Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting information

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

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Materials

Two-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA), oligo(ethylene glycol) methacrylate (OEGMA, M_n = 500 g/mol), and 2-(Diisopropylamino)ethyl methacrylate (DP) were purchased from Sigma-Aldrich and purified by passing through basic alumina column. Six-acetylthiohexyl acrylate (ATA) was synthesized and purified according to the protocol given in previous reports (ref. 6). All other chemicals and solvents were used as received. Distilled water used in this study was purified with a Millipore Milli-Q system.

Characterizations

^1H NMR spectra of copolymers were taken with a JNM-GSX300 spectrometer operating at 300 MHz (JEOL, Tokyo, Japan) to confirm successful synthesis and determine the chemical composition of the synthesized copolymers.

Molecular weight and polydispersity of the synthesized copolymers were determined by gel permeation chromatography (GPC) at 40 °C (DMF including 10mM LiBr, 1 mL/min) with a TOSOH TSK-GEL a-2500 and a-4000 and (Tosoh, Tokyo, Japan) connected to a RI-2031 refractive index detector (JASCO International Co., Ltd., Tokyo, Japan).

Transmittance/absorbance of a solution at 500 nm was continuously recorded at a heating rate of 1.0 °C/min by a UV-Vis spectrometer V-550 (JASCO International Co., Ltd., Tokyo, Japan). Synthesized copolymers were dissolved in aqueous solution at the given concentration.

Dynamic light scattering (DLS) was performed with a DLS-8000 seriese (Otsuka Electronics Co., Ltd., Osaka, Japan) using a light scattering apparatus equipped with He-Ne laser and temperature controller. All samples were kept at given temperatures to reach the equilibrium prior to the measurements. We obtained the diameter data using the Marquardt method (cumulative number = 80).

Atomic force microscope (AFM) measurements were performed using the SPM-9500J3 (Shimadzu Co., Kyoto, Japan) in non-contact mode using Si_3N_4 cantilever (spring constant; 42 N/m). A substrate of silicon wafer was washed with ethanol and water before use. The micelles were dropped on the silicon wafers by spin-coating method.

Fluorescence spectra were recorded using a Fluorescence spectrometer F-2500 (Hitachi High-Technologies Corporation, Tokyo, Japan).

Preparation of P(MEO$_2$MA-*co*-X)s (X = OEGMA, ATA, and DP) at different contents

P(MEO$_2$MA-*co*-OEGMA)s, P(MEO$_2$MA-*co*-ATA), and P(MEO$_2$MA-*co*-DP) were prepared at different contents (5, 10, and 15 mol%) by reversible addition-fragmentation chain transfer (RAFT) polymerization. A typical polymerization method of P(MEO$_2$MA-*co*-OEGMA) (OEGMA: 10 mol% in feed) was shown below. MEO$_2$MA (0.90 g, 4.78 mmol), OEGMA (0.27 g, 0.53 mmol), 4-Cyanopentanoic acid dithiobenzoate (CTP) (7.42 mg, 2.66×10^{-2} mmol), and 4,4'-azobis-4-cyanovaleric acid (ACVA) (2.98 mg, 1.06×10^{-2} mmol) ($[MEO_2MA]_0/[OEGMA]_0/[CTP]_0/[ACVA]_0 = 180/20/1/0.4$) were dissolved in 4 mL methanol. After degassing with nitrogen gas for 30 min, the mixture was allowed to polymerize for 6 h at 60 °C. The polymerization container was soaked into liquid nitrogen to stop the reactions. The resulting P(MEO$_2$MA-*co*-OEGMA) was purified by reprecipitation using diethyl ether and was dried under reduced pressure.

These copolymers were used for estimation of LCSTs of 4-componential copolymers of P(MEO$_2$MA-*co*-OEGMA-*co*-ATA-*co*-DP)s. The characterizations (compositions, M_n, M_w/M_n, ^{1}H NMR, and the relationships between LCSTs and contents of X) of the P(MEO$_2$MA-*co*-X)s are shown in Table S1 and Fig. S1~8.

Table S1 Characterization of Copolymers

	In feed			In copolymer^b				
Monomer numbers^a	OEGMA (mol%)	ATA (mol%)	DP (g/mol)	OEGMA (mol%)	ATA (mol%)	DP (g/mol)	M_n^{c} (g/mol)	M_w/M_n^{c} (-)
P(MEO$_2$MA$_{84}$)	-	-	-	-	-	-	16100	1.18
P(MEO$_2$MA$_{77}$-*co*-OEGMA$_9$)	10	-	-	10.8	-	-	19400	1.25
P(MEO$_2$MA$_{71}$-*co*-OEGMA$_{19}$)	20	-	-	21.3	-	-	23100	1.27
P(MEO$_2$MA$_{62}$-*co*-OEGMA$_{27}$)	30	-	-	30.3	-	-	25400	1.28
P(MEO$_2$MA$_{88}$-*co*-ATA$_3$)	-	5	-	-	3.0	-	17400	1.30
P(MEO$_2$MA$_{82}$-*co*-ATA$_6$)	-	10	-	-	6.9	-	17100	1.31
P(MEO$_2$MA$_{74}$-*co*-ATA$_8$)	-	15	-	-	9.3	-	15900	1.27
P(MEO$_2$MA$_{86}$-*co*-DP$_4$)	-	-	5	-	-	4.1	17200	1.21
P(MEO$_2$MA$_{82}$-*co*-DP$_7$)	-	-	10	-	-	8	17200	1.29
P(MEO$_2$MA$_{69}$-*co*-DP$_9$)	-	-	15	-	-	11.3	15200	1.28

[a] The monomer numbers were calculated by GPC and ^{1}H NMR. [b] Determined by ^{1}H NMR.

[c] Determined by GPC using 10 mM LiBr DMF.

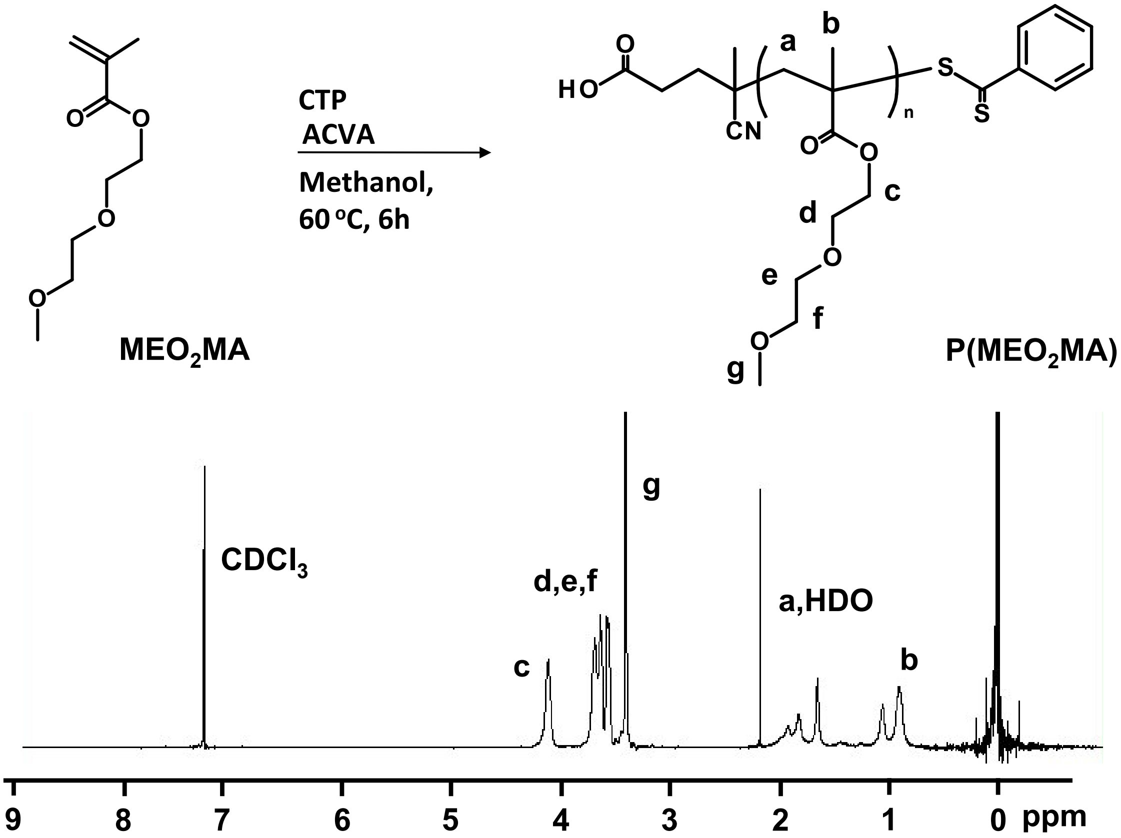


Fig. S1 ^{1}H NMR spectra of P(MEO$_2$MA).

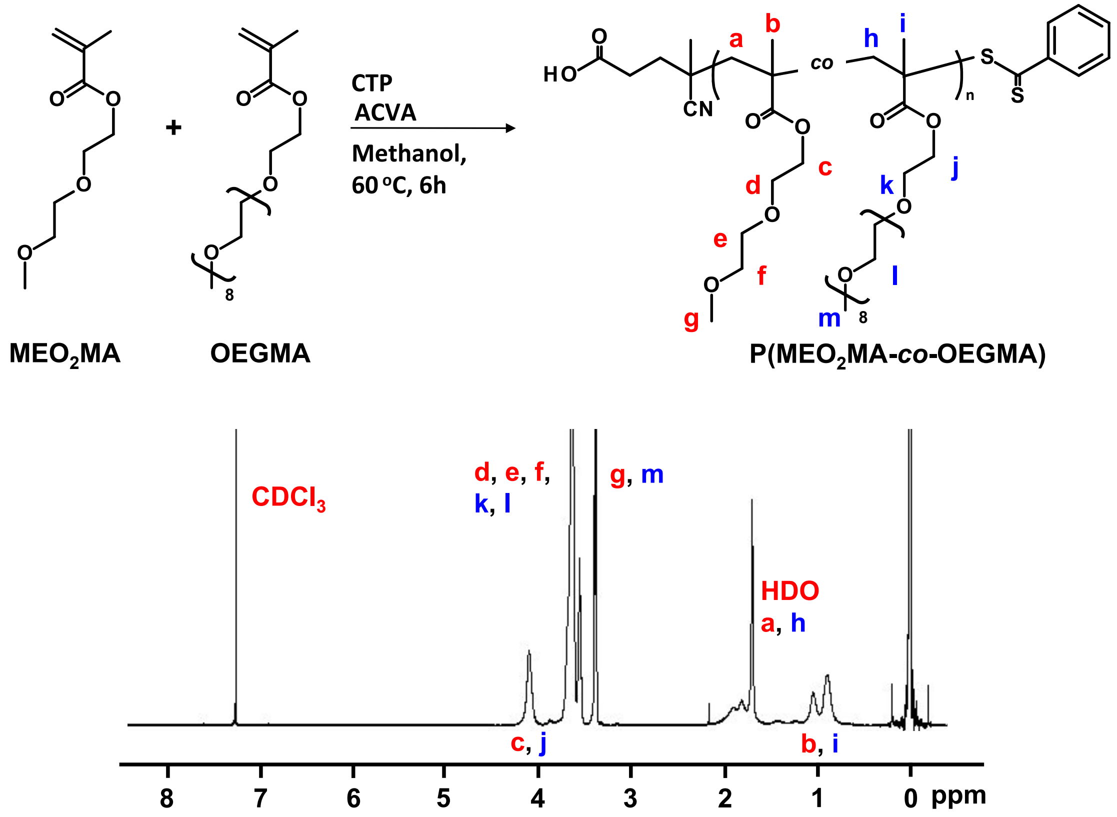


Fig. S2 ^{1}H NMR spectra of P(MEO_2MA_{62}-*co*-$OEGMA_{27}$).

