Electronic Supporting Information for:

Degradable Thermoresponsive Polymers which Display Redox-Responsive LCST Behaviour.

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Experimental

General

All chemicals were used as supplied unless stated. Methanol, hexane, ethyl acetate, dichloromethane, toluene, acetone, 40-60 °C petroleum ether, tetrahydrofuran, glacial acetic acid (analytical reagent grade) were all purchased from Fischer Scientific at laboratory reagent grade unless otherwise stated. Deuterated chloroform (99.9 atom % D), dithranol (≥ 98.5%), sodium trifluoracetate (≥ 98.5%), 2,2'-dithiodipyridine (≥ 99.0%), 2-mercaptoethanol (≥ 99.0%), 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (≥ 97%), N, N'-diisopropylcarbodiimide (99.0%), 4,4'-azobis(4-cyanovaleric acid) (≥ 98.0%), N-isopropylacrylamide (97.0%), ethanolamine (≥ 99.0%), tributyl phosphine (97.0%), triethylamine (≥ 99.0%), tris(2-carboxyethyl)phosphine hydrochloride (≥ 98.0%) and mesitylene (analytical standard) were all purchased from Sigma-Aldrich. Fresh ovine (defibrinated) erythrocytes were supplied by TCS Biosciences Ltd UK. The as-supplied Erythrocyte suspension was centrifuged (1950 x g, 5 min, 25 °C) and the top layer (containing any plasma and its constituents) removed and replaced with an equivalent volume of PBS. When not in use erythrocytes were stored in this form at 4 °C.

Analytical and Physical Methods

NMR spectroscopy (\(^1\)H, \(^{13}\)C) was conducted on a Bruker DPX-300 spectrometer 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. MALDI-ToF (matrix-assisted laser desorption ionisation time-of-flight mass spectrometry) was obtained using a Bruker Daltonics Ultraflex II MALDI-ToF mass spectrometer,
equipped with a nitrogen laser delivering 2 ns laser pulses at 337 nm. Positive ion ToF detection was performed using an accelerating voltage of 25 kV. Samples were prepared by mixing dithranol (2 μL of a 10 mg.mL$^{-1}$ solution), sodium trifluoroacetate (2 μL of a 10 mg.mL$^{-1}$ solution) and the analyte solution (2 μL of a 10 mg.mL$^{-1}$ solution). 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 (including 2% triethylamine) was used as the eluent at a flow rate of 1 mL.min$^{-1}$. Data were analysed using Cirrus 3.2 software and molecular weight determined relative to narrow molecular weight PMMA standards (200 - 1.0 × 10$^6$ 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. Cloud point analysis of disulfide-containing polycondensation polymers was performed on a Perkin Elmer Lambda-35 UV/Vis spectrometer at 550 nm. Glass cuvettes with a 1 cm path length were used and a heating rate of 1 °C.min$^{-1}$ was applied.
Procedures

Hydroxyethyl pyridyl disulfide  2,2’-dithiodipyridine (2.50 g, 11 mmol) was dissolved in methanol (15.9 mL). Glacial acetic acid (0.23 mL) was then added. To this mixture, a solution of 2-mercaptoethanol (0.51 mL, 7.5 mmol) in methanol (6.8 mL) was added dropwise over 45 minutes with continuous stirring. 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. Separation by column chromatography (hexane:ethyl acetate, 50:50) yielded the title product as a pale yellow oil. Isolated yield 0.66 g, 32%.

\[^1H\] NMR (CDCl\textsubscript{3}) δ ppm: 8.49 (1H, ddd, \(J_{1-2} = 4.97\) Hz, \(J_{1-3} = 1.71\) Hz, \(J_{1-4} = 0.90\) Hz, \(H^1\)); 7.56 (1H, td, \(J_{3-1} = 1.80\) Hz, \(J_{3-2, 3-4} = 7.54\) Hz, \(H^3\)); 7.38 (1H, dt, \(J_{4-1, 4-2} = 0.90\) Hz, \(J_{4-3} = 8.04\) Hz, \(H^4\)); 7.14 (1H, ddd, \(J_{2-1} = 5.00\) Hz, \(J_{2-3} = 7.38\) Hz, \(J_{2-4} = 1.02\) Hz, \(H^2\)); 5.75 (1H, s, br., \(H^7\)); 3.78 (2H, t, \(J_{6-5} = 5.15\) Hz, \(H^6\)); 2.93 (2H, t, \(J_{5-6} = 5.21\) Hz, \(H^5\)).

\[^{13}C\] NMR (CDCl\textsubscript{3}) δ ppm: 159.08 (C\textsuperscript{5}); 149.89 (C\textsuperscript{1}); 136.89 (C\textsuperscript{3}); 122.01-121.58 (C\textsuperscript{2}, C\textsuperscript{4}); 58.17 (C\textsuperscript{7}); 42.70 (C\textsuperscript{6}).

