Electronic Supplementary Information (ESI) document

Temperature-responsive methacrylamide polyampholytes.

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1. Polymer synthesis

1.1 Synthesis of P(DMAPMAm-*stat*-MAA-*stat*-tBMAm) copolymers (W1-W4)

DMAPMAm (5.13 g, 30.12 mmol), MAA (2.60 g, 30.18 mmol), tBMAm (0.69 g, 4.85 mmol), CETCPA (0.10 g, 0.33 mmol), ACVA (4.70 mg, 16.77 µmol) and the internal standard 1,3,5-trioxane (0.53 g, 5.85 mmol) were each added to volumetric flasks (10 mL, 5 mL, 5 mL, 1 mL, and 5 mL, respectively) and diluted with water. For tBMAm, CETCPA and ACVA, approximately 1 mL of EtOH was also added to each flask to help solublise the components. Once the volumetric flasks had been made up to the mark

and shaken to form an homogeneous mixture, small portions of each stock solution were each added to 25 mL screw cap vials as shown in Table S1.

Table S1. Volumes of stock solutions added to reaction vessels during the synthesis of polymers **W1**-**W4**.

Reaction	DMAPMAm stock solution (mL)	MAA stock solution (mL)	tBMAm stock solution (mL)	CETCPA stock solution (mL)	ACVA stock solution (mL)	IS stock solution (mL)	Water (mL)
W1	2.59	2.45	2.31	2.17	0.24	0.67	1.85
W2	1.30	1.23	1.16	1.09	0.24	0.67	1.23
W3	0.00	0.80	1.59	2.37	0.24	0.66	0.62
W4	1.20	1.19	1.19	1.18	0.24	0.66	0.01

The contents of the vials were gently agitated to mix all components and a small aliquot was taken for NMR analysis (t=0 time point). A rubber septa was then fitted to the top of each vial and oxygen was removed *via* a nitrogen purge for 30 min. The vials were then placed in a temperature controlled oil bath that had been pre-heated to 70 °C for 19 h. After this time the vials were removed from the oil bath, cooled to room temperature and opened to air to quench the polymerisation. The resultant polymers were isolated by dialysis (SpectraPor tubing RC, MWCO 3.5 kDa) and were dialysed against Milli-Q grade water. Water was replaced 6 times over the course of the dialysis. Cleaned samples were then transferred to round bottomed flasks, frozen and then dried using a freeze dryer. All polymers were isolated as fluffy white solids. Monomer consumptions, overall polymer conversions and the ratio of monomers in the final polymers were calculated from ^{1}H NMR spectra by comparison of the integrals of the vinylic protons at the beginning and the end of polymerisations against the integral of the internal standard at ~5.2 ppm, as shown in Table 2 of the main manuscript.

1.2 Synthesis of P(DMAPMAm-*stat*-MAA-*stat*-tBMAm) copolymer (D1)

DMAPMAm (2.62 g, 15.38 mmol), MAA (1.32 g, 15.31 mmol), tBMAm (1.09 g, 7.73 mmol), CETCPA (59.0 mg, 192.12 µmol), ACVA (2.70 mg, 9.61 µmol) and the internal standard 1,3,5-trioxane (0.17 g, 1.92 mmol) were added to a 25 mL glass screw cap vial and 1,4-dioxane (5.0 g, 50 wt. %) was added. The mixture was gently agitated to solubilise all components and a small aliquot was taken for NMR analysis (t=0 time point). A rubber septa was then fitted to the top of the vial and oxygen was removed *via* a nitrogen purge for 30 min. The vial was then placed in a temperature controlled oil bath that had been pre-heated to 70 °C for 1.5 h. After this time it was observed that the reaction mixture was cloudy. The polymerisation was left for a further 17 h before the vial was removed from the oil bath, cooled to room temperature and opened to air to quench the polymerisation. At this point, the contents of the vial were completely solid, and so water was added try to solubilise all components for analysis by NMR to determine conversion. Due to the heterogeneous nature of the mixture and the insolubility of tBMAm in water, this analysis could not be completed. The resultant polymer was isolated by dialysis (SpectraPor tubing RC, MWCO 3.5 kDa) and was dialysed first against Milli-Q grade water, then 25% acetone/water (to help remove the large excess of

tBMAm), followed by Milli-Q grade water once more. Dialysate was replaced 6 times over the course of the dialysis. The polymer sample was then transferred to round bottomed flasks, frozen *via* immersion in liquid nitrogen and dried using a freeze dryer. The polymer **D1** was isolated as fluffy white solid. **D1**: 0.58 g (11.5% conversion). GPC: M_n 7 700, M_w 19 400, $Đ$ 2.50.

