Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

For

Copper-mediated controlled radical polymerization under biological conditions: SET-LRP in blood serum

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Materials

N-Isopropylacrylamide (NIPAM, 97%) was obtained from Sigma-Aldrich and was recrystallized from hexane to remove the inhibitor. 2-Hydroxyethyl acrylate (HEA, 96%, Sigma-Aldrich), poly(ethylene glycol) methyl ether acrylate ($PEGA_{480}$, average M_n 480, Sigma-Aldrich), *N*, *N*-dimethylacetamide (DMA, 99.5%, Sigma-Aldrich) were de-inhibited by passing through a column of basic alumina prior to use.

Sheep serum (USA origin, sterile-filtered, suitable for cell culture, Sigma-Aldrich) was directly used as the solvent for polymerization unless otherwise stated. The sheep serum was stored under -20 °C in the freezer and transferred to the cold room to defrost at 4 °C overnight prior to use.

Tris(2-(dimethylamino)ethyl)amine (Me_6TREN) was synthesized according to literature procedure and stored under a nitrogen atmosphere prior to use.[1, 2] The water soluble initiator 2, 3-dihydroxypropyl 2-bromo-2-methylpropanoate was synthesized according to literature procedure.[3]

Copper(I) bromide (Cu(I)Br, 98%, Sigma-Aldrich) was washed sequentially with acetic acid and ethanol and dried under vacuum.

Membrane dialysis (1K MWCO) was obtained from Spectrum Laboratories.

All other reagents and solvents were obtained at the highest purity available from Sigma-Aldrich and used without further purification unless otherwise stated.

Instruments and analysis

^1H and ^{13}C NMR spectra were recorded on Bruker DPX-300 and DPX-400 spectrometers using deuterated solvents obtained from Aldrich. Monomer conversion for NIPAM polymerization was calculated by comparing the integral of vinyl protons with isopropyl protons.

SEC was conducted on Varian 390-LC system in DMF (5 mM NH_4BF_4) at 50 ^oC, equipped with refractive index, UV and viscometry detectors, 2 × PLgel 5 mm mixed-D columns (300 × 7.5 mm), 1 × PLgel 5 mm guard column (50 × 7.5 mm) and autosampler. Narrow linear poly(methyl methacrylate) standards in range of 200 to 1.0×10^6 $g \cdot mol^{-1}$ were used to calibrate the system. All samples were passed through 0.45 μm PTFE filter before analysis.

Infrared absorption spectra were recorded on a Bruker VECTOR-22 FTIR spectrometer using a Golden Gate diamond attenuated total reflection cell.

RP-HPLC was carried out using Phenomenex Luna 100 Å (5 μm) 250 x 4.6 mm columns. The HPLC system was an Agilent 1260 infinity series stack equipped with an Agilent 1260 binary pump, mixer and degasser. Samples were injected using an Agilent 1260 autosampler and detection was achieved using an Agilent 1260 variable wavelength detector, connected in series. UV detection was monitored at $\lambda = 280$ nm. The mobile phases used were:

Mobile phase A: 90% v/v water, 10% v/v MeCN (far UV) and 0.05% TFA.

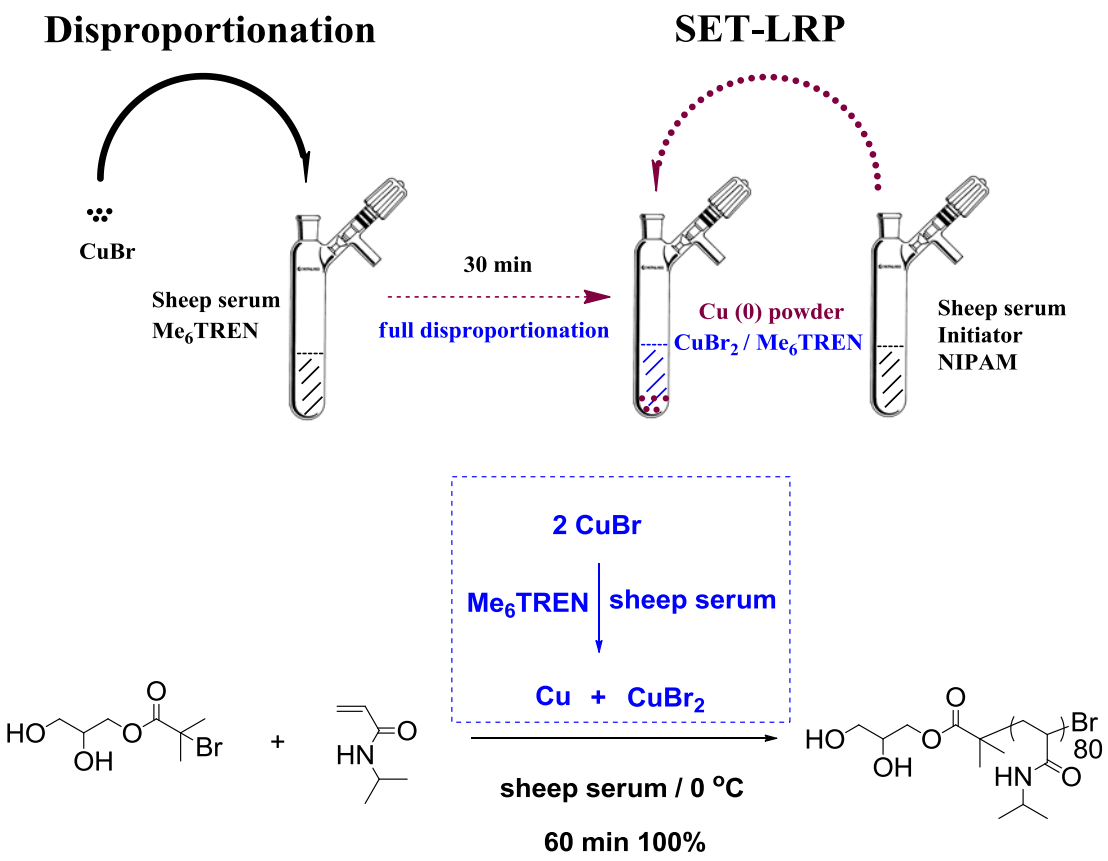
Mobile phase B: 100% MeCN (far UV) and 0.04% TFA.

Time (min)	(%A)	(%B)	Flow (mL/min)
0	90	10	1.0
27	40	60	1.0
35	40	60	1.0
36	90	10	1.0
45	90	10	1.0

The use of this gradient method included thorough washing of the columns whereby the concentration of mobile phase B was increased to 60% to prevent the build of contaminants on the column system.