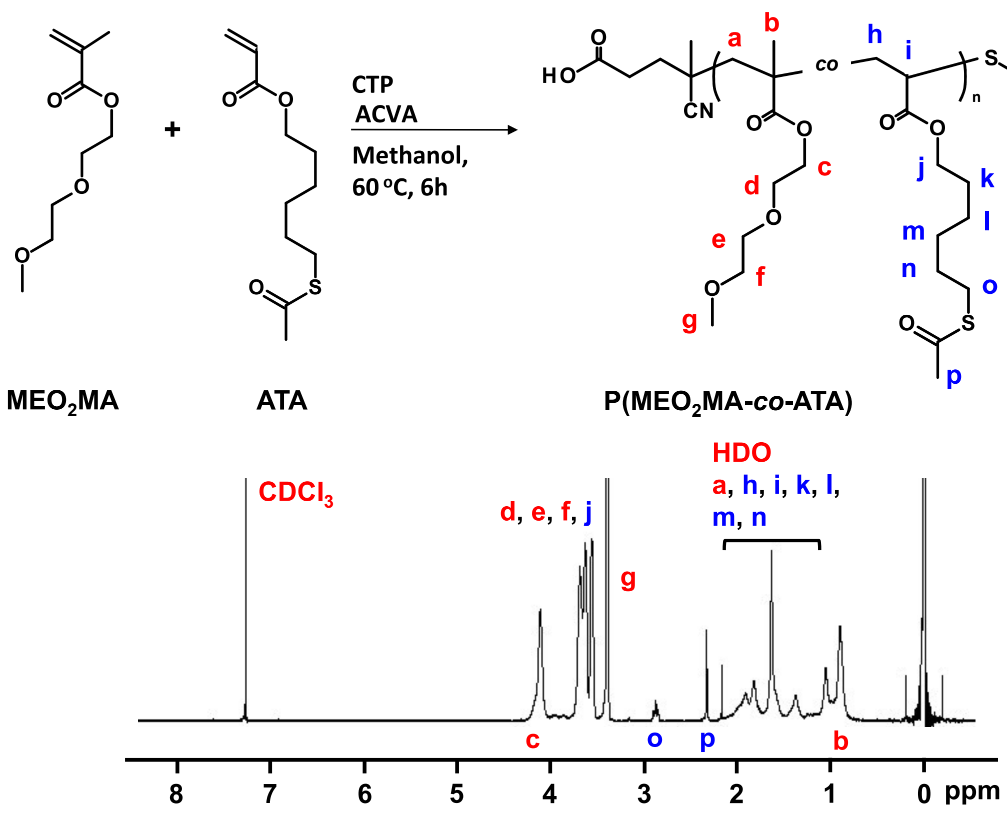


Fig. S3 ^{1}H NMR spectra of P(MEO_2MA_{74}-*co*-ATA_8).

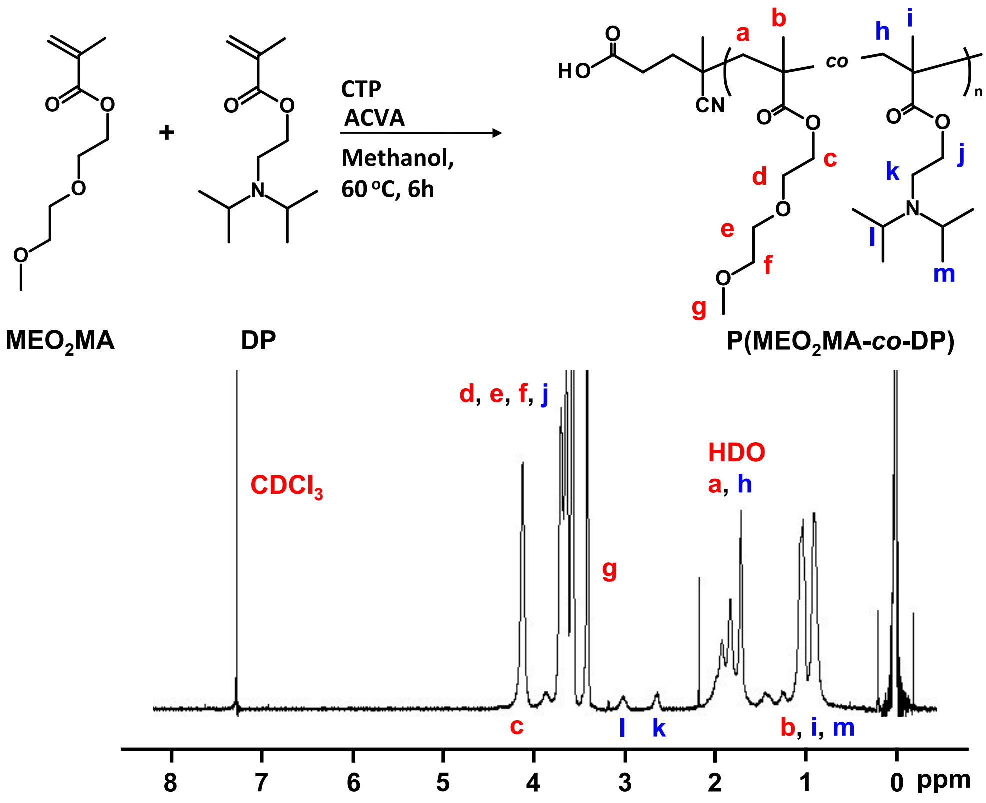


Fig. S4 ^{1}H NMR spectra of P(MEO$_2$MA$_{69}$-*co*-DP$_9$).

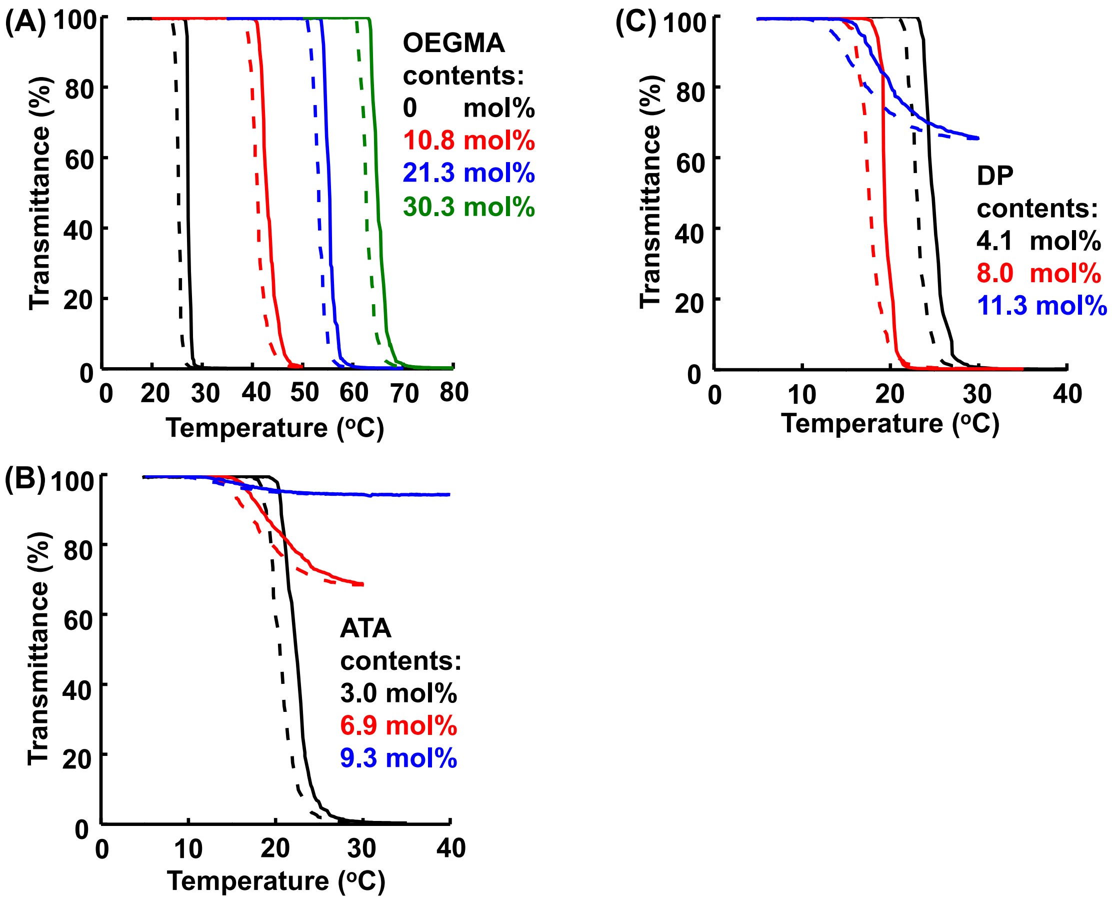


Fig. S5 Transmittance change of 0.1 wt% (A) P(MEO_2MA-*co*-OEGMA)s, (B) P(MEO_2MA-*co*-ATA)s, and (C) P(MEO_2MA-*co*-DP)s in milliQ as a function of temperature. Solid line: heating and dot line: cooling at 1.0 °C/min.

