Supporting Information

Macromolecular Hydrogen Sulfide Donors Trigger Spatiotemporally Confined Changes in Cell Signaling

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Experimental Details

Materials. Oligo(ethylene glycol) methyl ether methacrylate (OEGMA) (M_n = 300 g mol^{-1}) and methyl methacrylate (MMA) were purchased from Sigma-Aldrich and passed through a column of basic alumina in order to remove inhibitor before use. Initiators, α,α′-azobisisobutyronitrile (AIBN) and 1,1′-azobis(cyclohexanecarbonitrile) (ACHN), were purchased from Sigma-Aldrich and purified by recrystallization from methanol and chloroform/methanol respectively before use. The RAFT agent, 4-cyano-2-propyl benzodithioate (CPBDT) was purchased from Sigma-Aldrich at the highest purity available (>97% HPLC) and used as received. Sodium sulfide (Na_2S) anhydrous was purchased from Alfa Aesar (Cat. No. 65122) and hydrochloric acid 32% was purchased from Ajax Finechem. Solvents (except anhydrous solvents) were purchased from Merck Millipore and used as received.

All other chemicals such as reagents and anhydrous solvents for synthesis were purchased from Sigma-Aldrich at the highest purity available and used without further purification (unless otherwise stated). A Reveleris® Flash Chromatography System fitted with GRACE® silica cartridges was used for purification of monomer and intermediates. TLC was performed on Merck Silica 60F_{254} plates.

Characterization

^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained with a Bruker UltraShield 400 MHz spectrometer at 25°C running Bruker Topspin Software. Spectra were recorded for

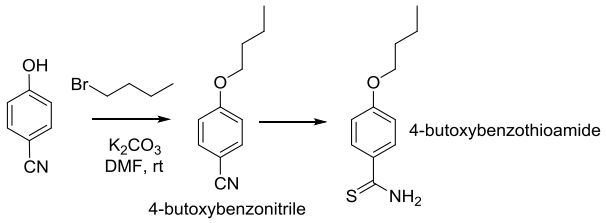
samples dissolved in deuterated solvent (chloroform, $CDCl_3$ or dimethylsulfoxide, DMSO) and chemical shifts are reported as parts per million from external tetramethylsilane. Monomer conversions were obtained from the ^1H NMR spectra.

Gel Permeation Chromatography (GPC) was performed using a Shimadzu modular system comprised of a DGU-12A degasser, an SIL-20AD automatic injector, a 5.0 μm bead-size guard column (50 × 7.8 mm), followed by three KF-805L columns (300 × 8 mm, bead size: 10 μm, pore size maximum: 5000 Å), a SPD-20A ultraviolet detector, and an RID-10A differential refractive-index detector. The temperature of columns was maintained at 40 °C using a CTO-20A oven. The eluent was *N*,*N*-dimethylacetamide (DMAc, HPLC grade, with 0.03% w/v LiBr) and the flow rate was kept at 1 mL/min using a LC-20AD pump. A molecular weight calibration curve was produced using commercial narrow molecular weight distribution polystyrene standards with molecular weights ranging from 500 to 2 x 10^6 g mol^{-1}. Polymer solutions at approx. 2 mg mL^{-1} were prepared and filtered through 0.45 μm filters prior to injection.

Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR) spectra were obtained using a Shimadzu IR Tracer-100 Fourier Transform Infrared spectrometer fitted with a GladiATR 10 single reflection accessory. Data was processed using LabSolutions IR™ software in the mid IR region of 4000-500 cm^{-1} at a resolution of 8 cm^{-1} by averaging 128 scans (collected via a mercury cadmium tellyuride detector).

Dynamic Light Scattering (DLS) measurements were carried out on a Malvern Zetasizer Nano ZS Series running DTS software (laser, 4 mW, λ = 633 nm; angle 173°). The polydispersity index (PDI) was used to describe the average diameters and size distribution of prepared micelles from a Cumulants analysis of the measured intensity autocorrelation function. Samples were filtered using 0.45 μm Nylon syringe filter to remove contaminants / dust prior to measurement.

Synthesis of 4-butoxybenzothioamide

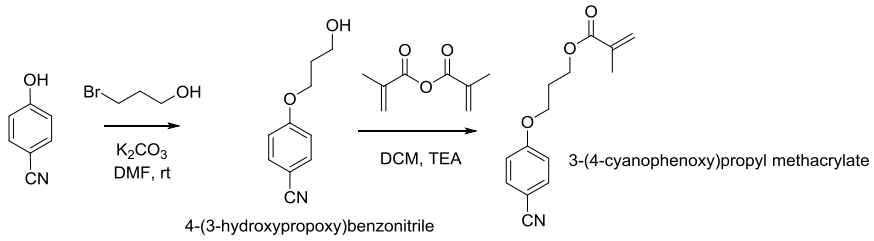


4-Cyanophenol (2.19 g, 0.018 mol) was suspended together with 4-bromobutane (3.01 g, 0.022 mol) and potassium carbonate (3.73 g, 0.027 mol) in dimethylformamide (DMF) (50 mL) and stirred for 30 hours. The product mixture was then diluted with water to dissolve the salts and unreacted phenol and then extracted with ethyl acetate (× 2). The organic layers were combined and then washed with 0.2 M HCl (× 2), water (× 2) and saturated brine (× 1). After drying with $MgSO_4$ (anhydrous), removal of the solvent and drying under a stream of nitrogen, the crude product, 4-butoxybenzonitrile, was isolated as a clear, colourless oil (3.0 g, 95%). This was found to be sufficient purity for the next reaction. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.96 (t, *J* 7.4 Hz, 3H, CH_3), 1.42 – 1.53 (m, 2H, C*H*$_2$CH$_3$), 1.73 – 1.80 (m, 2H, C*H*$_2$CH$_2$CH$_3$), 3.98 (t, *J* 6.5 Hz, 2H, CH_2OAr), 6.90 – 6.93 (m, 2H, 2 × ArH), 7.50 – 7.56 (m, 2H, 2 × ArH) ppm.

The following reaction was adapted from the procedure of Manaka and Sato.[1] To a solution of 4-butoxybenzonitrile (2.5 g, 14.3 mmol) in anhydrous DMF (30 mL) was added magnesium chloride hexahydrate ($MgCl_2.6H_2O$) (2.9 g, 14.4 × 10^{-3} mol) and 70% sodium hydrosulfide hydrate (2.26 g, 28.6 × 10^{-3} mol). The green slurry was left to stir at room temperature for 2 hours. The slurry was then added to water (100 mL) and the resulting yellow precipitate was collected by filtration. The product was then suspended in 1.0 M HCl aqueous and left to sit for 30 minutes. The resulting yellow solid was then filtered and washed several times with water to give 4-butoxybenzothioamide of sufficient purity by ^{1}H NMR (96% pure, 2.90 g, 97 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.98 (t, *J* 7.4 Hz, 3H,

CH_3), 1.45 -1.53 (m, 2H, C*H*$_2$CH$_3$), 1.75 – 1.82 (m, 2H, C*H*$_2$CH$_2$CH$_3$), 4.01 (t, *J* 6.5 Hz, 2H, CH_2OAr), 6.87 – 6.89 (m, 2H, 2 × ArH), 7.09 (br s, 1H, NH), 7.48 (br s, 1H, NH), 7.87 – 7.89 (m, 2H, 2 × ArH) ppm. ^{1}H NMR (400 MHz, DMSO) δ: 0.93 (t, *J* 7.4 Hz, 3H, CH_3), 1.38 -1.48 (m, 2H, C*H*$_2$CH$_3$), 1.67 – 1.74 (m, 2H, C*H*$_2$CH$_2$CH$_3$), 4.02 (t, *J* 6.6 Hz, 2H, CH_2OAr), 6.92 – 6.95 (m, 2H, 2 × ArH), 7.93 – 7.95 (m, 2H, 2 × ArH), 9.30 (s, 1H, NH), 9.62 (s, 1H, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.0, 19.3, 31.2, 68.1, 114.2, 129.4, 131.8, 162.8, 200.7 ppm.

Synthesis of 3-(4-cyanophenoxy) propyl methacrylate (CPPMA) monomer



4-Cyanophenol (2.19 g, 0.018 mol) was suspended together with 3-bromo-1-propanol (3.07 g, 0.022 mol) and potassium carbonate (3.73 g, 0.027 mol) in DMF (50 mL) and stirred for 30 hours. The product mixture was then diluted with water to dissolve the salts and unreacted phenol and extracted with ethyl acetate (× 2). The organic layers were combined and then washed with 0.2 M HCl (× 2), water (× 2) and saturated brine (× 1). After drying with $MgSO_4$ (anhydrous) and removal of the solvent, the crude product was found to contain impurities based on multiple and successive alkylations with bromopropanol. The product was therefore purified using a Reveleris® Flash Chromatography System fitted with a GRACE® silica cartridge, with the gradient solvent system, 2:8 EA/Hexane with 1% MeOH → 1:1 EA/Hexane with 1% MeOH, affording the product, 4-(3-hydroxypropoxy)benzonitrile, as a white waxy solid (1.64 g, 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.63 (br m, 1H, CH_2*OH*), 2.08 (quintet, *J* 6.0 Hz, 2H, CH_2C*H*$_2$$CH_2$), 3.89 (m, 2H, CH_2CH_2C*H*$_2$OH), 4.19 (t,

J 6.0 Hz, 2H, CH_2OAr), 6.96 – 7.00 (m, 2H, 2 × ArH), 7.59 – 7.62 (m, 2H, 2 × ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 31.8, 59.3, 65.5, 103.7, 115.2, 119.3, 134.0, 162.3 ppm.

To a solution of 4-(3-hydroxypropoxy)benzonitrile (1.16 g, 6.55 mmol), triethylamine (TEA) (1.37 mL, 0.99 g, 9.83 mmol) and 4-dimethylaminopyridine (DMAP) (0.100g, 0.82 mmol) in dry DCM (40 mL) was added methacrylic anhydride dropwise (1.52 g, 1.46 mL, 9.83 mmol) under nitrogen. The reaction mixture was left to stir under nitrogen overnight at room temperature. The reaction mixture was then diluted with more DCM and washed successively with 0.2 M HCl (× 2), water (× 2), saturated aqueous sodium bicarbonate (× 2), water (× 2) and saturated brine (× 1). The organic phase was then dried with $MgSO_4$ (anhydrous) and the solvent removed under reduced pressure. The crude product was purified using a Reveleris® Flash Chromatography System fitted with a GRACE® silica cartridge, with the gradient solvent system, 1:9 EA/Hexane→1:1 EA/Hexane, affording the product, 3-(4-cyanophenoxy)propyl methacrylate, as a clear, colourless oil (1.4 g, 90%). ^1H NMR (400 MHz, $CDCl_3$) δ: 1.94 (m, 3H, -CH_3=CH_2), 2.20 (quintet, *J* 6.0 Hz, 2H, $CH_2CH_2CH_2$), 4.11 (t, *J* 6.1 Hz, 2H, $CH_2CH_2CH_2O$-CO-), 4.35 (t, *J* 6.1 Hz, 2H, CH_2OAr), 5.57 – 5.58 (m, 1H, =CH), 6.10 – 6.11 (m, 1H, =CH), 6.92 – 6.96 (m, 2H, 2 × ArH), 7.56 – 7.60 (m, 2H, 2 × ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 18.4, 28.5, 61.3, 65.0, 104.2, 115.3, 119.3, 125.8, 134.1, 136.3, 162.1, 167.4 ppm.

The monomer was found to polymerize into an insoluble crystalline material on standing. After purification and analysis it was therefore stored as a solution in ethyl acetate with 2 ppm of added inhibitor (mono methyl ether of hydroquinone, MEHQ). The solvent was evaporated off and the residual monomer weighed prior to polymerizing.

Synthesis of [POEGMA$_{46}$]-S(C=S)Ph (1)

The polymerization was carried out using the following stoichiometry of $[CPBDT]_0$:$[OEGMA]_0$:$[AIBN]_0$ = 1:80:0.125. OEGMA (2.12 g, 7.08 × 10^{-3} mol), CPBDT

RAFT agent (19.4 mg, 8.76×10^{-5} mol), AIBN (1.80 mg, 1.10×10^{-5} mol) and acetonitrile (ACN) (8 mL) were placed in a round bottom flask equipped with a magnetic stirrer bar and capped with a rubber septum. The reaction mixture was deoxygenated for 30 min at 0 °C by sparging with N_2. The deoxygenated mixture was placed into a pre-heated oil bath at 70 °C and the polymerization left to stir for 6 h. The resulting mixture was then allowed to cool in an ice bath for about 15 min to terminate polymerization and then exposed to air. The monomer conversion was determined by ^{1}H NMR. The resonances integrated to obtain conversions for OEGMA polymerizations were the vinyl peaks at 5.4 and 5.9 ppm (monomer only) and the OCH_2- peaks at 3.8 – 4.1 ppm (monomer and polymer). To remove all traces of unreacted monomer the polymerization solution was firstly transferred to dialysis tubing (Cellu Sep, nominal MWCO 3500 g mol^{-1}) and dialysed against acetone with at least 5 exchanges of solvent. This was followed by multiple precipitation and centrifugation steps, using petroleum ether/diethyl ether 1:1 as the precipitant. Residual solvent was then removed by blowing a gentle stream of nitrogen over the polymer solution overnight, followed by drying with a high vacuum line. The final product, $POEGMA_{46}$-S(C=S)Ph **1**, was analysed by ^{1}H NMR and GPC. Polymerization results: conversion = 60 %; M_n 9,672 g/mol, PDI 1.12 (GPC-DMAc); M_n 14,020 g/mol (^{1}H NMR estimate); M_n 14,620 g/mol (theoretical M_n). The ^{1}H NMR spectrum of **1** with main peak assignments is provided as Figure S1.

