Supporting Information

Fluorescent Heterotelechelic Single-Chain Polymer Nanoparticles: Synthesis, Spectroscopy and Cellular Imaging

Daniel N. F. Bajj, Michael V. Tran, Hsin-Yun Tsai, Hyungki Kim, Nathan R. Paisley, W. Russ Algar* and Zachary M. Hudson*

Department of Chemistry, The University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z1

*algar@chem.ubc.ca
*zhudson@chem.ubc.ca

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**S1. Materials**

**General**

Pentafluorophenol was from Matrix Chemical Co. Dichloromethane was from Sigma-Aldrich and dried by distillation over P_2O_5. Triethylamine (Et_3N) and dioxane were from Sigma-Aldrich and dried by refluxing over CaH_2 overnight followed by distillation under nitrogen onto 4 Å molecular sieves. Anhydrous DMF was from Sigma-Aldrich and stored over 4 Å molecular sieves. Jeffamine M-1000 was from Huntsman Chemicals and freeze-dried from benzene. Folic Acid-PEG_100-Alkyne was from Biochempeg Scientific and used as received. BTA-NH_2 and fluorescein-NH_2 were synthesized using literature methods. Bovine Serum Albumin (BSA) was from Amresco. PBS buffers were from Gibco Life Technologies. The primary PBS buffer composition was pH 7.2, 1.54 mM KH_2PO_4, 2.71 mM Na_2HPO_4, 155 mM NaCl, and is denoted as 1× PBS. For samples with adhered cells (excluding microinjections), the PBS buffer composition was pH 7.2, 1.47 mM KH_2PO_4, 8.06 mM Na_2HPO_4, 138 mM NaCl, 2.67 mM KCl, 0.49 mM MgCl_2, 0.90 mM CaCl_2 and is denoted as PBS(+, +). All other reagents and solvents were from Sigma-Aldrich and used as received. Dialysis was carried out using a 3.5 kDa MWCO Spectra/Por 3 standard regenerated cellulose membrane. Amicon Ultra-0.5 mL centrifugal filters with a 30 kDa MWCO were used for spin filtration.

**Preparation of FITC-labeled Bovine Serum Albumin (BSA-FITC)**

A BSA solution was prepared by dissolving 39 mg (0.59 µmol) in 4 mL of 50 mM borate buffer pH 9.3 in a glass vial. Next, 235 µL of FITC solution (2.5 mM in 50 mM borate buffer pH 9.3) was added to the BSA solution. The sample was gently mixed in the dark at room temperature for 2 h. The labeled protein was dialyzed in 3.5 kDa MWCO dialysis tubing over two days against 1 L of water at 4 °C with one water change to remove unreacted FITC. The calculated labelling ratio after purification was ~0.1:1 FITC:BSA.

**Avidin Filter Paper Functionalization and P6 Binding Test**

Whatman #1 cellulose filter paper was functionalized with biotin using a literature method. The filter paper was first soaked in a 50 mM solution of NaIO_4 in H_2O for 2 h under protection from light, then washed three times with H_2O and once with EtOH. The papers were then incubated in a 5 mM solution of biotin hydrazide in H_2O overnight, then washed with H_2O. They were then soaked in a 50 mM solution of NaBH_3CN in H_2O for 1 h and washed with H_2O three times. Papers were then soaked in a 1 mg/mL avidin solution for 20 min and washed with H_2O to yield an avidin-functionalized surface. Next, 10 µL of the purified SCPN-Biotin solution (50 µM) SCPN-P6 was spotted onto one avidin-functionalized filter
paper and one control filter paper that had only been subjected to NaIO₄ treatment. The substrates were then allowed to sit at room temperature under protection from light for 1 h, followed by washing with excess H₂O then EtOH. The binding of SCPN-P₆ to the paper was then determined by fluorescence spectroscopy and imaging.