IR cm\textsuperscript{-1}: 3306 (br., O-H); 3043 (aryl-H); 2917 (alkyl C-H).

MS (ESI +) m/z: 188.1 [M+H]\textsuperscript{+}; 210.0 [M+Na]\textsuperscript{+}. 
Figure S1. NMR characterisation of hydroxyethyl pyridyl disulfide: (a) $^1$H and (b) $^{13}$C.
2-(pyridyldisulfanyl)ethyl 4-cyano-4-(phenylcarbonothioylthio)pentanoate. 4-
cyano-4-(phenylcarbonothioylthio)pentanoic acid (0.48 g, 172 mmol) was dissolved
in dichloromethane (3 mL) and added to a round bottom flask containing a stir bar. A
solution of hydroxyethyl pyridyl disulfide (0.66g, 352 mmol) in dichloromethane (17
mL) was added. N, N’-diisopropylcarbodiimide (0.69 mL, 442 mmol) was added to
the solution dropwise over a period of 5 minutes. The flask was covered with
aluminium foil and the mixture left to stir at ambient conditions for 40 hours. The
solution was concentrated under vacuum and purified by column chromatography
(dichloromethane inc. 5% methanol). Further purification by column chromatography
(hexane:ethyl acetate 70:30) was required. The product was dried under vacuum
(overnight) leaving the title product as a pink solid. Isolated yield 0.12 g, 16%.

$^1$H NMR (CDCl$_3$) $\delta$ppm: 8.47 (1H, m, H$_1$); 7.88 (2H, dt, $J_{10-12}$ = 1.33 Hz, $J_{10-11}$ = 7.31
Hz, H$_{10}$); 7.65 (2H, m, H$_2$, H$_3$); 7.55 (1H, dt, $J_{12-11}$ = 1.65 Hz, $J_{12-10}$ = 7.44 H$_{12}$); 7.38
(2H, tt, $J_{11-10}$ = 7.35 Hz, $J_{11-12}$ = 1.62 Hz, H$_{11}$); 7.10 (1H, m, H$_4$); 4.35 (2H, t, $J_{6,5}$ =
6.36 Hz, H$_6$); 3.04 (2H, t, $J_{5,6}$ = 6.33 Hz, H$_5$); 2.63 – 2.40 (4H, m, H$_7$, H$_8$); 1.91 (3H,
s, H$_9$).

$^{13}$C NMR (CDCl$_3$) $\delta$ppm: 171.24 (C$_8$); 163.87 (C$_5$); 149.76 (C$_4$); 144.53 (C$_{15}$); 137.19
(C$_3$); 133.08 (C$_{18}$); 128.61 (C$_{17}$); 126.70 (C$_{16}$); 121.03 (C$_2$); 120.03 (C$_4$); 118.47 (C$_{13}$);
62.78 (C$_7$); 45.71 (C$_{11}$); 37.17 (C$_6$); 33.33 (C$_{10}$); 29.71 (C$_9$); 24.20 (C$_{12}$).

IR cm$^{-1}$: 3055 (aryl C-H); 2932 (alkyl C-H); 1737 (C=O).