Synthesis of [$POEGMA_{46}$]-*block*-[$PCPPMA_{10}$-*co*-$PMMA_{99}$]-S(C=S)Ph (4)

The polymerization was carried out using the stoichiometry of [POEGMA Macro RAFT]$_0$:[Monomers]$_0$:[AIBN]$_0$ = 1:188:0.129. The molar feed ratio of MMA to CPPMA was 9:1 Briefly, $POEGMA_{46}$-S(C=S)Ph (**4**) (0.435 g, 3.10×10^{-5} mol), MMA (0.532 g, 5.31×10^{-3} mol), CPPMA (0.130 g, 5.31×10^{-4}), AIBN (0.64 mg, 3.86×10^{-6} mol) and anhydrous 1,4-dioxane (1.8 mL) were transferred to a glass vial equipped with a magnetic stirrer bar and capped with a rubber septum. The reaction mixture was deoxygenated with nitrogen for 30

min at 0 °C by sparging with N_2. The deoxygenated solution was then placed into a pre-heated oil bath at 70 °C and the block copolymerization was allowed to proceed with stirring for 15 hours. The polymerization was terminated by placing the sample in an ice bath for 15 min. The monomer conversion was determined by ^{1}H NMR. The resonances integrated to obtain conversions for CPPMA were the CH_2 resonance (triplet) at 4.2 ppm from the CPPMA monomer only vs aromatic peaks at 6.8 ppm – 7.0 ppm (monomer and polymer). The MMA vinyl peaks at 5.4 and 5.9 ppm (monomer only) could be ascertained and the average value was compared to a broad resonance at 3.5 ppm corresponding to the methoxy hydrogens of the polymerized MMA. The product polymer was recovered by multiple precipitations into diethyl ether/petroleum ether. The purified block polymer was then dried under high vacuum after evaporating off a majority of the solvent with a stream of nitrogen. The final product [POEGMA$_{46}$]-*block*-[PCPPMA$_{10}$-*co*-PMMA$_{99}$]-S(C=S)Ph **4** was analysed by ^{1}H NMR and GPC. Polymerization results: conversion (CPPMA) = 50 %; conversion (MMA) = 50 %; M_n 19,850 g/mol, PDI 1.14 (GPC-DMAc); M_n 26,400 g/mol (^{1}H NMR estimate); M_n 25,500 g/mol (theoretical M_n). The ^{1}H NMR spectrum of **4** with peak assignments is provided as Figure S2. Overlayed GPC traces showing block extension are displayed in Figure S3.

RAFT end group removal from [POEGMA$_{46}$]-*block*-[PCPPMA$_{10}$-*co*-PMMA$_{99}$]-S(C=S)Ph (4)

A solution of [POEGMA$_{46}$]-*block*-[PCPPMA$_{10}$-*co*-PMMA$_{99}$]-S(C=S)Ph, **4**, (0.430 g, 1.63×10^{-5} mol, 1 equivalent), 1-ethylpiperidine hypophosphite (EPHP) (0.030 g, 1.70×10^{-4}, ~10 equivalents), AIBN (1.0 mg, 6.09×10^{-6}, 0.37 equivalents) and anhydrous toluene (3.0 mL) were added to a Schlenk vessel with a magnetic stirrer bar and capped with a rubber septum. The reaction mixture was deoxygenated with nitrogen for 30 min and then placed into a pre-heated oil bath at 80 °C with stirring for 2 hours. The radical induced reduction process was terminated by placing the sample in an ice bath for 15 min. The product was then recovered by multiple precipitations into diethyl ether/petroleum ether (1:2). After drying under a

stream of nitrogen the conversion for thiocarbonylthio end group removal was determined by ^{1}H NMR to be only 90%. The procedure was therefore repeated, as described above. However, after precipitation and drying the product was found to contain reaction by-products, most likely from EPHP. These were removed by washing the polymer, dissolved in chloroform, with water and brine. Decolourisation of the polymer and ^{1}H NMR analysis showed that the RAFT end groups and reaction by-products had been adequately removed. Specifically, three distinct signals in the ^{1}H NMR spectrum (δ 7.35, 7.50 and 7.90 ppm), representing the aromatic benzodithioate, were found to be removed on radical induced reduction. The final product was also analysed by GPC to monitor possible side reactions such as termination. Results: conversion >95 %; M_n 22,014 g/mol, PDI 1.15 (GPC-DMAc). The ^{1}H NMR spectrum before and after RAFT end group removal is provided as Figure S4.

Thionation of [POEGMA$_a$]-*block*-[PCPPMA$_b$-*co*-PMMA$_c$]-H to give [POEGMA$_a$]-*block*-[PTHA$_b$-*co*-PMMA$_c$]-H (7)

The reaction was adapted from the procedure of Manaka and Sato.[1] [POEGMA$_a$]-*block*-[PCPPMA$_b$-*co*-PMMA$_c$]-H (0.320 g) from the previous step was dissolved in anhydrous DMF (2 mL) followed by the successive addition of magnesium chloride hexahydrate ($MgCl_2.6H_2O$) (120 mg, 5.90×10^{-4} mol) and 70% sodium hydrosulfide hydrate (100 mg, 1.2×10^{-3} mol). The green slurry was left to stir at room temperature for 4 hours. The slurry was then added to 0.2 M aqueous HCl (5 mL) and the resulting yellow gum stirred for several minutes. The crude product was then extracted using chloroform and washed with 0.2 M HCl (× 2), water (× 2), and saturated brine (× 1). The organic phase was then dried with $MgSO_4$ (anhydrous) and the solvent removed under reduced pressure, leaving behind a yellow glassy polymer. The final product [POEGMA$_a$]-*block*-[PTHA$_b$-*co*-PMMA$_c$]-H, **7**, was also analysed by GPC to monitor possible side reactions and termination. Results: M_n 23,300 g/mol, PDI 1.16 (GPC-DMAc). The ^{1}H NMR spectrum before and after thionation is provided as Figure S5.

Synthesis of [POEGMA$_{37}$-*co*-PCPPMA$_5$]-S(C=S)Ph (2b)

The synthesis was carried out using the following stoichiometry: $[CPBDT]_0$:$[Monomers]_0$:$[AIBN]_0$=1:81:0.125. The molar feed ratio of OEGMA to CPPMA was 9:1. Briefly, OEGMA (1.81 g, 6.02×10^{-3} mol), CPPMA (0.164 g, 6.69×10^{-4} mol), CPBDT RAFT agent (18.3 mg, 8.27×10^{-5} mol), AIBN (1.72 mg, 1.05×10^{-5} mol) and acetonitrile (ACN) (9 mL) were placed in a round bottom flask equipped with a magnetic stirrer bar and capped with a rubber septum. The reaction mixture was deoxygenated for 30 min at 0 °C by sparging with N_2. The reaction mixture was placed into a pre-heated oil bath at 70 °C and the polymerization was allowed to proceed with stirring for 6 h. The resulting mixture was then cooled in an ice bath for 15 min to terminate polymerization and then was opened to air. The monomer conversion was determined by ^{1}H NMR. The resonances integrated to obtain conversions for CPPMA were the CH_2 resonances (triplet) at 4.2 ppm, from CPPMA monomer only, vs. aromatic peaks at 6.8 ppm – 7.0 ppm (CPPMA monomer and polymer). By taking the CPPMA polymer and monomer integral values into account, the integral value for polymerized OEGMA (under the broad resonance at 3.9 ppm) could be determined, as well the integral value for OEGMA vinyl protons at 5.4 and 5.9 ppm (monomers only) to allow the determination of OEGMA conversion.

To remove all traces of unreacted monomer the polymerization solution was firstly transferred to dialysis tubing (Cellu Sep, Nominal MWCO 3500) and dialysed against acetone, with at least 5 exchanges of solvent. This was followed by multiple precipitation and centrifugation steps, using petroleum ether/diethyl ether 1:1 as the precipitant. Residual solvent was then removed by blowing a gentle stream of nitrogen over the polymer solution overnight, followed by placing the sample on a high vacuum line. The final product [POEGMA$_{37}$-*co*-PCPPMA$_5$]-S(C=S)Ph **(2b)** was analysed by ^{1}H NMR and GPC. Polymerization results: conversion (CPPMA) = 40 %; conversion (OEGMA) = 36 %; M_n

8,690 g/mol, PDI 1.14 (GPC-DMAc); M_n 12,550 g/mol (^{1}H NMR estimate); M_n 8,880 g/mol (theoretical M_n). The ^{1}H NMR spectrum of **2b** with peak assignments is displayed as Figure S6.

Synthesis of [POEGMA$_{37}$-*co*-PCPPMA$_5$]-*block*-[PMMA$_{96}$]-S(C=S)Ph (3)

Block extension of [POEGMA$_{37}$-*co*-PCPPMA$_5$]-S(C=S)Ph Macro RAFT agent **2b** with MMA was carried out using the following stoichiometry: [POEGMA Macro RAFT]$_0$:[MMA]:[AIBN]=1:175:0.115. Briefly, [POEGMA$_{37}$-*co*-PCPPMA$_5$]-S(C=S)Ph **2b** (0.568 g, 4.53×10^{-5} mol), MMA (0.775 g, 7.75×10^{-3} mol), AIBN (0. 90 mg, 5.50×10^{-6} mol) and anhydrous dioxane (2.0 mL) were transferred to a glass vial equipped with a magnetic stirrer bar and capped with a rubber septum. The reaction mixture was deoxygenated with nitrogen for 30 min. The deoxygenated solution was then placed into a pre-heated oil bath at 70 °C and the block copolymerization was allowed to proceed with stirring for 14 hours. The polymerization was terminated by placing the sample in an ice bath for 15 min. The monomer conversion was determined by ^{1}H NMR of the polymerization mixture, by comparing the integral ratio of the MMA vinyl peaks at 5.4 and 5.9 ppm (monomer only) to the broad resonance at 3.9 ppm (macro RAFT agent only) at t=0 hrs vs t=14 hrs. The product polymer was recovered by multiple precipitations into diethyl ether/petroleum ether. The purified block polymer was then dried under high vacuum after blowing off a majority of the solvent with a stream of nitrogen. The final product [POEGMA$_{37}$-*co*-PCPPMA$_5$]-*block*-[PMMA$_{96}$]-S(C=S)Ph **3** was analysed by ^{1}H NMR and GPC. Polymerization results: conversion (MMA) = 55 %; M_n 16,100 g/mol, PDI 1.11 (GPC-DMAC); M_n 22,160 g/mol (^{1}H NMR estimate); M_n 22,190 g/mol (theoretical M_n). The ^{1}H NMR spectrum of **3** with peak assignments is displayed as Figure S7. Overlayed GPC traces showing block extension are displayed in Figure S8.

RAFT thiocarbonylthio end group removal from [POEGMA$_{37}$-*co*-PCPPMA$_5$]-*block*-[PMMA$_{96}$]-S(C=S)Ph (3)

A solution of [POEGMA)$_{37}$-*co*-PCPPMA$_5$]-*block*-[PMMA$_{96}$]-S(C=S)Ph **3** (0.580 g, 2.6×10^{-5} mol, 1 equivalent), 1-ethylpiperidine hypophosphite (EPHP) (0.050 g, 2.6×10^{-4}, ~10 equivalents), ACHN (2.5 mg, 1.15×10^{-5}, 0.4 equivalents) and anhydrous toluene (1.5 mL) were added to a schlenk vessel with a magnetic stirrer bar and capped with a rubber septum. The reaction mixture was deoxygenated with nitrogen for 30 min and then placed into a pre-heated oil bath at 100 °C with stirring for 2 hours. The radical induced was terminated by placing the sample in an ice bath for 15 min. The product was recovered by multiple precipitations into diethyl ether/petroleum ether. Decolourisation of the polymer and ^{1}H NMR analysis showed the RAFT end groups had been adequately removed however the ^{1}H NMR spectrum also showed reaction by-products, most likely from EPHP. These were removed by washing the polymer, dissolved in chloroform, with water and brine. Three distinct signals in the ^{1}H NMR spectrum, δ 7.4, 7.5 and 7.9 ppm, representing the aromatic benzodithioate protons were found to be removed on radical induced reduction. The final product [POEGMA$_a$-*co*-PCPPMA$_b$]-*b*-[PMMA$_c$]-H was also analysed by GPC to monitor possible termination. Conversion = 100 %; M_n 17,175 g/mol, PDI 1.15 (GPC-DMAc). The ^{1}H NMR spectrum before and after RAFT end group removal is displayed as Figure S9.

Thionation of [POEGMA$_a$]-*block*-[PCPPMA$_b$-*co*-PMMA$_c$]-H to give [POEGMA$_a$]-*block*-[PTHA$_b$-*co*-PMMA$_c$]-H (6)

The reaction was adapted from the procedure of Manaka and Sato.[1] [POEGMA$_a$-*co*-CPPMA$_b$]-*block*-[PMMA$_c$]-H (0.31 g) from the previous step was dissolved in anhydrous DMF (3 mL) followed by the successive addition of magnesium chloride hexahydrate ($MgCl_2.6H_2O$) (120 mg, 5.90×10^{-4} mol) and 70% sodium hydrosulfide hydrate (100 mg, 1.2×10^{-3} mol). The green slurry was left to stir at room temperature for 4 hours. It was then added to 0.2 M HCl aqueous (5 mL) and the resulting yellow gum stirred for several minutes.

The crude product was then extracted out using chloroform and washed with 0.2 M HCl (× 2), water (× 2), and saturated brine (× 1). The organic phase was then dried with $MgSO_4$ (anhydrous) and the solvent removed under reduced pressure, leaving behind a yellow glassy polymer. The final product [$POEGMA_a$-*co*-$PTHA_b$]-*block*-[$PMMA_c$]-H **6** was also analysed by GPC to monitor possible termination. Conversion = >95 %; M_n 20,292 g/mol, PDI 1.16 (GPC-DMAc). The ^{1}H NMR spectrum before and after thionation is displayed as Figure S10.

