Electronic Supporting Information

Sol-gel reversible metallo-supramolecular hydrogels based on a thermoresponsive double hydrophilic block copolymer

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1-Reagents

All chemicals were purchased from Aldrich unless otherwise noted. *N,N*-Dimethylformamide (DMF, 99.8%), *N,N*-dimethylacrylamide (DMA, 99%), 4,4'-azobis(4-cyanovaleric acid (ACPA, > 98%), 1,4-dioxane (> 99.5%), *N*-isopropylacrylamide (NIPAM, > 99%), 2,2'-azobis(2-methyl-propionamidine)dihydrochloride (V-50, 98%), sodium bicarbonate ($NaHCO_3$, Fischer Scientific) and acetic anhydride (98%, Merck), were used as received. Dichloromethane (DCM, HPLC grade, Fisher Chemical), tetrahydrofuran (THF, > 99.8%) were dried over dry solvent stations GT S100. Pure water was obtained from a Millipore Direct Q system and had a conductivity of 18.2 MΩ cm at 25°C. *N*-acryloyl-2-methylalanine

(NAMA) was synthesized according to a reported procedure.[1] Terpyridine-terminated trithiocarbonate chain transfer agent (Tpy-CTA) was synthesized following a reported procedure.[2]

2-General Characterization

Nuclear magnetic resonance (NMR) spectroscopy. NMR spectra were recorded on a Bruker AC-400 spectrometer for ^{1}H-NMR (400 MHz). Chemical shifts are reported in ppm relative to the deuterated solvent resonances.

Size exclusion chromatography (SEC). The average molar masses (number-average molar mass $\overline{M_n}$, weight-average molar mass $\overline{M_w}$) and dispersity ($Đ = \overline{M_w}/\overline{M_n}$) values were measured by SEC using *N,N*-dimethylformamide (with LiBr at 1g.L^{-1}) as an eluent, and carried out using a system equipped with a guard column (Polymer Laboratories, PL gel 5 μm) followed by two columns (Polymer Laboratories, 2 PL gel 5 μm MIXED-D columns) and with a Waters 410 differential refractometer (DRI) and a Waters 481 UV detector operating at 309 nm and 290 nm. The instrument operated at a flow rate of 1.0 mL.min^{-1} at 60°C and was calibrated with narrow linear polystyrene (PS) standards ranging in molar mass from 580 g.mol^{-1} to 483 000 g.mol^{-1}. Molar mass and dispersities were calculated using Waters EMPOWER software.

Infra-red spectroscopy characterization (FT-IR). FT-IR spectra of copolymers were recorded using a Nicolet iS5 FT-IR spectrometer operating with an attenuated total reflection (iD5 ATR Diamond) gate. Spectra were analyzed with OMNIC software.

Mass spectrometry analysis. MALDI-TOF (Matrix-Assisted Laser Desorption and Ionization Time Of Flight) mass spectrometry analysis was performed on a Bruker UltraFlex II MALDI-TOF instrument equipped with a nitrogen laser operating at 337 nm, pulsed ion extraction source and reflectron. Spectra were recorded in the reflectron mode with an acceleration voltage of 19 kV and a delay of 200 ns. 500 single shot acquisitions were summed to give the spectra and the data were analyzed using FlexAnalysis software. Samples were prepared by dissolving the matrix (*trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile, DCTB) in the solvent (DCM, 30 mg.mL^{-1}) and mixing with the polymer (10 mg.mL^{-1}) in the ratio 1:10 (v/v). 1 μL was spotted directly onto a thin layer of sodium trifluoroacetate (NaTFA) in acetone (concentration 19 mg.mL^{-1}) that had been deposited to act as a cationizing agent.

UV-Vis spectrophotometry characterization. UV-Vis measurements were performed on a Varian Cary 100 Scan spectrophotometer. Samples of copolymers in pure water of concentration 0.5% w/v were prepared and then introduced into a quartz cuvette.

