Electronic Supplementary Material (ESI) for Polymer Chemistry


**Supporting Information:**

# Glutathione-Triggered Disassembly of Isothermally Responsive Polymer Nanoparticles obtained by Nanoprecipitation of Hydrophilic Polymers

Daniel J. Phillips, Joseph P. Patterson, Rachel K. O'Reilly and Matthew I. Gibson*

Department of Chemistry, University of Warwick, Coventry, CV4 7AL, UK

*Corresponding author

Phone: +44 247 652 4803

Fax: +44 247 652 4112

Email: m.i.gibson@warwick.ac.uk

## 1. Materials

All chemicals were used as supplied unless stated. Methanol, hexane, ethyl acetate, dichloromethane, toluene, acetone, 40-60 °C petroleum ether, tetrahydrofuran, diethyl ether and glacial acetic acid (analytical reagent grade) were all purchased from Fisher Scientific at laboratory reagent grade unless otherwise stated. Deuterated chloroform (99.9 atom % D), aldrithiol-2 (98.0 %), 2-mercaptoethanol (≥ 99.0 %), dodecane thiol (≥ 98.0 %), 2-(boc-amino) ethanethiol (97.0 %), tribasic potassium phosphate (reagent grade, ≥ 98.0 %), carbon disulfide, 2-bromo-2-methylpropionic acid (98.0 %), benzyl bromide (98.0 %), 4-dimethylaminopyridine (≥ 99.0 %), *N,N*'-diisopropylcarbodiimide (99.0 %), *N*-isopropylacrylamide (97.0 %), *N*-hydroxyethyl acrylamide (97.0 %), mesitylene (analytical standard), glutathione (99.0 % reduced) and 1,6-diphenyl-1,3,5-hexatriene (98.0 %) were all purchased and used as received from Sigma-Aldrich.

## 2. Analytical Methods

NMR spectroscopy ($^{1}H$, $^{13}C$) was conducted on a Bruker DPX-400, Bruker DRX-500,a Bruker AV III-600 or a Bruker AV II-700 spectrometer using deuterated chloroform as solvent. All chemical shifts are reported in ppm (δ) relative to tetramethylsilane (TMS).

High resolution mass spectra were recorded on a Bruker Electrospray Ultra-High Resolution tandem TOF mass spectrometer using electrospray ionization (ESI) in positive mode on samples prepared in methanol. Degraded sample mass spectral analysis was carried using a Bruker MaXis UHR-Q-TOF mass spectrometer using ESI in positive mode over a scan range of 500 – 7000 m/z. Samples were prepared in methanol, diluted 20-fold in 50:50 methanol: water and introduced by direct infusion at 90 $\mu L.hr^{-1}$. Source conditions were: end plate

offset at -500 V; capillary at -4500 V; nebulizer gas ($N_2$) at 1.6 bar; dry gas ($N_2$) at 8 L.min$^{-1}$; dry temperature at 180 °C. Ion tranfer conditions were: ion funnel RF at 400 Vpp; multiple RF at 400 Vpp; quadruple low mass set at 455 m/z; collision energy at 5.0 eV; collision RF at 1200 Vpp; ion cooler RF at 250-600 Vpp; transfer time set at 121 µs; pre-pulse storage time set at 15 µs. Calibration was completed with sodium formate (10mM). FTIR spectra were acquired using a Bruker Vector 22 FTIR spectrometer with a Golden Gate diamond attenuated total reflection cell. A total of 64 scans were collected on samples in their native (dry) state. UV-visible spectra were obtained using an Agilent Cary 60 UV-visible spectrophotometer at a "medium" scan-speed. SEC analysis was performed on a Varian 390-LC MDS system equipped with a PL-AS RT/MT autosampler, a PL-gel 3 µm (50 × 7.5 mm) guard column, two PL-gel 5 µm (300 × 7.5 mm) mixed-D columns using DMF with 5 mM $NH_3BF_4$ at 50 °C as the eluent at a flow rate of 1.0 mL min$^{-1}$. The GPC system was equipped with ultraviolet (UV) (set at 280 nm) and differential refractive index (DRI) detectors. Narrow molecular weight PMMA standards (200 - 1.0 × $10^6$ g mol$^{-1}$) were used for calibration using a second order polynomial fit. The cloud points were measured using an Optimelt MPA100 system (Stanford Research Systems). The recorded turbidimetry curve was normalized between values of 0 and 1. The cloud point was defined as the temperature corresponding to a normalized absorbance of 0.5. A polymer concentration of 0.5 mg.mL$^{-1}$ and a constant heating rate of 1 °C.min$^{-1}$ were used for all experiments. Fluorescence measurements were performed using a Biotech Synergy HT and processed using the Gen5 software package. Particle size analysis was determined by Dynamic Light Scattering using a Malvern Zetasizer Nano ZS instrument. A 4 mW He-Ne 633 nm laser module was used and scattered light was measured at 173° (back scattering). The attenuator and position was selected automatically by the instrument.

Cryogenic Transmission Electron Microscopy samples (0.5 mg/mL in water) were examined using a Jeol 2010F TEM operated at 200 kV and imaged using a GatanUltrascan 4000 camera. Images were captured using Digital Micrograph software (Gatan). A 3 μL droplet of the sample solution held at 50 °C was rapidly transferred to a holey carbon-coated copper grid, and the grid was blotted to remove excess solution. Subsequently, the grid was plunged into liquid ethane to vitrify the sample. The temperature of the cryogenic stage was maintained below -170 °C, using liquid nitrogen, during imaging. Where appropriate, particle size analysis was performed using ImageJ.

## 3. Synthetic Procedures

### 3.1. Synthesis of hydroxyethylpyridyl disulfide[1]

Aldrithiol-2 (10 g, 45.4 mmol) was dissolved in methanol (50 mL) and glacial acetic acid (1.5 mL) was then added. To this mixture, a solution of 2-mercaptoethanol (2.12 mL, 30.3 mmol) in methanol (10 mL) was added dropwise over 45 minutes with continuous stirring. After this time, the reaction mixture had turned bright yellow. The reaction mixture was left to stir overnight under ambient conditions. The solution was concentrated under vacuum to leave the crude product as a yellow oil. Purification by column chromatography (hexane: ethyl acetate, 85:15) yielded the title product as a pale yellow oil (3.40 g, 60%).

