SUPPORTING INFORMATION

to

Timed-Release Polymer Nanoparticles

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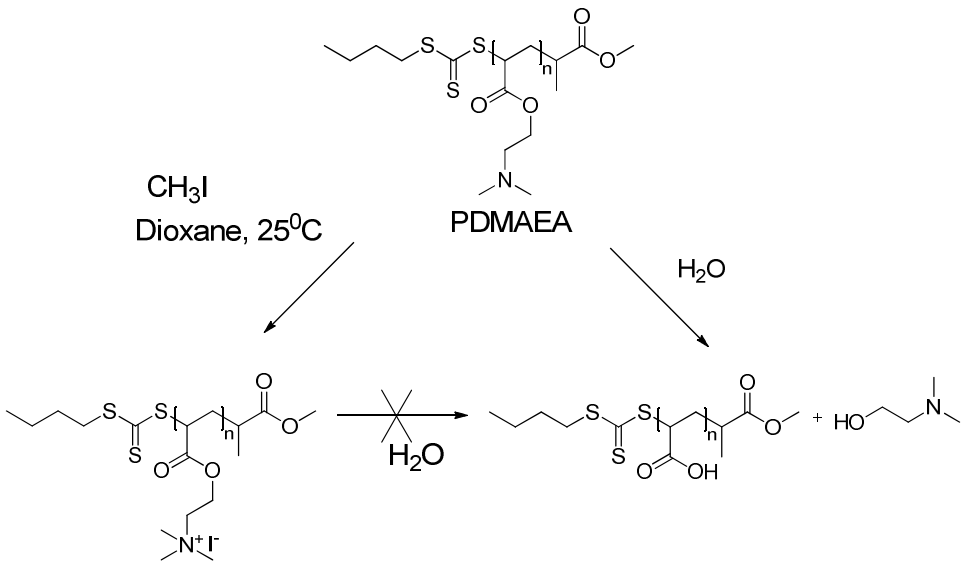
Scheme S1. Self-catalyzed hydrolysis of Poly(2-dimethylaminoethyl acrylate).



Scheme S2. Synthesis of folic acid functional copolymer H

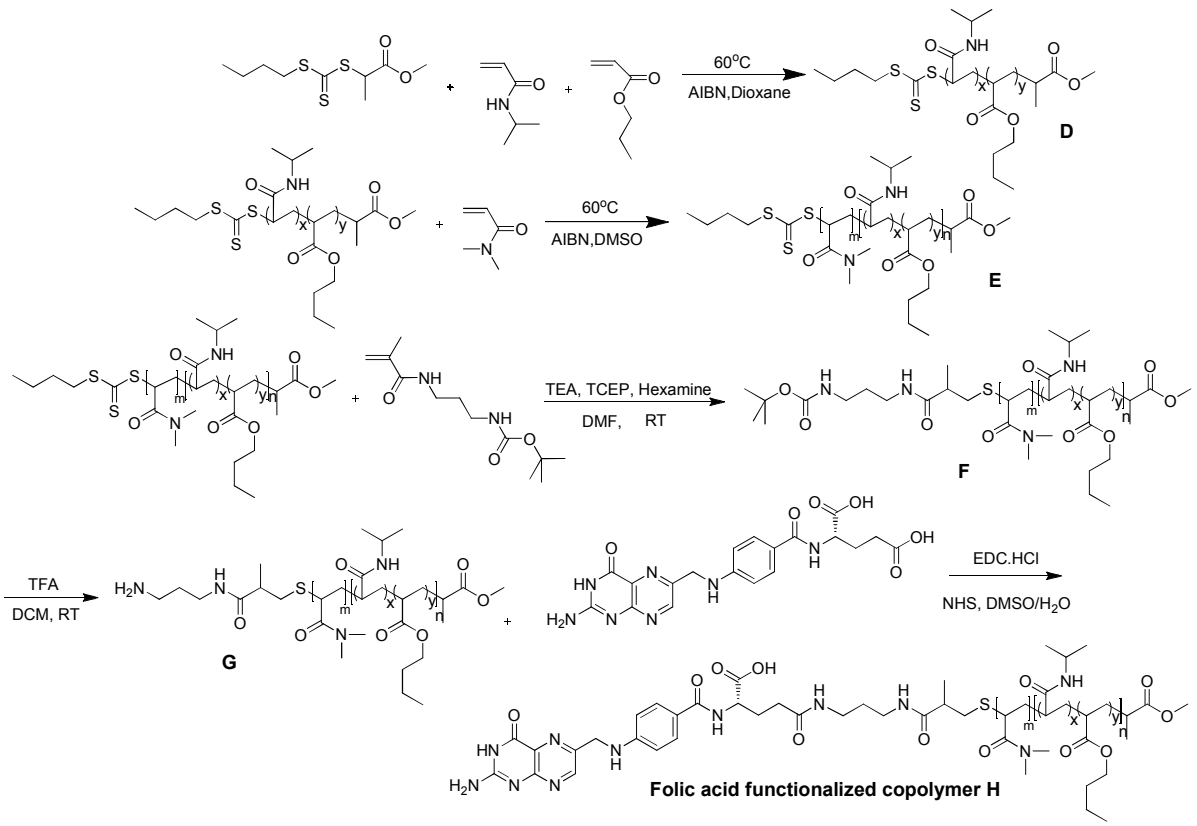


Table S1. Molecular weights, polydispersities (PDI), and ^{1}H NMR data of RAFT polymerization of thermo responsive block copolymers at 60 ^{o}C in Dioxane.

Polymer code [a]		SEC[b]				^{1}H NMR					
		RI		Triple detection[c]		Repeating units				M_n[i]	Percentage of BA or STY (%)[k]
		M_n	PDI	M_n	M_w	NIPAM[d]	DMAEA	BA[e]	STY[f]		
A	$P(DMA_{96}$-b-$(NIPAM_{87}$-co-$DMAEA_{25}))$	34500	1.23	23800	24000	87	25[g]	-	-	23175	-
B1	$P(DMA_{96}$-b-$(NIPAM_{88}$-co-$DMAEA_{25}$-co-$BA_6))$	36200	1.24	28500	28900	88	25[h]	6	-	24057	5.04
B2	$P(DMA_{96}$-b-$(NIPAM_{91}$-co-$DMAEA_{25}$-co-$BA_{12}))$	36700	1.26	24000	24300	91	25[h]	12	-	25165	9.38
C1	$P(DMA_{96}$-b-$(NIPAM_{84}$-co-$DMAEA_{22}$-co-$STY_5))$	34300	1.32	24800	25700	84	22[g]	-	5	22927	4.50
C2	$P(DMA_{96}$-b-$(NIPAM_{40}$-co-$DMAEA_{15}$-co-$STY_{13}))$	24700	1.27	24100	24900	40	15[g]	-	13	17786	19.11

[a] PDMA (Macro-CTA) with repeating unit = 96; Mn = 9769 calculated from ^{1}H NMR. [b] SEC data measured in DMAc solution with 0.03 wt% of LiCl and using PSTY standards for calibration. [c] Triple detection data measured based on the dn/dc calculated from the polymers' concentration. [d] Repeating units of NIPAM (N_{NIPAM}) determined by ^{1}H NMR were calculated based on 96 repeating unit of Macro-CTA by the integral area of a peak at 1.08 ppm ($I_{1.08}$) and a peak in the range 2.85-3.07 ppm ($I_{2.85\text{-}3.07}$) using the following equation: $N_{NIPAM} = 96 \times I_{1.08} / I_{2.85\text{-}3.07}$

[e] Repeating units of BA (N_{BA}) determined by ^{1}H NMR were calculated based on 96 repeating unit of Macro-CTA by the integral area of a peak at 0.88 ppm ($I_{1.08}$) and a peak in the range 2.85-3.07 ppm ($I_{2.85\text{-}3.07}$) using the following equation: $N_{BA} = (96 \times 2 \times I_{0.88} / I_{2.85\text{-}3.07}) - 1$

[f] Repeating units of STY (N_{STY}) determined by ^{1}H NMR were calculated based on 96 repeating unit of Macro-CTA and the N_{NIPAM} by the integral area of the peak in the range 6.20-7.18 ppm ($I_{6.20\text{-}7.18}$) and the peak in the range 2.85-3.07 ppm ($I_{2.85\text{-}3.07}$) using the following equation: $N_{STY} = [(96 \times 6 \times I_{6.20\text{-}7.18})/I_{2.85\text{-}3.07} - N_{NIPAM}]/5$. [g] Repeating units of DMAEA (N_{DMAEA}) determined by ^{1}H NMR were calculated based on 96 repeating unit of Macro-CTA and the N_{NIPAM} by the integral area of the peak in the range 2.85-3.07 ppm ($I_{2.85\text{-}3.07}$) and the peak in the range 3.95-4.30 ppm ($I_{3.95\text{-}4.30}$) using the following equation: $N_{DMAEA} = [(96 \times 6 \times I_{3.95\text{-}4.30} / I_{2.85\text{-}3.07}) - N_{NIPAM}]/2$. [h] Repeating units of DMAEA (N_{DMAEA}) determined by ^{1}H NMR were calculated based on 96 repeating unit of Macro-CTA, and the N_{NIPAM} and N_{BA} by the integral area of the peak in the range 2.85-3.07 ppm ($I_{2.85\text{-}3.07}$) and the peak in the range 3.95 - 4.30 ppm ($I_{3.95\text{-}4.30}$) using the following equation: $N_{DMAEA} = [(96 \times 6 \times I_{3.95\text{-}4.30}/I_{2.85\text{-}3.07}) - (N_{NIPAM} + 2\,N_{BA})]/2$

[i] Molecular weight determined by ^{1}H NMR were calculated based on the repeating units of N_{NIPAM}, N_{BA} or N_{STY} ,N_{DMAEA}, and the molecular weight of macro-CTA: $Mn = (N_{NIPAM} \times 113) + (N_{BA} \times 128.2)$ or $(N_{STY} \times 104) + (N_{DMAEA} \times 143) + 9769$. [k] Percentages of BA or STY were calculated based on N_{DMAEA} ,N_{NIPAM} ,N_{BA} or N_{STY}: Percentage of BA = $N_{BA}/(N_{DMAEA} + N_{NIPAM} + N_{BA})$ and percentage of STY = $N_{STY}/(N_{DMAEA} + N_{NIPAM} + N_{STY})$.

Table S2: Hydrodynamic diameter (D_h), Polydispersities (PDIs) of thermoresponsive block copolymers and their Oligo DNA 9-27 complexes in Milli-Q water at N/P Ratio 0.5, 1, 2, 5, and 10 with 1 μg DNA. Measurements were carried out on DLS machine at 37 ^oC.