1.3 Synthesis of P(DMAPMAm-*stat*-MAA-*stat*-tBMAm) (D2)

DMAPMAm (2.27 g, 13.31 mmol), MAA (1.14 g, 13.33 mmol), tBMAm (1.65 g, 11.68 mmol), CETCPA (58.40 mg, 191.25 µmol), ACVA (2.70 mg, 9.56 µmol) and the internal standard 1,3,5-trioxane (170.8 mg, 1.91 mmol) were added to a 25 mL glass screw cap vial and 1,4-dioxane (5.0 g, 50 wt. %) was added. The mixture was gently agitated to solubilise all components and a small aliquot was taken for NMR analysis (t=0 time point). A rubber septa was then fitted to the top of the vial and oxygen was removed *via* a nitrogen purge for 30 min. The vial was then placed in a temperature controlled oil bath that had been pre-heated to 70 °C for 1.5 h. After this time it was observed that the reaction mixture was cloudy. The polymerisation was left for a further 17 h before the vial was removed from the oil bath, cooled to room temperature and opened to air to quench the polymerisation. At this point, the contents of the vial were completely solid, and so water was added try to solubilise all components for analysis by NMR. Due to the heterogeneous nature of the mixture and the insolubility of various components in water, this analysis could not be completed. The resultant polymer was isolated by dialysis (SpectraPor tubing RC, MWCO 3.5 kDa) and was dialysed first against Milli-Q grade water, then 25% acetone/water (to help remove the large excess of tBMAm), followed by Milli-Q grade water once more. Dialysate was replaced 6 times over the course of the dialysis. The polymer sample was then transferred to round bottomed flasks, frozen *via* immersion in liquid nitrogen and dried using a freeze dryer. The polymer **D2** was isolated as fluffy white solid. **D2**: 0.56 g (11.1% conversion). GPC: M_n 7 700, M_w 20 100, $Đ$ 2.59.

1.4 Synthesis of P(DMAPMAm-*stat*-MAA-*stat*-tBMAm) (D3)

DMAPMAm (1.92 g, 11.29 mmol), MAA (0.97 g, 11.23 mmol), tBMAm (2.11 g, 14.94 mmol), CETCPA (58.0 mg, 187.29 µmol), ACVA (2.6 mg, 9.36 µmol) and the internal standard 1,3,5-trioxane (171.6 mg, 1.87 mmol) were added to a 25 mL glass screw cap vial and 1,4-dioxane (5.0 g, 50 wt. %) was added. The mixture was gently agitated to solubilise all components and a small aliquot was taken for NMR analysis (t=0 time point). A rubber septa was then fitted to the top of the vial and oxygen was removed *via* a nitrogen purge for 30 min. The vial was then placed in a temperature controlled oil bath that had been pre-heated to 70 °C for 1.5 h. After this time it was observed that the reaction mixture was cloudy. The polymerisation was left for a

further 17 h before the vial was removed from the oil bath, cooled to room temperature and opened to air to quench the polymerisation. At this point, the contents of the vial were completely solid, and so water was added try to solubilise all components for analysis by NMR. Due to the heterogeneous nature of the mixture and the insolubility of various components in water, this analysis could not be completed. The resultant polymer was isolated by dialysis (SpectraPor tubing RC, MWCO 3.5 kDa) and was dialysed first against Milli-Q grade water, then 25% acetone/water (to help remove the large excess of tBMAm), followed by Milli-Q grade water once more. Dialysate was replaced 6 times over the course of the dialysis. The polymer sample was then transferred to round bottomed flasks, frozen *via* immersion in liquid nitrogen and dried using a freeze dryer. The polymer **D3** was isolated as fluffy white solid. **D3**: 0.39 g (7.8% conversion). GPC: M_n 6 500, M_w 15 800, $Đ$ 2.42.