HRMS (ESI +) m/z: 471.0302 [M+Na]$^+$; expected 471.0300
Figure S2. NMR characterisation of 2-(pyridyl disulfanyl)ethyl 4-cyano-4-(phenylcarbonothioylthio)pentanoate: (a) $^1$H and (b) $^{13}$C (note: peaks corresponding to solvent are not labelled on $^{13}$C NMR for clarity).
Figure S3. HRMS of 2-(pyridylsulfanyl)ethyl 4-cyano-4-(phenylcarbonothioylthio)pentanoate. Top = observed spectrum; bottom = predicted spectrum for [C$_{20}$H$_{20}$N$_2$O$_2$S$_4$Na]$^+$. 
General procedure for the polymerisation of N-isopropylacrylamide with 2-(2-cyanopropyl) dithiobenzoate. N-isopropylacrylamide (1.0 g, 8.85 mmol), 2-(2-cyanopropyl) dithiobenzoate (10.0 mg, 88.5 μmol) and 4,4’-azobis(4-cyanovaleric acid) (4.96 mg, 17.7 μmol) were added to a Schlenk tube fitted with stir bar and rubber septum and dissolved in methanol:toluene (50:50) (3 mL). Mesitylene (0.35 mL) was added as internal reference and the mixture stirred (5 mins). An aliquot of this starting mixture was removed for $^1$H NMR analysis. The mixture was degassed by three freeze-pump-thaw cycles, the vessel back-filled with nitrogen and placed in an oil bath thermostated at 70 °C. After 24 hours, the reaction mixture had turned orange and was quenched in liquid nitrogen. An aliquot was removed and conversion determined by $^1$H NMR. The product was purified three times by precipitation from acetone into 40-60 °C petroleum ether and the solid isolated by centrifugation. The solid was dried under vacuum to yield a pale pink/orange solid. Conversion (NMR): 65.8%; $M_n$ (theoretical): 7400 g.mol$^{-1}$; $M_n$ (SEC): 9800 g.mol$^{-1}$; $M_w/M_n$ (SEC): 1.37.

$^1$H NMR (CDCl$_3$) δ ppm: 7.90 (H$_7$); 7.55 (H$_8$); 7.35 (H$_9$); 5.85-6.65 (H$_4$); 3.8-4.2 (H$_5$); 2.0-2.4 (H$_3$); 1.32 (H$_1$); 1.00-1.20 (H$_6$).

![Structure of polyNIPAM](image)

Figure S4. Structure of polyNIPAM
Polymerisation of \( N \)-isopropylacrylamide with 2-(pyridyldisulfanyl)ethyl 4-cyano-4-(phenylcarbonothioylthio)pentanoate. \( N \)-isopropylacrylamide (500.0 mg, 4.42 mmol), 4,4’-azobis(4-cyanvaleric acid) (10.0 mg, 88.5 \( \mu \)mol) and 2-(pyridyldisulfanyl)ethyl 4-cyano-4-(phenylcarbonothioylthio)pentanoate (79.0 mg, 0.18 mmol) were added to a Schlenk tube equipped with stir bar and rubber septum and dissolved in methanol:toluene (50:50) (1.8 mL). Mesitylene (0.18 mL) was added as an internal standard and the mixture stirred (5 mins). A control-sample was removed and analysed by \(^1\)H NMR. The mixture was degassed three times by freeze-pump thaw, the vessel back-filled with nitrogen and placed in an oil bath thermostated at 70 \( ^\circ \)C for 52 hours. The reaction was quenched in liquid nitrogen and a sample removed and analysed by \(^1\)H NMR to determine conversion. Purification was performed as per the method described in section 2.3.3 to yield the title product as a waxy orange substance. SEC was conducted directly on the polymer and also following addition of 1 drop of tributylphosphine to remove the end groups as described in the main paper. Conversion (NMR): 19.8\%; \( M_n \) (SEC): 1300 g.mol\(^{-1}\); \( M_w/M_n = 1.87 \) (SEC). Following addition of tributyl phosphine: \( M_n \) (SEC): 1300 g.mol\(^{-1}\); \( M_w/M_n = 1.35 \) (SEC)

General Procedure for the Polycondensation of Telechelic Poly(\( N \)-isopropylacrylamide). A 40 mg.mL\(^{-1}\) solution of polymer in tetrahydrofuran was prepared. In a Schlenk tube equipped with a stir bar and rubber septum, an aliquot of the polymer solution (4 mg, 2.51 \( \mu \)mol) was degassed three times by freeze-pump-thaw. A separate solution containing ethanolamine (2.51 \( \mu \)mol) and triethylamine (5.02 \( \mu \)mol) was also degassed by three freeze-pump thaw cycles. The amine solution
was added to the polymer solution by a syringe purged with dry nitrogen, and the solution left to stir at room temperature for 24 hours. After this time, the mixture had turned from orange to yellow and become viscous. A sample of this mixture was analysed by SEC. $M_n$ (SEC): 3600 g.mol$^{-1}$; $M_w/M_n = 9.3$ (SEC).

**Measurement of Erythrocyte Haemolysis.** A 500 μL aliquot of erythrocytes was added to 500 μL of the polymer in PBS and mixed by inversion. The samples were incubated at 25 °C for 120 minutes before analysis. Haemolysis was measured as described below, with all measurements completed in triplicate.