Synthesis of [$POEGMA_{58}$-*co*-$PCPPMA_7$]-S(C=S)Ph (2a)

The polymerization was carried out using the following stoichiometry: $[CPBDT]_0$:$[Monomers]_0$:$[AIBN]_0$=1:65:0.09. The molar feed ratio of OEGMA to CPPMA was 9:1. Briefly, OEGMA (0.868 g, 2.89×10^{-3} mol), CPPMA (0.080 g, 3.26×10^{-4} mol), CPBDT RAFT agent (11.0 mg, 4.97×10^{-5} mol), AIBN (0.70 mg, 4.27×10^{-6} mol) and DMSO (2 mL) were placed in a glass vial equipped with a magnetic stirrer bar and capped with a rubber septum. The reaction mixture was deoxygenated for 30 min at 0 °C by sparging with N_2. The polymerization mixture was placed into a pre-heated oil bath at 70 °C and the polymerization was run with stirring for 16 h. The resulting mixture was then allowed to cool to 0 °C for about 15 min to terminate polymerization and then opened to air. The monomer conversion was determined by ^{1}H NMR. The resonances integrated to obtain conversions for CPPMA were the CH_2 resonance (triplet) at 4.2 ppm from CPPMA monomer only vs. aromatic peaks at 6.8 ppm – 7.0 ppm (CPPMA monomer and polymer). By taking the CPPMA polymer and monomer integral values into account, the integral value for polymerized OEGMA (under the broad resonance at 3.9 ppm) could be determined, as well the integral value for OEGMA vinyl protons at 5.4 and 5.9 ppm (monomers only). This allowed the determination of OEGMA conversion. To remove all traces of unreacted monomer multiple precipitation and centrifugation steps, were carried out using petroleum ether/diethyl ether 1:1 as the precipitant. Residual solvent was then removed by blowing with

a gentle stream of nitrogen over the polymer solution overnight, followed by drying under a high vacuum line. The final product [POEGMA$_{58}$-*co*-PCPPMA$_7$]-S(C=S)Ph **2a** was analysed by ^{1}H NMR and GPC. Polymerization results: conversion (CPPMA) = 86 %; conversion (OEGMA) = 88 %; M_n 17,290 g/mol, PDI 1.17 (GPC-DMAc); M_n 19,240 g/mol (^{1}H NMR estimate); M_n 17,040 g/mol (theoretical M_n). The ^{1}H NMR spectrum of **2a** with peak assignments is displayed as Figure S11.

RAFT thiocarbonylthio end group removal from [POEGMA$_{58}$-*co*-PCPPMA$_7$]-S(C=S)Ph (2a)

A solution of [POEGMA$_{58}$-*co*-PCPPMA$_7$]-S(C=S)Ph **2b** (0.250 g, 1.32×10^{-5} mol, 1 equivalent), 1-ethylpiperidine hypophosphite (EPHP) (0.025 g, 1.32×10^{-4}, ~10 equivalents), ACHN (1.3 mg, 5.28×10^{-6}, 0.4 equivalents) and anhydrous dioxane (1.5 mL) were added to a schlenk vessel with a magnetic stirrer bar and capped with a rubber septum. The reaction mixture was deoxygenated with nitrogen for 30 min and then placed into a pre-heated oil bath at 100 °C with stirring for 2 hours. The radical induced was terminated by placing the sample in an ice bath for 15 min. The product was recovered by multiple precipitations into diethyl ether/petroleum ether. Decolourisation of the polymer and ^{1}H NMR analysis showed the RAFT end groups had been adequately removed however the ^{1}H NMR. Reaction by-products, most likely from EPHP were removed by washing the polymer dissolved in chloroform with water and brine. Residual solvent was then removed by blowing a gentle stream of nitrogen over the polymer solution overnight followed by placing the sample on a high vacuum line. Three distinct signals in the ^{1}H NMR spectrum, δ 7.4, 7.5 and 7.9 ppm, representing the aromatic benzodithioate were found to be removed on radical induced reduction. The final product [POEGMA)$_a$-*co*-PCPPMA$_b$]-H was also analysed by GPC to monitor termination. Conversion = 100 %; M_n 18,210 g/mol, PDI 1.23 (GPC-DMAc). The ^{1}H NMR spectrum before and after RAFT end group removal is displayed as Figure S12.

Thionation of [POEGMA$_a$-*co*-PCPPMA$_b$]-H to give [POEGMA$_a$-*co*-PTHA)$_b$]-H (5)

The reaction was adapted from the procedure of Manaka and Sato.[1] [$POEGMA_a$-*co*-$CPPMA_b$]-H prepared in the previous step (0.200 g) was dissolved in anhydrous DMF (1.5 mL) followed by the successive addition of magnesium chloride hexahydrate ($MgCl_2.6H_2O$) (60 mg, 2.97×10^{-4} mol) and 70% sodium hydrosulfide hydrate (50 mg, 6.0×10^{-4} mol). The green slurry was left to stir at room temperature for 4 hours. It was then added to 0.2 M HCl aqueous (5 mL) and the resulting yellow solution was stirred for several minutes. The crude product was then extracted out using chloroform and washed with 0.2 M HCl (× 2), water (× 2), and saturated brine (× 1). The organic phase was then dried with $MgSO_4$ (anhydrous) and the solvent removed under reduced pressure, leaving behind a yellow viscous polymer. The final product $[POEGMA)_a$-*co*-$p(THA)_b]$-H **5** was also analysed by GPC to monitor termination. Conversion = 100 %; M_n 19,600 g/mol, PDI 1.25 (GPC-DMAc). The ^{1}H NMR spectrum before and after thionation is displayed as Figure S13.

Overlayed GPC traces of [$POEGMA_{58}$-*co*-$PCPPMA_7$]-S(C=S)Ph **2b**, after RAFT removal and thionation (to give **5**) are displayed as Figure S14.

Synthesis of control polymer [$POEGMA_a$]-*block*-[$PMMA_b$]-H (8) used for cellular work

The polymerization was carried out using the following stoichiometry of $[CPBDT]_0$:$[OEGMA]_0$:$[AIBN]_0$ = 1:80:0.125. Briefly, OEGMA (1.36 g, 4.53×10^{-3} mol), CPBDT RAFT agent (12.6 mg, 5.67×10^{-5} mol), AIBN (1.16 mg, 7.08×10^{-6} mol) and acetonitrile (ACN) (6 mL) were placed in a round bottom flask equipped with a magnetic stirrer bar and capped with a rubber septum. The reaction mixture was deoxygenated for 30 min at 0 °C by sparging with N_2. The deoxygenated mixture was placed into a pre-heated oil bath at 70 °C and the polymerization left to stir stirring for 6 h. The resulting mixture was then allowed to cool with ice for about 15 min to terminate polymerization and then opened to air. The monomer conversion was determined by ^{1}H NMR. The resonances integrated to obtain conversions for OEGMA polymerizations were the vinyl peaks at 5.4 and 5.9 ppm

(monomer only) and the OCH_2- peaks at 3.8 – 4.1 ppm (monomer and polymer). To remove all traces of unreacted monomer the polymerization solution was firstly transferred to dialysis tubing (Cellu Sep, Nominal MWCO 3500) and dialysed against acetone, with at least 5 exchanges of solvent. This was followed by multiple precipitation and centrifugation steps, using petroleum ether/diethyl ether 1:1 as the precipitant. Residual solvent was then removed by blowing a gentle stream of nitrogen over the polymer solution overnight followed by drying with a high vacuum line. The final product, [$POEGMA_{33}$]-S(C=S)Ph, was analysed by ^{1}H NMR and GPC. Polymerization results: conversion = 30 %; M_n 8,800 g/mol, PDI 1.14 (GPC-DMAc); M_n 9,750 g/mol (^{1}H NMR estimate); M_n 7,200 g/mol (theoretical M_n).

Block extension of [$POEGMA_{33}$]-S(C=S)Ph macro RAFT was carried out with MMA and CPPMA using the following stoichiometry: [POEGMA Macro RAFT]$_0$:[Monomers]:[AIBN]=1:134:0.11. Briefly, [$POEGMA_{33}$]-S(C=S)Ph (0.230 g, 2.34×10^{-5} mol), MMA (0.314 g, 3.14×10^{-3} mol), AIBN (0.43 mg, 2.6×10^{-6} mol) and anhydrous 1,4 dioxane (1.0 mL) were transferred to a glass vial equipped with a magnetic stirrer bar and capped with a rubber septum. The reaction mixture was deoxygenated with nitrogen for 30 min. The deoxygenated solution was then placed into a pre-heated oil bath at 70 °C and the block polymerization was allowed to proceed with stirring for 15 hours. The polymerization was terminated by placing the sample in an ice bath for 15 min. The monomer conversion was determined by ^{1}H NMR. The MMA vinyl peaks at 5.4 and 5.9 ppm (monomer only) could be ascertained and the average value was compared to a broad resonance at 3.5 ppm accounting for polymerized MMA. The product polymer was recovered by multiple precipitations into diethyl ether/petroleum ether. The purified block polymer was then dried under high vacuum after blowing off a majority of the solvent with a stream of nitrogen. The final product [$POEGMA_{33}$]-*b*-[$PMMA_{120}$]-S(C=S)Ph was analysed by ^{1}H NMR

and GPC. Polymerization results: conversion (MMA) = 80 %; M_n 18,180 g/mol, PDI 1.17 (GPC-DMAc); M_n 22,100 g/mol (^{1}H NMR estimate); M_n 20,900 g/mol (theoretical M_n).

A solution of [POEGMA$_{33}$]-*b*-[PMMA$_{120}$]-S(C=S)Ph (0.297 g, 1.34×10^{-5} mol, 1 equivalent), 1-ethylpiperidine hypophosphite (EPHP) (0.025 g, 1.32×10^{-4}, ~10 equivalents), ACHN (1.3 mg, 5.28×10^{-6}, 0.4 equivalents) and anhydrous dioxane (1.5 mL) were added to a schlenk vessel with a magnetic stirrer bar and capped with a rubber septum. The reaction mixture was deoxygenated with nitrogen for 30 min and then placed into a pre-heated oil bath at 100 °C with stirring for 2 hours. The radical induced was terminated by placing the sample in an ice bath for 15 min. The product was recovered by multiple precipitations into diethyl ether/petroleum ether. Decolourisation of the polymer and ^{1}H NMR analysis showed the RAFT end groups had been adequately removed however the ^{1}H NMR. Reaction by-products, most likely from EPHP were removed by washing the polymer dissolved in chloroform with water and brine. Residual solvent was then removed by blowing a gentle stream of nitrogen over the polymer solution overnight followed by placing the sample on a high vacuum line. Three distinct signals in the ^{1}H NMR spectrum, δ 7.4, 7.5 and 7.9 ppm, representing the aromatic benzodithioate were found to be removed on radical induced reduction. The final product [POEGMA$_a$]-*co*-[PMMA$_b$]-H was also analysed by GPC to monitor termination. Conversion = 100 %; M_n 18,180 g/mol, PDI 1.19 (GPC-DMAc).

Methods

Micelle formation of thioamide block copolymers 6 and 7

10 mg of block copolymer, [POEGMA$_a$-*co*-PTHA$_b$]-*block*-[PMMA$_c$]-H **6** or [POEGMA$_a$]-*block*-[PTHA$_b$-*co*-PMMA$_c$]-H **7**, was dissolved in 1,4-dioxane (1 mL). Subsequent micelle formation was afforded by injecting 10 mL of PBS (pH 7.4) into each polymer-dioxane solution, under vigorous stirring at room temperature. The rate of addition was 2.0 mL per hour. The micelle solution was filtered through a 0.45 μm Nylon syringe filter to remove

contaminants / dust prior to measurement and then analysed with dynamic light scattering (DLS). The DLS number distributions for each are displayed as Figure S15.

Determination of hydrogen sulfide release using an amperometric sensor

The H_2S-generating capability of polymers was examined using amperometric approach using an H_2S selective micro-sensor manufactured by Unisense. The working concept behind the sensor has been published by P. Jeroschewski et al.[2]

Calibration of the sensor was performed after the sensor signal had stabilized over a pre-polarization period (usually 2 hours or more). A 2.0 mM stock solution of Na_2S was prepared anaerobically by dissolving a known quantity of the salt into N_2-flushed, deionized water in a closed container. The acidic calibration buffer was prepared by adding aqueous HCl to PBS at pH 7.4. A pH value < 4 (normally 3.8) was deemed acceptable for use. This solution was also deoxygenated for at least 10 minutes by bubbling with N_2 gas at a rate that was found to not cool the acidic buffer too significantly. 20 mL of the acidic buffer was transferred to a nitrogen-flushed bottle equipped with a stirrer and the bottle capped with a septum. The sensor was then immersed into the solution, through the septum. This was facilitated via a specialized opening on the septum which enabled the bottle to be capped after the sensor tip had been carefully passed through. Once the signal stabilized to a low, stable reading, this was taken as the zero [H_2S] value. Calibration points within the expected range of measurement were collected by injecting known amounts of Na_2S stock solution using a micro-syringe into the stirred calibration buffer solution. The current increased upon addition of the first aliquot and reached a plateau after several seconds. Further calibration values were obtained as subsequent aliquots were added, six in total, ranging from 10 – 160 µL. The recorded data was used to generate a linear calibration curve for [H_2S] vs. current (amps). Hydrolytic or L-cysteine-mediated H_2S release from the polymers was measured using the calibrated sensor. Firstly, stock solutions were prepared containing (a) 10 mg of polymer in

1,4-dioxane (1 mL) and (b) L-cysteine (400 mM) in PBS at pH 7.4. Subsequent micelle formation was then afforded by manually injecting the polymer-dioxane solution dropwise through a septa into 10 mL of vigorously stirring deoxygenated PBS at pH 7.4 and room temperature (22°C). [The buffer solution was deoxygenated by bubbling with N_2 gas]. The rate of addition of polymer solution was maintained to ensure complete addition over 5 minutes. This more rapid procedure for micelle formation was chosen to minimise H_2S generation during the micelle preparation stage. After addition of the polymer, the sensor was then immersed into the micelle solution. As with the calibration, a septum was used for the recording experiments to minimise exposure to air on immersing the sensor tip into the solution. For those experiments examining L-cysteine mediated release of H_2S, after approximately 30 seconds of recording the hydrolytic release, 100 µL of L-cysteine solution was injected into the stirring solution using a micro-syringe. The times at which the tip was immersed, the experiment was started and the L-cysteine was added, were noted.

Determination of H_2S release via Methylene Blue Formation

The specific reagents required for the methylene blue assay were zinc acetate (1% w/v in deoxygenated water), *N*-*N*-dimethyl-1,4-phenylenediamine sulfate (20 mM) dissolved in 7.2 M HCl and ferric chloride (30 mM) dissolved in 1.2 M HCl. The acidic buffers were prepared from 32% HCl using deoxygenated (N_2-flushed) water. The reagent stock solutions were kept in the dark under nitrogen.

