Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

Polymers with acyl-protected perthiol chain termini as convenient building blocks for doubly responsive H_2S-donating nanoparticles

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Synthesis and Characterization Methods

A. Materials

Oligo(ethylene glycol) methyl ether methacrylate (OEGMA) with M_n = 500 g mol^{-1} (denoted as $OEGMA_{500}$ where relevant) was purchased from Sigma-Aldrich and deinhibited by percolating over a column of basic alumina. 2-(dimethylamino)ethyl methacrylate (DMAEMA), butyl methacrylate (BMA) 2-(diisopropylamino)ethyl methacrylate (DIPMA) were purchased from Sigma-Aldrich and passed through a column of basic alumina in order to remove inhibitor prior to polymerization. Azobisisobutyronitrile (AIBN) was purified by recrystallization from methanol before use. 2-cyanoprop-2-yl benzodithioate (CPBD), ethanolamine, L-cysteine and dipyridyl disulfide (aldrithiol^{TM}-2) were purchased from Sigma-Aldrich at the highest purity available and used as received. Thiobenzoic acid (94%) was purchased from Alfa Aesar and used as received. Petroleum ether (b.p. 40 - 60 °C), diethyl ether, toluene, acetonitrile, chloroform and dioxane were purchased from Merck Millipore and used as received.

B. Synthetic Methods

B.1 Synthesis of P[OEGMA]-S(C=S)Ph, ***1a***

The synthesis of P[OEGMA]-S(C=S)Ph was carried out using the following stoichiometry: $[CPBD]_0$:$[OEGMA_{500}]_0$:$[AIBN]_0$=1:14.5:0.1. Briefly, $OEGMA_{500}$ (2.00 g, 4.01 × 10^{-3} mol), CPBD RAFT agent (6.14× 10^{-2} g, 2.77 × 10^{-4} mol), AIBN (0.45 × 10^{-2} g, 2.74 × 10^{-5} mol) and toluene (3 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 25 min at 0 °C by sparging with N_2. The deoxygenated and sealed reaction vessel was placed into a pre-heated oil bath at 70 °C and the polymerization was run with stirring for 4 h. The resulting mixture was then allowed to cool to 0 °C for about 15 min to halt polymerization. The monomer conversion was determined by ^{1}H NMR. The polymer was purified of unreacted monomer by first precipitating into a 50/50 (v/v) mixture of diethyl ether and petroleum ether (bp 40-60 °C), followed by two subsequent precipitations into petroleum ether (bp 40-60 °C). The product was then placed in a vacuum oven overnight to remove remaining solvent. The final product was analysed by ^{1}H NMR and GPC. The molecular weight and average monomer chain length calculated from ^{1}H NMR were determined to be 6813 g mol^{-1} and 13 units, respectively. The polydispersity index (determined by GPC) was 1.13.

B.2 Synthesis of P[OEGMA-block-BMA]-S(C=S)Ph, ***2a***

The synthesis of P[OEGMA-*block*-BMA]-S(C=S)Ph was carried out using the following stoichiometry: $[P[OEGMA]\text{-}S(C=S)Ph]_0:[BMA]_0:[AIBN]_0=1:50:0.13$. P[OEGMA]-S(C=S)Ph (0.104 g, 1.49×10^{-5} mol), BMA (0.10 g, 7.14×10^{-4} mol), and AIBN (0.32×10^{-3} g, 1.92×10^{-6} mol) were added to a glass vial equipped with a magnetic stirrer bar and capped with a rubber septum. The reaction mixture was deoxygenated for 30 min by sparging with N_2. The deoxygenated and sealed reaction vessel was placed into a pre-heated oil bath at 70 °C and the polymerisation was run with stirring for 4 h. The resulting mixture was then allowed to cool to 0 °C for about 15 min to halt polymerisation. The monomer conversion was determined by ^{1}H NMR. The polymer was purified via three precipitation and centrifugation steps (using a 50/50 (v/v) mixture of petroleum ether (bp 40-60 °C) and diethyl ether as the precipitant) to remove any traces of unreacted monomer. The product was then placed in a vacuum oven overnight to remove remaining solvent. The final product was analysed by ^{1}H NMR and GPC. The molecular weight and average monomer chain length calculated from ^{1}H NMR were determined to be 9710 g mol^{-1} and 19 units, respectively. The polydispersity index determined by GPC was 1.11.

B.3 Synthesis of P[OEGMA-co-DMAEMA]-S(C=S)Ph (Precursor for block polymer)

P[OEGMA-*co*-DMAEMA]-S(C=S)Ph was synthesized according to a previously reported procedure[1]. The stoichiometry was $[CPADB]_0:[OEGMA_{500}]_0:[DMAEMA]_0:[AIBN]_0$ = 1:50:6:0.08. Briefly, $OEGMA_{500}$ (10.0 g, 2×10^{-2} mol), DMAEMA (0.38 g, 2.40×10^{-3} mol), CPADB RAFT agent (0.09 g, 4.00×10^{-4} mol), AIBN (0.52×10^{-2} g, 3.20×10^{-5} mol) and toluene (20 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 1 h by sparging with N_2. The deoxygenated and sealed reaction vessel was placed into a pre-heated oil bath at 70 °C and the polymerisation was run with stirring for 6.15 h. The resulting mixture was then allowed to cool to 0 °C for about 15 min to halt polymerisation. The monomer conversion was determined by ^{1}H NMR. The polymer was purified via three precipitation and centrifugation steps (using a 50/50 (v/v) mixture of petroleum ether (bp 40-60 °C) and diethyl ether as the precipitant) to remove any traces of unreacted monomer. The product was then placed in a vacuum oven overnight to remove any remaining solvent. The final product was analysed by ^{1}H NMR and GPC. The molecular weight and average monomer chain lengths calculated from ^{1}H NMR

were determined to be 14,200 g mol^{-1}, and 27 units of OEGMA and 3 units of DMAEMA, respectively. The polydispersity index determined by GPC was 1.12.

B.4 Synthesis of P[OEGMA-co-DMAEMA-block-DIPMA]-S(C=S)Ph, ***3a***

The synthesis of P[OEGMA-*co*-DMAEMA-*block*-DIPMA]-S(C=S)Ph was conducted according to a previously reported procedure[1]. The stoichiometry was [P[OEGMA-*co*-DMAEMA]-S(C=S)Ph]$_0$:[DIPMA]$_0$:[AIBN]$_0$=1:100:0.08. P[OEGMA-*co*-DMAEMA]-S(C=S)Ph (1.31 g, 9.38 × 10^{-5} mol), DIPMA (2.00 g, 9.38 × 10^{-3} mol), and AIBN (0.12 × 10^{-2} g, 7.50 × 10^{-6} mol) were added to a glass vial equipped with a magnetic stirrer bar and capped with a rubber septum. The reaction mixture was deoxygenated by sparging with nitrogen for 1 h. The deoxygenated solution was placed into a pre-heated oil bath at 70 °C and the polymerisation was allowed to proceed with stirring for 12 h. The polymerisation was stopped by placing the sample in an ice bath for 15 min. The product was recovered by dialysis with acetone. The purified block polymer was then dried in vacuo. P[OEGMA-*co*-DMAEMA-*block*-DIPMA]-S(C=S)Ph was analysed by ^{1}H NMR and GPC.

B.5 Modification of the thiocarbonylthio end group from P[OEGMA]-S(C=S)Ph to form P[OEGMA]-S-S-Py, ***1b***

A mixture of P[OEGMA]-S(C=S)Ph (0.10 g, 1.43× 10^{-5} mol) and aldrithiol^{TM}-2 (0.03 g, 1.43 × 10^{-4} mol) in acetonitrile (1 mL) was deoxygenated with nitrogen for 15 min. A solution of ethanolamine (35 μL, 5.71 × 10^{-4} mol) in acetonitrile (965μL) was deoxygenated with nitrogen for 15 min. The solution of ethanolamine in acetonitrile (100 μL) was transferred into the mixture of P[OEGMA]-S(C=S)Ph and aldrithiol^{TM}-2 in acetonitrile. The deoxygenated reaction mixture was then stirred for 2 h at room temperature to convert the P[OEGMA]-S(C=S)Ph to P[OEGMA]-S-S-Py (i.e. convert the dithioester endgroup to pyridyl-2-dithiol endgroup). Dialysis was conducted overnight to ensure removal of the *N*-(2-hydroxyethyl)benzothioamide side product and the excess aldrithiol/ethanolamine. The resulting polymer was dried under air and in vacuo, and then analysed by ^{1}H NMR spectroscopy to confirm the removal of the dithioester end group and the formation of pyridyl-2-dithiol endgroup. Three distinct signals in the ^{1}H NMR spectrum (δ 7.4, 7.55 and 7.9 ppm, corresponding to the aromatic benzodithioate) were lost, while three new peaks appeared (δ 7.1, 7.65, 8.45 ppm, representing the aromatic pyridyl group).

B.6 Modification of the thiocarbonylthio end group from P[OEGMA-block-BMA]-S(C=S)Ph to form P[OEGMA-block-BMA]-S-S-Py, ***2b***

A mixture of P[OEGMA-*block*-BMA]-S(C=S)Ph (0.10 g, 9.63×10^{-6} mol) and aldrithiol™-2 (0.02 g, 9.62×10^{-5} mol) in acetonitrile (1 mL) was deoxygenated with nitrogen for 15 min. A solution of ethanolamine (233 μL, 3.85×10^{-4} mol) in acetonitrile (9767 μL) was deoxygenated with nitrogen for 15 min. The solution of ethanolamine in acetonitrile (100 μL) was transferred into the mixture of P[OEGMA-*block*-BMA]-S(C=S)Ph and aldrithiol™-2 in acetonitrile. The deoxygenated reaction mixture was then stirred for 2 h at room temperature to convert the P[OEGMA-*block*-BMA]-S(C=S)Ph to P[OEGMA-*block*-BMA]-S-S-Py (i.e. convert the dithioester endgroup to pyridyl-2-dithiol endgroup). Dialysis was conducted overnight to ensure removal of the *N*-(2-hydroxyethyl)benzothioamide side product and the excess aldrithiol and ethanolamine. The resulting polymer was dried under air and in vacuo, and then analysed by ^{1}H NMR spectroscopy to confirm the removal of the dithioester end group and the formation of pyridyl-2-dithiol endgroup. Three distinct signals in the ^{1}H NMR spectrum (δ 7.35, 7.5 and 7.85 ppm, corresponding to the aromatic benzodithioate) were lost and replaced with three new peaks (δ 7.1, 7.6, 8.45 ppm, corresponding to the aromatic pyridyl group).

