Supporting Information

Synthetic aptamer-polymer hybrid constructs for programmed drug delivery into specific target cells

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Methods:

Materials and Reagents

All chemicals were used as received from Sigma-Aldrich (St. Louis, MO) unless otherwise specified. Tetrahydrofuran (THF) was collected from a dry solvent system and used immediately thereafter. Benzyl alcohol was dried over calcium hydride and distilled before titration with potassium naphthalenide in THF (0.3 M). Ethylene oxide (EO) was degassed through several freeze-pump-thaw cycles and distilled to a flame-dried buret immediately before use. Potassium naphthalenide was prepared from potassium metal and recrystallized naphthalene in dry THF and allowed to stir with a glass-coated stir-bar for 24 hours at room temperature before use. Ethylene glycol vinyl glycidyl ether (EGVGE) was synthesized by reacting ethylene glycol vinyl ether with epichlorohydrin in basic conditions, distilled to a buret and degassed in vacuo before use. Allyl glycidyl ether (AGE) was purchased from TCI-America (Portland, OR), degassed through several freeze-pump-thaw cycles, and distilled from butyl magnesium chloride to a buret for storage. $(Bim)_3$ and $(BimC_4A)_3$ were provided by Prof. M.G. Finn at the Georgia Institute of Technology. All DNA oligos (Table S1) were synthesized, modified and purified by Integrated DNA Technologies (Coralville, IA). Nucleolin was purchased from Abcam (Cambridge, MA). Human α-thrombin was purchased from Haematologic

Technologies (Essex Junction, VT). Immunoglobulin E (IgE) was purchased from Athens Research and Technology (Athens, GA). Gelstar and MEGM (Mammary Epithelial Cell Growth Medium) BulletKit were purchased from Lonza (Allendale, NJ). M-270 carboxylic acid-coated magnetic bead and LIVE/DEAD Viability/Cytotoxicity Kit for mammalian cells were purchased from Life Technologies (Carlsbad, CA). iQ SYBR Green Supermix was purchased from Bio-Rad Laboratories (Hercules, CA) for quantitative PCR experiments. Dulbecco's Phosphate Buffered Saline solution (DPBS, calcium and magnesium-free), agarose and 10× PBS were purchased from Thermo Fisher Scientific (Waltham, MA). Dulbecco's Modified Eagle's Medium (DMEM) was purchased from American Type Culture Collection (ATCC, Manassas, VA). Fetal bovine serum (FBS) and penicillin-streptomycin were used as received from Life Technologies (Carlsbad, CA).

Synthesis of poly(EGVGE)-b-(EO) (1)

All polymerizations were carried out on a Schlenk line in custom thick-walled glass reactors fitted with ACE-threads under an argon atmosphere. The reactors were dried under vacuum then refilled with argon five times. Under an argon atmosphere, benzyl alcohol initiator was added by gas-tight syringe through a 6-mm Puresep (Herefordshire, UK) septum. THF was then added by opening the valve of the already-connected buret on the reactor. The potassium alkoxide initiator was formed by titration of benzyl alcohol with potassium naphthalenide under argon until a green color persisted in solution, indicating the deprotonation of all alcohols. EGVGE was first added into the reactor, and polymerization was carried out at 45 °C for 20 hrs. EO was then added and the reactor was maintained at 45 °C for an additional 48 h until the polymerization was terminated with isopropanol. Polymers were precipitated in hexane and dried in vacuo before characterization (yield > 95%). ^{1}H NMR of Poly(EGVGE)-b-(EO) (1) (500 MHz, $CDCl_3$): δ 3.54–3.71 (broad m, –C$\underline{H}_2$–C$\underline{H}$–O–C$\underline{H}_2$–C$\underline{H}$(CH_2–O–C$\underline{H}_2$–C$\underline{H}_2$–O–CH=CH_2)–O–), 3.81/3.82 (two broad peaks, –CH_2–CH(C$\underline{H}_2$–O–CH_2–CH_2–O–CH=CH_2)–O–), 4.00/4.19 (doublet of doublets, –O–CH=C$\underline{H}_2$), 4.51–4.55 ppm (four singlets, Ph–C$\underline{H}_2$–O–), 6.49 (m, –O–C$\underline{H}$=CH_2), 7.32 (overlap with residual $CHCl_3$, 1H on $\underline{Ph}$–CH_2–O–), 7.38 (s, 4H on $\underline{Ph}$–CH_2–O–). ^{13}C

NMR of Poly(EGVGE)-b-(EO) (500MHz, $CDCl_3$): δ 61.6 (s, –$\underline{C}H_2$–OH of chain ends), 67.4 (s, –CH_2–CH($\underline{C}H_2$–O–CH_2–CH_2–O–CH=CH_2)–O–), 69.8–72.6 (broad m, –$\underline{C}H_2$–$\underline{C}H_2$–O–$\underline{C}H_2$–CH(CH_2–O–$C\underline{H}_2$–$C\underline{H}_2$–O–CH=CH_2)–O–), 73,4 (s, Ph–$\underline{C}H_2$–O–), 77.2 (triplet, $\underline{C}DCl_3$), 78.6 (s, –O–CH_2–$\underline{C}$H(CH_2–O–CH_2–CH_2–O–CH=CH_2)–O–), 86.7 (s, –O–CH=$\underline{C}H_2$), 127.6/128.4 (doublet, 5C on $\underline{Ph}$–CH_2–O–), 151.8 (s, –O–$\underline{C}$H=CH_2). See **Figure S14** and **Figure S15A** for characterization data.

Alkyne functionalization of poly(EGVGE)-b-(EO) (2)

Poly(EGVGE)-b-(EO) (**1**) (1.0 g, 0.087 mmol) was placed in a 50 mL round-bottom flask sitting on an ice bath. 10 ml of dry toluene was added and the mixture was stirred with a magnetic stir-bar until the polymer was fully dissolved in the solvent. Crushed potassium hydroxide (48 mg, 0.87 mmol) was then slowly added to the mixture at 0 °C to avoid generating excess heat. After 2 hours, propargyl bromide (260 mg, 1.7 mmol) was added dropwise and the reaction flask was moved to a 50 °C oil bath and stirred for an additional 48 hours. After cooling the reaction mixture, the potassium bromide precipitate was filtered and the solvent was evaporated. The polymer was then washed several times with water and precipitated in hexane. ^{1}H NMR of the alkyne-functionalized Poly(EGVGE)-b-(EO) (**2**) (500 MHz, $CDCl_3$): δ 2.44 (singlet, –O–CH_2–C=C$\underline{H}$) 3.54–3.71 (broad m, –C$\underline{H}_2$–C$\underline{H}$–O–C$\underline{H}_2$–C$\underline{H}$(CH_2–O–C$\underline{H}_2$–C$\underline{H}_2$–O–CH=CH_2)–O–), 3.81/3.82 (two broad peaks, –CH_2–CH(C$\underline{H}_2$–O–CH_2–CH_2–O–CH=CH_2)–O–), 4.00/4.19 (doublet of doublets, –O–CH=C$\underline{H}_2$), 4.21/4.22 (doublet, –O–C$\underline{H}_2$–C=CH), 4.51–4.55 ppm (four singlets, Ph–C$\underline{H}_2$–O–), 6.49 (m, –O–C$\underline{H}$=CH_2), 7.32 (overlap with residual $CHCl_3$, 1H on $\underline{Ph}$–CH_2–O–), 7.38 (s, 4H on $\underline{Ph}$–CH_2–O–). See **Figure S15B** for characterization data.

Alkyne functionalized, hydroxyl-grafted poly(EGGE)-b-(EO) (3)

Alkyne-functionalized poly(EGVGE)-b-(EO) (**2**) (1.0 g, 0.087 mmol) was placed in a 50 mL round-bottom flask. 10 ml of methanol was added and the mixture was stirred with a magnetic stir-bar. Para-toluene sulfonic acid (34 mg, 0.087 mmol) was

added and the reaction was stirred at room temperature for 3 hours. The resulting polymer was washed several times with water and precipitated in hexane (yield > 95%). ^{1}H NMR of the alkyne functionalized poly(EGGE)-b-(EO) with deprotected alcohols along the backbone (**3**) (500 MHz, $CDCl_3$): δ 2.44 (singlet, –O–CH_2–C=C<u>H</u>) 3.54–3.71 (broad m, –C<u>H</u>$_2$–C<u>H</u>–O–C<u>H</u>$_2$–C<u>H</u>(CH_2–O–C<u>H</u>$_2$–C<u>H</u>$_2$–OH)–O–), 4.21/4.22 (doublet, –O–C<u>H</u>$_2$–C=CH), 4.51–4.55 ppm (four singlets, Ph–C<u>H</u>$_2$–O–), 7.32 (overlap with residual $CHCl_3$, 1H on <u>Ph</u>–CH_2–O–), 7.38 (s, 4H on <u>Ph</u>–CH_2–O–). See **Figure S15C** for characterization data.

Synthesis of the Polymer Scaffold with Cleavable Coumarin Payload (4)

Alkyne-functionalized, vinyl-deprotected poly(EGGE)-b-(EO) (**3**) (100 mg, 0.090 mmols alcohol) and carboxylic acid-functionalized coumarin [7-(diethylamino)-2-oxo-2*H*-chromene-3-carboxylic acid][1] (36 mg, 0.14 mmols) were dissolved in 5 mL dry methylene chloride. A solution of *N,N'*-dicyclohexylcarbodiimide (29 mg, 0.14 mmols) in 2 mL dry methylene chloride was added dropwise at 0 °C. The solution was allowed to warm to room temperature and stirred for 24 hours. The solution was then concentrated to ~ 2 mL and precipitated into hexanes. The precipitate was collected and further purified by preparatory gel-permeation chromatography (GPC) to yield the desired material as a fluorescent powder (40 mg). (yield ~ 88%) See NMR and GPC data in **Figure S15D** and **Figure S16**, respectively.

