Electronic Supplementary Material (ESI) for Polymer Chemistry.

Starfish Supporting Information

SI Table 1

SI table 1 Theoretical and experimentally determined copolymer composition, number average molecular weights (Mn), and molar mass distributions (D) for a series of HEMA and O950 copolymers prepared by RAFT

Sam. #	n_f HEMA (feed)	n_f O950 (feed)	$^a n_f$ HEMA (exp.)	$^a n_f$ O950 (exp.)			$^b M_n$ (kDa) (Theo.)	$^c M_n$ (kDa) (exp.)	Đ
1	75	25	83	17	50	10	13800	15800	1.07
2	50	50	62	38	50	10	22400	20400	1.08
3	25	75	55	45	50	10	25200	28000	1.18
4	50	50	59	41	12.5	10	6100	6500	1.2
5	50	50	57	43	25	10	12300	10300	1.09
6	50	50	60	40	50	10	23200	19600	1.08
7	50	50	64	36	100	10	42800	35700	1.14

a As determined by 500 mhz 1H NMR spectroscopy in CDCl3 by evaluation of O950 Methoxy resonance at 3.39 ppm [A] to the combined ester region at 4.1 ppm [B] using the formulas: 1H O950 = [A]/3 and 1H HEMA =([B]-[A]2/3)/2.

b As determined by HPLC by comparison of the individual comonomer peeks before and after polymerization. Theoretical values were calculated based on the relative molar feed ratios, individual comonomer conversions and the initial ratio monomer to CTA using the equation Mn Theory = ($[HEMA]_o$*Conversion HEMA*FWHEMA+ $[O950]_o$*Conversion O950*FWO950)/[CTA]o +FWCTP

c As determined by size exclusion chromatography using Tosoh SEC TSK-GEL α-3000 and α-4000 columns (Tosoh Bioscience, Montgomeryville, PA) connected in series to an Agilent 1200 Series Liquid Chromatography System (Santa Clara, CA) and Wyatt Technology miniDAWN TREOS, 3 angle MALS light scattering instrument and Optilab TrEX, refractive index detector (Santa Barbara, CA). HPLC-grade DMF containing 0.1 wt.% LiBr at 60 ^oC was used as the mobile phase at a flow rate of 1 mL/min

Supporting information Table 2. Table 1: Summary of theoretical and experimentally determined molecular weights and Đ values for polymeric brushes synthesized from 10, arm gCTA with short EB segments.

DP EB Target	DP/arm Exp	Mn, Brush	Mn, Arm	Đ
gCTA	-	15,700	-	1.19
20	15	49,000	2,600	1.19
35	24	69,000	4,100	1.27
50	41	105,000	6,900	1.28

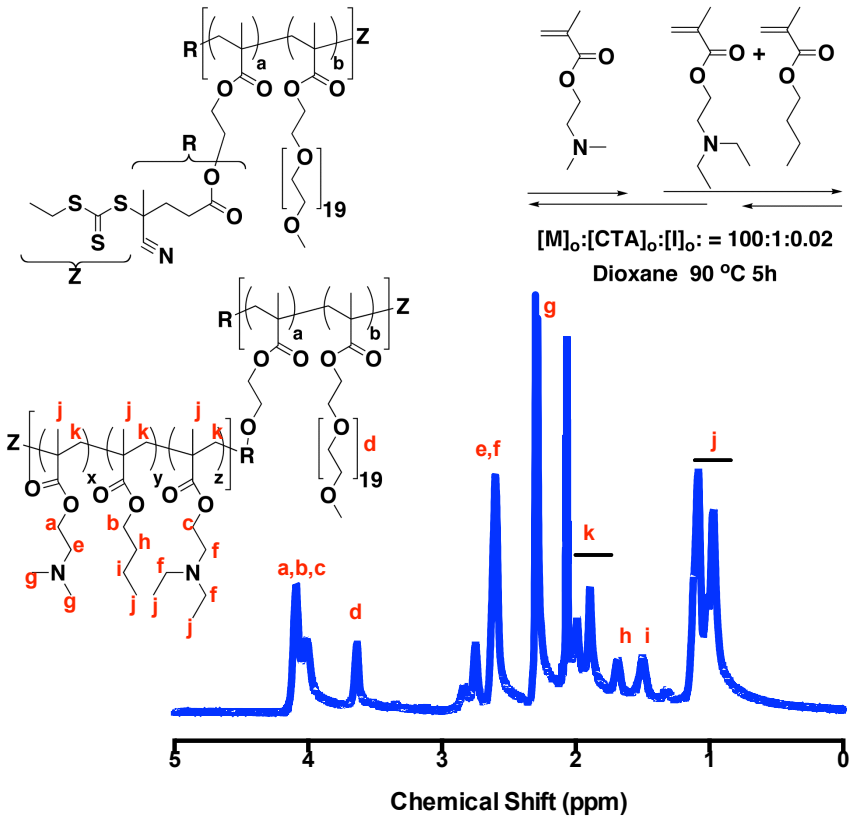
a As determined by size exclusion chromatography using Tosoh SEC TSK-GEL α-3000 and α-4000 columns (Tosoh Bioscience, Montgomeryville, PA) connected in series to an Agilent 1200 Series Liquid Chromatography System (Santa Clara, CA) and Wyatt Technology miniDAWN TREOS, 3 angle MALS light scattering instrument and Optilab TrEX, refractive index detector (Santa Barbara, CA). HPLC-grade DMF containing 0.1 wt.% LiBr at 60 °C was used as the mobile phase at a flow rate of 1 mL/min.

