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Supporting Information

for

Poly(*N*-(2-Hydroxypropyl) Methacrylamide)-Valproic Acid Conjugates as Model Block Copolymer Nanocarriers

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Materials and measurements

All reagents and solvents were obtained from commercial sources and used as received. The HCT116 cells were purchased from the American Type Culture Collection (Manassas, VA). 2-Propylvaleric acid (valproic acid, VPA, 97%), 4-dimethylaminopyridine (DMAP, 99%), 2,2′-azobis(2-methylpropionitrile) (AIBN, 98%), and pyrene (99%) were purchased from Sigma-Aldrich. *N,N*-Dimethylacetamide (DMAc, 99%), methacryloyl chloride (97%), and 1-amino-2-propanol (97%) were purchased from VWR International. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC•HCl, 95%) was purchased from Combi-Blocks. 4-Cyano-4[(dodecylsulfanylthiocarbonyl)-sulfanyl]-pentanoic acid (CDTPA) was synthesized according to a previous report.[44] All ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz)

spectra were recorded on a Varian Mercury 500 spectrometer with chemical shifts referenced to residual signals from $CDCl_3$ (7.26 ppm) or DMSO-d_6 (2.50 ppm). Gel permeation chromatography (GPC) was conducted in DMAc with 0.05 M LiCl at 50 °C with a flow rate of 1.0 mL min^{-1} (Pump: Agilent 1260 Infinity Isocratic Pump G1310B, Columns: Guard + two ViscoGel I-series G3078 mixed bed columns, molecular weight range 0–20 × 10^3 and 0–100 × 10^4 g mol^{-1}). Detection consisted of a Wyatt Optilab T-rEX refractive index detector operating at 658 nm and a Wyatt miniDAWN TREOS laser light scattering detector (operating at 50 mW, 658 nm with detection angles of 49°, 90°, and 131°). Polymer d*n*/dc values were determined online assuming 100% mass recovery during GPC analysis. Absolute molecular weights and molecular weight distributions were calculated using the Wyatt ASTRA software. Transmission electron microscopy **(**TEM) analysis was conducted as follows: five microliters of the sample was applied onto a formvar-coated 200-mesh Cu grid that was freshly glow discharged (Pelco easiGlow™, Ted Pella, Inc.). The grids were stained with a 2% aqueous solution of uranyl acetate and observed on a Hitachi H7000 microscope operating at 100 kV. The images were recorded with a slow-scan CCD camera (Veleta 2k × 2k). Dynamic light scattering (DLS) analysis was conducted on a Zetasizer Nano-ZS (Malvern) at room temperature. Fluorescence measurements were conducted with 150 μL of sample on black 96-well polypropylene microplates (Greiner Bio-One) with an excitation wavelength of 340 nm and λ_{maxem} = 396 nm. Calibration curves were constructed using the integrated SoftMax Pro software. Gas chromatograms were recorded on an Agilent 6550 Series II equipped with a 30 m x 0.32 mm ID Agen-Tubular column with a 0.25 mm HP-1 (polydimethylsiloxane) stationary phase film. Detection consisted of a flame ionization detector (FID) operating at 250 °C. Gas flows were maintained at the following flow rate: hydrogen at 40 mL/min, air at 450 mL/min, and helium at 45 mL/min. The inlet temperature was maintained at 250 °C, and the column was heated according to the following profile: 70°C initial temperature, 10 °C/min ramp for 5 min, 120 °C isothermal for 2 min. Chromatogram peak integrations were calculated using Agilent ChemStation software.

Synthesis of *N*-(2-hydroxypropyl)methacrylamide (HPMA)