Synthesis of the Polymer Scaffold with Cleavable Doxorubicin Units (7)

First, 1.0 g (0.091 mmol) of alkyne-functionalized poly(EGGE)-b-(EO) (**3**) was mixed with succinic anhydride (450 mg, 4.5 mmol) and 4-dimethylaminopyridine (110 mg, 0.91 mmol) in a 100 mL round-bottom flask. The mixture was dried in vacuo overnight before 10 mL of dichloromethane was added. The heterogeneous solution was stirred at 20 °C for 8 hrs before being filtered. The functionalized polymer (***6***) was then precipitated out of ethyl ether.

Second, the doxorubicin-conjugated polymer was synthesized by a slight modification to the procedure of Yoo *et al.*,[2] because the grafted carboxylic acid

groups react more with primary alcohols on doxorubicin rather than the secondary ones. Specifically, 9-fluorenylmethyl chloroformate (Fmoc)-protected doxorubicin (16 mg, 0.022 mmols) and a carboxylic acid-containing copolymer (**6**) were added to 1 mL dry methylene chloride. Dicyclohexyl carbodiimide (6 mg, 0.029 mmol) and a catalytic amount of 4-dimethylaminopyridine (1 mg) were dissolved in a separate aliquot of 1 mL dry dichloromethane and added dropwise to the Fmoc-Dox/polymer solution. The reaction was allowed to stir 24 hours, after which time the reaction mixture was quenched by addition of 10 mL 1 N HCl and extracted 3× with chloroform. The organic layers were combined, the solvent was removed and the product mixture purified by preparatory GPC using chloroform with 0.25 mole % triethylamine as the eluent to yield a red powder. The product was redissolved in *N*,*N*-dimethylformamide and 250 μL piperidine was added, yielding a deep purple solution. The reaction mixture was allowed to stir for 10 minutes, the solvent was removed under vacuum, and the product was redissolved in chloroform and purified by preparatory GPC using chloroform with 0.25 mole % triethylamine as the eluent to yield a red powder as the final product. See NMR data in **Figure S17.**

Synthesis of poly[(allyl glycidyl ether)-co-(ethylene oxide)] (8)

All polymerizations were carried out on a Schlenk line in custom thick-walled glass reactors fitted with ACE-threads under an argon atmosphere. The reactors were dried under vacuum then refilled with argon five times. Under an argon atmosphere, benzyl alcohol initiator was added by gas-tight syringe through a 6-mm Puresep (Herefordshire, UK) septum. THF was then added by opening the valve of the already-connected buret on the reactor. The potassium alkoxide initiator was formed by titration of benzyl alcohol with potassium naphthalenide under argon until a green color persisted in solution, indicating the deprotonation of all alcohols. Allyl glycidyl ether (AGE) and EO were simultaneously added into the reactor, and polymerization was carried out at 45 °C for 20 hrs until the polymerization was terminated with isopropanol. Then the resulting copolymers were precipitated in hexane and dried in vacuo before characterization (yield > 95%). ^{1}H NMR of

Poly(EO-*co*-AGE) (500 MHz, $CDCl_3$): δ 1.55 (d, –O–CH=CH–C<u>H</u>$_3$), 3.47–3.72 (broad m, –C<u>H</u>$_2$–C<u>H</u>–O–C<u>H</u>$_2$–C<u>H</u>(C<u>H</u>$_2$–O–CH_2–CH=CH_2)–O– and –C<u>H</u>$_2$–C<u>H</u>–O–C<u>H</u>$_2$–C<u>H</u>(C<u>H</u>$_2$–O–CH=CH–CH_3)–O–), 3.79/3.87 (two broad peaks, –CH_2–CH(C<u>H</u>$_2$–O–CH=CH–CH_3)–O–), 4.01 (d, –O–C<u>H</u>$_2$–CH=CH_2), 4.38 (m, –O–CH=C<u>H</u>–CH_3), 4.53–4.56 (four singlets, Ph-C<u>H</u>$_2$–O–), 5.18/5.28 (doublet of doublets, –O–CH_2–CH=C<u>H</u>$_2$), 5.91 (m, –O–CH_2–C<u>H</u>=CH_2), 5.97 (d, –O–C<u>H</u>=CH–CH_3), 7.30 (overlap with residual $CHCl_3$, 1H on <u>Ph</u>–CH_2–O–), 7.36 (s, 4H on <u>Ph</u>–CH_2–O–).^{13}C NMR of Poly(EO-*co*-AGE) (500 MHz): δ 70.9 (–<u>C</u>H$_2$–<u>C</u>H$_2$–O–<u>C</u>H$_2$–CH(<u>C</u>H$_2$–O–CH_2–CH=CH_2)–O–), 72.0 (–O–<u>C</u>H$_2$–CH=CH_2), 79.4 (–CH_2–<u>C</u>H(CH_2–O–CH_2–CH=<u>C</u>H$_2$)–O–), 100.3 (–O–CH=<u>C</u>H–CH_3), 116.3 (–O–CH_2–CH(CH_2–O–CH_2–CH=<u>C</u>H$_2$)–O–), 127.5/128.6 (5C, <u>P</u>h–CH_2–O–), 135.6 (–CH_2–CH(CH_2–O–CH_2–<u>C</u>H=CH_2)–O–), 138.8 (1C, <u>P</u>h–CH2–O–), 146.8 (–CH_2–CH(CH_2–O–<u>C</u>H=CH–CH_3)–O–).

Synthesis of the Polymer Scaffold with Non-cleavable Coumarin Payload (10)

Poly(allyl glycidyl ether)$_6$-b-(ethylene oxide)$_{120}$ ($PAGE_6$-b-PEO_{120}) (**8**) (2.2g, 0.37 mmol) and m-chloroperbenzoic acid (0.65g, 5.5 mmol) were dissolved in methylene chloride (**Scheme S2**). The solution was then stirred overnight at room temperature and the product (**9**) was purified by precipitation in diethyl ether. The resulting polymer has approximately three epoxides and three allyl group grafted along the backbone. The epoxidized polymer was then reacted with three equivalents of an amine functional coumarin dye (*N*-(2-aminoethyl)-7-(diethylamino)-2-oxo-2*H*-chromene-3-carboxamide)[3] in dry THF at room temperature for 36 hours to yield a material with grafted coumarin units. (yield ~ 52%) See NMR data in **Figure S18**.

Characterization of Polymer Scaffold

^{1}H NMR spectroscopy was carried out on a Bruker (Billerica, MA) AC 500 spectrometer in deuterated chloroform ($CDCl_3$). Size exclusion chromatography (SEC) was performed on a Waters (Milford, MA) chromatograph with four Viscotek (Malvern, Worcestershire, UK) columns (two I-MBHMW-3078, I-series mixed-bed,

high-molecular-weight columns and two I-MBLMW-3078, I-series mixed-bed, low-molecular-weight columns) for fractionation, a Waters 2414 differential refractometer and 2996 photodiode array detector for detection of eluent, with chloroform with 0.1% triethylamine at room temperature used as the mobile phase. Gas chromatography was carried out on a Shimadzu (Kyoto, Japan) GC-2014 using a flame ionization detector and a Restek (Bellefonte, PA) column (SHRXI-5MS) for separation. DSC measurements were performed using a TA Instruments Q2000 modulated differential scanning calorimeter with 50-position auto-sampler and mass flow control in the temperature range from −80 to 120°C at a heating rate of 10 K/min and cooling rate of −5 K/min under nitrogen.

Ligand-Accelerated CuAAC Conjugation

2 μL of 50 mM Cu(II) salt ($CuSO_4$) in water and 2 μL of 100 mM ligand ($(Bim)_3$ or $(BimC_4A)_3$) in 3:1 dimethyl sulfoxide:tert-butanol (DMSO/t-BuOH) were mixed, after which 2 μL of 0.5 mM azide-modified DNA oligos in water, 1.25 mM polymer with an alkyne end group in DMSO/t-BuOH and 2 μL of 10× PBS were added to the mixture. 4 μL of a freshly prepared sodium ascorbate solution in water (200 mM) was subsequently added and completely mixed. The solution was shaken at room temperature overnight and used for gel electrophoresis or RT-PCR without further purification. For affinity measurements and confocal microscopy experiments, the reaction was diluted with 500 μL of water, and the conjugate was filtered and purified three times using Amicon Ultra (Millipore, Billerica, MA) centrifugal filters. The purified conjugate was quantified via UV-vis measurement at 260 nm.

Gel Electrophoresis

All CuAAC reaction products were analyzed via electrophoresis on a 2% agarose gel with 1× Gelstar staining dye in 1× TBE buffer (89 mM Tris borate, 2 mM Na_2EDTA, pH 8.3) at 150 V for 40 mins. Each sample contained 0.25 μL of CuAAC reaction product and 5 μL of 15% glycerol. In parallel, we ran 1.5 μL of low molecular weight DNA ladder (20 base) and 5 μL of 15% glycerol, and 0.25 μL each of 50 μM unconjugated aptamer and 500 μM unconjugated polymer mixed with 5 μL of 15%

glycerol as negative controls. Gel images were taken with a Kodak Gel Logic 200 imaging system equipped with Kodak molecular imaging software (Carestream Health, Rochester, NY).

Quantitative PCR Experiments

CuAAC conjugate were also quantified by PCR. PCR reactions contained 10 μL iQ SYBR Green Supermix, 8.8 μL PCR water, 0.1 μL of 0.1 mM forward primer, 0.1 μL of 0.1 mM reverse primer and 1 μL of diluted CuAAC product or an equal concentration of unconjugated aptamer, with fluorescence signal monitored using the iQ 5 multi-color real time-PCR Detection System (Bio-Rad Laboratories). C_T (threshold cycle) values were subsequently determined for each sample.