All reactions were carried out using standard Schlenk techniques under an inert atmosphere of oxygen-free nitrogen, unless otherwise stated.

SET-LRP of NIPAM in sheep serum at 0 °C *via* disproportionation of Cu(I)Br / Me_6TREN



S Scheme 1. SET-LRP of NIPAM in sheep serum at 0 °C *via* disproportionation of Cu(I)Br / Me_6TREN.

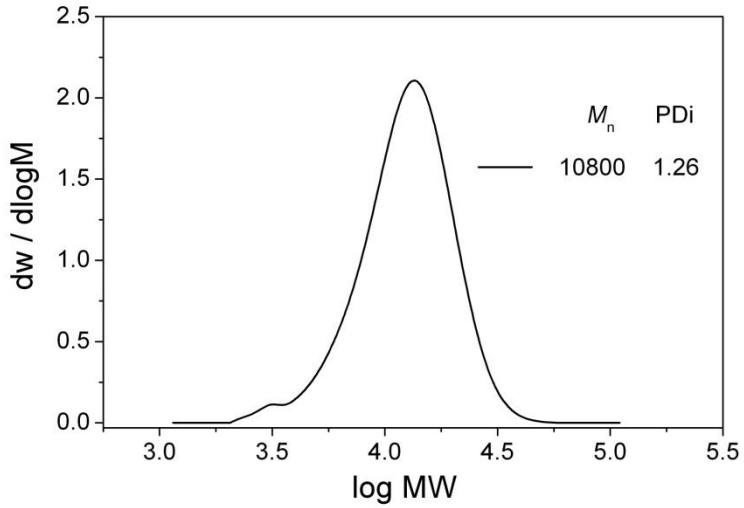
A magnetic stir bar, sheep serum (2 mL) and Me_6TREN (9 µL, 0.035 mmol) were charged to Schlenk tube fitted with a rubber septum and the solution was deoxygenated via nitrogen bubbling for ten minutes. Cu(I)Br (10 mg, 0.07 mmol) was then carefully added under slight positive pressure of nitrogen. The nitrogen bubbling was continued for another 10 min and then the blue suspension with purple red colour copper(0) powder was allowed to stir at ambient temperature under nitrogen protection for 15 min. After that, the tube was immersed into ice / water bath.

At the same time, to another 100 mL single neck round bottom bottle fitted with a magnetic stir bar and a rubber septum, sheep serum (4 mL), 2, 3-dihydroxypropyl 2-bromo-2-methylpropanoate (21 mg, 0.087 mmol) and NIPAM (0.79 g, 7 mmol) were charged and the mixture was bubbled with nitrogen for 15 min. Subsequently, the

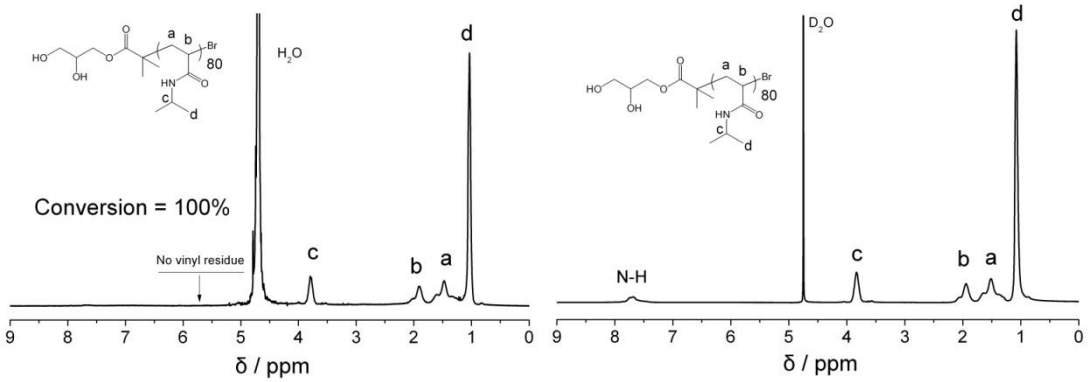
degassed monomer / initiator serum solution was transferred *via* cannula to the Schlenk tube with Cu(0) / $CuBr_2$ / Me_6TREN catalyst. The tube was sealed and the mixed solution was allowed to polymerize at 0 °C for 60 min. Sample of the reaction mixture was then removed for analysis. The sample for ^{1}H NMR was directly diluted with D_2O, which confirmed 100% conversion according to the total disappearance of vinyl groups. Catalyst residues and protein precipitate were removed by filtering through a column of neutral alumina prior to DMF SEC analysis.

Then 20 mL DMF was added to the remaining polymer / serum solution, which caused the residual protein to precipitate. The suspension was directly transferred to a 50 mL centrifuge tube for centrifugation at 11000 rpm for 20 min. After that the up-layer clear liquid was transferred to one dialysis tube for dialysis against water for 3 days. The polymer product could then be recovered by freeze drying.

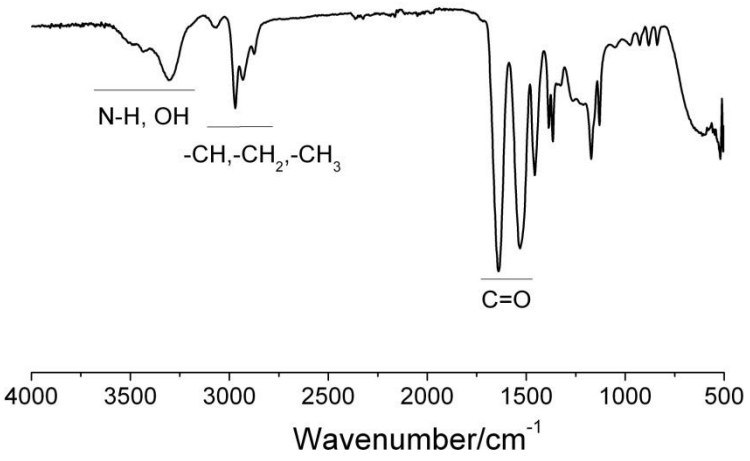
S Figure 1. Visual observation of the disproportionation of CuBr / Me_6TREN in sheep serum. Conditions: sheep serum= 2 mL; Cu(I)Br = 10 mg, 0.07 mmol; Me_6TREN = 9 μL, 0.035 mmol. Picture was taken 1 min after mixing CuBr with the other reagents.