**$^{1}$H NMR** (400 MHz, $CDCl_3$) $\delta_{ppm}$: 8.50 (1H, d, $J_{1\text{-}2}$ = 5.04 Hz, $H^1$); 7.59 (1H, td, $J_{3\text{-}2}$, $J_{3\text{-}4}$ = 8.04 Hz $J_{3\text{-}1}$ = 2.00 Hz, $H^3$); 7.41 (1H, d, $J_{4\text{-}3}$ = 8.04 Hz, $H^4$); 7.15 (1H, ddd, $J_{2\text{-}3}$ = 8.04 Hz, $J_{2\text{-}1}$ = 5.04 Hz, $J_{2\text{-}4}$ = 1.00 Hz, $H^2$); 5.72 (1H, s, br., $H^7$); 3.81 (2H, t, $J_{6\text{-}5}$ = 5.28 Hz, $H^6$); 2.95 (2H, t, $J_{5\text{-}6}$ = 5.28 Hz, $H^5$).

**$^{13}$C NMR** (400 MHz, $CDCl_3$) $\delta_{ppm}$: 159.1 ($C^5$); 149.9 ($C^1$); 136.9 ($C^3$); 121.9 ($C^4$); 121.5 ($C^2$); 58.3 ($C^7$); 42.7 ($C^6$).

**FTIR** $cm^{-1}$: 3303 (br., O-H stretch); 3048 (aryl-H stretch); 2922 (alkyl C-H stretch).

**MS** (ESI +) m/z: 188.1 $[M+H]^+$; 210.0 $[M+Na]^+$

### 3.2 Synthesis of 2-(dodecylthiocarbonothioylthio)-2-methylpropanoic acid

2-(dodecylthiocarbonothioylthio)-2-methylpropanoic acid was prepared using a method similar to that already reported.[2] Dodecane thiol (4.00 g, 19.76 mmol) was added dropwise to a stirred suspension of tribasic potassium phosphate (4.20g, 19.76 mmol) in acetone (60 mL) over 25 minutes. Carbon disulfide (4.10 g, 53.85 mmol) was added and the solution turned bright yellow. After stirring for ten minutes 2-bromo-2-methylpropionic acid (3.00 g, 17.96 mmol) was added and a precipitation of KBr was noted. After stirring for 16 hours, the solvent was removed under reduced pressure and the residue was extracted into $CH_2Cl_2$ (2 x 200 mL) from 1M HCl (200 mL). The organic extracts were washed with water (200 mL) and brine (200 mL) and further dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica using an eluent comprising 75:24:1 40 – 60 °C petroleum ether: diethyl ether: acetic acid to yield a bright yellow solid (4.01 g, 61 %).

**$^1$H NMR** (500 MHz, $CDCl_3$) $\delta_{ppm}$: 3.22 (2H, t, $J_{12\text{-}11}$ = 7.55 Hz, $H^{12}$); 1.66 (6H, s, $H^{13}$); 1.60 (2H, p, $J_{11\text{-}10,\ 11\text{-}12}$ = 7.70 Hz, $H^{11}$); 1.31 (2H, p, $J_{10\text{-}9,\ 10\text{-}11}$ = 7.70 Hz, $H^{10}$); 1.24-1.19 (16H, m, $H^{2\text{-}9}$); 0.81 (3H, t, $J_{1\text{-}2}$ = 7.25 Hz, $H^1$).

**$^{13}$C NMR** (500 MHz, $CDCl_3$) $\delta_{ppm}$: 220.9 ($C^{13}$); 177.2 ($C^{16}$); 55.5 ($C^{14}$); 37.1 ($C^{12}$); 31.9, 29.7, 29.6, 29.6, 29.5, 29.4, 29.1, 22.7 ($C^{2\text{-}9}$); 29.0 ($C^{10}$); 27.8 ($C^{11}$); 25.3 ($C^{15}$); 14.1 ($C^1$).

**FTIR** $cm^{-1}$: 2955 (alkyl-H stretch); 1712 (C=O stretch); 1069 (S-(C=S)-S stretch).

**HRMS** (ESI +) m/z: 365.1632 $[M+H]^+$; expected 365.1637 ($C_{17}H_{33}O_2S_3$).

### 3.3 Synthesis of 2-(pyridyldisulfanyl) ethyl 2-(dodecylthiocarbonothioylthio)-2-methylpropanoate[1]

Hydroxyethyl pyridyl disulfide (1.61 g, 8.57 mmol), 2-(dodecylthiocarbonothioylthio)-2-methylpropanoic acid (2.50 g, 6.86 mmol) and 4-dimethylaminopyridine (0.25g, 2.06 mmol) were dissolved in dichloromethane (50 mL). *N,N'*-Diisopropylcarbodiimide (1.08 g, 8.57 mmol) was added to the solution under ice and dropwise over a period of 20 minutes. The mixture was left to warm to room temperature and stirred for 24 hours. The observed precipitate was removed by gravity filtration and the solvent concentrated *in vacuo*. The resulting yellow residue was purified by column chromatography using silica gel as the stationary phase and 49:1 hexane: ethyl acetate mixture as eluent to yield a yellow/orange oil (1.07 g, 29 % yield).

**$^{1}$H NMR** (600 MHz, $CDCl_3$) $\delta_{ppm}$: 8.47 (1H, d, $J_{19\text{-}18}$ = 4.14 Hz, $H^{19}$); 7.73 (1H, d, $J_{16\text{-}17}$ = 7.86 Hz, $H^{16}$); 7.66 (1H, t, $J_{17\text{-}16,\ 17\text{-}18}$ = 7.71 Hz, $H^{17}$); 7.10 (1H, t, $J_{18\text{-}17,\ 18\text{-}19}$ = 6.24 Hz, $H^{18}$); 4.36 (2H, t, $J_{14\text{-}15}$ = 6.36 Hz, $H^{14}$); 3.27 (2H, t, $J_{12\text{-}11}$ = 7.56 Hz, $H^{12}$); 3.03 (2H, t, $J_{15\text{-}14}$ = 6.36 Hz, $H^{15}$); 1.70 (3H, s, $H^{13}$); 1.65 (2H, p, $J_{11\text{-}12,\ 11\text{-}10}$ = 7.50 Hz, $H^{11}$); 1.37 (2H, p, $J_{10\text{-}11,\ 10\text{-}9}$ = 7.50 Hz, $H^{10}$); 1.23-1.31 (16 H, m, $H^{2\text{-}9}$); 0.88 (3H, t, $J_{1\text{-}2}$ = 6.96 Hz, $H^{1}$).