1-Amino-2-propanol (24.3 mL, 0.311 mol, 1.0 equiv.) was added to a round bottom flask with sodium bicarbonate (34 g, 0.40 mol, 1.3 equiv.) and dry ethyl acetate (85 mL). The solution was stirred, purged with nitrogen, and cooled in an ice bath. Methacryloyl chloride (29.5 mL, 0.304 mol, 0.980 equiv.) in anhydrous ethyl acetate (40 mL) was cooled to -20 °C and added dropwise to the reaction. The reaction mixture was stirred for an hour at room temperature, then anhydrous sodium sulfate (10 g) was added. After stirring the mixture for an additional 10 min, the solution was filtered to remove the salts. The filtrate was concentrated and stored at -20 °C overnight to crystallize the product. The crystals were collected by filtration, washed with cold acetone and dried under high vacuum to yield pure HPMA (yield: 20 g, 45%). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 6.22 (s, 1H), 5.74(s, 1H), 5.35 (s, 1H), 3.95 (m, 1H), 3.49-3.55 (m, 1H), 3.16-3.22 (m, 1H), 2.46 (s, 1H), 1.98 (s, 3H), 1.23 (d, 3H).

RAFT homopolymerization of HPMA

HPMA (1.0 g, 7.0 mmol), AIBN (1.2 mg, 0.0070 mmol), and CDTPA (0.028 g, 0.070 mmol) were dissolved in DMAc (2.3 mL) in a Schlenk flask. The molar ratios of [HPMA]/[CDTPA]/[AIBN] were 100/1/0.1. The flask was degassed by three cycles of freeze-pump-thaw and then placed in a preheated oil bath at 70 °C. The polymerization was quenched after 5 h by placing the flask in an ice bath and exposing it to air. The polymer was purified by precipitation into diethyl ether (3×) and dried under vacuum at room temperature.

Synthesis of VPMA

Valproic acid (1.2 g, 8.4 mmol, 1.2 equiv.) was dissolved in dichloromethane (20 mL) and cooled to 0 °C. EDC (2.0 g, 10 mmol, 1.5 equiv.) was added and stirred for 15 min. HPMA (1.0 g, 7.0 mmol, 1.0 equiv.) and DMAP (0.12 g, 1.0 mmol, 0.15 equiv.) were added, and the temperature was slowly increased to room temperature. The mixture was stirred for 20 h, then washed with 5% citric acid solution. The organic layer was collected and dried over anhydrous magnesium sulfate. The solution was filtered and solvent was removed using a rotary evaporator. The desired product was collected as white crystals (1.7 g, yield: 90%). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 0.91 (m, 3H),

1.25-1.65 (m, 7H), 1.60 (m, 4H), 1.97 (s, 3H), 2.39 (m, 1H), 3.41 (m, 1H), 3.55 (m, 1H), 5.25 (m, 1H), 5.35 (s, 1H), 5.7 (s, 1H), 6.20 (s, 1H). ^{13}C NMR ($CDCl_3$) δ (ppm) 176.69, 168.09, 139.54, 119.59, 61.50, 44.59, 34.65, 34.58, 20.50, 20.46, 18.39, 17.78, 13.87, 13.84. ESI-HRMS calcd. for $C_{12}H_{16}O_2$ $[M + H]^+$: 270.2064. Found: 270.2063.

Chain extension of PHPMA macroCTA with HPMA and VPMA

A series of chain extensions at varying initial feed ratios of [HPMA]:[VMPA]:[CTA] were performed at 70 °C as denoted in Table 1. In a typical procedure, PHPMA macroCTA (0.3 g, 0.04 mmol), VPMA (0.81 g, 3.0 mmol), HPMA (0.43 g, 3.0 mmol), and AIBN (1.4 mg, 0.0085 mmol) were dissolved in DMAc ($[M]_o$ = 2 M) in a Schlenk flask equipped with a magnetic stirring bar. The flask was degassed by three cycles of freeze-pump-thaw and then placed in a preheated oil bath at 70 °C. The reaction was quenched after 3 h by exposing the solution to air. The polymer was purified by precipitation into diethyl ether.

Preparation of polymeric micelles

Micelles were prepared by dialysis from a good solvent into a non-solvent. In a typical procedure, PHPMA-*b*-P(HPMA-*co*-VPMA) in DMF (10 mg/mL) was transferred to a dialysis bag with a molecular weight cutoff of 3500 g/mol. The mixture was dialyzed against deionized (DI) water for 3 days and lyophilized. After dialysis, 1 mL of the solution was filtered (0.45 μm) into a cuvette and the size was measured by DLS and TEM.

