity of Nile Red decreased about 75% and totally disappeared at pH 3, which were consistent with the results of UV absorbance. Since the polymer nanoparticles could be swelled at acidic condition or under UV light irradiation, the combined stimulation of pH 5 and UV light irradiation could be applied to enhance the release of encapsulated molecules from the nanoparticles. Figure S10 shows that the release could be enhanced indeed upon the combined stimulation compared with those under single stimulation. The controlled release of Nile Red from the nanoparticles self-assembled from the copolymers with DF of 9% and 17% could be also realized

View Article Online
DOI: 10.1039/C5RA26894J

under pH stimulation just like that from the copolymer with DF of 4%. It is noted that the random copolymer system provided a burst release of encapsulated molecules at acidic pH, while a sustained release could be achieved from carboxylic acid functionalized copolymer micelles at basic pH.[41]

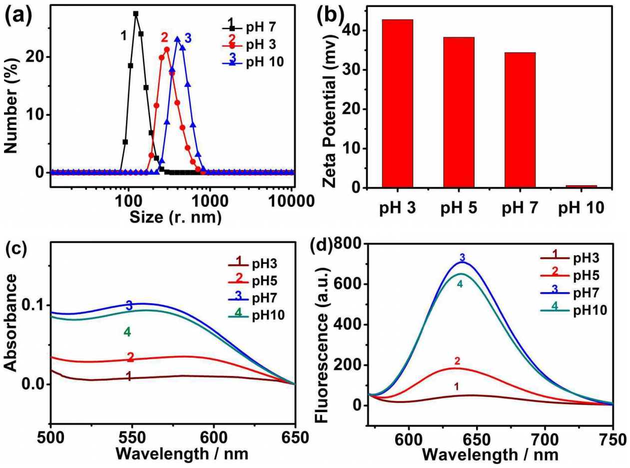


Figure 7. (a) and (b) DLS curves and zeta potentials of the nanoparticles self-assembled from PDMAEMA-AZO with DF of 4% at different pH, respectively. (c) and (d) UV-vis absorption and fluorescence spectra of Nile Red loaded in the nanoparticles at different pH, respectively.

3.6 Thermo-responsive Behaviors of Nanoparticles .

PDMAEMA, as we know, exhibits a reversible thermo-sensitive phase transition in aqueous solution. Below LCST, PDMAEMA is hydrophilic, while it exhibits hydrophobic above LCST since the hydrogen bonding between the polymer chains and water will be broken at high temperature. Figure 8a shows the TEM image of the nanoparticles self-assembled from the copolymer with DF of 4% when heated to 60 °C (above the LCST), from which it can be seen that the size decreased to 80 nm, due to the shrinkage of the nanoparticles where the hydrophobic interactions between N,N-dimethylaminoethyl groups could be dominated. Figure 8b shows the DLS curve of the nanoparticles when heating to 60 °C, where the D_h decreased to 105 nm. The thermo-responsive morphological changes of nanoparticles self-assembled from the polymers with DF of 9% and 17% are shown in Figure S5 and S6, from which it can be seen that the nanoparticles were also shrunk at high temperature.

The absorbance and fluorescence of loaded Nile Red in the polymer nanoparticles upon heating and cooling process were also investigated. Upon heating to 60 °C, the absorption band of Nile Red changed little, as indicated no leakage of Nile Red (Figure S13). Figure 8c shows that the fluorescence intensity of Nile Red loaded in nanoparticles of the copolymer with DF of 4% increased when increasing temperature from 20 to 60 °C since the hydrophobicity of PDMAEMA increased.[42] Figure 8d shows that the fluorescence intensity could be recovered to the initial value upon cooling due to the increase of hydrophilicity of PDMAEMA. The reversible fluorescence change of loaded Nile Red in the nanoparticles of the copolymers with DF of 9% and 17% could be also observed during the process of heating and cooling, shown in Figure S15.

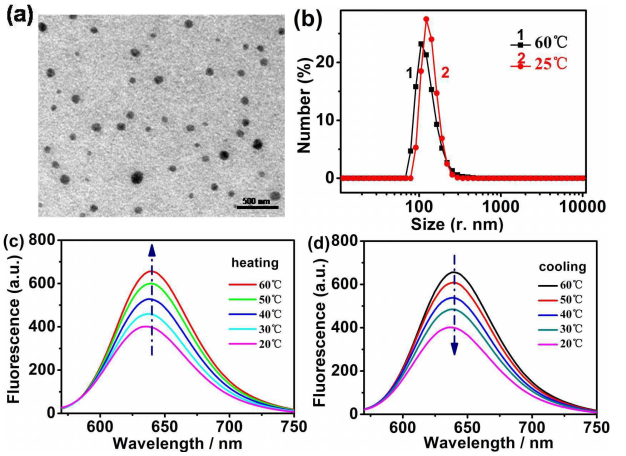


Figure 8. (a) and (b) TEM image and DLS curve of the nanoparticles self-assembled from PDMAEMA-AZO with DF of 4% when heating to 60 °C. (c) and (d) Fluorescence spectra (λ_{ex}=550 nm) of the nanoparticles loaded with Nile Red through a heating process from 20 to 60 °C and the subsequent cooling process from 60 to 20 °C, respectively.