Differential scanning calorimetry (DSC). DSC measurement was performed on a TA Instruments Q100 connected to a computer in aluminum pans under nitrogen otherwise noted. The DSC instrument was calibrated using an indium standard. Sample was heated from 15 °C to 50 °C at a heating rate of 10 °C.min^{-1} and under a static nitrogen atmosphere, followed by cooling to 15°C at the same rate after an isotherm at 50°C during 2 minutes. Thermal transitions was obtained from the maximum of the endotherm (heating scan).

Dynamic light scattering (DLS). DLS measurements were performed on a Malvern Instruments Nanosizer fitted with a 4 mW He–Ne laser operating at 633 nm with an angle detection (173°). The data were processed using the CONTIN method of analysis. Individual measurements were made at 20°C and 45°C after an equilibration time of two minutes. The sample was prepared by dissolving PDMA macroCTA agents or block copolymers in pure water (5 g.L^{-1}) and the polymer solution was filtered using nylon membrane filter to 0.22 μm porosity.

Rheology. Shear rheological experiments were performed on a controlled stress rheometer DHR3 (TA Instruments) with a cone-plate geometry (diameter 20 mm, angle about 4°). The temperature was controlled with a Peltier system. Evaporation was avoided by covering the geometry with a mineral oil. For dynamic shear measurements, the linear regime was first defined during a stress sweep at a frequency f of 1Hz and 20°C. The frequency dependence of the viscoelastic moduli, elastic G' and viscous G" modulus, were determined between 5.10^{-3} and 10 Hz. The temperature dependence was performed at 5°C fixing the frequency at 1Hz. The time dependence after a temperature step was also investigated measuring G' and G" at a f = 1Hz. The flow behavior of samples was also investigated measuring the viscosity η of the samples between 10^{-3} and 100 s^{-1} of the shear rate.

3-Synthesis of the terpyridine functionalized poly(*N,N*-dimethylacrylamide) PDMA macromolecular chain transfer agent (Tpy-PMDA-CTA)

A magnetic stirrer was charged to a Schlenk tube along with DMA (3.9206 g, 39.5 mmol), Tpy-CTA (0.229 g, 0.215 mmol), ACPA (0.0089 g, 0.031 mmol) using the ratio of $[DMA]_0/[Tpy\text{-}CTA]_0/[ACPA]_0 = 180/1/0.142$ with DMF (24 µL, 0,1%v) used as internal standard and 1,4-dioxane (20 mL). The polymerization mixture was degassed with argon for 30 minutes and placed in a thermostated oil bath at 80 °C to initiate the polymerization. The immersion in the oil bath defines t = 0 and a sample was removed using a deoxygenated syringe as a reference point. The polymerization was stopped and the reaction mixture was subjected to ^{1}H-NMR spectroscopy to determine monomer conversion and, to SEC analysis to determine the molar mass and the dispersity. The DMA conversion was determined by comparing the integrals of vinylic protons from DMA at 5.55 ppm with the integral of C***H***O from DMF at 8 ppm. The monomer conversion mediated by Tpy-CTA was determined to be 54 % in 30 min. DMF and 1,4-dioxane were removed using a rotary evaporator and polymer was purified by a series of precipitations in cold ether, filtered and dried in a vacuum oven at 25 °C. **SEC** (DMF-LiBr, 60°C) : $\overline{M_{n,SEC,Tpy\text{-}PDMA\text{-}CTA}} = 14100$ g.mol^{-1}, $Đ_{Tpy\text{-}PDMA\text{-}CTA} = 1.19$.