A 2.0 mM stock solution of Na_2S was prepared anaerobically by dissolving a known quantity of the salt into N_2-flushed, deionized water, in a closed container.

To prepare the calibration curve, six aliquots of between 6.5 and 100 µL of Na_2S solution were injected into 4 mL screw-cap vials each containing a stirrer bar, deoxygenated H_2O (1.0 mL), zinc acetate (1% w/v), *N*-*N*-dimethyl-1,4-phenylenediamine sulfate (200 µL, 20 mM) in 7.2 M HCl and ferric chloride (200 µL, 30 mM) in 1.2 M HCl. The mixtures were stirred in

the dark for 20 minutes to allow colour to develop. To generate a linear calibration curve for absorbance vs [H_2S], the absorbance at 670 nm for each solution was measured with a UV-Vis spectrophotometer using a quartz cuvette with a path length of 1 cm, against a blank sample containing all components except the Na_2S aliquot.

H_2S release from the macromolecular donors was measured using the methylene blue method and calibration curve. Stock solutions of L-cysteine (400mM) were prepared as they were for the amperometric testing, and the polymeric micelles were prepared by the same methodology. At certain time points after addition of the cysteine aliquot (7, 12, 22, 38 and 80 minutes), the stopper was removed and 1 mL of micellar solution was sampled using a pipette. This aliquot was added to a 4 mL screw-cap vial containing a stirrer bar, zinc acetate (1% w/v), *N*-*N*-dimethyl-1,4-phenylenediamine sulfate (200µL, 20 mM) in 7.2 M HCl and ferric chloride (200 µL, 30 mM) in 1.2 M HCl. Once the 1 mL sample had been removed from the micellar solution, the septum was quickly re-inserted so as to minimise loss of H_2S and oxidation of the contents. In order to develop the colour, the mixtures were quickly capped and stirred in the dark for 20 minutes. The absorbance at 670 nm for each sampled solution was measured against a blank sample (made up with 1mL of water instead of the sampled micelle solution).

Considerations for H_2S kinetics

H_2S is a weak acid and when it is dissolved in water ionization equilibriums are established with its two anions, hydrosulfide HS^- and sulfide S^{-2}, according to equations (1) and (2):

$$H_2S + H_2O \underset{}{\overset{K_1}{\rightleftharpoons}} HS^- + H_3O^+ \quad (1)$$

$$HS^- + H_2O \underset{}{\overset{K_2}{\rightleftharpoons}} S^{-2} + H_3O^+ \quad (2)$$

The proportions of molecular H_2S, HS^- and S^{-2} which are established in water are therefore determined by the equilibrium constants (K_1 and K_2) for the first and second ionizations of the sulfide species according to (3) and (4):

$$K_1 = \frac{[HS^-][H^+]}{[H_2S]} \qquad (3) \qquad\qquad K_2 = \frac{[S^{-2}][H^+]}{[HS^-]} \qquad (4)$$

The total sulfides generated $[\text{Sulfides}]_{tot}$ represents all sulfides generated from a donor according to (5):

$$[\text{Sulfides}]_{tot} = [H_2S] + [HS^-] + [S^{-2}] \quad (5)$$

A high pK_2 (~14 at 25°C)[3] and low K_2 value (~10^{-14}) for equilibrium (2) results in a negligible percentage of $[S^{-2}]$ existing in the relevant pH range for most studies (i.e., when pH < 9 and $[H^+] > 10^{-9}$). Therefore, the second equilibrium can be neglected in our H_2S calculations.[4]

The total concentration of dissolved sulfides in solution is thus simplified to (6):

$$[\text{Sulfides}]_{tot} = [H_2S] + [HS^-] \qquad (6)$$

And therefore from equilibrium (1):

$$[\text{Sulfides}]_{tot} = [H_2S] + \frac{K_1\,[H_2S]}{[H^+]} \qquad (7)$$

$$[H_2S] = [\text{Sulfides}]_{tot} / \left(1 + \frac{K_1}{[H^+]} \right) \qquad (8)$$

$$\frac{[H_2S]}{[\text{Sulfides}]_{tot}} = \frac{[H^+]}{K_1 + [H^+]} \qquad (9)$$

Since $K_1 = 10^{-pK_1}$ and $[H^+] = 10^{-pH}$

$$\frac{[H_2S]}{[\text{Sulfides}]_{tot}} = 1 / \left(10^{\,pH - pK_1} + 1\right) \qquad (10)$$

$$[\text{Sulfides}]_{tot} = [H_2S] \times \left(10^{\,pH - pK_1} + 1\right) \qquad (11)$$

All H_2S measurements in the amperometric study were conducted at 22°C. Given that 25°C is the standard temperature most often associated with literature regarding the pK_1 of aqueous H_2S and that this value can vary as a function of temperature, we used the following equation (12) from literature[3] (where T is in Kelvin) to derive a more accurate pK_1 value of 7.02 for aqueous H_2S.

$$pK_1 = 32.55 + (1519.44/T) - 15.672\log T + 0.02722T \quad (12)$$

From equation (10) one can estimate that at a pH < 4, $[H_2S] / [\text{Sulfides}]_{tot} \approx 1$ and that $[\text{Sulfides}]_{tot} \approx [H_2S]$ and therefore that equilibrium (1) lies virtually all the way to the left, favouring H_2S over HS^-. This would be the case under conditions of methylene blue test, in which pH < 4, with dye formation representative of $[\text{Sulfides}]_{tot} \approx [H_2S]$. However the amperometric sensor only detects partial pressure of H_2S and since the test is carried out in situ, at a pH of 7.4, this only accounts for one component of the total sulfides generated, $[\text{Sulfides}]_{tot}$. At pH 7.4, the $[\text{Sulfides}]_{tot}$ generated = $[H_2S]_{measured} / 0.296$ (or = $[H_2S]_{measured} \times 3.40$). Throughout our amperometric studies we therefore calculate a value of $[\text{Sulfides}]_{tot}$ for each polymer based on equation (11) to represent the true H_2S-generating capability of the polymers.

Determination of hydrogen sulfide release using an amperometric sensor

Calibration of the sensor was performed after the sensor signal had stabilized over a pre-polarization period (usually 2 hours or more). A 2.0 mM stock solution of Na_2S was prepared anaerobically by dissolving a known quantity of the salt into N_2-flushed, deionized water in a closed container. The acidic calibration buffer was prepared by adding aqueous HCl to PBS at pH 7.4. A pH value < 4 (normally 3.8) was deemed acceptable for use. This solution was also deoxygenated for at least 10 minutes by bubbling with N_2 gas at a rate that was found to not cool the acidic buffer too significantly. 20 mL of the acidic buffer was transferred to a nitrogen-flushed bottle equipped with a stirrer and the bottle was capped with a septum. The

sensor was then immersed into the solution, through the septum. This was facilitated via a specialized opening on the septum which enabled the bottle to be capped after the sensor tip had been carefully passed through. Once the signal stabilized to a low, stable reading, this was taken as the zero [H_2S] value. Calibration points within the expected range of measurement were collected by injecting known amounts of Na_2S stock solution using a micro-syringe into the stirred calibration buffer solution. The current increased upon addition of the first aliquot and reached a plateau after several seconds. Further calibration values were obtained as subsequent aliquots were added, six in total, ranging from 10 – 160 μL. The recorded data was used to generate a linear calibration plot for [H_2S] vs. current (amps).

Hydrolytic or L-cysteine-mediated H_2S release from the polymers was measured using the calibrated sensor. Firstly, stock solutions were prepared containing (a) 10 mg of polymer in 1,4-dioxane (1 mL) and (b) L-cysteine (400 mM) in deoxygenated PBS at pH 7.4. [The buffer solution was deoxygenated by bubbling with N_2 gas]. Subsequent micelle formation was then afforded by manually injecting the polymer-dioxane solution dropwise through a septa into 10 mL of vigorously stirring deoxygenated PBS at pH 7.4 and room temperature (22°C). The rate of addition of polymer solution was maintained to ensure complete addition over 5 minutes. This more rapid procedure for micelle formation was chosen to minimize H_2S generation during the micelle preparation stage. After addition of the polymer, the sensor was immersed into the micelle solution. As with the calibration, a septum was used for the experiments to minimize exposure to air on immersing the sensor tip into the solution. For those experiments examining L-cysteine mediated release of H_2S, after approximately 30 seconds of recording the hydrolytic release, 100 μL of L-cysteine solution was injected into the stirring solution using a micro-syringe. The times at which the tip was immersed, the experiment was started and the L-cysteine was added were noted.

Determination of H_2S release via methylene blue formation

The specific reagents required for the methylene blue assay were zinc acetate (1% w/v in deoxygenated water), *N*-*N*-dimethyl-1,4-phenylenediamine sulfate (20 mM) dissolved in 7.2 M HCl and ferric chloride (30 mM) dissolved in 1.2 M HCl. The acidic solutions were prepared from 32% HCl using deoxygenated (N_2-flushed) water. The reagent stock solutions were kept in the dark under nitrogen.

A 2.0 mM stock solution of Na_2S was prepared anaerobically by dissolving a known quantity of the salt into N_2-flushed, deionized water, in a closed container.

To prepare the calibration curve, six aliquots of between 6.5 and 100 µL of Na_2S solution were injected into 4 mL screw-cap vials each containing a stirrer bar, deoxygenated H_2O (1.0 mL), zinc acetate (1% w/v), *N*-*N*-dimethyl-1,4-phenylenediamine sulfate (200 µL, 20 mM) in 7.2 M HCl and ferric chloride (200 µL, 30 mM) in 1.2 M HCl. The mixtures were stirred in the dark for 20 minutes to allow colour to develop. To generate a linear calibration curve for absorbance vs [H_2S], the absorbance at 670 nm for each solution was measured with a UV-Vis spectrophotometer using a quartz cuvette with a path length of 1 cm, against a blank sample containing all components except the Na_2S aliquot.

H_2S release from the macromolecular donors was measured using the methylene blue method and calibration curve. Stock solutions of L-cysteine (400mM) were prepared as they were for the amperometric testing, and the polymeric micelles were prepared by the same methodology. At certain time points after addition of the cysteine aliquot (7, 12, 22, 38 and 80 minutes), the stopper was removed and 1 mL of micellar solution was sampled using a pipette. This aliquot was added to a 4 mL screw-cap vial containing a stirrer bar, zinc acetate (1% w/v), *N*-*N*-dimethyl-1,4-phenylenediamine sulfate (200µL, 20 mM) in 7.2 M HCl and ferric chloride (200 µL, 30 mM) in 1.2 M HCl. Once the 1 mL sample had been removed from the micellar solution, the septum was quickly re-inserted so as to minimize loss of H_2S

and oxidation of the contents. In order to develop the color, the mixtures were quickly capped and stirred in the dark for 20 minutes. The absorbance at 670 nm for each sampled solution was measured against a blank sample (made up with 1mL of water instead of the sampled micelle solution).

Preparation of Macromolecular Solutions for Cell Studies

Micelle solutions of polymers (macromolecular control **8** and macromolecular H_2S-donor **6**) were prepared ~1 hour prior to cell experiments, by dropwise addition of the polymer dissolved in THF (10 mg/mL THF) into nitrogen-sparged PBS 7.4 (10mg of polymer solution/10mL PBS). The THF was then removed using a centrifugal filter unit (Amicon Ultra-4, Ultracell – 3K, regenerated cellulose 3,000 NMWL). For this, an aliquot of micelle solution (500 μL) underwent three spin cycles of 20 minutes at 5000-6000 rpm. The solution was reconstituted to the original volume with nitrogen-sparged PBS 7.4 after each spin cycle. The final concentration of micelle solution was 1 mg/mL.

For the high-content ratiometric FRET imaging experiments and high-content SF4 time course studies, the micelle solutions were diluted 1:10 in PBS prior to addition to the cells (final concentration 0.1 mg/mL).

For the intracellular H_2S release imaging studies (using SF4 probe) the micelle solutions were diluted 1:1 in OptiMEM prior to addition to the cells (final concentration 0.5mg/mL).

Cell culture for high-content ratiometric FRET and SF4 time-course study

HEK293 cells were grown in DMEM supplemented with 5% v/v FBS, and all assay plates were coated with poly-D-lysine (5 $\mu g/cm^2$) prior to use.

High-content ratiometric FRET imaging

HEK293 cells were seeded in black, optically clear 96-well plates and grown to 70% confluency prior to transfection with 90 ng/well FRET biosensor using linear polyethyleneimine (PEI).[5] NucEKAR Cerulean-Venus (Addgene plasmid 18681) and

cytoEKAR Cerulean-Venus (Addgene plasmid 18679) were gifts from K Svoboda.[6] CytoCKAR (Addgene plasmid 14870) and pmCKAR (MyrPalm-CKAR, Addgene plasmid 14862) were gifts from A Newton.[7,8]

At 48 hours following transfection, fluorescence imaging was performed using a high-content GE Healthcare INCell 2000 Analyzer with a Nikon Plan Fluor ELWD 40x (NA 0.6) objective and FRET module as described previously.[9] For CFP/YFP (cytoCKAR, pmCKAR) or Cerulean/Venus (nucEKAR, cytoEKAR) emission ratio analysis, cells were sequentially excited using a CFP filter (430/24) with emission measured using YFP (535/30) and CFP (470/24) filters, and a polychroic optimized for the CFP/YFP filter pair (Quad3). HEK293 cells were imaged every 90 sec (image capture of 20 wells per 90 sec). Baseline signaling was measured in cells over 6 min, followed by the addition of polymer (either macromolecular control **8** or macromolecular H_2S-donor **6**) or PBS, and the response was measured over 1 hr. For all wells, a positive control for signaling (used to generate a maximal increase in the signaling pathway: 200 nM phorbol 12,13-dibutyrate (PDBu) for ERK or 200 nM PDBu with phosphatase inhibitor cocktail for PKC) was measured to allow only cells that responded to the positive control to be selected for analysis. Data were analyzed using in-house scripts written for the FIJI distribution of ImageJ[10], as described previously.[9] Data are expressed as the mean ± S.E.M from 170-292 cells.