4. Conclusions

A series of novel amphiphilic azobenzene-functionalized polymers have been successfully prepared by incorporating the photo-responsive azobenzene unit into the pH- and thermo-responsive PDMAEMA through quaternization reaction. The LCST increased with the increase of the functionalization degree. The amphiphilic random copolymers could self-assemble into micellar nanoparticles in water. The CAC values decreased with the increase of the functionalization degree. Upon UV light irradiation, the trans azobenzene changed to the cis form and thus the polarity of the nanoparticles increased, while the nanoparticle could only swell a little. Although the loaded Nile Red in the polymer nanoparticles could not be released under UV light irradiation, the fluorescence intensity of Nile Red changed reversibly when altering UV and visible light irradiation for the reversible change of the polarity. Under acidic condition, the nanoparticles swelled a lot due to the protonation of the tertiary amine groups in PDMAEMA segments and the loaded Nile Red could be released. Under alkaline condition, the nanoparticles could be shrunk and aggregated owing to the deprotonation of PDMAEMA segments and the loaded Nile Red could be released only a little. When the temperature increased, the nanoparticles shrank, while the Nile Red was still encapsulated in the nanoparticles. The fluorescence intensity of loaded Nile Red could be increased upon heating and decreased upon cooling due to the reversible changes between hydrophobicity and hydrophilicity of PDMAEMA. The

View Article Online
DOI: 10.1039/C5RA26894J

polymer nanoparticles self-assembled from the photo-, pH- and thermo-responsive azobenzene-functionalized PDMAEMA may find their applications in the fields of sensing, catalysis, separation and controlled release.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No. 51373025 and 21074010), the Program for New Century Excellent Talents in University (NCET-11-0582), and the Fundamental Research Funds for the Central Universities (FRE-TP-12-004B).

