Electronic Supporting Information for:

“Isothermal” LCST Transitions Triggered by Bioreduction of Single Polymer End Groups.

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Experimental Section

Materials

All chemicals were used as supplied unless otherwise stated. Acetone, dichloromethane (DCM), diethyl ether, 1,4-dioxane (analytical reagent grade), ethyl acetate, glacial acetic acid (analytical reagent grade), hexane, methanol, 40-60% petroleum ether, tetrahydrofuran (THF) and toluene were all supplied by Fisher Scientific at laboratory reagent grade unless otherwise stated. Aldrithiol-2 (98%), 2-bromo-2-methylpropionic acid (98%), carbon disulfide (≥ 99.9%), deuterated chloroform (99.9 atom % D) di(ethyleneglycol) methyl ether methacrylate (DEGMA) (95%), 1-dodecanethiol (≥ 98%), ethanolamine (≥ 98%), L-glutathione reduced (≥ 99.8%), mesitylene (≥ 99.8%), 2-mercaptoethanol (≥ 99%), N,N’-diisopropylcarbodiimide (99%), potassium phosphate tribasic (≥ 98%), oligo-(ethyleneglycol) methyl ether methacrylate (OEGMA<sub>475</sub>), sodium carbonate anhydrous (≥ 99%), sodium chloride (≥ 99%), 1-thioglycerol (≥ 97%), thionyl chloride (97%), tributylphosphine (97.0%), triethylamine (≥ 99%) and tris(2-carboxyethyl)phosphine hydrochloride (≥ 98%) and 2,2’-Azobis(2-methylpropionitrile) (AIBN) were all supplied by Sigma-Aldrich.

Physical and Analytical Methods

NMR spectroscopy (<sup>1</sup>H,<sup>13</sup>C) measurements were conducted on Bruker DPX-300, DRX-500 or AV III-600 spectrometers using deuterated chloroform as solvent. All chemical shifts are reported in ppm (δ) relative to tetramethylsilane (TMS). Mass spectral analyses were recorded on an Esquire2000 mass spectrometer using electrospray ionisation (ESI) in positive mode. FTIR spectra were acquired using a Bruker Vector 22 FTIR spectrometer with a Golden Gate diamond attenuated total reflection cell. 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 held at 30 °C and the instrument equipped with a differential refractive index and a Shimadzu SPD-M20A diode array detector. Tetrahydrofuran or N,N-Dimethylformamide (including 2% triethylamine) was used as the eluent at a flow rate of 1 mL min⁻¹. Data was analysed using Cirrus 3.2 software and molecular weight determined relative to narrow molecular weight PMMA standards (200 -1.0 × 10⁶ g mol⁻¹). The cloud point was measured using an Optimelt MPA100 system (Stanford Research Systems). The recorded turbidimetry curve was normalised between values of 0 and 1. The transition temperature was defined as that corresponding to a normalised absorbance of 0.5. A constant heating rate of 1 °C min⁻¹ was used in all experiments and resulting curves were smoothed when plotted. Kinetic turbidity plots were obtained using a BioTek Synergy HT microplate reader, with constant temperature UV readings taken at 650 nm.

Procedures

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\begin{align*}
\text{i)} & \quad C_{11}H_{23}SH + C_{12}S + \text{Br-CO-OH} \xrightarrow{K_3PO_4} C_{12}H_{25}S\backslash S\backslash O\text{COOH} \\
\text{ii)} & \quad \text{N-S-S-N} + \text{HO-SH} \xrightarrow{} \text{HO-S-S-N} + \text{N-S} \\
\text{iii)} & \quad C_{12}H_{25}S\backslash S\backslash O\text{COOH} \xrightarrow{i)} \frac{O}{\text{Cl-S-Cl}} \xrightarrow{ii)} \frac{\text{HO-S-S-N}}{C_{12}H_{25}S\backslash S\backslash O\text{CO-}} \xrightarrow{\text{S-N}}
\end{align*}
\]

**Scheme S1** - Synthetic strategy for the synthesis of pyridyl disulfide-functionalised RAFT agent; PADE (Propanoic acid, 2-[[[(dodecylthio)thioxomethyl]thio]-2-methyl-2-(2-pyridinyl)dithio)ethyl ester).
Synthesis of hydroxyethyl pyridyl disulfide (HEPDS).

