Reversible Fluorescence Modulation through Energy Transfer with ABC Triblock Copolymer Micelles as Scaffold

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Supplementary information

Experimental details, ^{1}H NMR and ^{13}C NMR spectra, GPC curves, determination of CMC, AFM image and particle size distribution, absorption spectra for monomer and triblock copolymer in solutions, surface tension vs. concentration curve for triblock copolymer in water, illustration for light-induced structural variation, fluorescence emission spectra, photographs and emission spectra of micelles upon UV or visible light irradiation, fluorescence intensity changes of NBD/micelle dispersion upon alternate irradiation with UV and visible light, calculation of Förster Critical Radius and determination of experimental energy transfer efficiency, and illustration for effective and noneffective FRET volume in a micelle core. (34 pages)

1. Experimental Section

Synthesis routes:
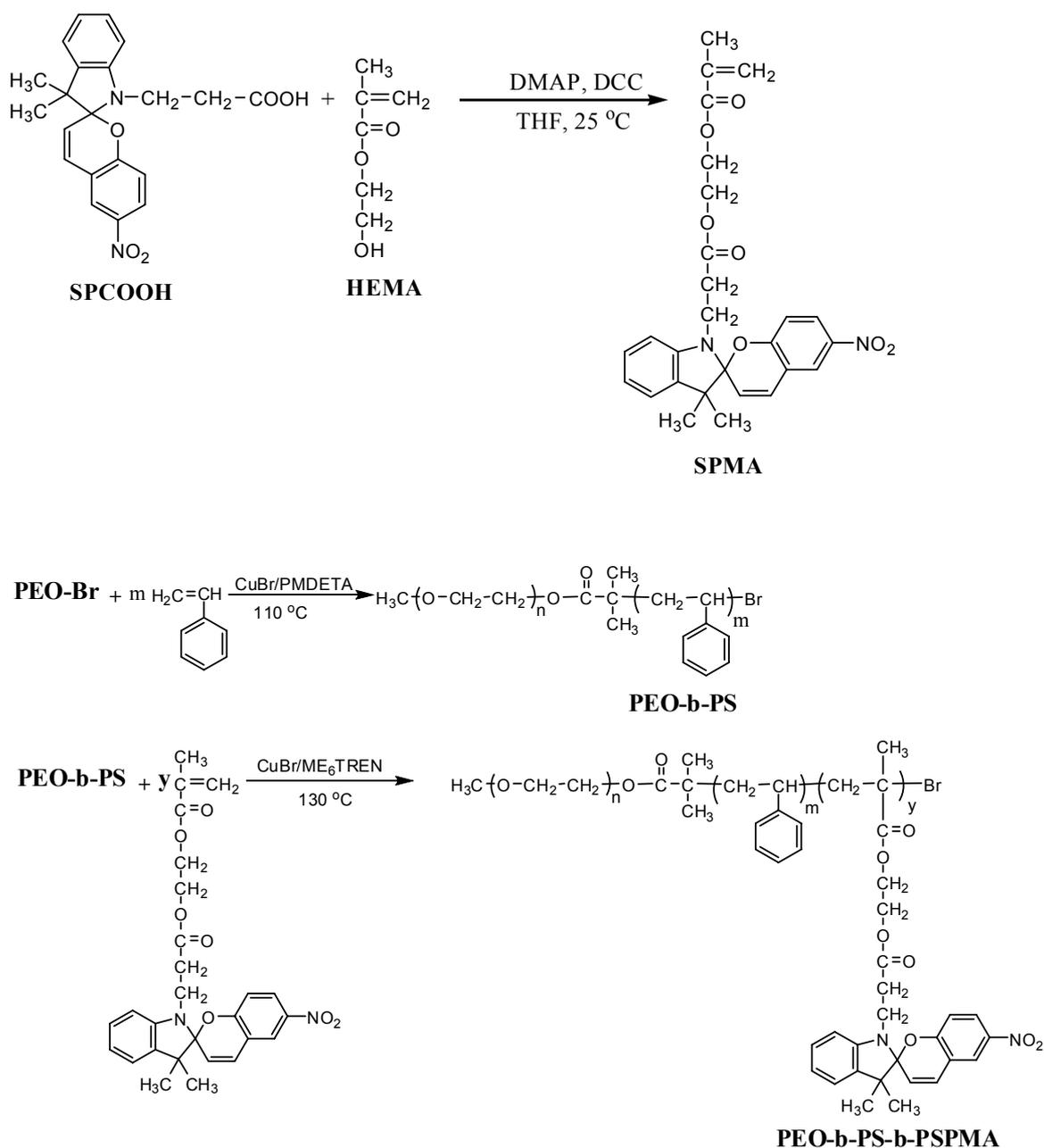
Scheme S1. Synthesis route for spiropyran-containing methacrylate monomer (SPMA) and the triblock copolymer PEO-*b*-PS-*b*-PSPMA. HEMA: 2-hydroxyethyl methacrylate; SPCOOH: carboxyl-containing spiropyran.

Materials

DCC (N,N'-dicyclohexylcarboiimide) (99%, Alfa), bipyridine (bpy) (99%, Alfa) N,N,N',N'',N'''-pentamethyldiethylenetriamine (PMDETA) (99%, Alfa), DMAP (4-dimethylamino-pyridine) (99%, Alfa) were used as received. PEO (M_n= 5000, Alfa) was dried by azeotropic distillation with toluene before use. Chlorobenzene (99%, Acros Organics) was washed with concentrated sulphuric acid to remove thiophenes, followed by washing twice with water, once with 5 wt% sodium carbonate solution, and again with water before drying with anhydrous calcium chloride and distillation. Styrene (St) was received from Aldrich (99%), stirred with CaH_2 overnight, and distilled prior to use. 2-Bromoisobutyryl bromide (98%, Alfa) was freshly distilled at room temperature under vacuum. CuBr (99%, Alfa) were purified by washing with glacial acetic acid and methanol, and then drying under vacuum. Dichloromethane (DCM) was washed with sulfuric acid and then distilled over CaH_2. 2-hydroxyethyl methacrylate (HEMA) (97%, Aldrich) was dissolved in water (25 vol % of monomer) and washed four times with an equal volume of hexane and then dried over $MgSO_4$ and distilled under vacuum prior to use. Tetrahydrofuran was distilled over CaH_2. Petroleum ether, benzene, and other reagents were analytical reagents and used without further purification, except as noted.

Tris(2-(dimethylamino)- ethyl)amine (Me_6TREN) was synthesized following the previously reported procedure. (Queffelec, J.; Gaynor, S. G.; Matyjaszewski, K. *Macromolecules*, **2000**, *33*, 8629-8639.).

The 7-nitro-N-octylbenzo-furazan-4-amine (NBD) was prepared in the present work as described below (Galinier, F.; Bertorelle, F.; Fery-Forgues, S. *Comptes Rendus Acad. Sci. Ser.*

II C **2001**, *4*, 941-950.). 3 mmol (300 mg) 4-chloro-7-nitro-benzo-furazan (NBD-Cl, Alfa)

were dissolved in 2 ml of toluene. Then, 6 mmol fatty amine diluted in 2 ml toluene were

added dropwise under efficient stirring in an ice bath. The mixture was stirred for 2 h at room

temperature. The solution obtained was purified by silica thin layer chromatography using the

mixture CH_2Cl_2: toluene (3:1) as eluent. The red product was extracted from the silica with

dichloromethane, filtered, dried and recrystallized in ethyl ether, yielding bright red crystals

(48 % yield) of excellent spectroscopic purity.

Synthesis of the carboxyl-containing spiropyran (SPCOOH)

During the synthesis process, all the reaction vessels were wrapped with aluminum foil, so

as to ensure the reaction was performed in the dark. The carboxyl-containing spiropyran

l-(β-carboxyethyl)-3',3'-dimethyl-6 -nitrospiro (indoline-2',2 [2*H*-1] benzopyran) (referred to

as SPCOOH) was synthesized as follows: 2,3,3-trimethylindolenine (0.06 mol),

3-iodopropanoic acid (0.06 mol) and ethyl methyl ketone (5ml) were heated under nitrogen at

100°C for 3 h. The resulting solid material was dissolved in water, and the solution was

washed with chloroform. Evaporation of water gave l-(β-carboxyethyl)-

2,3,3-trimethylindolenine iodide (73% yield). The above-obtained iodide (0.04 mol),

5-nitrosalicylaldehyde (0.04 mol), and piperidine (3.8 ml, 0.04 mol) were dissolved in ethyl

methyl ketone, and the red solution was refluxed for 3 h. On standing overnight, the product

precipitated as a yellow crystalline powder. This was filtered and washed with methanol to

give the product SPCOOH (75% yield). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 1.0-1.3 (6H, CH_3),

2.6 (2H, CH_2), 3.4-3.5 (2H, CH_2), 5.8-5.9 (2H, olefinic protons), 6.6-8.2 (7H, aromatic), 12.0

(1H, COOH). ^{13}C NMR (400 MHz, DMSO-d_6, δ): 19.4, 25.5, 33.1, 52.4, 106.5, 115.4, 118.8,

119.2, 121.7, 122.7, 125.7, 127.6, 128.1, 135.6, 140.5, 146.1, 159.0, 172.8. Elem. Anal.: calcd.

for $C_{21}H_{20}N_2O_5$: C, 66.31; H, 5.30; N, 7.36; found: C, 66.01; H, 5.37; N, 7.20.

Synthesis of SPMA from SPCOOH

SPCOOH (3.8g, 10mmol), HEMA (2.6g, 20mmol) and DMAP (0.272g, 2mmol) were

added to a 100 ml round-bottomed flask equipped with a pressure-equalized dropping funnel,

magnetic stirrer and a nitrogen inlet. Dry THF (80 ml) was added to the flask and the solution

was cooled to 0°C, a red/brown solution resulted. A solution of DCC (2.06g, 10mmol) was

prepared in dry THF (20 ml) in a small round-bottomed flask. The DCC solution was added to

the SPCOOH/HEMA solution via the pressure-equalized dropping funnel over 45 minutes. The

flask was maintained at 0°C for 2 hours, then was raised gradually to 25°C over 24hours. The

product was filtrated with cold (0°C) dry THF (3×50 ml) giving a red filtrate. Most of solvent

was evaporated from the filtrate under vacuum, then, the residual was washed by distilled

water to give a red/purple precipitate. The precipitate was dissolved in benzene and filtrated

again, afterwards most of the solvent was evaporated, the solution was precipitated in a large

amount of petroleum ether, finally a fine red/purple precipitate of purified 2-[1-

acrylate-3',3'-dimethyl-6-nitrospiro(indoline-2',2-[2H-1]-benzopyran)]ethyl methacrylate

(SPMA) was obtained. The target product was dried in vacuum oven overnight at room

temperature. (^{1}H NMR spectrum shown in Figure S3, Supporting information). ^{1}H NMR (400

MHz, CDCl$_3$, δ): 1.0-1.3 (6H, CH$_3$), 1.8-1.9 (3H, CH$_3$), 2.6-2.7 (2H, CH$_2$), 3.5-3.6 (2H, CH$_2$),

4.2 (4H, CH$_2$), 5.8-5.9 (2H, olefinic protons in spiropyran), 5.6 and 6.1 (2H, vinylic), 6.6-8.1

(7H, aromatic). ^{13}C NMR (400 MHz, CDCl$_3$, δ): 17.7, 19.3, 25.3, 33.0, 38.6, 52.4, 61.8, 106.2,

115.0, 118.0, 119.3, 121.4, 122.2, 125.3, 127.8, 135.4, 140.6, 145.7, 153.2, 158.9, 166.5, 171.1.