1.5 Synthesis of P(DMAPMAm-*stat*-MAA-*stat*-tBMAm) (D4)

DMAPMAm (1.59 g, 9.32 mmol), MAA (0.80 g, 9.28 mmol), tBMAm (2.62 g, 18.56 mmol), CETCPA (57.30 mg, 185.78 µmol), ACVA (2.50 mg, 9.29 µmol) and the internal standard 1,3,5-trioxane (167.4 mg, 1.86 mmol) were added to a 25 mL glass screw cap vial and 1,4-dioxane (5.0 g, 50 wt. %) was added. The mixture was gently agitated to solubilise all components and a small aliquot was taken for NMR analysis (t=0 time point). A rubber septa was then fitted to the top of the vial and oxygen was removed *via* a nitrogen purge for 30 min. The vial was then placed in a temperature controlled oil bath that had been pre-heated to 70 °C for 1.5 h. After this time it was observed that the reaction mixture was cloudy. The polymerisation was left for a further 17 h before the vial was removed from the oil bath, cooled to room temperature and opened to air to quench the polymerisation. At this point, the contents of the vial were completely solid, and so water was added try to solubilise all components for analysis by NMR. Due to the heterogeneous nature of the mixture and the insolubility of various components in water, this analysis could not be completed. The resultant polymer was isolated by dialysis (SpectraPor tubing RC, MWCO 3.5 kDa) and was dialysed first against Milli-Q grade water, then 25% acetone/water (to help remove the large excess of tBMAm), followed by Milli-Q grade water once more. Dialysate was replaced 6 times over the course of the dialysis. The polymer sample was then transferred to round bottomed flasks, frozen *via* immersion in liquid nitrogen and dried using a freeze dryer. The polymer **D4** was isolated as fluffy white solid. **D4**: 0.32 g (6.4 % conversion). GPC: M_n 4 800, M_w 11 500, $Đ$ 2.40.

1.6 Synthesis of P(DMAPMAm-*stat*-MAA-*stat*-tBMAm) (A1-A4)

DMAPMAm (6.40 g, 37.62 mmol), MAA (3.24 g, 37.62 mmol), tBMAm (0.86 g, 6.07 mmol), CETCPA (0.12 g, 0.40 mmol), ACVA (5.70 mg, 20.33 µmol) and the internal standard 1,3,5-trioxane (0.37 g, 4.07 mmol) were each added to volumetric flasks (10 mL,

5 mL, 5 mL, 5 mL, 1 mL, and 5 mL, respectively) and diluted with glacial acetic acid (AcOH). Particular care was taken during the addition of AcOH to the flask containing DMAPMAm, which was kept in an ice bath during this process. Once the volumetric flasks had been made up to the mark and shaken to form a homogeneous mixture, small portions of each stock solution were each added to 25 mL screw cap vials as shown in Table S2.

Table S2. Volumes of stock solutions added to reaction vessels during the synthesis of polymers **A1**-**A4**.

Reaction	DMAPMAm stock solution (mL)	MAA stock solution (mL)	tBMAm stock solution (mL)	CETCPA stock solution (mL)	ACVA stock solution (mL)	IS stock solution (mL)	Water (mL)
A1	2.59	2.45	2.31	2.17	0.24	1.20	3.28
A2	1.30	1.23	1.16	1.09	0.24	1.19	2.65
A3	0.00	0.80	1.59	2.37	0.24	1.19	2.04
A4	1.20	1.19	1.19	1.18	0.24	1.18	1.43

The contents of the vials were gently agitated to mix all components and a small aliquot was taken for NMR analysis (t=0 time point). A rubber septa was then fitted to the top of each vial and oxygen was removed *via* a nitrogen purge for 30 min. The vials were then placed in a temperature controlled oil bath that had been pre-heated to 70 °C for 21 h. After this time the vials were removed from the oil bath, cooled to room temperature and opened to air to quench the polymerisation. The resultant polymers were isolated by dialysis (SpectraPor tubing RC, MWCO 3.5 kDa) and were dialysed against Milli-Q grade water. Water was replaced 6 times over the course of the dialysis. Cleaned samples were then transferred to round bottomed flasks, frozen and then dried using a freeze dryer. All polymers were isolated as fluffy white solids. Monomer consumptions, overall polymer conversions and the ratio of monomers in the final polymers were calculated from ^{1}H NMR spectra by comparison of the integrals of the vinylic protons at the beginning and the end of polymerisations against the integral of the internal standard at ~5.2 ppm, as shown in Table 2 of the main manuscript.