**$^{13}$C NMR** (600 MHz, $CDCl_3$) $\delta_{ppm}$: 221.5 ($C^{13}$); 177.8 ($C^{16}$); 159.9 ($C^{19}$); 149.7 ($C^{23}$); 137.1 ($C^{21}$); 120.8 ($C^{22}$); 119.8 ($C^{20}$); 63.4 ($C^{17}$); 55.8 ($C^{14}$); 37.2 ($C^{18}$); 37.0 ($C^{12}$); 31.9, 29.7, 29.6, 29.6, 29.4, 29.3, 29.1, 22.7 ($C^{2\text{-}9}$); 28.9 ($C^{10}$); 27.9 ($C^{11}$); 25.3 ($C^{15}$); 14.1 ($C^{1}$).

**FTIR** $cm^{-1}$: 3049 (aryl-H stretch); 2923 (alkyl-H stretch); 1735 (C=O stretch); 1063 (S-(C=S)-S stretch).

**HRMS** (ESI +) m/z: 534.1661$[M+H]^+$; expected 534.1657 ($C_{24}H_{40}NO_2S_5$).

### 3.4 Synthesis of benzyl 2-[(*tert*-butoxycarbonyl)amino]ethyl trithiocarbonate

2-(Boc-amino)ethanethiol (1.00 g, 5.64 mmol) was added dropwise to a stirred suspension of tribasic potassium phosphate (1.32 g, 6.21 mmol) in acetone (20 mL) over 25 minutes. Carbon disulfide (1.29 g, 16.90 mmol) was added and the solution turned bright yellow. After stirring for ten minutes benzyl bromide (0.97 g, 5.64 mmol) was added. After stirring for 16 hours, the solvent was removed under reduced pressure and the residue was purified by column chromatography on silica using a 40 – 60 °C petroleum ether – ethyl acetate gradient to yield a bright yellow solid (1.20 g, 62 %).

**$^{1}$H NMR** (700 MHz, $CDCl_3$) $\delta_{ppm}$: 1.47 (9H, s, $H^1$); 3.46 (2H, t, $J_{5\text{-}6}$ = 6.44Hz, $H^5$); 3.57 (2H, t, $J_{6\text{-}5}$ = 6.44 Hz, $H^6$); 4.64 (2H, s, $H^8$); 4.84 (1H, s, br., $H^4$); 7.29 - 7.37 (5H, m, $H^{10\text{-}12}$).

**$^{13}$C NMR** (700 MHz, $CDCl_3$) $\delta_{ppm}$: 223.3 ($C^7$); 155.9 ($C^3$); 135.0 ($C^9$); 129.4, 128.9, 128.0 ($C^{10\text{-}12}$); 79.9 ($C^2$); 41.8 ($C^8$); 39.3 ($C^5$); 36.7 ($C^6$); 28.5 ($C^1$).

**FTIR** $cm^{-1}$: 3368 (N-H stretch); 2975, 2922, 2873 (Alkyl C-H stretch); 1681 (C=O stretch); 1523 (Aryl C=C stretch); 1070 (C=S stretch).

**HRMS** (ESI +) m/z: 366.0623 $[M+Na]^+$; expected 366.0627 ($C_{15}H_{21}NO_2S_3Na$).

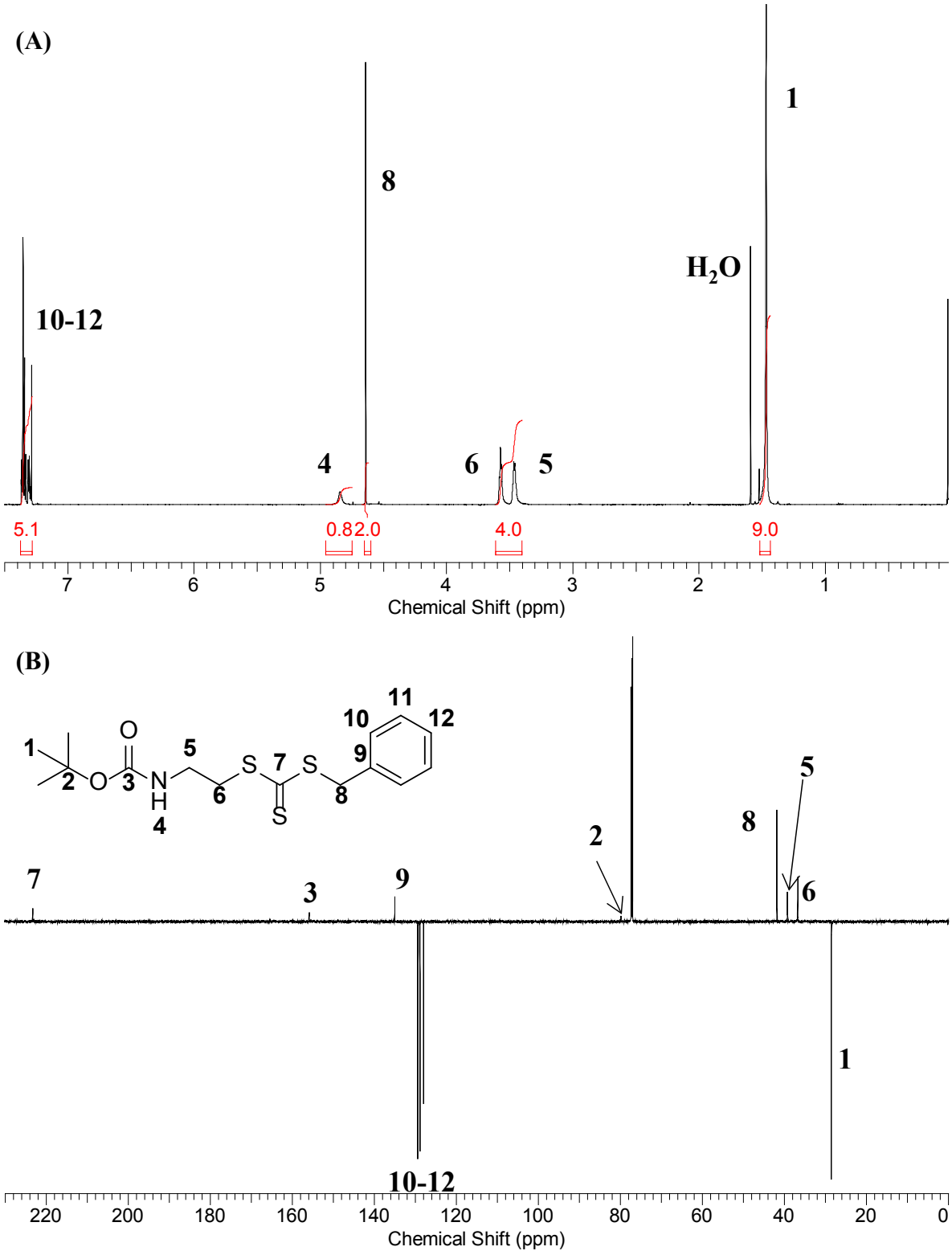



**Figure S1.** A) $^{1}H$ NMR and B) $^{13}C$ NMR spectra of benzyl 2-[(*tert*-butoxycarbonyl)amino]ethyl trithiocarbonate