Critical micelle concentration determination

The CMC of PHPMA-*b*-P(HPMA-*co*-VPMA) conjugate was determined using pyrene as a model fluorescent probe. The concentrations of copolymer **4** were varied from 3.00 mg/mL to 3.18 × 10^{-6} mg/mL, with a fixed pyrene concentration of 6 × 10^{-6} M. The fluorescence emission spectra were recorded from 360 to 600 nm (Fig. S6 a, λ_{ex} = 340 nm). The fluorescence intensity at 393 nm was plotted as a function of

log(concentration), and the intersection of the extrapolated linear regions at high and low concentrations were used to determine the CMC.

Degradation study of the block copolymer-based micelles at acidic and physiological pH

Block copolymer micelle (10 mg) was dissolved in 2 mL of phosphate buffer solution (pH 5) and stirred in a sealed vial at 37 ºC. Aliquots (0.2 mL) were removed at predetermined time points from the polymer solution and lyophilized. Each sample was characterized using ^{1}H NMR spectroscopy, DLS, and gas chromatography. The control degradation study for the block copolymer was conducted in phosphate buffer solution (pH 7.4).

Cytotoxicity assay

PHPMA$_{53}$ and PHPMA$_{53}$-*b*-P(HPMA$_{15}$-*co*-VPMA$_{28}$) were dissolved in DMSO at 80 mg/ml and diluted two-fold with DMSO (×5). HCT116 cells were seeded into 96 well plates at a density of 7.5 x 10^{3} cells/well and allowed to attach overnight before the addition of the test compounds at a final concentration of 0.5% DMSO. Plates were incubated for 72 h at 30 °C in a humidified incubator with 5% CO_2, after which cell viability was assessed using the CellTiter 96® Non-Radioactive Cell Proliferation Assay (Promega, Madison WI) according to the manufacturer's instructions.

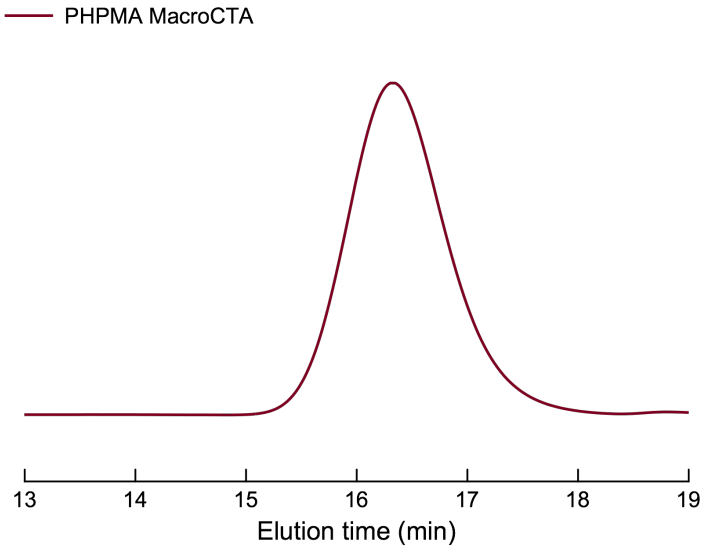
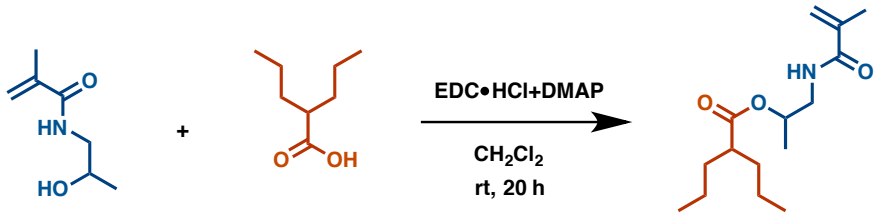


Figure S1. Gel permeation chromatogram of PHPMA macroCTA with M_n = 8.0 kg/mol and M_w/M_n = 1.1.



Scheme S1. Synthesis of HPMA-valproic acid conjugate, VPMA.