^{1}H-NMR (Tpy-PDMA-CTA, 400MHz, $CDCl_3$), δ in ppm: 0.88 (t, PDMA-S-C(=S)S-$(CH_2)_{11}$-$C\boldsymbol{H}_3$); 1-2,7(m, -OCH_2-$C\boldsymbol{H}_2$-$C\boldsymbol{H}_2$-$C\boldsymbol{H}_2$-, PDMA-S-C(=S)S-CH_2-$(C\boldsymbol{H}_2)_{10}$-CH_3; -$C\boldsymbol{H}_2$-CH-C(=O)-$N(CH_3)_2$; -$C(C\boldsymbol{H}_3)_2$-CO-NH-; 2.7-3.2 (m, -C$\boldsymbol{H}$-C(=O)-$N(CH_3)_2$); -O-$(CH_2)_4$-$C\boldsymbol{H}_2$-NH-; PDMA-S-C(=S)S-$C\boldsymbol{H}_2$-$(CH_2)_{10}$-CH_3; -$N(C\boldsymbol{H}_3)_2$); 4.21 (t, 2H, -$OC\boldsymbol{H}_2$-CH_2-); 7.32 (ddd, 2H, -C(N)=CH-C$\boldsymbol{H}$=); 7.84 (dt, 2H, =N-CH-CH-C$\boldsymbol{H}$-); 8.00 (s, 2H, -OC=C$\boldsymbol{H}$-); 8.60 (dd, 2H, -C(N)=C$\boldsymbol{H}$-); 8.68 (dd, 2H, =N-CH-C$\boldsymbol{H}$=). **MALDI-TOF** (DCTB, NaTFA): $\overline{M_{n,\text{Tpy-PDMA-CTA}}}$ = 9530 g.mol^{-1}, Đ$_{\text{Tpy-PDMA-CTA}}$ = 1.04.

4-RAFT copolymerization of NIPAM and NAMA mediated by Tpy-PDMA-CTA in dispersed media

Dispersion copolymerization of NIPAM (3.43 g) and NAMA (1.0266 g) was performed in water at 70°C in the presence of PDMA macroCTA (Tpy-PDMA-CTA,1.5 g), of DMF as internal standard, and of basic aqueous solution (255.9 g of a solution containing 0.360 g of $NaHCO_3$ and 300 g of deionized water) of 2,2'-azobis(2-methyl-propionamidine)dihydrochloride (V50, 12.1 mg) using the ratio of [Tpy-PDMA$_{91}$-CTA]$_0$/[NIPAM]$_0$/[NAMA]$_0$/[V50]$_0$=1/194/42/0.3. The solution was purged with argon for 30 min in an ice bath, and then placed in an oil bath thermostated at 70°C to initiate the polymerization. Twenty five minutes later the opalescence appeared and the reaction was continued for 200 min, quenched by immersion of the flask in iced water and then was lyophilized. The complete monomer conversion was checked by ^{1}H-NMR spectroscopy in D_2O by the relative integration of the protons of DMF at 8 ppm and the vinylic protons of NIPAM and NAMA between 5.9-6.3 ppm.

^{1}H-NMR (Tpy-PDMA$_{91}$-*b*-P(NIPAM-*co*-NAMA), 400 MHz, D_2O), δ in ppm: 0,88-2,7 (m, PDMA-S-C(=S)S-$(CH_2)_{11}$-$C\boldsymbol{H}_3$; PDMA-S-C(=S)S-CH_2-$(C\boldsymbol{H}_2)_{10}$-CH_3; -$C\boldsymbol{H}_2$-CH-CO-$N(CH_3)_2$; -$C(C\boldsymbol{H}_3)_2$-CO-NH-; -NH-$CH(C\boldsymbol{H}_3)_2$; -CH-CO-NH-$C(C\boldsymbol{H}_3)_2$-COOH; -C$\boldsymbol{H}$-CO-NH-$CH(CH_3)_2$;

-C***H***-CO-NH-C(CH$_3$)$_2$-COOH; -C***H***$_2$-CH-CO-NH-CH(CH$_3$)$_2$; -C***H***$_2$-CH-CO-NH-C(CH$_3$)$_2$-COOH; -C***H***-CO-N(CH$_3$)$_2$); 2.7-3.2 (m, -O-(CH$_2$)$_4$-C***H***$_2$-NH-; PDMA-S-C(=S)S-C***H***$_2$-(CH$_2$)$_{10}$-CH$_3$; -N(C***H***$_3$)$_2$; 4.00 (m, -NH-C***H***(CH$_3$)$_2$); 7.32 (ddd, 2H, -C(N)=CH-C***H***=); 7.84 (dt, 2H, =N-CH-CH-C***H***-); 8.00 (s, 2H, -OC=C***H***-); 8.60 (dd, 2H, -C(N)=C***H***-); 8.68 (dd, 2H, =N-CH-C***H***=). **FTIR** (ν in cm^{-1}): 1716 ($\nu_{C=O}$(NAMA)); 1616 (($\nu_{C=O}$(DMA)).

