Direct synthesis and aqueous solution behavior of pH-responsive three-layer zwitterionic branched copolymer nanoparticles

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Supporting Information

Experimental Details

Materials. All chemicals were purchased from Sigma Aldrich and used as received unless specified. Specifically, poly (ethylene glycol) methyl ether (Mn~2000 g/mol), CuCl (anhydrous, ≥ 99.99 %), 2,2′-bipyridyl (≥ 99%), (*tert*-butyl methacrylate (98%), 2-*N,N*-dimethylaminoethylmethacrylate (98 %), triethylamine (≥ 99%), 2-bromo-2 methylpropionyl bromide (98 %) and trifluoroacetic acid (99%) were used. All used solvents were HPLC grade.

Synthesis of PEO macroinitiator (α-methyl-ω-2-bromoisobutyryl polyethylene glycol 2000)

Monomethoxypolyethylene glycol (20.0 g, 0.01 mol) was charged in a two-neck round-bottom flask (250ml) and dried under vacuum for 2 hours at 100 ^{o}C. Then the dried polymer was dissolved with 200 mL of $CHCl_3$. Triethylamine (3.0 g, 0.03 mol) was added to the polymer solution, and the mixture was cooled to 0 ^{o}C using an ice bath. 2-Bromo-2 methylpropionyl bromide (6.9 g, 0.03 mol) was added dropwise via syringe, and the mixture was stirred overnight at room temperature. The polymer solution was washed three times with 10% $NaHCO_3$ aqueous solution and the organic mixture was dried over $MgSO_4$. The polymer solution was concentrated by rotary evaporation and precipitated into a 10-fold excess of diethyl ether. The precipitate was then dried under vacuum. ^{1}H NMR of product in d_6-DMSO indicated full conversion of the end-hydroxyl group (δ = 4.57 ppm, triplet, 1H) to ester (δ = 4.25 ppm, triplet, 2H).

Synthesis of branched triblock copolymer, PEO_{45}-*b*-poly(*N,N*-dimethylaminoethylmethacrylate)$_{25}$-*b*-poly(tert-butylmethacrylate$_{25}$-co-ethylene glycoldimethacrylate$_{0.97}$)

The typical synthetic procedure was as follows: Into a Schlenk flask was added the PEO macroinitiator (2.5 g, 0.00116 mol), *N,N*-dimethylaminoethylmethacrylate (4.5 g, 0.028 mol) and solvent (isopropanol/water (92.5/7.5 v/v; 10mL)). The reaction mixture was then degassed via nitrogen sparge for 60 minutes under stirring to completely remove the oxygen. Then, the reaction mixture was heated to 40 ^{o}C. CuCl (0.114 g, 0.00115 mol) and bipyridine (0.45g, 0.00288mol) were added and the reaction solution turned dark brown. The polymerization was carried out at 40 ^{o}C under stirring and a nitrogen atmosphere. Samples were taken periodically from the reaction mixture during polymerization for ^{1}H NMR analysis to measure the monomer conversion. Meanwhile, in a 100 mL round bottom flask, *t*-butylmethacrylate (4.0 g, 0.028 mol), ethyleneglycoldimethacrylate (0.22 g, 0.0111 mol) and 4 mL of the water-alcohol solvent mixture were added, and this mixture sparged with nitrogen for 1 hour. After the conversion of dimethylmethacrylate monomer reached above 95%, the mixture with *t*-butylmethacrylate monomer was added into the reaction flask rapidly using a syringe, taking care not to admit any air into the vessel. A sample was taken immediately after the addition of the *t*-

butylmethacrylate monomer solution for ^{1}H NMR analysis. The block copolymerization reaction was carried at 40 °C and samples were taken periodically from the reaction mixture for ^{1}H NMR analysis. The polymerization was terminated by the addition of THF (20mL) into the reaction mixture and exposing the sample to air. The mixture was passed through an Al_2O_3 column to remove the copper species and the ABC branched block copolymer was obtained after re-precipitation in cold hexane three times and drying in a vacuum oven overnight.