S2. Synthetic Procedures

**Pentafluorophenylacrylate (1)**

PFP (PFPA) was synthesized using a variation of a literature method.⁶ 5.0 g (27 mmol, 1.0 equiv.) of pentafluorophenol was added to a dried 100 mL Schlenk flask under a nitrogen atmosphere. Dry CH₂Cl₂ (30 mL) was added via syringe and the mixture was stirred to yield a clear colorless solution. Dry Et₃N (4.6 mL, 32 mmol, 1.2 equiv.) was added dropwise to yield a clear pale yellow solution. The mixture was cooled to 0 °C and acryloyl chloride (2.6 mL, 32 mmol, 1.2 equiv.) was then added, resulting in the formation of a bright yellow color and precipitate. The solution was left stirring for 12 h at room temperature under protection from light. The solution was then filtered to yield a clear yellow organic layer, which was washed with 0.01 M HCl (2 × 30 mL) and H₂O (2 × 30 mL) then dried over MgSO₄ (s) and filtered. Removal of the solvent yielded an orange oil, which was purified by vacuum distillation under nitrogen to yield a clear colorless liquid. Yield: 5.78 g, 89%. ¹H-NMR (400 MHz, CDCl₃): δ 6.72 (dd, J = 17.3, 0.9 Hz, 1H, OCH=CH₂), 6.37 (dd, J = 17.3, 10.5 Hz, 1H, OCH=CH₂), 6.18 (dd, J = 10.5, 0.9 Hz, 1H, OCH=CH₂).
4,4′-azobis(4-cyanovaleric (20″-O-(S)-camptothecin) ester (2)

4,4′-azobis(4-cyanovaleric acid) (ACVA, 42 mg, 0.15 mmol, 1.0 equiv.), 125 mg (0.36 mmol, 2.4 equiv.) of (S)-camptothecin and 42 mg (0.34 mmol, 2.3 equiv.) of 4-dimethylaminopyridine (DMAP) were added to a dry 100 mL round-bottom flask under N₂. Dry CH₂Cl₂ (25 mL) was added via syringe, and the mixture was stirred to yield a cloudy yellow solution. The mixture was cooled to 0 °C and 66 mg (0.34 mmol, 2.3 equiv.) of N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC · HCl) dissolved in dry CH₂Cl₂ (15 mL) was added dropwise. The mixture was left stirring under nitrogen for 24 h, yielding a clear pale yellow solution containing small amounts of insoluble white solid. The reaction was washed with saturated NaHCO₃ (15 mL), H₂O (15 mL) and brine (15 mL), then dried over MgSO₄, filtered and concentrated to give a yellow solid. Purification by column chromatography on silica (1:1 CH₂Cl₂:acetone) yielded the product as a yellow solid. Yield: 78 mg, 55%. ¹H-NMR (400 MHz, CDCl₃): δ 8.39 (d, J = 6.6 Hz, 2H, Ar-H), 8.23 (d, J = 8.4 Hz, 2H, Ar-H), 7.94 (d, J = 8.2 Hz, 2H, Ar-H), 7.87-7.76 (m, 2H, Ar-H), 7.67 (t, J = 7.5 Hz, 2H, Ar-H), 7.20 (d, J = 4.0 Hz, 2H, Ar-H), 5.68 (m, 2H, -N-CH₂), 5.49-5.32 (m, 2H, -N-CH₂), 5.28 (d, J = 8.2 Hz, 4H, COO-CH₂), 2.87-2.04 (m, 12H, aliphatic), 1.72 (d, J = 18.0 Hz, 6H, -CH₃), 0.97 (q, J = 7.5 Hz, 6H, -CH₃); ¹³C-NMR (100 MHz, CDCl₃): δ 170.58, 167.35, 157.32, 152.83, 146.98, 145.63, 145.62, 132.21, 131.39, 129.2-128.0 (m, br, 5C), 120.86, 117.56, 97.11, 71.86, 67.24, 53.94, 50.16, 32.87, 31.89, 29.43, 23.96, 7.76 ppm. HRMS (ESI) m/z: [M+H]⁺ calcd for [C₅₂H₄₅N₈O₁₀]: 941.3259, found: 941.3257.
poly(PFPA)$_{78}$ (P1)