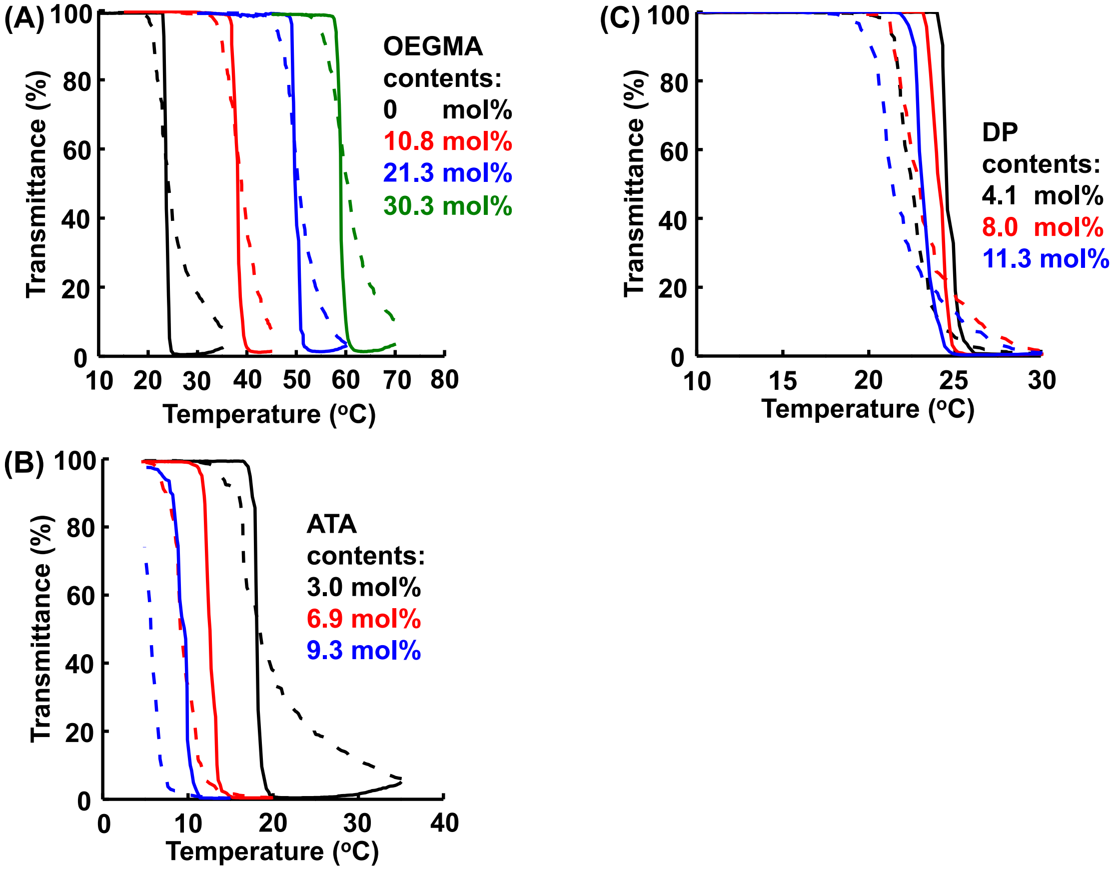


Fig. S6 Transmittance change of 0.1 wt% (A) P(MEO$_2$MA-*co*-OEGMA)s, (B) P(MEO$_2$MA-*co*-ATA)s, and (C) P(MEO$_2$MA-*co*-DP)s in pH 7.4 PBS as a function of temperature. Solid line: heating and dot line: cooling at 1.0 °C/min.

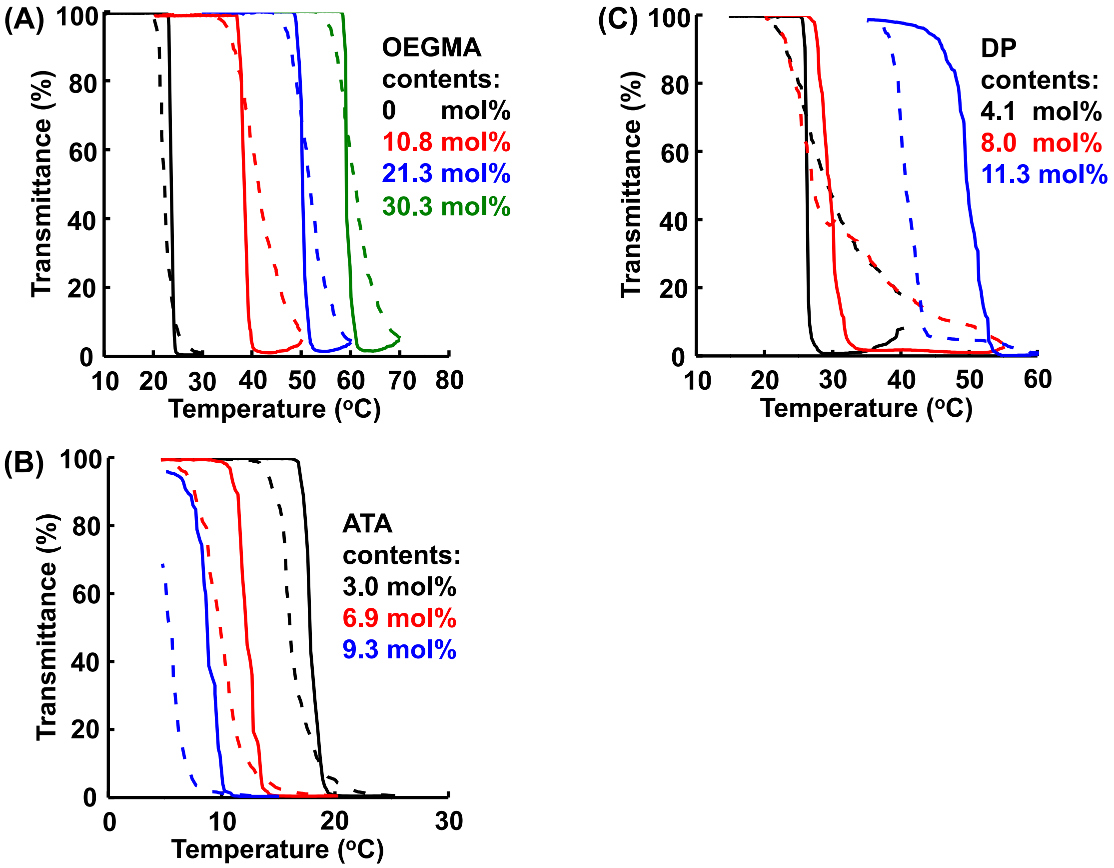


Fig. S7 Transmittance change of 0.1 wt% (A) P(MEO$_2$MA-*co*-OEGMA)s, (B) P(MEO$_2$MA-*co*-ATA)s, and (C) P(MEO$_2$MA-*co*-DP)s in pH 5.5 CH$_3$COOH/CH$_3$COONa buffer solution (adjusted 150 mM by NaCl) as a function of temperature. Solid line: heating and dot line: cooling at 1.0 °C/min.

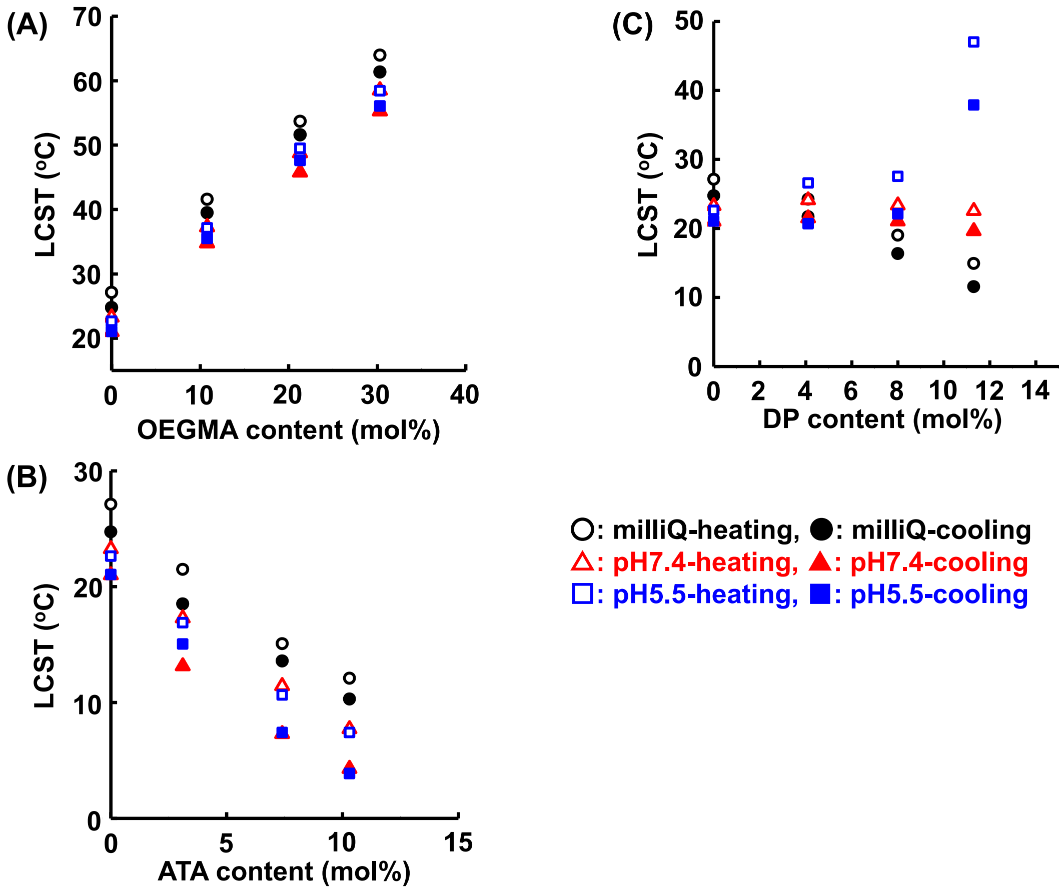


Fig. S8 LCSTs of 0.1 wt% (A) P(MEO$_2$MA-*co*-OEGMA)s, (B) P(MEO$_2$MA-*co*-ATA)s, and (C) P(MEO$_2$MA-*co*-DP)s in (circle) milliQ, (triangle) pH7.4 PBS, and (square) pH 5.5.