Elem. Anal.: calcd. for $C_{27}H_{28}N_2O_7$: C, 65.84; H, 5.73; N, 5.69; found: C, 65.21; H, 5.57; N, 5.72.

Synthesis of PEO-Br Macroinitiator

The bromotelechelic PEO macroinitiator (PEO-Br) was synthesized by reacting PEO with 2-bromoisobutyryl bromide according to the literature (Jankova K, Chen XY, Kops J, *Macromolecules*, 1998; 31:538-541.). ^{1}H NMR (400 MHz, CDCl$_3$, δ): 1.94 (6H, C(CH$_3$)$_2$Br), 3.35 (3H, OCH$_3$), 3.42–3.63 (4nH, CH$_2$CH$_2$O), 4.33 (2H, COOCH$_2$).

Synthesis of PEO-*b*-PS Diblock Copolymer by ATRP

PEO-*b*-PS diblock copolymers were synthesized by solution polymerization in chlorobenzene. In a typical run, a glass tube was charged with 0.25 g (0.05 mmol) of PEO-Br macroinitiator, 1.16g (11.2 mmol) of St, 0.071g (0.05 mmol) of CuBr, 0.0260g (0.15 mmol) of PMDETA, and 1.125g of chlorobenzene. After degassing with three freeze–pump–thaw cycles, the tube was sealed under vacuum and then immersed in a thermostated oil bath at 110°C. After a certain time, the tube was withdrawn and broke to stop the reaction. The reaction mixture was diluted with tetrahydrofuran (THF). After the solution was filtrated with the activated Al$_2$O$_3$ column (to remove the catalyst) and then precipitated into ether, the PEO-*b*-PS diblock copolymer was obtained and dried in a vacuum oven overnight at room temperature. ^{1}H NMR (400 MHz, CDCl$_3$, δ): 1.24-1.59 (2mH, CH$_2$), 1.70-1.95 (mH, CH), 3.40 (3H, OCH$_3$), 3.42-3.63 (4nH, CH$_2$CH$_2$O), 6.40-7.23 (5mH, aromatic); $M_{n,NMR} = 1.37 \times 10^4$, $M_{n,GPC} = 1.64 \times 10^4$, $PDI = 1.15$; *conversion* = 38% (calculated through ^{1}H-NMR).

Synthesis of PEO-*b*-PS-*b*-PSPMA Triblock Copolymer by ATRP

PEO-*b*-PS-*b*-PSPMA Triblock copolymers were synthesized by solution polymerization

in chlorobenzene. In a typical run, a glass tube was charged with 0.30g of PEO-*b*-PS macroinitiator, 0.251g (0.51mmol) of SPMA, 0.0086g (0.06 mmol) of CuBr, 0.0141g (0.06 mmol) of ME$_6$TREN, and 1.5g of chlorobenzene. After degassing with three freeze–pump–thaw cycles, the tube was sealed under vacuum and then immersed in a thermostated oil bath at 130°C. After 6h, the tube was withdrawn and broke to stop the reaction. The reaction mixture was diluted with tetrahydrofuran (THF). After the solution was passed through a column with activated Al$_2$O$_3$ (to remove the catalyst) and then precipitated into ether, afterwards, the precipitate was again dissolve in THF and re-precipitated in ethanol to remove the unreacted SPMA monomer. And the PEO-*b*-PS-*b*-PSPMA triblock copolymer was obtained and dried in a vacuum oven overnight at room temperature. ^{1}H NMR (400 MHz, CDCl$_3$, δ): 1.10-1.18 (6H, CH$_3$), 1.19-1.30 (9yH, CH$_3$), 1.24-1.59 (2mH, CH$_2$), 1.70-1.95 (mH, CH; 2yH, CH$_2$), 3.40 (3H, OCH$_3$), 3.42-3.63 (4nH, CH$_2$CH$_2$O), 4.0-4.3 (4yH, CH$_2$), 5.8-5.9 (2yH, olefinic protons), 6.40-7.23 (5mH, aromatic; 5yH, aromatic), 7.92-8.01 (2yH, aromatic); $M_{n,NMR}$ = 1.52×10^4, $M_{n,GPC}$ = 1.99×10^4, PDI = 1.14.

Micelle Formation in Water

Aqueous micelle dispersions were prepared with the following procedures. First the PEO-*b*-PS-*b*-PSPMA triblock block copolymer (0.1g) was dissolved in tetrahydrofuran (THF) (ca. 4 ml) in a 100-ml volumetric flask, and the purified water (Milli-Q, doubly distilled, 40 ml) was added into the THF solution under stirring, then THF was removed at 25°C under vacuum with a rotary evaporator. The solution was then diluted with water to 100.0 ml to obtain a micelle dispersion with the concentration of 1.0g/l.

Introduction of NBD dye into micelles in aqueous solution

The prepared NBD dye was dissolved in dichloromethane to obtain a 10^{-4} M of solution. A

given amount of the solution was transferred into a 10 ml flask, and then the solvent was evaporated under vacuum. The flask with dried NBD dye was then filled with the aqueous dispersion of micelles, and was stirred for 48 hours at 50 °C to let the dye molecules move into the micelles, subsequently it was cooled to room temperature.

Characterization and determination

^{1}H-NMR spectra and ^{13}C-NMR spectra were recorded at 25°C on an INOVA-400 NMR spectrometer with tetramethylsilane (TMS) as an internal standard. Elemental analysis was performed on a Vario EL Elemental Analyzer. The number average molecular weight (M_n) and polydispersity (M_w/M_n) were measured at 30°C on a Waters 2410 gel permeation chromatography (GPC) using THF as the eluent (1.0 ml/min) and polystyrene as the standard. The calibration curve was established by using polystyrene (PS) as the standard. Surface tension measurements were made by the platinum ring method. The error was ±0.1 mN/m. UV-vis spectra were recorded on a Hitachi U-3010 UV-vis spectrophotometer at room temperature. Fluorescence spectra were recorded on a Hitachi F-4500 Fluorescence spectrophotometer at room temperature. The micelle size and distribution was determined by dynamic light scattering on a Malvern Nano-ZS90 particle size analyzer and atomic force microscope (AFM, Seiko SII 400) in the tapping mode.

2. Characterizations:

a. Carboxyl-containing spiropyran (SPCOOH):
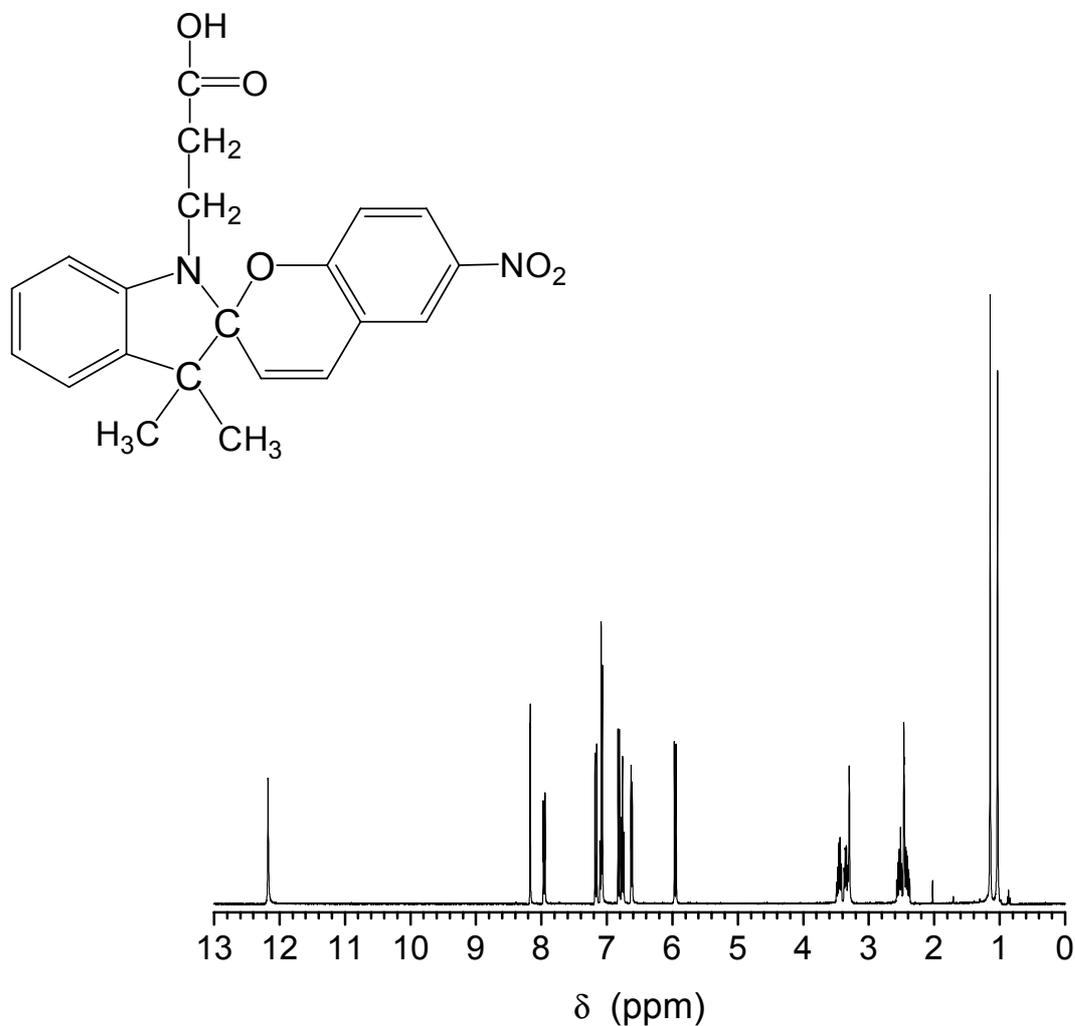
Figure S1. ^{1}H NMR spectrum of SPCOOH. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 1.0-1.3