2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid 3-azido-1-propanol ester (12.5 mg, 0.028 mmol, 1.0 equiv.) was added to a dried 5 mL Schlenk bomb with stir bar under a nitrogen atmosphere. Pentafluorophenyl acrylate (1.0 g, 4.2 mmol, 150 equiv.), 2,2’-azobis(2-methylpropionitrile) (AIBN) in dioxane (0.46 mg, 2.8 μmol, 0.1 equiv.; added as 92 μL of a 5 mg/mL stock solution in dioxane) and 1.1 mL of dioxane were added under nitrogen flow, and the mixture was stirred to give a yellow solution. The mixture was subjected to three freeze-pump-thaw cycles then submerged in a preheated 80 °C oil bath for 45 min with stirring. It was then submerged in liquid N$_2$ to stop the polymerization and exposed to air. The viscous yellow liquid was precipitated in MeOH to yield a yellow solid, which was redispersed in 1 mL THF and re-precipitated twice in MeOH. The polymer product was dried in a vacuum oven for 24 h to yield a yellow powder. $M_n = 19000$, $D = 1.34$, $DP = 78$. The refractive index increment $dn/dc$ was measured as 0.0403 at 35 °C in THF.

poly(PFPA)$_{150}$ (P2)

Prepared in analogy with P1, but using a 1:130:0.15 RAFT Agent:Monomer:Initiator ratio and a 2 h reaction time at 80 °C. $M_n = 37600$, $D = 1.40$, $DP = 150$. 

\[ \text{poly(PFPA)} \]
CPT-poly(PFPA)_{78} (P3)

30 mg (0.032 mmol, 20 equiv.) of 2, 30 mg (1.6 μmol, 1.0 equiv.) of P1 and 256 μL (3.2 μmol, 2.0 equiv.) of lauroyl peroxide solution (5 mg/mL in 1:1 dry DMF/dioxane) were added to a 5 mL Schlenk bomb with stir bar. Additional 1:1 dry DMF/dioxane (800 μL) was added to dissolve all solids and give a clear yellow solution. The reaction was subjected to three freeze-pump-thaw cycles then placed under nitrogen atmosphere. The solution was then submerged in a preheated 80 °C oil bath for 2 h, then submerged in liquid N₂ and opened to air. The polymer was isolated by preparatory SEC using inhibitor-free THF as mobile phase. Yield: 20 mg, 65%. The incorporation of CPT was then verified by ¹H NMR and UV-visible spectroscopy (Figure S4). Molecular weight (M_n) and dispersity (D) were unchanged relative to P1 by SEC.
CPT-SCP-N\textsubscript{3} (P4)

20 mg (1.0 μmol, 1.0 equiv.) of P3 was charged into a dry 25 mL round-bottom flask and placed under an N\textsubscript{2} atmosphere. Dry DMF (1.5 mL) was added via syringe, and the mixture was stirred to yield a clear yellow solution. The solution was heated to 50 °C in an oil bath, then 5.2 mg (8.3 μmol, 8.0 equiv.) BTA-NH\textsubscript{2} dissolved in 1.0 mL dry DMF was added. The mixture was stirred for 2 h, then a solution containing 5.2 mg (8.3 μmol, 8.0 equiv.) fluorescein-NH\textsubscript{2} in 1.0 mL dry DMF was added, and the mixture was stirred for an additional 2 h. 93 mg (93 μmol, 89 equiv.) of Jeffamine M-1000 dissolved in 1.0 mL of dry DMF was then added and the solution stirred for 24 h under nitrogen atmosphere and protection from light. The pure polymer was dialyzed against THF (3.5 kDa MWCO), then the solvent was removed to yield a brown sticky solid. The solid was dissolved with 500 μL Milli-Q H\textsubscript{2}O to yield a clear yellow solution with green fluorescence. Further purification was performed using a 30 kDa MWCO spin filter. 60 mg of polymer was dissolved into 1.0 mL of Milli-Q H\textsubscript{2}O and volume reduced to 100 μL with 30 min of centrifugation at 14000 rcf, then diluted back to 1.0 mL with Milli-Q H\textsubscript{2}O. This process was repeated three times, then drying in a vacuum centrifuge overnight yielded a sticky brown solid. Yield: 40 mg, 50%. \textsuperscript{1}H NMR: Figure S5; \(M_n\) (NMR) = 78,800.
SCPN-\(\mathrm{N}_3\) (P5)

Prepared analogously to P4, using 50 mg of P2 and 15, 7.5 and 167 equivalents of BTA-\(\mathrm{NH}_2\), fluorescein-\(\mathrm{NH}_2\) and Jeffamine M-1000 respectively relative to the number of polymer chains. The resulting product was a glassy brown solid. Yield: 120 mg, 59%. \(^1\)H NMR: Figure S7; \(M_n\) (NMR) = 151,000.
Bioconjugation of P5 with Biotin to form SCPN-Biotin (P6) Stock Solution