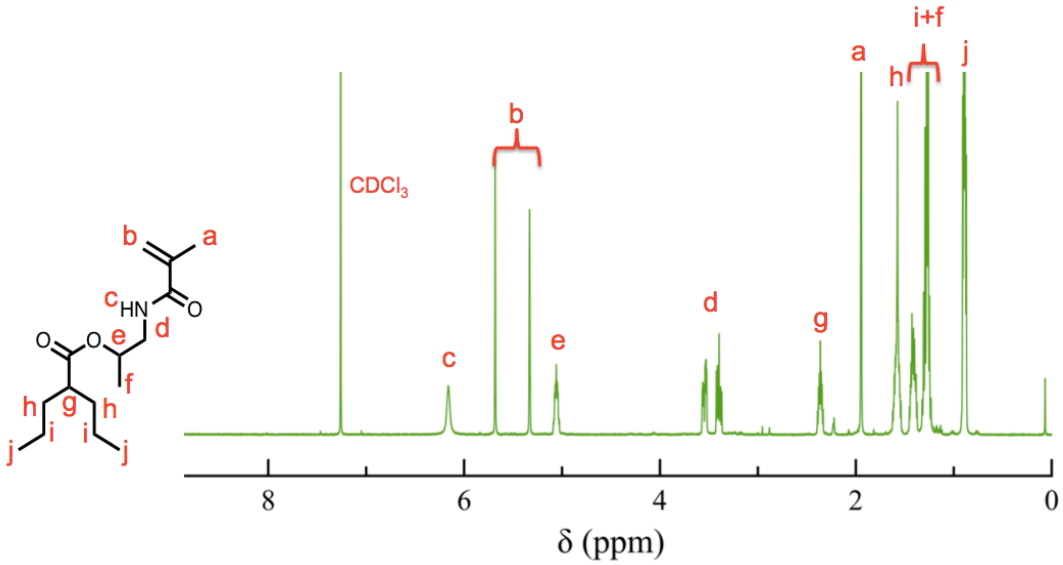


Figure S2. ^{1}H NMR spectrum of VPMA (500 MHz, $CDCl_3$, 25 °C).

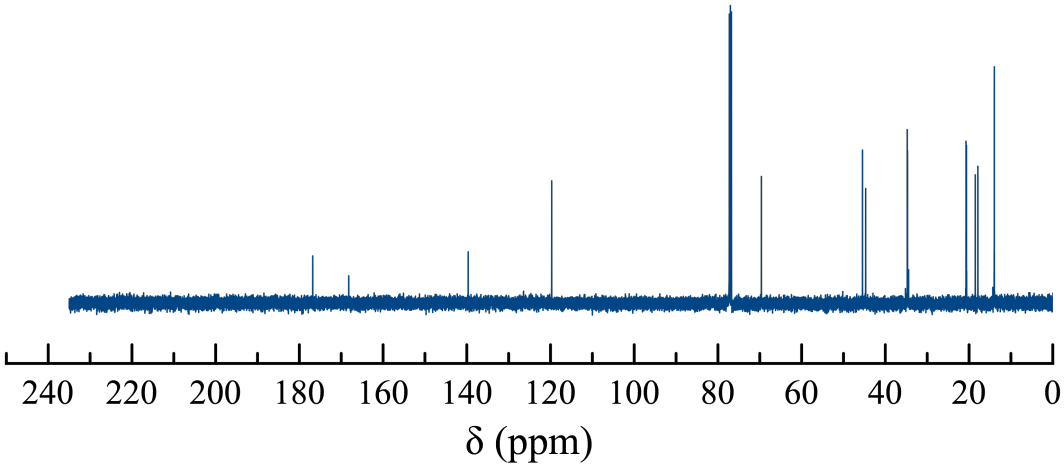


Figure S3. ^{13}C NMR spectrum of VPMA (125 MHz, $CDCl_3$, 25 °C).