Synthesis of linear triblock copolymer, PEO_{45}-*b*-poly(N,N-dimethyl aminoethylmethacrylate)$_{25}$-*b*-poly(tert-butylmethacrylate)$_{25}$

The synthesis and monomer conversion monitoring for the poly(*N,N*-dimethylmethacrylate) block was carried out exactly as described above. In 100 mL round bottom flask, *t*-butylmethacrylate (4.0 g, 0.028 mol) and 4 mL of the water-alcohol solvent mixture were added, and this mixture was sparged with nitrogen for 1 hour. After the conversion of DMA monomer reached above 95%, the mixture with *t*-butylmethacrylate was added into the reaction flask rapidly using a syringe and taking care not to admit any air into the vessel. A sample was taken immediately after the addition of the *t*-butylmethacrylate monomer solution for ^{1}H NMR analysis. The block copolymerization reaction was carried at 40 °C and samples were taken periodically from the reaction mixture for ^{1}H NMR analysis. The polymerization was stopped by adding THF into the reaction mixture (20mL) and exposing the sample. The mixture was passed through an Al_2O_3 column to remove the copper species and the ABC linear block copolymer was obtained after re-precipitation in cold hexane three times and drying in a vacuum oven overnight.

Preparation of ABC copolymer nanoparticles

The copolymer was dissolved in 8 mL of THF (1 mg/mL or 10 mg/mL) and 2 mL of water was added dropwise. After the addition of water, the mixture was stirred for 30 minutes. The copolymer particles in the water/THF mixture were then exhaustively dialysed against water for three days (7000 g/mol *M*w cut-off dialysis tubing).

Hydrolysis of poly(*tert*-butylmethacrylate) block of ABC block copolymers to form poly(methacrylic acid)

0.5g of ABC (linear or branched) block copolymer were dissolved in 30 mL of trifluoroacetic acid/chloroform mixture (30/70 v/v) and left under stirring overnight at room temperature. Then, the polymer solution was concentrated on rotary evaporator to afford a solid residue, which subsequently was washed several times with diethyl ether and dried overnight under vacuum overnight at room temperature. ^{1}H NMR of product in d_6-DMSO indicated full hydrolysis of the tert-butyl ester groups ($\delta = 1.41$ ppm, singlet, 9H) to acid ones ($\delta = 12.3$ ppm, singlet, 1H).

Analysis

^{1}H-NMR characterization of linear and branched ABC tri-block copolymers

All ^{1}H-NMR spectra were recorded on copolymer solutions in CD_3Cl, d_8-THF, acetone-d_6, or D_2O on a Bruker Avance 400MHz NMR spectrometer. The copolymer sample in D_2O was prepared from copolymer solution in acetone-d6, which was diluted with D_2O and acetone-d_6 was evaporated by bubbling with N_2. The copolymer composition and number average molecular weight were calculated using the intensity ratio of the macro-initiator signal (3.65 ppm, $-CH_2-CH_2-O-$, 4H) to the signals of the poly(*N,N*-dimethylaminoethylmethacrylate) (2.3 ppm, $(CH_3)_2N-$, 6H) and the poly-(*tert*-butylmethacrylate) (1.41 ppm, $(CH_3)_3C-$, 9H).

GPC characterization

GPC chromatograms were obtained for the PEO macroinitiator prior to polymerization, PEO-DMA diblock copolymer prior to addition of tBuMA and EGDMA and the final PEO-PDMA-P(tBuMA/EGDMA) branched copolymer and are overlayed in Figure S5 to demonstrate the molecular weight evolution during polymerization. Molecular weights, molecular weight distributions and Mark-Houwink α-values were measured using a Viscotek TDA-302 triple-detection gel permeation chromatography equipped with two ViscoGel HHR-N columns and a guard column with a mobile phase of THF at 35°C and a flow rate of 1 mL min^{-1}.