N/P ratio	Polymer B1/oligo DNA complexes		Polymer B2/oligo DNA complexes		Polymer C1/oligo DNA complexes		Polymer C2/oligo DNA complexes	
	D_h (nm)	PDI	D_h (nm)	PDI	D_h (nm)	PDI	D_h (nm)	PDI
0.5	30.33 ± 6.58	0.413	27.16 ± 2.14	0.356	11.10 ± 1.01	0.453	17.20 ± 4.29	0.389
1	29.49 ± 3.44	0.337	27.35 ± 1.23	0.218	24.03 ± 1.07	0.400	24.53 ± 0.86	0.243
2	14.75 ± 4.24	0.450	24.21 ± 0.24	0.240	9.021 ± 2.40	0.430	24.20 ± 1.33	0.361
5	17.72 ± 8.74	0.488	25.56 ± 0.70	0.168	8.872 ± 2.74	0.560	23.75 ± 0.58	0.214
10	27.21 ± 0.20	0.048	29.12 ± 1.02	0.071	29.27 ± 0.69	0.080	20.76 ± 0.54	0.110
Polymer only[a]	27.75 ± 1.67	0.063	29.87 ± 0.53	0.047	28.02 ± 1.82	0.087	21.22 ± 0.78	0.100

[a] For polymer only, the polymer concentration was 0.5 mg/mL. B1; P(DMA_{96}-b-($NIPAM_{88}$-co-$DMAEA_{25}$-co-BA_6)), B2; P(DMA_{96}-b-($NIPAM_{91}$-co-$DMAEA_{25}$-co-BA_{12})), C1; P(DMA_{96}-b-($NIPAM_{84}$-co-$DMAEA_{22}$-co-STY_5)), C2; P(DMA_{96}-b-($NIPAM_{40}$-co-$DMAEA_{15}$-co-STY_{13})). Data were reported as soluble polymers in Milli-Q water were incubated with DNA at N/P Ratio 0.5, 1, 2, 5, and 10 at below their LCST for 30 min prior to be measured at 37 ^oC on DLS machines. Data were reported as an average of five measurements. The mean standard of deviation of polymer particle sizes was calculated from five measurements.

Table S3. Molecular weights, polydispersities (PDIs), and ^{1}H NMR data of RAFT polymerization for synthesis of copolymer D and E at 60 ^{o}C.

Polymer code		SEC[a]				^{1}H NMR			
		RI		Triple detection[b]		Repeating units			M_n
		M_n	PDI	M_n	M_w	NIPAM[c]	BA[d]	DMA[e]	
D	P(NIPAM$_{97}$-co-BA$_{13}$)	28700	1.16	15900	16100	97	13	-	12880[f]
E	P(DMA$_{99}$-b-(NIPAM$_{97}$-co-BA$_{13}$))	38500	1.19	26900	27200	-	-	99	22694[g]

[a] SEC data measured in DMAc solution with 0.03 wt% of LiCl and using PSTY standards for calibration.
[b] Triple detection data measured based on the dn/dc calculated from the polymers' concentration.
[c] Repeating units of NIPAM (N_{NIPAM}) determined by ^{1}H NMR were calculated by the integral area of a peak at 1.12 ppm ($I_{1.12}$) and a peak at 3.64 ppm ($I_{3.64}$) using the following equation: $N_{NIPAM} = I_{1.12} /(2 \times I_{3.64})$
[d] Repeating units of BA (N_{BA}) determined by ^{1}H NMR were calculated by the integral area of a peak at 0.89 ppm ($I_{0.89}$) and a peak at 3.64 ppm ($I_{3.64}$) using the following equation: $N_{BA} = (I_{0.89} / I_{3.64}) - 1$
[e] Repeating units of DMA (N_{DMA}) determined by ^{1}H NMR were calculated based on N_{BA} by the integral area of the peak in the range 2.86-3.07 ppm ($I_{2.86\text{-}3.07}$) and the peak at 0.89 ppm ($I_{0.89}$) using the following equation: $N_{DMA} =[((N_{BA} + 3) \times I_{2.86\text{-}3.07})/(I_{0.89} \times 6)]$
[f] Molecular weight determined by ^{1}H NMR were calculated based on the repeating units of N_{NIPAM}, N_{BA} and the molecular weight of MCEBTTC: $Mn = (N_{NIPAM} \times 113) + (N_{BA} \times 128.2) + 252.42$
[g] Molecular weight determined by ^{1}H NMR were calculated based on the repeating units of N_{DMA} and the molecular weight of macro-CTA: $Mn = (N_{DMA} \times 99.131) + 12880$

Table S4. Lower critical solution temperature (LCST), hydrodynamic diameter (D_h), Polydispersities (PDI) for thermoresponsive block copolymers G and folic acid functionalized copolymer (H) determined by dynamic light scattering (DLS).

Polymer code	DLS		
	LCST (^oC)[a]	D_h (nm)[b]	PDI[b]
G	13 – 15	33.83 ± 0.65	0.017
H	15 – 19	31.66 ± 0.98	0.089

[a]LCST determined by DLS (10 mg/mL)

[b]Hydrodynamic diameter (D_h) and Polydispersity (PDI) determined by DLS (10 mg/mL) at 37 ^oC.

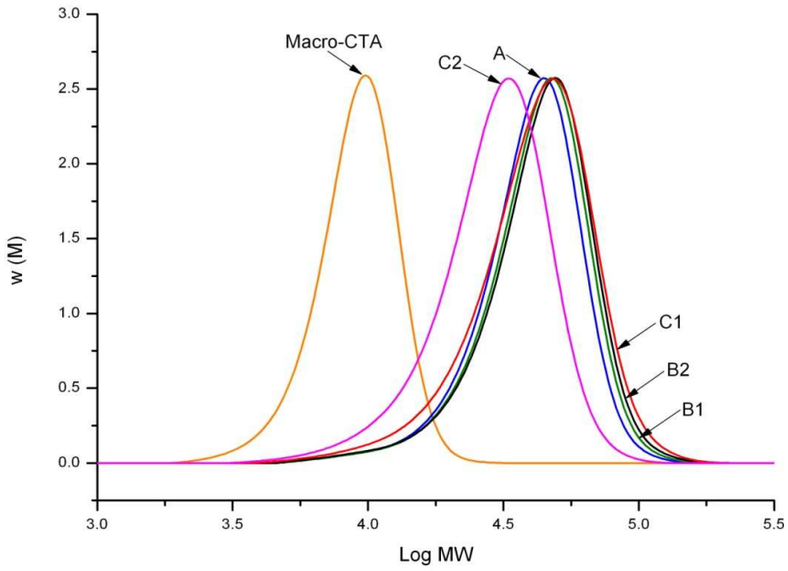


Figure S1. Size Exclusion Chromatography (SEC) traces of macro CTA; $PDMA_{96}$, A; P(DMA_{96}-b-($NIPAM_{87}$-co-$DMAEA_{25}$)), B1; P(DMA_{96}-b-($NIPAM_{88}$-co-$DMAEA_{25}$-co-BA_6)), B2; P(DMA_{96}-b-($NIPAM_{91}$-co-$DMAEA_{25}$-co-BA_{12})), C1; P(DMA_{96}-b-($NIPAM_{84}$-co-$DMAEA_{22}$-co-STY_5)), C2; P(DMA_{96}-b-($NIPAM_{40}$-co-$DMAEA_{15}$-co-STY_{13})). The data were measured by DMAc SEC. The intensity for different distribution curves was normalized.

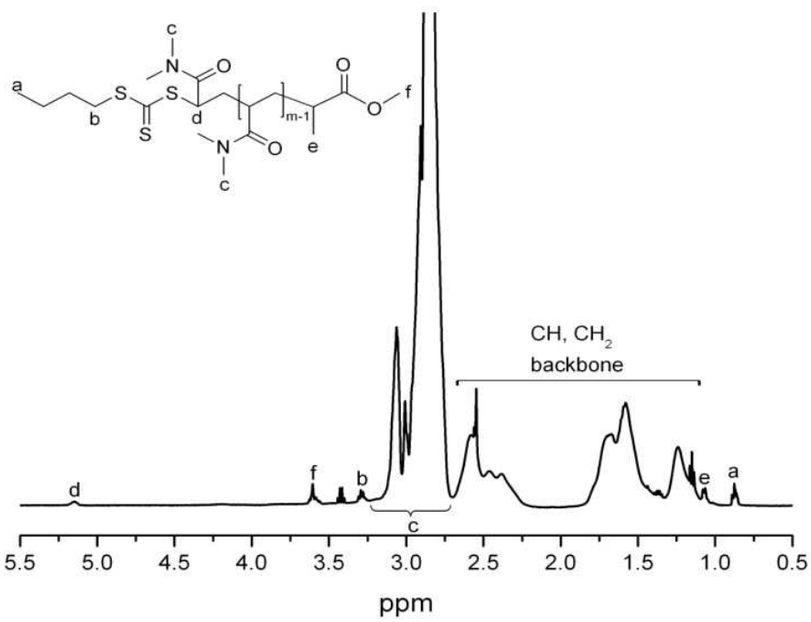


Figure S2 ^{1}H NMR spectrum of Macro-CTA: $PDMA_{96}$ in $CDCl_3$.

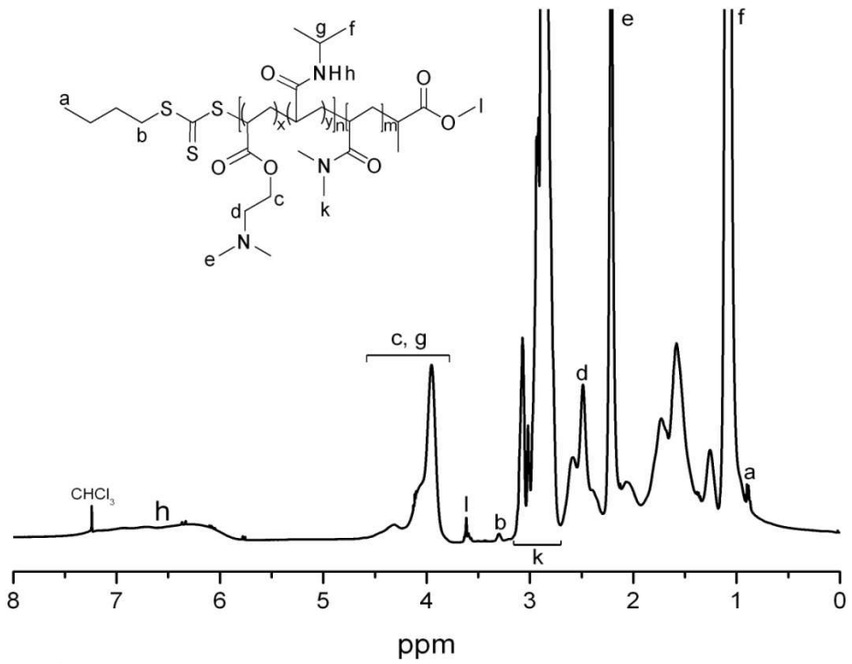


Figure S3. ^{1}H NMR spectrum of A: P(DMA_{96}-b-($NIPAM_{87}$-co-$DMAEA_{25}$)) in $CDCl_3$.