Aldrithiol-2 (9.99 g, 45 mmol) was dissolved in methanol (60 mL). Glacial acetic acid (1 mL) was added and the solution stirred for 10 min. To this solution, with stirring, 2-mercaptopropanoic acid (1.54 mL, 22.5 mmol) in methanol (10 mL) was added dropwise over 10 min. The reaction was left for 20 hours to give a yellow solution. The solvent was removed under reduced pressure to yield a yellow oil. The product was purified by column chromatography, using a 1:1 mixture of hexane and ethyl acetate as eluent to yield a pale yellow oil (2.73 g, 66.3% yield.)

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ ppm: 8.54 (1H, dd, $J_{1,2} = 5.5$ Hz, $J_{1,3} = 0.66$ Hz, H$_1$); 7.61 (1H, dt, $J_{3,2,3,4} = 7.8$ Hz, $J_{3,1} = 1.2$ Hz, H$_3^3$); 7.43 (1H, dd, $J_{4,3} = 8.0$ Hz, $J_{4,2} = 0.65$ Hz, H$_3^4$); 7.18 (1H, ddd, $J_{2,3} = 7.5$ Hz, $J_{2,1} = 5.0$ Hz, $J_{2,4} = 1.2$ Hz, H$_3^2$); 5.75 (1H, s, broad, H$_7^1$); 3.83 (2H, t, $J_{6,5} = 5.0$ Hz, H$_5^6$); 2.98 (2H, t, $J_{5,6} = 5.1$ Hz, H$_5^6$)

$^{13}$C NMR (500 MHz, CDCl$_3$) $\delta$ ppm: 159.11 (C$_5^5$); 149.89 (C$_1^1$); 136.85 (C$_3^3$); 122.00-121.55 (C$_2^2$,C$_4^4$); 58.21 (C$_7^7$); 42.75 (C$_6^6$)

IR cm$^{-1}$: 3300 (broad, OH); 3040 (aryl-H); 2915 (alkyl-H)

MS (ESI+) m/z: 188.0 [M+H]$^+$; 210.0 [M+Na]$^+$

Synthesis of 2-(dodecylthiocarbonothioylthio)-2-methylpropanoic acid (DMPA).

To a suspension of tribasic potassium phosphate (8.39 g, 39.52 mmol) in acetone, 1-dodecanethiol (9.5 mL, 39.52 mmol) was added and the solution stirred for 30 minutes. Carbon disulfide (6.5 mL, 107.79 mmol) was added and the solution turned bright yellow. After stirring for a further 20 minutes, 2-bromo-2-methylpropionic acid (6 g, 35.93 mmol) was added and the mixture left to stir for 21 hours. After this time the solution contained a yellow precipitate. The solvent was removed under reduced vacuum and the residue
dissolved in 1M HCl (400 mL) and extracted with DCM (2 x 400 mL). The organic extracts were combined and washed with water (1 x 400 mL), brine (1 x 400 mL) and dried over magnesium sulphate. The solvent was removed under reduced vacuum leaving a yellow oil which crystallised on standing. The compound was dissolved in DCM and purified by column chromatography using a petroleum ether, diethyl ether gradient. The product fractions were combined and the solvent removed under reduced vacuum to yield the title product as a yellow solid (4.24 g, 32.4% yield)

**1H NMR** (600 MHz, CDCl₃) δ ppm: 3.28 (2H, t, J₁₂₋₁₁₋₁₀ = 7.4 Hz, H¹²); 1.72 (6H, s, H¹³); 1.67 (2H, p, J₁₁₋₁₂,₁₁₋₁₀ = 7.4 Hz, H¹¹); 1.38 (2H, p, J₁₀₋₁₁₋₁₀₋₉ = 8 Hz, H¹⁰); 1.28 (16H, m, J=7.2 Hz); 0.88 (3H, t, J₁₋₂ = 7.1 Hz, H¹)