(6H, CH$_3$), 2.6 (2H, CH$_2$), 3.4-3.5 (2H, CH$_2$), 5.8-5.9 (2H, olefinic protons), 6.6-8.2 (7H,

aromatic), 12.0 (1H, COOH).
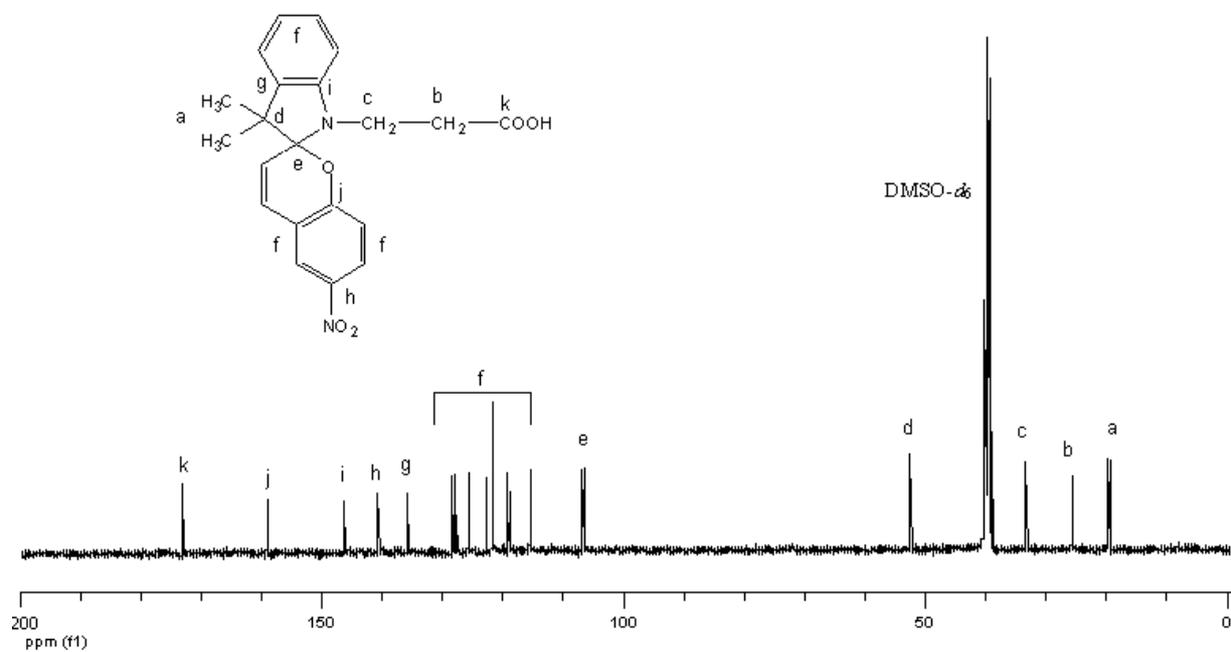
Figure S2. ^{13}C NMR spectrum of SPCOOH

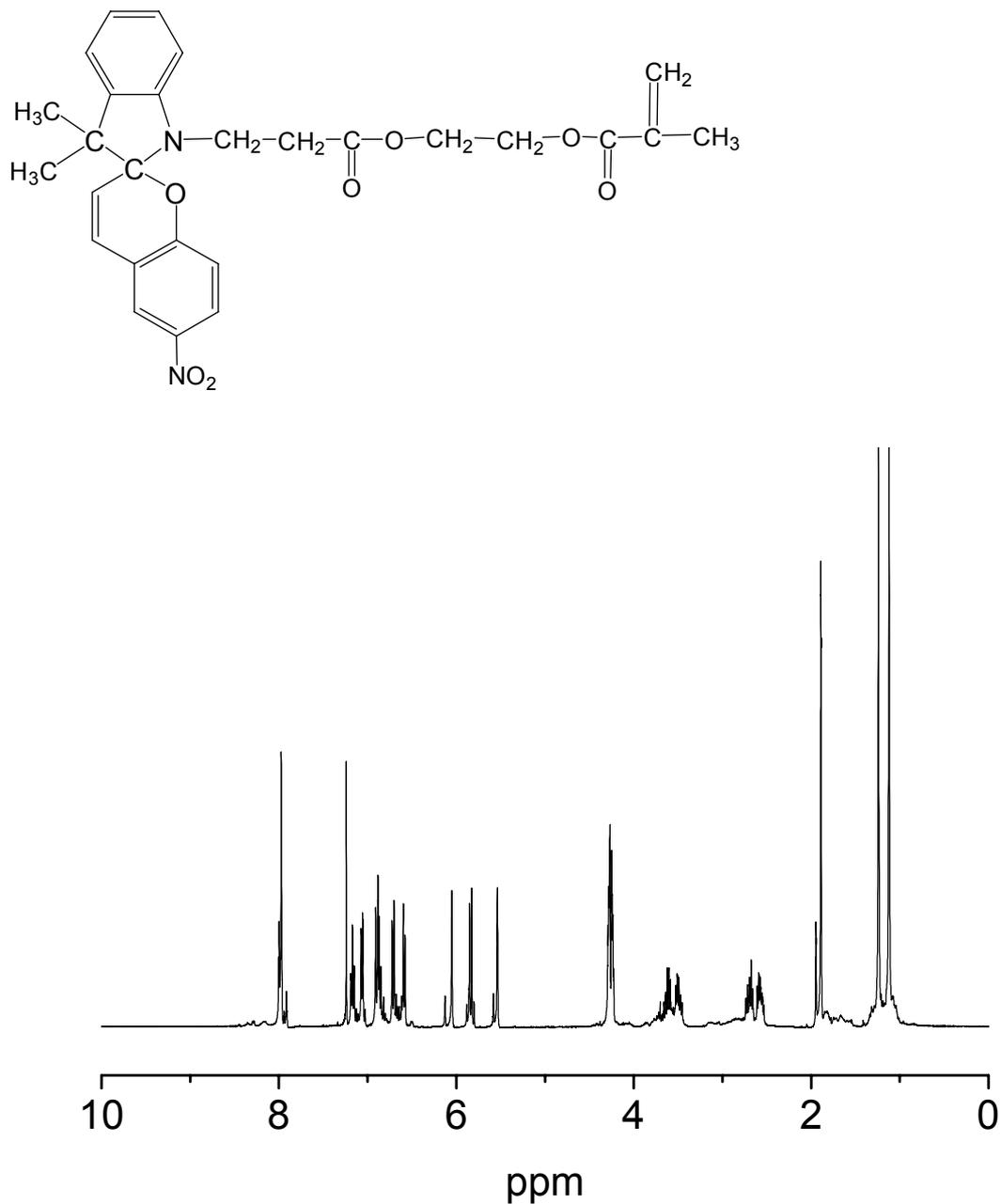b. Spiropyran-containing methacrylate monomer (SPMA)

Figure S3. ^{1}H NMR spectrum of spiropyran-linked methacrylate (SPMA) monomer. ^{1}H NMR (400 MHz, CDCl$_3$, δ): 1.0-1.3 (6H, CH$_3$), 1.8-1.9 (3H, CH$_3$), 2.6-2.7 (2H, CH$_2$), 3.5-3.6 (2H, CH$_2$), 4.2 (4H, CH$_2$), 5.8-5.9 (2H, olefinic protons in spiropyran), 5.6 and 6.1 (2H, vinylic), 6.6-8.1 (7H, aromatic).
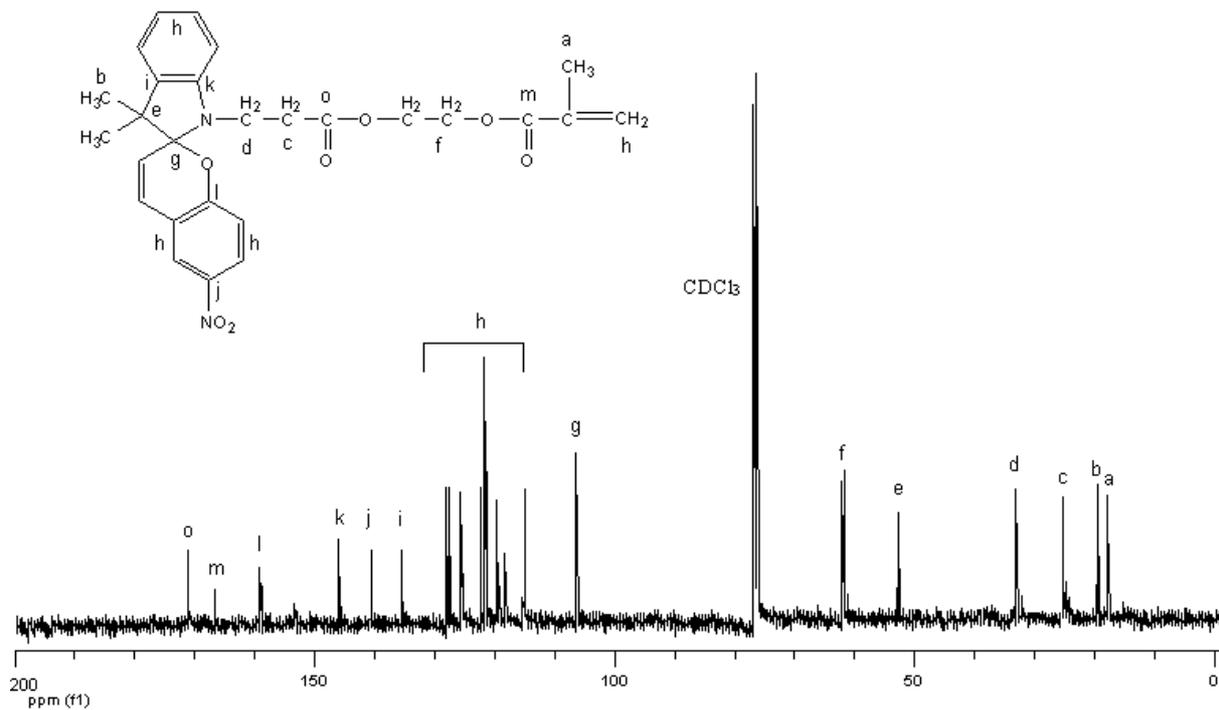
Figure S4. ^{13}C NMR spectrum of spiropyran-linked methacrylate (SPMA) monomer

c. Block copolymers

(I)

(II)