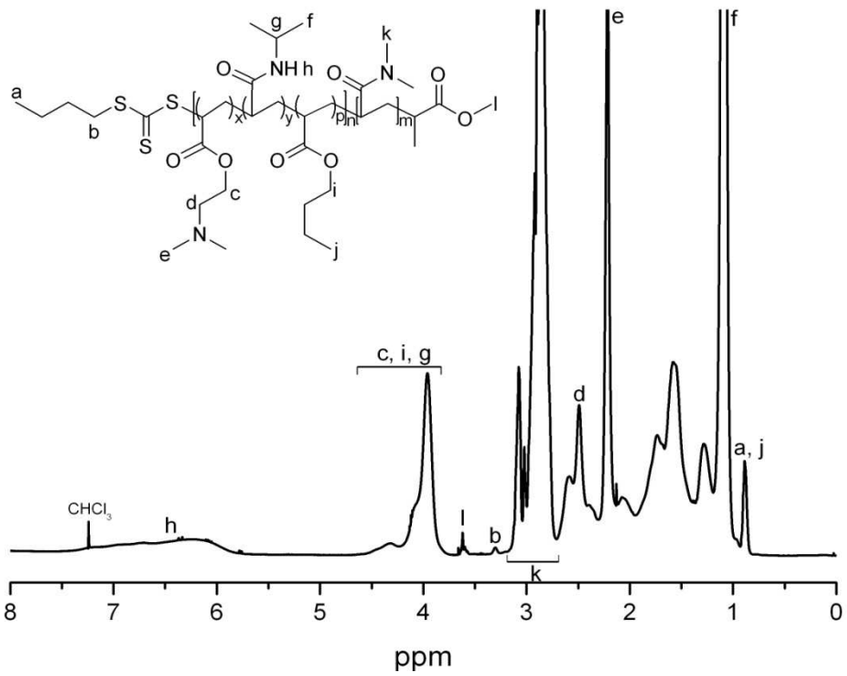


Figure S4. ^{1}H NMR spectrum of B1: P(DMA_{96}-b-($NIPAM_{88}$-co-$DMAEA_{25}$-co-BA_6)) in $CDCl_3$.

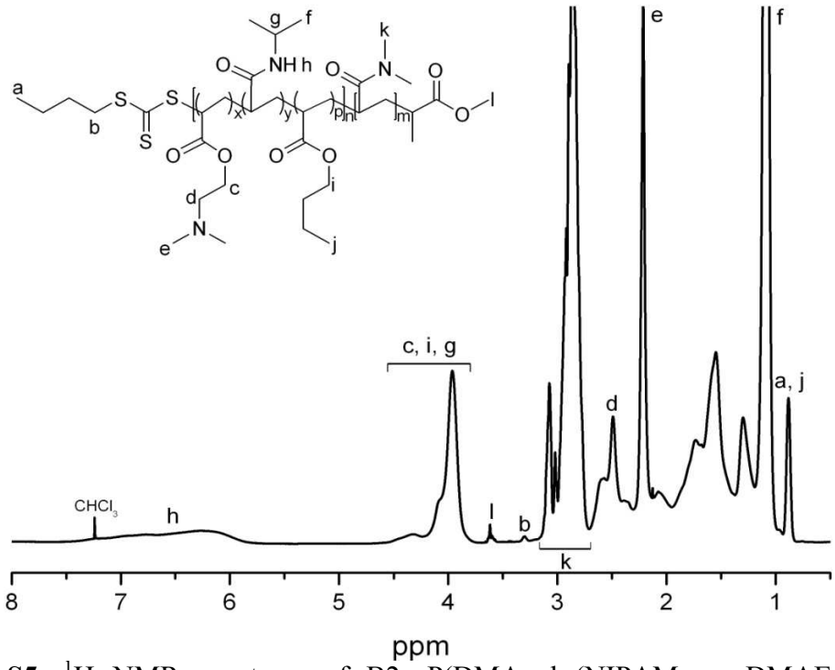


Figure S5. ^{1}H NMR spectrum of B2: P(DMA_{96}-b-($NIPAM_{91}$-co-$DMAEA_{25}$-co-BA_{12})) in $CDCl_3$.

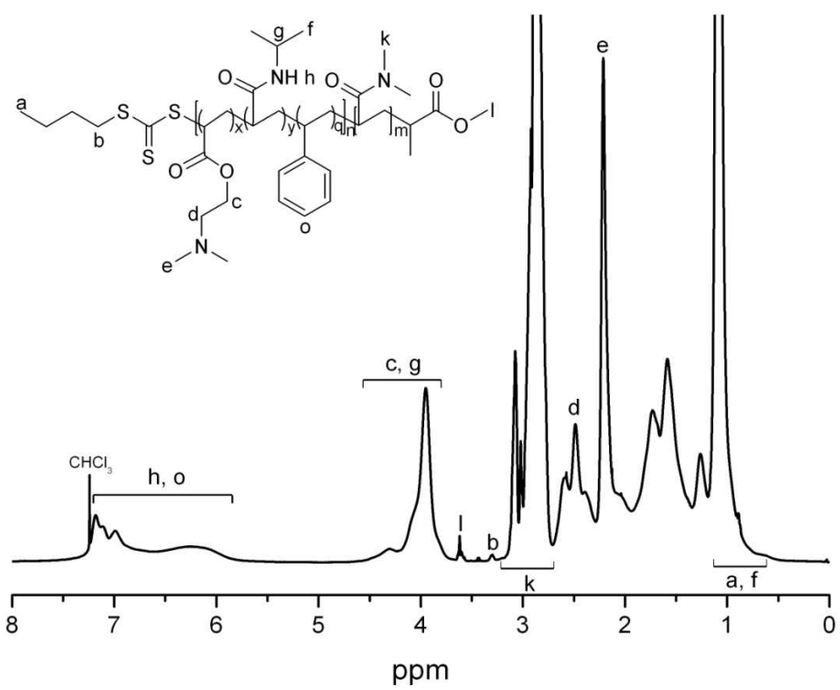


Figure S6. ^{1}H NMR spectrum of C1: P(DMA_{96}-b-($NIPAM_{84}$-co-$DMAEA_{22}$-co-STY_5)) in $CDCl_3$.

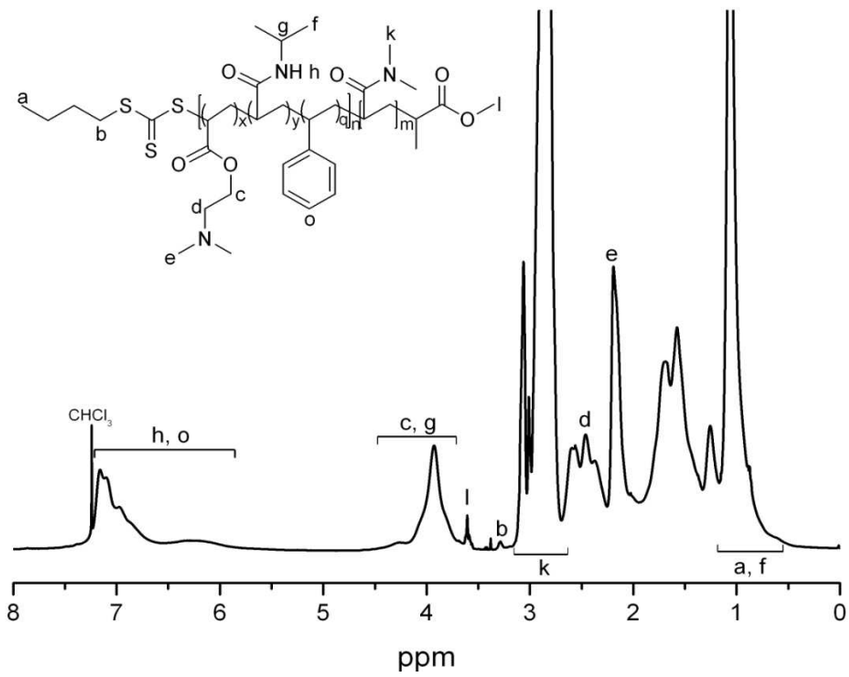


Figure S7. ^{1}H NMR spectrum of C2: P(DMA_{96}-b-($NIPAM_{40}$-co-$DMAEA_{15}$-co-STY_{13})) in $CDCl_3$.

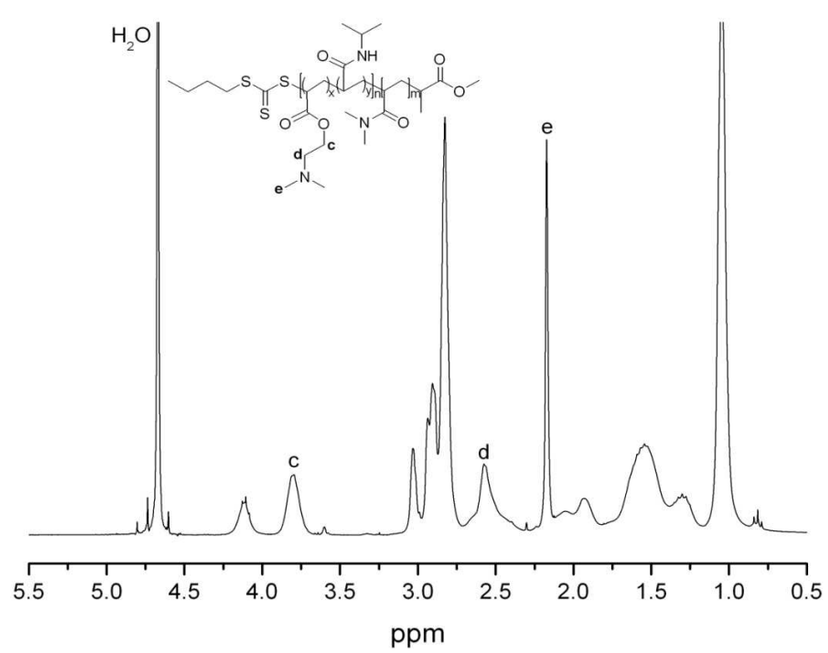


Figure S8. ^{1}H NMR spectrum of A: P(DMA_{96}-b-($NIPAM_{87}$-co-$DMAEA_{25}$)) after being dissolved in D_2O and immediately measured at 25 ^{o}C.