B.7 Modification of the thiocarbonylthio end group from P[OEGMA-co-DMAEMA-block-DIPMA]-S(C=S)Ph to form P[OEGMA-co-DMAEMA-block-DIPMA]-S-S-Py, ***3b***

A mixture of P[OEGMA-*co*-DMAEMA-*block*-DIPMA]-S(C=S)Ph (1.00 g, 4.51×10^{-5} mol) and aldrithiol™-2 (0.10 g, 4.51×10^{-4} mol) in acetonitrile (1 mL) was deoxygenated with nitrogen for 15 min. A solution of ethanolamine (110 μL, 1.80×10^{-3} mol) in acetonitrile (890 μL) was deoxygenated with nitrogen for 15 min. The solution of ethanolamine in acetonitrile (100 μL) was transferred into the mixture of P[OEGMA-*co*-DMAEMA-*block*-DIPMA]-S(C=S)Ph and aldrithiol™-2 in acetonitrile. The deoxygenated reaction mixture was then stirred for 2 h at room temperature to convert the P[OEGMA-*co*-DMAEMA-*block*-DIPMA]-S(C=S)Ph to P[OEGMA-*co*-DMAEMA-*block*-DIPMA]-S-S-Py (i.e. convert the dithioester endgroup to pyridyl-2-dithiol endgroup). Dialysis was conducted overnight to ensure removal of the *N*-(2-hydroxyethyl)benzothioamide side product and the excess aldrithiol and ethanolamine. The resulting polymer was dried under air and in vacuo, and then analysed by ^{1}H NMR spectroscopy to confirm the removal of the dithioester end group and the formation of pyridyl-2-dithio endgroup. Three distinct signals in the ^{1}H NMR spectrum (δ 7.3, 7.5 and

7.85 ppm, corresponding to the aromatic benzodithioate) were lost and replaced with new peaks (δ 7.1, 7.55-7.7, 8.45 ppm, corresponding to the aromatic pyridyl group).

B.8 Thiol-disulfide exchange reaction of P[OEGMA]-S-S-Py with thiobenzoic acid to form P[OEGMA]-S-S-(C=O)Ph, ***1c***

P[OEGMA]-S-S-Py prepared as above (B.5) (0.06 g, 8.79 × 10^{-6} mol) was dissolved in chloroform (900 µL). Thiobenzoic acid (1.94 mg, 1.40× 10^{-5} mol) was added into the solution with stirring and allowed to react at room temperature for 1 h to convert the P[OEGMA]-S-S-Py to P[OEGMA]-S-S-(C=O)Ph (i.e., to convert the pyridyl-2-thiol endgroup to the acyl-protected perthiol endgroup). Dialysis was conducted overnight to ensure removal of the 2-mercaptopyridine side product and the excess thiobenzoic acid. The resulting polymer was dried under air and in vacuo, and then analysed by ^{1}H NMR spectroscopy to confirm the removal of the pyridyl-2-thiol endgroup and the formation of the acyl-protected perthiol endgroup. Three distinct signals in the ^{1}H NMR spectrum (δ 7.1, 7.65, 8.45 ppm corresponding to the aromatic pyridyl group) were lost and replaced with new peaks (δ 7.5, 7.65, 8.0 ppm, corresponding to the benzoyl protecting group).

B.9 Thiol-disulfide exchange reactions of P[OEGMA-block-BMA]-S-S-Py with thiobenzoic acid to form P[OEGMA-block-BMA]-S-S-(C=O)Ph, ***2c***

P[OEGMA-*block*-BMA]-S-S-Py prepared as above (B.6) (0.05 g, 5.15 × 10^{-6} mol) was dissolved in chloroform (1 mL). Thiobenzoic acid (1.14 mg, 8.22× 10^{-6} mol) was added into the solution with stirring and allowed to react at room temperature for 1h to convert the P[OEGMA-*block*-BMA]-S-S-Py to P[OEGMA-*block*-BMA]-S-S-(C=O)Ph (i.e. convert pyridyl-2-thiol endgroup to the acyl-protected perthiol endgroup). Dialysis was conducted overnight to ensure removal of the 2-mercaptopyridine side product and the excess thiobenzoic acid. The resulting polymer was dried under air and in vacuo, and then analysed by ^{1}H NMR spectroscopy to confirm the removal of the pyridyl-2-thiol endgroup and the formation of the acyl-protected perthiol endgroup. Three distinct signals in the ^{1}H NMR spectrum (δ 7.1, 7.6, 8.45 ppm, corresponding to the aromatic pyridyl group) were lost and replaced with new peaks (δ 7.5, 7.6, 8.0 ppm, corresponding to the aromatic benzoyl protecting group).

Micelles were formed in pH 7.4 PBS buffer by dissolving 7 mg (0.71 µmoles) of polymer directly into PBS at pH 7.4 (5 mL) and then confirming formation by DLS.

B.10 Thiol-disulfide exchange reactions of P[OEGMA-co-DMAEMA-block-DIPMA]-S-S-Py with thiobenzoic acid to form P[OEGMA-co-DMAEMA-block-DIPMA]-S-S-(C=O)Ph, ***3c***

P[OEGMA-*co*-DMAEMA-*block*-DIPMA]-S-S-Py prepared as above (B.7) (0.46 g, 2.08×10^{-5} mol) was dissolved in chloroform (2 mL). Thiobenzoic acid (4.58 mg, 3.32×10^{-5} mol) was added into the solution with stirring and allowed to react at room temperature for 1h to convert the P[OEGMA-*co*-DMAEMA-*block*-DIPMA]-S-S-Py to P[OEGMA-*co*-DMAEMA-*block*-DIPMA]-S-S-(C=O)Ph (i.e., to convert the pyridyl-2-thiol endgroup to the acyl-protected perthiol endgroup). Dialysis was conducted overnight to ensure removal of the 2-mercaptopyridine side product and the excess thiobenzoic acid. The resulting polymer was dried under air and in vacuo, and then analysed by ^{1}H NMR spectroscopy to confirm the removal of the pyridyl-2-thiol endgroup and the formation of the acyl-protected perthiol endgroup. Three distinct signals in the ^{1}H NMR spectrum (δ 7.1, 7.55-7.7, 8.45 ppm, corresponding to the aromatic pyridyl group) were lost and replaced with new peaks (δ7.4-7.6, 8.0, 8.2 ppm, corresponding to the aromatic benzoyl protecting group). Micelle formation was conducted in pH 7.4 PBS buffer. 25 mg of polymer was dissolved in acetone (0.3 mL) and then added into 5 mL of buffer solution with stirring. Acetone was removed by evaporation with a stream of air. Micelle formation was confirmed by DLS.

B.11 Control polymer (mixture of P[OEGMA]-S(C=O)Ph, P[OEGMA]-S-S-$C_3H_7NO_2$ and P[OEGMA]-S-S-P[OEGMA]) formed via exposure of P[OEGMA]-S-S-(C=O)Ph, ***1c****, to L-cysteine.*

H_2S-donating homopolymer P[OEGMA]-S-S-(C=O)Ph (5 mg, 0.71 µmoles, 143 µM) was dissolved in PBS at pH 7.4 in the presence of L-cysteine (4.65 molar equiv., 3.3 µmoles, 660 µM) over 72 h in an open open vessel. Dialysis was then conducted to ensure removal of the side products and the excess L-cysteine. The resulting polymer was dried under air and in vacuo, and then analysed by ^{1}H NMR spectroscopy to confirm the final products (mixture of P[OEGMA]-S(C=O)Ph, P[OEGMA]-S-S-$C_3H_7NO_2$ and P[OEGMA]-S-S-P[OEGMA]).

C. Analysis Methods

C.1 ^{1}H Nuclear Magnetic Resonance Spectroscopy

^{1}H NMR spectra were recorded at 400 MHz on a Bruker UltraShield 400 MHz spectrometer 7 running Bruker Topspin, version 1.3. Spectra were recorded in $CDCl_3$.

C.2 Gel Permeation Chromatography (GPC)

GPC was performed using a Shimadzu modular system comprised of a SIL-20AD automatic injector, a RID-10A differential refractive-index detector and a 50 × 7.8 mm guard column followed by three KF-805L columns (300 × 8 mm, bead size: 10 μm, pore size maximum: 5000 Å). *N,N*-Dimethylacetamide (DMAc, HPLC grade, 0.03% w/v LiBr) at 50 °C was used as the eluent with a flow rate of 1 mL min^{-1} . Samples were filtered through 0.45 μm PTFE filters before injection. The GPC calibration was performed with narrow-polydispersity polystyrene standards ranging from 500 to 2 × 10^6 g mol^{-1} .

C.3 Dynamic Light Scattering (DLS)

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), used to describe the average diameters and size distribution of prepared micelles, was determined via a cumulants analysis of the measured intensity autocorrelation function using the DTS software. Samples were filtered using 0.45 μm PTFE syringe filter to remove contaminants / dust prior to measurement.

C.4 Fluorescence spectroscopy

Fluorescence spectra were obtained using a fluorescence spectrophotometer (Shimadzu RF-5301 PC). Slit widths were set at 5 nm for excitation and at 3 or 5 nm for emission, with sensitivity set at low.

C.5 Amperometric H_2S Sensing

The H_2S-generating capability of the polymers was examined using an amperometric H_2S microsensor manufactured by Unisense A/S.

Calibration of the sensor was performed after the sensor signal had stabilized over a prepolarization 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 prepared by adding aqueous HCl to PBS at pH 7.4, giving a pH value < 4 (normally 3.8). This was deoxygenated for 20 minutes by bubbling with N_2 gas. 20 mL of the acidic buffer was then transferred to a nitrogen-flushed bottle equipped with a stirrer, which was capped with a septum. The sensor was then immersed

into the solution via a specialized opening on the septum which enabled the bottle to be capped after the sensor tip had been passed through. Once the signal stabilized to a low, stable reading, the value was recorded and assumed to correspond to the zero [H_2S] value. Calibration points within the expected range of measurement were collected by injecting known amounts of Na_2S stock solution into the stirred calibration buffer solution using a micro-syringe. 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)[2].

L-cysteine mediated H_2S-generating capability of polymers was examined as follows. Polymer (0.71 µmoles) in deoxygenated pH 7.4 PBS (5 mL) and L-cysteine (3.3 µmoles) in deoxygenated PBS at pH 7.4 were prepared separately. The sensor was immersed into the polymer solution, using the septum to minimize exposure to air on immersing the sensor into the solution. 50 µL of L-cysteine solution was then injected slowly into the stirring polymer solution using a micro-syringe. The time of cysteine addition was recorded.