Deprotection of P(MEO_2MA-*co*-ATA)s for thiol groups.

Deprotection of ATA in the copolymers was made according to the protocol given in previous reports (Ref. 6). A typical method of deprotection was shown below. P(MEO_2MA_{82}-*co*-ATA_6) (100 mg, 4.33×10^{-4} mmol) was dissolved in 2 mL methanol. Propylamine (4.45 mg, 7.53×10^{-2} mmol) and dithiotheitol (3.87×10^{-2} mg, 2.51×10^{-4} mmol) were dissolved in 2 mL methanol. After degassing with nitrogen gas for 20 min, solutions were mixed by syringe work and the mixture was allowed to react for 2 h at 50 °C. The resulting copolymer was purified by reprecipitation using degassed diethyl ether. After removing the diethyl ether, small amount of acetone was added to the synthesis tube to collect the sticky copolymer. The solution of the copolymer was replaced to sample tube and was dried under pressure. The sample was stored with oxygen scavenger (ageless) to prevent the construction of S-S bond. The efficiency of the deprotection of ATA was 36.7 % (calculated by ^{1}H NMR in Fig. S9) and was controlled by the amount of reducing materials and reactive condition (e.g. it reached to be 67.7 % using 3 times higher amount of propylamine/dithiotheitol, reaction time 4h).

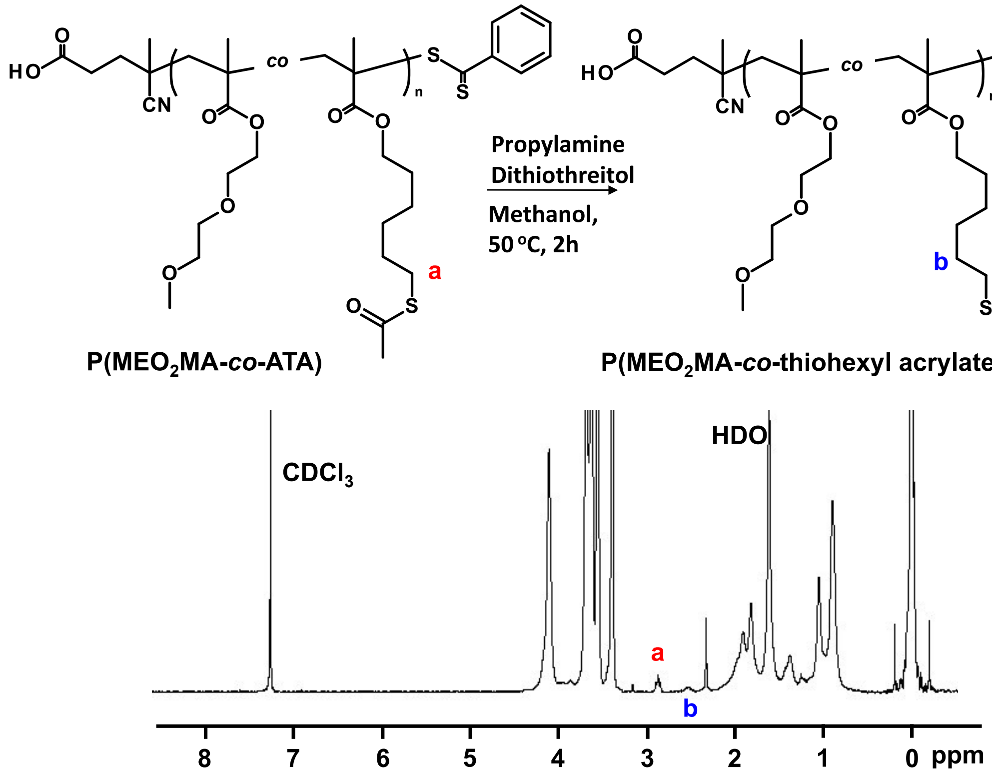
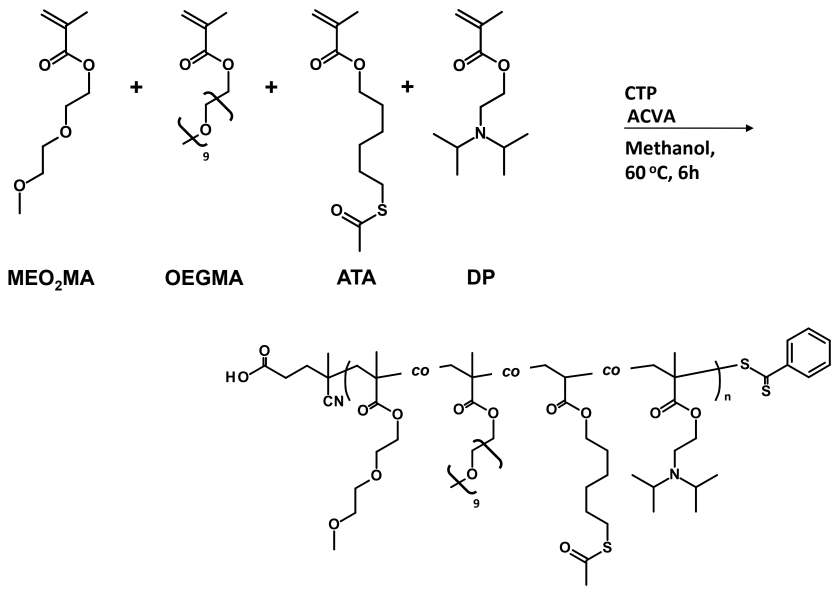


Fig. S9 Deprotection of of P(MEO$_2$MA$_{82}$-*co*-ATA$_7$).

Preparation of 4-componential copolymer of (MEO_2MA-*co*-OEGMA-*co*-ATA-*co*-DP) (4C) and block copolymer of P(MEO_2MA-*co*-OEGMA)-*b*-P(MEO_2MA-*co*-OEGMA-*co*-ATA-*co*-DP) (2C*b*4C)

Four-componential copolymers of (MEO_2MA-*co*-OEGMA-*co*-ATA-*co*-DP) (4C) having different chain length (4C-long and 4C-short) were prepared as shown below. The compositions of the monomers were designed from the results of Fig. S5~8 to show the LCST at 35 °C in PBS. For preparation of 4C-long, MEO_2MA (0.60 g, 3.19 mmol), OEGMA (0.27 g, 0.53 mmol), ATA (0.18 g, 0.80 mmol), DP (0.17 g, 0.80 mmol), CTP (7.42 mg, 2.66×10^{-2} mmol), and 4,4'-azobis-4-cyanovaleric acid (ACVA) (2.98 mg, 1.06×10^{-2} mmol) ($[MEO_2MA]_0/[OEGMA]_0/[ATA]_0/[DP]_0/[CTP]_0/[ACVA]_0$ = 120/20/30/30/1/0.4) were dissolved in 4 mL methanol. After degassing with nitrogen gas for 30 min, the mixture was allowed to polymerize for 6 h at 60 °C. The copolymer of 4C-long was purified by same condition with above copolymers (Scheme S1 and Fig. S10). The 4C-short was prepared using same condition with 4C-long but half amount of the monomers.



P(MEO$_2$MA-*co*-OEGMA-*co*-ATA-*co*-DP) (Macro-CTA)

Scheme S1 Preparation of P(MEO$_2$MA-*co*-OEGMA-*co*-ATA-*co*-DP) (4C-Short or 4C-Long).