5-Cyclization of NAMA units of Tpy-PDMA-*b*-P(NIPAM-*co*-NAMA) copolymer

0.1432 g of Tpy-PDMA-*b*-P(NIPAM-*co*-NAMA) (4.025 μmol) and 10 mL of acetic anhydride (0.105 mol) were added to 10 mL of dry THF. The reaction mixture was stirred and deoxygenated by bubbling argon for 1 h. at room temperature. Then, the solution was stirred for 25 h. at 70°C. The filtrate was concentrated under vacuum and the filtrate residue was purified by two precipitations in cold ether. The solid was filtered and dried under vacuum. The copolymer Tpy-PDMA-*b*-P(NIPAM-*co*-VDM) was characterized by SEC, ^{1}H NMR spectroscopy and IR spectroscopy. **SEC** (DMF-LiBr, 60°C): $\overline{M_{n,SEC,Tpy\text{-}PDMA\text{-}b\text{-}P(NIPAM\text{-}co\text{-}VDM)}}$ = 43800 g.mol^{-1}, Đ$_{\text{Tpy-PDMA-}b\text{-P(NIPAM-}co\text{-VDM)}}$ = 1.42. **^{1}H-NMR** (400 MHz, D$_2$O), δ in ppm: 0,88-2,7 (m, 2302H, PDMA-S-C(=S)S-(CH$_2$)$_{11}$-C***H***$_3$; PDMA-S-C(=S)S-CH$_2$-(C***H***$_2$)$_{10}$-CH$_3$; -C***H***$_2$-CH-CO-N(CH$_3$)$_2$; -C(C***H***$_3$)$_2$-CO-NH; -NH-CH(C***H***$_3$)$_2$; -OCO-C(C***H***$_3$)$_2$-N=; -C***H***-CO-NH-CH(CH$_3$)$_2$; -C***H***-C=N-C(CH$_3$)$_2$-; -C***H***$_2$-CH-CO-NH-CH(CH$_3$)$_2$; -C***H***$_2$-CH-C=N-C(CH$_3$)$_2$-; -C***H***-CO-N(CH$_3$)$_2$); 2.7-3.2 (m, 550H, -O-C***H***$_2$-(CH$_2$)$_4$-NH-; -O-(CH$_2$)$_4$-C***H***$_2$-NH-; PDMA-S-C(=S)S-C***H***$_2$-(CH$_2$)$_{10}$-CH$_3$; -N(C***H***$_3$)$_2$; 4 (m, 192H, -NH-C***H***(CH$_3$)$_2$); 7.32 (ddd, 2H, -C(N)=CH-C***H***=); 7.84 (dt, 2H, =N-CH-CH-C***H***-); 8.00 (s, 2H, -O-C=C***H***-); 8.60 (dd, 2H, -C(N)=C***H***-); 8.68 (dd, 2H, =N-CH-C***H***=). $\overline{DP_{n,PDMA,NMR}}$ = 91, $\overline{DP_{n,PNIPAM,NMR}}$ = 192, $\overline{DP_{n,VDM,NMR}}$ = 28, $\overline{M_{n,NMR,Tpy\text{-}PDMA\text{-}b\text{-}P(NIPAM\text{-}co\text{-}VDM)}}$ = 35074 g.mol^{-1} (= $\overline{DP_{n,PNIPAM,NMR}} \times M_{PNIPAM}$ + $\overline{DP_{n,PVDM,NMR}} \times M_{VDM}$ + $M_{extr.}$, with M_{NIPAM} = 112.01 g.mol^{-1}, M_{VDM} = 138.15 g.mol^{-1} and

$M_{extr.=}$ 9700 g.mol^{-1}). **FTIR** (ν in cm^{-1}): 1817.96 ($\nu_{C=O}$(azlactone)); 1628 ($\nu_{C=N}$(azlactone)); 1616 (($\nu_{C=O}$(DMA)).