C.6 H_2S kinetics considerations

H_2S is a weak acid, which can be ionized into HS^- and S^{2-}. The ionization equilibrium between H_2S and HS^-/S^{2-} has to be established.[2]

The total concentration of sulfides in solution can be described as $[sulfides]_{tot} = [H_2S] + [HS^-]$. By using the equilibrium constants K_1 and K_2 (K_1= equilibrium constant between H_2S and HS^-, K_2= equilibrium constant between HS^- and S^{2-}), the total concentration of dissolved sulfide species generated in solution was simplified into $[sulfides]_{tot} = [H_2S] \times (10^{pH-pK1} + 1)$. The pK_1 value was also affected by temperature measurement[2]: $pK_1 = 32.55 + (1519.44/T) - 15.672\log(T) + 0.02722T$. T is in Kelvin. All amperometric H_2S release measurements were conducted at 25°C (298.15 K).

Measurement in pH 7.4 at 25°C (298.15 K) is calculated from $[sulfides]_{tot} = [H_2S]_{detected} \times 3.40$.
Measurement in pH 5.0 at 25°C (298.15 K) is calculated from $[sulfides]_{tot} = [H_2S]_{detected} \times 1.01$

*C.7 H_2S release test from P[OEGMA]-S-S-(C=O)Ph (**1c**) using SF4 probe*

A stock solution of SF4 was prepared fresh in a 1:1 mixture of DMF and DMSO (6.1 mM). An aliquot (110 µL) was then diluted into water to a final volume of 5 mL and concentration

of 0.134 mM. Separately, 5.0 mg of homopolymer was dissolved into 300 µL of THF and this was mixed with 1 mL of SF4 solution (0.134 mM) and 300 µL of PBS (7.4), the final volume was therefore 1.6 mL. The solution was transferred to a quartz cuvette and the fluorescence measured (λ_{ex} = 488 nm; λ_{em} = 490-700 nm), representing the H_2S donor without added L-cysteine. L-cysteine-mediated H_2S release from the polymers was then was investigated by adding 40 µL of L-cysteine solution (5 mL stock solution with a concentration of 33 mM in degassed PBS at pH 7.4). The solution was left to mix for 2 minutes and then the fluorescence re-measured. Negative controls were also measured using pyridyl terminated polymer (with and without cysteine), in place of the H_2S-donating homopolymer. As a positive control, a sample was measured using Na_2S: A stock solution of Na_2S was initially prepared fresh in degassed PBS 7.4 (10 mM) and then an aliquot (10 µL) of this was mixed with 1 mL of SF4 solution (0.134 mM) and 600 µL of PBS (7.4).

C.8 SF4 detection of H_2S donating polymer (B.10) in human embryonic kidney (HEK) cells.

Human embryonic kidney (HEK293) cells were cultured in DMEM supplemented with 5% heat-inactivated FBS. Cell were plated onto poly-D-lysine (5µg/cm^2) coated 8-well optical µ-slides (Ibidi, Germany) 24 h prior to imaging. On the day of imaging, cells were labelled with 10µM SF4 fluorescent probe and Hoechst nuclear stain 33342 in OptiMem media for 30 min at 37°C.

H_2S donor polymer (acyl protected perthiol end-group (P[OEGMA-*co*-DMAEMA-*block*-DIPMA]-S-S-(C=O)Ph) **3c**) or control polymer (formed by previous exposure of **1c** to L-cysteine to) were diluted to 10µM in OptiMem media and added to cells at a final concentration of 100nM (2.2 ng/ml and 0.8 ng/ml for H_2S donor and control polymer, respectively) for 60 min at 37°C in 5% CO_2. Cells were then transferred to physiological saline (HBSS, pH7.4, supplemented with Mg^{2+} and Ca^{2+}) and imaged on Leica SP8 confocal microscope (humidified, 37°C) with a HCX PL APO 63x (NA 1.40) oil objective (Pinhole 2AU). Images were acquired by sequential excitation with UV (405 diode) and Argon (488 nm) lasers and emission at 450±40m (NucBlue Hoechst stain, blue) and 550±50nm (SF4 dye, green), using identical laser intensity and gain settings.

Chart S1. Various protected perthiol end-group modified polymers synthesized during this study: (**1c**) poly(oligoethylene glycol methyl ether methacrylate)-S-S-(C=O)Ph; (**2c**) poly(oligoethylene glycol methyl ether methacrylate-*block*-butyl methacrylate)-S-S-(C=O)Ph; (**3c**) poly(oligoethylene glycol methyl ether methacrylate-*co*-*N,N*-(dimethylamino)ethyl methacrylate-*block*- *N,N*-(diisopropylamino)ethyl methacrylate)-S-S-(C=O)Ph.

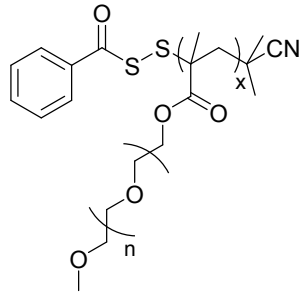
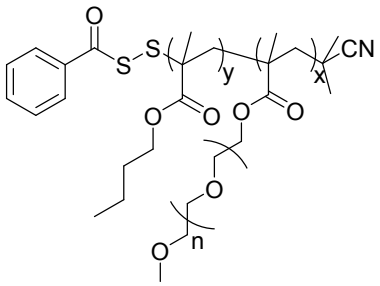
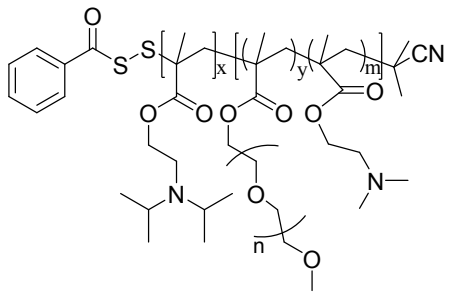
1c	**2c**	**3c**
		

Table S1. Characterisation of polymers synthesized

Polymer	M_n (g mol^{-1})[a]	M_n (g mol^{-1})[b]	PDI
P[OEGMA]-S(C=S)Ph, **1a**	6813	7041	1.13
P[OEGMA]-S-S-Py, **1b**	-	7041	1.13
P[OEGMA]-S-S-(C=O)Ph, **1c**	-	7844	1.15
P[OEGMA-b-BMA]-S(C=S)Ph, **2a**	9710	10672	1.11
P[OEGMA-*b*-BMA]-S-S-Py, **2b**	-	10625	1.12
P[OEGMA-*b*-BMA]-S-S-(C=O)Ph, **2c**	-	11564	1.15
P[OEGMA-co-DMAEMA-b-DIPMA]-S(C=S)Ph, **3a**	22200	21280	1.24
P[OEGMA-*co*-DMAEMA-*b*-DIPMA]-S-S-Py, **3b**	-	22803	1.22
P[OEGMA-*co*-DMAEMA-*b*-DIPMA]-S-S-(C=O)Ph, **3c**	-	25847	1.30

[a] Molecular weight determined by ^{1}H NMR spectroscopy.

[b] Molecular weight determined by gel permeation chromatography.

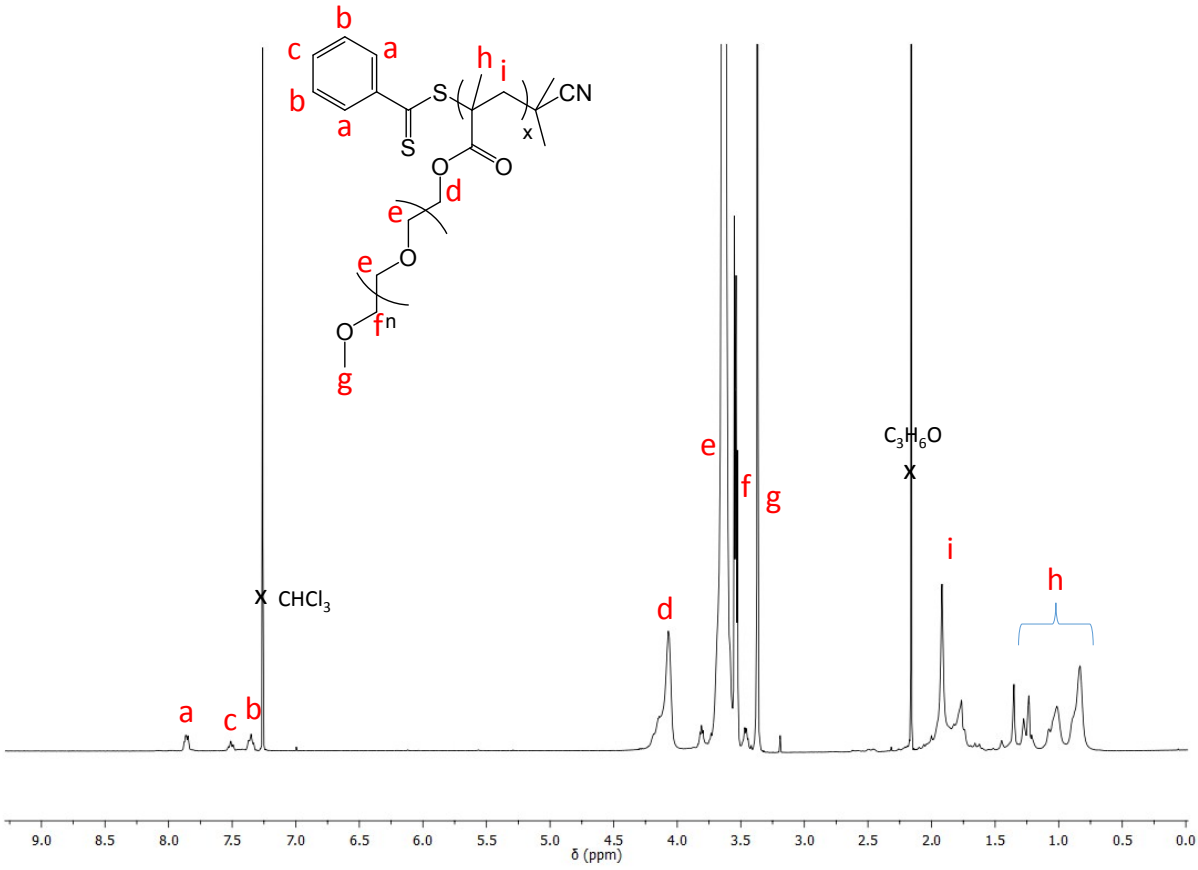


Figure S1. ^{1}H NMR spectrum of P[OEGMA]-S(C=S)Ph (Table S1, **1a**), recorded in $CDCl_3$ (400 MHz).