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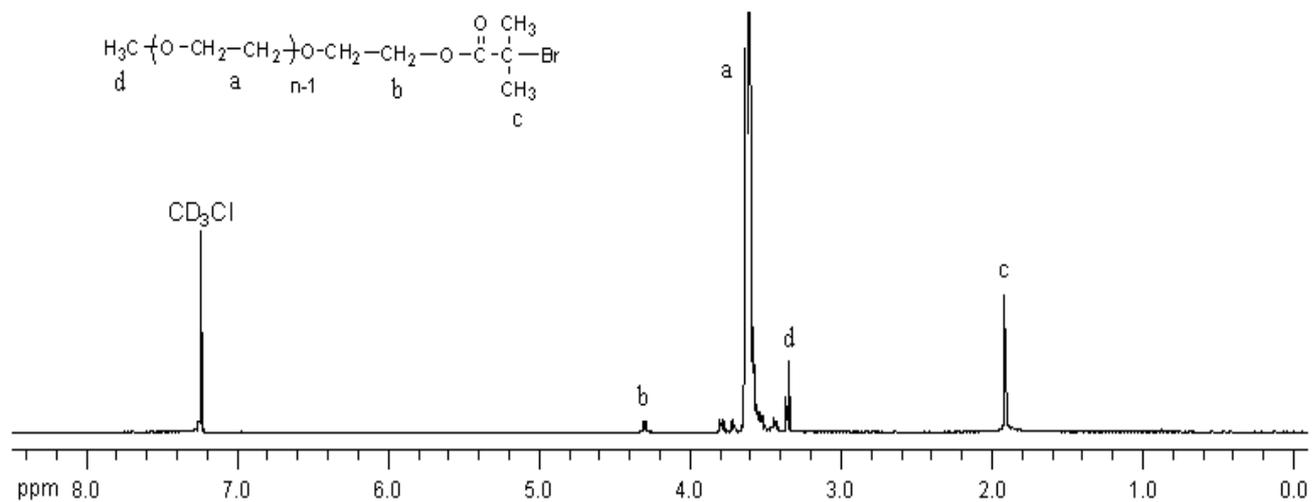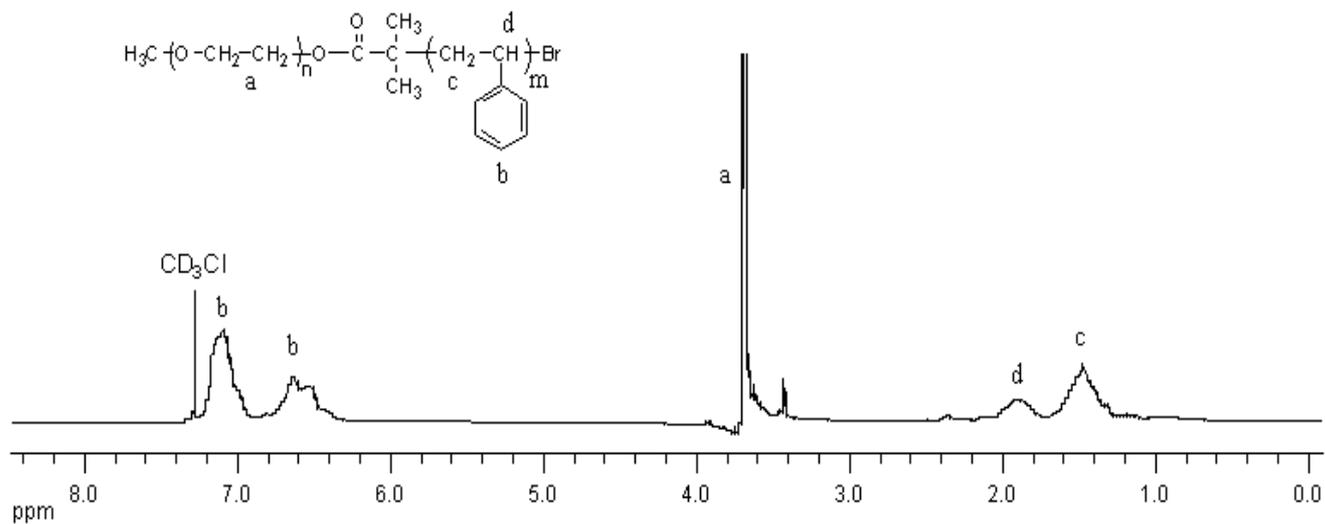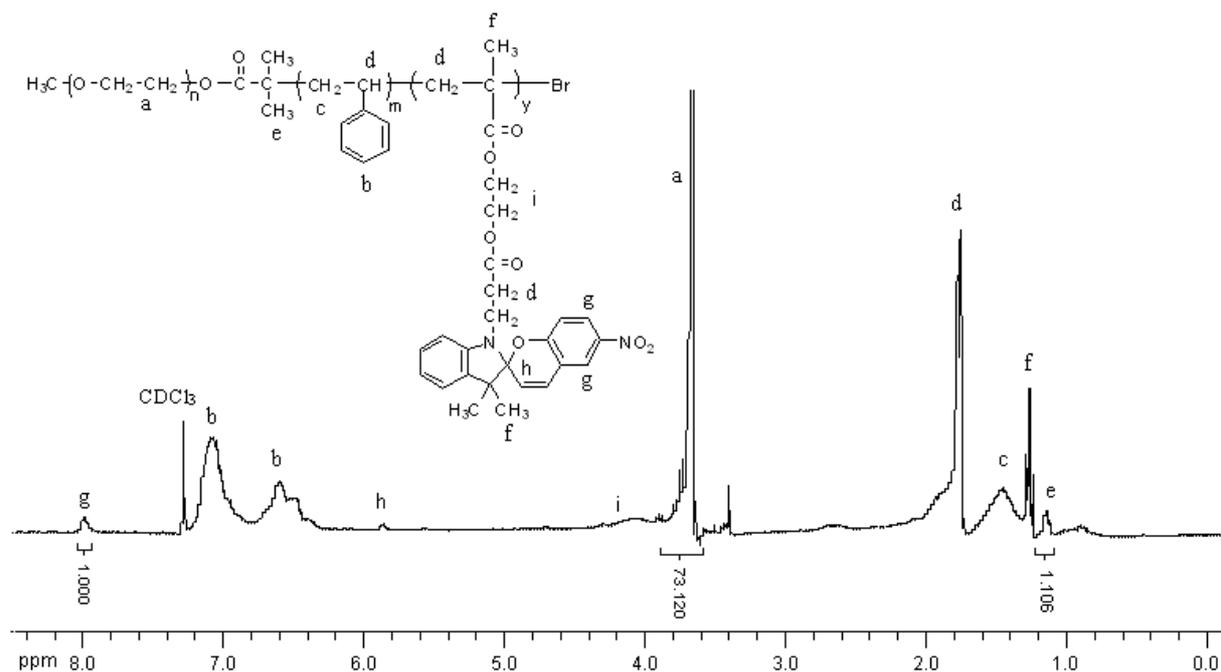
(III)
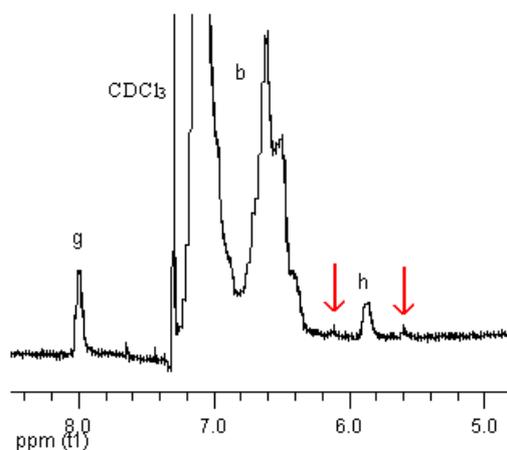
(IV)

Figure S5. ^{1}H NMR spectra of PEO$_{110}$-Br macroinitiator (I), PEO$_{110}$-*b*-PS$_{85}$ Diblock Copolymer (II), PEO$_{110}$-*b*-PS$_{85}$-*b*-PSPMA$_3$ Triblock copolymer (III). For the triblock copolymer, the number average molecular weight of each block are Mn(EO) =4.8 kg/mol, Mn(S)= 8.8 kg/mol, and Mn(SPMA)= 1.5kg/mol by ^{1}H NMR spectroscopy.

In addition, the expanded region from 5.0 to 8.0 is given in (IV). By comparison of Figure S1 on page 9 and Figure S3 on page 11, we can assign the peak at ca. 5.85 ppm (h) in