Dynamic Light Scattering

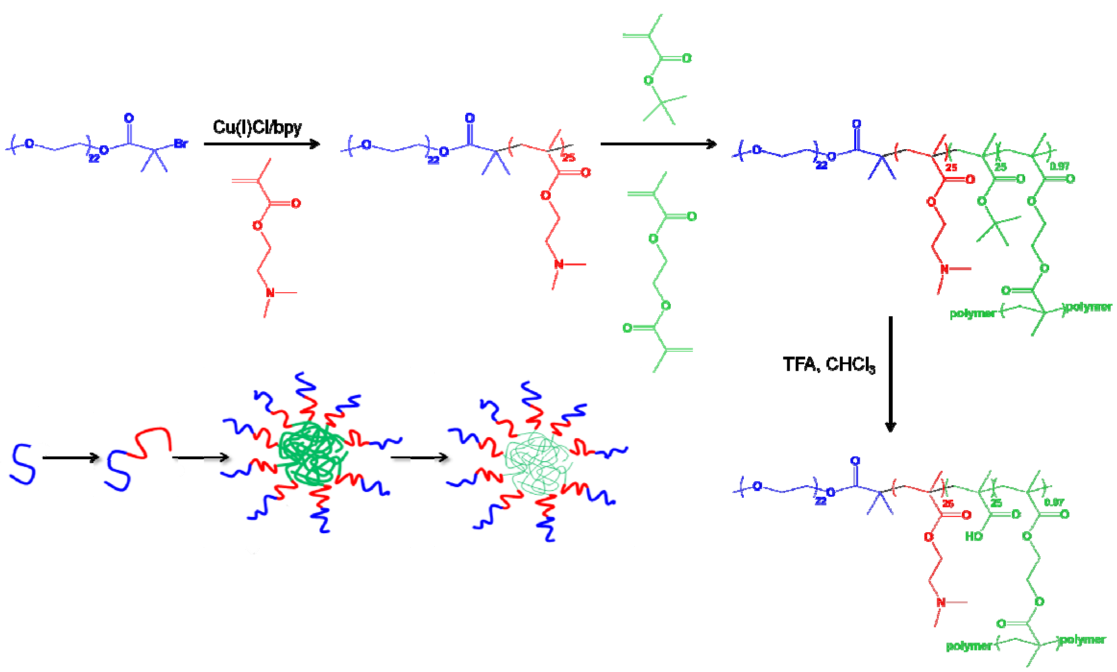
Prior to DLS measurements, the copolymer samples were ultrafiltered using 0.2 μm Teflon PTFE filter. DLS measurements were carried out using Malvern Nano-S DLS instrument. The PDI values were calculated from the cumulants analysis of the DLS measured intensity correlation function. The instrument was calibrated and PDI calculations were checked using standard mono-disperse 60nm polystyrene latex.

Cryo-TEM

Samples for thin film cryo-TEM were loaded onto plasma treated (30 s) holey-carbon grids and prepared using a GATAN cryoplunge into liquid ethane. This set-up incorporates a humidity chamber consisting of a cylindrical perspex column sealed at the top and bottom where the release shutter system operates the closure of the base of the chamber. An internal humidity of ~ 98% was maintained by means of a warm water soaked sponge inserted into the chamber 15 minutes before the start of the sample preparation. The samples were transferred using a Gatan 626 cryotransfer system and examined using a JEOL 2100 TEM operating at 200 keV. Images were obtained using a Ultrascan 4000 camera and analysed by GATAN Digital Micrograph.

Negative stain TEM

TEM was carried out using a JEOL 2100 TEM operating at 200 keV. Samples were stained with 1% aqueous uranyl acetate.



Scheme S1. Schematic and composition of the branched ABC copolymer synthesis.

Polymer	M_n/Da	M_w/Da	M_w/M_n	α
Linear ABC	12,000	15,000	1.3	0.48
Branched ABC	37,000	156,000	4.2	0.29

Table S1. TD-GPC data for linear and branched copolymers after purification.