Preparation of Solutions of SF4

The solutions of SF4 were prepared fresh in DMF/DMSO (5 mM) and diluted to give a 5 µM solution in Hank's Balanced Salt Solution (HBSS) for the high-content SF4 time course study or in OptiMEM for the intracellular H_2S release imaging studies.

High-content SF4 time-course study

HEK293 cells were seeded in triplicate in black, optically clear 96-well plates and grown to 90% confluency. Cells were washed once with HBSS and nuclei were stained using Hoechst

nuclear stain 33342 for 10 min at 37°C. Fluorescence imaging was performed using a high-content PerkinElmer Operetta with an Olympus LUCPlanFLN 20x (NA 0.45) objective. Nuclei were visualized using the Hoechst 33342 filter set (excitation 360-400, emission 410-480), and SF4 fluorescence was visualized using the EGFP filter set (excitation 460-490, emission 500-550). Images were taken every 2 min.

Cells were washed with HBSS, then loaded with 5 μM SF4 probe (in HBSS) for 10 min at 37°C. Baseline fluorescence was determined for 8 min followed by the addition of the macromolecular H_2S donor **6** (or macromolecular control **8**), then images were taken every 2 min for 1 hr at 37°C. Data were automatically analyzed by determining the mean SF4 fluorescence per well using Harmony High Content Imaging and Analysis software (v3.5.2). SF4 fluorescence was PBS vehicle-subtracted and expressed relative to the baseline fluorescence for each experimental condition. Data are expressed as the mean ± S.E.M from three independent experiments.

Cell culture for intracellular imaging of H_2S release using SF4 probe

H460 lung carcinoma cells were maintained in T75 flasks in exponential growth as a monolayer in DMEM (Gibco by Life Technologies) supplemented with 10% fetal bovine serum and 1% L-glutamine, and incubated at 37 ºC in 5% CO_2. Cells were plated into poly-D-lysine-coated μ-dishes (ibidi, 35 mm) at 20,000 cells per dish and allowed to attach for three days.

Intracellular imaging of H_2S release using SF4 probe

On the day of imaging, the culture medium was removed and cells were washed with PBS once. Cells were treated with freshly prepared micelle solutions (macromolecular control **8** or macromolecular H_2S donor **6**) diluted 1:1 with OptiMEM for 60 minutes, followed by 1 μM Hoechst nuclear stain 33342 for 10 min prior to imaging. The micelle solution was removed and the cells were washed with PBS once. The cells were then incubated with 5 μM SF4 (H_2S

probe) and images were collected at 5 min intervals using a Nikon Eclipse Ti-E microscope. The cells were excited using a GFP filter cube for SF4 fluorescence and a DAPI cube for H33342 nuclear stain.

Characterization

Table S1 Copolymers prepared incorporating CPPMA.[a]

Macro RAFT Agent	Time (h)	$[M]_0$ / $[RAFT]_0$	Feed[b]		Final[c]		% Conversion[d]		M_n ×10^3 (GPC)[e] (g/mol)	M_n ×10^3 (NMR)[f] (g/mol)	M_n ×10^3 (theo.)[g] (g/mol)	$Đ$[h]
			OEGMA	CPPMA	OEGMA	CPPMA	OEGMA	CPPMA				
1	6	80	100	0	100	-	60	-	9.7	14.0	14.6	1.12
2a	16	65	90	10	90	10	88	86	17.3	19.2	17.0	1.17
2b	6	81	90	10	89	11	36	40	8.7	12.2	8.9	1.14
block copolymer			**MMA**	**CPPMA**	**MMA**	**CPPMA**	**MMA**	**CPPMA**				
3	14	139	100	-	100	-	-	55	16.1	17.1	16.5	1.11
4	15	188	91	9	91	9	50	50	19.8	26.5	25.4	1.14

[a] Polymerizations performed at 70 °C; $[\text{Total Monomers}]_0 = [M]_0$; for block copolymers **3** and **4,** [RAFT] = [MacroRAFT]. [b] mol % of monomers in the feed. [c] % composition of purified polymer as calculated using ^{1}H NMR spectroscopy. [d] % monomer conversion determined using ^{1}H NMR spectroscopy. [e] determined by GPC analysis in DMAc using polystyrene standards. [f] determined by ^{1}H NMR spectroscopy via integration of aromatic RAFT end groups versus polymer resonances. [g] theoretical number-average molecular weight: M_n (theo.) = (([Monomer 1]$_0$/[RAFT]$_0$) × conversion × (MW of Monomer 1)) + (([Monomer 2]$_0$/[RAFT]$_0$) × conversion × (MW of Monomer 2)) + (MW of RAFT Agent). [h] dispersity index, $Đ$, as determined by GPC analysis in DMAc.

Table S2 Characterization of copolymers incorporating thioamide (THA) moeities and precursor polymers.

	Polymer	M_n ×10^3 (GPC)[b] (g/mol)	$Đ$[c]	Composition (%)[a]		
				OEGMA (a)	CPPMA/THA (b)	
RAFT Polymer	[POEGMA$_{58}$-*co*-PCPPMA$_7$]-S(C=S)Ph (**2a)**	17.3	1.17	90	10	
RAFT removed	[POEGMA$_a$-*co*-PCPPMA$_b$]-H	18.2	1.23	90	10	
Thionated	[POEGMA$_a$-*co*-PTHA$_b$]-H (**5)**	19.6	1.25	90	10	
				OEGMA (a)	**CPPMA/THA (b)**	**MMA (c)**
RAFT Block	[POEGMA$_{37}$-*co*-PCPPMA$_5$]-*b*-[PMMA$_{96}$]-S(C=S)Ph **(3)**	16.1	1.11	28	4	69
RAFT removed	[POEGMA$_a$-*co*-PCPPMA$_b$]-*b*-[PMMA$_c$]-H	17.2	1.15	26	4	70
Thionated	[POEGMA$_a$-*co*-PTHA$_b$]-*b*-[PMMA$_c$]-H **(6)**	20.3	1.16	33	4	64
RAFT Block	[POEGMA$_{46}$]-*b*-[PCPPMA$_{10}$-*co*-PMMA$_{99}$]-S(C=S)Ph **(4)**	19.8	1.14	29	6	65
RAFT removed	[POEGMAa]-*b*-[PCPPMA$_b$-co-PMMA$_c$]-H	22.0	1.15	25	6	69
Thionated	[POEGMA$_a$]-*b*-[PTHA$_b$-*co*-PMMA$_c$]-H **(7)**	23.3	1.16	27	7	66

[a] After RAFT end group removal specific number of monomer units in the block copolymer (originally estimated using ^{1}H NMR spectroscopy via integration of aromatic RAFT end groups versus polymer resonances) could not be determined anymore, therefore, a, b and c refer to % molar composition as calculated using ^{1}H NMR spectroscopy where a + b + c = 100. [b] determined by GPC analysis in DMAc using polystyrene standards. [c] Dispersity index, $Đ$, as determined by GPC analysis in DMAc.

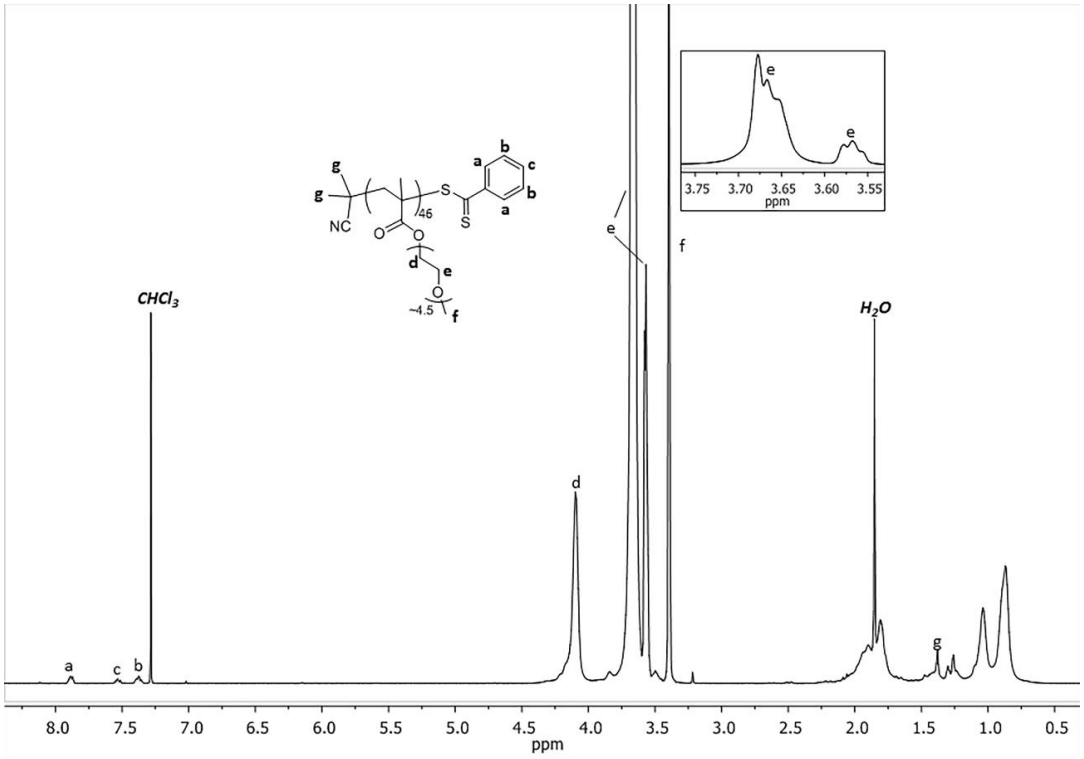


Figure S1: ^{1}H NMR spectrum of [POEGMA$_{46}$]-S(C=S)Ph **1** (400 MHz, $CDCl_3$) with main peak assignments. Box shows expanded region 3.55 - 3.75 ppm.

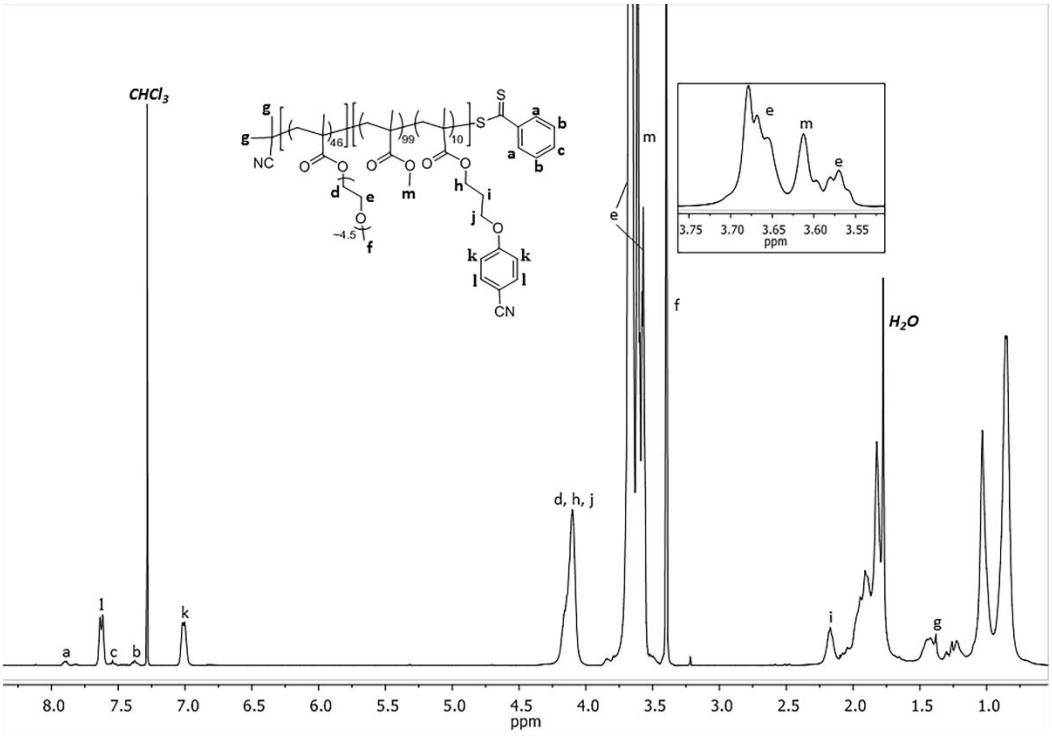


Figure S2: ^{1}H NMR spectrum of [POEGMA$_{46}$]-*block*-[PCPPMA$_{10}$-*co*-PMMA$_{99}$]-S(C=S)Ph **4** (400 MHz, $CDCl_3$) with main peak assignments. The inset figure (box) is the expanded region, 3.55 - 3.75 ppm, showing the –OCH_3 protons of PMMA segment (h) and –CH_2–O– ethylene glycol protons from POEGMA segment (e).

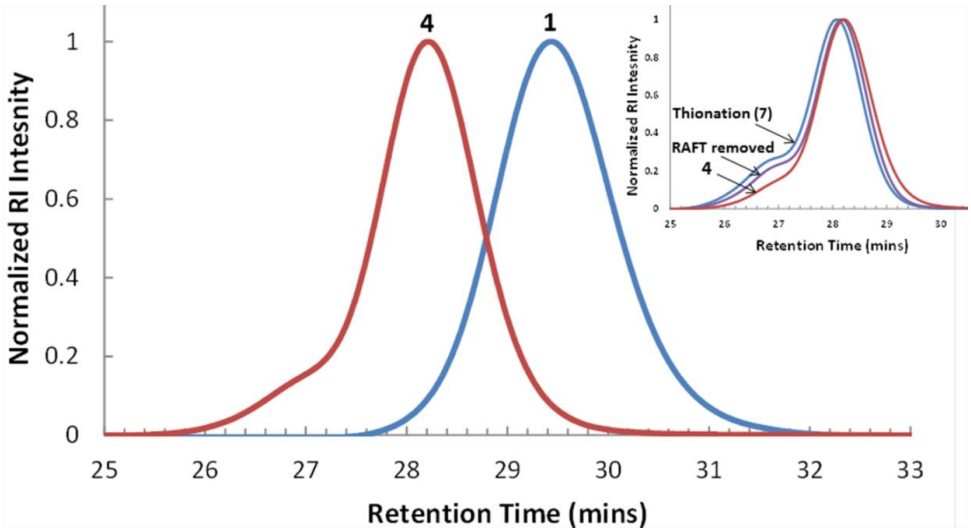


Figure S3. GPC (DMAC) chromatograms showing block extension of [POEGMA$_{46}$]-S(C=S)Ph **1** to give [POEGMA$_{46}$]-*block*-[PCPPMA$_{10}$-*co*-PMMA$_{99}$]-S(C=S)Ph **4**. The inset figure shows the GPC traces after the successive steps of RAFT removal and thionation to give **7**.