Affinity Measurements

Each sample's K_d was measured via a fluorescence-based binding assay[4]. CuAAC reaction products or mixtures of unconjugated aptamer and polymer scaffold in 1: 10 ratio were diluted to several different concentrations (from 0 to 100 nM, 50 nM and 25 nM for nucleolin, thrombin and IgE, respectively) in 90 μL of binding buffer. This buffer consisted of 1× PBS, pH 7.4 containing 1 mM $MgCl_2$ and 0.025% Tween20 (v/v) for nucleolin and IgE, and 100 mM Tris, 20 mM $MgCl_2$, 150 mM NaCl, pH 7.4 and 0.025% Tween20 (v/v) for thrombin. These dilutions were heated at 95 °C for 10 min, immediately cooled on ice for 10 min and then incubated for another 10 min at room temperature. We measured the K_d by incubating these heat-treated samples with target protein-coated M-270 magnetic beads (2×10^9 beads/mL, 2.8 μm in diameter) in a total volume of 100 μL for 1 hour at room temperature. 1 μL of nucleolin (2.34×10^5 molecules/bead), 2.5 μL of thrombin (1.05×10^5 molecules/bead) or 2.5 μL of IgE (5.97×10^4 molecules/bead) were immobilized onto the beads through the EDC-NHS coupling process using the manufacturer's protocol, and quantified with the NanoOrange Protein Quantitation Kit (Life Technologies). After incubating with our samples, the magnetic beads were thoroughly washed three times with binding buffer, and magnetic separation was used to eliminate unbound molecules. Bound molecules were released into 55 μL of

water or DMSO by heating the beads at 95 °C for 10 min. 50 μL of each eluate was transferred to a black 96-well microplate (Microfluor 2, Thermo Scientific), and released molecules were quantified by fluorescence measurement using a microplate reader (Tecan, San Jose, CA). Eluates in water and DMSO were used for fluorescence measurements of Cy5-labeled aptamer and coumarin-linked polymer scaffold, respectively. We calculated K_d from the calibrated curve fitting using the equation $Y = B_{max} \times X / (K_d + X)$, where X is the concentration of ssDNA and B_{max} is the fluorescence value at saturation.

Kinetic Fluorescence Measurement of Coumarin Release

To monitor kinetics of enzyme-dependent coumarin release, coumarin-loaded nucleolin APHs (**5**) were dissolved in 1× PBS, pH 7.4, and PLE (150 units/mL) was then added to produce a solution containing 500 nM conjugate and 1.5 U PLE in 100 μL. As a control, we prepared 100 μL of 10 μM free coumarin in 1× PBS, pH 7.4 containing 1.5 U PLE in 100 μL. At a set time, fluorescence spectra of these samples were immediately recorded in a black 96-well microplate with a Tecan Infinite M1000 microplate reader. The excitation wavelength was set at 410 nm and emission was recorded from 420–700 nm with excitation and emission slits of 5 nm. Time-dependent changes in fluorescence intensity at 470 nm were also measured every minute over 3 hours with different amounts of PLE, from 0 to 1.5 U.

Cell Culture and Sample Preparation

MCF-7 cells were cultured as recommended by the ATCC in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. MCF10A cells were cultured in MEGM complete growth medium as recommended by the ATCC. All cell cultures were grown in a humidified incubator maintained at 37 °C and 5% CO_2. For live-cell imaging, cells were harvested with a 0.25% Trypsin, 0.53 mM EDTA solution and seeded on 35-mm glass bottom dishes 24 hours before the experiment. The medium was then removed, and the cells were washed twice with pre-warmed DPBS. The washed cells were incubated with 500 nM solutions of each sample for 5 mins, thoroughly washed with pre-warmed DPBS twice, and then incubated with fresh

medium at 37 °C for 4 hours. At a set time, all samples were immediately observed via confocal microscopy.

Confocal Microscopy Measurement

Confocal microscopy measurements were performed with an Olympus (Tokyo, Japan) FluoView 500, mounted on an Olympus IX81 inverted microscope with a 40× oil immersion objective. 405-nm diode laser and 633-nm HeNe laser lines were used to excite coumarin (emission filter 465–495 nm) and Cy5 (emission filter >630 nm), respectively. All images were analyzed using ImageJ software. Cells were grown on MatTek plates (Ashland, MA). During kinetic experiments, all cells were kept at 37 °C and 5% CO_2.

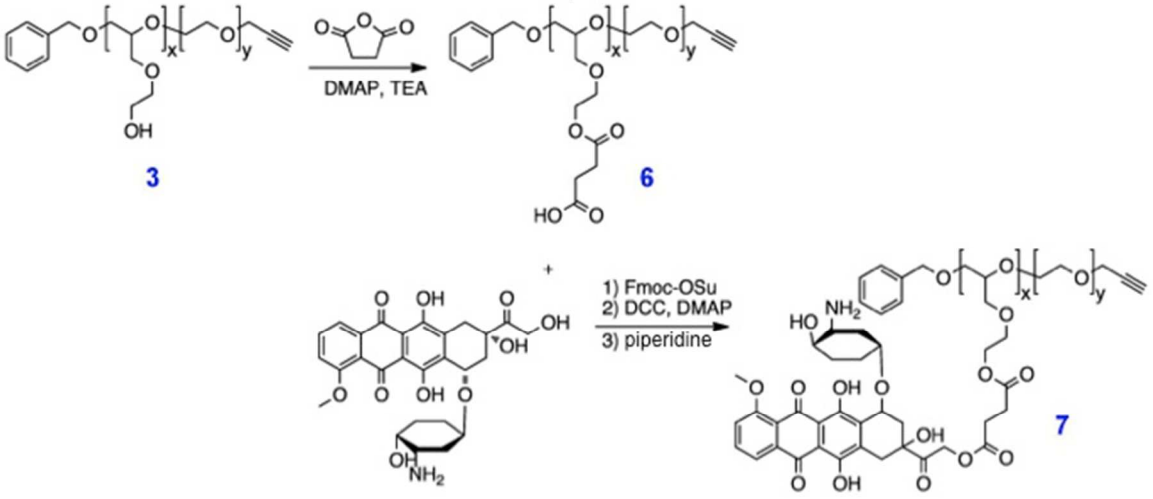
APH-Mediated DOX Delivery

In order to completely remove unreacted DOX-loaded polymers after the CuAAC reaction, APHs were purified using complementary DNA-coated, M-270 magnetic beads. Specifically, the reaction sample was diluted 10× with binding buffer and incubated with magnetic beads for 10 min at RT. The magnetic beads were washed twice with binding buffer, and bound APHs were then released into water by heating at 95 °C for 10 min. APH concentration was determined by UV-vis and Cy5 fluorescence measurements. MCF-7 cells (1×10^5 cells/mL) were grown in 96-well plates for 48 hrs and thoroughly washed twice with pre-warmed DPBS. Washed MCF-7 cells were treated with 500 nM unconjugated nucleolin aptamer, unconjugated polymer with DOX or DOX-loaded nucleolin APH in DMEM and incubated at 37 °C for four days. All experiments were performed in triplicate. Cell viability was measured via LIVE/DEAD Viability/Cytotoxicity Kit (Life Technologies), in which viable cells and nonviable cells were stained by calcein acetoxymethyl ester (green) and ethidium homodimer-1 (red), respectively. Staining was performed according to the manufacturer's protocol, and cell viability was subsequently analyzed using a microplate reader (Tecan).

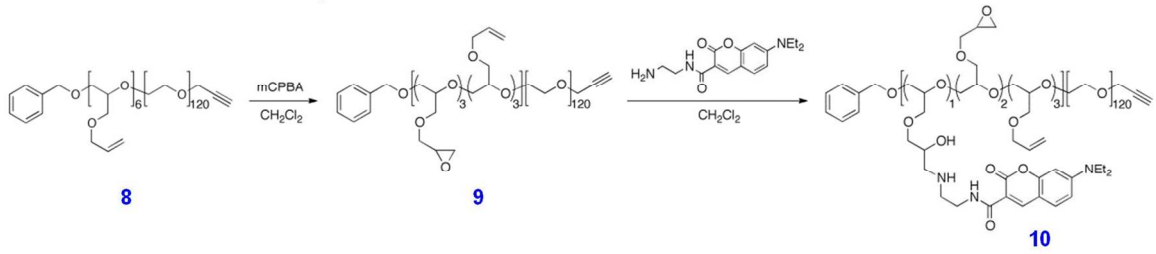
Table S1: DNA Sequences used in the experiments

Name	Sequence	Description
NCL-Apt1	5’-GGAAGAGATGGCGACGGTGGT GGTGGTTGTGGTGGTGGTGGAGCTGAT CCTGATGG-Azide-3’	Nucleolin aptamer sequence flanked by primer binding sites (azide tag at 3’ terminus)
Th-Apt1	5’-GGAAGAGATGGCGAC GGTTGGTGTGGTTGG AGCTGATCCTGATGG-Azide-3’	Thrombin aptamer sequence flanked by primer binding sites (azide tag at 3’ terminus)
IgE-Apt1	5’-GGAAGAGATGGCGACCACGTTT ATCCGTCCCTCCTAGTGGCGTGAGCTGA TCCTGATGG-Azide-3’	IgE aptamer sequence flanked by primer binding sites (azide tag at 3’ terminus)
FP	5'-GGAAGAGATGGCGAC-3'	Forward primer
RP	5'-CCATCAGGATCAGCT-3'	Reverse primer
NCL-Apt2	5'-Cy5-GGTGGTGGTGGTTGTGGTGGTGGTGGT TTTTTTTTTTTTT-Azide-3'	Nucleolin aptamer sequence with T_{14} sequence at 3’ terminus (Cy5 at 5’ terminus and azide tag at 3’ terminus)
Th-Apt2	5'-Cy5-GGGGCACGTTTATCCGTCCCTCCTAGTG GCGTGCCCCTTTTTTTTTTTTT-Azide-3'	Thrombin aptamer sequence with T_{15} sequence at 3’ terminus (Cy5 at 5’ terminus and azide tag at 3’ terminus)
IgE-Apt2	5'-Cy5-GGGGCACGTTTATCCGTCCCTCCTAGTG GCGTGCCCCTTTTTTTTTTTTT-Azide-3'	IgE aptamer sequence with T_{13} sequence at 3’ terminus (Cy5 at 5’ terminus and azide tag at 3’ terminus)

Scheme S1: Synthetic mechanism of doxorubicin conjugation to the polymer scaffold via enzymatically-cleavable linkage



Scheme S2: Synthetic mechanism of coumarin conjugation to the polymer scaffold via non-cleavable linkage



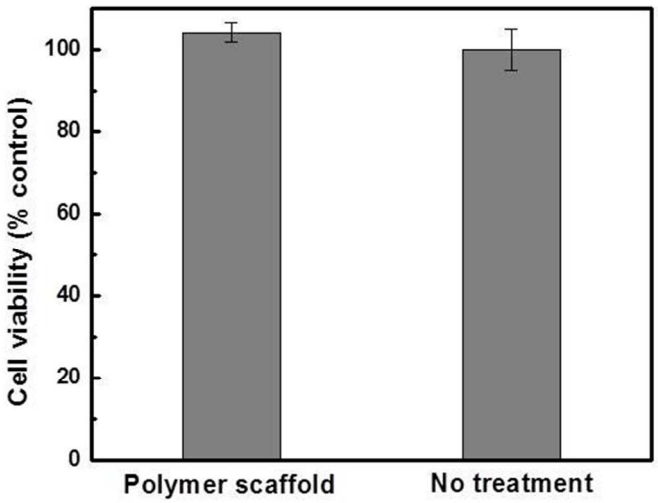


Figure S1: 5 μM polymer scaffold incubated with MCF-7 cells showed no big difference in cell viability compared to controls, which confirm that the polymer scaffold itself is not cytotoxic to the cells.

