Supplementary Material (ESI) for Chemical Communications

One-step synthesis of low polydispersity, biotinylated poly(*N*-isopropylacrylamide) by ATRP

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Materials

All chemicals were purchased from Aldrich or Acros and used as received. DMSO-d_6 was purchased from Cambridge Isotope Laboratories. Copper chloride (CuCl) was purified by stirring in glacial acetic acid overnight and rinsing with ethanol and diethyl ether. Recombinant streptavidin was kindly provided by Prof. Thomas Ward, U. de Neuchâtel, Switzerland.

Analytical Techniques

^{1}H NMR spectra were recorded on either a Bruker ARX500 or ARX600 spectrometer. GPC was conducted on a Shimadzu HPLC system equipped with a refractive index detector RID-10A and two 300 mm Polymer Laboratories PLgel 5 μm mixed-D columns (with guard column). DMF with 0.1 M LiBr at 40 °C was used as the eluent. Near-monodisperse poly(methyl methacrylate) standards (Polymer Laboratories) were employed for calibration. Chromatograms were processed with the EZStart 7.2 chromatography software. UV measurements were performed on a BioMate 5

(Thermospectronic) instrument. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using 10-20% precast gradient gels. Samples were diluted in TRIS buffer containing SDS, bromophenol blue and glycerol. No dithiothreitol (DTT) was added. Staining was accomplished with Coomassie Blue.

Methods

Synthesis of Biotin initiator 3: The synthesis of biotin alcohol (**2**) was accomplished with modification of a literature procedure.[1] Biotin (1.50 g, 6.14 mmol) was reacted with DSC (1.57 g, 6.14 mmol) and TEA (1.0 mL, 7.37 mmol) in DMF (30 mL) at room temperature for 8 h. 2-(2-aminoethoxy)ethanol (0.62 mL, 6.14 mmol) was then added, and the reaction mixture stirred 12 h. After removing the solvent *in vacuo*, the residue was redissolved in THF and filtered. The biotinylated alcohol **2** was purified by recrystallizations in 2:1 ether:methanol to give a white solid. **2** (0.65 g, 1.8 mmol) was then dissolved in DMF (10 mL) and reacted with 2-chloropropionic acid (0.20 g, 1.8 mmol) in the presence of DCC (0.37 g, 1.8 mmol) and DMAP (0.029 g, 0.24 mmol) for 12 h at room temperature. The solid residue was filtered out, and the solvent evaporated *in vacuo*. The product was isolated after column chromatography (dichloromethane : methanol = 9:1) as a white solid. Overall yield for both steps = 72%. δ_H(500 MHz; CD_3OD) 7.99 (1 H, bs), 4.53 (1 H, q), 4.48 (1 H, dd), 4.31–4.28 (3 H, m), 3.71–3.69 (2 H, m), 3.55 (2 H, t), 3.35 (2 H, dd), 3.22–3.19 (1 H, m), 2.92 (1 H, dd), 2.70 (1 H, d), 2.21 (2 H, t), 1.76–1.55 (8 H, m) and 1.46–1.40 (2 H, m); δ_C(500 MHz, CD_3OD) 176.2, 171.7, 70.6, 69.6, 66.1, 63.4, 61.6, 57.0, 41.0, 40.3, 36.8, 36.7, 29.7, 29.5, 26.8 and 21.8.

Polymerization of NIPAAm from the biotinylated initiator 3 for kinetic study. A Schlenk tube was charged with CuCl (3.5 mg, 35 mmol), $CuCl_2$ (1.2 mg, 8.8 mmol) and

NIPAAm (600 mg, 5.3 mol) in an argon atmosphere. Degassed DMSO-d_6 (0.90 mL) and Me_6TREN (12.2 μL, 44.2 mmol) were added and the mixture dissolved. Then 0.200 mL of initiator stock solution (prepared by dissolving 14.0 mg of **3** in 0.300 mL of DMSO-d_6) was syringe-transferred to start the polymerization. The polymerization mixture was sampled periodically for ^{1}H NMR and GPC analysis. The kinetic data derived from ^{1}H NMR and GPC analysis is tabulated in Table S1 below; the GPC traces for each time point are shown in Figure S1.

Table S1. Kinetic study data.