6-Preparation of micelles and hydrogels based on Fe(II)-bis-Tpy-PDMA-*b*-P(NIPAM-*co*-VDM)

Tpy-PDMA$_{91}$-*b*-P(NIPAM$_{192}$-*co*-VDM$_{28}$) was dissolved in pure water at a desired concentration (from 0.5% w/v to 8% w/v), then stirred 12 hours at room temperature. A series of micelles and hydrogels were prepared from copolymer solutions at 20°C upon addition of a determined volume of aqueous solution containing $FeCl_2,4H_2O$ (half an equivalent of metal ions with respect to the amount of terpyridine groups). The samples were stirred 24 hours at room temperature.

7-Figures

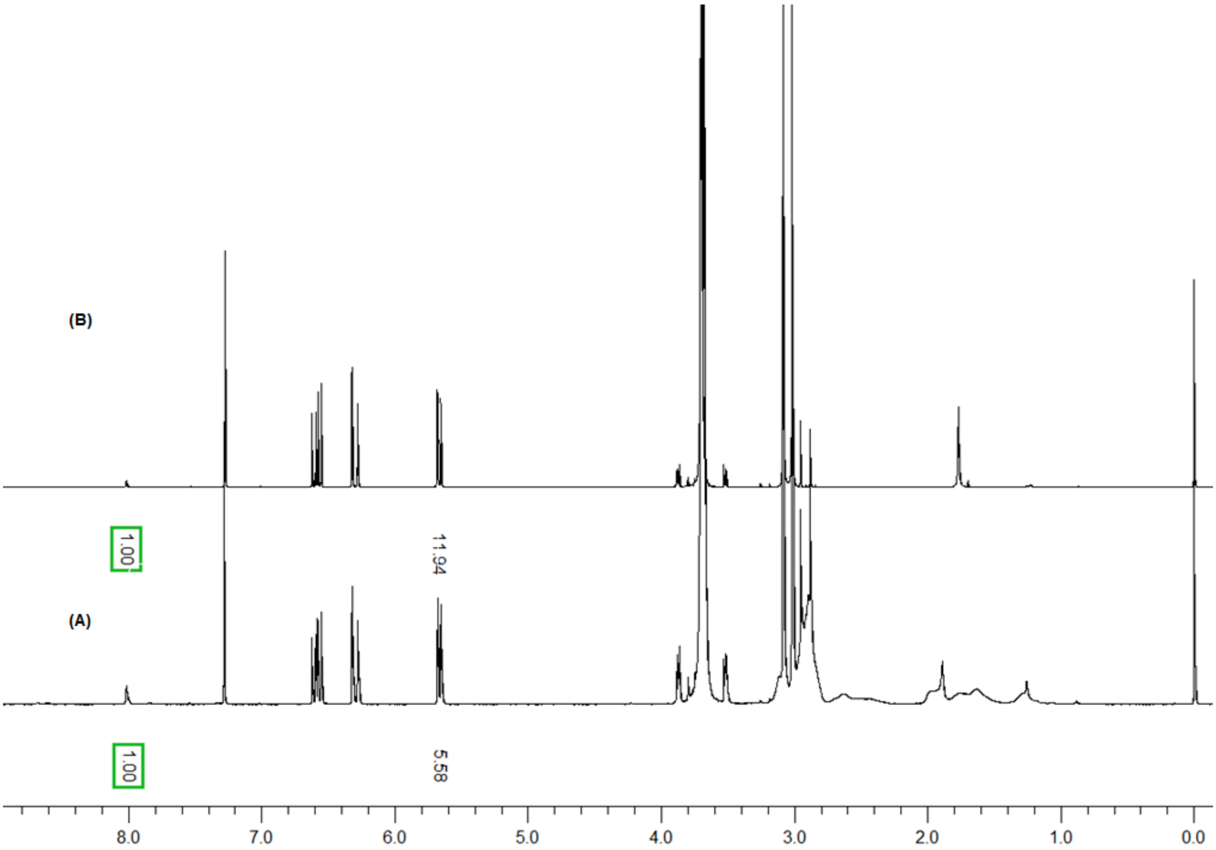