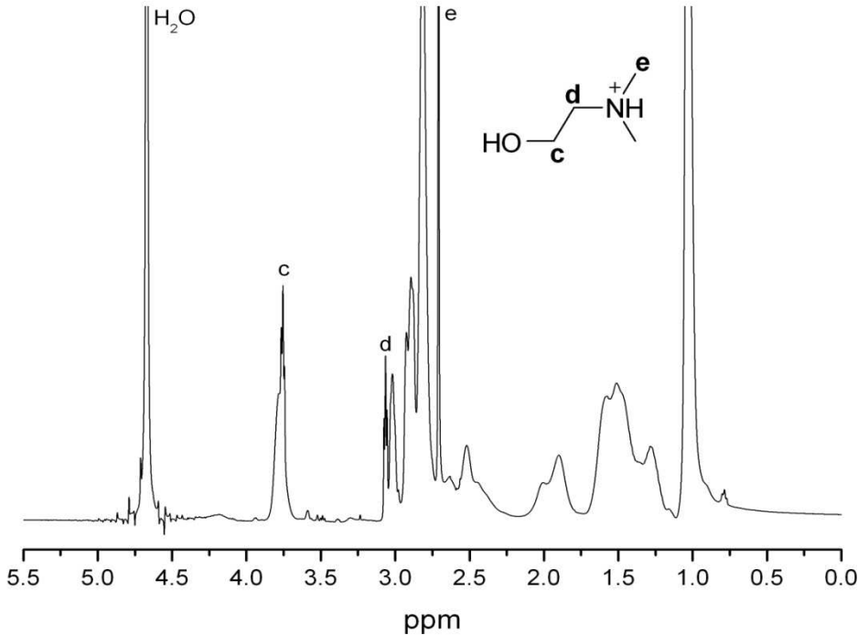


Figure S9. ^{1}H NMR spectrum of A; P(DMA$_{96}$-b-(NIPAM$_{87}$-co-DMAEA$_{25}$)) after being dissolved and kept in D_2O for 80 h. The spectrum was measured at 25 ^{o}C.

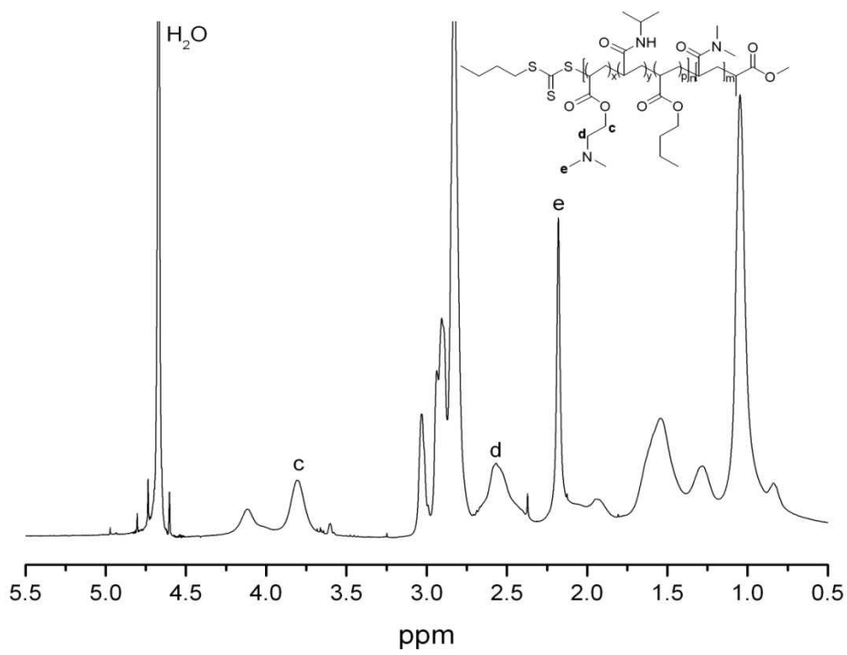


Figure S10. ^{1}H NMR spectrum of B1: P(DMA$_{96}$-b-(NIPAM$_{88}$-co-DMAEA$_{25}$-co-BA$_{6}$)) after being dissolved in D_2O and immediately measured at 25 ^{o}C.

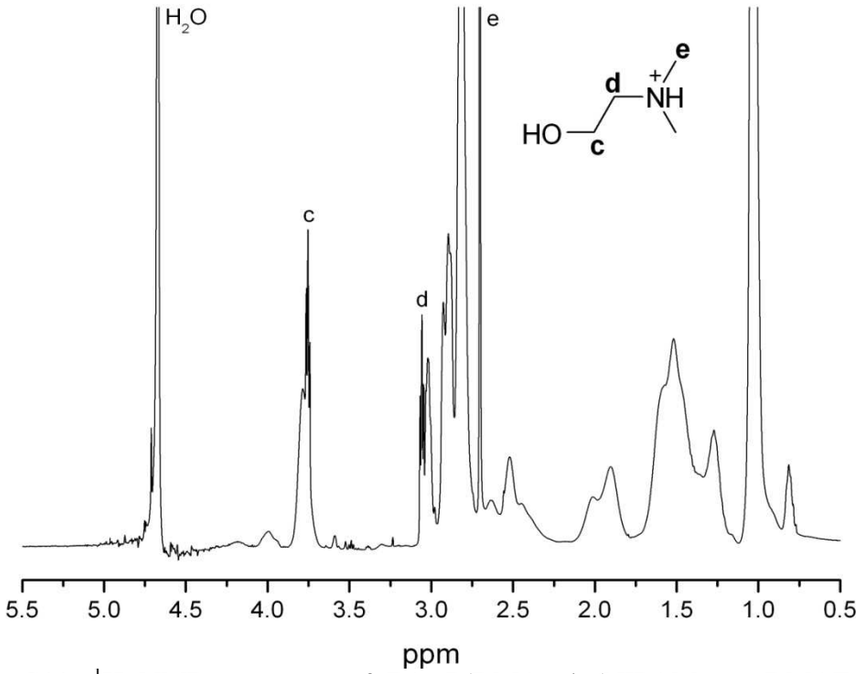


Figure S11. ^{1}H NMR spectrum of B1: P(DMA$_{96}$-b-(NIPAM$_{88}$-co-DMAEA$_{25}$-co-BA$_{6}$)) after being dissolved and kept in D_2O for 80 h. The spectrum was measured at 25 ^{o}C.

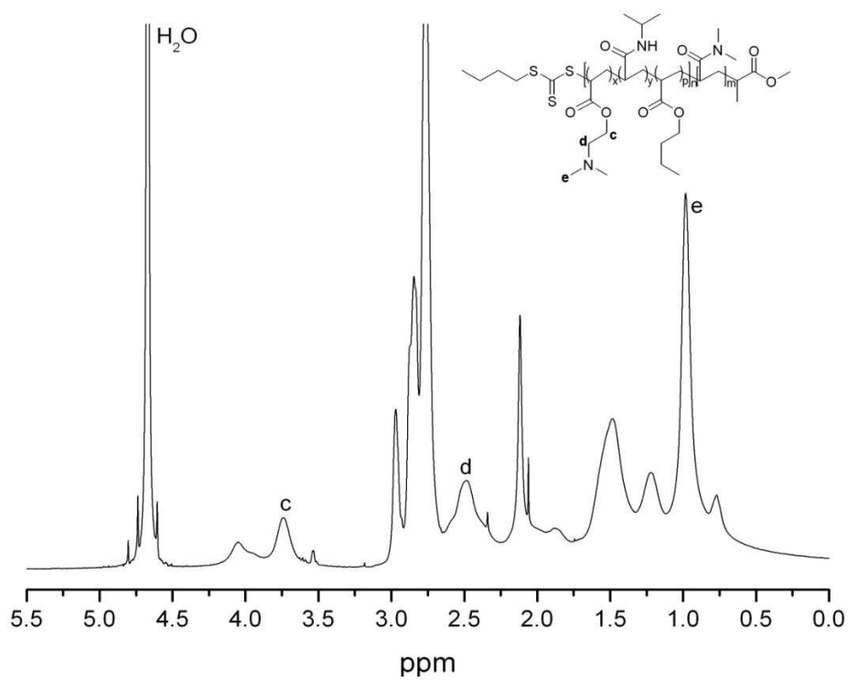


Figure S12. ^{1}H NMR spectrum of B2: P(DMA$_{96}$-b-(NIPAM$_{91}$-co-DMAEA$_{25}$-co-BA$_{12}$)) after being dissolved in D_2O and immediately measured at 15 ^{o}C.

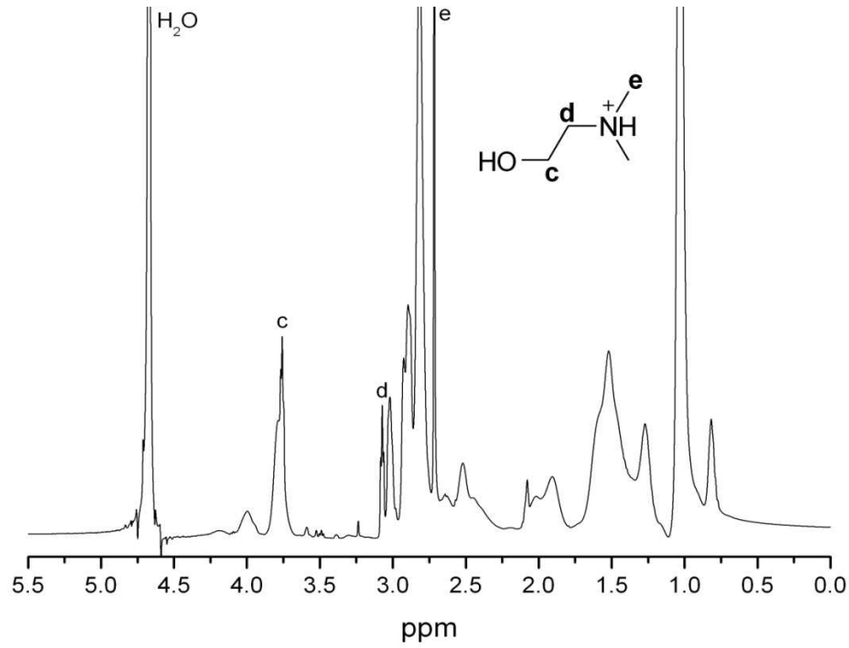


Figure S13. ^{1}H NMR spectrum of B2: P(DMA_{96}-b-($NIPAM_{91}$-co-$DMAEA_{25}$-co-BA_{12})) after being dissolved and kept in D_2O for 80 h. The spectrum was measured at 25 ^{o}C.