To a 1.5 mL Eppendorf tube was added 187 µL of 130 µM P5 (24 nmol, 1.0 equiv.) in H$_2$O and 233 µL of pH 7 phosphate buffer. 23 µL of 2.2 mM biotin-PEG$_4$-alkyne (50 nmol, 2.1 equiv.) in H$_2$O was then added. 2.5 µL of 20 mM CuSO$_4$ (50 nmol, 2.1 equiv.) in H$_2$O and 5.0 µL of 50 mM tris(3-hydroxypropyltriazolylmethyl)amine (250 nmol, 10 equiv.) in H$_2$O were combined, then added to the reaction. Lastly, 25 µL of 100 mM sodium ascorbate (2.5 µmol, 100 equiv.) in H$_2$O was added. The reaction tube was sealed with Parafilm, wrapped in aluminum foil and placed on a laboratory shaker for 2 h. The mixture was then purified using a 30 kDa MWCO spin filter to yield the purified product as a 500 µL solution (50 µM) in H$_2$O. Calculated $M_n = 152,000$ based on 100% end-group conversion.
Bioconjugation of P4 with Folic Acid (FA) to form CPT-SCPN-FA (P7) Stock Solution

To a 1.5 mL Eppendorf tube was added 195 µL of 128 µM P4 (25 nmol, 1.0 equiv) in H$_2$O and 250 µL of pH 7 phosphate buffer. 52 µL of 2 mM folic acid-PEG$_{100}$-alkyne (100 nmol, 4.2 equiv) in H$_2$O was then added. 2.5 µL of 20 mM CuSO$_4$ (50 nmol, 2.1 equiv.) in H$_2$O and 5.0 µL of 50 mM tris(3-hydroxypropyltriazolymethyl)amine (250 nmol, 10 equiv.) in H$_2$O were combined, then added to the reaction. Lastly, 25 µL of 100 mM sodium ascorbate (2500 nmol, 100 equiv.) in H$_2$O was added. The reaction tube was sealed with Parafilm, wrapped in aluminum foil and placed on a shaker for 2 h. The mixture was then purified using a 30 kDa MWCO spin filter to yield the purified product as a 500 µL 50 µM solution in H$_2$O. Calculated $M_n = 83,600$ based on 100% end-group conversion.
S3. Characterization Details

**Size Exclusion Chromatography**
SEC experiments used chromatography-grade THF at polymer concentrations of 0.5–2 mg mL\(^{-1}\) and a Malvern OMNISEC GPC instrument. GPC columns were packed with porous poly(styrene-co-divinylbenzene) particles regulated at a temperature of 35 °C. Signal response was measured using differential viscometer, differential refractive index, photodiode array and right-angle and low angle light scattering detectors. Calibration of interdetector distances was performed using a polystyrene standard from Malvern Inc. Refractive index increments \((dn/dc)\) were determined using 100% mass recovery methods from Malvern OMNISEC software version 10.2 with each polymer sample being run at least five times to ensure reproducibility of the calculated refractive index increment. Preparatory scale size exclusion chromatography was carried out on a Toyopearl HW-55F resin using inhibitor-free THF as the mobile phase.

**Atomic Force Microscopy**
Atomic force microscopy (AFM) images were obtained using an Asylum Instruments Cypher S AFM system in tapping mode at scan rates of 3.0 Hz. Samples were prepared by spin-coating solutions of polymer onto freshly cleaved mica at 2500 rpm for 30 s at concentrations of ~0.001 mg mL\(^{-1}\). Images were obtained using Asylum Research AC55TS Cr/Au (5/65) coated Si probes, with typical resonance frequencies, \(f = 1600\) kHz, and spring constants, \(k = 85\) N/m.

**Dynamic Light Scattering (DLS)**
DLS measurements were obtained using a Wyatt DynaPro Titan instrument (Wyatt Technologies, Santa Barbara, CA, USA) in deionized water at 25 °C using 10 mm optical path length quartz cuvettes. SCPN samples were dissolved at the specified concentration and subjected to ultrasonication for 30 minutes to reduce aggregation, then passed through a 0.45 μm PTFE syringe filter prior to measurement.