Notes and references

1 J. Gohy and Y. Zhao, *Chem. Soc. Rev*., 2013, **42**, 7117-7129.
2 D. Roy, W. Brooks and B. Sumerlin, *Chem. Soc. Rev*., 2013, **42**, 7214-7243.
3 S. Dai, P. Ravib and K. Tam, *Soft Matter*, 2008, **4**, 435-449.
4 J. Hu, G. Zhang and S. Liu, *Chem. Soc. Rev*., 2012, **41**, 5933-5949.
5 P. Theato, B. Sumerlin, R. O'Reilly and T. Epps, *Chem. Soc. Rev.*, 2013, **42**, 7055-7056.
6 Y. Leng, J. Zhao, P. Jiang and J. Wang, *ACS Appl. Mater. Interfaces*, 2014, **6**, 5947-5954.
7 K. Kim, S. Meeuwissen, R. Nolte and J. Hest, *Nanoscale*, 2010, **2**, 844-858.
8 M. Motornov, J. Zhou, M. Pita, V. Gopishetty, I. Tokarev, E. Katz and S. Minko, *Nano. Lett.*, 2008, **8**, 2993-2997.
9 Z. Ge and S. Liu, *Chem .Soc. Rev.*, 2013, **42**, 7289-7325.
10 J. Jiang, X. Tong, D. Morris and Y. Zhao, *Macromolecules*, 2006, **39**, 4633-4640.
11 Y. Liu, J. Li, J. Ren, C. Lin and J. Leng, *Mater. Lett.*, 2014, **127**, 8-11.
12 D. Xiong, Z. He, Y. An, Z. Li, H. Wang, X. Chen and L. Shi, *Polymer*, 2008, **49**, 2548-2552.
13 S. Ganta H. Devalapally, A. Shahiwala and M. Amiji, *J. Control. Release*, 2008, **126**, 187-204.
14 O. Bertrand, E. Poggi, J. Gohy and C. Fustin, *Macromolecules*, 2014, **47**, 183-190.
15 J. Dong, Y. Wang, J. Zhang, X., S. Zhu, H. Yang and G. Wang, *Soft Matter*, 2013, **9**, 370-373.
16 J. Dong, R. Zhang, H. Wu, X. Zhan, H. Yang, S. Zhu and G. Wang, *Macromol. Rapid Commun.*, 2014, **35**, 1255-1259.
17 H. Wu, J. Dong, C. Li, Y. Liu, N. Feng, L. Xu, X. Zhan, H. Yang and G. Wang, *Chem. Commun.*, 2013, **49**, 3516-3518.
18 H. Wu, J. Dong, X. Zhan, H. Yang, Y. Zhao, S. Zhu and G. Wang, *RSC Adv.*, 2014, **4**, 35757-35761.
19 Z. Cao, H. Wu, J. Dong and G. Wang, *Macromolecules*, 2014, **47**, 8777-8783.
20 N. Feng, G. Han, J. Dong, H. Wu, Y. Zheng and G. Wang, *J. Colloid Interface Sci.*, 2014, **421**, 15-21.
21 N. Feng, J. Dong, G. Han and G. Wang, *Macromol. Rapid Commun.*, 2014, **35**, 721-726.
22 J. Niskanen, C. Wu, M. Ostrowski, G. Fuller, S. Hietala and H. Tenhu, *Macromolecule*s, 2013, **46**, 2331-2340.
23 X. Liu, P. Ni, J. He and M. Zhang, *Macromolecules*, 2010, **43**, 4771-4781.
24 J. Gohy and Y. Zhao, *Chem. Soc. Rev.*, 2013, **42**, 7117-7129.
25 J. Wang, S. Wang, Y. Zhou, X. Wang and Y. He, *ACS Appl. Mater. Interfaces*, 2015, **7**, 16889-16895.
26 J. Wang, Y. Zhou, X. Wang and Y. He, *RSC Adv.*, 2015, **5**, 9476-9481.
27 Y. Huang, R. Dong, X. Zhu and D. Yan, *Soft Matter*, 2014, **10**, 6121-6138.
28 H. Feil, Y. Bae, J. Feijen and S. Kim, *Macromolecules*, 1993, **26**, 2496-2500.
29 Y. Zheng, L. Zhong, W. Huang, Y. Zhou and D. Yan, *J. Polym. Sci. Polym. Chem.*, 2010, **48**, 4428-4438.
30 W. Su, X. Luo, H. Wang, L. Li, J. Feng, X. Zhang and R. Zhuo, *Macromol. Rapid Commun.*, 2011, **32**, 390-396.
31 X. Liu, P. Ni, J. He and M. Zhang, *Macromolecules*, 2010, **43**, 4771-4781.
32 T. Noda, A. Hashidzume and Y. Morishima, *Macromolecules*, 2001, **34**, 1308-1317.
33 W. Su, K. Han, Y. Luo , Z. Wang, Y. Li and Q. Zhang, *Macromol. Chem. Phys.*, 2007, **208**, 955-963.
34 Y. Zhao, *Macromolecules*, 2012, **45**, 3647-3657.
35 P. Han, S. Li, C. Wang, H. Xu, Z. Wang, X. Zhang, J. Thomas and M. Smet, *Langmuir*, 2011, **27**, 14108-14111.
36 D. Sackett and J. Wolff, *Anal. Biochem.*, 1987, **167**, 228-234.
37 Z. Feng, L. Lin, Z. Yan and Y. Yu, *Macromol. Rapid Commun.*, 2010, **31**, 640-644.
38 E. Blasco, B. Schmidt, C. Kowollik, M. Pinol and L. Oriol, *Polym. Chem.*, 2013, **4**, 4506-4514.
39 X. Tong, G. Wang, A. Soldera and Y. Zhao. *J. Phys. Chem. B*, 2005, **109**, 20281-20287.
40 X. Tang, X. Liang, L. Gao, X. Fan and Q. Zhou, *J. Polym. Sci. Polym. Chem.*, 2010, **48**, 2564-2570.
41 D. Basak and S. Ghosh, *ACS Macro Lett.* 2013, **2**, 799-804.
42 A. Goodwin, J. Mynar, Y. Ma, G. Fleming and J. Fréchet, *J. Am. Chem. Soc.*, 2005, **127**, 9952-9953.

View Article Online
DOI: 10.1039/C5RA26894J

Polymer nanoparticles self-assembled from photo-, pH- and thermo-responsive azobenzene-functionalized PDMAEMA

Tingting Yuan, Jie Dong, Guoxiang Han, Guojie Wang*

Polymer nanoparticles self-assembled from an amphiphilic azobenzene-functionalized PDMAEMA have been constructed, the morphological changes of which under stimulation of UV light, temperature and pH changes are demonstrated.