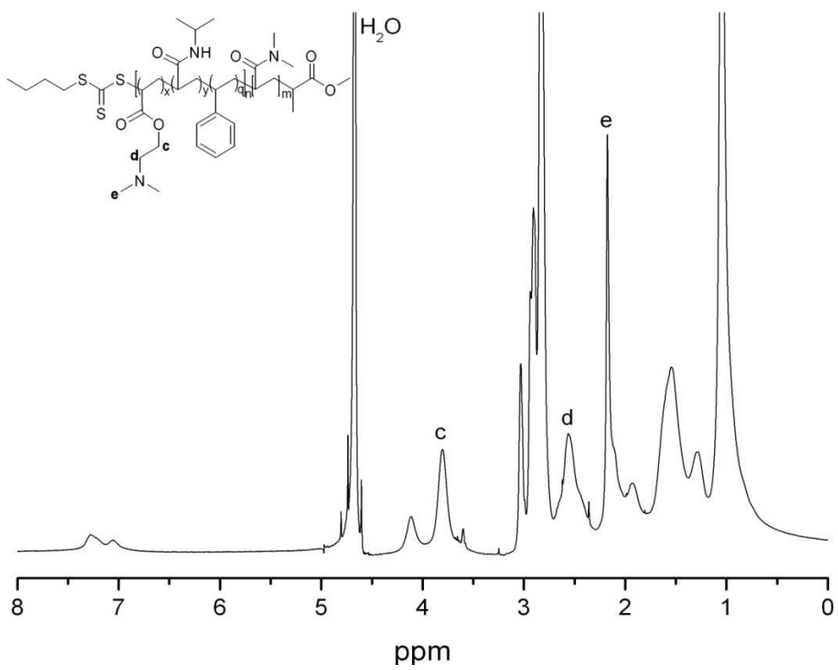


Figure S14. ^{1}H NMR spectrum of C1: P(DMA_{96}-b-($NIPAM_{84}$-co-$DMAEA_{22}$-co-STY_5)) after being dissolved in D_2O and immediately measured at 25 ^{o}C.

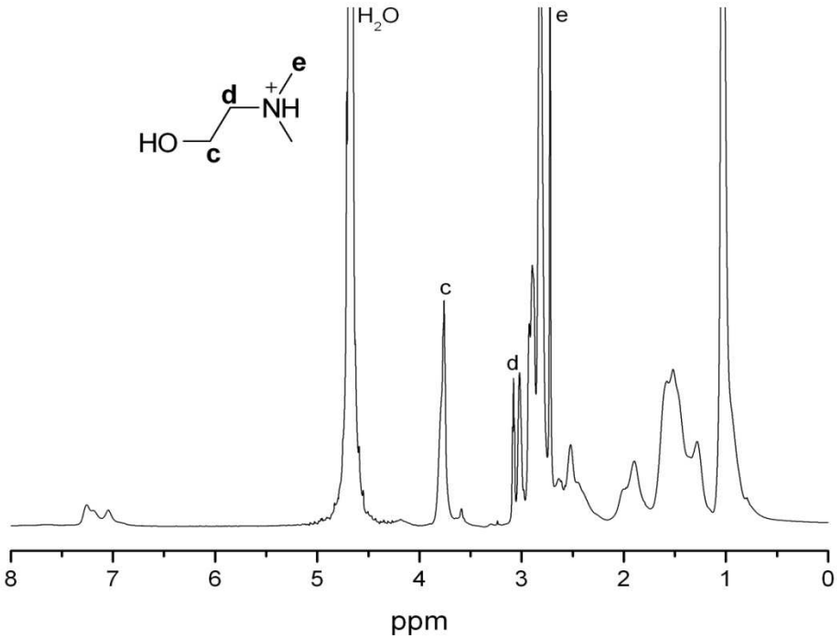


Figure S15. ^{1}H NMR spectrum of C1: P(DMA$_{96}$-b-(NIPAM$_{84}$-co-DMAEA$_{22}$-co-STY$_5$ after being dissolved and kept in D_2O for 80 h. The spectrum was measured at 25 ^{o}C.

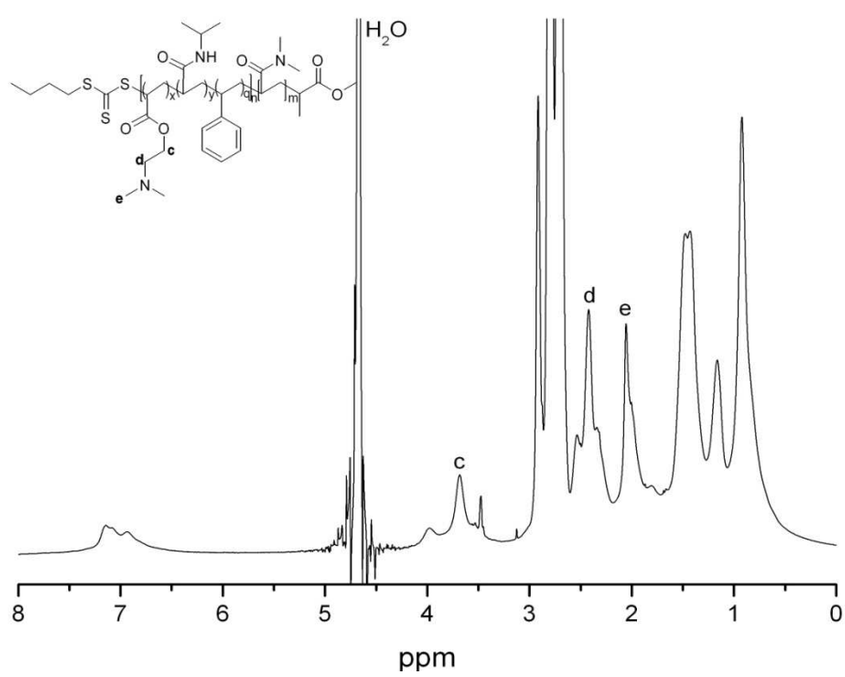


Figure S16. ^{1}H NMR spectrum of C2: P(DMA$_{96}$-b-(NIPAM$_{40}$-co-DMAEA$_{15}$-co-STY$_{13}$)) after being dissolved in D_2O and immediately measured at 15 ^{o}C.

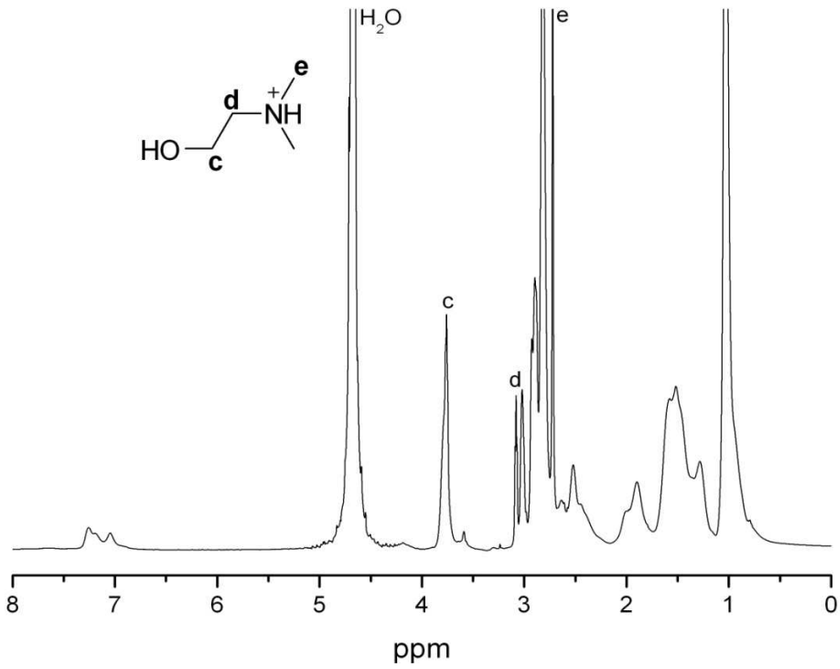


Figure S17. ^{1}H NMR spectrum of C2: P(DMA$_{96}$-b-(NIPAM$_{40}$-co-DMAEA$_{15}$-co-STY$_{13}$)) after being dissolved and kept in D_2O for 80 h. The spectrum was measured at 25 ^{o}C.

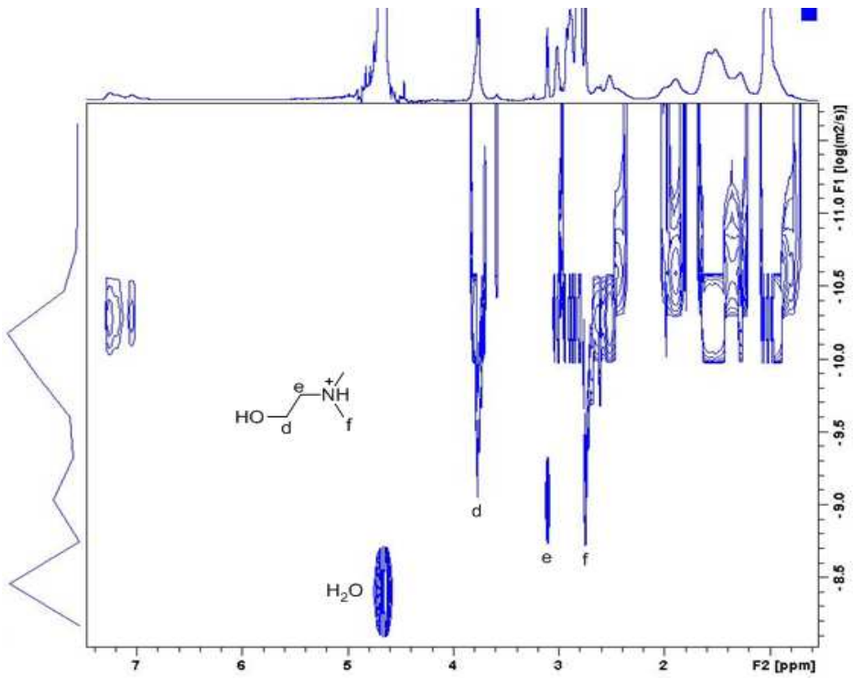


Figure S18. DOSY NMR spectrum of C1: P(DMA$_{96}$-b-(NIPAM$_{84}$-co-DMAEA$_{22}$-co-STY$_{5}$)) after being dissolved in D_2O for 80h. The spectrum was measured at 25 ^{o}C.