**Circular Dichroism Spectroscopy (CD)**
CD measurements were obtained using a Jasco J-815 CD spectrophotometer (Jasco Inc., Tokyo, Japan) at 25 °C using 1.0 cm optical path length quartz cuvettes, with \([SCP\text{N}] = 0.07\) mg/mL in deionized water.

**Spectral Characterization**
Absorbance and fluorescence excitation, emission, and anisotropy measurements were made with an Infinite M1000 Pro multifunction plate reader (Tecan, Morrisville, NC, USA) using 100 μL aliquots of
samples and half-area 96-well UV-transparent plates (Corning, Kennebunk, ME, USA). The path length for absorbance measurements was 0.6 cm. A defined path length was not applicable to fluorescence measurements because an optical fiber bundle was used for both delivery of excitation light and collection of fluorescence emission. Sample concentrations were 10 μM for fluorescein and FITC, 1.0 μM for BSA-FITC (with respect to FITC concentration), and 75 μM for SCPN-P7. The step size was 1 nm for collecting spectra, the bandwidth was 5 nm, 430 nm excitation was used to measure emission spectra, and 600 nm emission was used to measure excitation spectra. Measurements were made in 1× PBS buffer (see section S1 for recipe) between 24-26 °C. Spectra were also measured for a series of dilutions of P6 (data not shown).

**Quantum Yield Measurements**

Fluorescein dissolved in pH 9.5 borate buffer (50 mM) with 100 mM NaCl was used as a reference with a quantum yield of 0.93. Fluorescein, FITC, BSA-FITC, and SCPN-P7 samples were prepared in 1× PBS buffer between 24–26 °C. SCPN concentrations were from 0.25–2.5 µM. The quantum yield was determined for each material from the slopes of plots of integrated fluorescence intensity versus absorbance, relative to the slope for the fluorescein reference. The absorbance value was kept below 0.1 for the samples. The path length was 0.6 cm. The few data points for higher concentrations that appeared to deviate from linearity were excluded from calculations.

**Fluorescence Lifetime Measurements**

Fluorescence lifetime measurements were made using a FluoroCube time-correlated single photon counting instrument (Horiba, Edison, NJ). Samples were measured in 0.4–0.7 mL (1 cm path length) quartz fluorimeter cell (Starna Cells, Atascadero, CA). The samples were prepared to a final volume of 0.4 mL by diluting in 1× PBS buffer at room temperature. The four samples for measurement were FITC (2.5 µM), fluorescein (2.5 µM), BSA-FITC (250 nM with respect to fluorescein), and SCPN-P7 (100 µM). The samples were excited with a 453 nm peak wavelength nano-LED, with a pulse duration of 1.4 ns. The fluorescence emission was selected with a 500 nm longpass filter and measured on a picosecond photon detection module (Horiba, Edison, NJ).

**Fluorescence Anisotropy Measurements**

The fluorescence emission polarization module of the Infinite M1000 Pro plate reader was used for anisotropy measurements. Fluorescein (1.0 nM) dissolved in 0.1 M NaOH was used as the standard (27 mP) for calculating the G-factor. Excitation was at 470 nm (5 nm bandwidth) and emission measured at 520 nm (5 nm bandwidth) for fluorescein, FITC, BSA-FITC and SCPN. The same sample
concentrations, buffers, and temperatures were used for spectral characterization \((\textit{vide supra})\) and fluorescence anisotropy measurements.