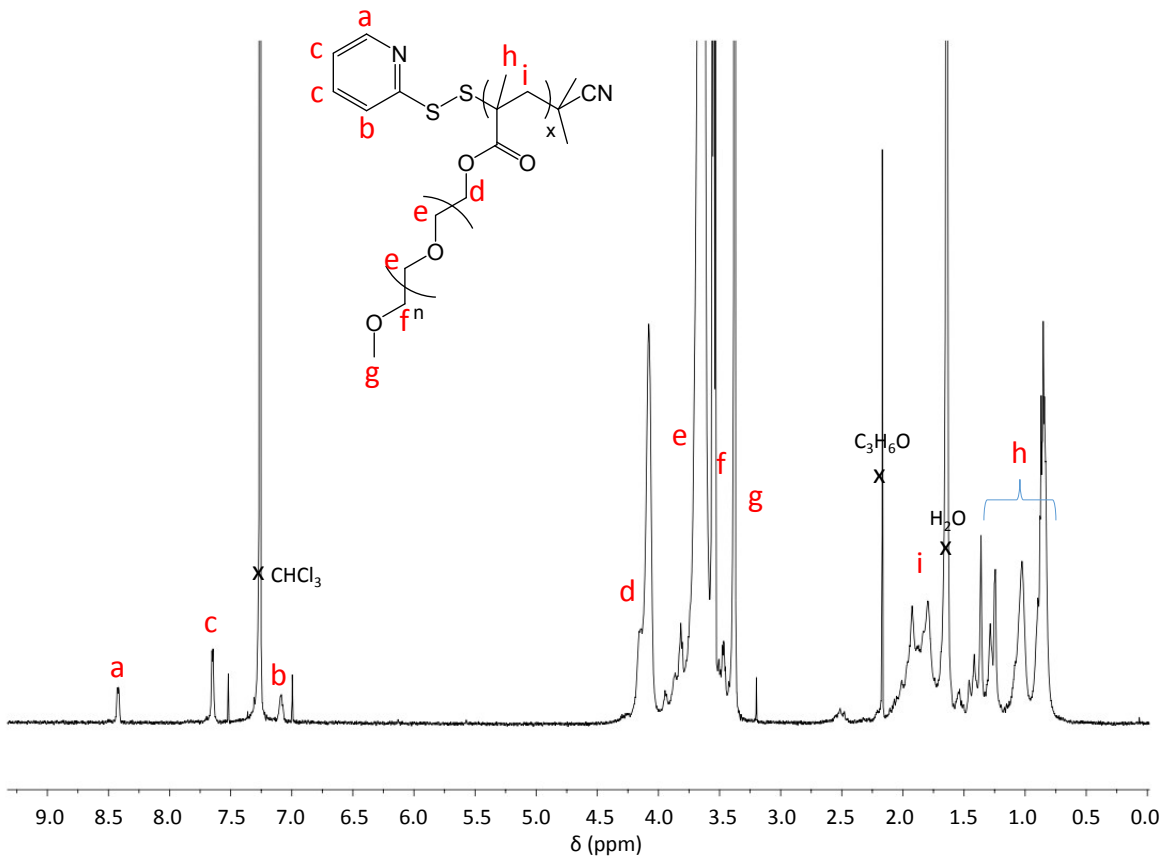


Figure S2. ^{1}H NMR spectrum of P[OEGMA]-S-S-Py (Table S1, **1b**), recorded in $CDCl_3$ (400 MHz).

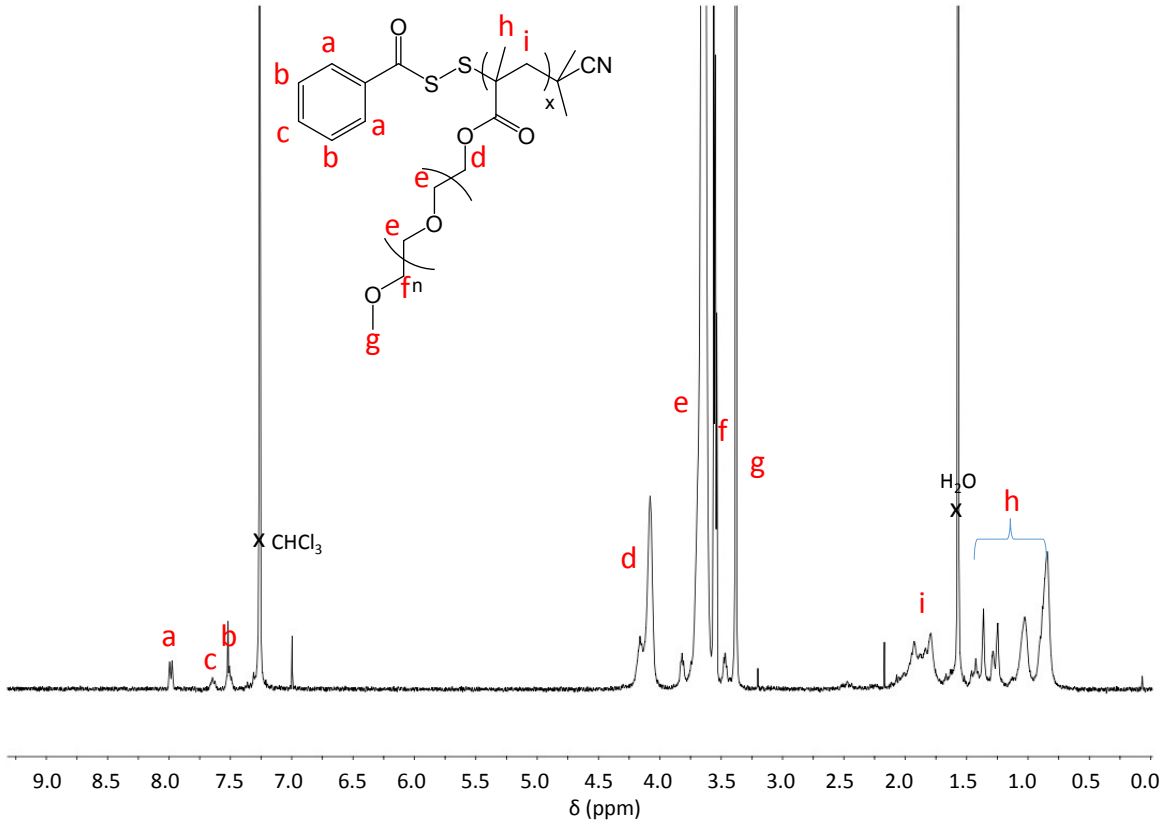


Figure S3. ^{1}H NMR spectrum of P[OEGMA]-S-S-(C=O)Ph (Table S1, **1c**), recorded in $CDCl_3$ (400 MHz).

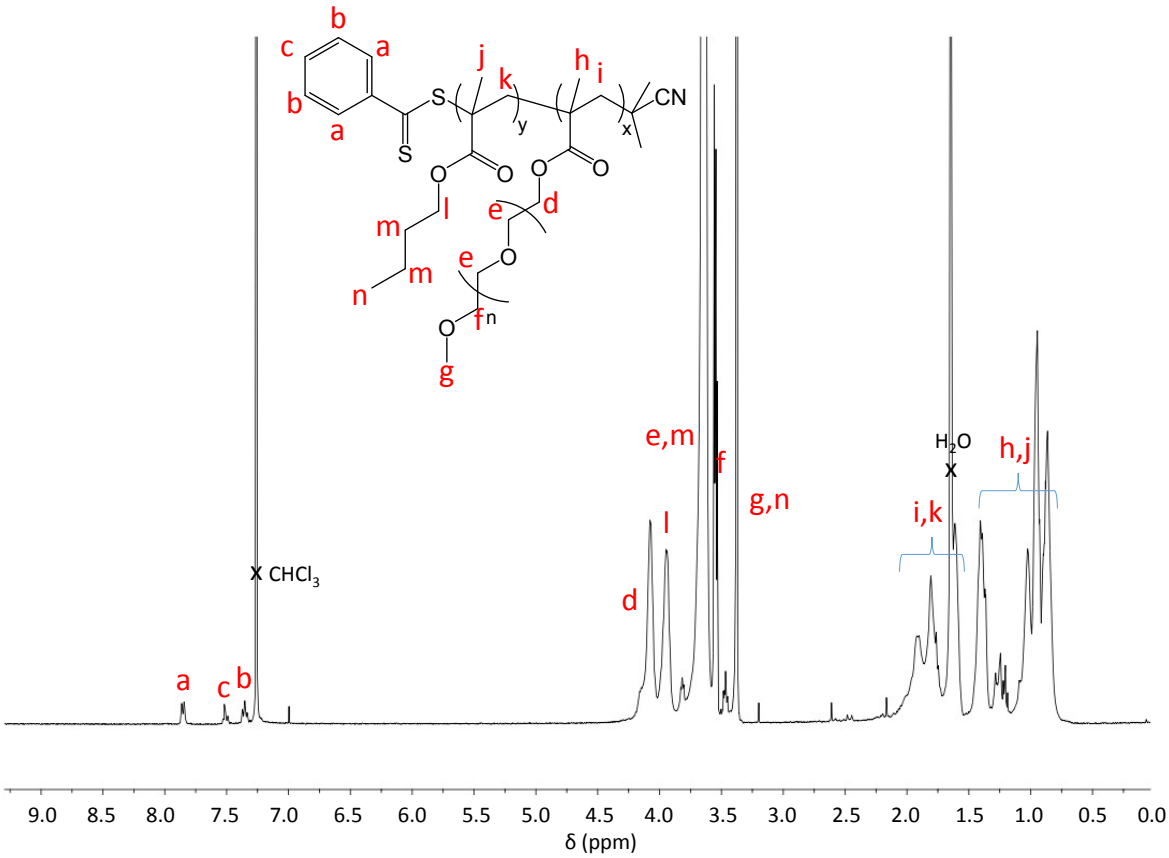


Figure S4. ^{1}H NMR spectrum of P[OEGMA-*block*-BMA]-S(C=S)Ph (Table S1, **2a**), recorded in $CDCl_3$ (400 MHz).