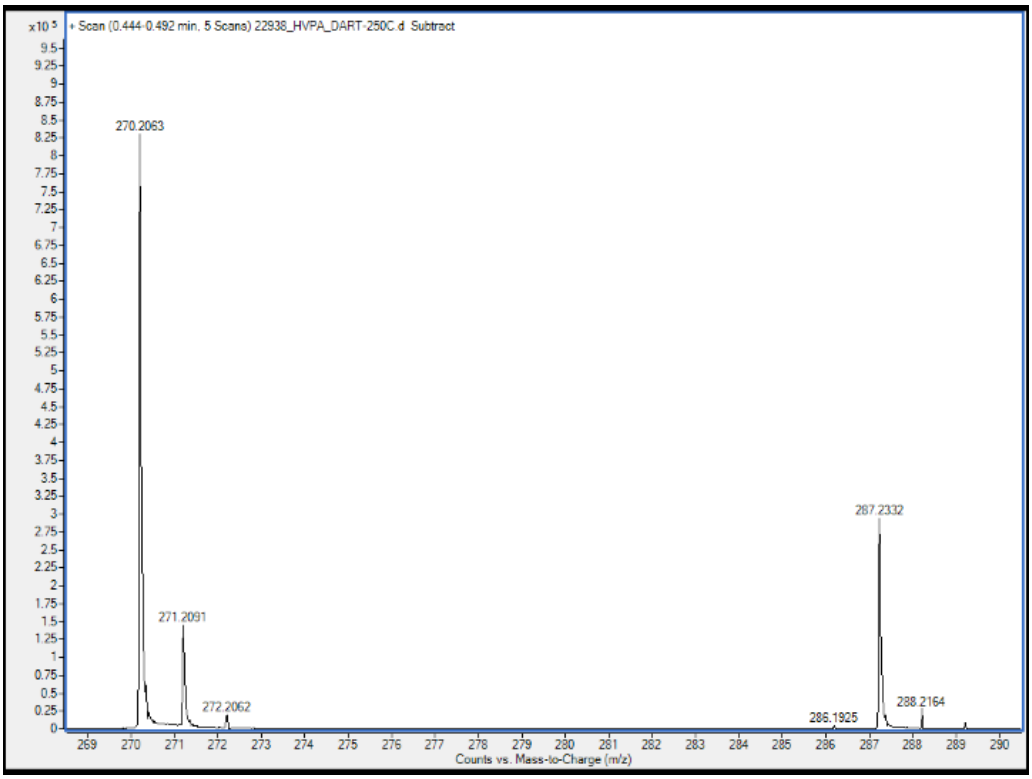


Figure S4. High-resolution mass spectrum of VPMA.

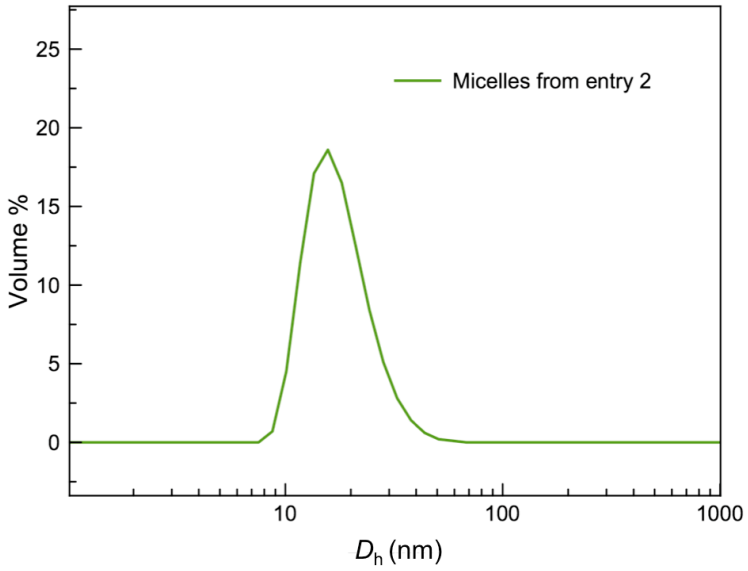


Figure S5. DLS plot of the micelles from entry **2** (Table 1).