A 40 μL aliquot of the treated erythrocyte solution was added to 400 μL PBS and centrifuged (1000 x g, 5 min, 4 °C) to remove intact cells. 50 μL of the supernatant is added to 150 μL PBS in a 96-well plate and an absorbance measurement at 450 nm recorded to assess the extent of haemoglobin leakage. 100% haemolysis samples were prepared by osmotic shock through addition of 500 μL H$_2$O to 500 μL erythrocytes suspension and the sample was vortexed vigorously. Control (0% haemolysis) samples were prepared by the addition of 500 μL PBS to 500 μL erythrocytes and left at room temperature (25 °C) for 60 minutes.

% Haemolysis was determined using Equation 1.

**Equation 1.** 

\[
\% \text{ Haemolysis} = 100\% \left( \frac{I_{T_{\text{raw}}} - I_{\text{Background}}}{I_{\text{water}}} \right)
\]
PolyNIPAM Synthesis

Well defined poly(NIPAM) was required to investigate its LCST behaviour as a function of chain length and was obtained by using 2-(2-cyanopropyl) dithiobenzoate, CPDB, as the RAFT agent with 4,4'-azobis(4-cyanovaleric acid), ACVA, as the chain transfer agent as shown in Scheme S1.

![Scheme S1. RAFT polymerization of NIPAM using CPDB and ACVA as RAFT agent and radical source, respectively. Conditions shown in Table S1.](attachment:image)

The progress of the polymerization was monitored by $^1$H NMR relative to an internal standard, mesitylene. Conversion could be determined by the decrease in the intensity of the vinyl peaks associated with the monomer, as shown in Figures S5 and S6. All polymerizations reached 70% conversion after approximately 50 hours reaction at 70 °C. Table S1 shows the molecular characteristics of the polyNIPAMs and Figure S7 shows the SEC traces. The data indicates that the obtained polymers were well defined, with low polydispersity indices.
Figure S5. $^1$H NMR spectra showing decrease in vinyl peaks during polymerisation, compared to the normalised mesitylene reference peak.

Figure S6. Monomer conversion verses time for the polymerisation of NIPAM, using the indicated ratios of monomer to RAFT agent. Reactions conducted using conditions shown in Table S1.
Table S1. Results of the polymerisation of NIPAM using CPDB as RAFT agent

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction Time/hrs</th>
<th>[M]:[CTA]</th>
<th>Conversion/%a</th>
<th>Theoretical $M_n$ g.mol$^{-1}$b</th>
<th>$M_w/M_n$ c</th>
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<tr>
<td>1</td>
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<td>100</td>
<td>65.8</td>
<td>7400</td>
<td>9800</td>
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<td>24</td>
<td>25</td>
<td>36.3</td>
<td>1000</td>
<td>1700</td>
</tr>
</tbody>
</table>

Solvent, 1:1 Methanol: Toluene, 3 mL. (a) Determined by $^1$H NMR, (b) Determined by $^1$H NMR and the monomer: CTA feed ratio, (c) Determined by SEC (THF inc. 2% TEA).

Figure S7. SEC analysis of polymers in Table S1.
Thermoresponsive Behaviour of PolyNIPAMs

Turbidimetry was used to determine the cloud points of the different polymers. The cloud point was defined as being the temperature where the normalised absorbance was half the maximum (0.5 in the graphs used here). The curves used to calculate these are shown in Figure S8 below.

Figure S8. Turbidimetry curves for pNIPAMs shown in Table S1.
Synthesis of pyridyl disulfide functionalised RAFT agent

The synthesis of 2-(pyridyl-disulfanyl) ethyl 4-cyano-4-(phenylcarbonothioylthio) pentanoate, a new RAFT agent, is shown in Scheme S2. The product was fully characterised by $^1$H and $^{13}$C NMR and high resolution mass spectrometry.

Scheme S2. Synthetic route for the preparation of the PDS-functionalised RAFT CTA. Experimental details included above.
Synthesis of Telechelic PolyNIPAM

SEC analysis of the telechelic polyNIPAM gave rise to a shoulder at high molecular weight which upon addition of the strong reducing agent, tributylphosphene (TBP), disappeared as shown in Figure S9. This was hypothesised to be due to the presence of base in the SEC solvent (triethylamine) which can remove the RAFT agent end group, allowing for either thiol-thiol or thiol-pyridyl disulfide coupling. MALDI-ToF analysis indicated quantitative incorporation of the desired end groups (Figure 2, main text) and all visible peaks are listed in Table S2.