S Figure 2. Molecular weight distribution of PNIPAM by SET-LRP in sheep serum at 0 °C *via* DMF SEC.

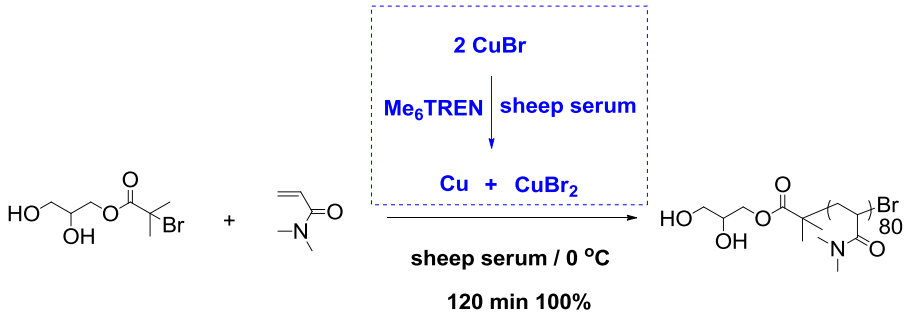


S Figure 3. ^{1}H NMR spectra for SET-LRP of NIPAM in sheep serum at 1 h (left) and PNIPAM (right) recovered after dialysis. D_2O was used as the NMR solvent.



S Figure 4. FTIR spectrum of PNIPAM (DP=80) by SET-LRP in sheep serum.

SET-LRP of DMA in sheep serum at 0 °C *via* disproportionation of Cu(I)Br / Me_6TREN



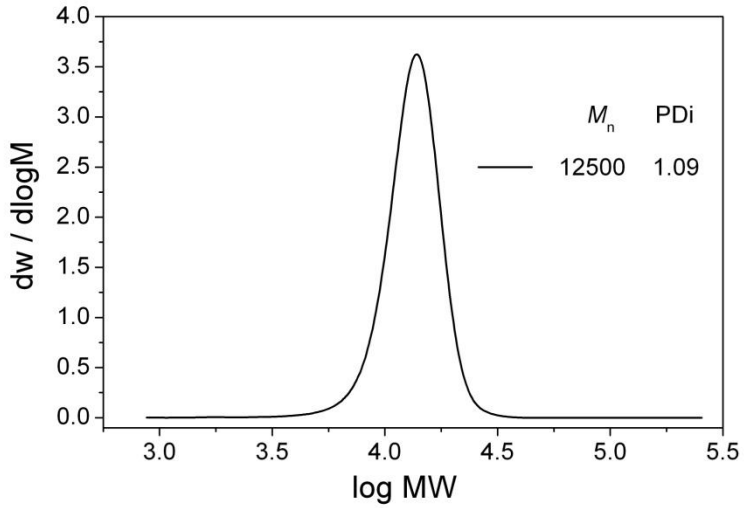
S Scheme 2. SET-LRP of DMA in sheep serum at 0 °C *via* disproportionation of Cu(I)Br / Me_6TREN.

The polymerization procedure is the same as described in SET-LRP of NIPAM (DP=80). The polymerizations was performed at the ratio of initiator/Cu(I)Br/ Me_6TREN (1: 0.8: 0.4) in sheep serum under ice / water bath (0 °C) for 2 h. The sample for ^{1}H NMR was directly diluted with D_2O, which confirmed 100% conversion at 2 h according to the total disappearance of vinyl groups. Catalyst residues and protein precipitate were removed by filtering through a column of neutral alumina prior to DMF SEC analysis.

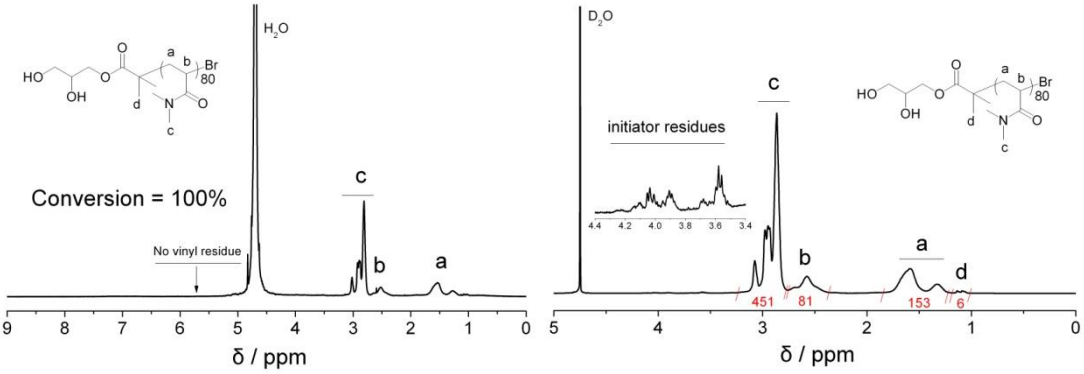
The charging amounts for each component are listed below.

S Table 1. Charging amounts for SET-LRP of DMA (DP=80) in sheep serum at 0 °C with [initiator]: [Cu(I)Br]: [Me_6TREN] = 1: 0.8: 0.4.

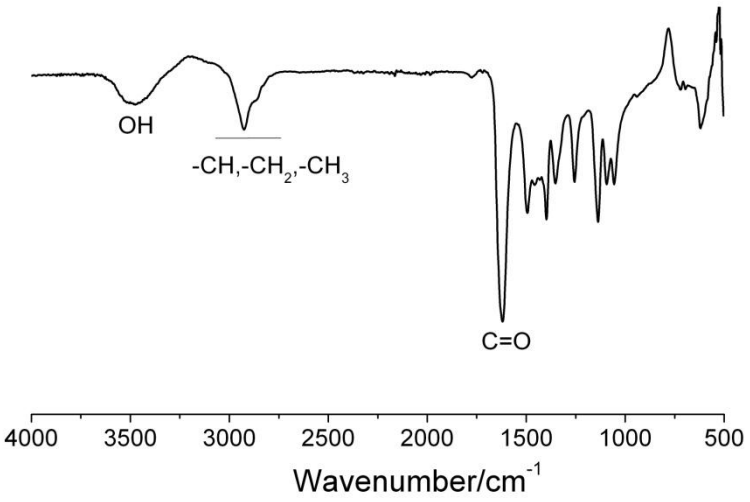
Initiator / monomer system			Catalyst system		
Initiator	NIPAM	Sheep serum	Cu(I)Br	Me_6TREN	Sheep serum
87 μmol	7 mmol	4 mL	70 μmol	35 μmol	2 mL



S Figure 5. Molecular weight distribution of PDMA after dialysis synthesized by SET-LRP in sheep serum at 0 °C *via* DMF SEC.