**Photobleaching Measurements**

Photobleaching measurements were done with the same inverted microscope used for cell imaging \((\textit{vide infra})\). Sample aliquots (5 µL) were measured in a clear flat-bottom 1536-well plate (Greiner Bio One, Kremsmünster, Austria) with the wells covered with universal optical sealing tape (#6575, Corning). Sample concentrations were adjusted to obtain similar initial fluorescence intensities (SCPN-\textbf{P7}, 75 µM; fluorescein, 4.1 µM; FITC, 4.4 µM; BSA-FITC, 1.0 µM with respect to fluorescein). Samples were prepared in 1× PBS buffer at room temperature. A 2 × 2 array of wells was illuminated and imaged with a 4× objective lens (NA 0.16). The excitation filter was 450/50 (center wavelength and bandwidth in nm), the dichroic mirror had a cut-off at 510 nm, and the emission filter was a longpass filter with a 500 nm cut-off (Chroma, Bellows Falls, VT). The excitation power was estimated to be ~62 mW at the sample. Images were acquired at 1 min intervals for 90 min and analyzed using ImageJ software (NIH, Bethesda, MD) with the Time Series Analyzer V3 plugin. The initial intensity for each sample was normalized to a value of unity. Photobleaching rates were determined by fitting the intensity versus time data with either a monoexponential decay function (FITC, fluorescein) or a biexponential decay function (FITC-BSA, SCPN-\textbf{P7}).

**Stern-Volmer Quenching Measurements**

Solutions (10 µM, 11 µL) of fluorescein, FITC, BSA-FITC, and SCPN-\textbf{P7} were prepared in PBS buffer. Stock solutions (100 mM) of KI, KNO\textsubscript{3}, and KCl were prepared in 1× PBS buffer between 24–26 °C. Fluorescein, FITC, BSA-FITC, and SCPN-\textbf{P7} (10 µM, 11 µL) were mixed with KI, KNO\textsubscript{3}, or KCl (100 mM, 0–88 µL) and diluted to 110 µL with PBS buffer. The final concentrations of fluorescein, FITC, BSA-FITC, and SCPN-\textbf{P7} were ~1.0 µM. The final concentrations of KI, KNO\textsubscript{3}, and KCl ranged between 0–80 mM. The KCl and KNO\textsubscript{3} were used as controls to rule out any effects from ionic strength rather than the expected quenching by iodide. After 25 min incubation in the dark at room temperature, aliquots (100 µL) of these samples were transferred to a 96-well plate with 100 µL of PBS buffer as a blank. The fluorescence intensities were measured with the following excitation/emission wavelengths for each sample: 492/512 nm for fluorescein, 594/521 nm for FITC, 502/524 nm for BSA-FITC, and 492/525 nm for SCPN-\textbf{P7}. The excitation and emission bandwidths were 5 nm in all cases. The quenching analysis with KI was done in triplicate. Fluorescence measurements were made with the Infinite M1000 Pro multifunction plate reader and transparent, nonbinding 96-well plates (Corning, Corning, NY). The slope of the Stern-Volmer plots for KCl and KNO\textsubscript{3} were approximately zero (data not shown).
Unfolding Measurements

Samples were prepared by diluting 22 μL of fluorescein (2.5μM), FITC (2.5μM), or SCPN-P7 (100 μM) in 1× PBS buffer with an equal volume of deionized water or DMF. Each sample was prepared in triplicate. The final concentrations of fluorescein, FITC, and SCPN-P7 in the samples were 1.25 μM, 1.25 μM, and 50 μM respectively. Samples were pipetted into a 384-well clear bottom plate (Thermo Scientific, Waltham, MA, USA) and the absorbance and fluorescence excitation and emission measurements were made with an Infinite M1000 Pro multifunction plate reader using 40 μL aliquots at 24–26 °C. The path length for absorbance measurements was 0.43 cm. The step size was 1 nm for collecting spectra, the bandwidth was 5 nm, 430 nm excitation was used to measure emission spectra, and 600 nm emission was used to measure excitation spectra.

S4. Cell Experiments

Cell Culture

SK-BR-3 cells (ATCC HTB-30 Manassas, VA, USA), a human breast cancer cell line, were cultured in a humidified incubator with 95% air/5% CO₂ at 37 °C. The culture medium was McCoy’s 5A (GE Healthcare, Chicago, IL, USA) supplemented with 10% v/v fetal bovine serum and 1× antibiotic and antimycotic (ThermoFisher, Waltham, MA, USA). Cells were cultured in T25 flasks and subcultured every 5–7 days.

A549 cells (ATCC CCL-185), a human lung carcinoma cell line, were used for microinjection experiments. Cells were subcultured once per week and grown in supplemented Ham’s F-12K media (Gibco, Grand Islands, NY, USA) and grown under the same incubator conditions as the SK-BR-3 cells.