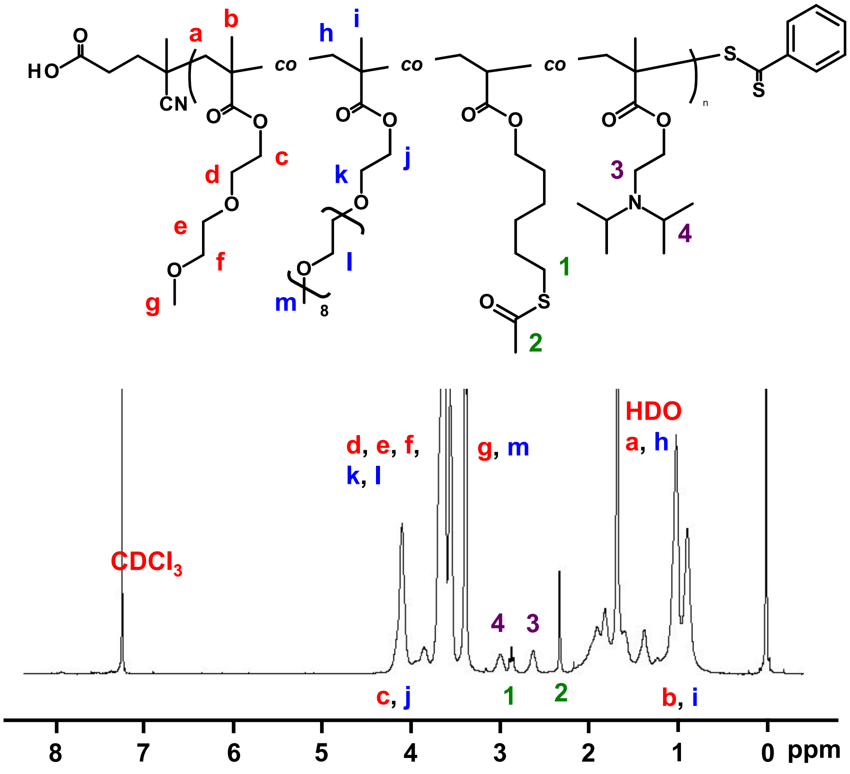
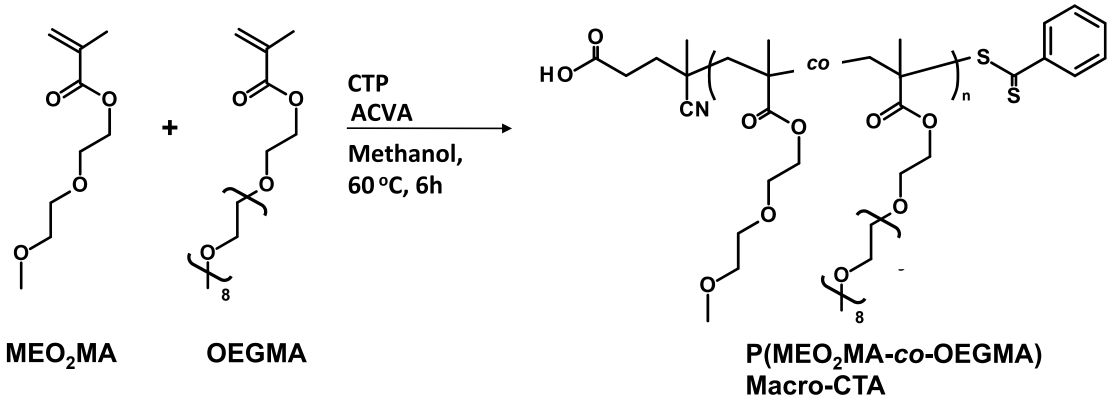
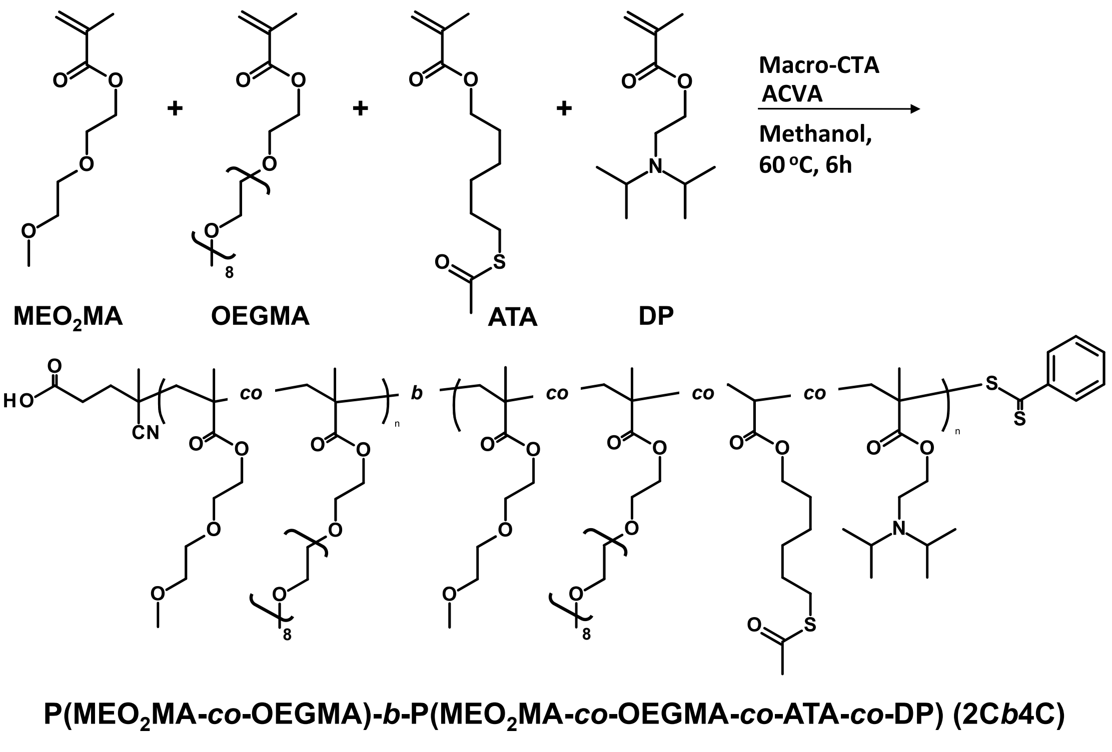


Fig. S10 NMR spectra of P(MEO$_2$MA$_{33}$-*co*-OEGMA$_5$-*co*-ATA$_4$-*co*-DP$_5$).

The Block copolymer of P(MEO_2MA-*co*-OEGMA)-*b*-P(MEO_2MA-*co*-OEGMA-*co*-ATA-*co*-DP) (2C*b*4C) was prepared having exactly same composition of 4C block with above copolymers of 4C (Scheme S2 and S3). The P(MEO_2MA-*co*-OEGMA) was first polymerized as macro-chain transfer agent (macro-CTA). MEO_2MA (3.16 g, 16.8 mmol), OEGMA (3.42 g, 7.20 mmol), CTP (27.9 mg, 0.10 mmol), and ACVA (11.0 mg, 4.40×10^{-2} mmol) ($[MEO_2MA]_0/[OEGMA]_0/[CTP]_0/[ACVA]_0$ = 168/72/30/30/1/0.4) were dissolved in 10 mL methanol. After degassing with nitrogen gas for 30 min, the mixture was allowed to polymerize for 6 h at 60 °C. The copolymer was purified by same condition above. Using the polymerized P(MEO_2MA-*co*-OEGMA) (2C) as macro-CTAs, block copolymer of 2C*b*4C was prepared. Macro-CTA of 2C (0.52 g, 2.66×10^{-2} mmol), MEO_2MA (0.60 g, 3.19 mmol), OEGMA (0.27 g, 0.53 mmol), ATA (0.18 g, 0.80 mmol), DP (0.17 g, 0.80 mmol), and ACVA (8.85 mg, 1.06×10^{-3} mmol) ($[MEO_2MA]_0/[OEGMA]_0/[ATA]_0/[DP]_0/[macro\text{-}CTP]_0/[ACVA]_0$ = 120/20/30/30/1/0.3) were dissolved in 8 mL methanol. After degassing with nitrogen gas for 30 min, the mixture was allowed to polymerize for 6 h at 60 °C. The block copolymer of 2C*b*4C was purified by same condition with above copolymers (Table S2 and Fig. S11). The order of polymerized blocks was very important. Interestingly, when the block copolymer was prepared from 4C block (*i.e.* 4C*b*2C), we could not obtain the block copolymer with fine structure (Table S3).



Scheme S2 Preparation of P(MEO_2MA-*co*-OEGMA) as macro-CTA for block copolymers (2C*b*4C).



Scheme S3 Preparation of P(MEO_2MA-*co*-OEGMA)-*b*-P(MEO_2MA-*co*-OEGMA-*co*-ATA-*co*-DP) (2C*b*4C).

Table S2 Characterization of copolymers (4C-Short, 4C-Long, and 2C*b*4C)

	P(MEO$_2$MA-*co*-OEGMA) block		P(MEO$_2$MA-*co*-OEGMA-co-ATA-co-DP) block							
	In feed	In copolymer	In feed			In copolymer				
Monomer numbers[a]	OEGMA (mol%)	OEGMA (mol%)	OEGMA (mol%)	ATA (mol%)	DP (mol%)	OEGMA (mol%)	ATA (mol%)	DP (mol%)	M_n[c] (g/mol)	M_w/M_n[c] (-)
P(MEO$_2$MA$_{48}$-*co*-OEGMA$_{20}$)	30	29.7	-	-	-	-	-	-	19400	1.16
P(MEO$_2$MA$_{48}$-*co*-OEGMA$_{20}$)-*b*-P(MEO$_2$MA$_{25}$-*co*-OEGMA$_4$-*co*-ATA$_3$-*co*-DP$_4$)	30	29.7	10	15	15	9.8	9.6	12.1	27600	1.31
P(MEO$_2$MA$_{33}$-*co*-OEGMA$_5$-*co*-ATA$_4$-*co*-DP$_5$)	-	-	10	15	15	11.0	8.6	10.2	10900	1.36
P(MEO$_2$MA$_{17}$-*co*-OEGMA$_2$-*co*-ATA$_3$-*co*-DP$_2$)	-	-	10	15	15	9.2	11.3	9.4	5800	1.35

[a] The monomer numbers were calculated by GPC and ^{1}H NMR. [b] Determined by ^{1}H NMR. [c] Determined by GPC using 10 mM LiBr DMF.

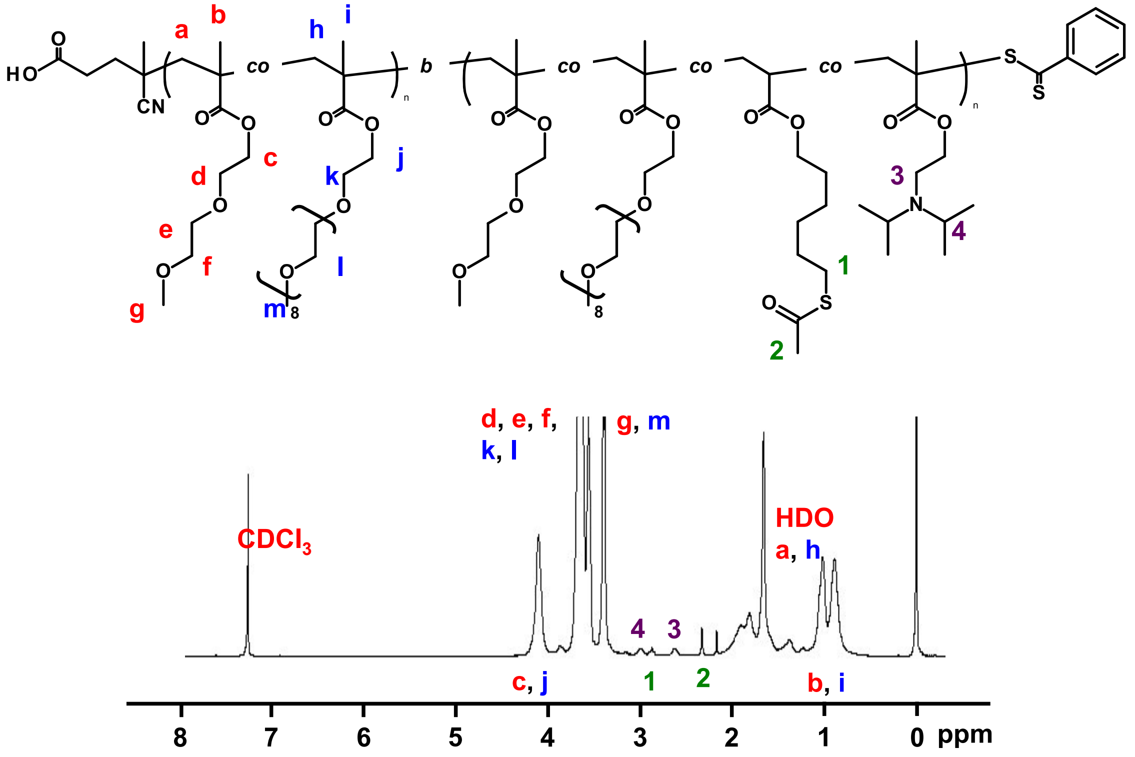


Fig. S11 ^{1}H NMR spectra of P(MEO$_2$MA$_{48}$-*co*-OEGMA$_{20}$)-*b*-P(MEO$_2$MA$_{25}$-*co*-OEGMA$_4$-*co*-ATA$_3$-*co*-DP$_4$).

