SUPPORTING INFORMATION

High-throughput physicochemical analysis of thermoresponsive polymers

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Reagents

All chemicals were purchased for Sigma-Aldrich, Australia and used as received, namely *N*-hydroxymethyl acrylamide (HMAM), *N*-isopropylacrylamide (NIPAM)**,** 4-cyano-4-(thiobenzoylthio) pentanoic acid (CTA 2)**,** 4′-azobis(4-cyanovaleric acid) (ACVA), dioxane, ethanol, deuterated dimethysulfoxide (DMSO-d6), *N,N*-dimethylacetamide and SYBR Green I (SG).

Experimental Details

Synthesis of poly(*N*-isopropylacrylamide) (pNIPAM). pNIPAM was synthesized by mixing NIPAM (1 g, 9 mmol) and CTA 2 (6.75 mg, 0.02 mmol) in a reaction vial with dioxane (5.9 mL). The mixture was then deoxygenated for 15 min with nitrogen and sealed with a rubber septum and copper wire. A stock solution of ACVA (27.5 mg, 98 µmol) was prepared in dioxane (1 mL) and after deoxgenation, 0.1 mL (9.8 µmol) was injected into the reaction vial. Polymerisation occurred over 24 h with the reaction stirring in an oil bath at 70 °C. The resulting polymer was initially dialysed with ethanol (3 days) then with water (3 days) at room temperature. Water was then removed via freeze-drying to give a white powder. The sample was denoted 0H.

Synthesis of pNIPAM-*co*-*N*-hydroxymethyl acrylamide (pNIPAM-*co*-HMAM) (2.5-12.5 mol % HMAM content). pNIPAM-*co*-HMAM copolymers (2.5 to 12.5 mol % HMAM) were synthesized using a similar methodology. As an example, synthesis of pNIPAM-*co*-HMAM (2.5 mol %) was achieved by mixing NIPAM (1 g, 9 mmol), HMAM (0.025 g, 0.2 mmol), and CTA 2 (6.75 mg, 0.02 mmol) in a reaction vial with dioxane (5.9 mL). The mixture was then deoxygenated for 15 min with nitrogen and sealed with a rubber septum and copper wire. A stock solution of ACVA (27.5 mg, 98 µmol) was prepared in dioxane (1 mL) and after deoxgenation, 0.1 mL (9.8 µmol) was injected into the reaction vial. Polymerisation occurred over 24 h with the reaction stirring in an oil bath at 70 °C. The resulting copolymer (0.5 g, yield 52%, calculated gravimetrically) was initially dialysed with ethanol (3 days) then with water (3 days) at room temperature. Water was then removed via freeze-drying to give a white powder. This procedure was repeated for 5, 7.5, 10 and 12.5 mol % HMAM.

Each sample was denoted 2.5H, 5H, 7.5H, 10H and 12.5H.

Characterization

Solution proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. NMR analysis was performed on pNIPAM and each pNIPAM-*co*-HMAM polymer (2.5-12.5 mol %) copolymer dissolved in DMSO-d6 using a Bruker 600 MHz spectrometer with a 5 mm triple resonance (HCN) probe for ^{1}H. The relative mol % of HMAM in each copolymer was determined experimentally by comparing the area beneath the peaks attributed to pNIPAM and HMAM as outlined in Figure S1.

Size exclusion chromatography (SEC). SEC was performed on a Shimadzu system equipped with a CMB-20A controller system, a SIL-20A HT autosampler, a LC-20AT tandem pump system, a DGU-20A degasser unit, a CTO-20AC column oven, a RDI-10A refractive index detector and 4× Waters Styragel columns (HT2, HT3, HT4, and HT5, each 300 mm × 7.8 mm^2, providing an effective molar mass range of $10^2 \times 10^7$). *N,N*-dimethylacetamide (containing

4.34 g/L lithium bromide) was used as an eluent with a flow rate of 1 mL/min at 80 °C. Number (Mn) and weight average (Mw) molar masses were evaluated using Shimadzu LC Solution software. The SEC columns were calibrated with low dispersity poly(methylmethacrylate) (PMMA) standards (Agilent Technologies calibration kit, P/N PL2020-0101, S/N 0006181883) ranging from 1,010 to 2,136,000 g/mol and molar masses are reported as PMMA equivalents. A 3rd-order polynomial was used to fit the log Mp vs. time calibration curve, which was near linear across the molar mass ranges ($R^2 = 0.999$).

Lower critical solution temperature (LCST) measurements using a real time thermocycler. PNIPAM and each pNIPAM-*co*-HMAM polymer (2.5-12.5 mol %) (20 µL) were placed in a Rotor-Gene Q real-time polymerase chain reaction (PCR) thermocycler and subjected to the following temperature profile: 25 °C for 3 min followed by a temperature ramp from 25 °C to 60 °C increasing at 0.5 °C /min. The light scattering measurements were made every 0.5 °C at an excitation wavelength of 470 nm and emission and wavelength of 510 nm. Raw data was processed using excel and plotted using Origin 7.0.
For fluorescence measurements the PNIPAM and each pNIPAM-*co*-HMAM polymer (2.5-12.5 mol %) (20 µL) were diluted in 5X SG made up in deionized water. The samples were then placed into the Rotor-Gene Q real-time thermocycler and subjected to the following temperature profile: 25 °C for 3 min followed by a temperature ramp from 25 °C to 60 °C increasing at 0.5 °C/min. The fluorescent measurements were made every 0.5 °C at an excitation wavelength of 470 nm and emission and wavelength of 510 nm.