Cell Imaging

Imaging was done with an IX83 inverted epifluorescence microscope (Olympus, Richmond Hill, ON, Canada) equipped with an X-Cite 120XL metal-halide light source (Excelitas Technologies, Mississauga, ON, Canada), an Orca-Flash 4.0 V2 sCMOS camera (C11440; Hamamatsu Photonics, Hamamatsu, SZK, Japan) and motorized filter wheels (Sutter Instruments, Novato, CA, USA), and MetaMorph/MetaFluor software (Molecular Devices, Sunnyvale, CA). For cell immunolabeling, the filter set was 450/50 (center line/bandwidth in nm) for the excitation filter, a 510 nm cut-off dichroic mirror, and 520/40 or 540/50 for the emission filter. For microinjection, the filter set was a 450/50 (center line/bandwidth in nm); a 470 nm
cut-off dichroic mirror (Chroma Technology Corp, Bellow Falls, VT, USA); and a 500 nm cut-off longpass filter (Thorlabs Inc., Newton, NJ, USA). ImageJ software was used for processing images.

**Growth, Preparation, and Microinjection of Cells**

An InjectMan 4 micromanipulator, FemtoJet 4i microinjector, and Femtotip II needles (Eppendorf, Mississauga, ON) were used for microinjection. Approximately $3 \times 10^5$ A549 cells were transferred to a cell culture dish (35 mm dish diameter, 14 mm diameter glass bottom, 0.08–0.12 mm thick glass bottom (Matsunami Glass Ind., Ltd., Osaka, Japan). Sterile cell culture dishes were treated with fibronectin (5 μg/mL) in an incubator for > 6 h prior to cell transfer. After transfer, the A549 cells were grown in supplemented McCoy’s 5A media (no phenol red, Gibco) for > 16 h. Immediately prior to microinjection, the media was removed, and the dish was filled with $1 \times$ PBS. Microinjections were done with a 200 hPa injection pressure and a 0.70 s injection time. The solution of SCPN-P7 (36.6 μM ≈ 3 mg/mL, in $1 \times$ PBS buffer) was syringe filtered using 0.22 μm membrane (Millex-GP, Merck Millipore Ltd., Tullagreen, Carrigtwohill, Co. Cork, Ireland), then centrifuged at ~17 000 rcf for 15 min prior to loading the supernatant into the microinjection needle. Brightfield and fluorescence images were taken before injection and at various time intervals following the injection.

**Cellular Labeling and Imaging**

SK-BR-3 cells were seeded in a 96-well, tissue culture-treated, film-bottom plate (Eppendorf, Mississauga, ON, Canada). Each well was seeded with ~1.5 × $10^4$ cells and the cells were allowed to grow for 2 days (5% CO$_2$, 37 °C, McCoy’s 5A media). Cells were washed with PBS(+, +), blocked with 1% w/v bovine serum albumin (BSA; Sigma Aldrich) in PBS(+, +) for 30 min, then incubated with 4–5 μg/mL biotinylated anti-HER2 antibody (Novus, Oakville, ON, Canada) supplemented with 1% w/v BSA for 1 h. After another wash with PBS(+, +), the cells were incubated with 0.4–1.0 mg/mL NeutrAvidin supplemented with 0.1% w/v BSA in PBS(+, +) for 1 h, then washed with PBS(+, +) to remove excess NeutrAvidin. The cells were labeled with ~5 μM (~0.8 mg/mL) SCPN-biotin (P6) supplemented with 0.5% w/v BSA in PBS(+, +) for 1 h, and washed with PBS(+, +) prior to imaging. The entire labeling procedure was done at room temperature.

**Cellular Viability Assay**

The cytotoxicities of CPT-SCPN-N$_3$ (P4) or CPT-SCPN-FA (P7) were determined with an MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) assay kit (Abcam, Toronto, ON, Canada). The SK-BR-3 cells were seeded in 96-well tissue culture-treated clear-bottom plate (ThermoFisher, Waltham, MA, USA) with a density of ~2750 cells/well and grown
overnight (5% CO₂, 37 °C). Cells were washed with PBS and incubated with 1 pM–16 μM (8×10⁻⁷–1.3 mg/mL) CPT-SCPN-N₃ (P₄) or CPT-SCPN-FA (P₇) for 2 h (5% CO₂, 37 °C). After another PBS wash, the cells were replenished with fresh medium (McCoy’s 5A) without phenol red and cultured for 3 days (5% CO₂, 37 °C). After the proliferation period, 20 μL MTS reagent solution was added to each well and incubated for 2 h (5% CO₂, 37 °C). The absorbance was measured with an Infinite M1000 Pro plate reader (Tecan Ltd., Morrisville, NC, USA) at 490 nm and 650 nm (background). The absorbance values for each sample were background subtracted (650 nm) and the cellular viabilities were reported as a percentage of negative control wells (non-treated cells). The assays were done in triplicate.
S5. $^1$H and $^{19}$F NMR Spectra