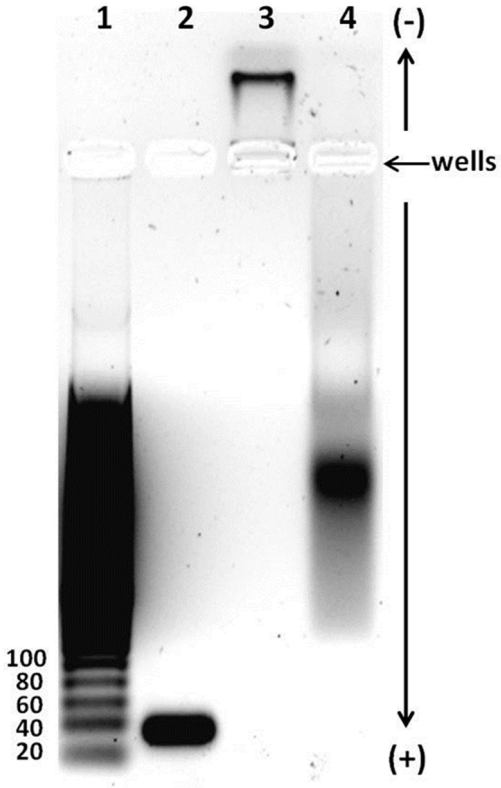


Figure S2: Gel electrophoresis showed the successful conjugation via $(BimC_4A)_3$ ligand-accelerated CuAAC to generate coumarin-loaded APH. Lanes: 1, 20-bp ladder; 2, unconjugated aptamer; 3, unconjugated polymer that grafts coumarin dyes; 4, coumarin-loaded APH synthesized with $(BimC_4A)_3$.

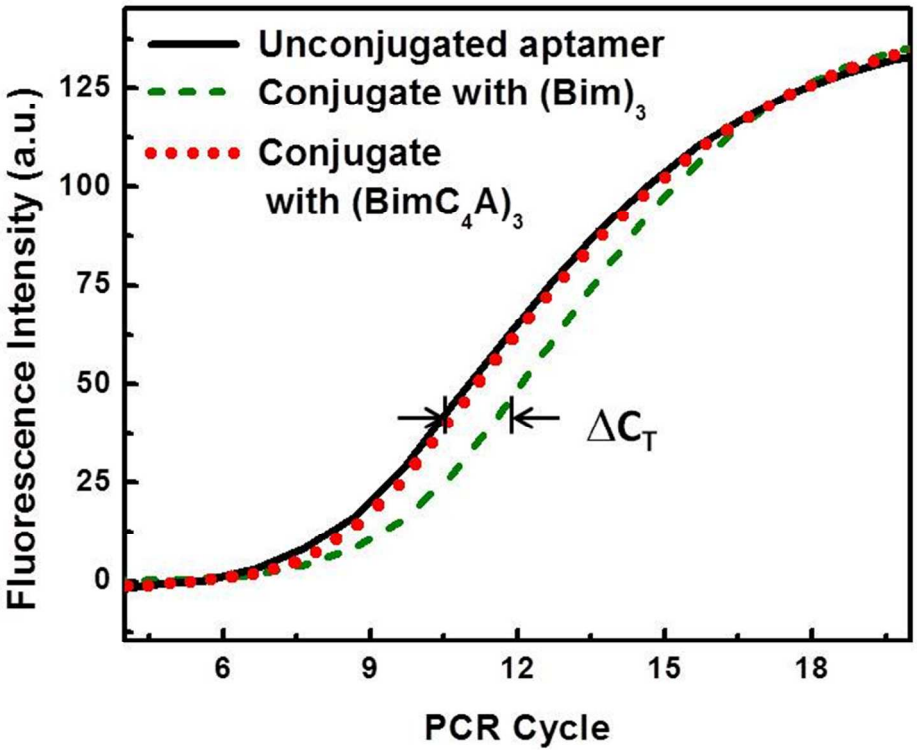


Figure S3: Quantitative PCR (qPCR) amplification profiles verify aptamer integrity in our CuAAC scheme. The amplification curve of APHs synthesized with $(BimC_4A)_3$ (red dotted line) overlaps with that of the unconjugated aptamer (black solid line), confirming that there is negligible damage resulting from conjugation. In contrast, APHs synthesized with $(Bim)_3$ (green dashed line) exhibit a difference in threshold cycle (ΔC_T) of ~1.11, indicating ~54% of the conjugated aptamers are damaged..

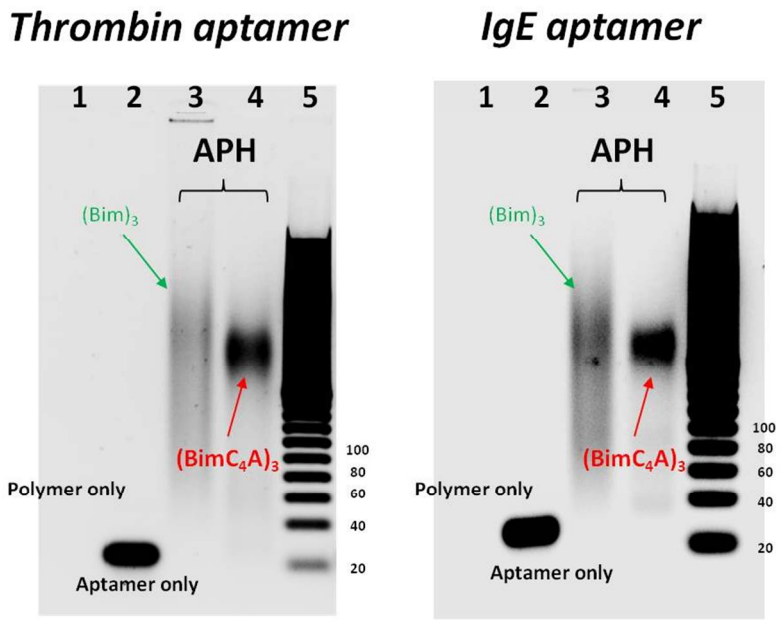


Figure S4: Gel electrophoresis confirmed that our CuAAC reaction with the $(BimC_4A)_3$ ligand is equally effective for the conjugation of intact aptamers for thrombin (left) or IgE (right). Lanes: 1, polymer only; 2, aptamer only; 3, $(Bim)_3$-conjugated APH; 4, $(BimC_4A)_3$-conjugated APH; 5, ladder.

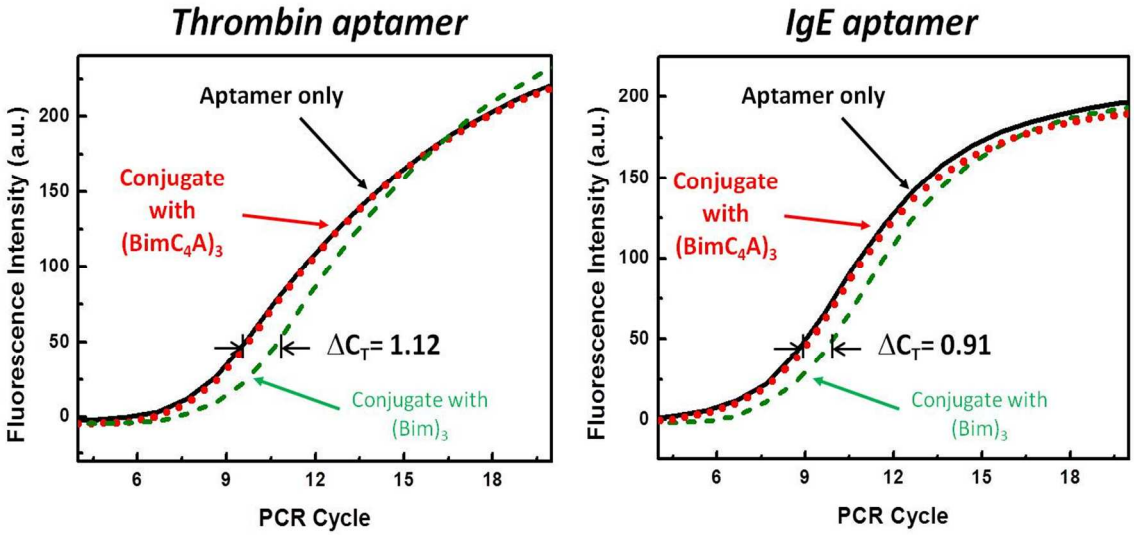


Figure S5: qPCR of thrombin (left) or IgE (right) APHs further confirms that CuAAC with $(BimC_4A)_3$ ligand offers a generalizable strategy for quantitative APH synthesis that leaves aptamers undamaged.