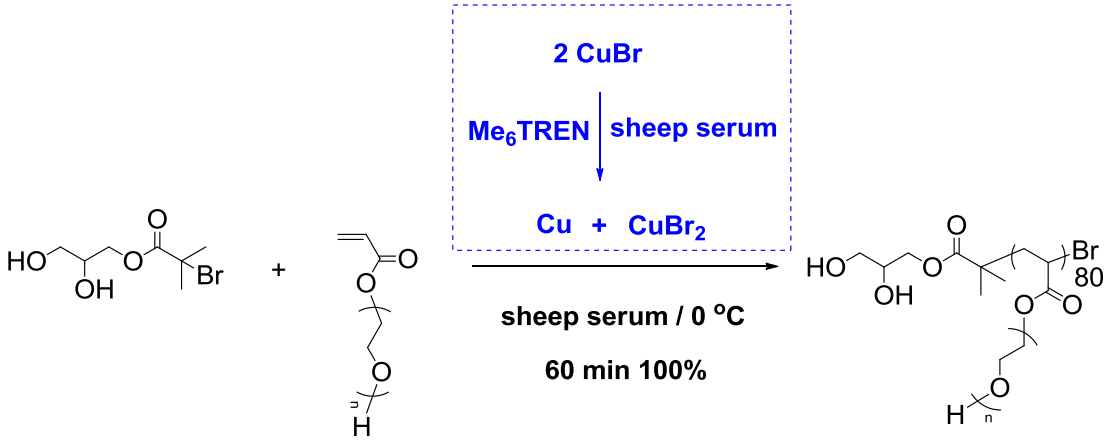


S Figure 6. ^{1}H NMR spectra for SET-LRP of DMA in sheep serum at 2 h (left) and PDMA (right) recovered after dialysis. D_2O was used as the NMR solvent.



S Figure 7. FTIR spectrum of PDMA (DP=80) by SET-LRP in sheep serum.

SET-LRP of PEG acrylate in sheep serum at 0 °C *via* disproportionation of Cu(I)Br / Me_6TREN



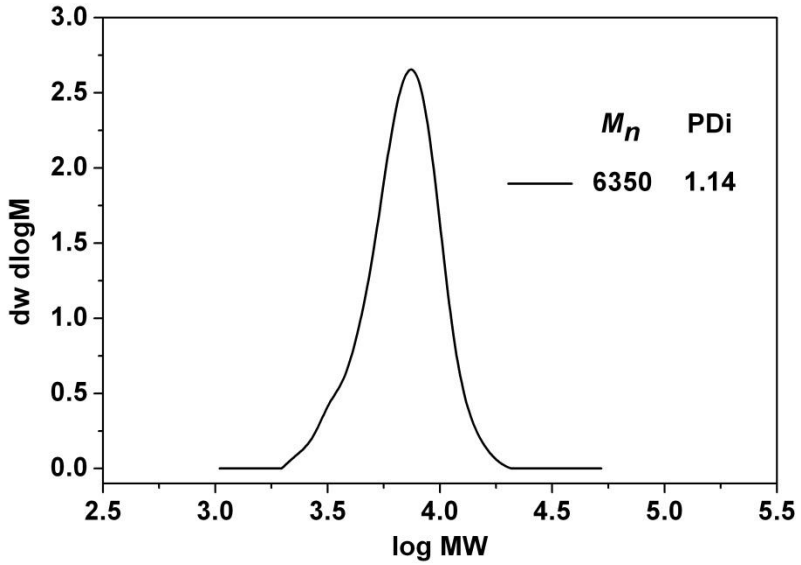
S Scheme 3.SET-LRP of PEGA in sheep serum at 0 °C *via* disproportionation of Cu(I)Br / Me_6TREN.

A magnetic stir bar, sheep serum (2.5 mL) and Me_6TREN (26 μL, 0.1 mmol) were charged to Schlenk tube fitted with a rubber septum and the solution was deoxygenated *via* nitrogen bubbling for ten minutes. Cu(I)Br (28.6 mg, 0.2 mmol) was then carefully added under slight positive pressure of nitrogen. The nitrogen bubbling was continued for another 15 min and then the blue suspension with purple red colour copper(0) powder was allowed to stir at ambient temperature under nitrogen protection for 15 min. Subsequently, the tube was immersed into ice / water bath.

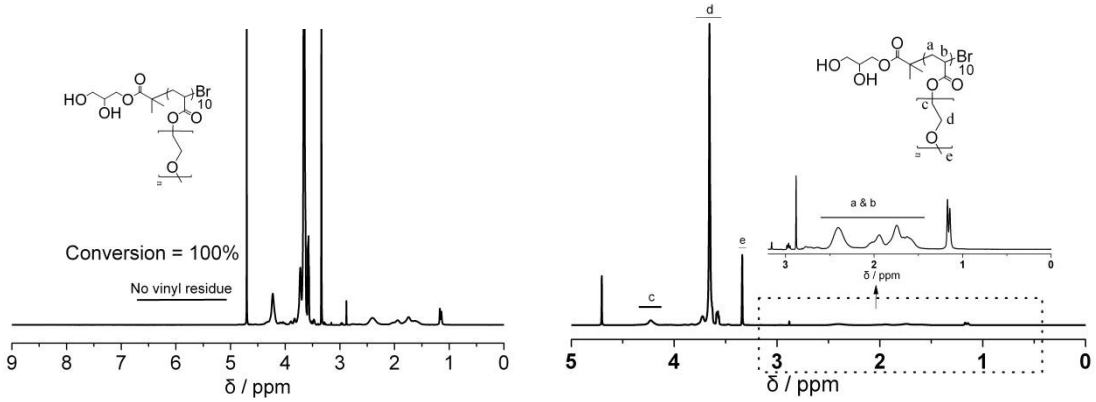
At the same time, to another 100 mL single neck round bottom bottle fitted with a magnetic stir bar and a rubber septum, sheep serum (4mL), 2, 3-dihydroxypropyl 2-bromo-2-methylpropanoate (60 mg, 0.25 mmol) and PEG acrylate (1.21g, 2.5 mmol) were charged and the mixture was bubbled with nitrogen for 15 min. After that, the degassed monomer / initiator serum solution was transferred *via* cannula to the Schlenk tube with Cu(0)/Cu(II)Br_2/Me_6TREN catalyst. The tube was sealed and the mixed solution was allowed to polymerize at 0 °C for 60 min. Sample of the reaction mixture was then removed for analysis. The sample for ^{1}H NMR was directly diluted with D_2O, which confirmed 100% conversion according to the total disappearance of

vinyl groups. Catalyst residues and protein precipitate were removed by filtering through a column of neutral alumina prior to DMF SEC analysis.