### 3.5 Synthesis of poly(*N*-isopropylacrylamide) using 2-(pyridyldisulfanyl) ethyl 2-(dodecylthiocarbonothioylthio)-2-methylpropanoate

In a typical procedure *N*-isopropylacrylamide (2.16 g, 19.09 mmol), 2-(pyridyldisulfanyl) ethyl 2-(dodecylthiocarbonothioylthio)-2-methylpropanoate (20.90 mg, 39.15 μmol) and 4,4'-azobis(4-cyanovaleric acid) (1.79 mg, 6.39 μmol) were dissolved in methanol: toluene (1:1) (4 mL) in a glass vial containing a stir bar. Mesitylene (100 μL) was added as an internal reference and the mixture stirred (5 mins). An aliquot of this starting mixture was removed for $^1$H NMR analysis. The vial was fitted with a rubber septum and degassed by bubbling with nitrogen gas (30 mins). The vial was then placed in an oil bath thermostated at 70 °C. After 3 hours the reaction mixture was opened to air and quenched in liquid nitrogen. An aliquot was removed and conversion determined by $^1$H NMR. The product was purified three times by precipitation from tetrahydrofuran into cold diethyl ether, isolated by centrifugation and dried under vacuum overnight to give a pale yellow solid. The overall monomer conversion was determined from the $^1$H NMR spectrum by measuring the decrease in intensity of the vinyl peaks associated with the monomer relative to mesitylene. Conversion (NMR): 73.3 %; $M_n$ (theoretical): 40400 g.mol$^{-1}$; $M_n$ (SEC) 25400 g.mol$^{-1}$; $M_w/M_n$ (SEC): 1.24.

### 3.6 Synthesis of poly(*N*-isopropylacrylamide) using benzyl 2-[(*tert*-butoxycarbonyl)amino]ethyl trithiocarbonate

*N*-isopropylacrylamide (1.00 g, 8.84 mmol), benzyl 2-[(*tert*-butoxycarbonyl)amino]ethyl trithiocarbonate (12.80 mg, 37.26 μmol) and 4,4'-azobis(4-cyanovaleric acid) (2.10 mg, 7.49 μmol) were dissolved in methanol: toluene (1:1) (4 mL) in a glass vial containing a stir bar. Mesitylene (200 μL) was added as an internal reference and the mixture stirred (5 mins). An aliquot of this starting mixture was removed for $^1$H NMR analysis. The vial was fitted with a

rubber septum and degassed by bubbling with nitrogen gas (30 mins). The vial was then placed in an oil bath thermostated at 70 °C. After 1.5 hours the reaction mixture was opened to air and quenched in liquid nitrogen. An aliquot was removed and conversion determined by $^1$H NMR. The product was purified three times by precipitation from tetrahydrofuran into cold diethyl ether, isolated by centrifugation and dried under vacuum overnight to give a pale yellow solid. The overall monomer conversion was determined from the $^1$H NMR spectrum by measuring the decrease in intensity of the vinyl peaks associated with the monomer relative to mesitylene. Conversion (NMR): 80.0 %; $M_n$ (theoretical): 21500 g.mol$^{-1}$; $M_n$ (SEC) 18000 g.mol$^{-1}$; $M_w/M_n$ (SEC): 1.13.

**3.7 Synthesis of poly(*N*-isopropylacrylamide-*co*-*N*-hydroxyethyl acrylamide)**

In a typical procedure *N*-isopropylacrylamide (1.25 g, 11.05 mmol), *N*-hydroxyethyl acrylamide (66.7 mg, 579.35 µmol), 2-(pyridyldisulfanyl) ethyl 2-(dodecylthiocarbonothioylthio)-2-methylpropanoate (14.5 mg, 27.16 µmol) and 4,4'-azobis(4-cyanovaleric acid) (2.50 mg, 8.92 µmol) were dissolved in methanol: toluene (1:1) (2.7 mL) in a glass vial containing a stir bar. Mesitylene (200 µL) was added as an internal reference and the mixture stirred (5 mins). An aliquot of this starting mixture was removed for $^1$H NMR analysis. The vial was fitted with a rubber septum and degassed by bubbling with nitrogen gas (30 mins). The vial was then placed in an oil bath thermostated at 70 °C. After 40 minutes the reaction mixture was opened to air and quenched in liquid nitrogen. An aliquot was removed and conversion determined by $^1$H NMR. The product was purified four times by precipitation from tetrahydrofuran into cold diethyl ether, isolated by centrifugation and dried under vacuum overnight to give a pale yellow solid. The overall monomer conversion was determined from the $^1$H NMR spectrum by measuring the decrease in intensity of the vinyl peaks associated with the monomer relative to mesitylene. Conversion

(NMR): 79.9 % (NIPAM); 89.2 % (HEA); $M_n$ (theoretical): 38700 g.mol$^{-1}$; $M_n$ (SEC) 56900 g.mol$^{-1}$; $M_w/M_n$ (SEC): 1.31.

## 4. Assay Conditions

### 4.1 DLS Assay

Nanoparticles (570 μL) were transferred to a low volume, disposable polycarbonate cuvette and incubated at 50 °C in the Zetasizer Nano ZS for 30 minutes. After this time 30 μL of a concentrated glutathione solution was added to give the desired final glutathione concentration. A measurement was recorded every 30 seconds (5 scans x 5 seconds).

### 4.2 Nanoparticle Fluorescence Assay

A generalised procedure for the fluorescence assay is as follows. Nanoparticles (190 μL) were incubated in a 96-well plate at 50 °C for 1 hour. 10 μL water or concentrated glutathione solution was added to give final glutathione concentrations of 0, 0.01 and 1 mM and the fluorescence monitored over a period of 2 hours. Excitation wavelength set at 360 nm, emission wavelength at 460 nm and the plate maintained at 50 °C throughout.

### 4.3 UV-Visible Spectrophotometric Experiments

For each polymer tested, 2.5 mL of a 0.5 mg.mL$^{-1}$ polymer solution was transferred to three disposable, polycarbonate cuvettes and a UV-visible spectrum recorded. Water or concentrated glutathione solution was added to give final glutathione concentrations of 0, 0.01 and 1 mM. The cuvettes were stirred at ambient conditions and their UV-visible spectra re-recorded after one hour and twenty hours.