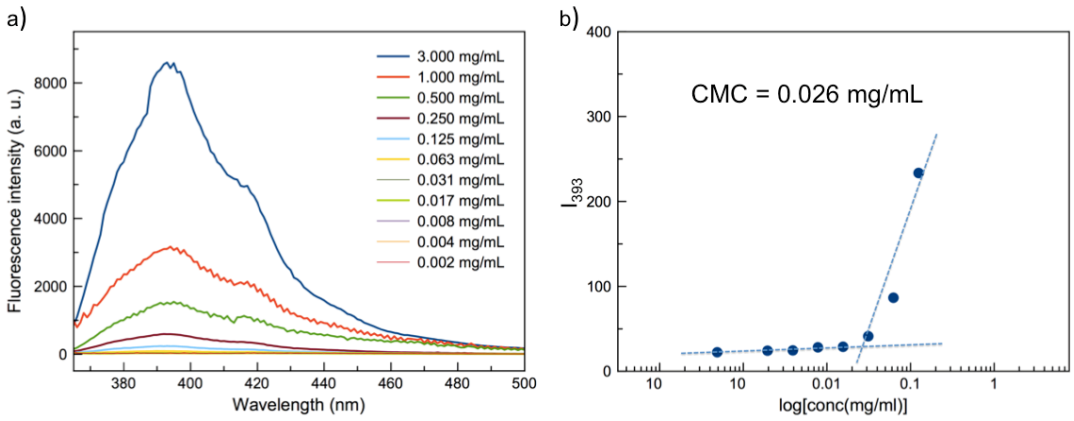


Figure S6. (a) Fluorescence spectra of the block copolymer **4** and pyrene (6 μm) in water at varying polymer concentrations; (b) Fluorescence intensity of pyrene emission band ($I_{393\ nm}$) as a function of block copolymer concentration in aqueous solution. The intersection point of the linear regions was taken as the critical micelle concentration.

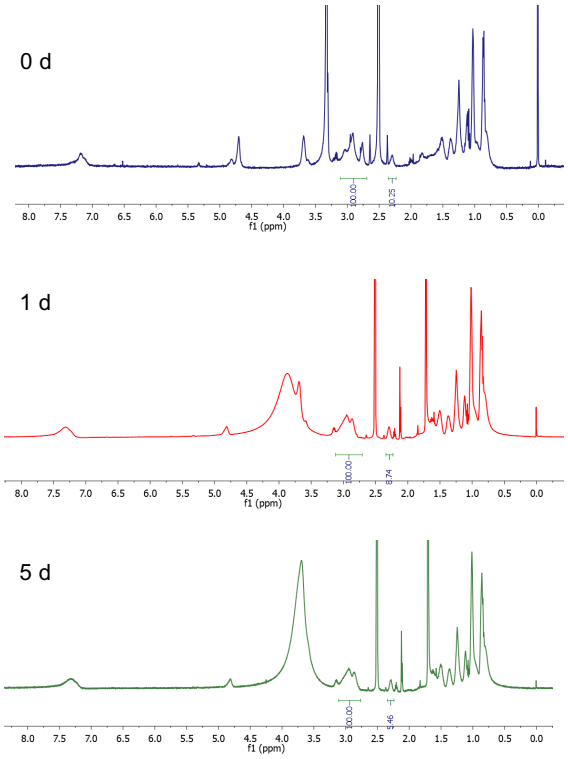


Figure S7. ^{1}H NMR spectra of PHPMA$_{53}$-*b*-P(HPMA$_{15}$-*co*-VPMA$_{28}$) after incubation in pH 5 buffer at 37 °C for 0 d (blue), 1 d (red), and 5 d (green). The gradual decrease of the signal attributed to the methine proton of VPMA (~2.25 ppm, relative to the integration of the backbone protons at ~3 ppm) indicates hydrolysis of the ester bond between PHPMA and VPA. After 5 d under these conditions, 46.7 % of VPA had been cleaved from the polymer.