b As determiend by ^{1}H NMR in $CDCl_3$ by comparison of the vinyl resonances present (4.86/5.39 for DMAEMA and 5.36/5.60 for HPMA) on the monomer to either the combined ester resonances at 3.26-3.60 ppm for DMAEMA) or the C**H**(OH)resonance at 3.85 ppm for HPMA.

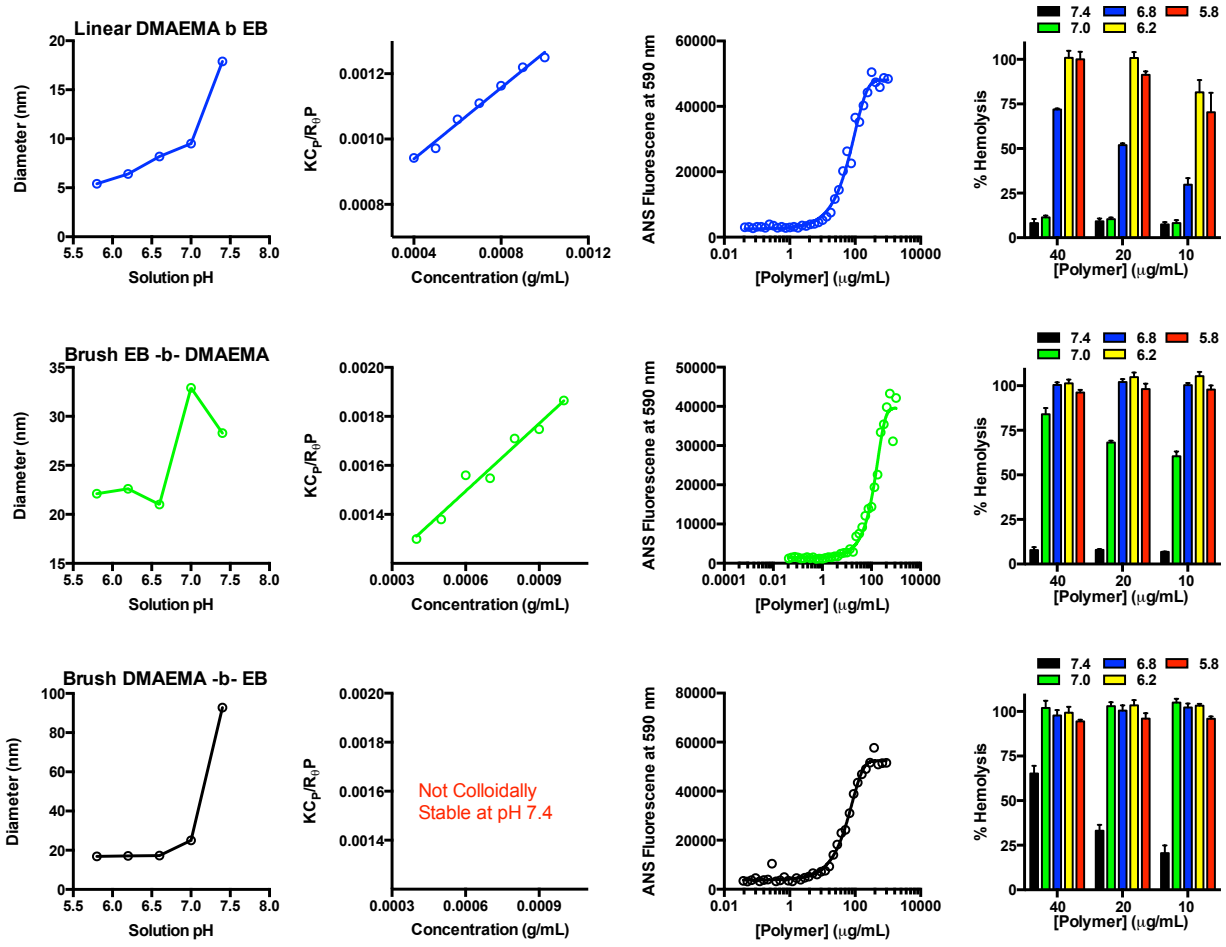
c As determined by the equation M_n theory = $[M]_o * C_M * FW_M / [ECT]_o$ where $[ECT]_o$ is the total concentration of ECT groups linked to the gCTA (including the chain end) as determined by UV spectroscopy in DMF using an extincition coefficient for ECT of 9400 L/mol.