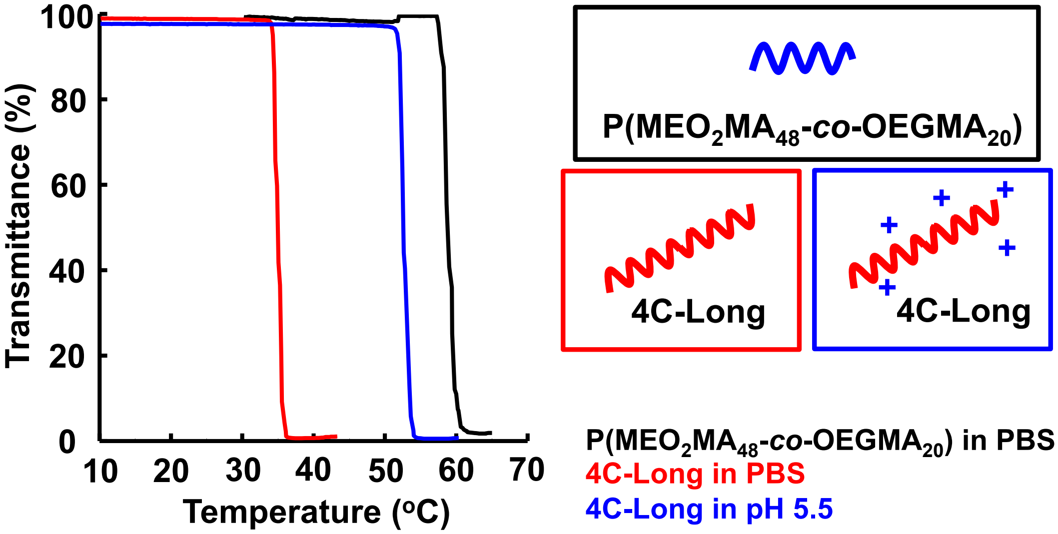


Fig. S12 (A) Transmittance change of 0.1 wt% of P(MEO_2MA_{48}-*co*-$OEGMA_{20}$) (black line: in pH 7.4 PBS) and P(MEO_2MA_{33}-*co*-$OEGMA_5$-*co*-ATA_4-*co*-DP_5) (red line: in pH 7.4 PBS, blue line: in pH 5.5) as a function of temperature.

Table S3 Characterization of block copolymers prepared from 4C block

	P(MEO_2MA-*co*-OEGMA-*co*-ATA-*co*-DP) block						P(MEO_2MA-*co*-OEGMA) block			
	In feed			In copolymer			In feed	In copolymer		
Monomer numbers[a]	OEGMA (mol%)	ATA (mol%)	DP (g/mol)	OEGMA (mol%)	DP (mol%)	ATA (g/mol)	OEGMA (mol%)	OEGMA (mol%)	M_n[c] (g/mol)	M_w/M_n[c] (-)
P(MEO_2MA_{67}-*co*-$OEGMA_{15}$-*co*-ATA_6-*co*-DP_{10})	15	10	15	15.2	5.8	10.6	-	-	23900	1.26
P(MEO_2MA_{67}-*co*-$OEGMA_{15}$-*co*-ATA_6-*co*-DP_{10})-*b*-POEGMA-1	15	10	15	15.2	5.8	10.6	100	100	Gel	-
P(MEO_2MA_{67}-*co*-$OEGMA_{15}$-*co*-ATA_6-*co*-DP_{10})-*b*-POEGMA-2	15	10	15	15.2	5.8	10.6	100	100	multi peaks	-
P(MEO_2MA_{67}-*co*-$OEGMA_{15}$-*co*-ATA_6-*co*-DP_{10})-*b*-POEGMA-3	15	10	15	15.2	5.8	10.6	100	100	25000	1.33
P(MEO_2MA_{67}-*co*-$OEGMA_{15}$-*co*-ATA_6-*co*-DP_{10})-*b*-P(MEO_2MA-*co*-OEGMA)	15	10	15	15.2	5.8	10.6	60	-	multi peaks	-

[a]The monomer numbers were calculated by GPC and ^{1}H NMR. [b]Determined by ^{1}H NMR.

[c]Determined by GPC using 10 mM LiBr DMF.

Preparation of mixed nanoparticles consisting of 4C-Short (or 4C-Long) and 2C*b*4C

The mixed nanoparticles were prepared using a mixture of solution of 4C-Short (or 4C-Long) and 2C*b*4C. The copolymers were dissolved in pH 7.4 PBS at 4 ^{o}C to be 0.5 wt% of total concentration at different mixture of ratios. The solution of copolymers was filtered at 4 ^{o}C using a 0.45 μm disposable filter (Millipore). Then, the temperature of solution was increased from 15 to 40 ^{o}C (increase of 5 ^{o}C for every 10 min.).

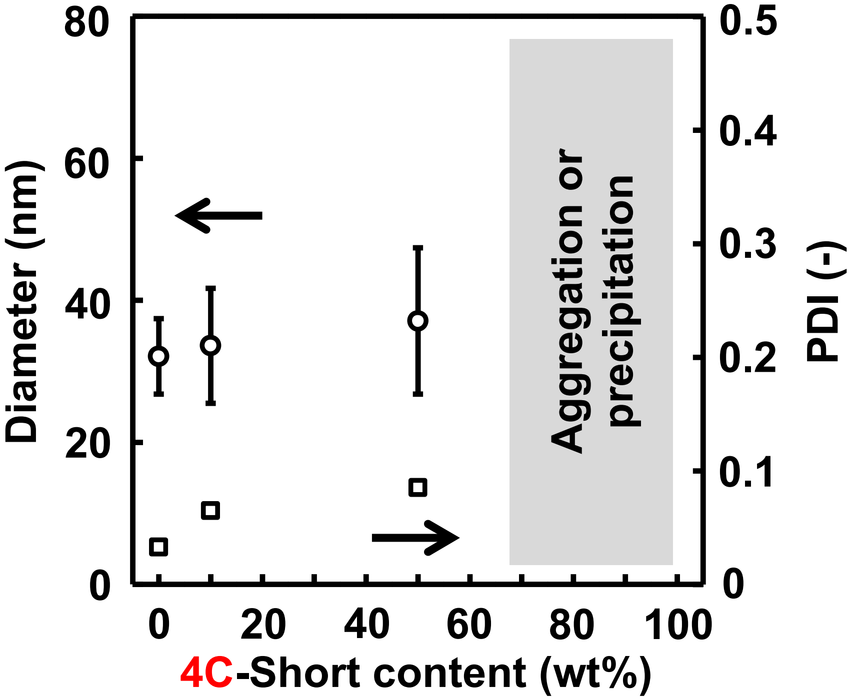
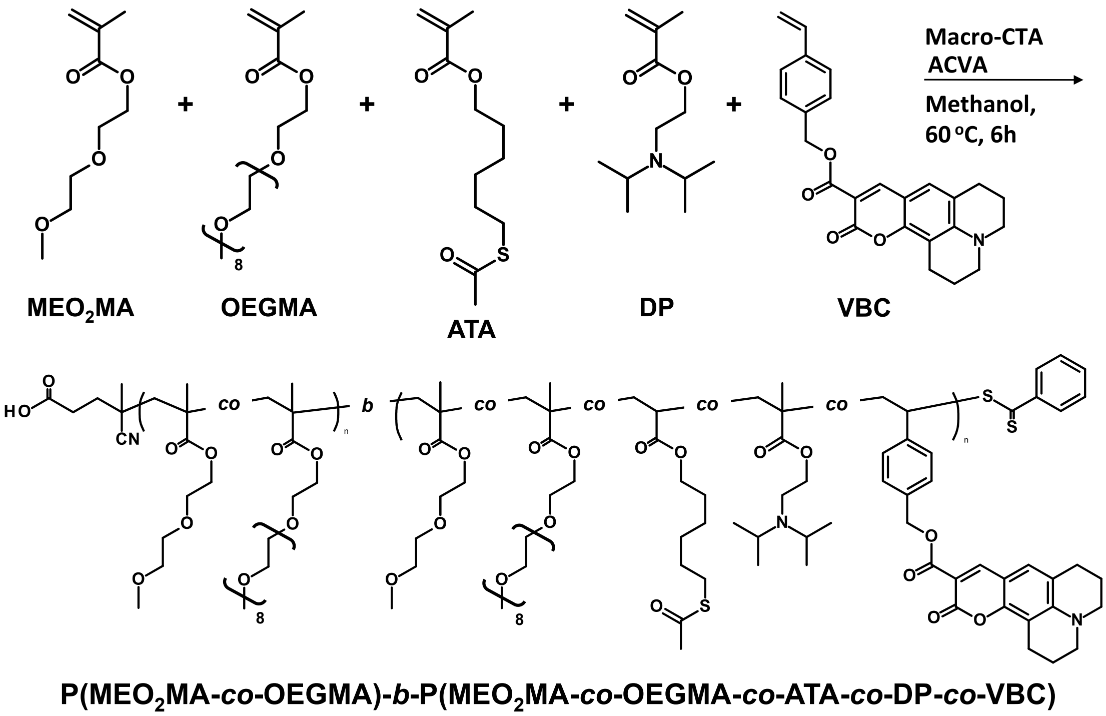
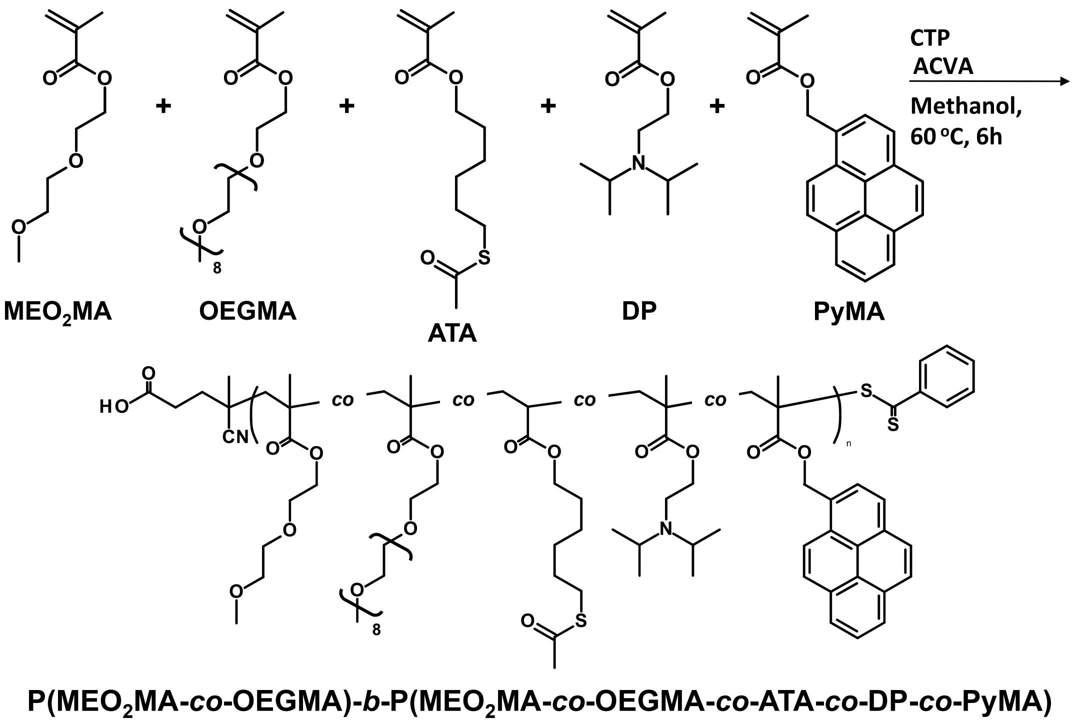