Time (h)	**Conv (%)**[a]	**M_n (th.)**[b]	**M_n (GPC)**	**PDI (GPC)**	**M_n (NMR)**[c]	**Area**[d] **2.8 ppm**	**Area**[e] **3.1 ppm**	**Area**[f] **3.8 ppm**	**Area**[g] **5.5 ppm**
0.25	13	3560	6500	1.15	4750	1.00	1.12	311	269
1.00	38	10400	16500	1.08	10600	1.00	1.02	241	147
2.00	52	13700	22500	1.07	9500	1.00	1.07	157	73
3.13	63	17200	26600	1.08	15400	1.00	0.95	215	79
4.58	71	19400	29000	1.10	24200	1.00	1.22	302	88
7.58	77	21100	30000	1.17	23000	1.00	0.95	265	61

[a] Calculated as [(Area 3.8 ppm) – (Area 5.5 ppm)] * 100 / (Area 3.8 ppm)
[b] M_n calculated from the monomer to initiator ratio: [[NIPAAm] / [initiator] * conversion] + MW(initiator)
[c] Calculated as [(Area 3.8 ppm) – (Area 5.5 ppm)] * MW (NIPAAm) / (Area 2.8 ppm)
[d] Integration for the ^{1}H NMR biotin signal at 2.8 ppm (1 initiator proton)
[e] Integration for the ^{1}H NMR biotin signal at 3.1 ppm (1 initiator proton)
[f] Integration for the ^{1}H NMR polymer + monomer signal at 3.8 ppm (1 polymer proton + 1 monomer proton)
[g] Integration for the ^{1}H NMR monomer signal at 5.5 ppm (1 monomer proton)

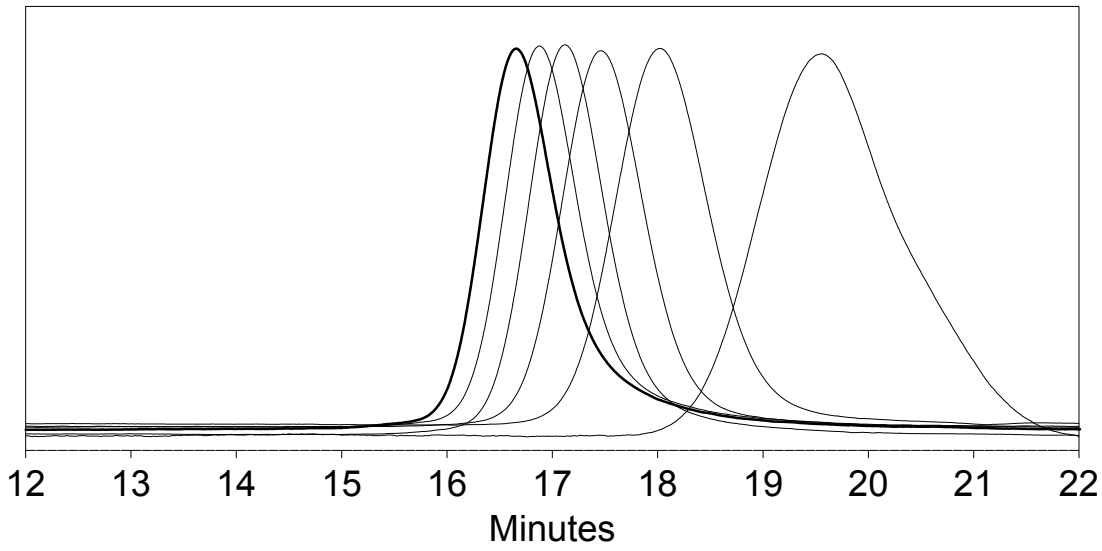

Figure S1. GPC chromatograms of pNIPAAm (synthesis conditions: $[NIPAAm]_0$ = 4M in DMSO-d_6, $[NIPAAm]_0$:[**3**]$_0$:$[CuCl]_0$:$[CuCl_2]_0$:$[Me_6TREN]_0$ = 240:1:1.6:0.4:2, 20 °C). From right to left, conversions (from ^{1}H NMR): 13%, 38%, 52%, 63%, 71%, and 77%.

Coupling of biotinylated pNIPAAm to streptavidin. Streptavidin (0.3 mg) and biotinylated pNIPAAm (3 mg) were dissolved in cold ultrapure water and incubated at room temperature for 1 hour. Formation of the conjugate was verified by SDS-PAGE.

HABA assay. The HABA assay was performed following standard procedure.[2]

References

1. K. Qi, Q. Ma, E. E. Remsen, C. G. Clark and K. L. Wooley, *J. Am. Chem. Soc.*, 2004, **126**, 6599.

2. G. T. Hermanson, *Bioconjugate Techniques*, Academic Press, New York, 1996.