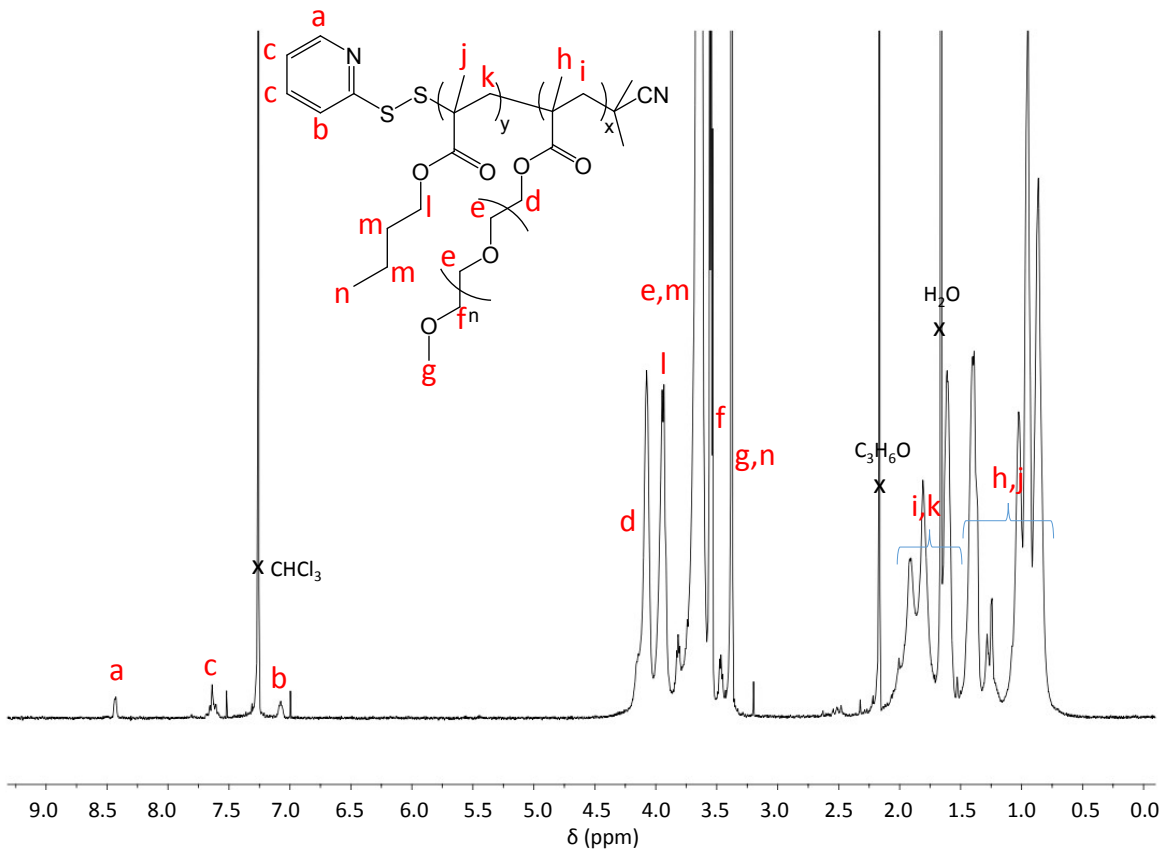


Figure S5. ^{1}H NMR spectrum of P[OEGMA-*block*-BMA]-S-S-Py (Table S1, **2b**), recorded in $CDCl_3$ (400 MHz).

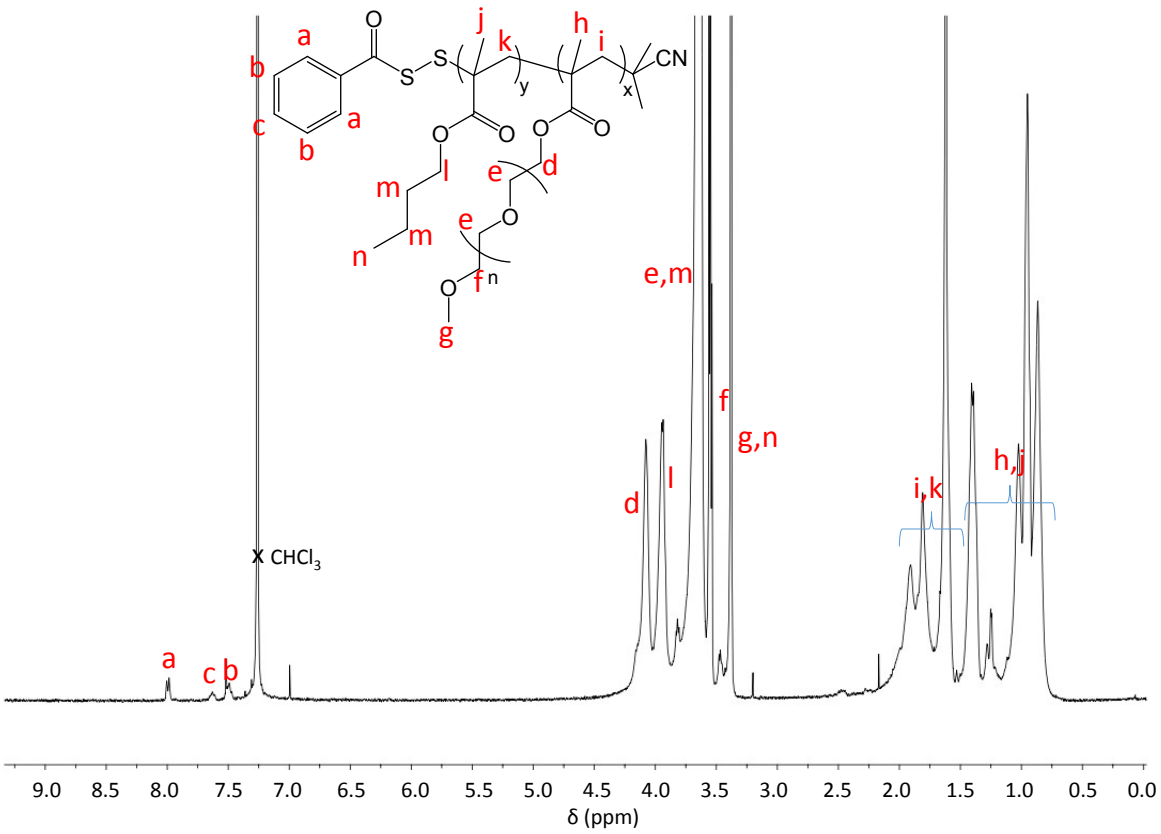


Figure S6. ^{1}H NMR spectrum of P[OEGMA-*block*-BMA]-S-S-(C=O)Ph (Table S1, **2c**), recorded in $CDCl_3$ (400 MHz).

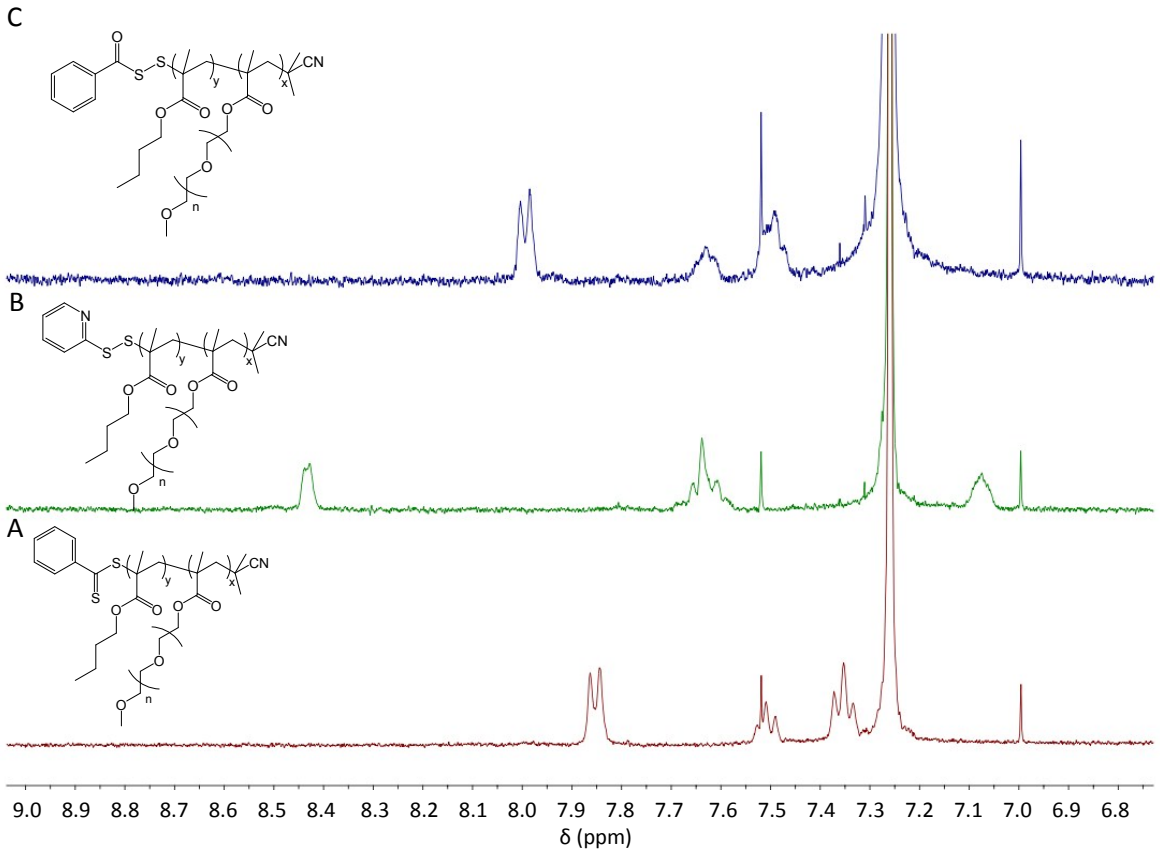


Figure S7. Enlarged ^{1}H NMR spectra (δ 9.0 - 6.8 ppm) corresponding to end-groups protons of (A) P[OEGMA-*block*-BMA]-S(C=S)Ph, **2a** (B) P[OEGMA-*block*-BMA]-S-S-Py, **2b** (C) P[OEGMA-*block*-BMA]-S-S-(C=O)Ph, **2c**, recorded in $CDCl_3$ (400 MHz). Refer to Table S1 for GPC characterisation of polymers.