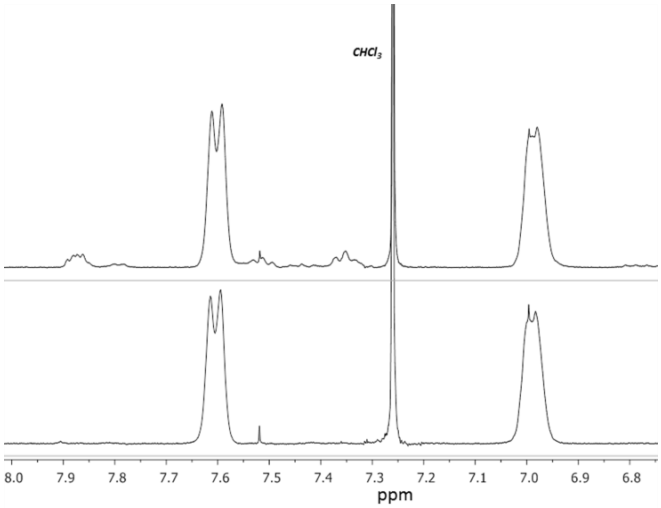


Figure S4: ^{1}H NMR spectra (400 MHz, $CDCl_3$) showing the aromatic benzodithioate signals of polymer **4** before (top) and after radical induced reduction process (bottom).

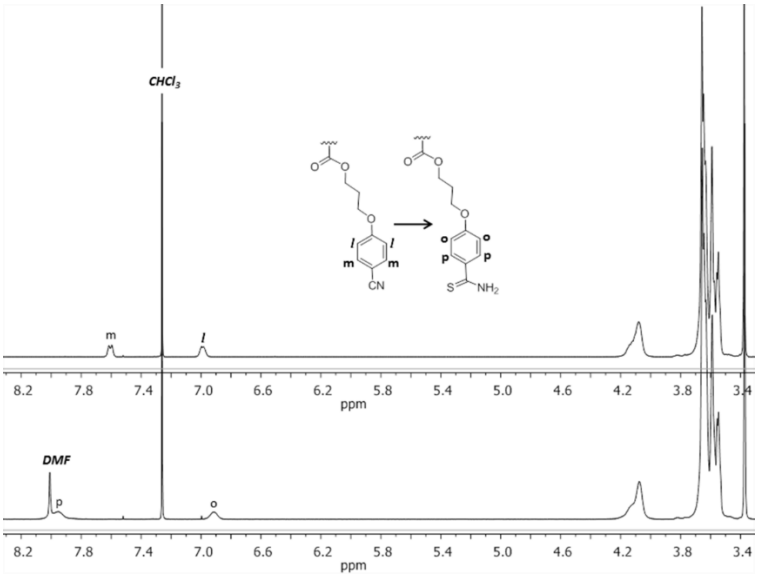


Figure S5: ^{1}H NMR spectra (400 MHz, $CDCl_3$) showing the aromatic signals of polymer before (top) and after thionation process to give **7,** (bottom).

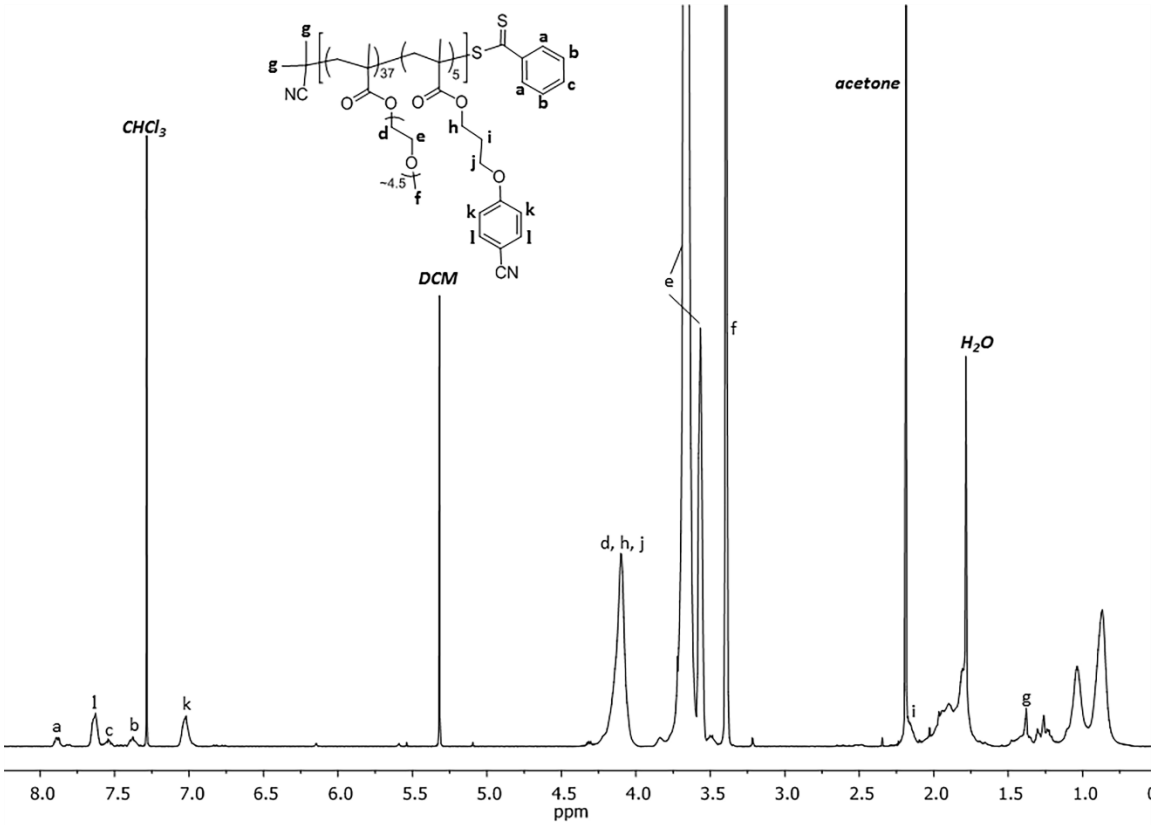


Figure S6: ^{1}H NMR spectrum of [$POEGMA_{37}$-*co*-$PCPPMA_5$]-S(C=S)Ph **2b** (400 MHz, $CDCl_3$) showing main peak assignments.

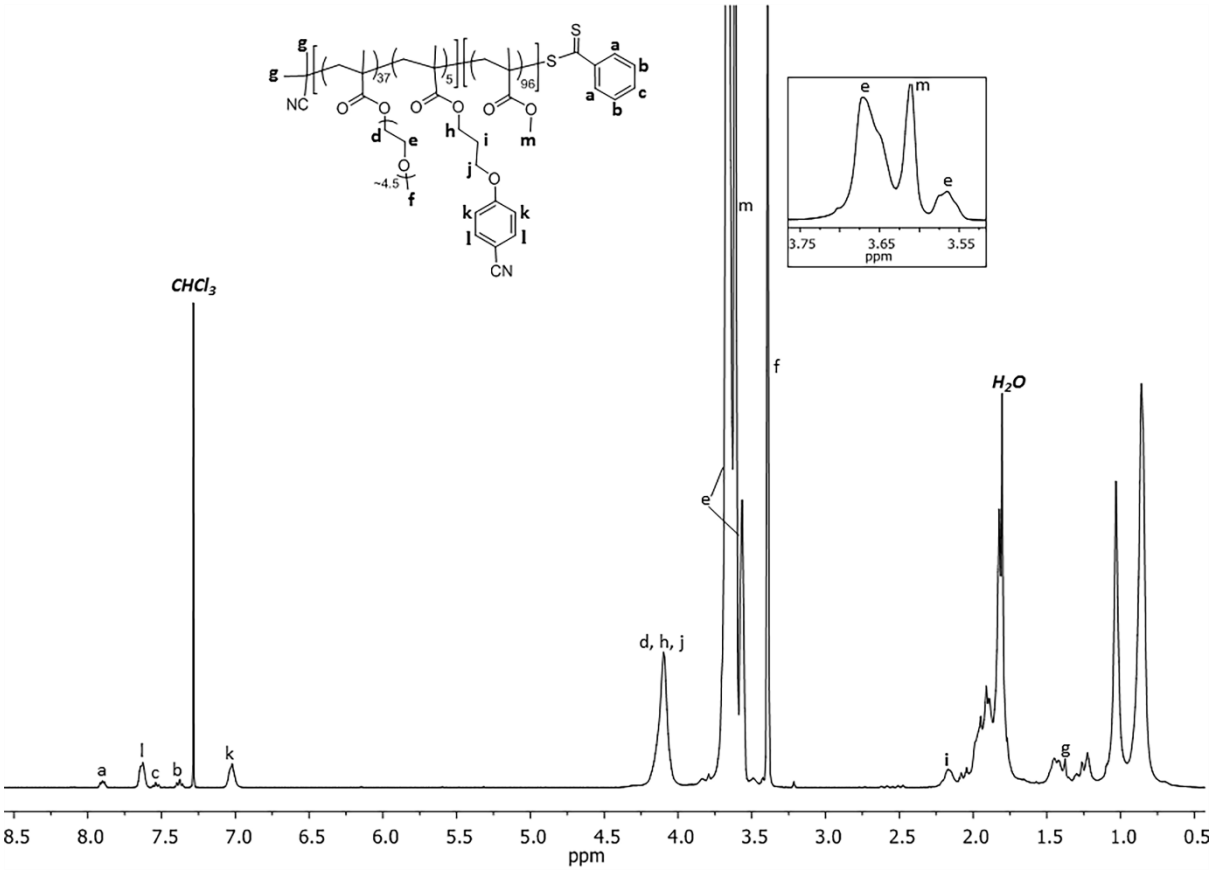


Figure S7: ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of [$POEGMA_{37}$-*co*-$PCPPMA_5$]-*block*-[$PMMA_{81}$]-S(C=S)Ph, **3,** with main peak assignments. The inset figure (box) is the expanded region, 3.50 - 3.75 ppm, showing the –OCH_3 protons of PMMA segment (m) and –CH_2–O– ethylene glycol protons (e) from POEGMA segment.

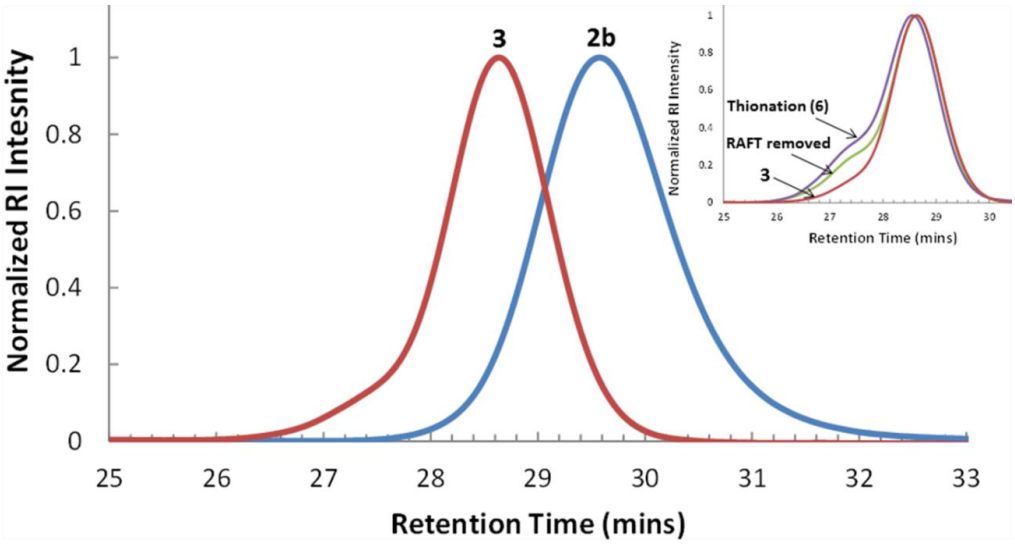


Figure S8. GPC chromatograms (DMAc) showing block extension of [$POEGMA_{37}$-*co*-$PCPPMA_5$]-S(C=S)Ph **2b** to give [$POEGMA_{37}$-*co*-$PCPPMA_5$]-*block*-[$PMMA_{96}$]-S(C=S)Ph **3**. The inset figure shows the GPC traces after successive steps of RAFT removal and thionation, to give **6**.

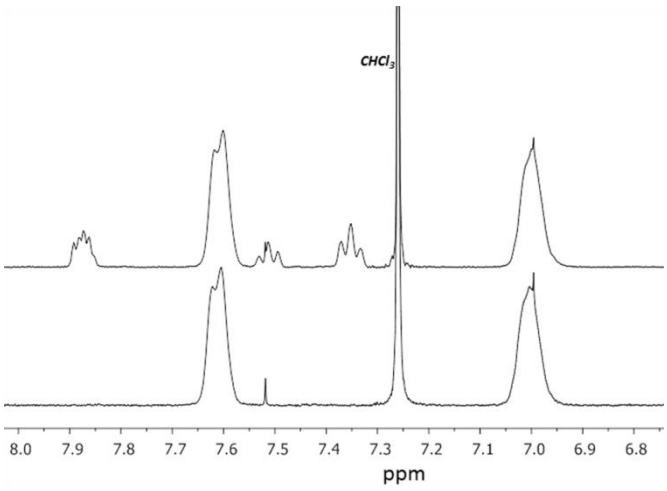


Figure S9: ^{1}H NMR spectra (400 MHz, $CDCl_3$) showing the aromatic benzodithioate signals of polymer **3** before (top) and after radical induced reduction process (bottom).