![Figure S9. SEC analysis of initial telechelic poly(NIPAM) before (black) and after (red) TBP reduction.](image-url)
Table S2. MALDI-ToF peak assignment. Mass corresponds to end groups plus the number of NIPAM repeat units and ion indicated.

<table>
<thead>
<tr>
<th>Entry</th>
<th>m/z</th>
<th>NIPAM Repeat Units</th>
<th>Ion</th>
<th>Entry</th>
<th>m/z</th>
<th>NIPAM Repeat Units</th>
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<td>15</td>
<td>Na⁺</td>
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Removal of RAFT Agent-derived Dithioester End-Group During Polycondensation.

The polycondensation reaction (described in main part of paper) required the removal of the RAFT-agent derived end-group from the telechelic macromonomer to generate a terminal thiol group. Figure S10 shows photodiode-array–coupled SEC analysis of pNIPAM$_{1700}$ (entry 3, Table S1) before and after addition of the primary amine. Before addition of the amine, there is clearly a large absorption at 300 nm, attributable to the RAFT-agent end group. Following addition of the amine, the absorbance at 300 nm decreases significantly demonstrating removal of the dithioester group under the conditions used.

Figure S10. UV/vis spectra of pNIPAM$_{1700}$ (entry 3, Table S1): (a) before and (b) after amine addition. X, y and z axes correspond to wavelength, RI response and retention time respectively. Note y-axis scale bar (fluorescence intensity is lower in (b) than (a)).
Effect of Oxygen on Polycondensation.

Figure S11 shows SEC traces of the macromonomer (black) and the resulting material following addition of triethylamine/ethanolamine, conducted in non-degassed solvent where a shoulder at approximately double molecular weight formed (red). This was hypothesised to be due to oxidative coupling of thiol end groups competing with the desired thiol-pyridyl disulfide reaction, scheme S3. Addition of tributylphosphine caused a reduction in molecular weight (blue) confirmed the presence of disulfide bonds. Performing the polycondensation in solvents which had been thoroughly degassed by freeze-pump-thaw cycles resulted in high molecular weight polymers which could only be formed by thiol-pyridyl disulfide coupling, as shown in the main text.

![Figure S11](image)

**Figure S11.** SEC traces of macromonomer polycondensation in the presence of oxygen: Initial telechelic macromonomer starting material (black); product polymer following attempted polycondensation reaction in presence of oxygen (red).
Scheme S3. Proposed reaction pathway in presence of oxygen: combination of two thiols to form a dimer.
Effect of Macromonomer Concentration

The polycondensation reaction was conducted using several macromonomer concentrations (see figure S12 for SEC traces). All traces were essentially identical, suggesting that varying the concentration is not a useful method for fine-tuning the molecular weight of the disulfide linked polymers. At all concentrations there was no evidence of mono-cyclic polymers which would elute at longer retention times, but larger, multicyclic structures could not be ruled out.

![Figure S12. Investigating “macro”-polymerisation at different polymer concentrations: 40 mg.mL\(^{-1}\) (black); 20 mg.mL\(^{-1}\) (red); 10 mg.mL\(^{-1}\) (blue) and 1 mg.mL\(^{-1}\) (olive).](image-url)
Biocompatibility

In vitro haemolysis testing is the first stage of toxicity testing used to assess the structural/membrane integrity of erythrocytes in the presence of a “foreign” compound.\cite{1} In essence, to be biocompatible no release of haemoglobin (haemolysis) should be observed upon incubation of the test compound with erythrocytes. For the purpose of this study the potential effect of temperature on biocompatibility, specifically whether increased haemolysis would be observed at a temperature above the LCST, was important. Testing was therefore performed at room temperature (i.e. below NIPAM LCST) and 42 °C (above NIPAM LCST, and a common temperature for hyperthermia treatments) on the polymers denoted in table S1 (entries 1 and 3). Phosphate-buffered saline (PBS) solution was used to maintain an appropriate osmotic pressure. The accepted cut-off for haemolytic activity in humans is approximately 10%.\cite{2} Fig. S13 shows the haemolysis relative to a positive control (incubation of erythrocytes with water) to be limited (< 10%) for both polymers at both temperatures, indicating that they are haemocompatible.

Figure S13. Haemolysis of ovine erythrocytes in presence of two molecular weight ($M_n$ (SEC): 9800 and 1700 g.mol$^{-1}$) poly(NIPAM) samples (table S1, entries 1 and 3) above and below the cloud point of the polymers.
References