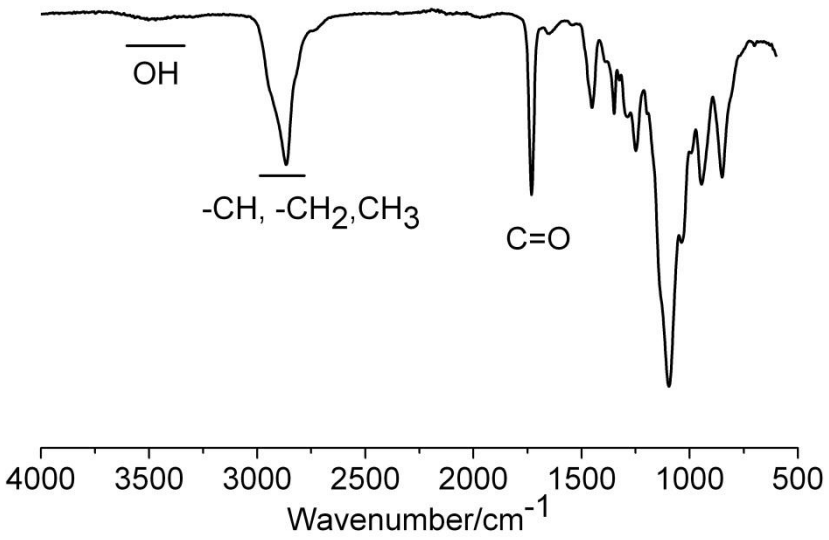
Then 20 mL DMF was added to the rest polymer / serum solution, which caused the protein precipitate. The suspension was directly transferred to one 50 mL centrifuge tube for centrifuge at 4000 rpm for 10 min. After that the up-layer clear liquid was transferred to one dialysis tube for dialysis against water for 3 days. The polymer product could then be recovered by freeze drying.



S Figure 8. Molecular weight distribution of PEG acrylate by SET-LRP in sheep serum at 0 °C *via* DMF SEC.

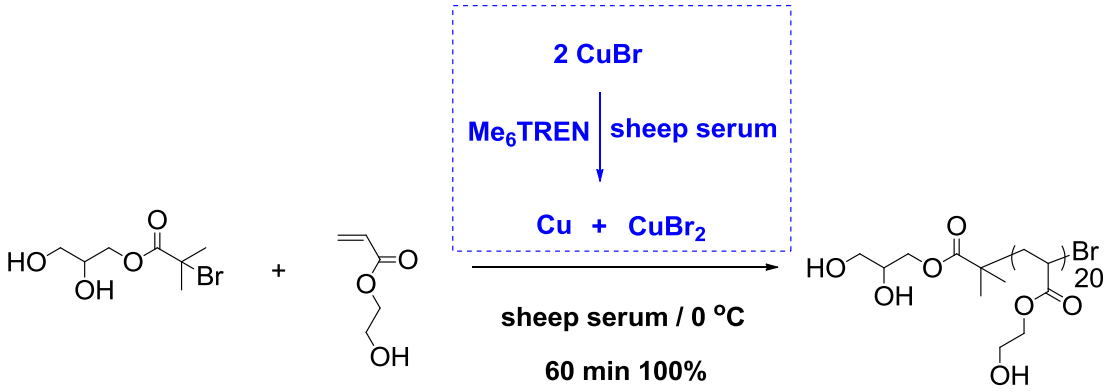


S Figure 9. ^{1}H NMR spectra for SET-LRP of PEG acrylate in sheep serum at 1 h (left) and PEG acrylate (right) recovered after dialysis. D_2O was used as the NMR solvent.



S Figure 10. FTIR spectrum of PEG acrylate (DP=10) by SET-LRP in sheep serum.

SET-LRP of HEA in sheep serum at 0 °C *via* disproportionation of Cu(I)Br / Me_6TREN



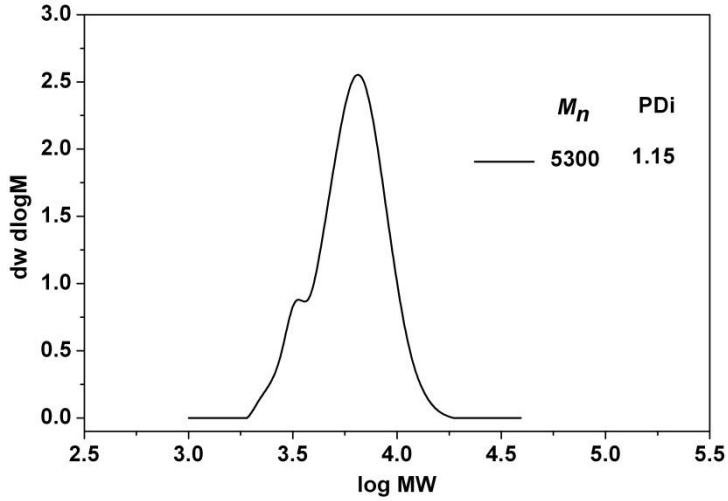
S Scheme 4.SET-LRP of HEA in sheep serum at 0 °C *via* disproportionation of Cu(I)Br/Me_6TREN.

The polymerization procedure is the same as described in SET-LRP of HEA (DP=20). The polymerizations was performed at the ratio of initiator/Cu(I)Br/Me_6TREN (1: 0.8: 0.4) in sheep serum under ice / water bath (0°C) for 1h. The sample for ^{1}H NMR was directly diluted with D_2O, which confirmed 100% conversion at 1h according to the total disappearance of vinyl groups. Catalyst residues and protein precipitate were removed by filtering through a column of neutral alumina prior to DMF SEC analysis.