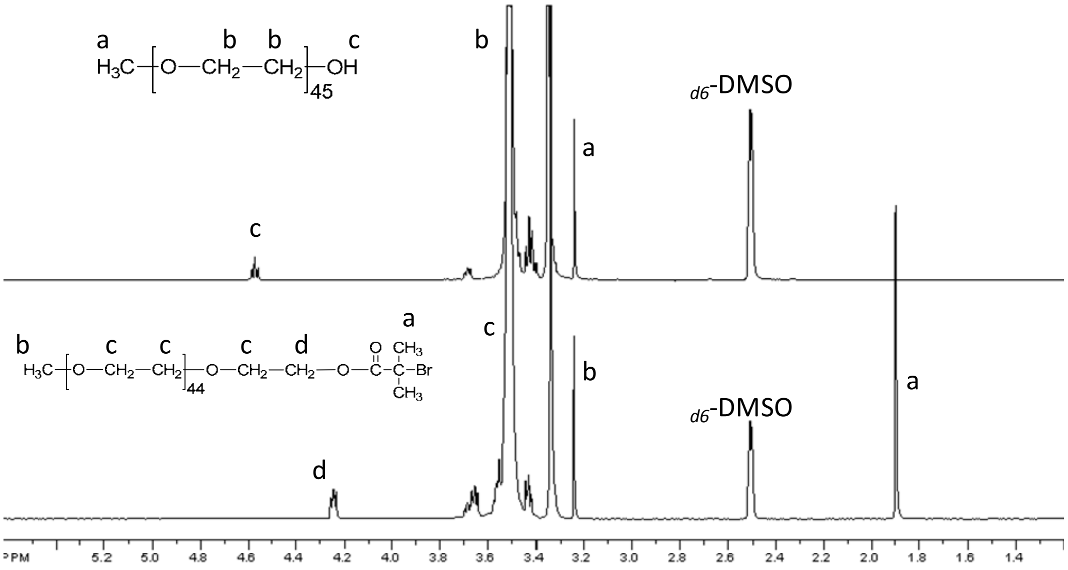


Figure S1. ^{1}H-NMR of (top) mPEO-OH and (bottom) macroinitiator prepared from mPEO-OH.

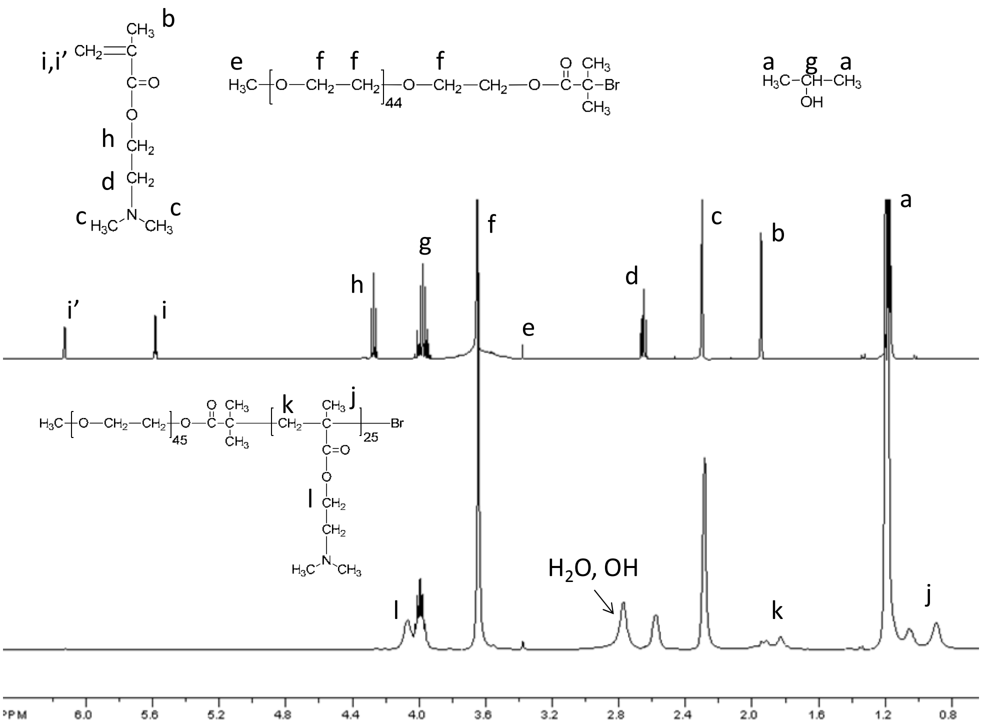


Figure S2. B-block polymerisation of ABC-branched copolymer. Top, at 0% conversion. Bottom, at >95% conversion.