Figure S1. Overlay of ^{1}H-NMR spectra (400 MHz, $CDCl_3$) of crude solution issued from the RAFT polymerization of DMA in DMF at 80 °C using Tpy-CTA as the chain transfer agent and ACPA with a $[DMA]_0/[Tpy\text{-}CTA]_0/[ACPA]_0$ = 180/1/0.142 ratio for a reaction time of (A) t = 0 and (B) t = 30 min.

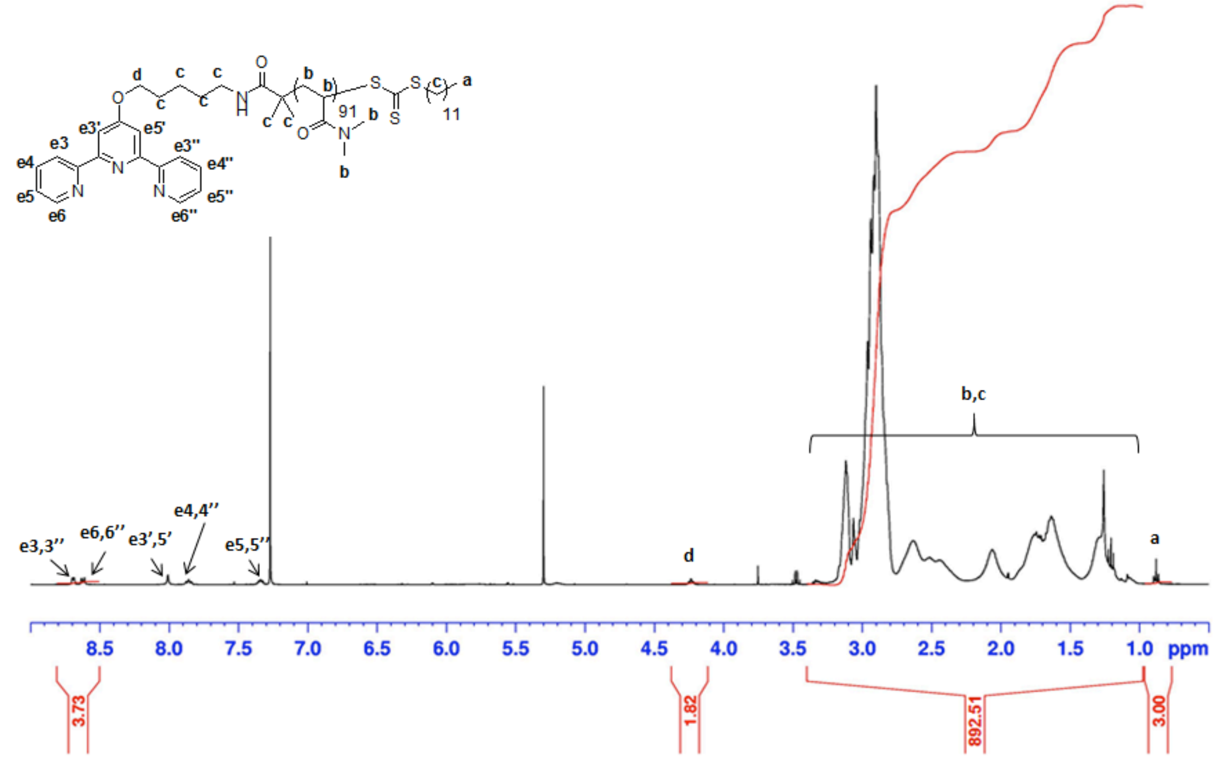

Figure S2. ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of purified Tpy-PDMA$_{91}$-CTA prepared by RAFT polymerization of DMA in DMF at 80 °C using Tpy-CTA as the chain transfer agent and ACPA as the source of radicals with a $[DMA]_0/[Tpy\text{-}CTA]_0/[ACPA]_0 = 180/1/0.142$ for a reaction time of 30 min.