## 5. Additional Data

### 5.1 Polymer Details

**Table S1** Polymers prepared in this study

| Polymer[a] | [NIPAM]:[HEA]:[CTA] | Conversion[b] (%) | $M_{n,Theo}$[d] (g.mol$^{-1}$) | $M_{n,SEC}$[e] (g.mol$^{-1}$) | $M_w/M_n$[e] (-) | CP - 0M GSH[f] (°C) | CP - 1 mM GSH[f] (°C) |
|---|---|---|---|---|---|---|---|
| pNIPAM-1 | 488:0:1 | 73.3 | 40500 | 25400 | 1.24 | 31.5 | 34.5 |
| $pNIPAM_{98}$-*co*-$HEA_2$ | 439:9:1 | 84.8/$N_d$[c] | - | 60300 | 1.31 | 33.5 | 33.5 |
| $pNIPAM_{95}$-*co*-$HEA_5$ | 404:21:1 | 79.9/89.2 | 38700 | 56900 | 1.31 | 35.3 | 37.1 |
| $pNIPAM_{90}$-*co*-$HEA_{10}$ | 407:45:1 | 76.1/88.8 | 39600 | 45600 | 1.25 | 38.2 | 39.3 |

[a]NIPAM = *N*-isopropylacrylamide, HEA = *N*-Hydroxyethyl acrylamide; [b]x/y = % conversion NIPAM/HEA (where appropriate); [c]Nd = Not determined due to low intensity of NMR peaks preventing quantitative determination. This polymer was not used further in the study; [d]Determined by $^1$H NMR relative to an internal standard (mesitylene); [e]Determined by SEC (DMF inc. 5 mM $NH_3BF_4$) relative to PMMA standards; [f]CP = Cloud Point at polymer concentration of 0.5 mg.mL$^{-1}$.

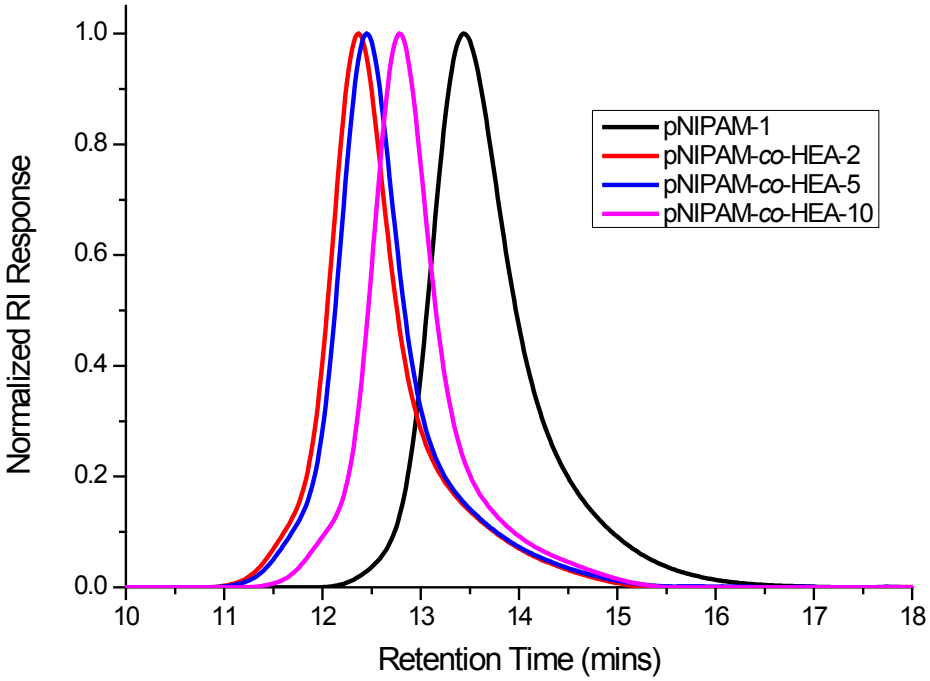



**Figure S2** SEC Characterization of polymers prepared in this study determined using DMF inc. 5 mM $NH_3BF_4$ as eluent, relative to PMMA standards.

## 5.2 Additional Cryogenic Transmission Electron Microscopy Images and Data Analysis

A variety of the cryo-TEM images taken of the nanoparticles prepared from pNIPAM-1 (Figure S3) together with data analysis (Figure S4) are shown below. Each individual image contained relatively few nanoparticles, so multiple images were used to assemble the histogram. Example cryo-TEM images (Figure S5A) and the DLS data (Figure S5B) for nanoparticles prepared from pNIPAM$_{95}$-*co*-HEA$_5$ are also shown.