d Polymerization conditions for synthesis of p(O950coHEMA) brushes from gCTA 1 with short EB segments are as follows: [Monomer]o:[gCTA-ECT]o:[ABCVA]o = [20,35,50]:1:0.01 at 20 weight per volume monomer in inhibitor free dioxane at 70 °C for 5 hours

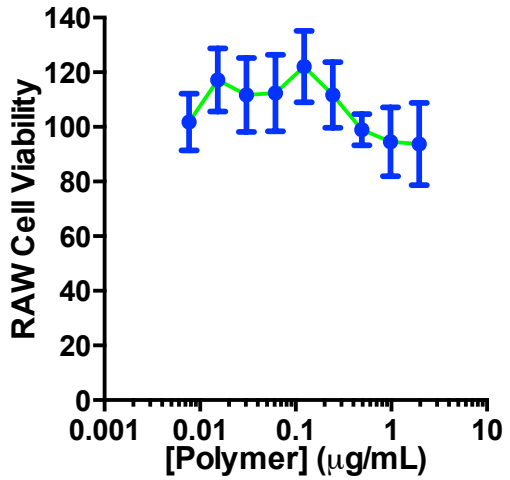
Si Figure 1. Synthetic scheme for the synthesis of poly(DMAEMA$_b$EB) and poly(EB$_b$DMAEMA) brushes via RAFT polymerization.



SI Figure 2 Aqueous solution characterization of the diblock copolymer brushes and the corresponding linear diblock copolymer control via dynamic and static light scattering as well as red blood cell lysis as a function of pH. Dynamic light scattering results showing that the poly(Eb40$_b$DMAEMA) (green traces) brushes have diameters of approximately 20 nm at low pH values where they exist as unimers while the linear control (blue traces) shows unimer sizes of approximately 5 nm. At increased pH values poly(Eb40bDMAEMA) show only slight increases in size to nm. The linear diblock copolymer increases in size to approximately 20 nm at pH 7.4. Static light scattering measurements conducted at pH 7.4 yield a molecular weight of 1,110,000 Da corresponding to anaggregation number of 1.2 for the poly(EB40$_b$DMAEMA) while linear control shows a molecular weight and aggregation number of 1,428,000 and 72 respectively which are consistent with diblock copolymer micelles. Red blood cell hemolysis results for the poly(Eb40$_b$DMAEMA) brush and linear control showing low levels of hemolysis at pH 7.4 with increasing levels of red blood cell lysis at pH 6.2 and 5.8. The diblock copolymer brush show enhanced endosomalytic activity at lower polymer concentrations.