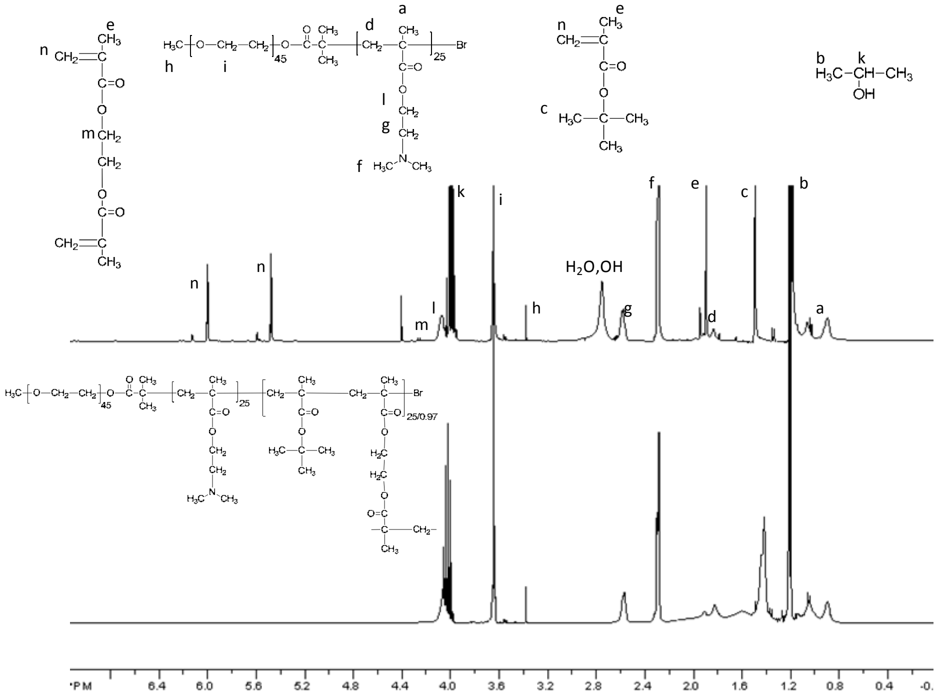


Figure S3. C-block polymerisation of ABC-branched copolymer. Top, at 0% conversion of tert-butyl acrylate. Bottom, at >95% conversion of monomers. The sharp peaks at 1.19 ppm and 3.98 ppm are residual isopropanol.

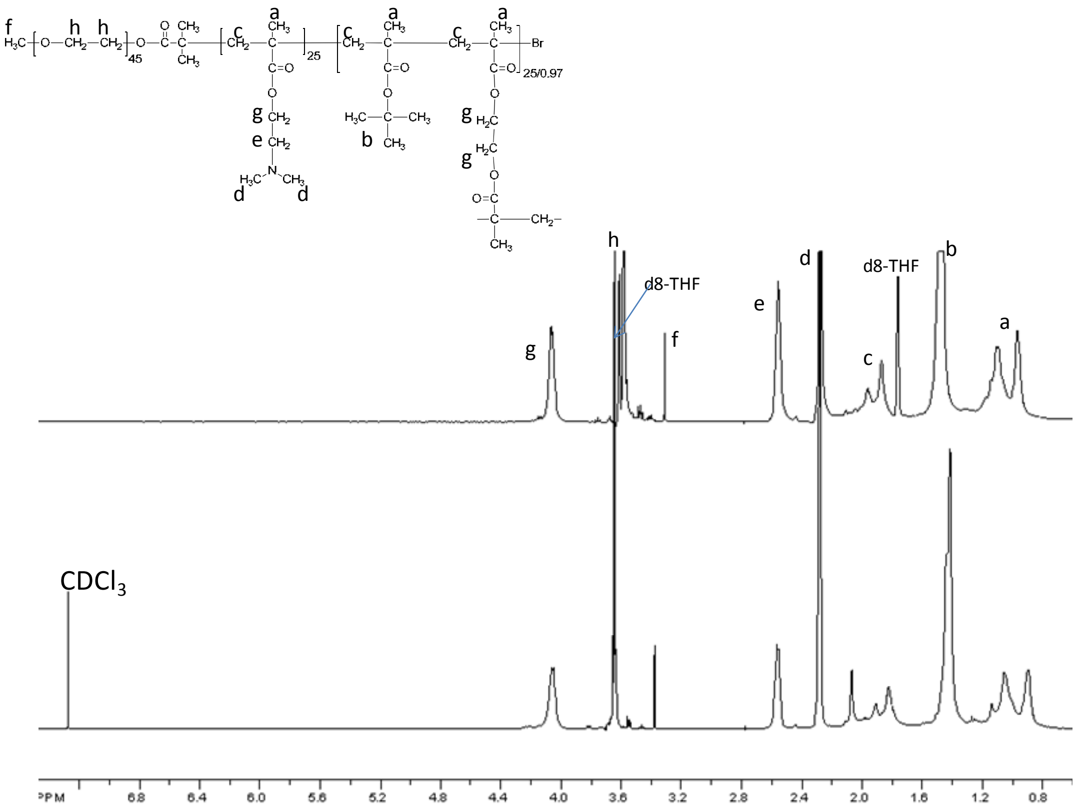


Figure S4. ^{1}H NMR spectra of branched ABC copolymer in d$_8$-THF (top) and CDCl$_3$ (bottom).