(IV) to the olefinic protons in spiropyran ring; and the small peaks (pointed by arrows) at 5.6 and 6.1ppm, could be residual methacrylate double bonds (if not the noises), however, their integral areas are only comparable to that of the noise at ca. 7.6 ppm, and they are very low compared to that of peak g (protons next to the nitro group in the aromatic ring of spiropyran), thus this tiny methacrylate double bonds would not influence the spectroscopic results.
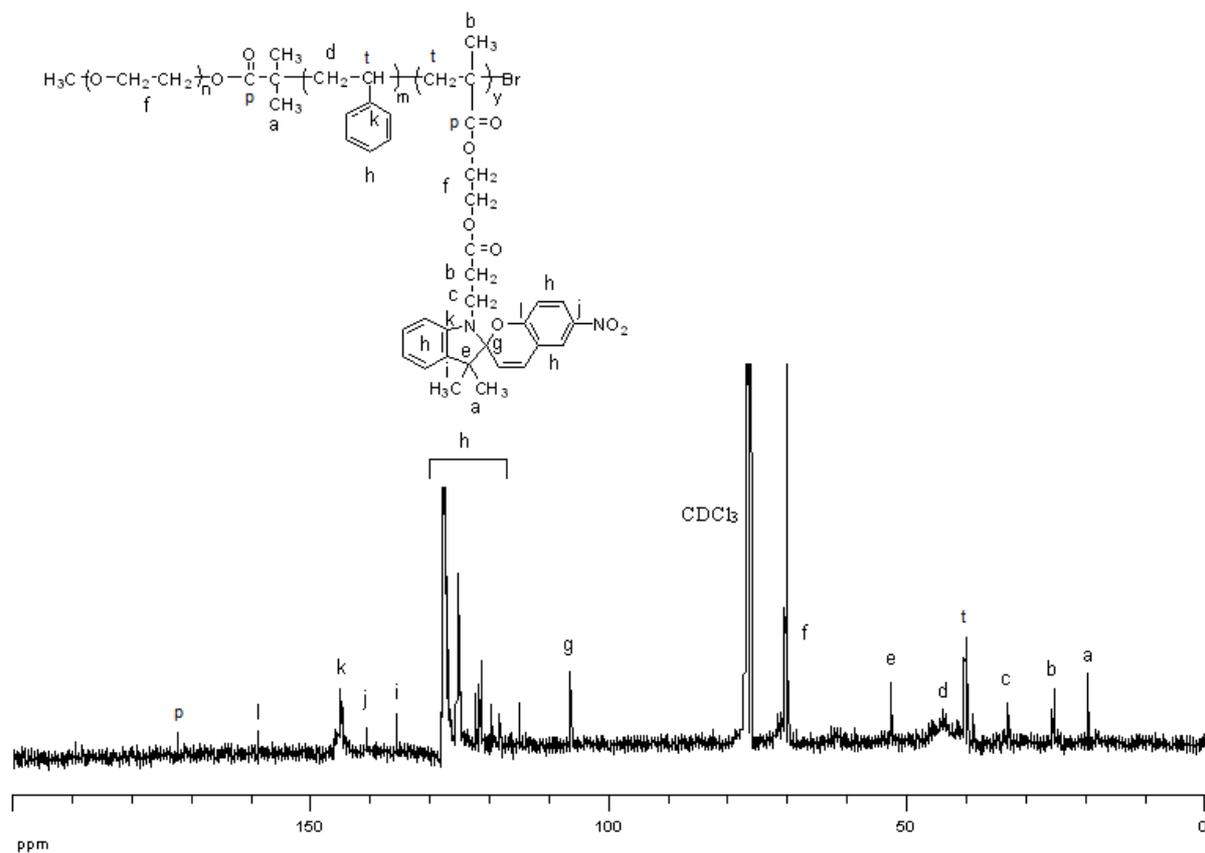
Figure S6. ^{13}C NMR spectra of PEO$_{110}$-*b*-PS$_{85}$-*b*-PSPMA$_3$ Triblock Copolymer
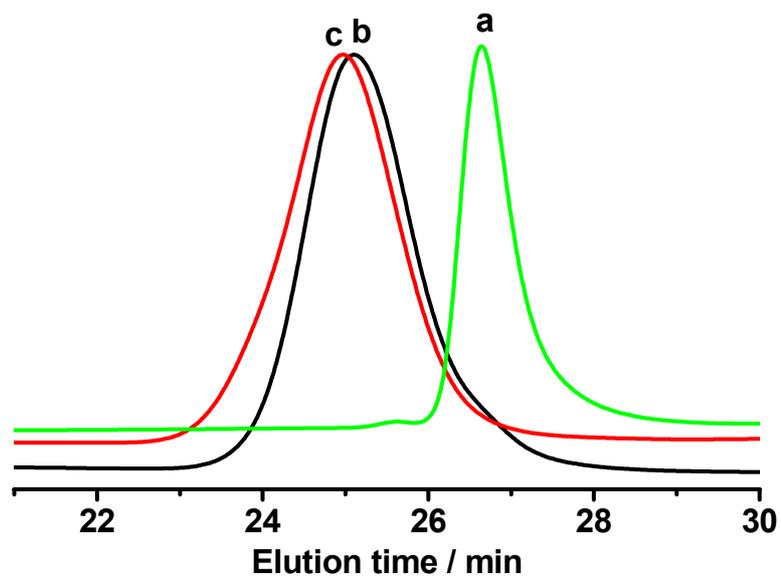
Figure S7. GPC curves for PEO (a), PEO-*b*-PS Diblock Copolymer (b) and PEO$_{110}$-*b*-PS$_{85}$-*b*-PSPMA$_3$ triblock copolymer (c). The polydispersity index for the triblock copolymer is 1.14.
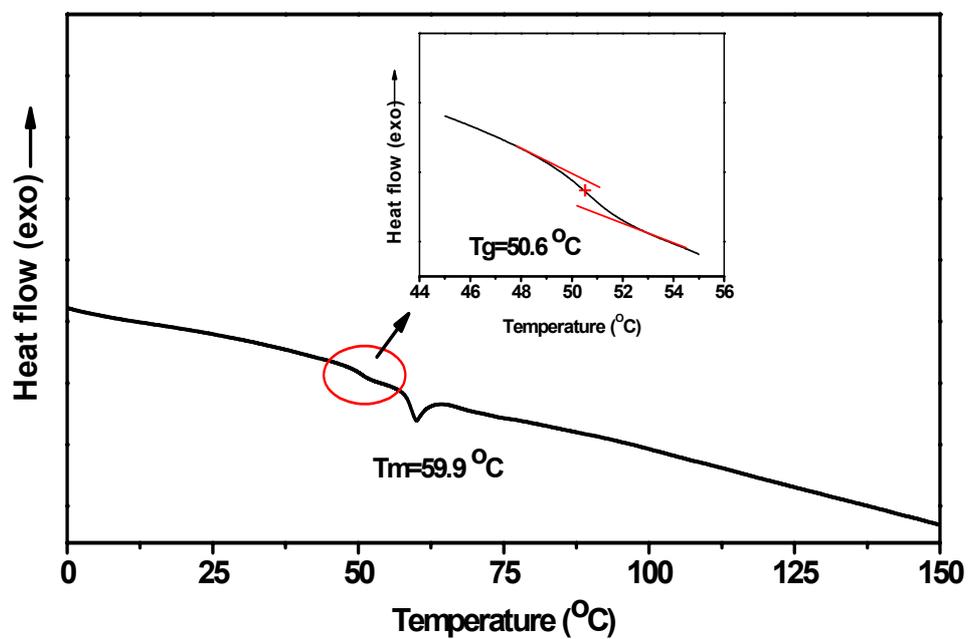
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Figure S8. DSC diagrams of the triblock copolymer at a rate of 5°C/min. The inset represents the glass transition region of the copolymer with an enlarged heat flow scale.
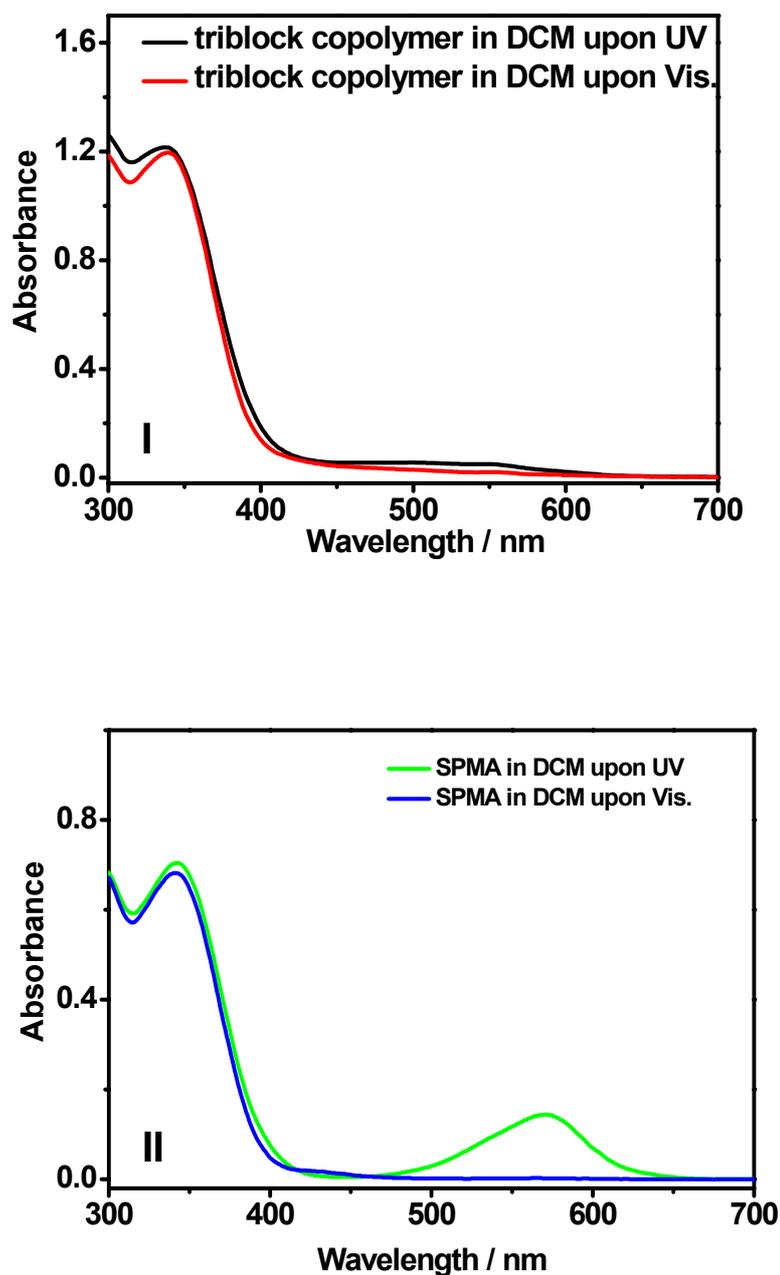
Figure S9 (A) Absorption spectra for triblock copolymer in dichloromethane (DCM) solution upon UV (30 min) and visible light irradiation (30 min) (I); and for spiropyran-linked methyl acrylate monomer (SPMA) in DCM solution upon UV (10 min) and visible light irradiation (20 min) (II).

For the absorption spectrum for ABC triblock copolymer in dichloromethane upon UV irradiation, there is no absorbance maximum from 450 nm to 700 nm, the absorbance value decreases very slowly from 500 nm to 700 nm.
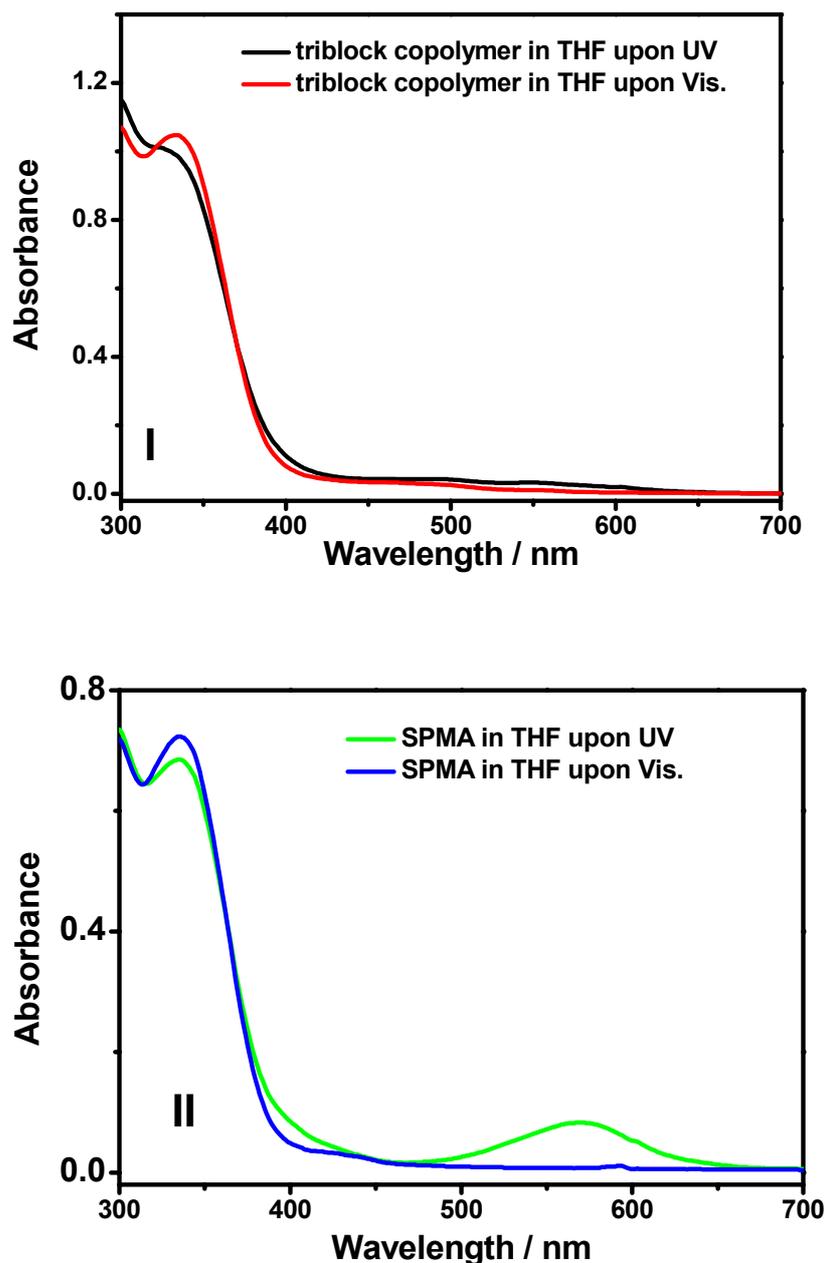
Figure S9 (B) Absorption spectra for triblock copolymer in THF solution upon UV (30 min) and visible light irradiation (30 min) (I); and for spiropyran-linked methyl acrylate monomer (SPMA) in THF solution upon UV (10 min) and visible light irradiation (20 min) (II).