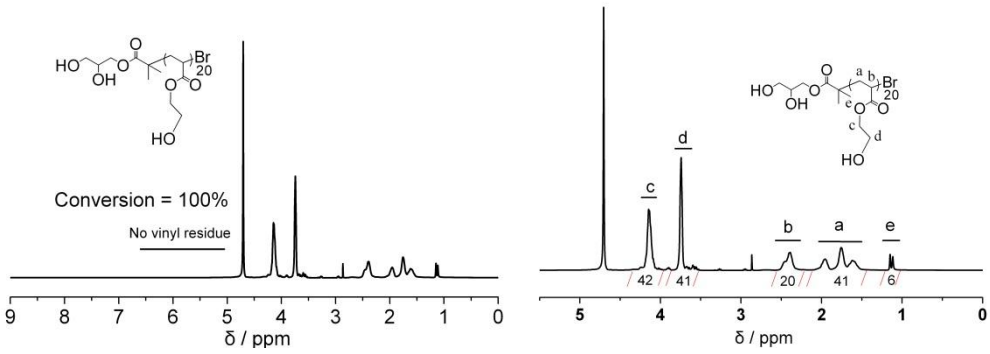
The charging amounts for each component are listed below.

S Table 2. Charging amounts for SET-LRP of HEA (DP=20) in sheep serum at 0°C with [initiator]: [Cu(I)Br]: [Me_6TREN] = 1: 0.8: 0.4.

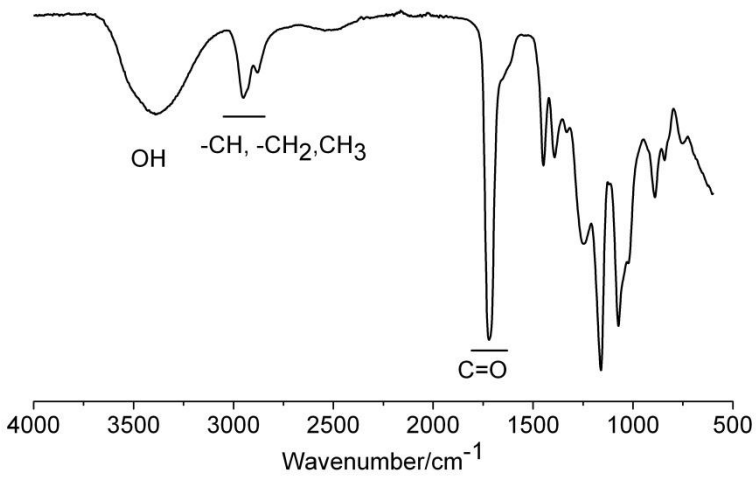
Initiator / monomer system			Catalyst system		
Initiator	HEA	Sheep serum	Cu(I)Br	Me_6TREN	Sheep serum
250 μmol	5 mmol	2.5 mL	200 μmol	100 μmol	4 mL



S Figure 11. Molecular weight distribution of HEA after dialysis synthesized by SET-LRP in sheep serum at 0 °C *via* DMF SEC.

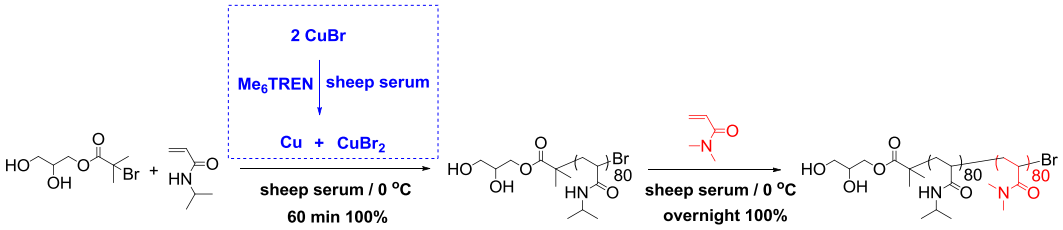


S Figure 12. ^{1}H NMR spectra for SET-LRP of HEA in sheep serum at 1 h (left) and HEA (right) recovered after dialysis. D_2O was used as the NMR solvent.



S Figure 13. FTIR spectrum of HEA (DP=20) by SET-LRP in sheep serum.

Synthesis of poly(NIPAM)$_{80}$-*b*-(DMA)$_{80}$ by SET-LRP in sheep serum



S Scheme 5. Synthesis of poly(NIPAM)$_{80}$-*b*-(DMA)$_{80}$ by SET-LRP in sheep serum with [initiator]: [Cu(I)Br]: [Me$_6$TREN] = 1: 0.8: 0.4 at 0 °C.

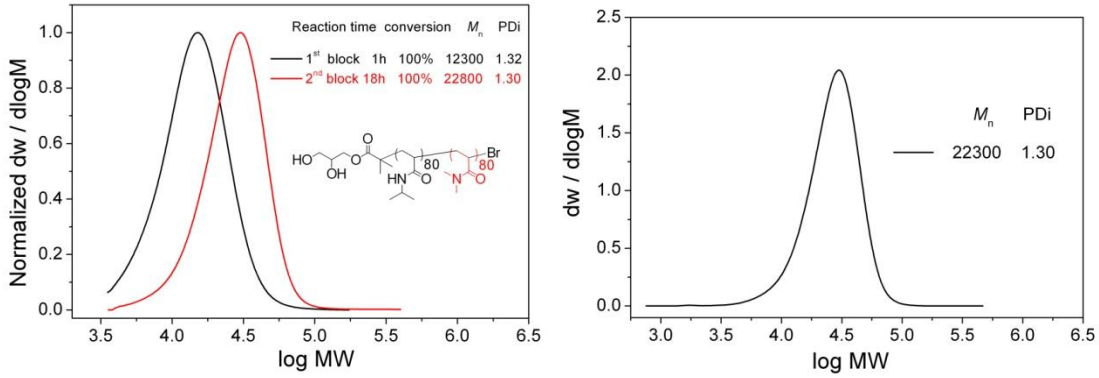
The procedure of the first block polymerization is the same as described in SET-LRP of NIPAM (DP=80). It was performed at the ratio of initiator / Cu(I)Br / Me$_6$TREN = 1: 08: 0.4 in sheep serum at 0 °C for 1 h. The charging amounts for each component are listed below.

S Table 3. Charging amounts for SET-LRP of NIPAM (DP=80) in sheep serum at 0 °C with [initiator]: [Cu(I)Br]: [Me$_6$TREN] = 1: 0.8: 0.4.