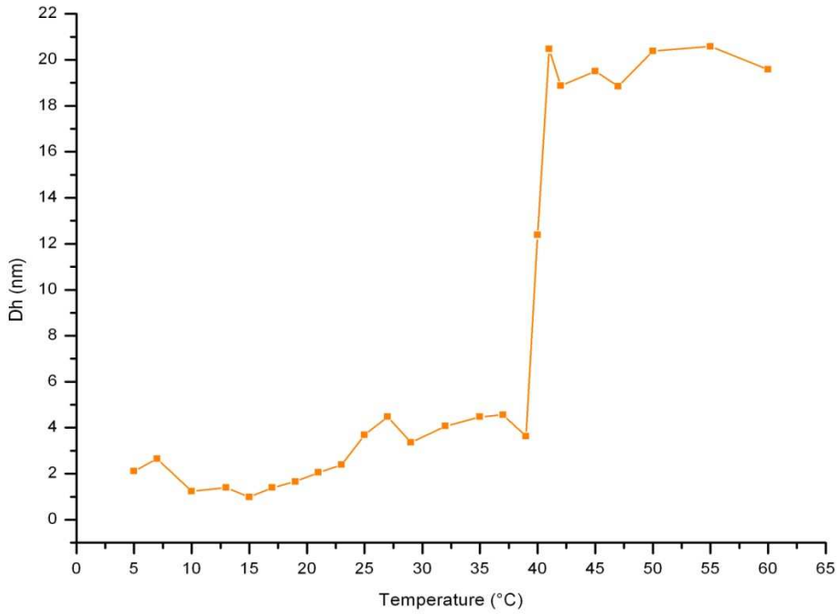


Figure S19. LCST of A: P(DMA_{96}-b-($NIPAM_{87}$-co-$DMAEA_{25}$)). The data were reported as average numbers from five measurements on DLS machine at concentration 10 mg/mL.

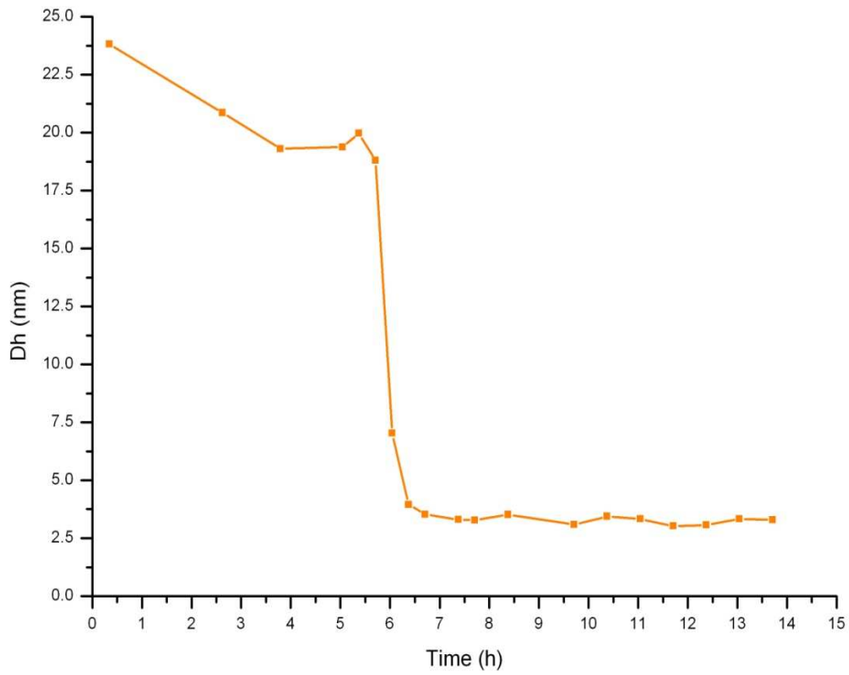


Figure S20. Degradation kinetic of A: P(DMA_{96}-b-($NIPAM_{87}$-co-$DMAEA_{25}$)). The data were averaged from five measurements on DLS machine at polymer solution concentration 5 mg/mL at 45 ^{o}C.

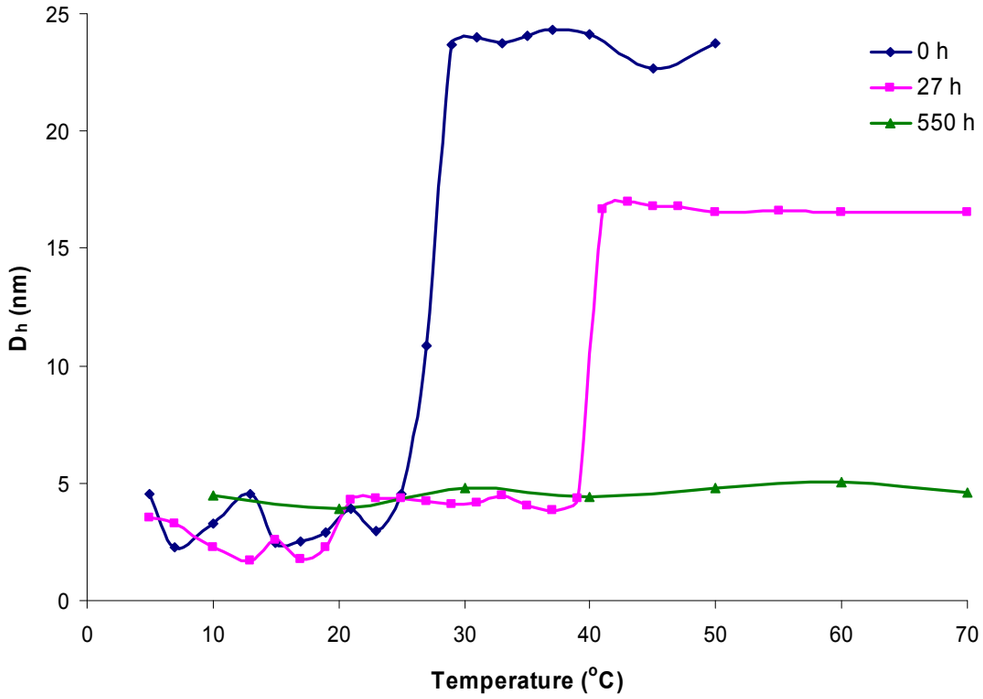


Figure S21. LCST of B1: P(DMA_{96}-b-($NIPAM_{88}$-co-$DMAEA_{25}$-co-BA_6)) at different times after the polymer was dissolved in water. The data were reported as average numbers from five measurements on DLS machine at concentration 10 mg/mL.

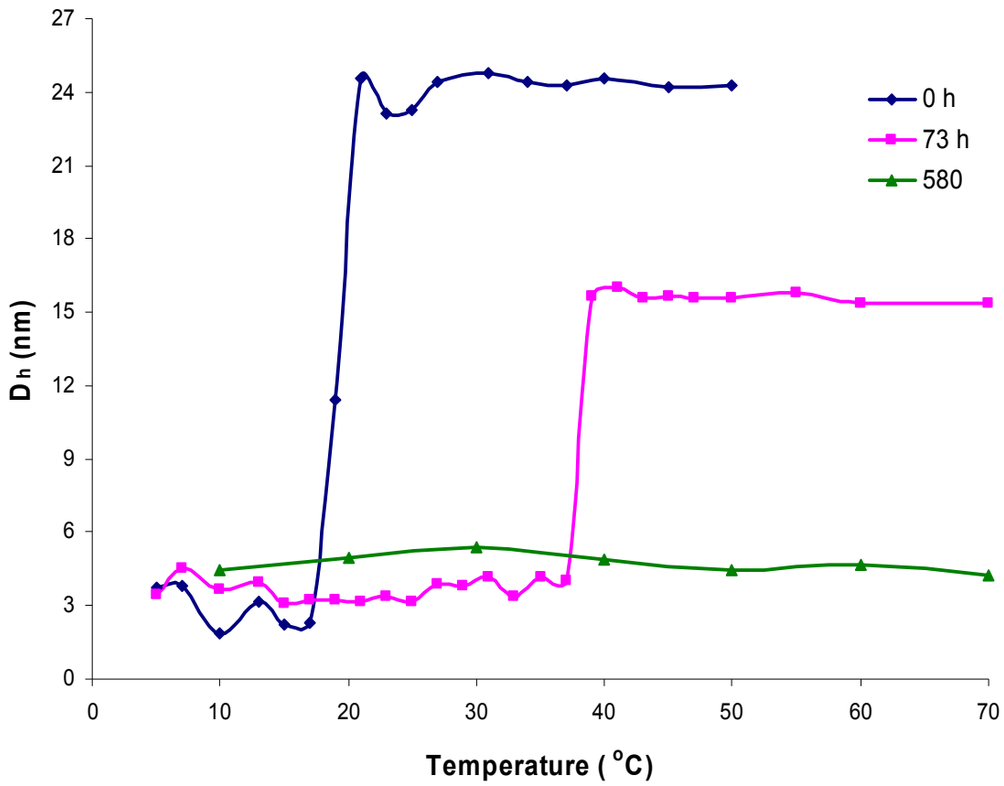


Figure S22. LCST of B2: P(DMA_{96}-b-($NIPAM_{91}$-co-$DMAEA_{25}$-co-BA_{12})) at different times after the polymer was dissolved in water. The data were reported as average numbers from five measurements on DLS machine at concentration 10 mg/mL.