Fig. S13 Diameter/PDI (40 °C) of 0.5 wt% mixture solution of P(MEO_2MA_{48}-*co*-$OEGMA_{20}$)-b-P(MEO_2MA_{25}-co-$OEGMA_4$-co-ATA_3-co-DP_4) and P(MEO_2MA_{17}-co-$OEGMA_2$-co-ATA_3-co-DP_2) in pH 7.4 PBS. The temperature of solution was directly increased from 20 to 40 °C.



Scheme S4 Preparation of P(MEO_2MA-*co*-OEGMA)-*b*-P(MEO_2MA-*co*-OEGMA-*co*-ATA-*co*-DP-*co*-VBC).



Scheme S5 **Preparation of P(MEO_2MA-*co*-OEGMA)-*b*-P(MEO_2MA-*co*-OEGMA-*co*-ATA-*co*-DP-*co*-PyMA).**

Table S4 Characterization of copolymers for fluorescent measurement

	P(MEO$_2$MA-*co*-OEGMA) block		P(MEO$_2$MA-*co*-OEGMA-co-ATA-co-DP-*co*-fluorecent) block									
	In feed	In copolymer	In feed				In copolymer					
Monomer numbers[a]	OEGMA	OEGMA	OEGMA	ATA	DP	Fluo.[b]	OEGMA	ATA	DP	Fluo.	M_{n}[c]	$M_{\mathrm{w}}/M_{\mathrm{n}}$[c]
	(mol%)	(mol%)	(mol%)	(mol%)	(mol%)	(mol%)	(mol%)	(mol%)	(mol%)	(mol%)	(g/mol)	(-)
P(MEO$_2$MA$_{48}$-*co*-OEGMA$_{20}$)	30	29.7	-	-	-	-	-	-	-	-	19400	1.16
P(MEO$_2$MA$_{48}$-*co*-OEGMA$_{20}$)-*b*-P (MEO$_2$MA$_{21}$-*co*-OEGMA$_2$-*co*-ATA$_3$-*co*-DP$_2$-*co*-VBC$_{0.7}$)	30	29.7	8	16	15	1	7.2	9.8	8.1	2.6	25800	1.26
P(MEO$_2$MA$_{34}$-*co*-OEGMA$_4$-*co*-ATA$_5$-*co*-DP$_5$-*co*-PyMA$_{0.8}$)	-	-	8	16	15	1	8.0	9.7	11.0	1.6	11100	1.35

[a] The monomer numbers were calculated by GPC and ^{1}H NMR. [b] Determined by ^{1}H NMR. [c] Determined by GPC using 10 mM LiBr DMF.

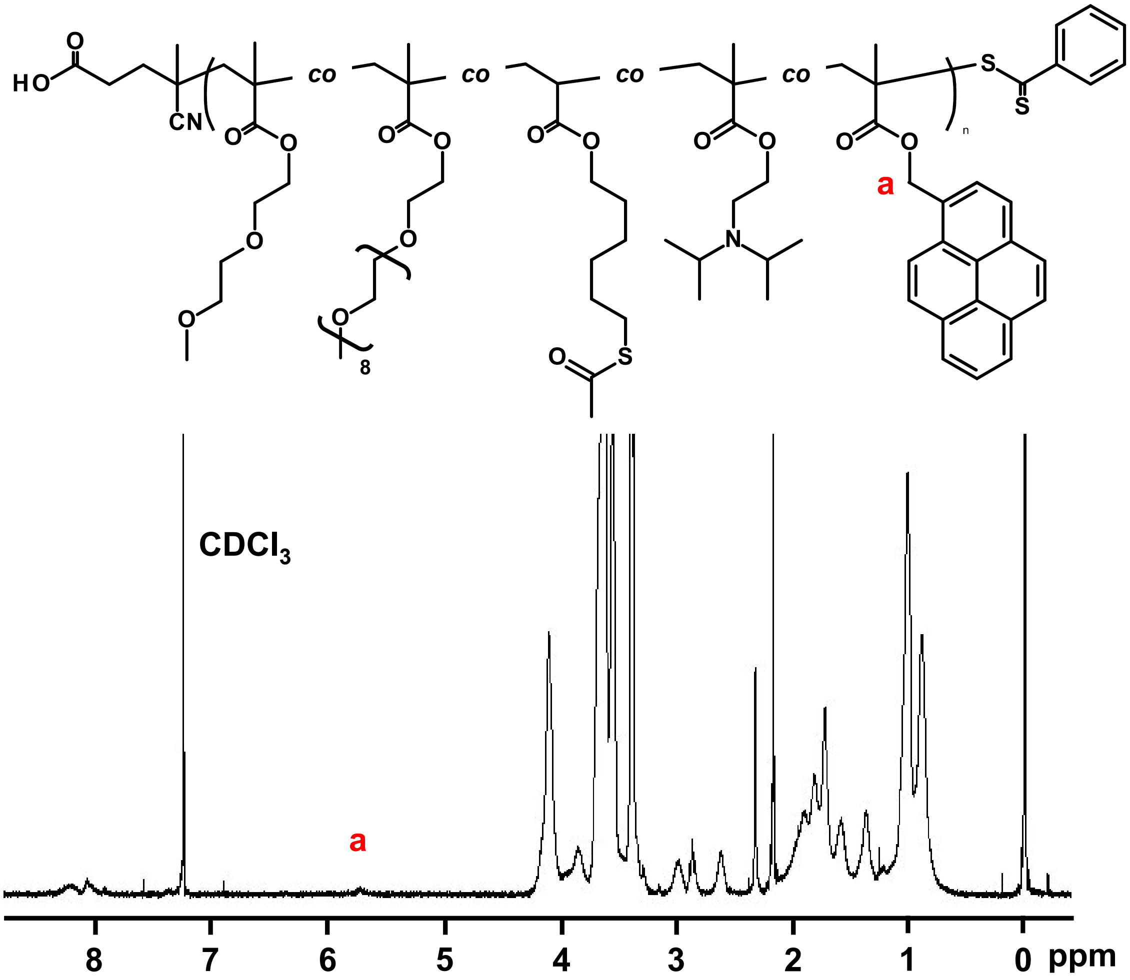


Fig. S14 NMR spectra of P(MEO$_2$MA$_{34}$-*co*-OEGMA$_4$-*co*-ATA$_5$-*co*-DP$_5$-*co*-PyMA$_{0.8}$).

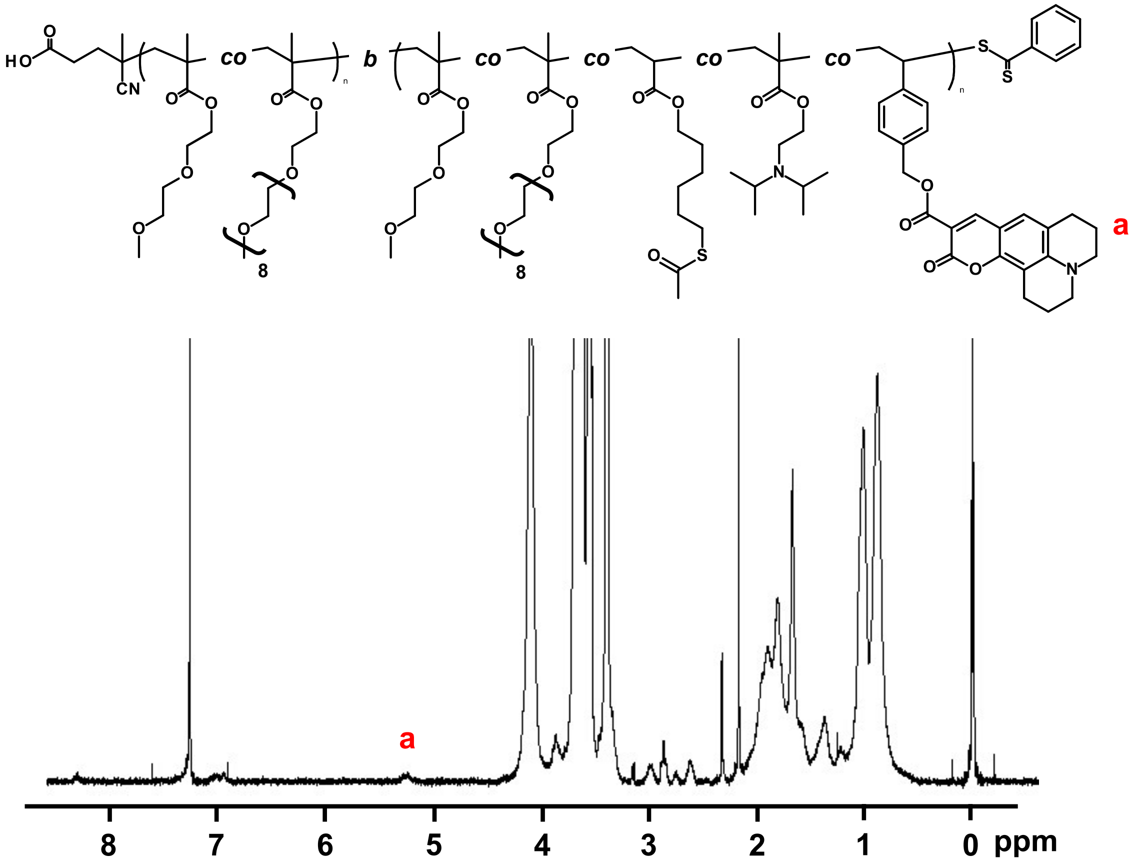


Fig. S15 NMR spectra of P(MEO$_2$MA$_{48}$-*co*-OEGMA$_{20}$)-*b*-P(MEO$_2$MA$_{23}$-*co*-OEGMA$_2$-*co*-ATA$_3$-*co*-DP$_2$-*co*-VBC$_{0.3}$).