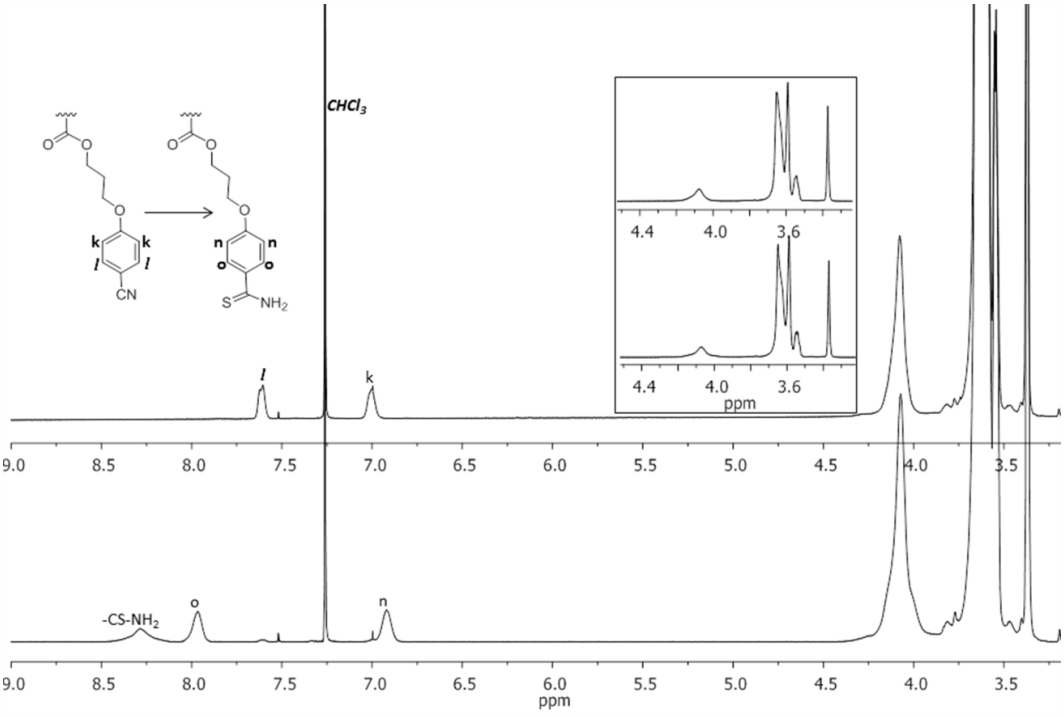


Figure S10: ^{1}H NMR spectra (400 MHz, $CDCl_3$) showing the aromatic signals of polymer before (top) and after (**6,** bottom) thionation process. The inset figure (box) is the expanded region, 3.40 – 4.40 ppm, showing little change in OEGMA:MMA ratio before and after thionation.

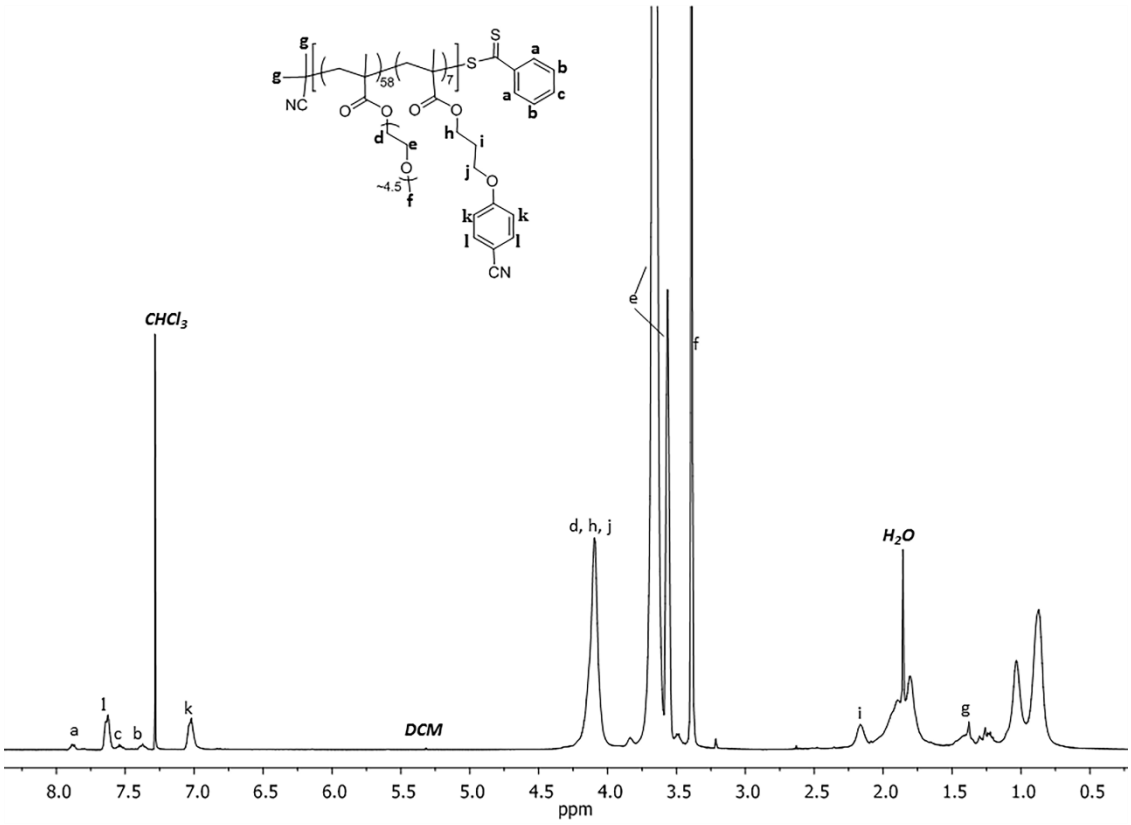


Figure S11: ^{1}H NMR spectrum of [POEGMA$_{58}$-*co*-PCPPMA$_{7}$]-S(C=S)Ph **2a** (400 MHz, $CDCl_3$) showing main peak assignments.

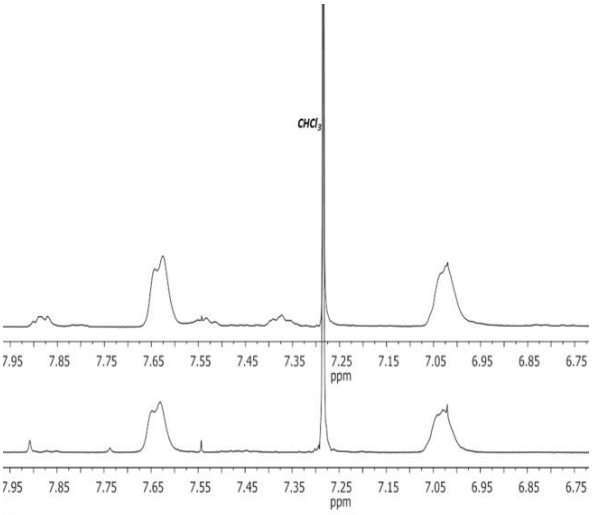


Figure S12: ^{1}H NMR spectra (400 MHz, $CDCl_3$) showing the aromatic benzodithioate signals of polymer **2a** before (top) and after radical induced reduction process (bottom).

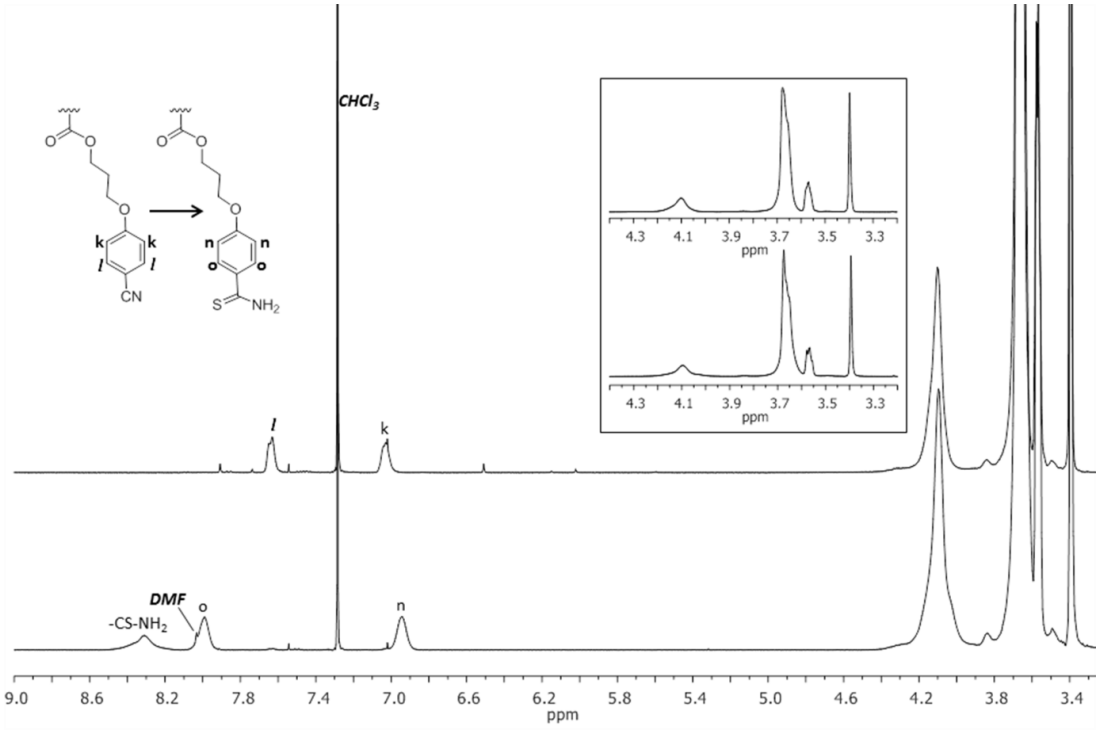


Figure S13: ^{1}H NMR spectra (400 MHz, $CDCl_3$) showing the aromatic signals of polymer before (top) and after thionation process (**5,** bottom). The inset figure (box) is the expanded region, 3.30 – 4.30 ppm, showing little change in OEGMA:MMA ratio before and after thionation.

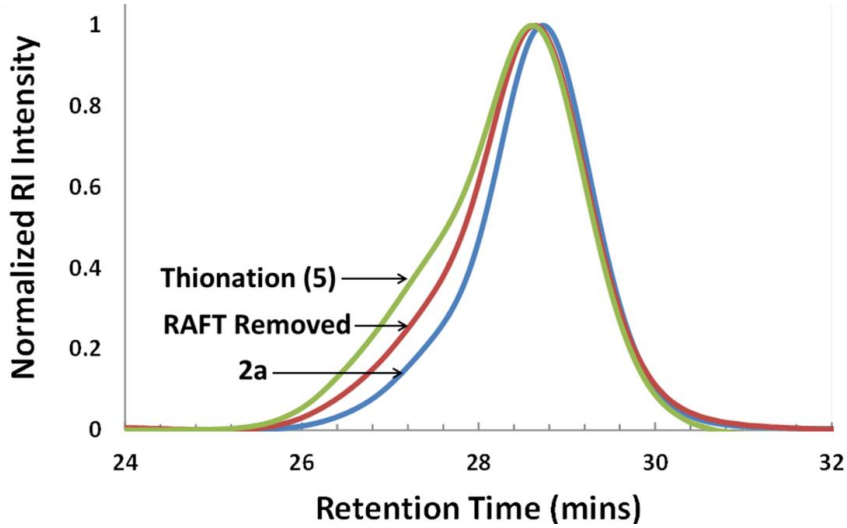


Figure S14. GPC (DMAc) chromatograms of [$POEGMA_{58}$-*co*-$PCPPMA_7$]-S(C=S)Ph **2b** and those of polymer after the successive steps of RAFT removal and thionation to give **5**.

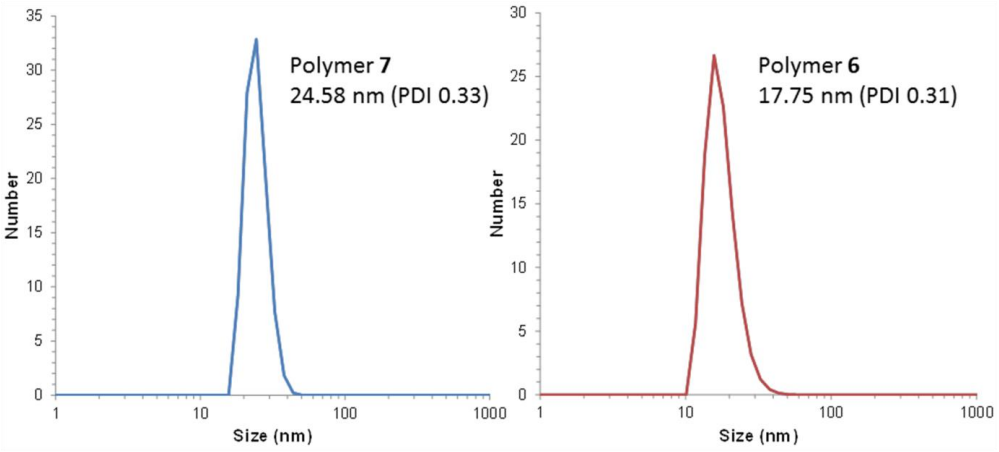


Figure S15. Number distribution of micelles in pH 7.4 PBS as determined by dynamic light scattering, formed from [POEGMA$_a$-*co*-PTHA$_b$]-*block*-[PMMA$_c$]-H, **6** (right) and [POEGMA$_a$]-*block*-[PTHA$_b$-*co*-PMMA$_c$]-H, **7** (left).