SI Fig 3. MTS assay for RAW cells incubated with polymer brush 3 for 24 hours over a range of polymer concentrations.9 6-well TC treated plate seeded with 5,000 RAW 264.7 cells/well in 100 uL/well complete medium (DMEM+10%FBS, 100U/mL penicillin, and 100 µg/mL streptomycin). After incubation for 16-18hrs at 37°C, 5% CO2, cell culture medium was replaced with complete medium containing polymersomes. After 24hr incubation, cells were then washed 1x with PBS and 100 µL/well of complete medium was added. 20 uL of MTS/PMS solution (Promega, G5430) was then added to each well and cells were incubated for 1hr before A490nm measurements were taken.



Dynamic light scattering Measurements. The architecture dependent solution properties for diblock copolymer brushes and linear polymer control were conducted using a combination of dynamic and static light scattering and fluorescence. As can be seen in in Figure x.xx at pH 5.6, where the EB40 formulation has been shown to be soluble, both polymeric brushes have diameters of approximately 20 nm while the linear control shows unimer sizes of approximately 5nm. Increasing the solution pH to 6.5 for the EB-b-DMAEMA brush, where the EB segment is tethered to the polymeric scaffold, causes an small increase in hydrodynamic diameter to around 33 nm which stabilizes at 28 nm at pH 7.4. This small increase in size from approximately 23 to 28 nm suggests that a negligible amount of self-association is occurring for these materials despite the pH change. In contrast the DMAEMA-b-EB brush, where the hydrophilic DMAEMA stabilizer is tethered to the polymeric scaffold, show a sharp increase in particle size to 92 nm at pH 7.4. This increase in particle size and loss of colloidal stability suggest that the hydrophobic EB40 segments are able to interact at elevated pH facilitating association of the polymeric brushes. The linear diblock copolymer control shows an increase in size from around 5 nm at pH 5.6 to 18 nm at pH 7.4, which is consistent with the formation of diblock copolymer micelles.

pH-responsive red blood cell lysis. In order to develop therapies based on biomacromolecules such as DNA and proteins that act on intracellular targets it is necessary to develop delivery systems capable of mediating cytoplasmic entry. Previously, we employed RAFT technology to develop a new series of diblock copolymers that show controlled and tunable red blood cell hemolysis profiles35,29,37,34. These materials were designed to be

physiologically inert and biocompatible under the typical extracellular conditions found in vivo but undergo a pH-induced morphology change rendering them membrane disruptive under acidic conditions found in endosomes and lysosomes. Based on these studies the polymeric brushes were evaluated to see what effect polymer architecture has on the ability of these materials to interact with biological membranes. Shown in Figure x.xx are the results of these studies for both polymer brushes and the linear control. At pH 7.4 both the EB-b-DMAEMA brush and the linear diblock copolymer control show negligible red blood cell lysis while the DMAEMA-b-EB brush shows strong hemolysis (62 at 40 μg/mL). This result is striking in that all previous formulations at a 60:40 molar composition of DEAEMA to BMA have shown negligible hemolysis at physiological pH. Upon reduction of the solution pH to 7.0 both brushes show a strong increase in hemolysis even at the lowest polymer concentrations evaluated (10 μg/mL) with further reductions in pH yielding quantitative red blood cell lysis. In comparison the linear diblock copolymer controls shows the anticipated red blood cell hemolysis profile with high levels of red blood cell lysis observed at pH 6.2 and 5.8. These results are especially significant for the EB-b-DMAEMA brush given that these materials exists as soluble SPNs where the hydrophilic DMAEMA arms stabilize the hydropohobic EB core and prevent associations. Despite the steric bulk of both the polymeric backbone and poly(DMAEMA) segments the EB segments show a strong ability to interact with biological membranes. The pH at which this interaction occurs can be precisely tailored by adjusting fraction of BMA in the EB segment with higher BMA contents shifting the hemolytic transition to lower pH values. These pH-responsive covalent SPNs could be particularly effective in an in vivo setting where high dilutions and numerous molecular interactions can destabilize nanoparticle assemblies. The kinetics of red blood cell lysis (not shown) was also observed to be significantly more rapid for the polymeric brushes relative to the linear control. Indeed complete red blood cell lysis was observed for the highest brush copolymer concentrations (40 g/mL) almost immediately upon mixing, which is similar to the hemolysis profiles of the low molecular weight surfactant control triton X surfactant. This result suggests the possibility that the large SPNs are able to act cooperatively to disrupt biological membranes.