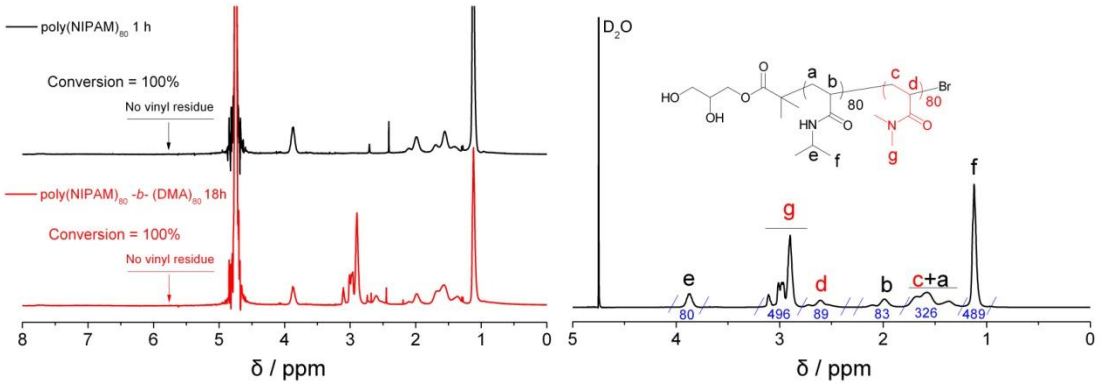
Initiator / monomer system			Catalyst system		
Initiator	NIPAM	Sheep serum	Cu(I)Br	Me$_6$TREN	Sheep serum
87 μmol	7 mmol	4 mL	70 μmol	35 μmol	2 mL

After 1 h, a solution of DMA (0.70 g, 7 mmol) in 4 mL sheep serum, previously degassed by nitrogen sparging for 15 min, was directly transferred via cannula to the Schlenk tube under nitrogen protection and polymerization overnight and the reaction was then stopped. The sample for ^{1}H NMR was directly diluted with D$_2$O. Catalyst residues and protein precipitate were removed by filtering through a column of neutral alumina prior to DMF SEC analysis.

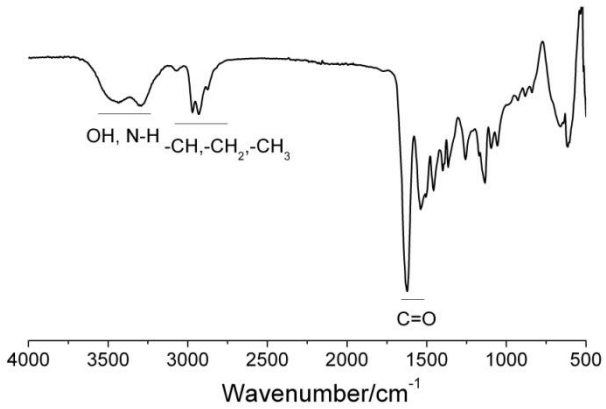
After reaction, 30 mL DMF was added to the rest polymer / serum solution, which caused the proteins to precipitate. The suspension was directly transferred to one 50 mL centrifuge tube for centrifuge at 11000 rpm for 20 min. After that the up-layer clear liquid was transferred to one dialysis tube for dialysis against water for 3 days. The polymer product could then be recovered by freeze drying.



S Figure 14. Molecular weight distributions of poly(NIPAM)$_{80}$-*b*-(DMA)$_{80}$ by SET-LRP in sheep serum at 0 °C *via* DMF SEC. Left: samples during polymerization; right: final product after dialysis.

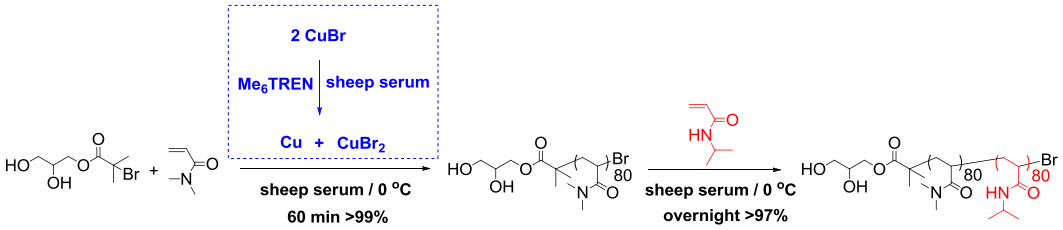


S Figure 15. ^{1}H NMR spectra for SET-LRP of poly(NIPAM)$_{80}$-*b*-(DMA)$_{80}$ in sheep serum during polymerization (left) and product (right) recovered after dialysis. D_2O was used as the NMR solvent.



S Figure 16. FTIR spectrum of poly(NIPAM)$_{80}$-*b*-(DMA)$_{80}$ by SET-LRP in sheep serum.

Synthesis of poly(DMA)$_{80}$-*b*-(NIPAM)$_{80}$ by SET-LRP in sheep serum



S Scheme 6. Synthesis of poly (DMA) $_{80}$ -*b*- (NIPAM) $_{80}$ by SET-LRP in sheep serum with [initiator]: [CuBr]: [Me$_6$TREN] = 1: 0.8: 0.4 at 0 °C.

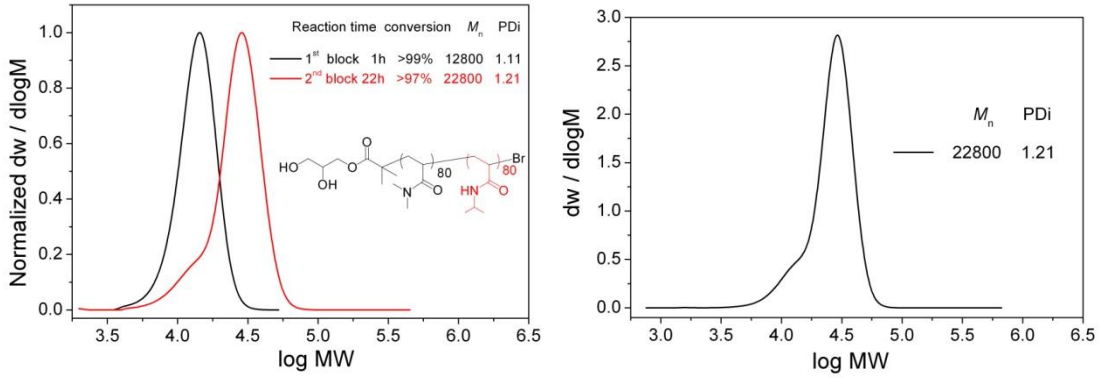
The procedure of the first block polymerization is the same as described in SET-LRP of NIPAM (DP=80). It was performed at the ratio of initiator / Cu(I)Br / Me$_6$TREN = 1: 08: 0.4 in sheep serum at 0 °C for 1 h. The charging amounts for each component are listed below.