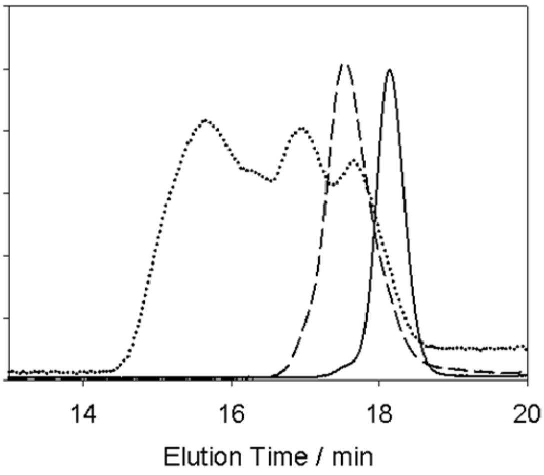


Figure S5. GPC data for the ABC branched copolymer. PEO-macroinitiator (—), PEO-DMA diblock copolymer (---) and branched PEO-PDMA-P(*t*BMA/EGDMA) copolymer (···).

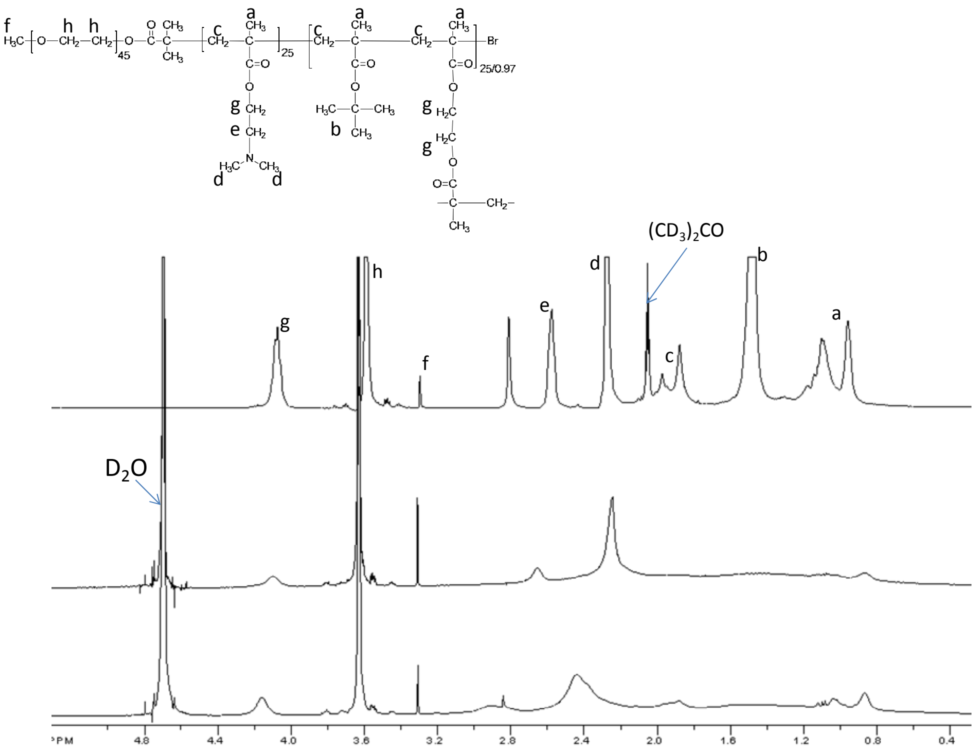


Figure S6. ^{1}H NMR spectra of branched ABC copolymer in (top) acetone-d6, (middle) D_2O and (bottom) acidified-D_2O. In D_2O, the t-BMA block is NMR invisible. In acidified D_2O, the DMA block is protonated and the proton resonances shift downfield accordingly. The t-BMA block remains NMR invisible.