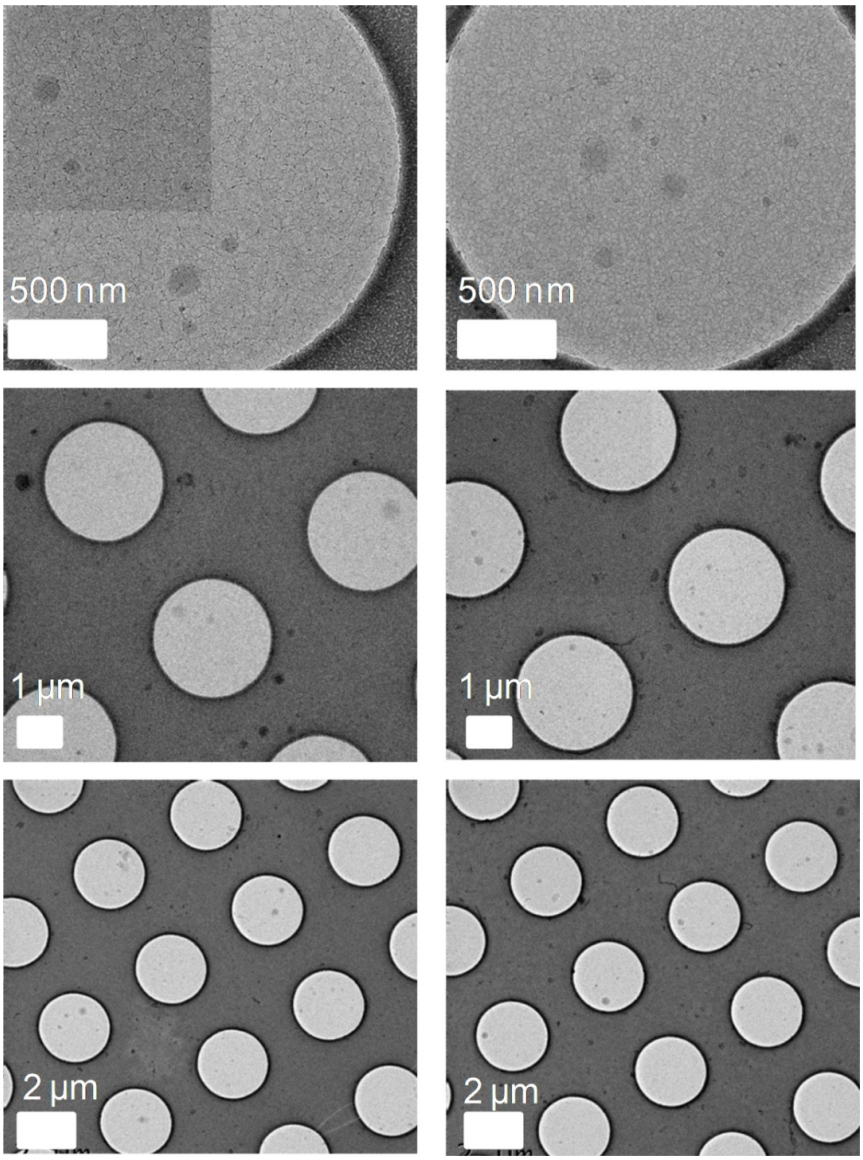



**Figure S3** Nanoparticles prepared from pNIPAM-1. Some discontinuity was noticed in the ice layer which has been previously attributed to over blotting.[3]

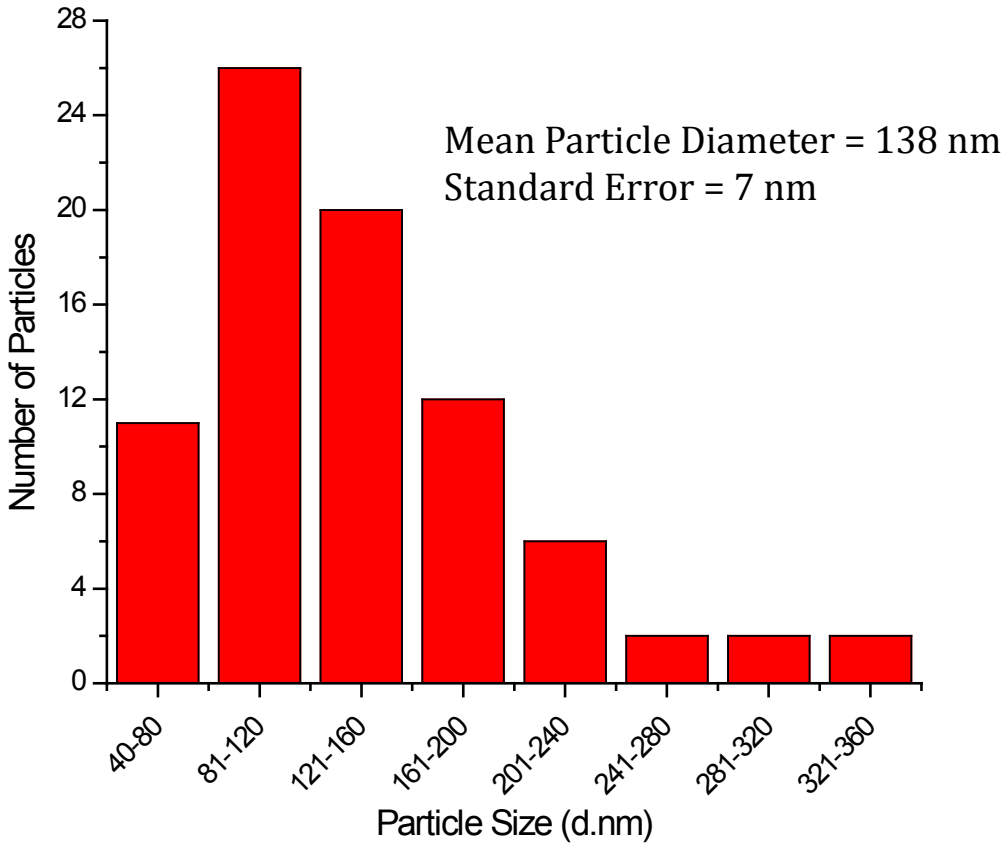



**Figure S4** Particle size analysis of nanoparticles prepared from pNIPAM-1. The particle size ($D_n$) of particles identified in cryo-TEM images was determined using ImageJ. The size distribution can be used to determine $D_w$ ($\sum D^2/\sum D$) = 168 nm or $D_z$ ($\sum D^6/\sum D^5$) = 281 nm size averages which are more appropriate for comparison with the DLS result of $D_H$ = 200 nm.