**13C NMR** (600 MHz, CDCl₃) δ ppm: 220.78 (C¹³); 178.88 (C¹⁶); 55.56 (C¹⁴); 37.07 (C¹²); 31.92-28.89 (C³⁻¹⁰); 27.81 (C¹¹); 24.41 (C¹⁵); 22.69 (C²); 14.13 (C¹)

**IR** cm⁻¹: 3100-3000 (broad)(Carboxylic OH); 2953.6 (aryl C-H); 2915.2 (alkyl C-H); 2848.0 (alkyl C-H); 1705.6 (Carboxylic C=O)

**MS** (ESI+) m/z: 365.0 [M+H]⁺; 387.1 [M+Na]⁺

**Synthesis of Propanoic acid, 2-[[dodecylthio]thioxomethyl]thio]-2-methyl-2-(2-pyridinyl)dithio)ethyl ester (PADE).**

To a stirred solution of DMPA (1.50 g, 4.11 mmol) in DCM (20 mL) thionyl chloride (1.5 mL, 20.6 mmol) was added over 30 minutes. The liberation of gasses was observed. After 4 hours of stirring the solution was concentrated under reduced vacuum. The resulting yellow oil was redissolved in DCM (5 mL) and to this, a solution of HEPDS (0.877 g, 4.68 mmol) and triethylamine (0.65 mL, 4.68 mmol) in DCM (15 mL) was added dropwise at 0 °C. The mixture was left to stir for 24 hours, after which time a white precipitate was observed. The
solution was washed twice with a sodium carbonate solution and four times with water, using a drop of saturated brine solution to promote separation. The organic layer was concentrated and purified by column chromatography using a 90:10 hexane: ethyl acetate mixture as eluent. The product containing band (as ascertained by thin layer chromatography) was collected and repurified on a column of basic alumina, prepared in ethyl acetate. Concentration under vacuum of the resulting solution produced the title product as a yellow oil (91 mg, 4% yield).

$^1$H NMR (500 MHz, CDCl$_3$) δ ppm: 8.47 (1H, dd, J$_{1-2}$ = 4.8 Hz, J$_{1-3}$ = 0.83 Hz, H$^1$); 7.73 (1H, dd, J$_{4-3}$ = 8.1 Hz, J$_{4-2}$ = 0.66 Hz, H$^4$); 7.66 (1H, dt, J$_{3-2,3-4}$ = 7.8 Hz, J$_{3-1}$ = 1.3 Hz, H$^3$); 7.10 (1H, ddd, J$_{2-3}$ = 7.4 Hz, J$_{2-1}$ = 4.8 Hz, J$_{2-4}$ = 1.0 Hz, H$^2$); 4.36 (2H, t, J$_{6-5}$ = 6.2 H$^6$); 3.27 (2H, t, J$_{8-9}$ = 6.3 Hz, H$^8$); 3.03 (2H, t, J$_{5-6}$ = 6.3 H$^5$); 1.70 (6H, s, H$^7$); 1.65 (2H, p, J$_{9-8,9-10}$ = 7.3 H$^9$); 1.37 (2H, p, J$_{10-9,10-11}$ = 7.8 Hz, H$^{10}$); 1.25 (16H, m, H$^{11-18}$); 0.88 (3H, t, J$_{19-18}$ = 6.5 Hz, H$^{19}$)

$^{13}$C NMR (600 MHz, CDCl$_3$) δ ppm: 221.49 (C$^{11}$); 172.80 (C$^8$); 159.91 (C$^5$); 149.69 (C$^1$); 137.08 (C$^3$); 120.77-119.77 (C$^2$,C$^4$); 63.37 (C$^9$); 55.80 (C$^6$); 37.23 (C$^6$); 37.01 (C$^{12}$); 31.92-28.52 (C$^{14-21}$); 27.86 (C$^{13}$); 25.35 (C$^{10}$); 22.69 (C$^{22}$); 14.13 (C$^{23}$)