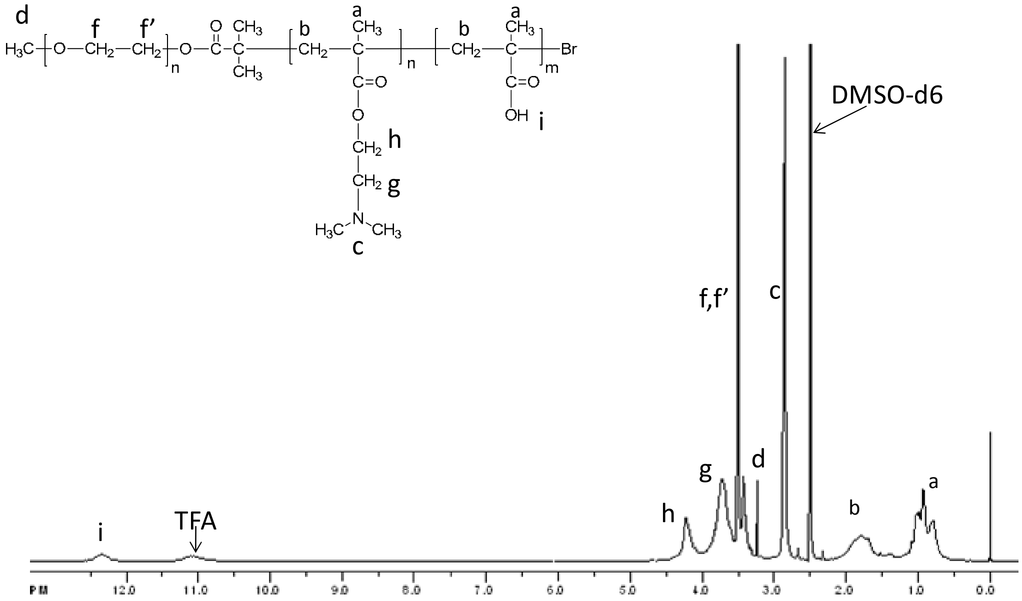


Figure S7. NMR of hydrolysed branched ABC polymer in d_6-DMSO.

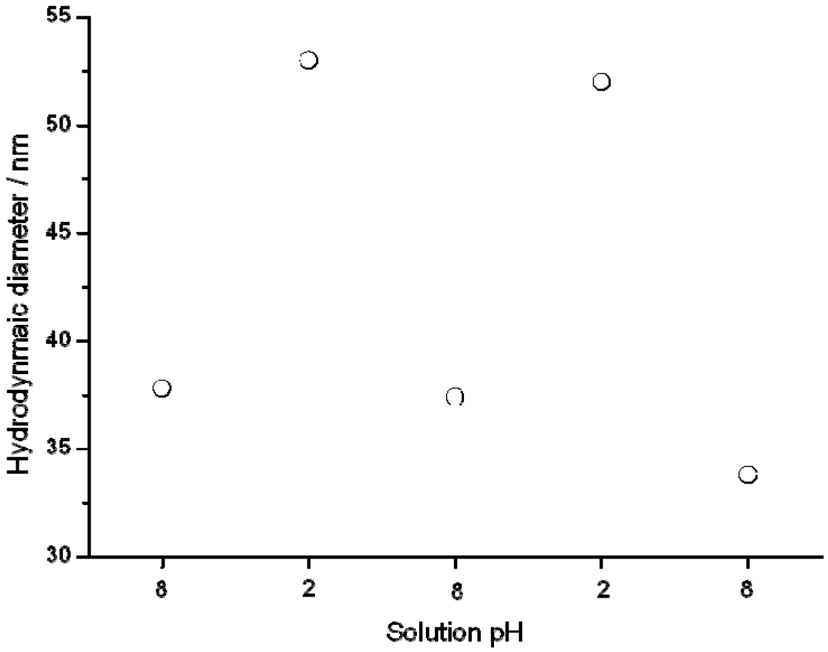


Figure S8. Hydrodynamic diameter of branched copolymer nanoparticles under cyclic pH switches between pH 8 and pH 2, as judged by DLS.