For the absorption spectrum for ABC triblock copolymer in THF upon UV irradiation, there is no absorbance maximum from 450 nm to 700 nm, the absorbance value decreases very slowly from 500 nm to 700 nm.
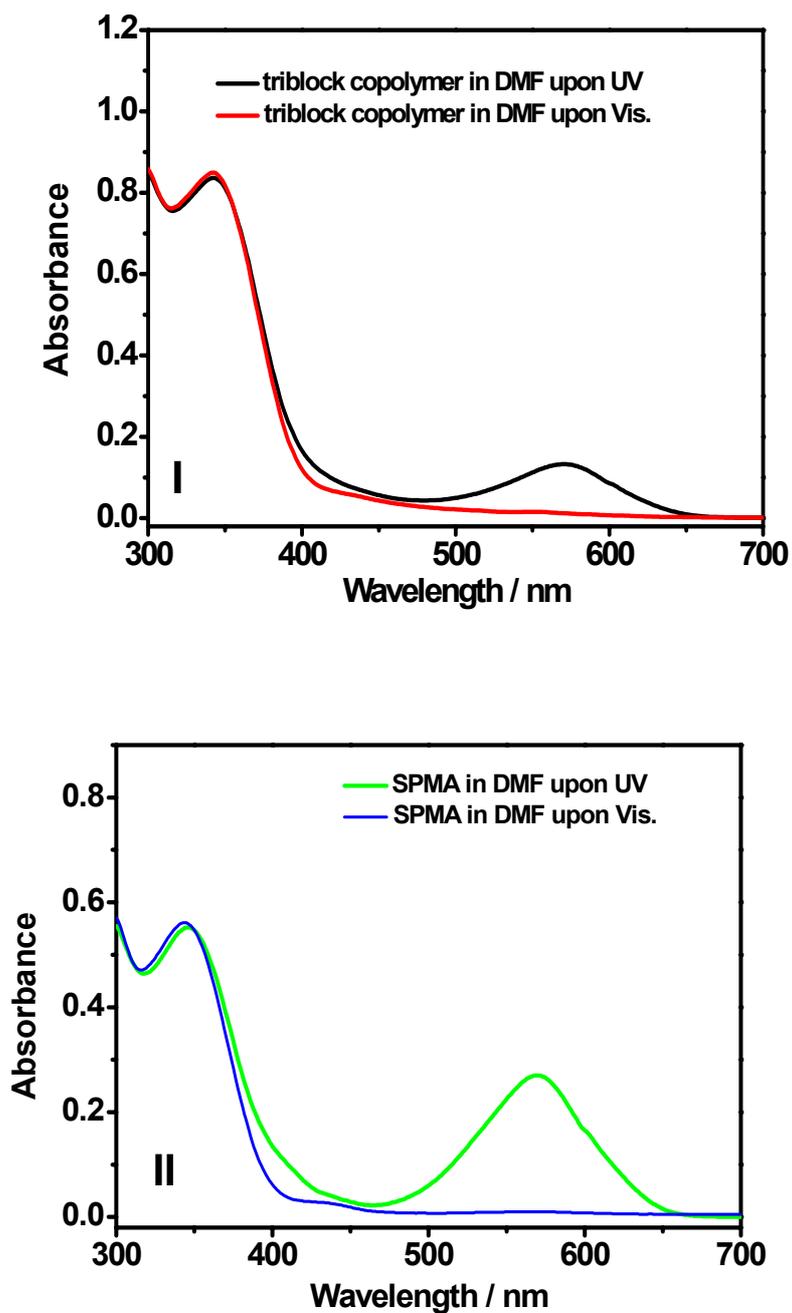
Figure S9 (C) Absorption spectra for triblock copolymer in dimethyl formamide (DMF) solution upon UV (10 min) and visible light irradiation (30 min) (I); and for spiropyran-linked methyl acrylate monomer (SPMA) in DMF solution upon UV (10 min) and visible light irradiation (20 min) (II).

Table S1. List of absorbance maximums for triblock copolymer and SP-linkage monomer (SPMA) in three organic solutions:

Items	λ_{max} (nm, upon vis.)	λ_{max} (nm, upon UV)
SPMA in DCM	341	342/570
Copolymer in DCM	338	338/---
SPMA in THF	336	336/570
Copolymer in THF	334	334/---
SPMA in DMF	344	346/569
Copolymer in DMF	342	342/570

Figure S9 and Table S1. Absorption properties for triblock copolymer and SPMA monomer in three organic solutions upon UV and visible light irradiation.

In all the three organic solvents, the spiropyran-linked monomer exhibits obvious ring-opening reaction (from SP to McH) upon UV irradiation, as evidenced by the occurrence of obvious absorption at 570 nm. However, in low-polar solvents (dichloromethane and THF), the triblock copolymer did not undergo SP-to-McH conversion upon UV as there is no obvious absorption band at 570 nm; while in high-polar solvent DMF, the triblock copolymer underwent SP-to-McH conversion upon UV. Currently the reason for these results are not clear, but these results can provide further evidence that the spiropyran have been covalently bound to the polymer chains; otherwise the mixture of PEO-PS diblock copolymer chains and free spiropyran molecules should exhibit the same or similar absorption behaviour as that of SPMA monomer in organic solutions.

3. Micelle formation of triblock copolymer in water, incorporation of NBD dye into micelles and light-induced fluorescence modulation.
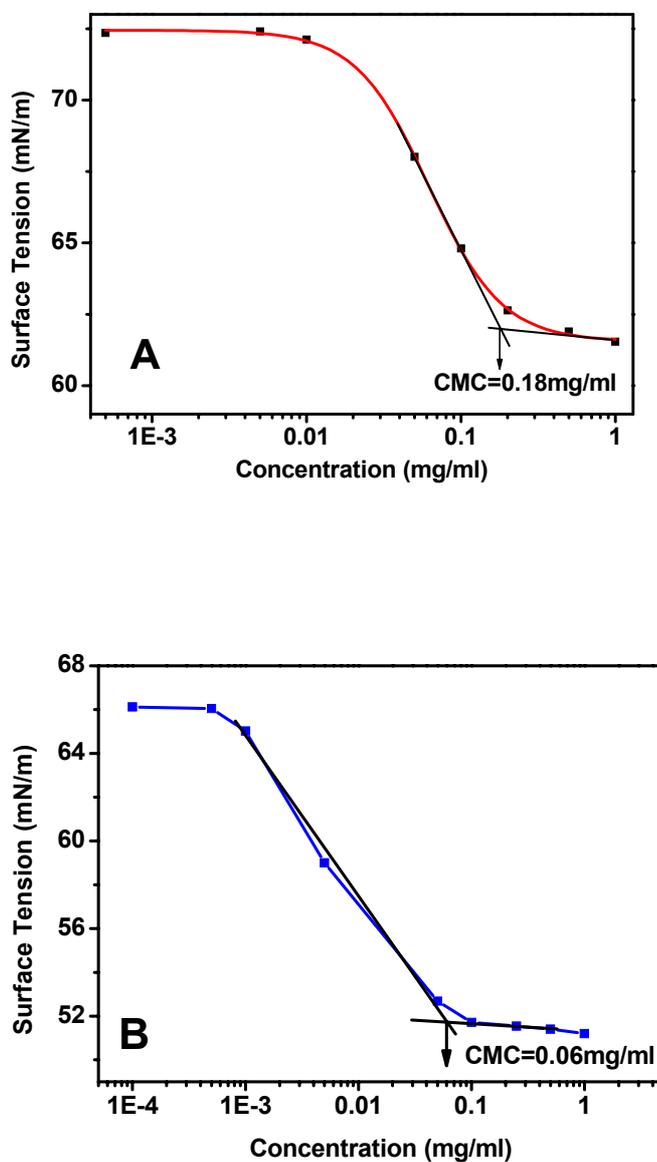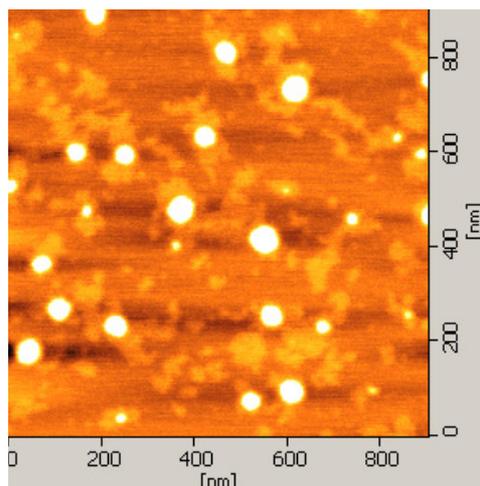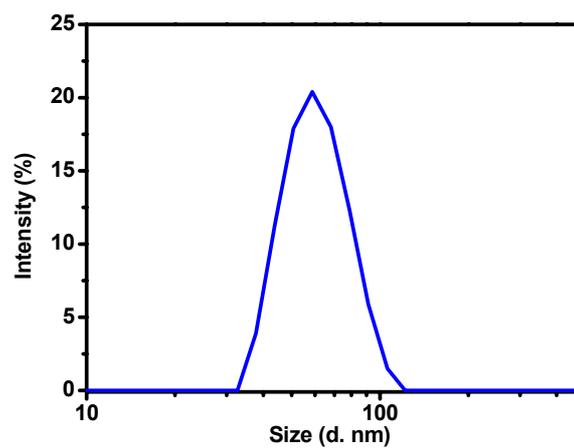

Figure S10. Relationship between concentration and surface tension for water dispersion of triblock copolymer at 25 °C (A) and 50 °C (B). The critical micelle concentration (CMC) can be obtained from the plots.