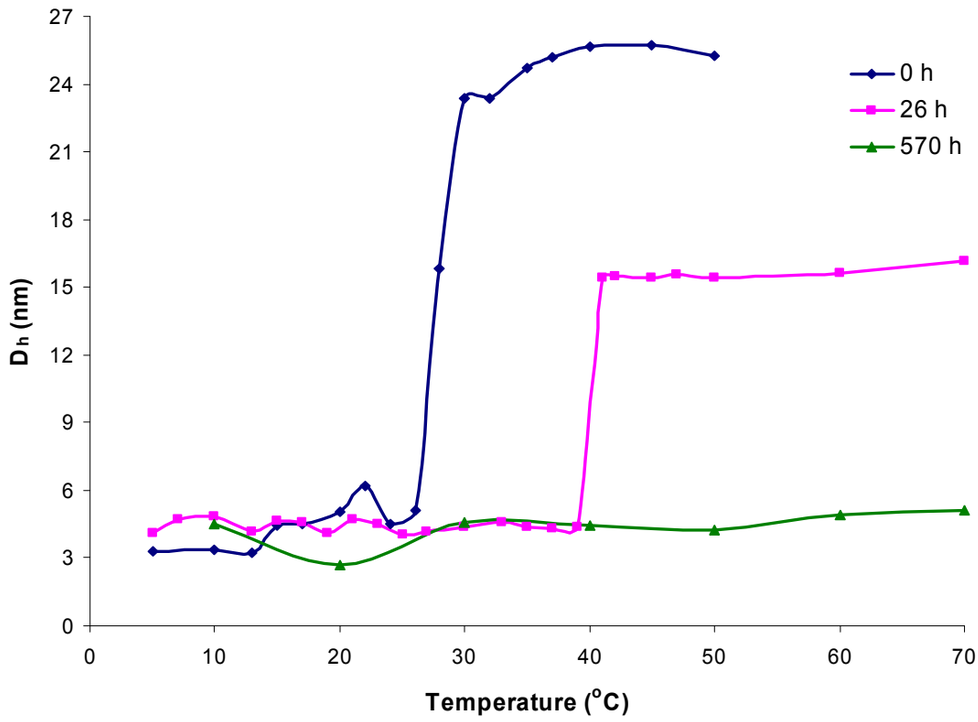


Figure S23. LCST of C1: P(DMA_{96}-b-($NIPAM_{84}$-co-$DMAEA_{22}$-co-STY_5 at different times after the polymer was dissolved in water. The data were reported as average numbers from five measurements on DLS machine at concentration 10 mg/mL

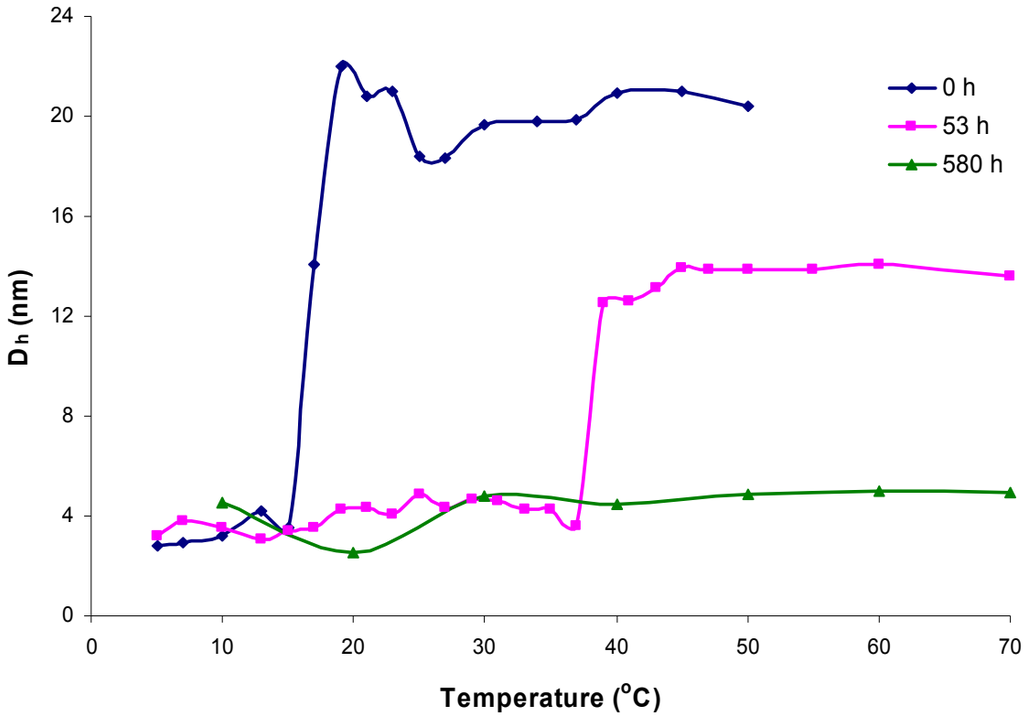


Figure S24. LCST of C2: P(DMA_{96}-b-($NIPAM_{40}$-co-$DMAEA_{15}$-co-STY_{13})) at different times after the polymer was dissolved in water. The data were reported as average numbers from five measurements on DLS machine at concentration 10 mg/mL.

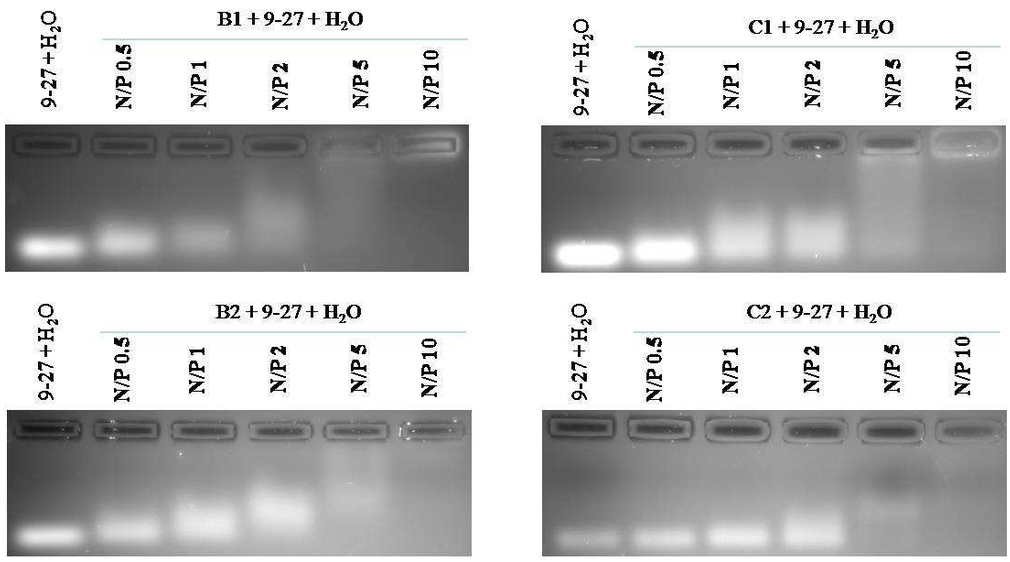


Figure S25. Agarose gel assay of Oigo DNA 9-27 / thermoresponsive block copolymers complexes in Milli-Q water at N/P Ratio 0.5, 1, 2, 5, and 10. B1: P(DMA_{96}-b-($NIPAM_{88}$-co-$DMAEA_{25}$-co-BA_6)), B2: P(DMA_{96}-b-($NIPAM_{91}$-co-$DMAEA_{25}$-co-BA_{12})), C1: P(DMA_{96}-b-($NIPAM_{84}$-co-$DMAEA_{22}$-co-STY_5)), C2: P(DMA_{96}-b-($NIPAM_{40}$-co-$DMAEA_{15}$-co-STY_{13})). Soluble copolymers in Milli-Q water were incubated with DNA at different N/P ratios at below their LCST for 30 min and then being heated up to 37 ^{o}C for 15 min before doing Agarose gel retardation assay.

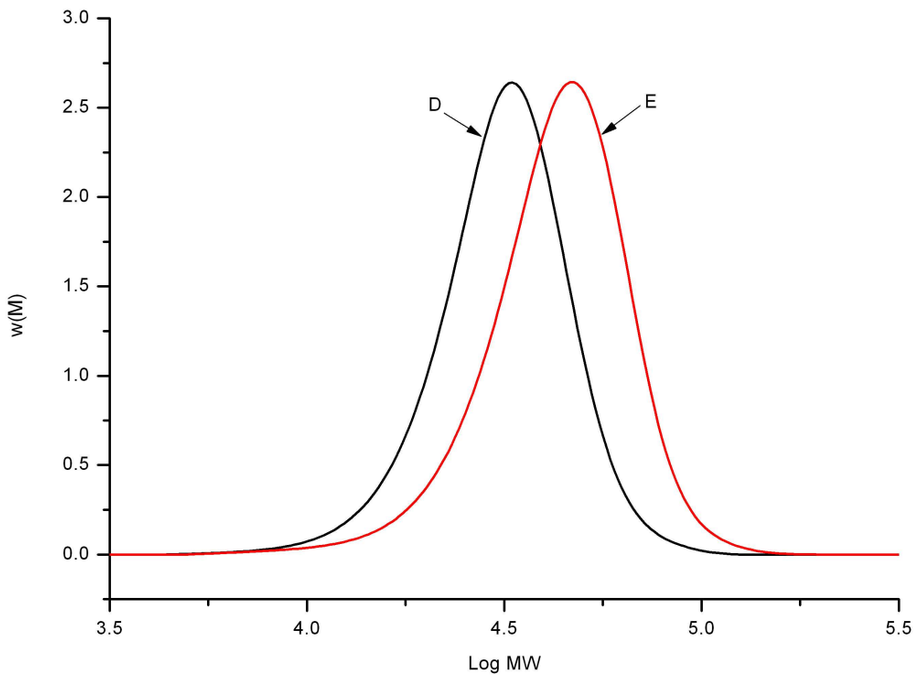


Figure S26. Size Exclusion Chromatography (SEC) traces of D: P($NIPAM_{97}$-co-BA_{13})) and E: P(DMA_{99}-b-($NIPAM_{97}$-co- BA_{13})). The samples were measured by DMAc SEC. The intensity for different distribution curves was normalized.

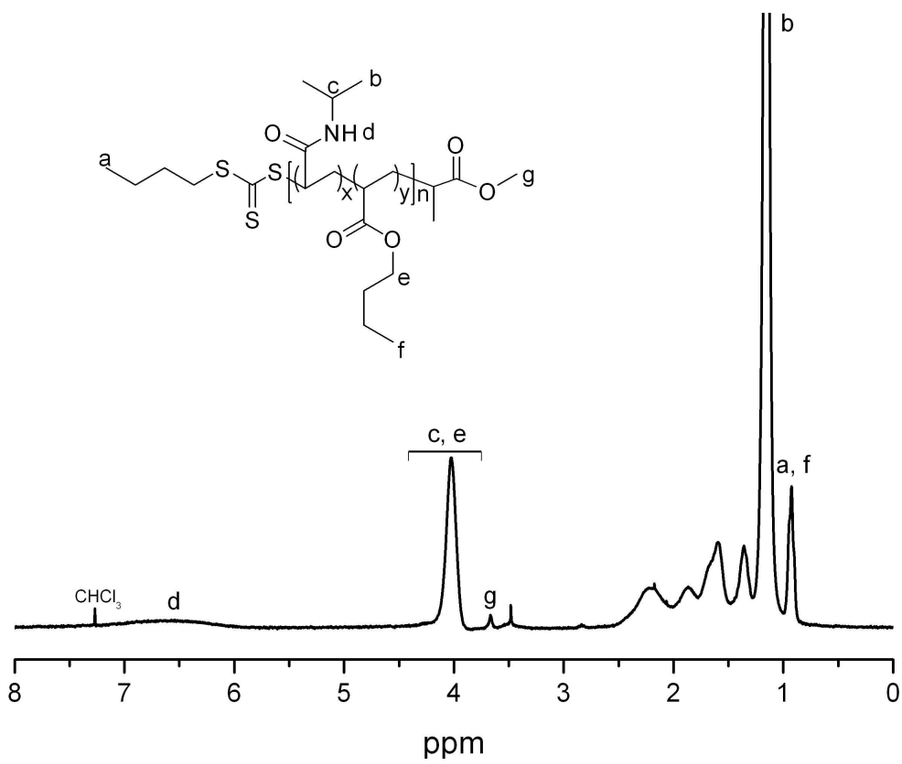


Figure S27. ^{1}H NMR spectrum of **D**: P(NIPAM$_{97}$-co-BA$_{13}$)) in $CDCl_3$