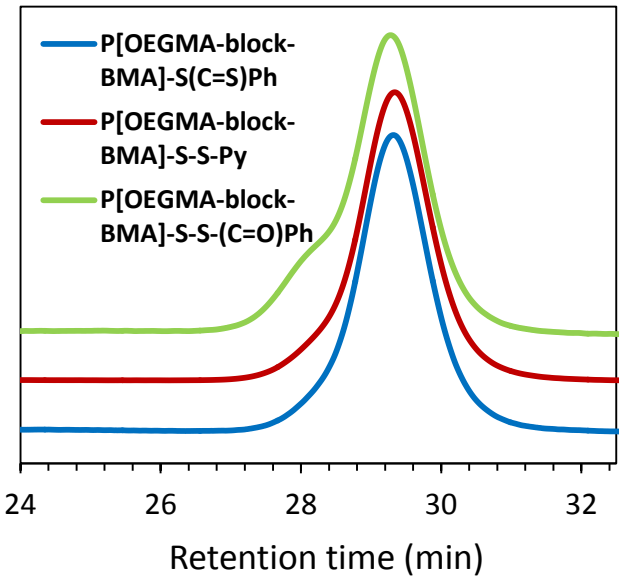


Figure S8. GPC chromatograms for P[OEGMA-*block*-BMA]-S(C=S)Ph (**2a**, blue line), P[OEGMA-*block*-BMA]-S-S-Py (**2b**, red line), P[OEGMA-*block*-BMA]-S-S-(C=O)Ph (**2c**, green line). Refer to Table S1 for GPC characterization of polymer.

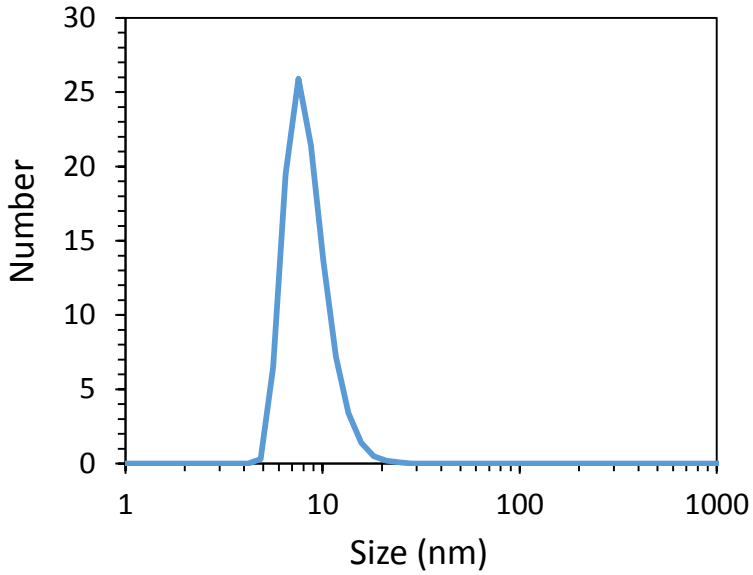


Figure S9. Size distribution (by number) for micelles of P[OEGMA-*block*-BMA]-S-S-(C=O)Ph (**2c**) in phosphate buffered saline at pH 7.4, as determined by dynamic light scattering. The average diameter was 9.1 nm with PDI = 0.17.

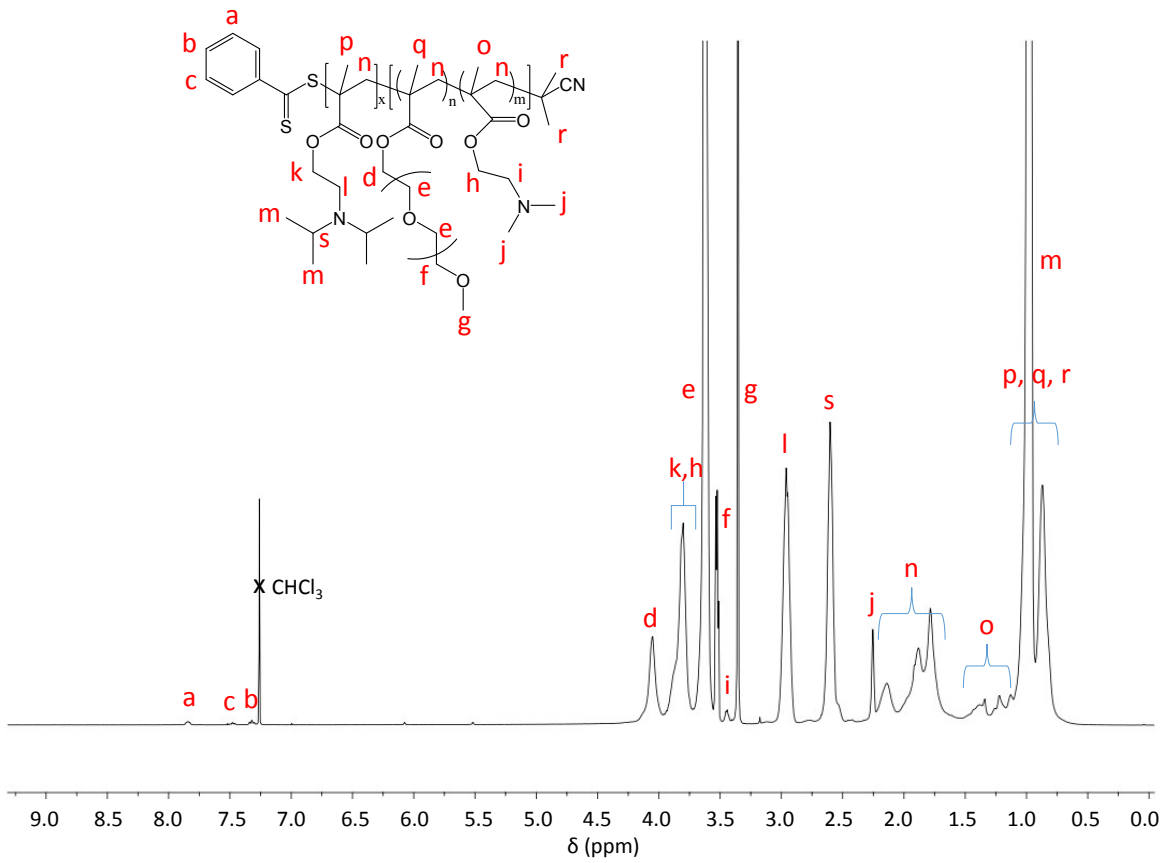


Figure S10. ^{1}H NMR spectrum of P[OEGMA-*co*-DMAEMA-*block*-DIPMA]-S(C=S)Ph (**3a**, Table S1), recorded in $CDCl_3$ (400 MHz).

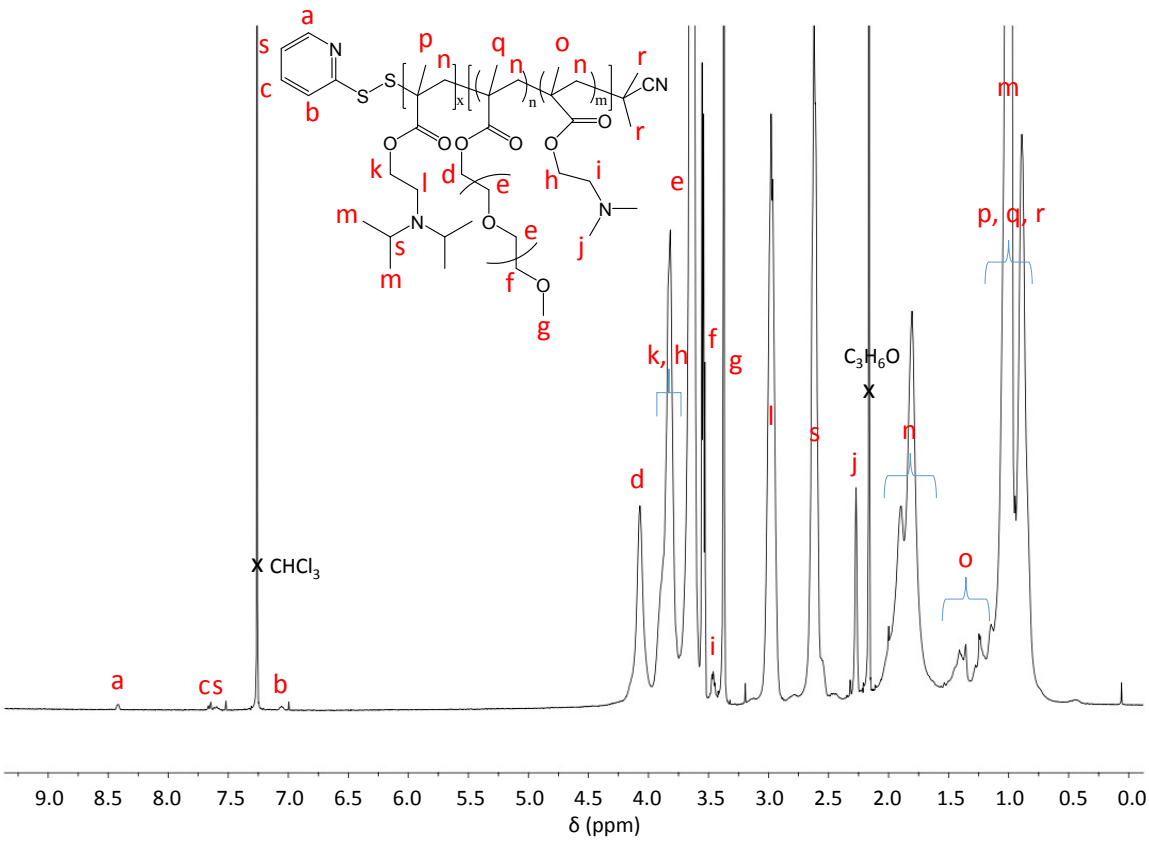


Figure S11. ^{1}H NMR spectrum of P[OEGMA-*co*-DMAEMA-*block*-DIPMA]-S-S-Py (**3b**, Table S1), recorded in $CDCl_3$ (400 MHz).