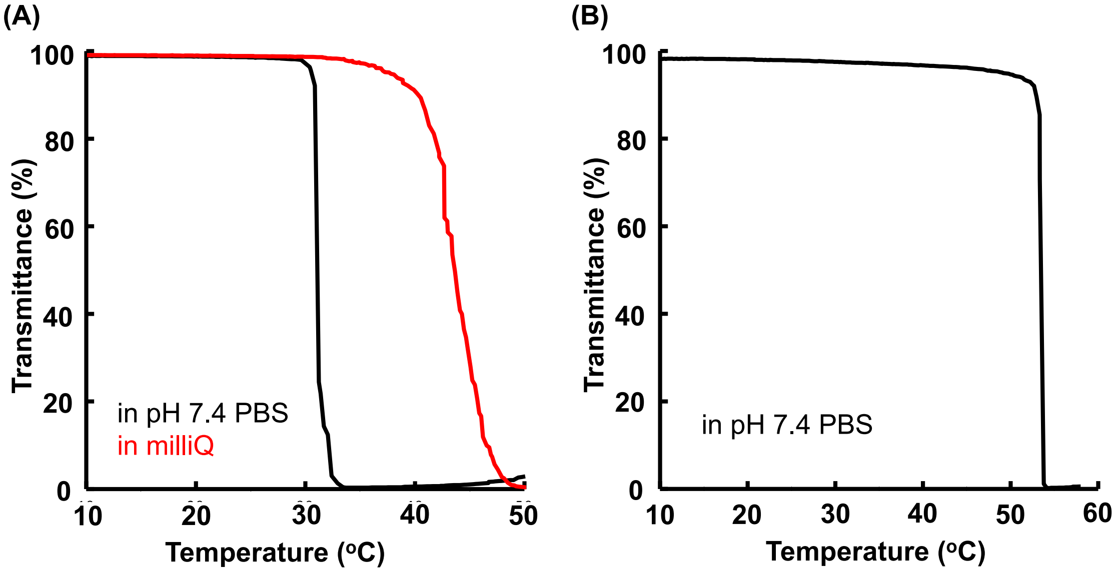


Fig. S16 (A) Transmittance change of 0.1 wt% P(MEO_2MA_{34}-*co*-$OEGMA_4$-*co*-ATA_5-*co*-DP_5-*co*-$PyMA_{0.8}$) as a function of temperature. Black line: in pH 7.4 PBS and red line: in milliQ. (B) Transmittance change of 0.1 wt% P(MEO_2MA_{48}-*co*-$OEGMA_{20}$)-*b*-P(MEO_2MA_{23}-*co*-$OEGMA_2$-*co*-ATA_3-*co*-DP_2-*co*-$VBC_{0.3}$) as a function of temperature in pH 7.4 PBS. The wavelength of 600 nm was used because of the adsorption on light of 500 nm by VBC units.

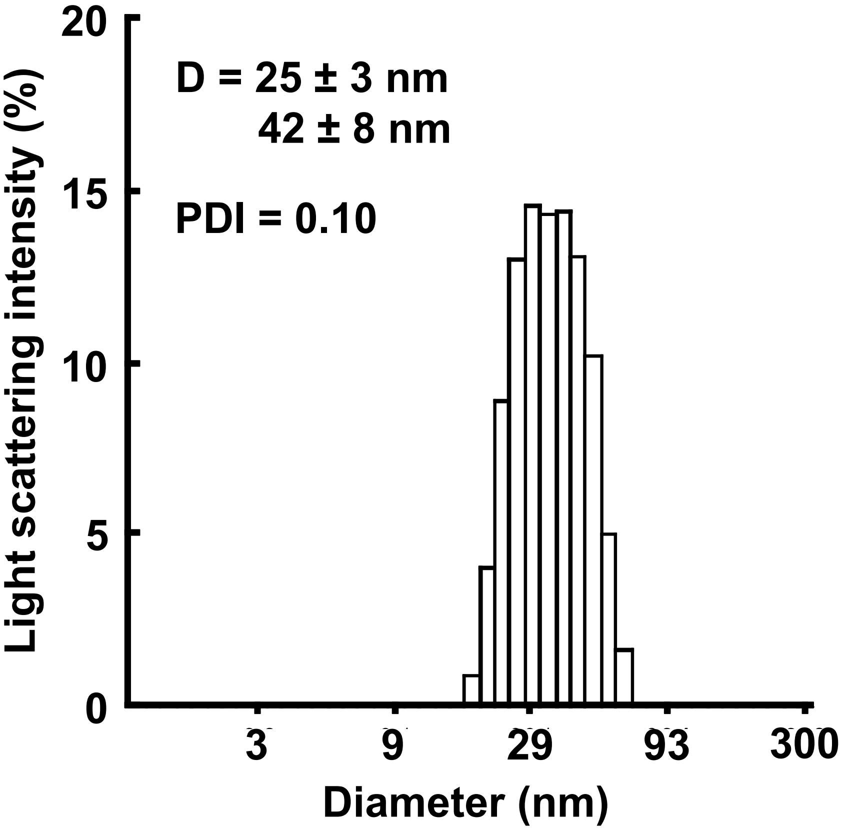


Fig. S17 Size distribution histogram for the mixed nanoparticles (2C*b*4C and 2C*b*4C/4C-Long) at 40 °C.

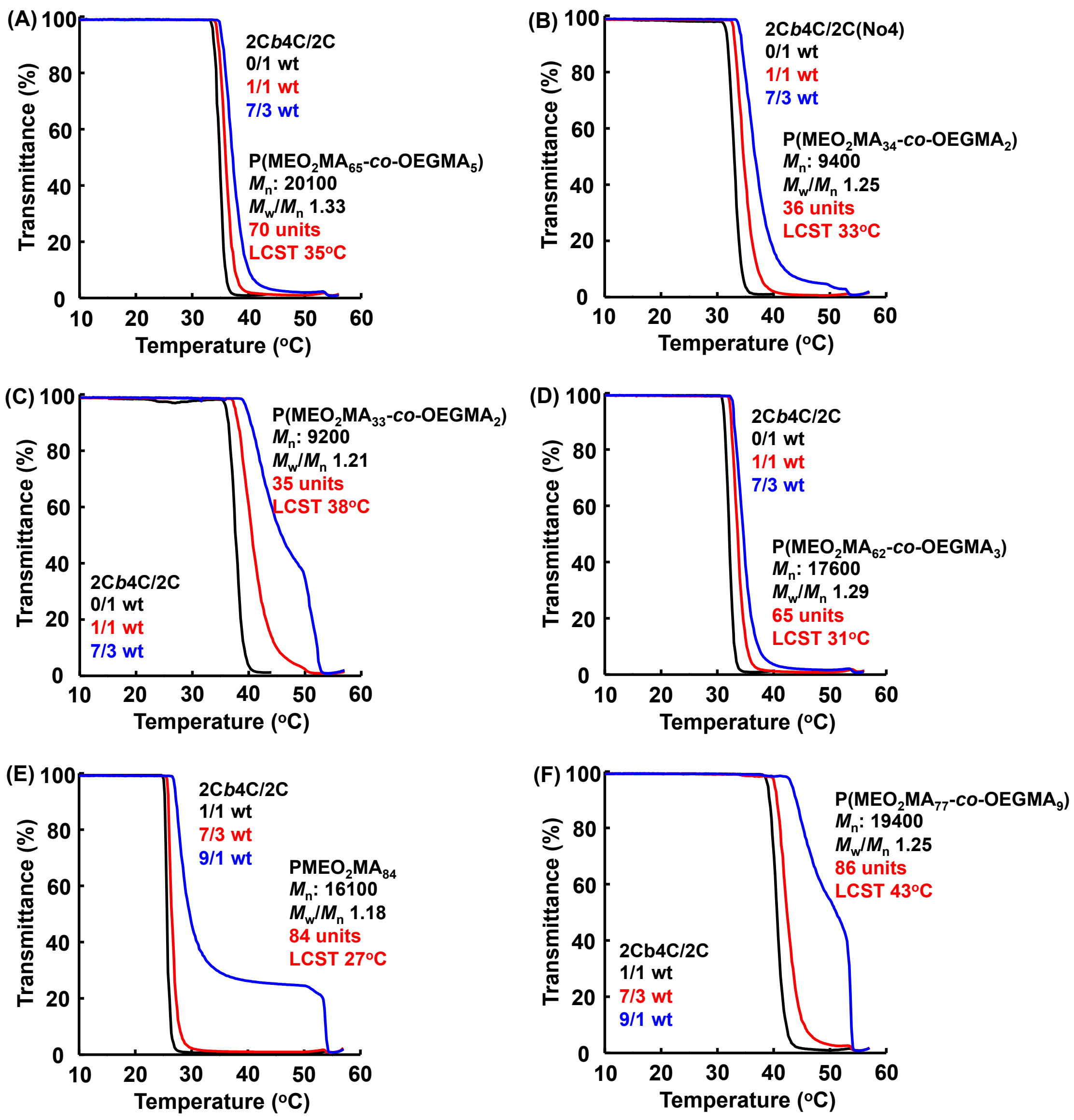


Fig. S18 Transmittance change of 0.5 wt% of mixture solution of 2C*b*4C and copolymers with different molecular weights and LCSTs in pH 7.4 PBS.