IR cm$^{-1}$: 2944.4 (aryl C-H); 2876.1 (alkyl C-H); 1573.0 (Ester C=O)

MS (ESI+) m/z: 534.0 [M+H]$^+$; 556.1 [M+Na]

**Polymerisation of Poly(ethylene glycol) Methyl Ether Methacrylate**

Poly(ethylene glycol) methyl ether methacrylate$_{475}$ (0.32 g, 0.67 mmol), Di(ethylene glycol) methyl ether methacrylate$_{2}$ (1.46 g, 7.75 mmol) 2-[[(dodecylthio)thioxomethyl]thio]-2-methyl-2-(2-pyridinyldithio)ethyl ester (45 mg, 84.3 µmol) and 4,4’-azobis(4-cyanovaleric acid) (4.72 mg, 16.8 µmol) were dissolved in dioxane (2 mL) in a Schlenk tube fitted with a rubber septum and containing a stir bar. The mixture stirred (5 mins). An aliquot of this starting
mixture was removed for $^1$H NMR analysis. The sample was degassed by a minimum of 3 freeze-pump-thaw cycles and back filled with nitrogen. The vial was then placed in an oil bath thermostated at 70 °C. After 4 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 dioxane into diethyl ether, isolated by centrifugation and dried under vacuum overnight to give a waxy, yellow solid. Conversion (NMR) 66%; $M_n$ (theoretical) 14200 g mol$^{-1}$; $M_n$ (THF-SEC) 28000 g mol$^{-1}$; $M_w/M_n$ (THF-SEC) 1.4.

**General Procedure for the Polymerisation of N-isopropylacrylamide**

N-isopropylacrylamide (0.25 g, 2.21 mmol), 2-(pyridyldisulfanyl) ethyl 2-(dodecylthiocarbonothioylthio)-2-methylpropanoate (59.0 mg, 11.05 µmol) and 4,4’-azobis(4-cyanovaleric acid) (6.19 mg, 2.21 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 4 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 toluene into diethyl ether, isolated by centrifugation and dried under vacuum overnight to give a yellow solid. Conversion (NMR): 91.7%; $M_n$ (theoretical): 2100 g mol$^{-1}$; $M_n$ (SEC) 2000; $M_w/M_n$ (SEC): 1.18.

**Functionalisation of terminal pyridyl disulfide groups with thiol.**
A solution (5 mg.mL\(^{-1}\)) of polymer in water was prepared. To this, thiol (10 eq.) was added and the solution stirred for 2 hours at ambient conditions. Excess thiol was removed by dialysis, and the polymer isolated by lyophilisation.

**Glutathione-mediated polymer degradation.**

End functionalised disulfide containing aqueous polymer solutions were prepared giving a concentration of 5 mg.mL\(^{-1}\). Aliquots of this solution (190 µL) were placed in separate wells of a 96-well microplate and to these, varying concentrations of glutathione solution were added (10 µL), so that the final concentrations were 5 µM, 50 µM, 500 µM and 5 mM and made up to a total volume of 200 uL with water. Absorbance was measured as a function of time at 30 °C for 120 minutes.
Characterisation data for synthesis of RAFT agent (PADE).

Hydroxyethyl pyridyl disulfide

Figure S1 – $^1$H (top) and $^{13}$C(lower) NMR spectra
2-(dodecylthiocarbonothioylthio)-2methylpropanoic acid $^1$H NMR

Figure S2 – $^1$H (top) and $^{13}$C(lower) NMR spectra
Propanoic acid, 2-[[dodecylthio]thioxomethyl]thio]-2-methyl-2-(2-pyridinylthio)ethyl ester

Figure S3 – $^1$H (top) and $^{13}$C(lower) NMR spectra
Additional Results

Effect of glutathione on cloud point.

\textbf{P1b} (POEGMA with thioglycerol end group) incubated with indicated concentrations of glutathione and turbidimetry profile obtained.

\textbf{Figure S4.} Turbidimetry profile of P1b as a function of glutathione concentration. [Polymer] = 5 mg.mL\(^{-1}\). Glutathione concentration indicated inset into graph.