Data Analysis

Light scattering and fluorescence measurements of each polymer were baseline corrected by subtracting all values from the average baseline fluorescence measurement made between 25 °C and 32 °C. Experimental data were then expressed as a percentage of the maximum normalized fluorescence intensity. Error bars represent the average and standard deviation of triplicate measurements.

Critical micelle temperature (CMT). The CMT of each pNIPAM-*co*-HMAM copolymer (2.5 to 12.5 mol %) was calculated from a plot of the SG fluorescence measured at different polymer concentrations (4600 µg/mL, 3100 µg/mL, 2160 µg/mL, 1470 µg/mL, 1000 µg/mL, 680 µg/mL, 470 µg/mL, 320 µg/mL, 220 µg/mL, 130 µg/mL, 100 µg/mL, 70 µg/mL, 50 µg/mL, 30 µg/mL, 20 µg/mL, 15 µg/mL, 10 µg/mL and 7 µg/mL) as a function of temperature as it was increased from 25 °C to 50 °C. The CMT was identified as the onset temperature at which fluorescence commenced.

Critical micelle concentration (CMC). The CMC of each pNIPAM-*co*-HMAM copolymer (2.5 to 12.5 mol %) was calculated from a plot of the SG fluorescence measured at different polymer concentrations (10,000 µg/mL, 6800 µg/mL, 4600 µg/mL, 3100 µg/mL, 2160 µg/mL, 1470 µg/mL, 1000 µg/mL, 680 µg/mL, 470 µg/mL, 320 µg/mL, 220 µg/mL, 130 µg/mL, 100 µg/mL, 70 µg/mL, 50 µg/mL, 30 µg/mL, 20 µg/mL, 15 µg/mL, 10 µg/mL and 7 µg/mL) plotted on a $\log_2$ scale. The CMC was identified from the intersection of the concentration x-axis, which was extrapolated from a line of best fit of the fluorescence values near the intersection.

Results

Table S1. The NIPAM/HMAM molar ratio used to generate different pNIPAM-*co*-HMAM copolymers (0 to 12.5 mol %). The molecular weights (Mn) and dispersity indicies (Đ's) measured by SEC.

Mol % HMAM	[NIPAM]/ [HMAM]	Mn (GPC)	Đ
0.0	100.0/0.0	N/A	N/A
2.5	98.5/2.5	26,000	1.4
5.0	95.0/5.0	43,000	1.6
7.5	92.5/7.5	44,000	1.6
10.0	90.0/10.0	52,000	1.5
12.5	88.5/12.5	43,000	1.6

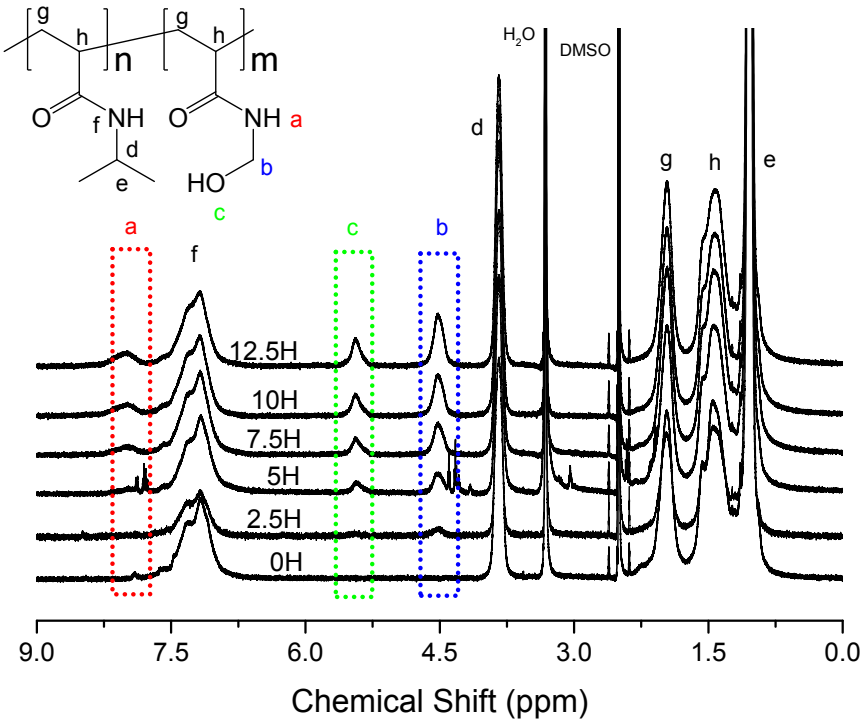

Figure S1. ^{1}H NMR spectra (600 MHz) for pNIPAM-*co*-HMAM copolymers (0-12.5 mol % HMAM), 0H, 2.5H, 5H, 7.5H, 10H and 12.5H.