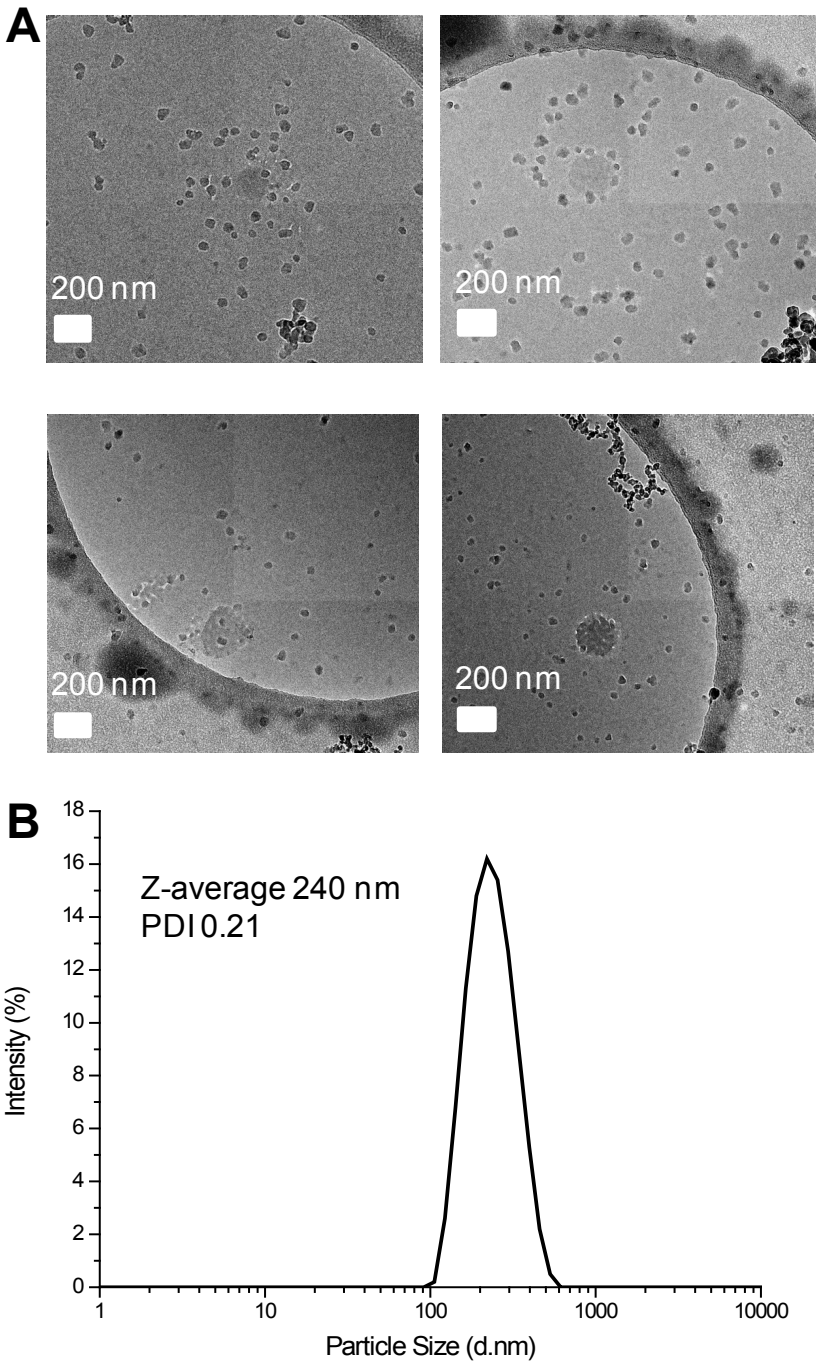
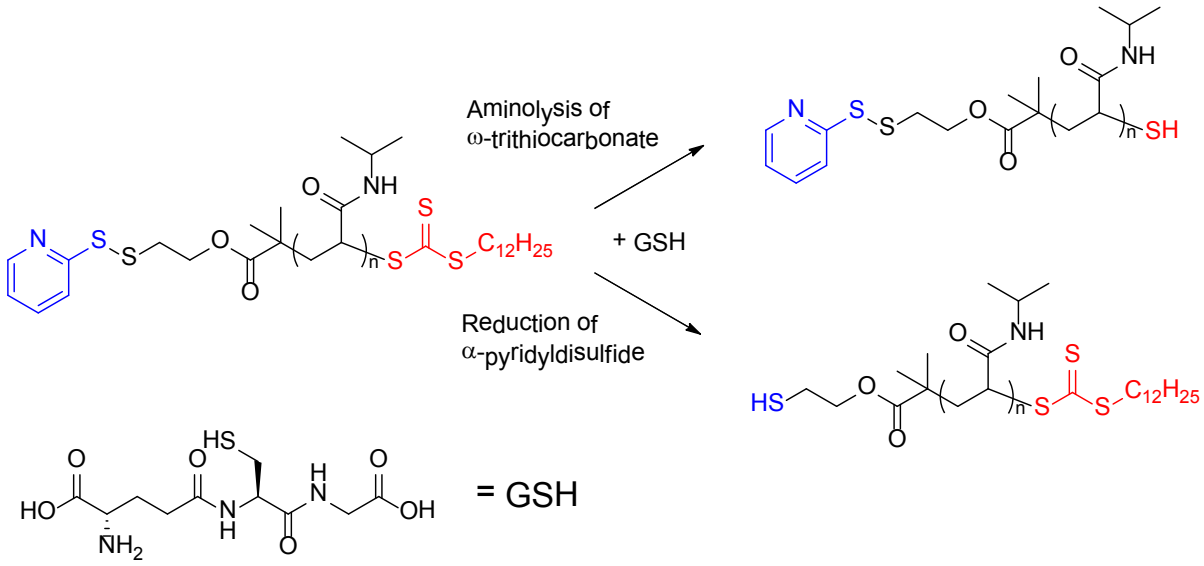



**Figure S5** (A) Cryogenic Transmission Electron Microscopy (magnification = 20000x) and (B) Dynamic Light Scattering characterization of nanoparticles prepared from pNIPAM$_{95}$-*co*-HEA$_5$. Both analysis techniques indicated the presences of particles of *ca.* 200 nm. For the cryo-TEM images the smaller objects are crystalline ice contamination on the surface of the vitrified ice layer where the 200 nm particles are embedded.

### 5.3 Stability of RAFT-Derived Trithiocarbonate End-Group to GSH.

GSH contains two functional groups capable of potentially altering the structure of a RAFT-derived polymer: (i) reduction of the pyridyl disulfide α-end-group by the cysteine residue and (ii) aminolysis of the trithiocarbonate ω-end-group by the glutamic acid residue (Scheme S1). The later reaction seems unlikely given the likelihood of GSH existing as a zwitterion in aqueous solutions. Hence, the amino group will be protonated (pKa ($NH_2$) = 8.75) and rendered non-nucleophilic.




**Scheme S1.** Potential reactions of glutathione with RAFT-derived pNIPAM.

To confirm that the isothermal particle disassembly process was triggered solely by the reduction reaction, additional UV-visible spectrophotometry experiments were performed given the trithiocarbonate end-group and pyridine thione (by-product of the reaction of a thiol with pyridyl disulfide) have distinct peaks in the UV-visible spectrum ($\lambda_{max}$ = 309 nm and 343 nm respectively). Thus, the UV-Vis spectra of PNIPAM-2 and PNIPAM-3 (see Table S2/Figure S6 for polymer details/SEC characterization), two polymers of similar molecular weights containing a trithiocarbonate end-group and one (PNIPAM-3) containing a pyridyl

disulfide end-group, were obtained as a function of GSH concentration at the same concentration (0.5 mg.mL$^{-1}$) at which the particles were prepared (Figure S6).