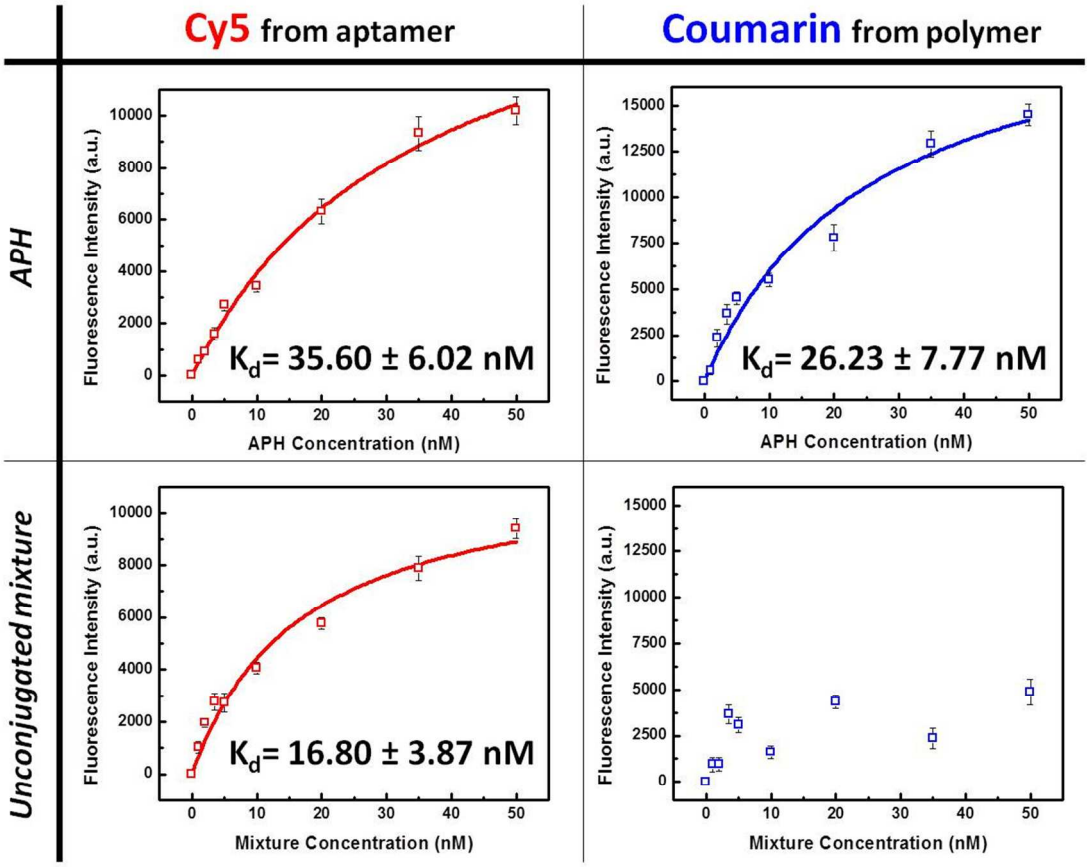


Figure S6: Affinity measurements for conjugated thrombin APH (top) versus an unconjugated mixture of thrombin aptamer and polymer (bottom) demonstrate that conjugation does not alter aptamer affinity, and that polymer binding is dependent on aptamer-mediated targeting.

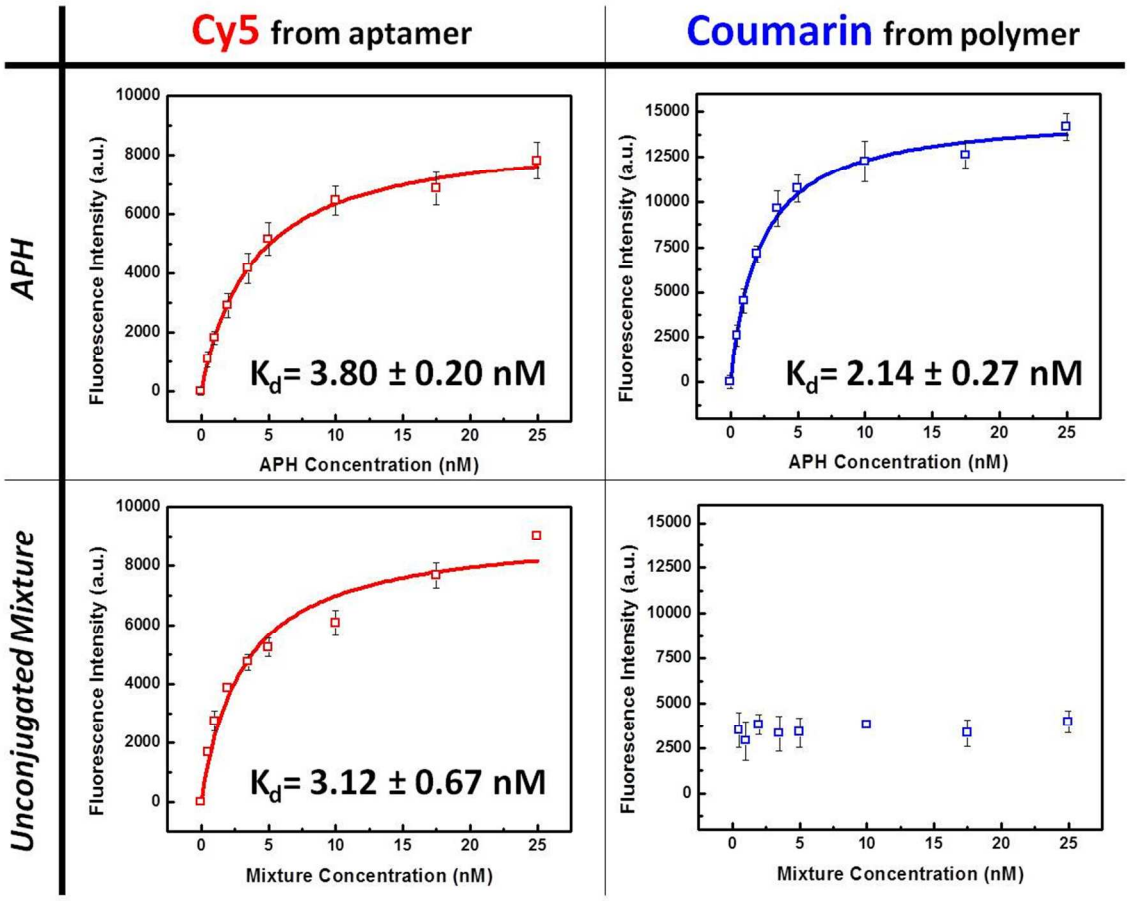


Figure S7: Affinity measurements for conjugated IgE APH (top) versus an unconjugated mixture of IgE aptamer and polymer (bottom) demonstrate that conjugation does not alter aptamer affinity, and that polymer binding is dependent on aptamer-mediated targeting.

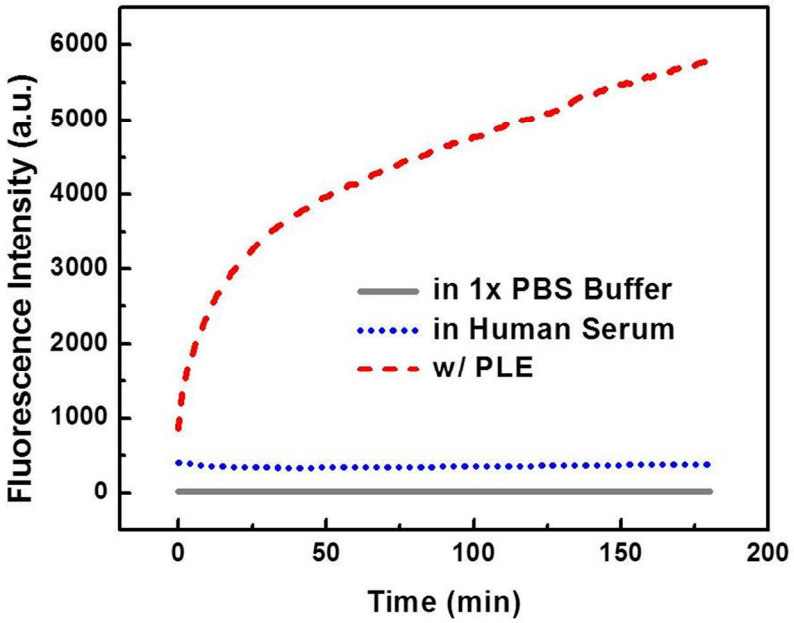


Figure S8: Measurement of coumarin payload release from the APH in 1x PBS (grey) or in undiluted human serum (blue). We observed no signal increase in human serum, indicating that the ester linkage between the payload and the APH should be stable under extracellular conditions, whereas it is selectively cleaved within an endosomal condition (red).

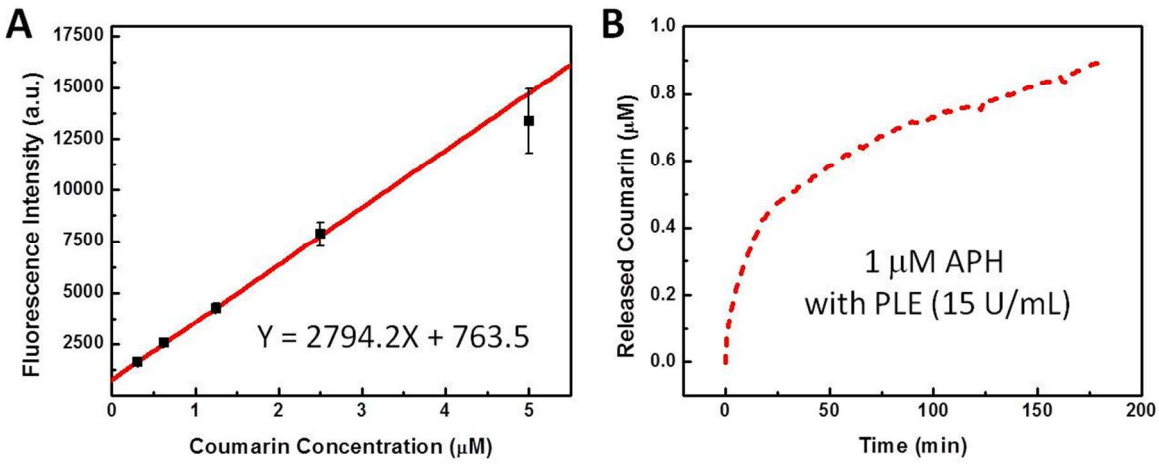


Figure S9: Quantification of time-dependent payload release induced by PLE. (A) Linear relationship between different coumarin concentrations and fluorescent intensities in the presence of PLE (15 U/mL). (B) The amount of coumarin dyes released from 1 μM APH induced by PLE (15 U/mL) was calculated and plotted as a function of time.