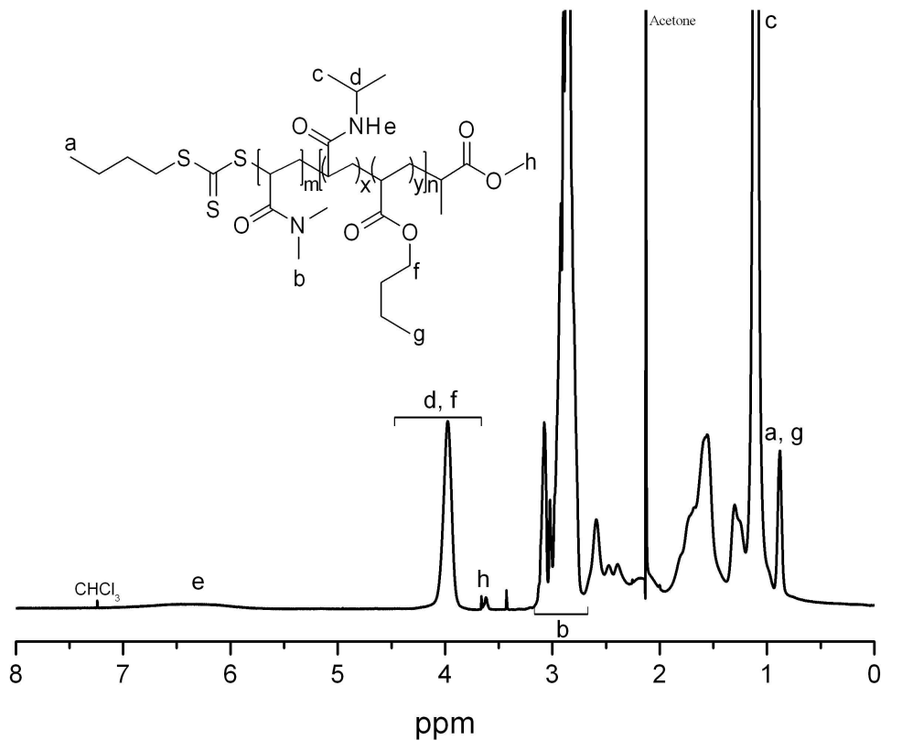


Figure S28. ^{1}H NMR spectrum of **E**: P(DMA$_{99}$-b-(NIPAM$_{97}$-co- BA$_{13}$)) in $CDCl_3$

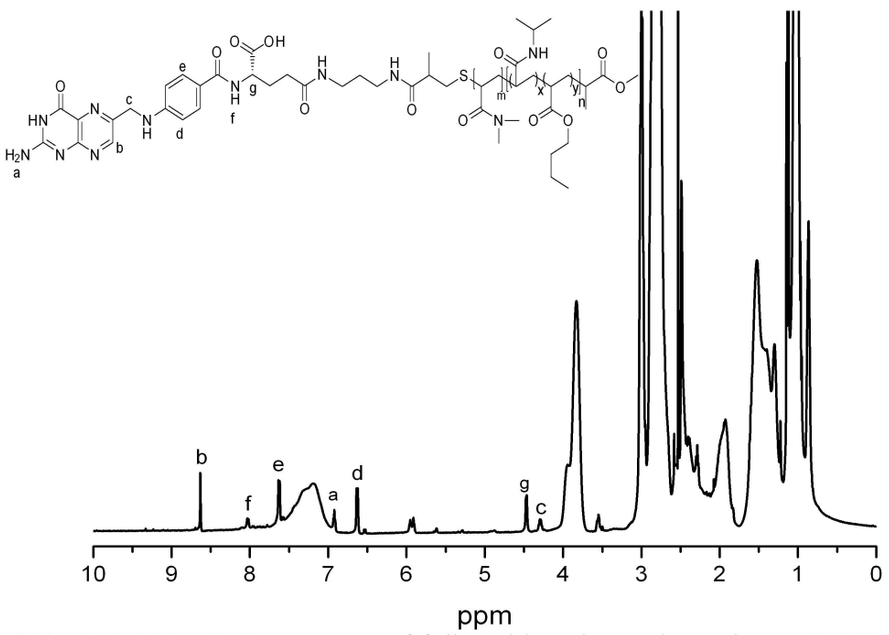


Figure S29 1D DOSY NMR spectrum of folic acid conjugated copolymer E, PDMA-b-P(NIPAM-co-BA) in DMSO-d_6.

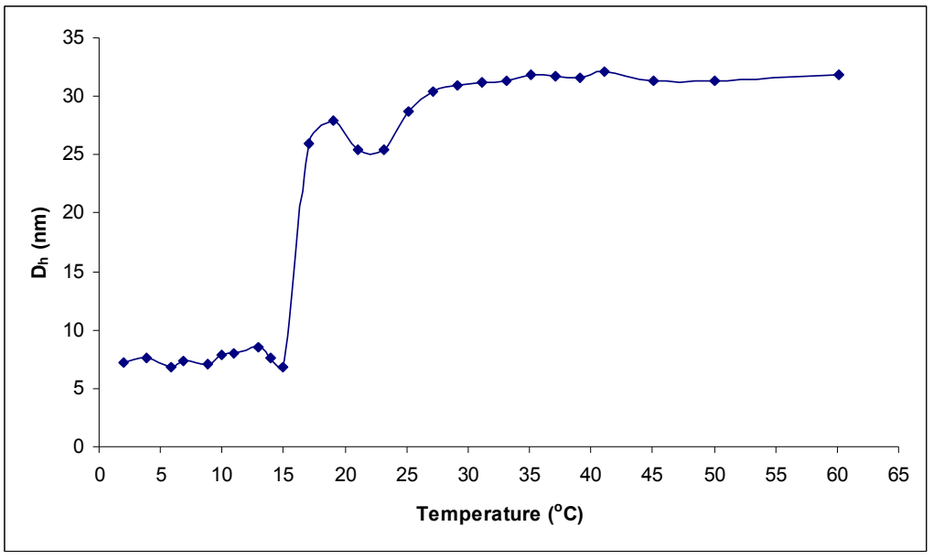


Figure S30. LCST of folic acid conjugated copolymer E, PDMA-b-P(NIPAM-co-BA) in DMSO-d_6. The polymer was dissolved in water. The data were reported as average numbers from five measurements on DLS machine at concentration 10 mg/mL. LCST = 15 – 19 ^{o}C.

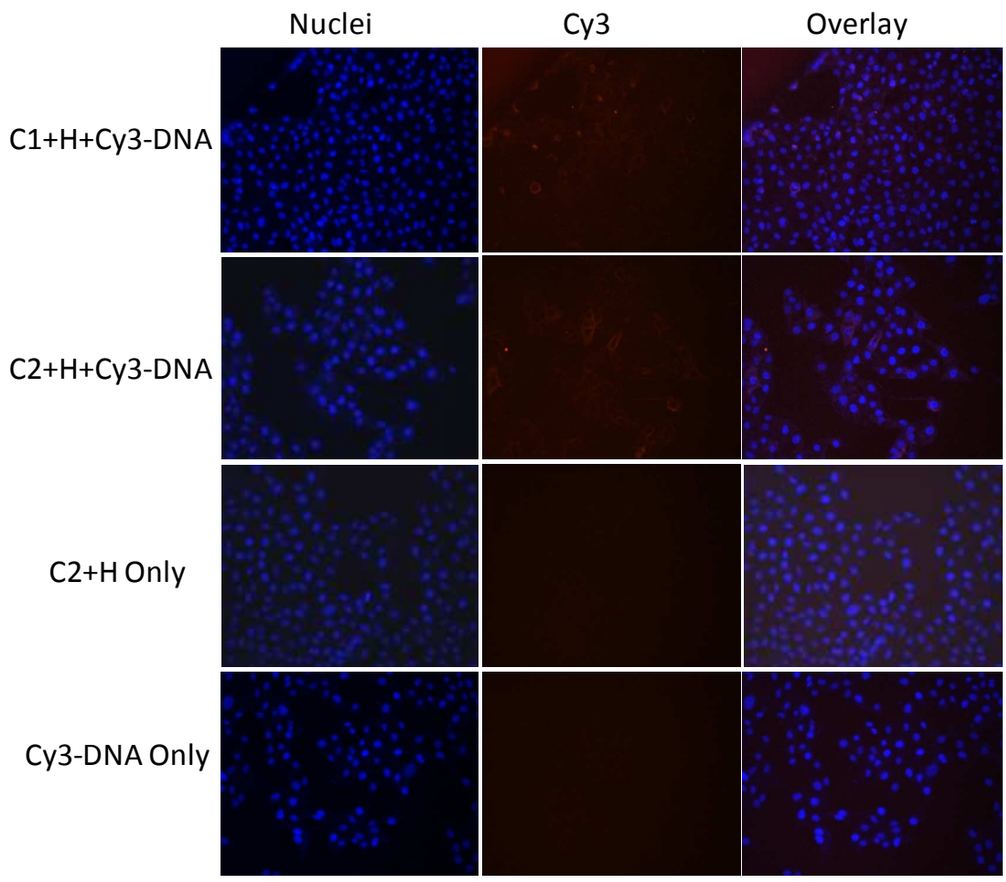


Figure S31: Fluorescent microscopy photos of the osteosarcoma U2OS cells which were dosed with 50 nM Cy3 oligo DNA and copolymer polyplexes and cultured in 24-well plate ($1x10^5$/well) in completed DMEM medium. The polyplexes were prepared in the ratio to siRNA as 50:1 in water. The mixtures were incubated in ice-bath for 10 minutes and then at 37^oC for 30 minutes. They were then added to the cells and were incubated for 10 hours before washing with PBS buffer and fixation with 4% paraffinformaldehyde. The cell nuclei were stained with Hoechst 33341 and cell uptake was viewed under fluorescent microscope.

Reference

1. Urbarni, C. N.; Monteiro, M. J. *Macromolecules* **2009**, *42,* 3884-3886.