(a)

(b)

Figure S11. AFM image (a) and particle sized distribution obtained by using light scattering for PEO-*b*-PS-*b*-PSPMA triblock copolymer micelles.
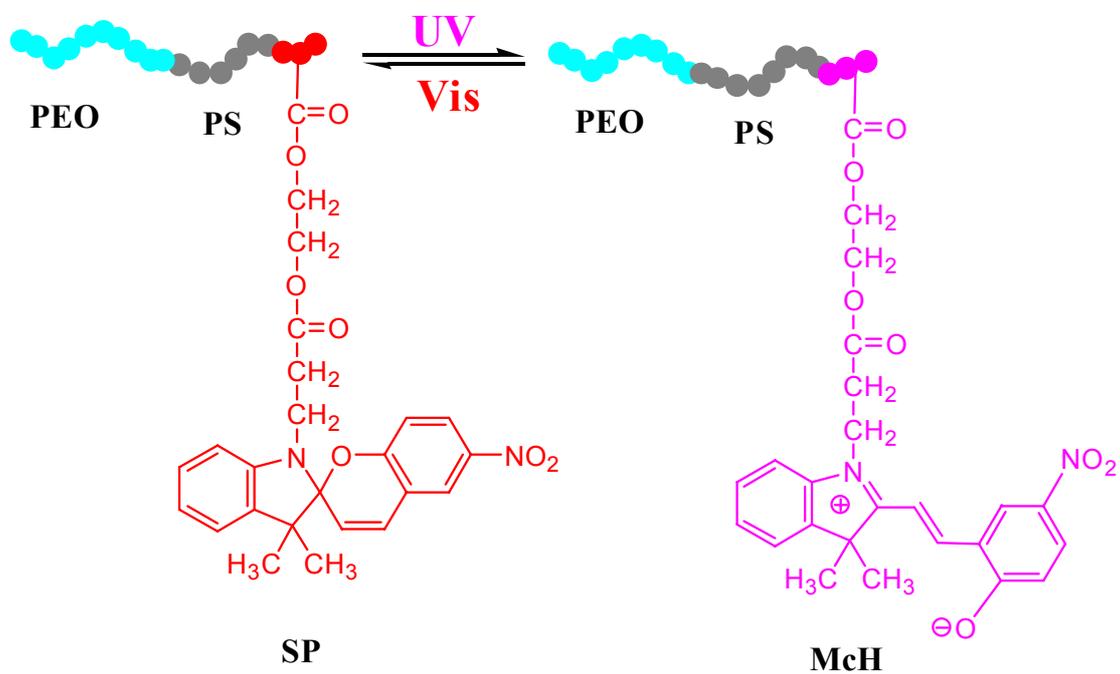
24

Figure S12. Light induced structural variation for spiropyran moieties in PEO-*b*-PS-*b*-PSPMA triblock copolymer micelles.
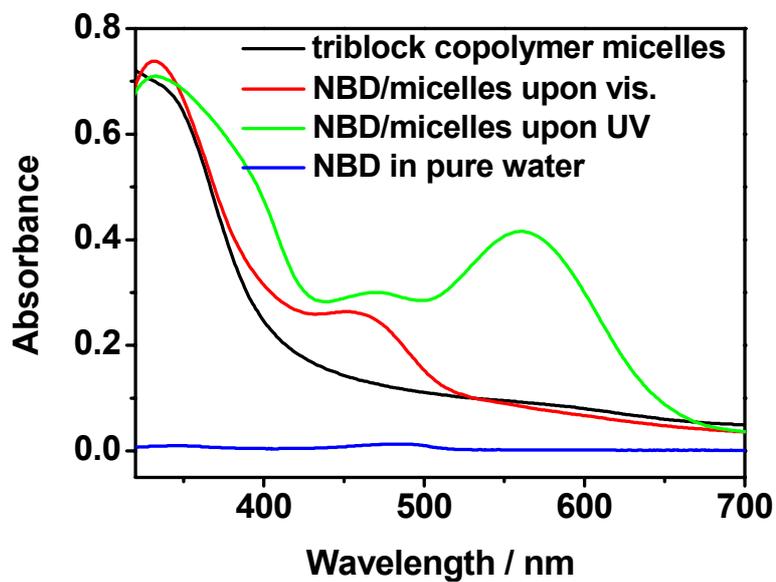
Figure S13. Absorption spectra for fluorescent dye in pure water and in PEO-*b*-PS-*b*-PSPMA micelle dispersion, and the determined concentration of micelles is 0.5mg/ml; the concentration of NBD in micelles is 1.27×10^{-5} mol/l; the NBD in pure water is saturated solution.
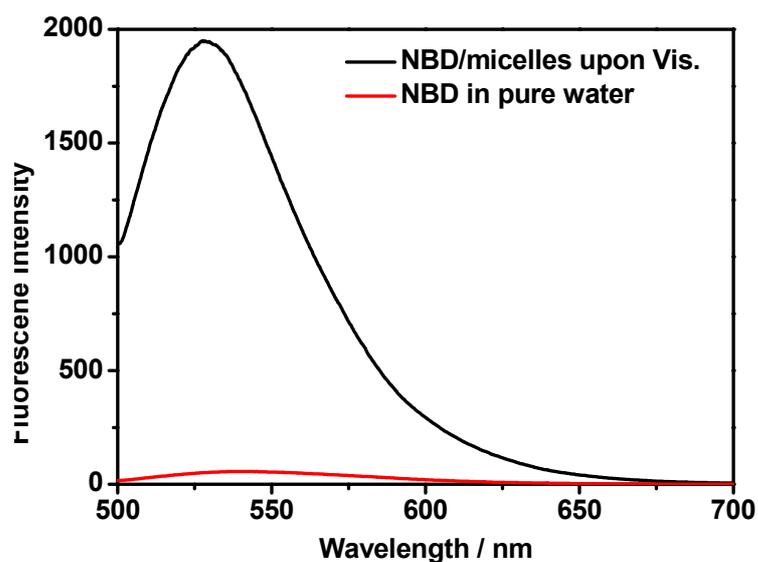
Figure S14. Fluorescence emission of NBD dye in pure water and incorporated in micelles. The fluorescence spectrum for NBD in pure water was recorded by using saturated water solution of NBD; the determined concentration of NBD in dispersion is 1.27×10^{-5} mol/l (excited at 490nm, 25°C).
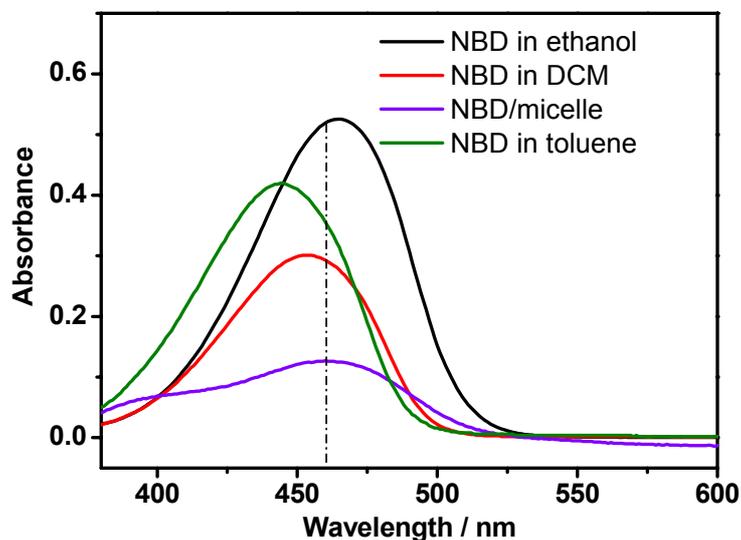
Figure S15. Absorption spectra for NBD dye in triblock copolymer micelle, in ethanol, in dichloromethane and in toluene solution. The absorption spectrum curve for NBD/micelle dispersion in this figure was adjusted by deducting the absorbance of the neat triblock copolymer micelles (with no NBD) in water to eliminate the light scattering effect of NBD/micelle system in water.

NBD dyes exhibit positive solvatochromism behaviour in environments with different solvent polarity (F. Galinier, F. Bertorelle and S. Fery-Forgues, *Comptes Rendus Acad Sci Ser II C*, 2001, **4**, 941.). The above absorption spectra show that, the absorbance maximum of NBD in the micelle system is between that of NBD in ethanol solution and in dichloromethane; however, the absorbance maximum for NBD in micelle exhibits 15 nm of red-shift compared with that of NBD in toluene which has similar polarity to polystyrene. This indicate that the micelle environment where NBD locates is a rather polar environment, thus we can say that the NBD dye can not enter into the PS core of very low polarity but locates in PEG corona, and most likely in the interfacial region of micelles, as the NBD in surfactant micelles (F. Galinier, F. Bertorelle and S. Fery-Forgues, *Comptes Rendus Acad Sci Ser II C*, 2001, **4**, 941.).
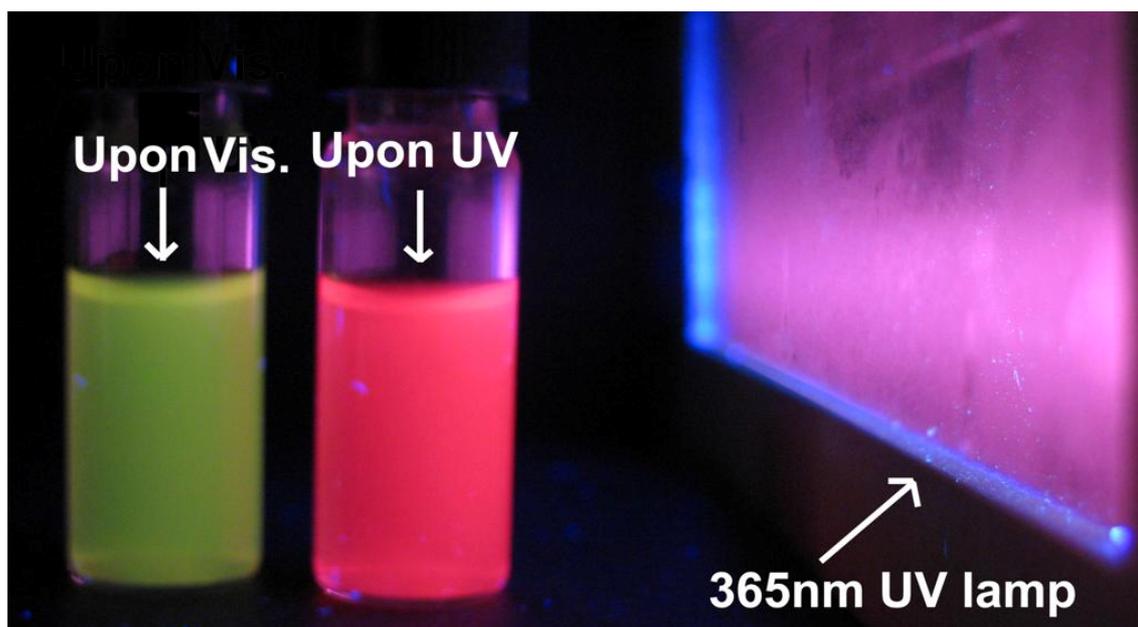
(a)
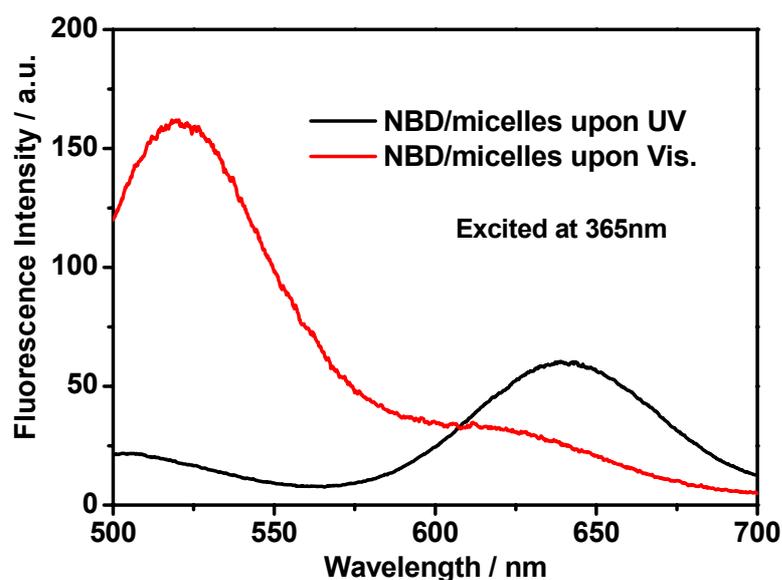
(b)

Figure S16. Photographs (a) and fluorescence spectra (b) for NBD/triblock copolymer micelle dispersion upon UV or visible light irradiation. Excited at 365nm, the dispersion still exhibits weaker fluorescence as shown in (b), thus we used 365nm light to excite the dispersion while taking photos, since the visible excitation light (490 nm) can interfere the appearance of the dispersion.