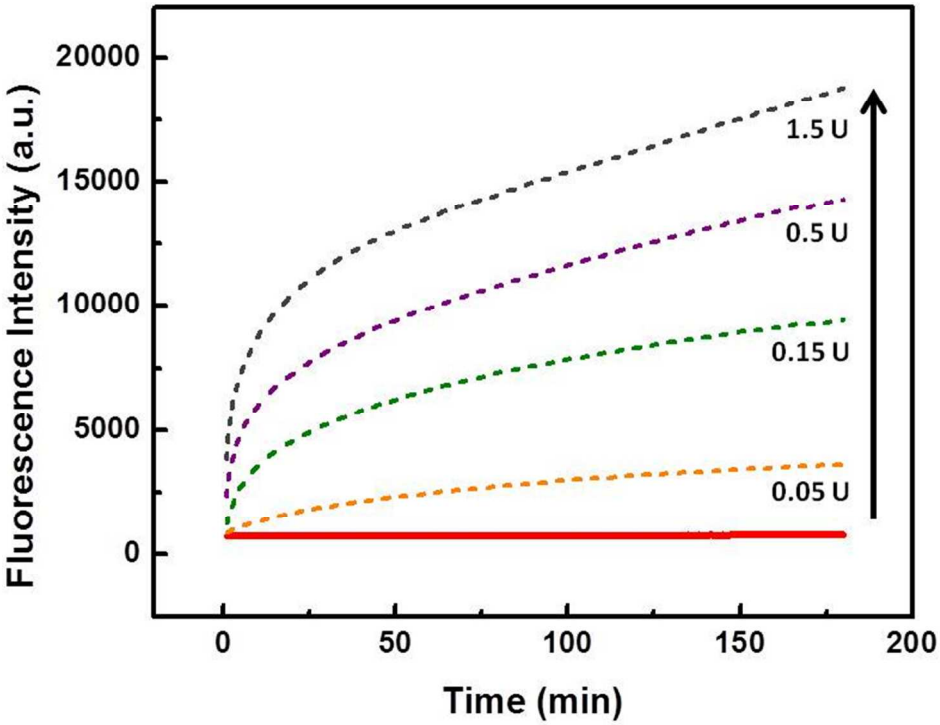


Figure S10: PLE-dependent payload release from the APH. PLE is required for coumarin release, based on the concentration-dependent fluorescence increase observed over time in enzyme-treated APH samples (dashed lines) relative to the stable, low signal observed without enzyme (red).

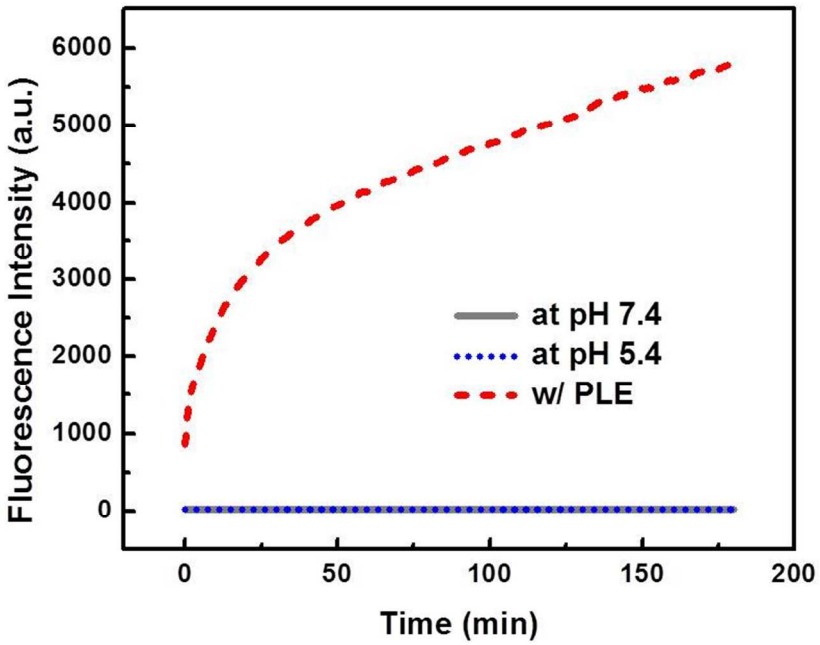


Figure S11: Measurement of coumarin release from the APH at different pH conditions. Unlike the fluorescence increase induced by PLE (red), no notable fluorescence increase of the APH was detected at a late endosomal pH, 5.4 (blue) and at a neutral pH, 7.4 (grey).

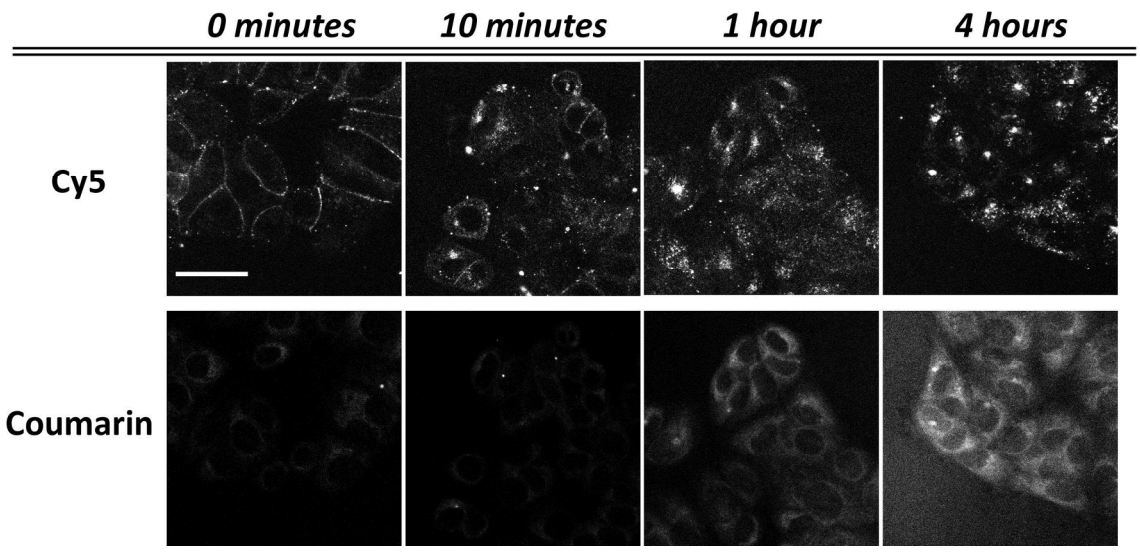


Figure S12: Live-cell imaging of APH-mediated, targeted coumarin delivery to MCF-7 cells through confocal fluorescence microscopy. Scale bar= 40 μm.

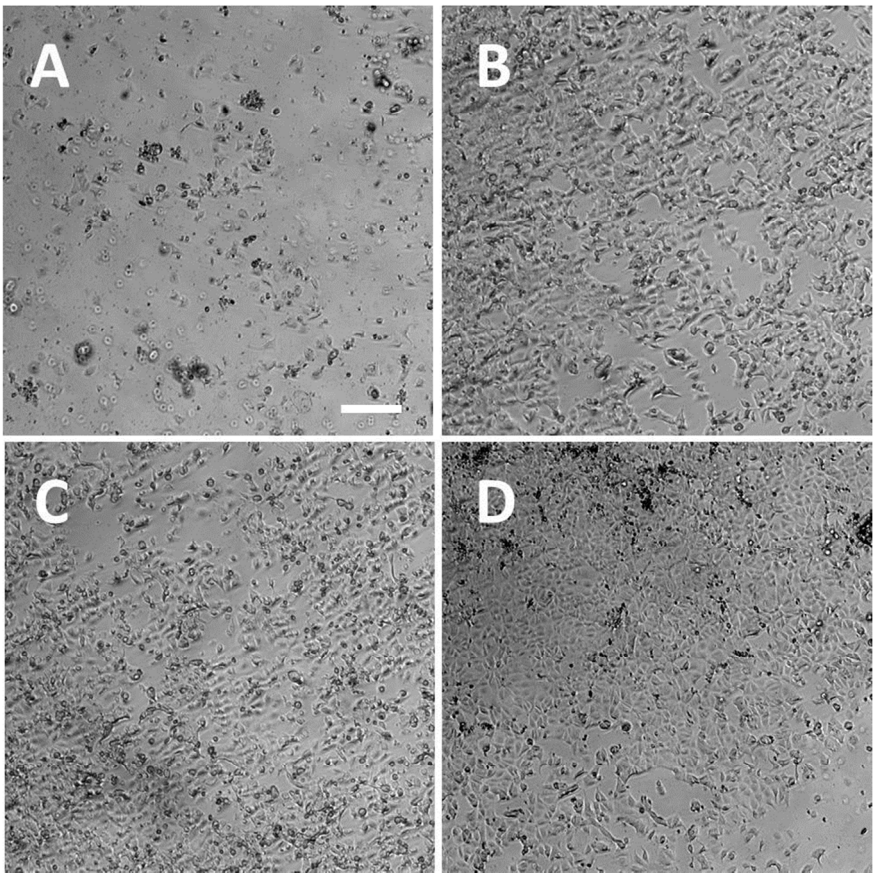


Figure S13: Optical microscopy images reveal efficient killing of MCF-7 cancer cells treated with (B) nucleolin-targeting, DOX-loaded APHs relative to cells treated with (C) unconjugated nucleolin-specific aptamers, (D) unconjugated DOX-loaded polymer scaffolds, and (E) culture media only. Scale bar = 100 μm.