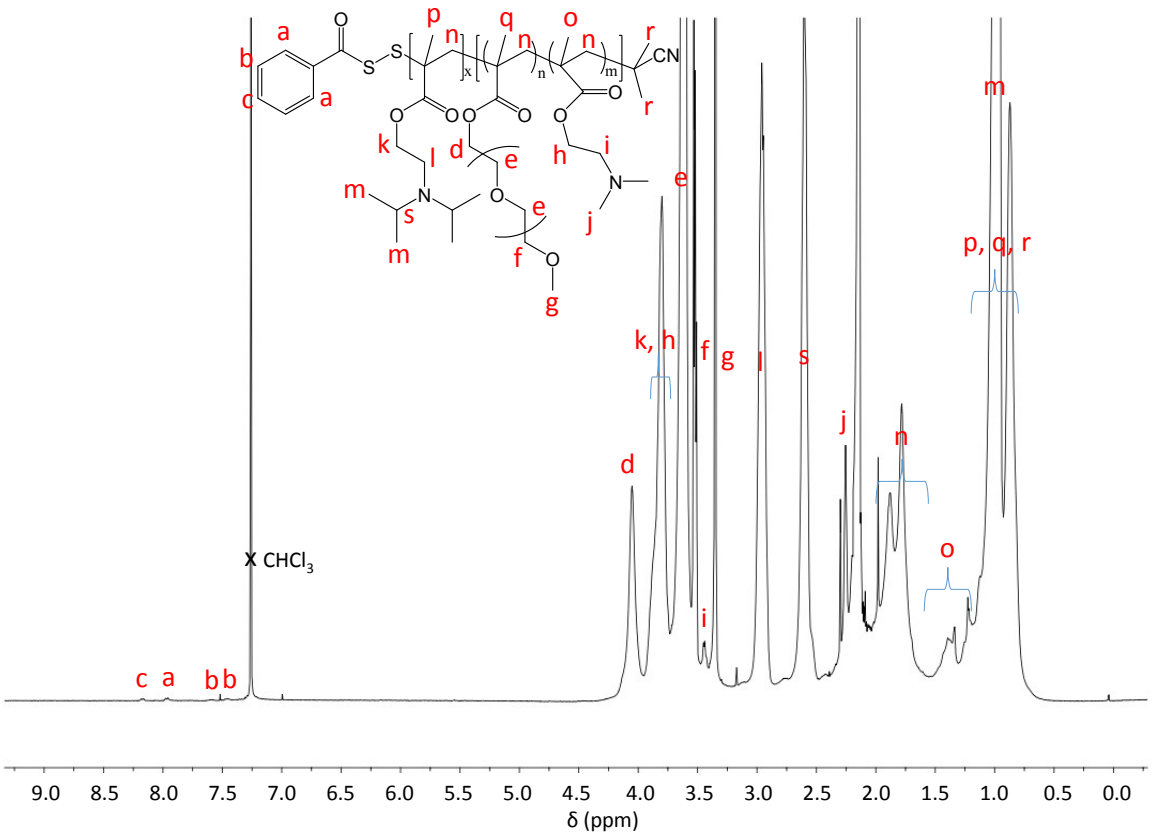


Figure S12. ^{1}H NMR spectrum of P[OEGMA-*co*-DMAEMA-*block*-DIPMA]-S-S-(C=O)Ph (**3c**, Table S1), recorded in $CDCl_3$ (400 MHz).

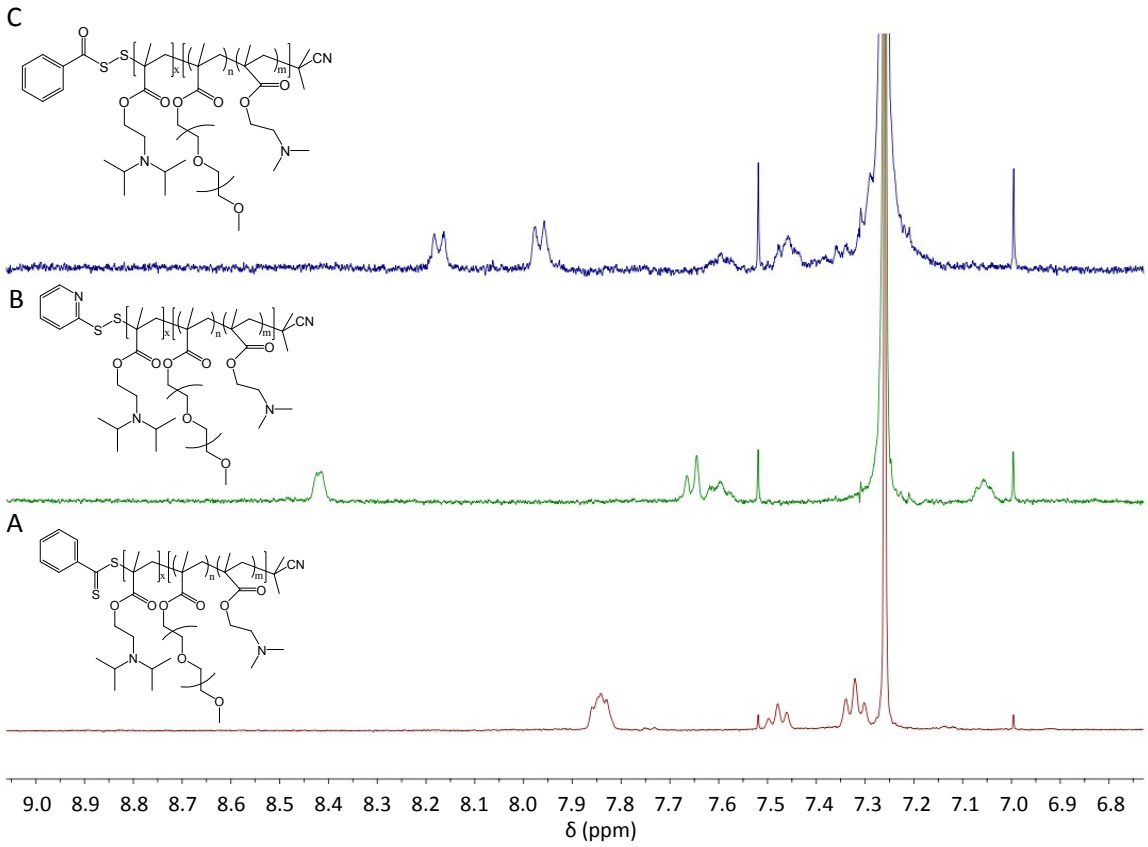


Figure S13. Enlarged ^{1}H NMR spectra (δ 9.0 - 6.8 ppm) corresponding to end-groups protons of (A) P[OEGMA-*co*-DMAEMA-*block*-DIPMA]-S(C=S)Ph, **3a** (B) P[OEGMA-*co*-DMAEMA-*block*-DIPMA]-S-S-Py, **3b** (C) P[OEGMA-*co*-DMAEMA-*block*-DIPMA]-S-S-(C=O)Ph, **3c**, recorded in $CDCl_3$ (400 MHz). Refer to Table S1 for GPC characterization of polymers.

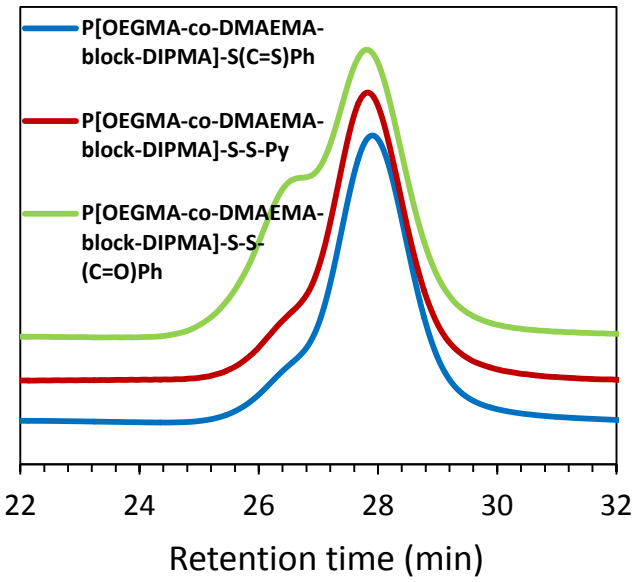


Figure S14. GPC chromatograms for P[OEGMA-*co*-DMAEMA-*block*-DIPMA]-S(C=S)Ph (**3a**, blue line), P[OEGMA-*co*-DMAEMA-*block*-DIPMA]-S-S-Py (**3b**, red line), P[OEGMA-*co*-DMAEMA-*block*-DIPMA]-S-S-(C=O)Ph (**3c**, green line). Refer to Table S1 for GPC characterisation of polymer.

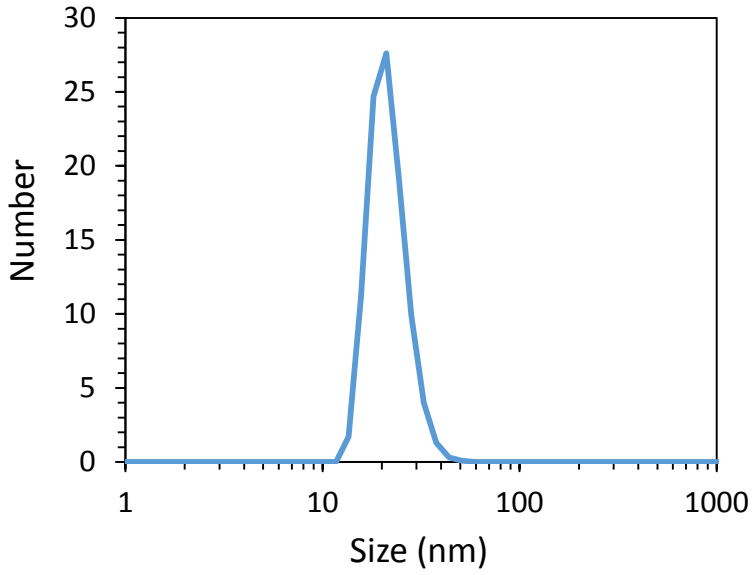


Figure S15. Size distribution (by number) for micelles of P[OEGMA-*co*-DMAEMA-*block*-DIPMA]-S-S-(C=O)Ph, **3c**, in phosphate buffered saline at pH 7.4, as determined by dynamic light scattering. The average diameter was 22 nm with PDI = 0.03.