4. Calculation of Förster Critical Radius (R_0) [1,3,4]

Determination of fluorescence quantum yield of the donor NBD

The quantum yield can be described as follows:

$$\Phi_D = \Phi_S \times \frac{F_D}{F_S} \times \frac{A_S}{A_D} \times \frac{(n_D)^2}{(n_S)^2}$$

Where Φ_S is the fluorescence quantum yield of the standard (rhodamine B in ethanol, 0.65, 25°C) [2], F_D and F_S are the integral area of fluorescence intensity of the donor and the standard at the same excitation wavelength, respectively; A_D and A_S are the absorbance of the donor and the standard at the defined excitation wavelength, respectively; n_S and n_D are the refractive index at 25°C of the solvent of standard (ethanol) and the matrix of donor (mainly PS), respectively.
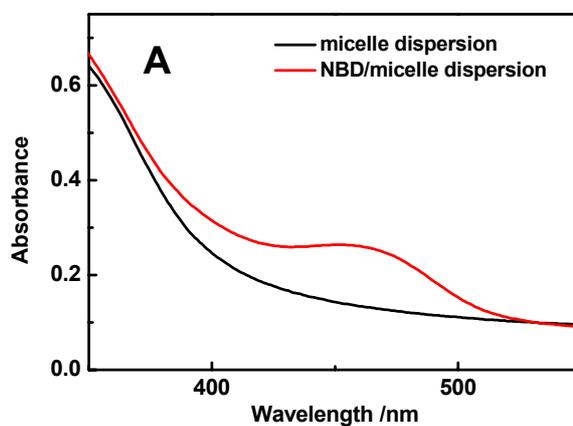

Figure S17. Absorption spectrum for dispersion of micelles and dispersion of NBD/ micelles. (NBD concentration: 1.27×10^{-5} mol/l).

To determine the quantum yield of NBD in micelle system, we first need to measure the

actual absorbance of NBD (at 490 nm) in micelle system. Since the NBD/micelle complex

dispersion exhibits relatively strong light scattering effect, to eliminate the error caused by

light scattering effect, we also measured the absorption spectrum for the micelle dispersion

(without NBD), and the actual absorbance value for NBD at 490nm can be obtained by the

deduction of the absorbance of neat micelle dispersion at 490nm from the apparent absorbance

value of NBD/micelle dispersion (Figure S17).

The Φ_D of NBD in micelle system was calculated to be 0.14.

Calculation of the Förster radii (R_0) [1,3,4] and determination of experimental energy transfer efficiency

The Förster's distance or critical distance R_0 is the characteristic distance, at which the

efficiency of energy transfer is 50%. The magnitude of R_0 is dependent on the spectral

properties of the donor and the acceptor molecules. If the wavelength λ is expressed in

nanometers, then $J(\lambda)$ is in units of $M^{-1}cm^{-1}nm^4$ and the Forster distance, R_0 in angstroms (Å),

is expressed as follows [Eq. (1)]:

$$R_0 = 0.2108 \times \left[\kappa^2 \times \Phi_D \times n^{-4} \times J(\lambda) \right]^{1/6} \qquad [\text{Eq. (1)}]$$

K^2 is the orientation factor for the emission and absorption dipoles and its value depends on

their relative orientation, n is the refractive index of the medium and Φ_D is the quantum yield

of the donor. $J(\lambda)$ is the overlap integral of the fluorescence emission spectrum of the donor

and the absorption spectrum of the acceptor (Figure S18) [Eq. (2)].

$$J(\lambda) = \int_0^\infty F_D(\lambda) \times \varepsilon_A(\lambda) \times \lambda^4 \times d\lambda \quad [\text{Eq. (2)}]$$

$F_D(\lambda)$ is the fluorescence intensity of the donor in the absence of acceptor normalized so

that $\int_0^\infty F_D(\lambda)d\lambda = 1$; $\varepsilon_A(\lambda)$ is molar extinction coefficient of the acceptor, λ is wavelength. In

current experimental conditions, for NBD/micelle system, the $J(\lambda)$ was calculated to be

3.54×10^{14} $M^{-1}cm^{-1}nm^4$. The Förster distance (R_0) has been calculated assuming random

orientation of the donor and acceptor molecules taking $K^2 = 2/3$, $n = 1.59$ (PS), and $\Phi_D = 0.14$.

For NBD (donor) and McH form of spiropyran (acceptor) in current experimental

situation, by using a commercial software Origin 7.0 as the integral tool, we calculated R_0

$= 27.9$Å. Energy transfer will be effective for 14.0 Å $\leq d \leq$ 41.9 Å ($R_0 \pm 50\% R_0$) [5].
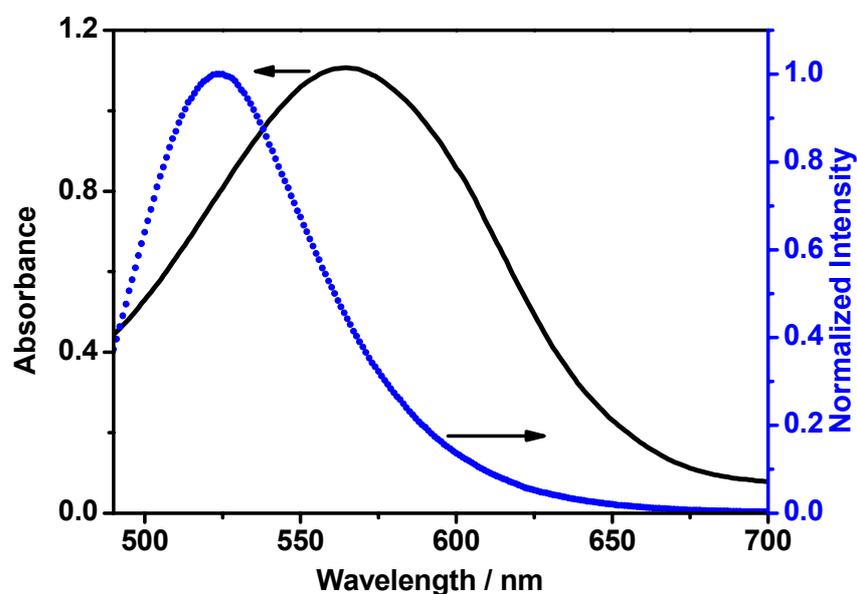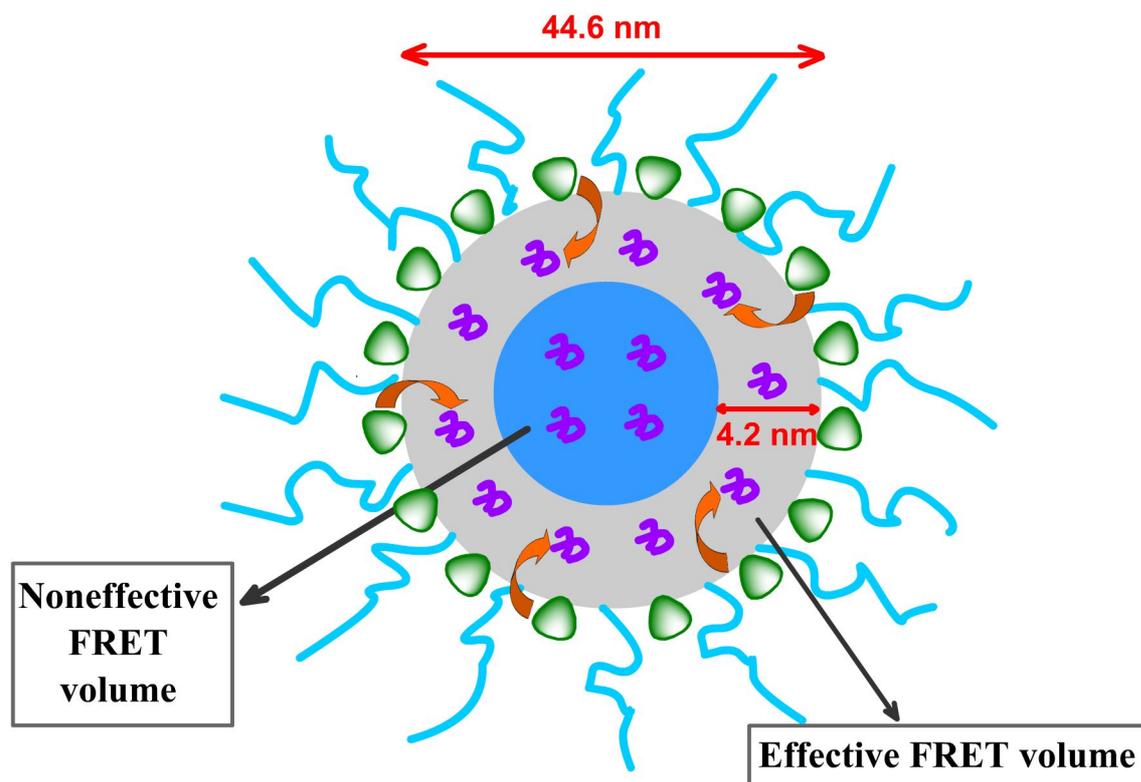

Figure S18. Fluorescence spectrum of NBD in micelle system (donor), and absorption

spectrum of spiropyran-containing triblock copolymer micelle (acceptor) in water (without

NBD) (with the light scattering effect eliminated).

Scheme S2. Illustration for effective and noneffective FRET volume in a micelle core.

For the core-corona micelles, we cannot access to small-angle neutron scattering approach for the determination of the core radius. However, based on the average overall diameter (51 nm) of micelles in dried state obtained by AFM, the weight ratio of hydrophilic block to hydrophobic blocks in a triblock copolymer, as well as the density of PEO and PS, we estimated the averaged core diameter as 44.6 nm by assuming the hydrophobic blocks were densely packed in water. Therefore, the effective volume accounts for about 47% of total core volume. FRET process can take place from fluorescent dye molecules to the spirpopyran moieties in the effective volume.

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