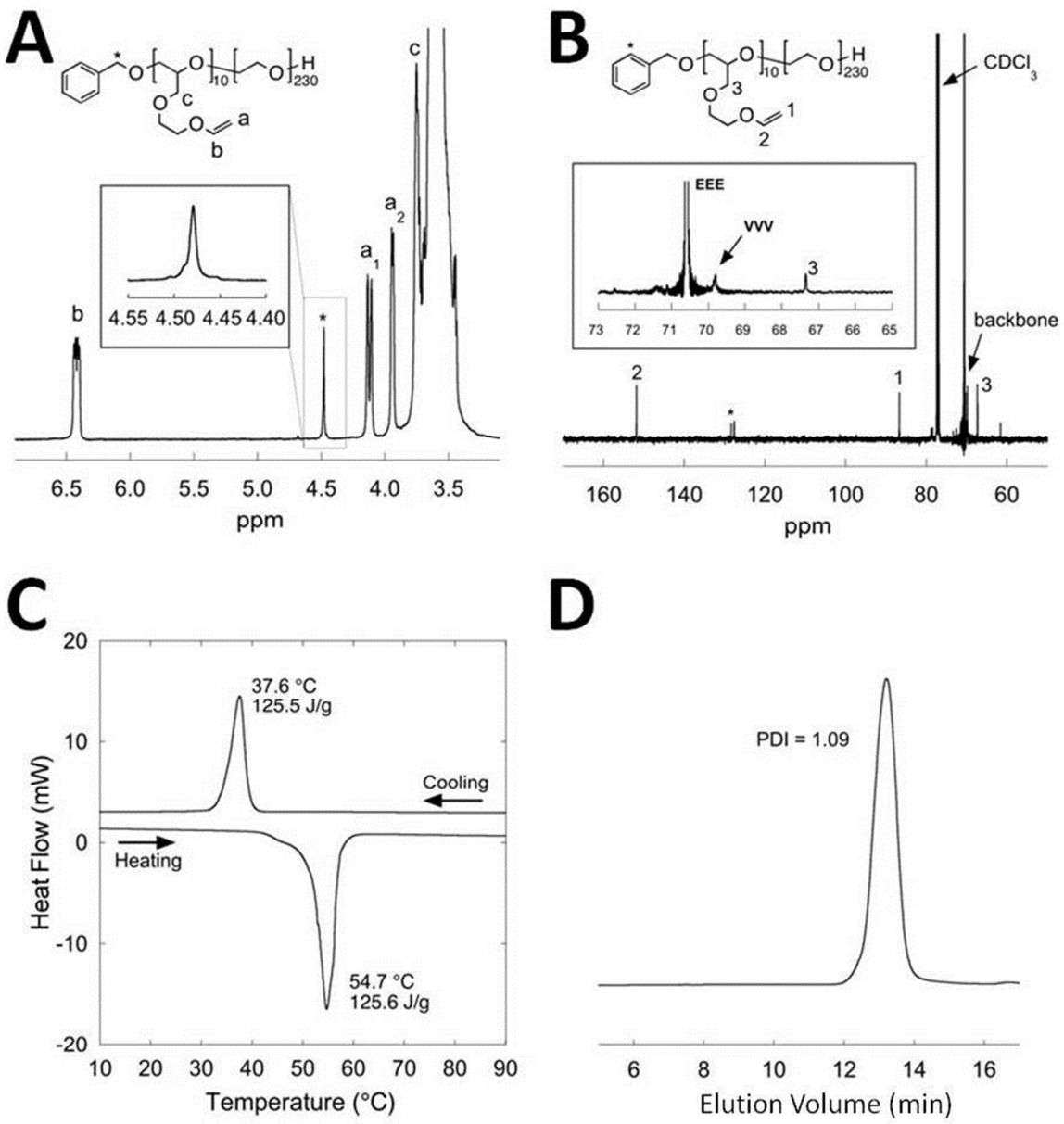


Figure S14: Confirming the structure of poly(EGVGE)-b-(EO)(**1**). (A) We calculated the degree of polymerization for each repeat unit with ^{1}H NMR spectroscopy. The singlet due to benzylic protons (inset) indicates EGVGE homopolymerization near the initiator[5]. (B) ^{13}C NMR triad analysis (inset) indicates that triads of homopolymer units (EEE and VVV) are dominant with negligible amounts of alternating triads[6]. (C) Sharp melting/crystallization peaks and quantitative amounts of ΔH based on differential scanning calorimetry measurement imply the existence of a long homo-polyethylene oxide block. (D) Gel permeation chromatography (GPC) indicates that the block-copolymer has a narrow polydispersity.

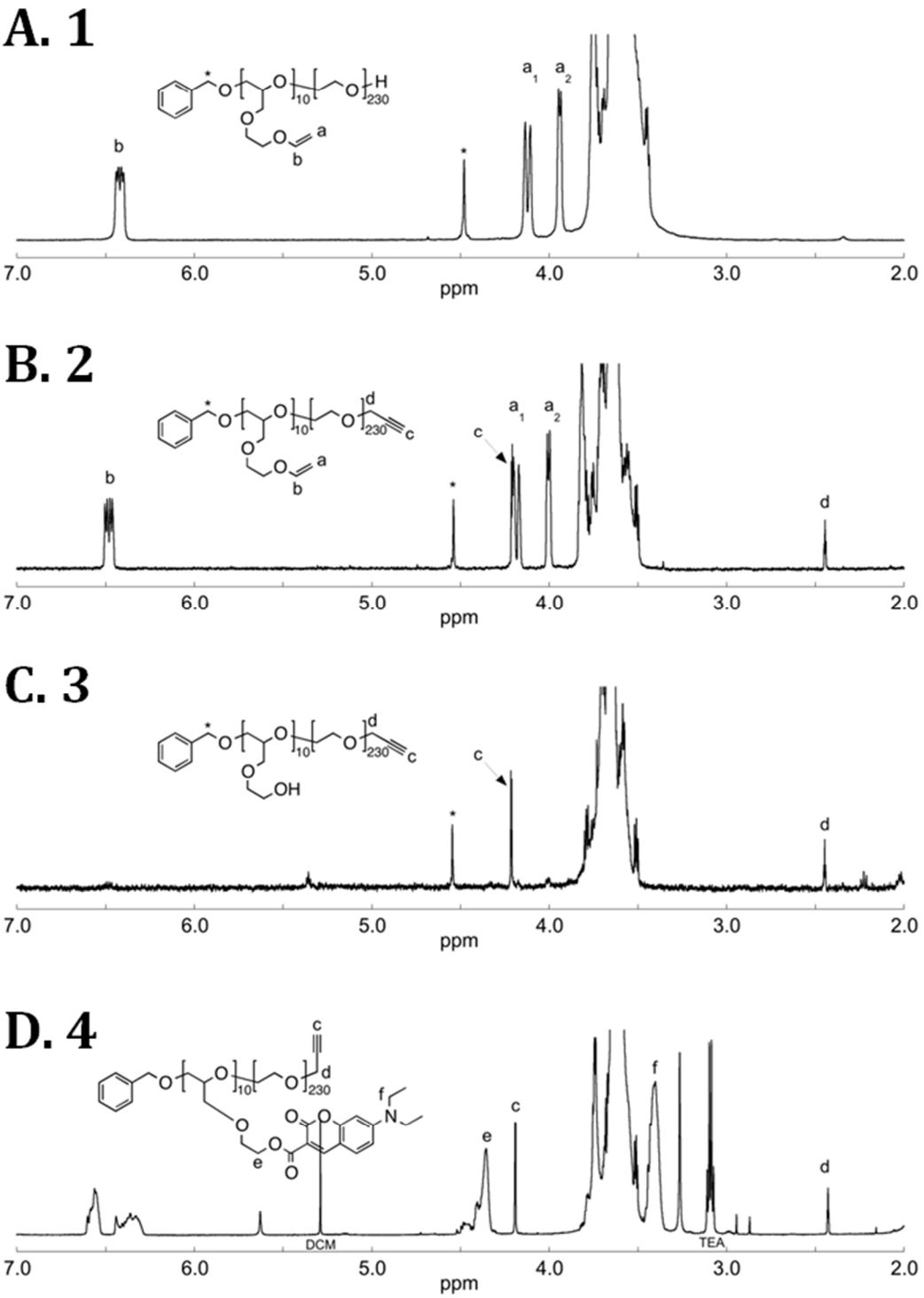


Figure S15: All functionalizations in **Scheme 1** were sequentially monitored by ^{1}H NMR spectroscopy after (A) polymerization, (B) alkyne functionalization of the end group, (C) alcohol deprotection, and (D) coumarin grafting via ester bond. In spectrum (D), the solvents dichloromethane (DCM) and triethylamine (TEA) slightly changed the chemical shift, and the unassigned peaks at 3.25 and 5.65 are from 1,3-dicyclohexylurea, a byproduct of dicyclohexylcarbodiimide coupling.