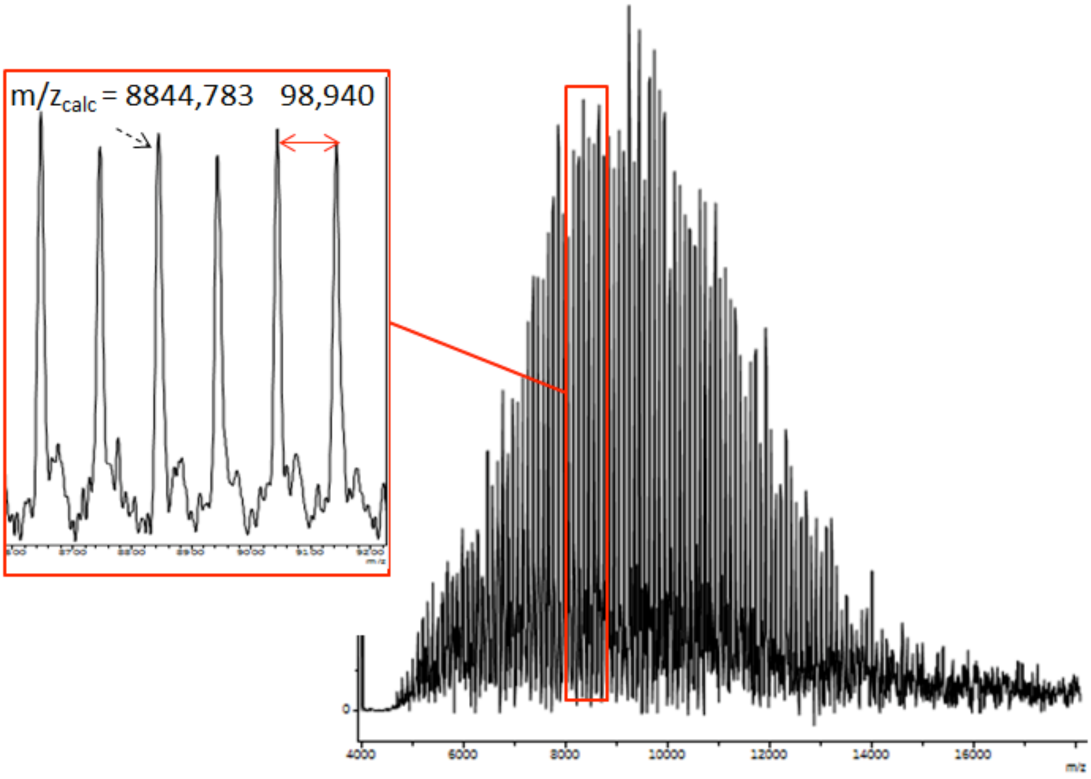

Figure S3. MALDI-TOF mass spectrum (matrix: *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) + sodium trifluoroacetate (NaTFA)) of the Tpy-PDMA$_{91}$-CTA.