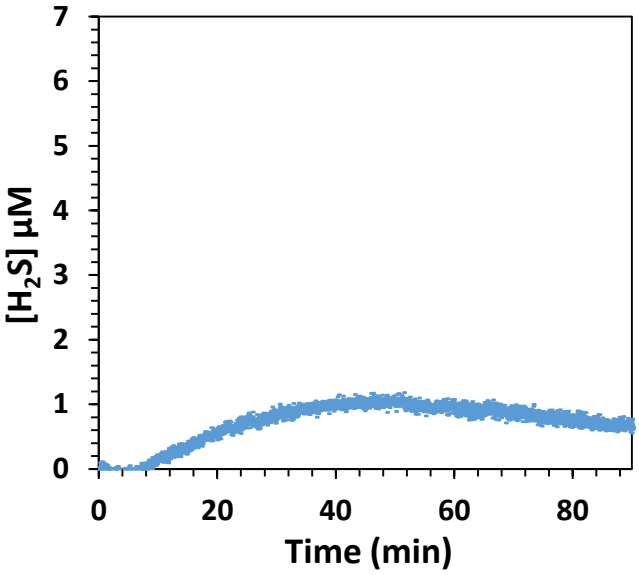
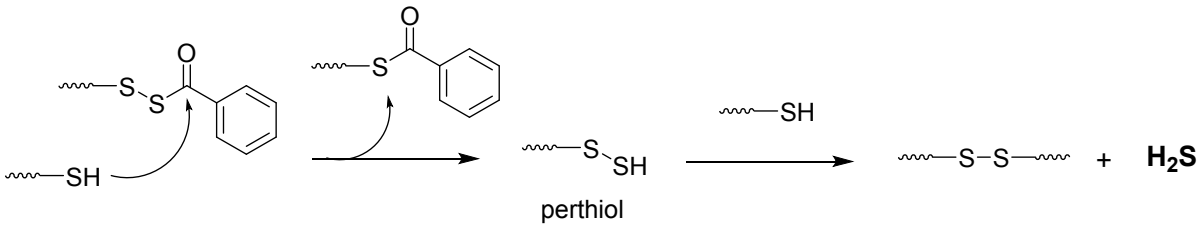


Figure S16. H_2S release from P[OEGMA-*block*-BMA]-S-S-(C=O)Ph, **2c**, (0.71 µmoles, 143 µM) in the presence of L-cysteine (33 mM, 100 µL, 3.3 µmoles). Note: L-cysteine was added at t = 2 min.

Table S2. Release characteristics of H_2S donor polymers, measured by Amperometry.

Polymer	L-cys[a] (Y/N)	pH[b]	P*eaking time*[c] (min)	P*eaking* $[H_2S]$[d] (μmol/L)	$[sulfides]_{tot}$[e] (μmol/L)	$[H_2S$ donors$]$[f] (μmol/L)	Sulfides (MPC) released[g] (%)
Chart S1, **1c**	Y	7.4	50	17	58	143	41
Chart S1, **2c**	Y	7.4	51	1	3.4	143	2.4
Chart S1, **3c**	Y	7.4	38	0.2	0.6	143	0.4
Chart S1, **3c**	Y	5.0	42	4.4	4.5	143	3.1

[a]Y = L-cysteine added (3.3 μmoles) during microsensor recording; N = L-cysteine not added. [b]Measured PBS pH condition. [c]Time taken to reach highest value of μmol/L on the curve of $[H_2S]$ vs time after L-cysteine added. [d]Highest μmol/L H_2S value attained on the curve of $[H_2S]$ vs time. [e]Total sulfides concentration = $[sulfides]_{tot} = [H_2S] + [HS^-]$, where $[sulfides]_{tot} = [H_2S] \times (10^{pH-pK1} + 1)$, (see supporting information C.6). [f]$[H_2S$ donors$] = n(H_2S$ donor polymers)/0.005, where 5mL = total volume in reaction vessel for microsensor testing. [g]% sulfides released (measured at peaking concentration) = $[sulfides]_{tot}/[H_2S$ donors$]$.



Scheme S1. Possible mechanism for formation of P[OEGMA]-S(C=O)Ph and P[OEGMA]-S-S-P[OEGMA] after H_2S is released from homopolymer P[OEGMA]-S-S-(C=O)Ph **1c**.

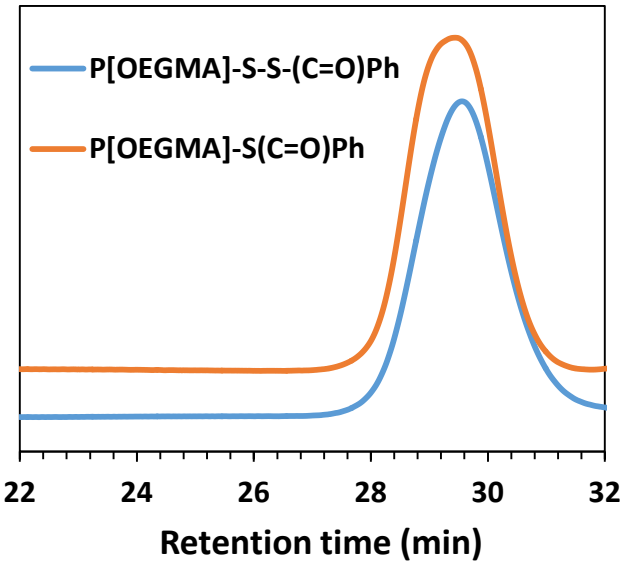


Figure S17. GPC chromatograms: blue line is P[OEGMA]-S-S-(C=O)Ph (**1c)** before exposure to L-cysteine; orange line is P[OEGMA]-S-S-(C=O)Ph (**1c)** after exposure to L-cysteine which forms a mixture of P[OEGMA]-S(C=O)Ph, P[OEGMA]-S-S-$C_3H_7NO_2$ and P[OEGMA]-S-S-P[OEGMA] (M_n = 9627 g mol^{-1} before and 10644 g mol^{-1} after treatment).

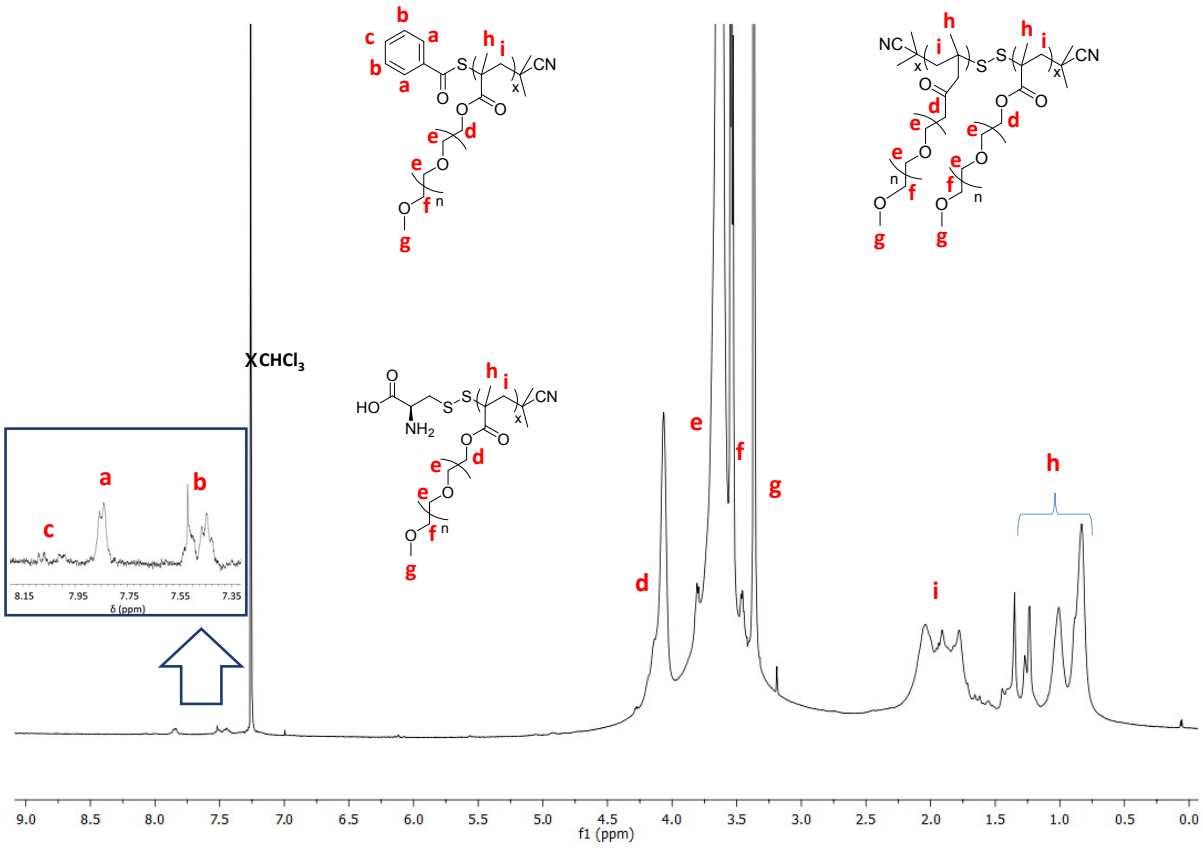


Figure S18. ^{1}H NMR spectrum of mixture of P[OEGMA]-S(C=O)Ph, P[OEGMA]-S-S-$C_3H_7NO_2$ and P[OEGMA]-S-S-P[OEGMA] after exposure of P[OEGMA]-S-S-(C=O)Ph, **1c**, to L-cysteine to release H_2S, recorded in $CDCl_3$ (400 MHz).

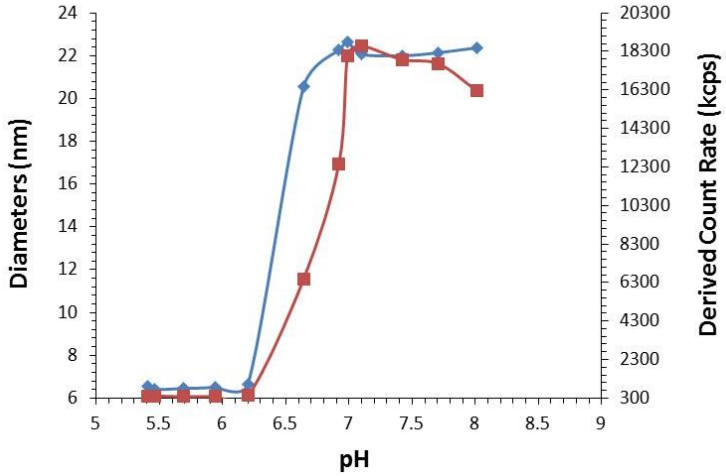


Figure S19. Hydrodynamic diameters (blue line) and derived count rate (red line) of P[OEGMA-*co*-DMAEMA-*block*-DIPMA]-S-S-(C=O)Ph, **3c**, self-assemblies as a function of solution pH.

References

(1) Yu, S. H.; Hu, J.; Ercole, F.; Truong N. P.; Davis, T. P.; Whittaker, M. R.; Quinn, J. F. *Macro Lett.*, **2015**, *4*, 1278.

(2) Ercole, F.; Mansfeld, F. M.; Kavallaris, M.; Whittaker, M. R.; Quinn, J. F.; Halls, M. L.; Davis, T. P. *Biomacromolecules*. **2016**, 17, 371.