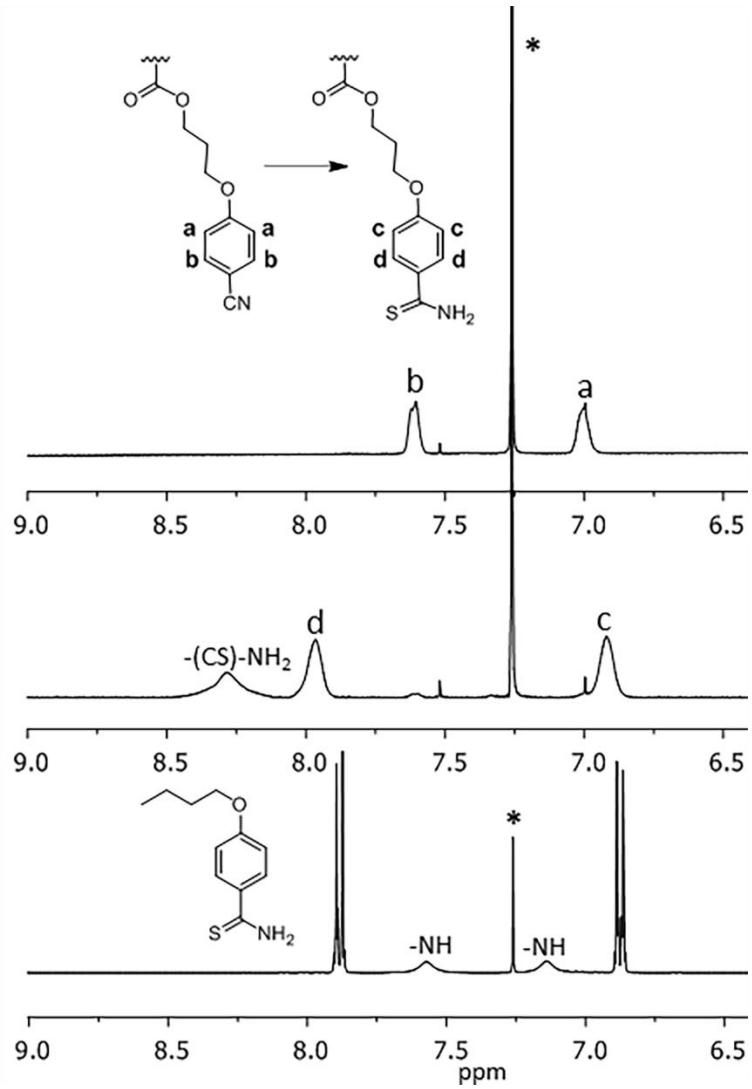


Figure S16 ^{1}H NMR spectra (400 MHz, $CDCl_3$) showing the aromatic proton signals for polymer **3** with the RAFT end group removed before (top) and after the thionation process (**6,** middle) vs. control thioamide molecule, 4-butoxybenzothioamide (bottom).

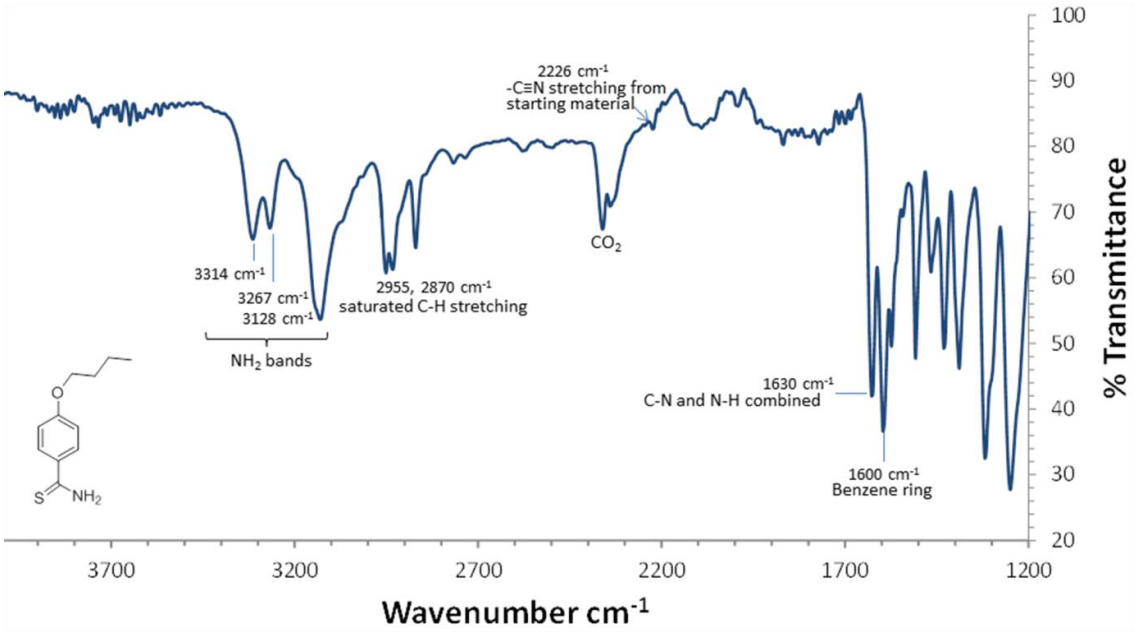


Figure S17. ATR-FTIR spectrum of 4-butoxybenzothioamide with assignments of characteristic IR frequencies.

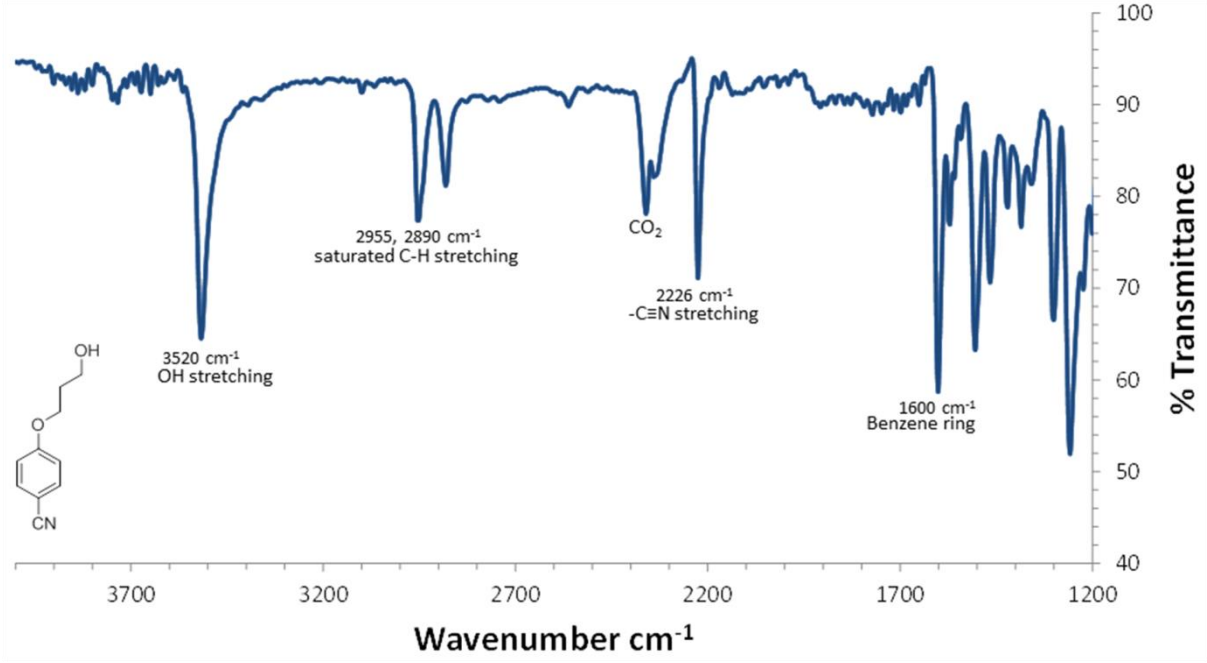


Figure S18. ATR-FTIR spectrum of 4-(3-hydroxypropoxy)benzonitrile with assignments of characteristic IR frequencies.

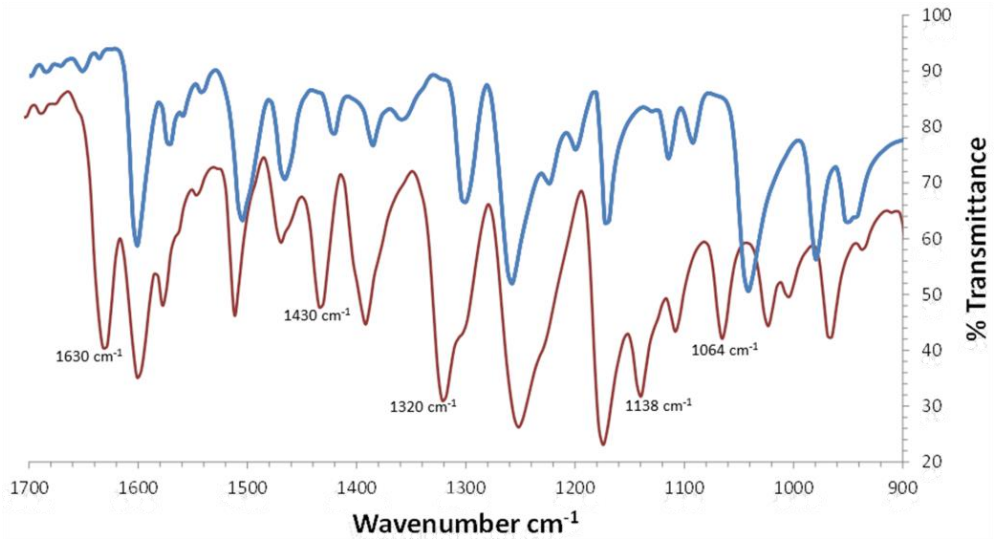


Figure S19. ATR-FTIR spectra of 4-(3-hydroxypropoxy)benzonitrile (top) and 4-butoxybenzothioamide (bottom) with assignments of characteristic IR frequencies in the fingerprint region for 4-butoxybenzothioamide.

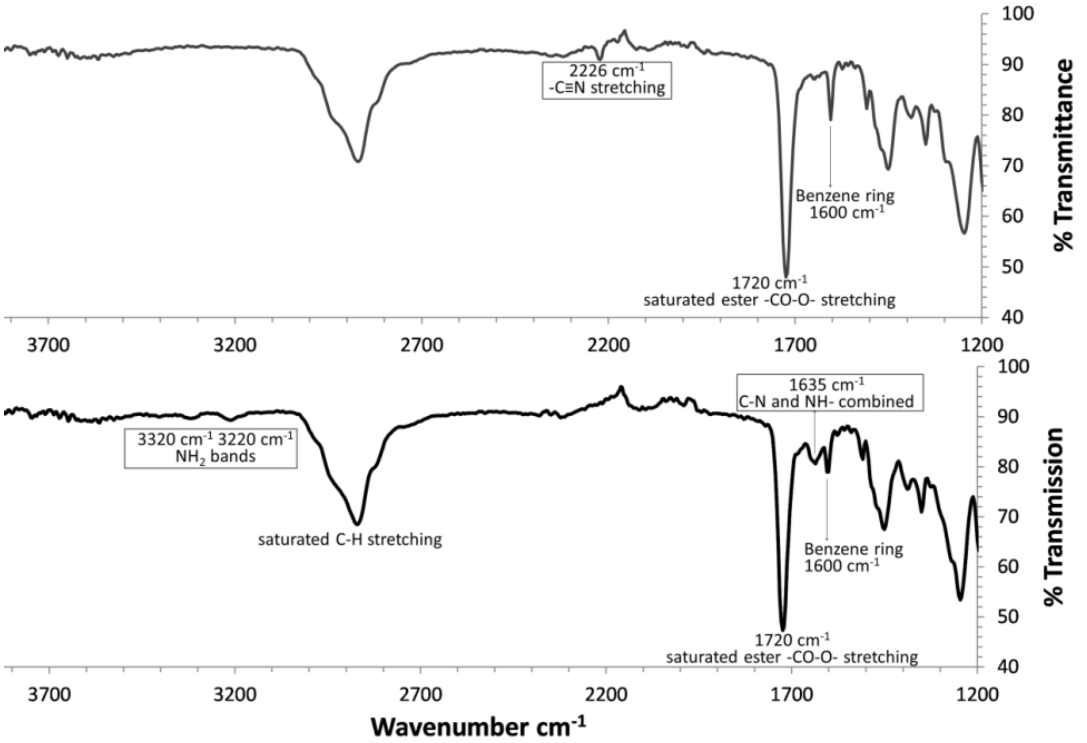


Figure S20. ATR-FTIR spectra of [POEGMA$_{58}$-*co*-PCPPMA$_7$]-S(C=S)Ph, **2a** (top) and [POEGMA$_a$-*co*-PTHA$_b$]-H **5** (bottom) showing characteristic IR frequencies in boxes.

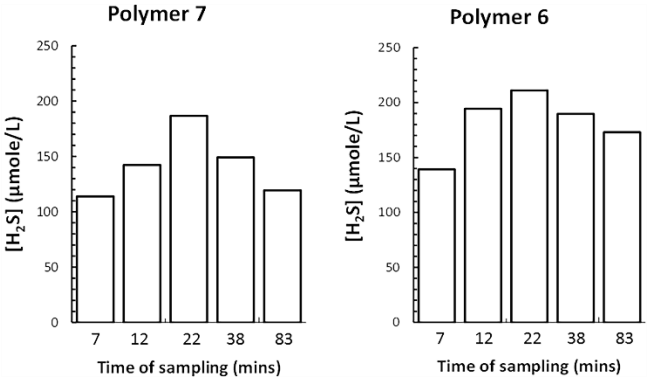


Figure S21 Release of H_2S from thioamide block copolymers **6** and **7** quantitated colorimetrically using the Methylene Blue (MB) method. Micelle solutions were prepared in PBS at pH 7.4. After the addition of 100 µL (400mM) of L-cysteine, 1 ml aliquots were removed at each time point and the H_2S content in each aliquot was measured by formation of MB^+.

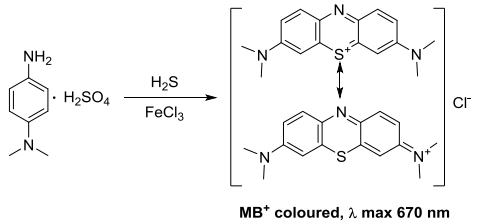
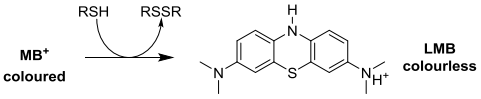


Figure S22. Formation of methylene blue (MB^+) from H_2S.



Scheme S23. Discoloration of methylene blue (MB^+) via reduction by thiols.

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