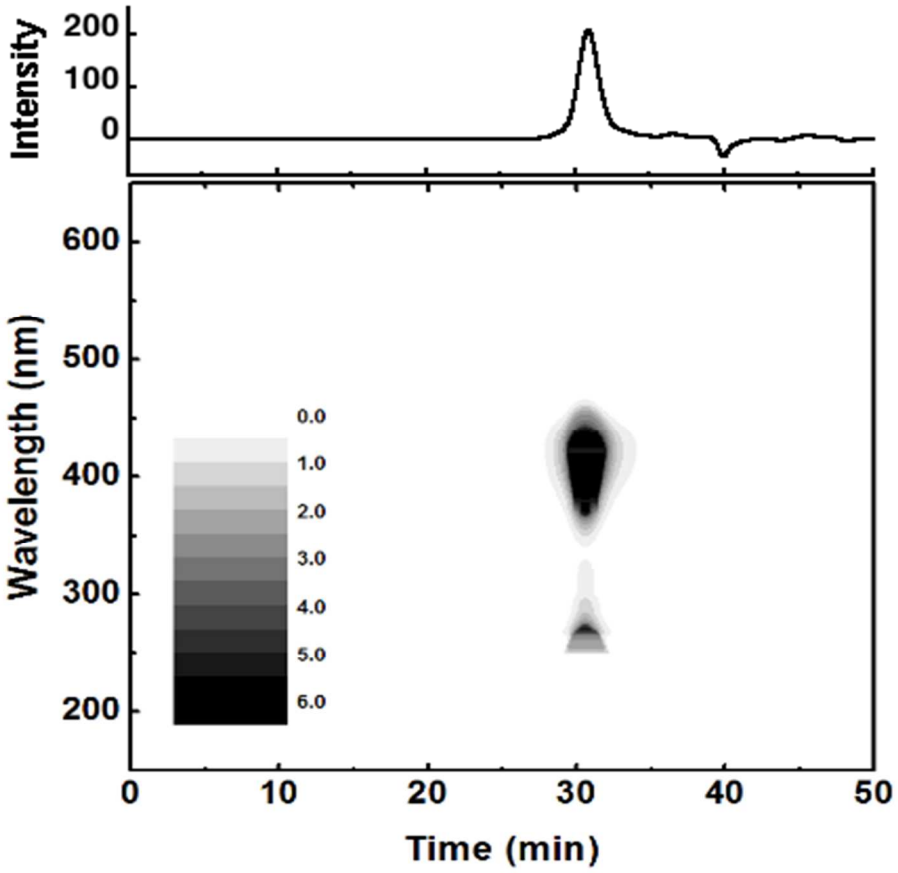


Figure S16: Dye functionalization of the polymer scaffold (**4**) was confirmed by combining reflective index (top) and UV-vis detection (bottom) in GPC. The identical elution volume of the polymer and coumarin indicates that the payloads were successfully grafted onto the polymer scaffold. The polydispersity index measured via RI detector was 1.09.

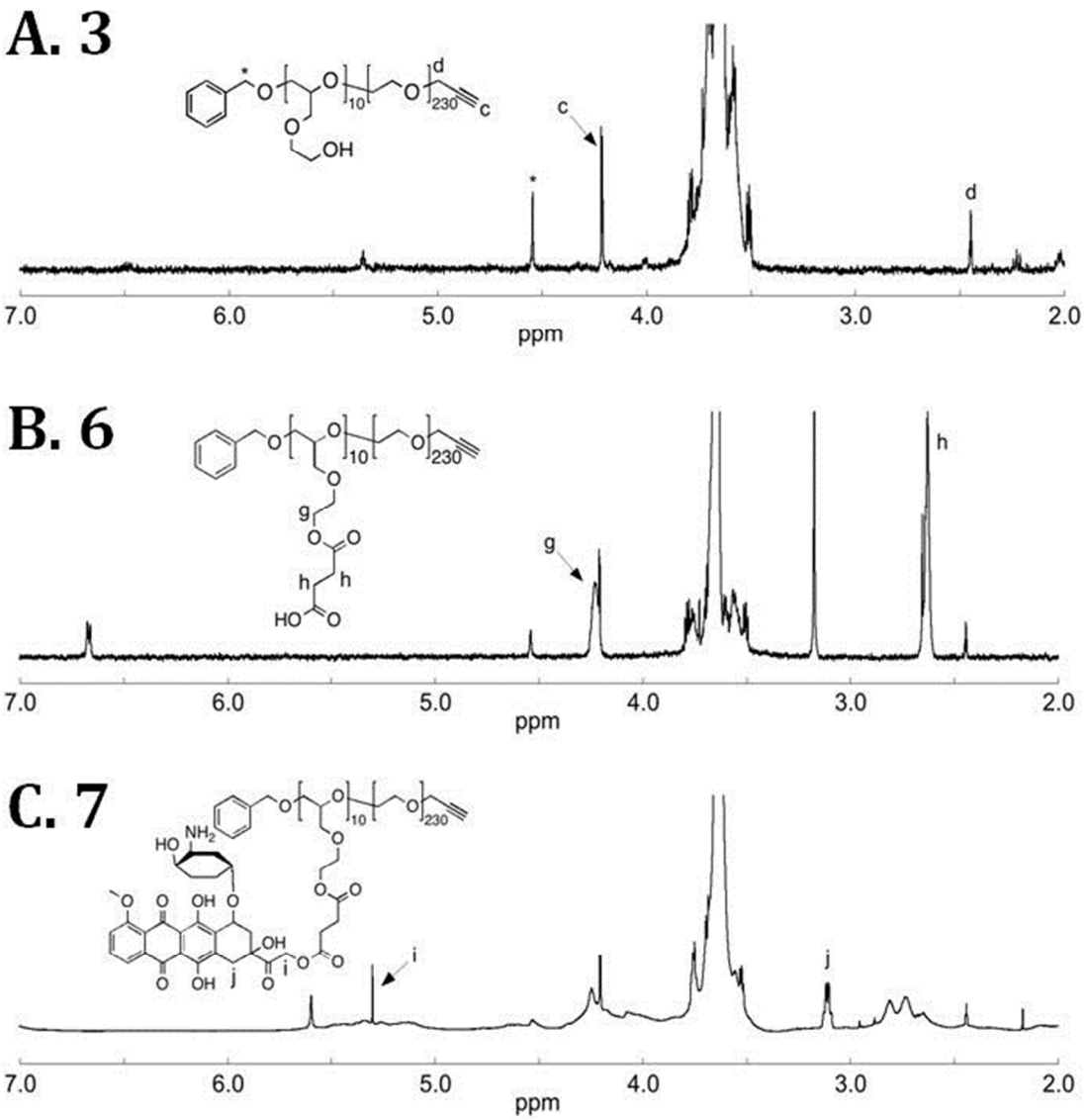


Figure S17: Functionalizations in **Scheme S1** were monitored by ^{1}H NMR spectroscopy for (A) the alcohol-deprotected polymer (**3**), (B) succinic anhydride conjugation (**6**), and (C) grafting of doxorubicin via an enzymatically cleavable linkage (**7**). The unassigned peaks at 3.15 and 6.68 are from residual succinic anhydride and 4-dimethylaminopyridine, respectively.

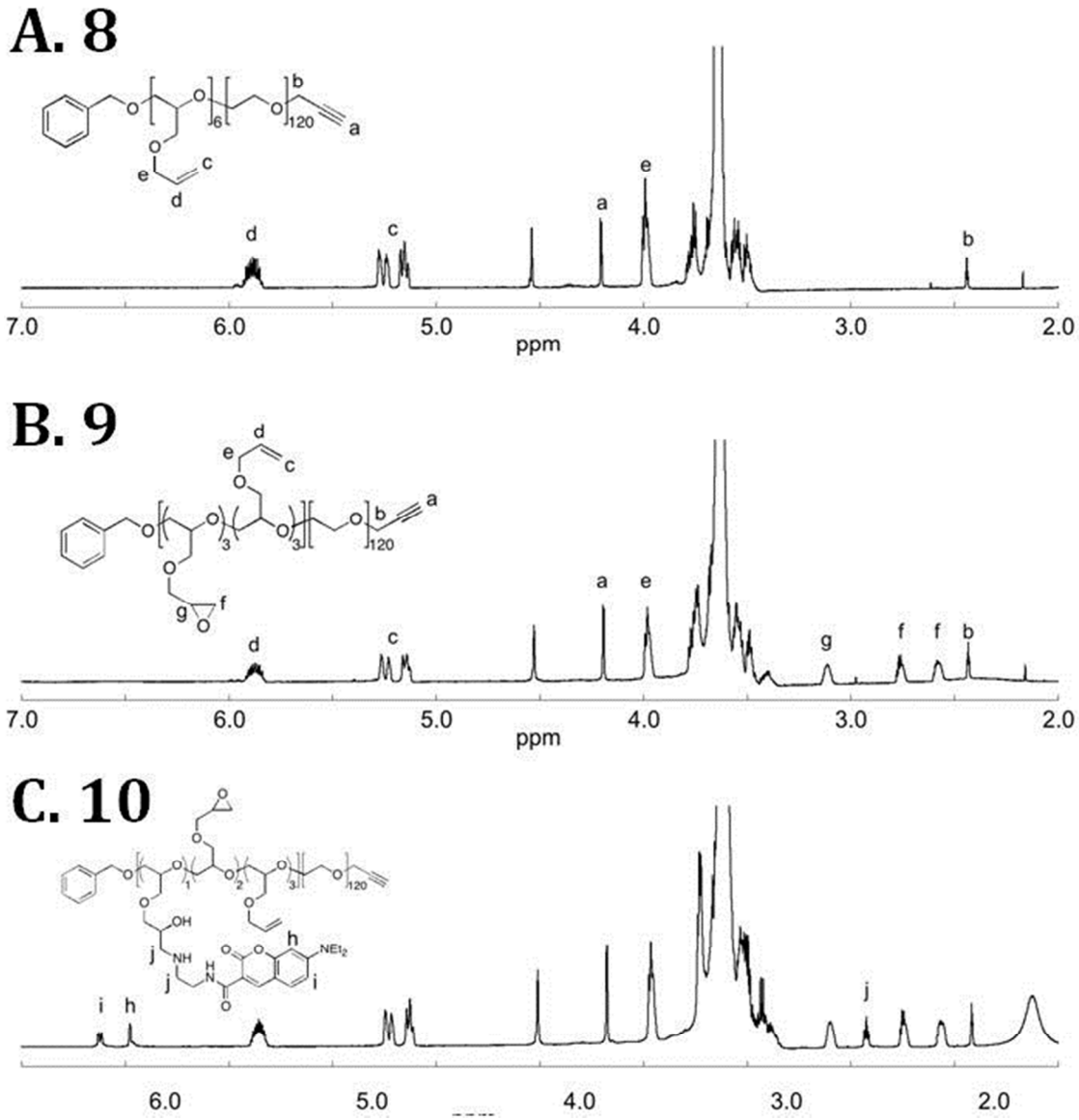


Figure S18: Functionalizations in **Scheme S2** were monitored by ^{1}H NMR spectroscopy for (A) the starting polymer, (B) partial epoxidation of allyl group, and (C) grafting of coumarin via non-cleavable linkages. The resulting polymer scaffold also has narrow polydispersity (PDI = 1.11) as measured by GPC.

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