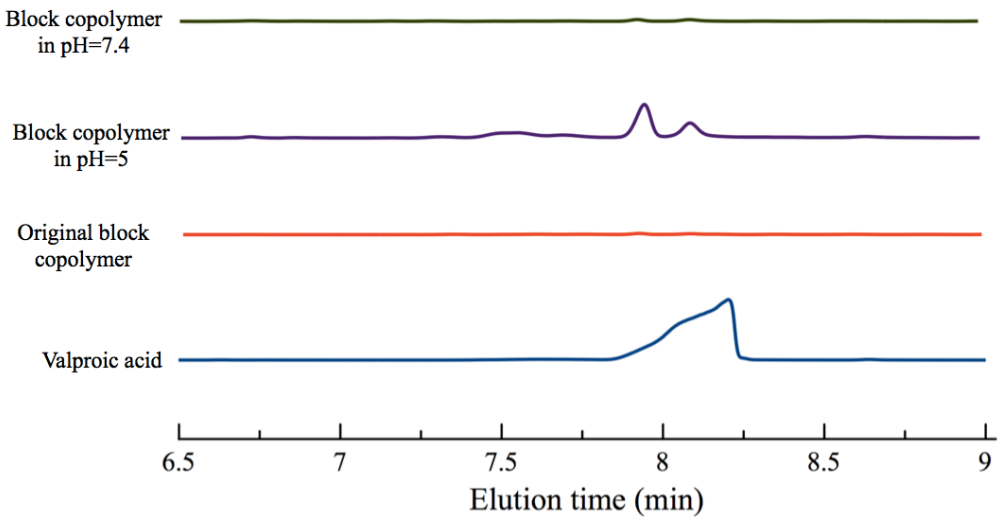


Figure S8. Gas chromatograms of valproic acid, the original block copolymer, and the block copolymer after being subjected to incubation at pH = 5 for 5 days and pH = 7.4 for 10 days at 37 °C.

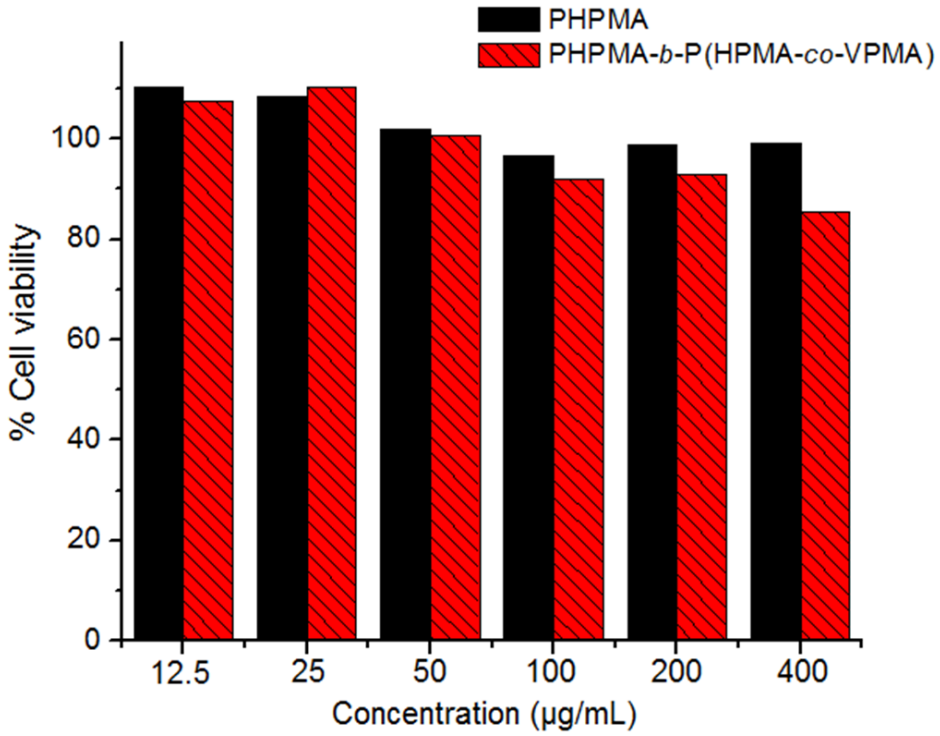


Figure S9. Effect of the presence of $PHPMA_{53}$ and $PHPMA_{53}$-*b*-P($HPMA_{15}$-*co*-$VPMA_{28}$) on cell survival percentage in HCT 116 cells based on MTT assay. Cells were treated with different concentrations of polymers (12.5-400 µg/mL) for 72 hours. Percent of cell viability was calculated by considering the value of carrier solvent treated samples (0.5% DMSO) as 100%. The experiment was repeated three times and the average of these trials was taken.