Endolytic, pH-Responsive HPMA-b-(L-Glu)
Copolymers Synthesized via Sequential Aqueous RAFT and Ring Opening Polymerizations

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**Determination of APMA Conjugation.** A ninhydrin assay was utilized for the determination of APMA content conjugated to the ω-terminus of poly(HPMA220), and a standard literature procedure was followed.1 Briefly, the ninhydrin assay solution was prepared by mixing 5 mL of ninhydrin (200 mg) dissolved in ethylene glycol and 5 mL of SnCl2 • 2H2O (8 mg) dissolved in citrate buffer (pH 5). Poly(HPMA220)-NH2 (4.3 mg) was diluted with 45.5 µL of DI H2O to give a 3 mM polymer solution. The poly(HPMA220)-NH2 solution (6 µL of 3 mM stock) was diluted with 200 µL of ninhydrin assay solution; the solution was vortexed and heated at 100 °C for 20 minutes. The solution was then diluted to 600 µL using DI H2O, and the absorbance at 570 nm was recorded using a Perkin-Elmer spectrophotometer. To determine APMA functionalization, a calibration curve was constructed utilizing D-alanine. Five different aliquots of 3 mM D-alanine
were diluted with 200 µL of ninhydrin assay solution and heated to 100 °C for 20 minutes. Each solution was diluted to 600 µL using DI H₂O, and the absorbance at 570 nm was recorded to create a calibration curve.

![Graph showing absorbance of ninhydrin at 570 nm versus amine concentration for D-alanine (open squares) and poly(HPMA₂₂₀)-NH₂ (black circle).]

**S1.** Absorbance of ninhydrin at 570 nm versus amine concentration for D-alanine (open squares) and poly(HPMA₂₂₀)-NH₂ (black circle).
S2. Fluorescence intensity of fPOPC at pH 7.4. fPOPC was incubated with poly(HPMA$_{220}$) and poly[HPMA$_{220}$-b-(L-Glu)$_{33}$] copolymers. Triton-X100 was utilized as the positive control. No observable increase in fluorescein release was determined at pH 7.4 when incubated with poly[HPMA$_{220}$-b-(L-Glu)] copolymers.
S3. Percent hemolysis release as a function of concentration for poly(HPMA\(_{220}\)) and poly[HPMA\(_{220}\)-b-(L-Glu)] copolymers at pH 7.4. Triton-X100 was utilized as the positive control.
S4. $^1$H NMR deprotection of (A) poly[HPMA$_{220}$-b-(L-Glu$_{33}$)], (C) poly[HPMA$_{220}$-b-(L-Glu$_{56}$)], and (B) poly[HPMA$_{220}$-b-(L-Glu$_{78}$)]. The absence of the benzyl group at 7.5 – 7.1 ppm and 5.1 – 4.9 ppm confirms successful deprotection of glutamic acid.

REFERENCES