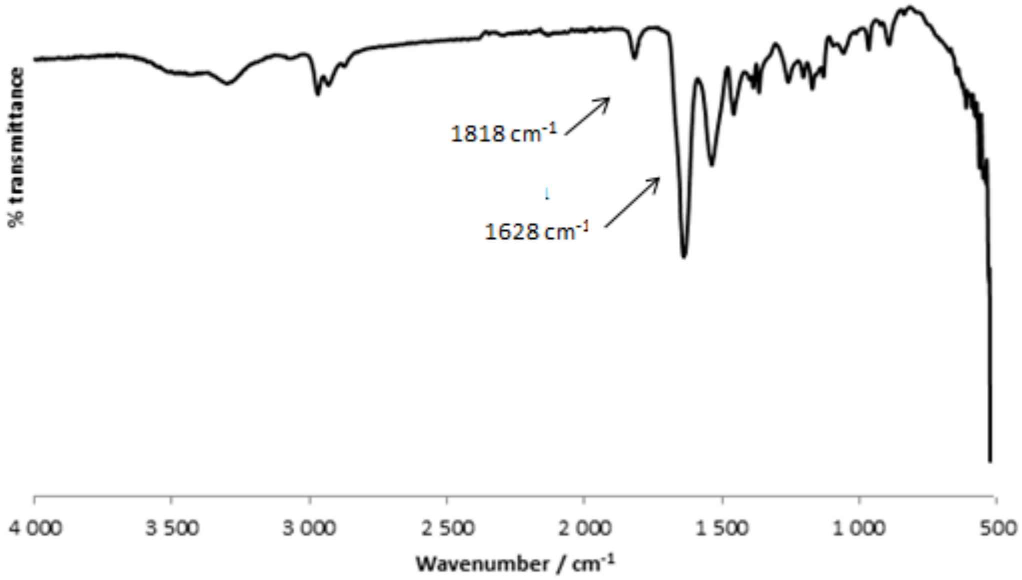

Figure S4. FT-IR spectrum of Tpy-PDMA$_{91}$-*b*-P(NIPAM$_{192}$-*co*-VDM$_{28}$) block copolymer.

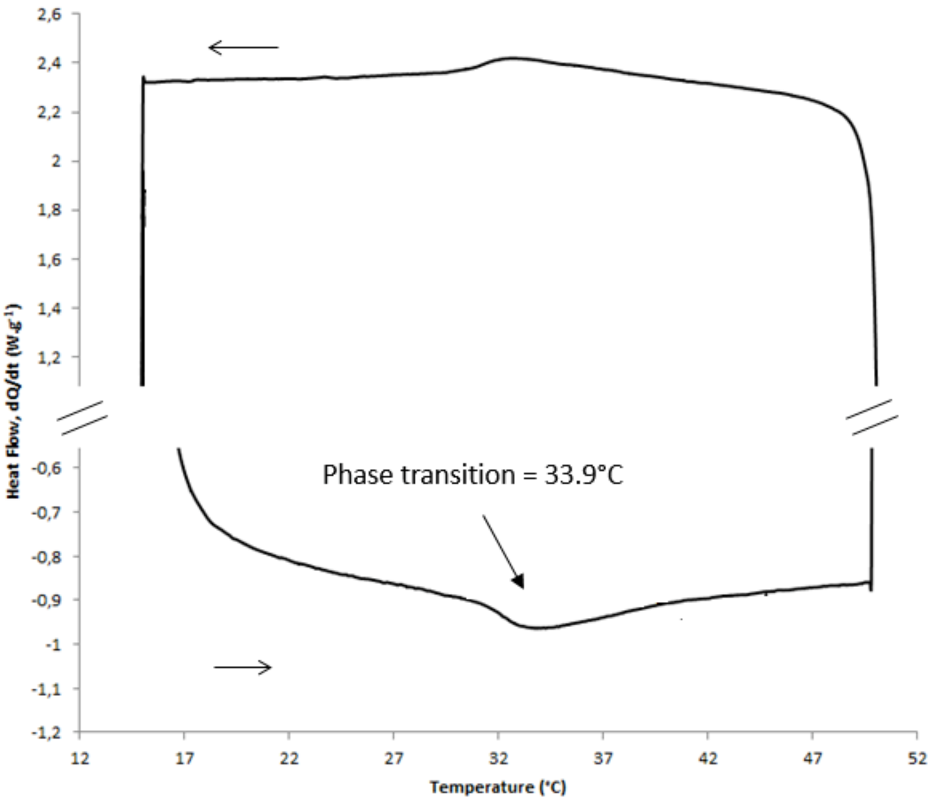

Figure S5. DSC thermogram of metallic bis-terpyridine complex [Fe(Tpy-PDMA$_{91}$-*b*-P(NIPAM$_{192}$-*co*-VDM$_{28}$))$_2$]$^{2+}$.

8-References

(1) M. E. Levere, H. T. Ho, S. Pascual, L. Fontaine, *Polym. Chem.,* 2011, **2**, 2878-2887.

(2) S. Piogé, C. A. Fustin, J. G. Gohy, *Macromol. Rapid Commun.,* 2012, **33**, 534-539.