S Table 4. Charging amounts for SET-LRP of DMA (DP=80) in sheep serum at 0 °C with [initiator]: [Cu(I)Br]: [Me$_6$TREN] = 1: 0.8: 0.4.

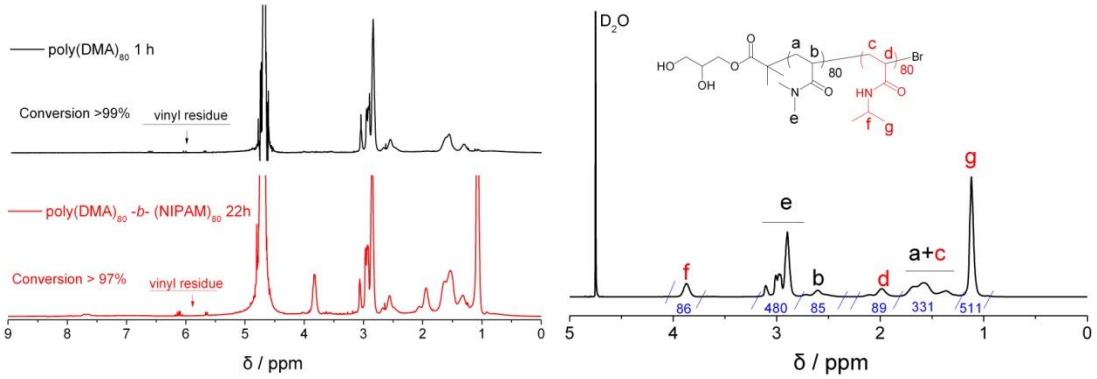
Initiator / monomer system			Catalyst system		
Initiator	DMA	Sheep serum	Cu(I)Br	Me$_6$TREN	Sheep serum
87 μmol	7 mmol	4 mL	70 μmol	35 μmol	2 mL

After 1 h, a solution of NIPAM (0.79 g, 7 mmol) in 4 mL sheep serum, previously degassed by nitrogen sparging for 15 min, was directly transferred via cannula to the Schlenk tube under nitrogen protection and polymerization overnight and the reaction was then stopped. The sample for ^{1}H NMR was directly diluted with D_2O. Catalyst residues and protein precipitate were removed by filtering through a column of neutral alumina prior to DMF SEC analysis.

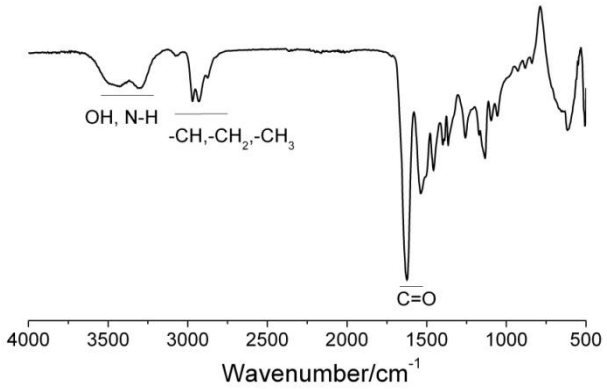
After reaction, 30 mL DMF was added to the rest polymer / serum solution, which caused the residual proteins to precipitate. The suspension was directly transferred to one 50 mL centrifuge tube for centrifuge at 11000 rpm for 20 min. After that the up-layer clear liquid was transferred to one dialysis tube for dialysis against water for 3 days. The polymer product could then be recovered by freeze drying.



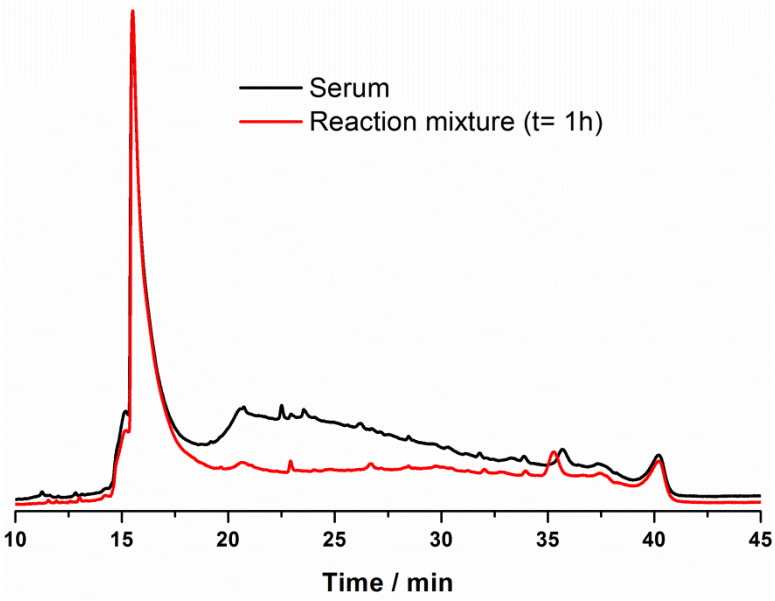
S Figure 17. Molecular weight distributions of poly(DMA)$_{80}$-*b*-(NIPAM)$_{80}$ by SET-LRP in sheep serum at 0 °C *via* DMF SEC. Left: samples during polymerization; right: final product after dialysis.



S Figure 18. ^{1}H NMR spectra for SET-LRP of poly(DMA)$_{80}$-*b*-(NIPAM)$_{80}$ in sheep serum during polymerization (left) and product (right) recovered after dialysis. D_2O was used as the NMR solvent.



S Figure 19. FTIR spectrum of poly(DMA)$_{80}$-*b*-(NIPAM)$_{80}$ by SET-LRP in sheep serum.



S Figure 20. HPLC analysis of commercial sheep serum before (black) and after (red) use as the medium for Cu(0)-mediated polymerisation.

References

1. M. Ciampolini and N. Nardi, *Inorg. Chem.* 1966, **5**, 41-44.
2. J. Queffelec, S. G. Gaynor and K. Matyjaszewski, *Macromolecules*, 2000, **33**, 8629-8639.
3. S. Perrier, S. P. Armes, X. S. Wang, F. Malet and D. M. Haddleton, *J. Polym. Sci., Part A: Polym. Chem.*, 2001, **39**, 1696-1707.