Figure S1. $^1$H NMR spectrum of 2 in CDCls.
Figure S2. $^1$H NMR spectra of poly(PFPA)$_{150}$ (P2) in CDCl$_3$. 
Figure S3. $^{19}$F NMR spectra of poly(PFPA)$_{150}$ (P2) in CDCl$_3$. 
Figure S4. Top: $^1$H NMR spectrum of CPT-poly(PFPA)$_{78}$ (P3) in CDCl$_3$ with enlarged aromatic region showing captothecin peaks (inset). Bottom: absorption spectra for P1 and P3 depicting the disappearance of trithiocarbonate absorbance at 302 nm and the appearance of camptothecin absorbance at 362 and 378 nm following reaction of P1 with 2. Absorption spectra were obtained via elution volume-resolved SEC photodiode array data to verify the camptothecin was bound to the polymer product.
Figure S5. $^1$H NMR spectra of P4 in methanol-\textit{d}_4.
Figure S6. $^{19}$F NMR monitoring of the amine substitution of P3 to give P4 in CDCl$_3$ (top) and methanol-$d_4$ (bottom). Aliquots are taken of the reaction mixture prior to amine addition (top) and after addition of BTA-NH$_2$, Fluorescein-NH$_2$ and Jeffamine M-1000 and stirring overnight (bottom).
Figure S7. $^1$H NMR of P5 in methanol-$d_4$. 
**S6. Gel Permeation Chromatography Data**

**Figure S8.** SEC refractive index traces for (A) P1 and (B) P2 at 2 mg mL\(^{-1}\) in THF.

**S7. DLS Data**

**Figure S9.** DLS intensity (top) and autocorrelation (bottom) data for P4 (A,C) and P5 (B,D).
S8. Additional Photophysical Data

Figure S10. Plots of fluorescence emission intensity versus absorbance for the SCPN-P7, fluorescein, FITC, and BSA-FITC. Error bars (smaller than the point size) represent one standard deviation of three replicate measurements. All measurements were made in 1× PBS buffer (see Section S1 for recipe), except the fluorescein reference standard (borate buffer, 50 mM, 100 mM NaCl, pH 9.5).

Figure S11. SCPN unfolding measurements in DMF (50% v/v with PBS) and PBS (0.5×, see section S1 for 1× recipe) at 24–26 °C: (A) absorption spectra; (B) fluorescence emission spectra. The fluorescence excitation spectra and integrated emission intensities can be found in Figure 5 in the main text.
S9. Additional Cell Labeling Data

Figure S12. Fluorescent labeling of SK-3 BR breast cancer cells with SCPN-Biotin.

| Brightfield | Negative Controls |
|-------------|-------------------|
| + SCPN Biotin + NeutrAvidin + Biotin-AntiHER2 | + SCPN Biotin + NeutrAvidin + SCPN Biotin Cells only |

Fluorescence

Figure S12. Fluorescent images. Middle row: fluorescent images. Bottom row: merged brightfield and fluorescence images. The sample is in the leftmost column and negative controls are in the other three columns. The "+" indicates that the cells were incubated with the respective material. Scale bars are 100 µm. This figure represents a lower contrast example of the experiment shown in Figure 6, performed several weeks later using the same stock solution.
Figure S13. Time series for brightfield, fluorescence and merged images of SCPN-P7 injected into A549 cells. Images were taken before injection, 1 min, 3 min, 10 min, and 30 min after the injection. (Images were also taken up to 1 h. These images are not shown but are analogous to the 30 min images.) Scale bars are 50 µm. The images for each time point were acquired with the same microscope settings and processed with the same brightness and contrast settings.
S10. References

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