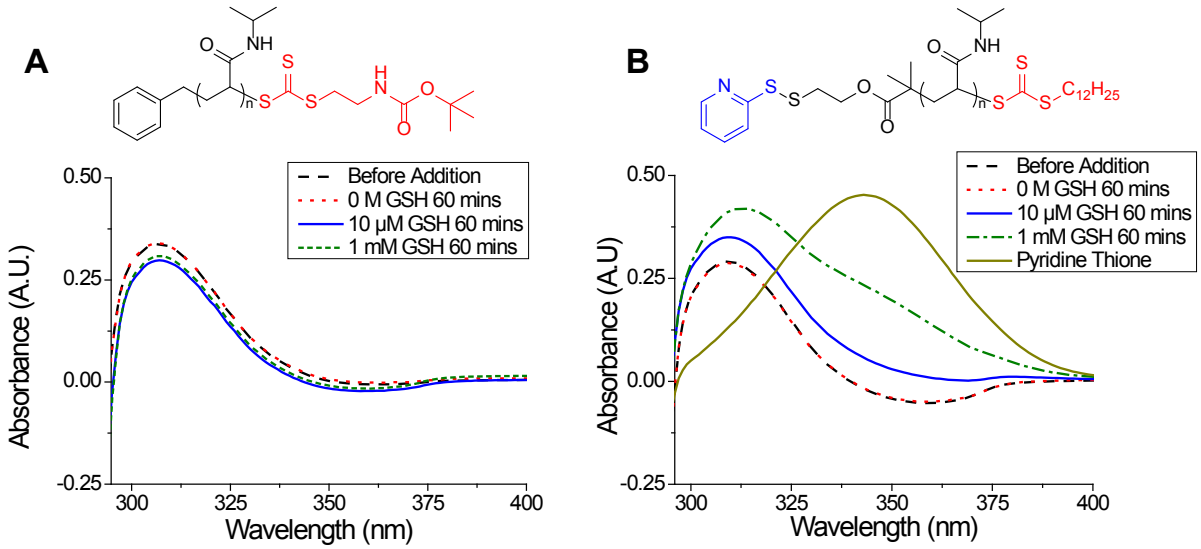



**Figure S6.** UV-visible spectra as a function of GSH concentration of (A) PNIPAM-2 and (B) PNIPAM-3. Concentration = 0.5 mg.mL$^{-1}$

At all concentrations of GSH there was no significant change in absorbance at 309 nm for either polymer after 1 hour indicating that the trithiocarbonate end-group is stable to aqueous solutions of GSH. The small increase in absorbance at 309 nm observed in Figure S4B can be attributed to the release of pyridine thione, whose spectrum exhibits some absorbance overlap with that of the trithiocarbonate. Spectra obtained after 20 hours revealed no additional change.

**Table S2** Polymers used for UV-Visible spectrophotometry experiments

| Polymer[a] | [M]:[CTA] | Conversion/%[b] | Theo. $M_n$ ( $^1$H NMR) | $M_n$ (SEC)[c] | Mw/Mn [c] |
|---|---|---|---|---|---|
| PNIPAM-2 | 237:1 | 80.0 | 21500 | 18000 | 1.13 |
| PNIPAM-3 | 450:1 | 27.4 | 14000 | 19200 | 1.19 |

[a]NIPAM = *N*-isopropylacrylamide; [b]Determined by $^1$H NMR relative to an internal standard (mesitylene); [c]Determined by SEC (DMF inc. 5 mM $NH_3BF_4$) relative to PMMA standards

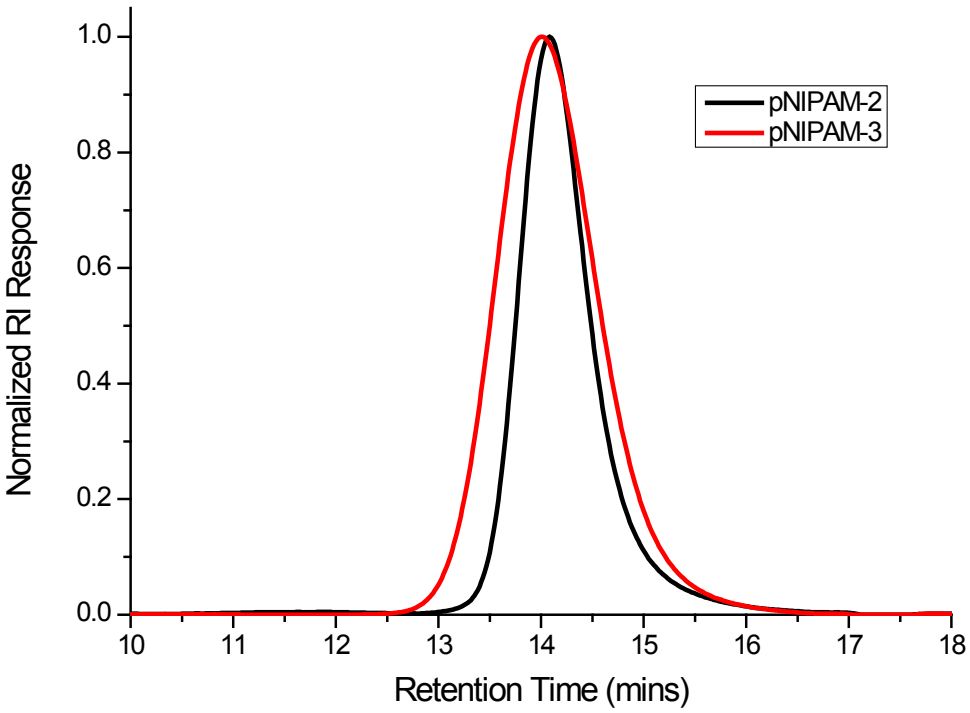



**Figure S5** SEC Characterization of polymers used for UV-visible spectrophotometry experiments. Determined using DMF inc. 5 mM $NH_3BF_4$ relative to PMMA standards.

### 5.4 Additional Fluorescence Experiment Figures

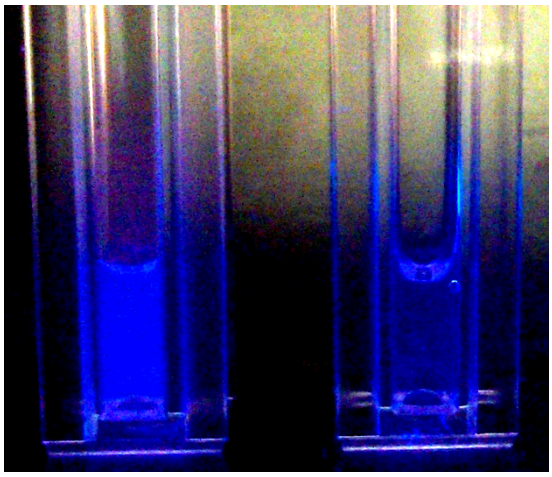


**Figure S6.** Side-by-side photos showing fluorescence quenching upon DPH release from nanoparticles due to action of glutathione (Figure 5 in Main Text).

### References

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[3] Cui, H.; Hodgdon, T. K.; Kaler, E. W.; Abezgauz, L.; Danino, D.; Lubovsky, M.; Talmon, Y.; Pochan, D. J. *Soft Matter* **2007,** *3,* 945-955.