1.7 Synthesis of P(DMAPMAm-*stat*-MAA-*stat*-tBMAm) (A5-A8)

Four aliquots of DMAPMAm [(a) 1.86 g, 10.92 mmol; (b) 1.61 g, 9.46 mmol; (c) 1.40 g, 8.22 mmol; (d) 1.13 g, 6.64 mmol] were each added to a 25 mL screw capped vial and 1 mL of glacial acetic acid (AcOH) was added. Particular care was taken during the addition of AcOH to these flasks, which were cooled under running water immediately after the addition of AcOH. MAA (3.17 g, 36.82 mmol), tBMAm (5.75 g, 40.71 mmol), CETCPA (0.18 g, 0.60 mmol), ACVA (7.90 mg, 28.19 µmol) and the internal standard 1,3,5-trioxane (0.53 g, 5.91 mmol) were each added to volumetric flasks (10 mL, 25 mL, 5 mL, 1 mL, and 5 mL, respectively) and diluted with glacial acetic acid (AcOH). Once the volumetric flasks had been made up to the mark and shaken to form a homogeneous mixture, small portions of each stock solution were each added to the DMAPMAm-containing 25 mL screw cap vials as shown in Table S3.

Table S3. Volumes of stock solutions added to reaction vessels during the synthesis of polymers **A5**-**A8**.

Reaction	MAA stock solution (mL)	tBMAm stock solution (mL)	CETCPA stock solution (mL)	ACVA stock solution (mL)	IS stock solution (mL)	Water (mL)
A5	2.97	3.46	1.21	0.24	1.21	3.89
A6	2.57	5.15	1.20	0.24	1.20	2.69
A7	2.18	6.79	1.19	0.24	1.19	1.51
A8	1.80	8.41	1.17	0.24	1.17	0.35

The contents of the vials were gently agitated to mix all components and a small aliquot was taken for NMR analysis (t=0 time point). A rubber septa was then fitted to the top of each vial and oxygen was removed *via* a nitrogen purge for 30 min. The vials were then placed in a temperature controlled oil bath that had been pre-heated to 70 °C for 19 h. After this time, 0.2 mL of a stock solution of ACVA (8 mg in 0.8 mL glacial acetic acid; purged with nitrogen for 15 min) was added to each of the vials and the reaction continued until a total time of 24.5 h for **A5** and **A6**; and 26 h for **A7** and **A8** had been reached. After this time, the vials were removed from the oil bath, cooled to room temperature and opened to air to quench the polymerisation. The resultant polymers were isolated by dialysis (SpectraPor tubing RC, MWCO 3.5 kDa) and were dialysed against Milli-Q grade water. Water was replaced 6 times over the course of the dialysis. Cleaned samples were then transferred to round bottomed flasks, frozen and then dried using a freeze dryer. All polymers were isolated as fluffy white solids. Monomer consumptions, overall polymer conversions and the ratio of monomers in the final polymers were calculated from ^{1}H NMR spectra by comparison of the integrals of the vinylic protons at the beginning and the end of polymerisations against the integral of the internal standard at ~5.2 ppm, as shown in Table 2 of the main manuscript.

2. Summary of the experimental parameters used during the synthesis of DMAPMAm-*stat*-MAA-*stat*-tBMAm copolymers

Table S4 Summary of the experimental parameters used during the synthesis of DMAPMAm-*stat*-MAA-*stat*-tBMAm copolymers **W1**-**W4**, **D1**-**D4** and **A1**-**A8** *via* RAFT polymerisation.

	Starting monomer ratios			Reaction conditions		
Entry	**%DMAP-MAm**	**%MAA**	**%tBMAm**	**Solvent**	**Time (h)**	**Polymer code**
1[a]	50	50	0	H_2O	19	**W1**
2[a]	47.5	47.5	5	H_2O	19	**W2**
3[a]	45	45	10	H_2O	19	**W3**
4[a]	42.5	42.5	15	H_2O	19	**W4**
5[b]	40	40	20	1,4-dioxane	18.5	**D1**
6[b]	35	35	30	1,4-dioxane	18.5	**D2**
7[b]	30	30	40	1,4-dioxane	18.5	**D3**
8[b]	25	25	50	1,4-dioxane	18.5	**D4**
9	50	50	0	AcOH	21	**A1**
10	47.5	47.5	5	AcOH	21	**A2**
11	45	45	10	AcOH	21	**A3**
12	42.5	42.5	15	AcOH	21	**A4**
13	40	40	20	AcOH	24.5	**A5**
14	35	35	30	AcOH	24.5	**A6**
15	30	30	40	AcOH	26	**A7**
16	25	25	50	AcOH	26	**A8**

[a] Trace amounts of EtOH was needed to solubilise some starting materials; [b] Precipitate formed after 1-2 h.

3. NMR spectra of polymers A1-A8

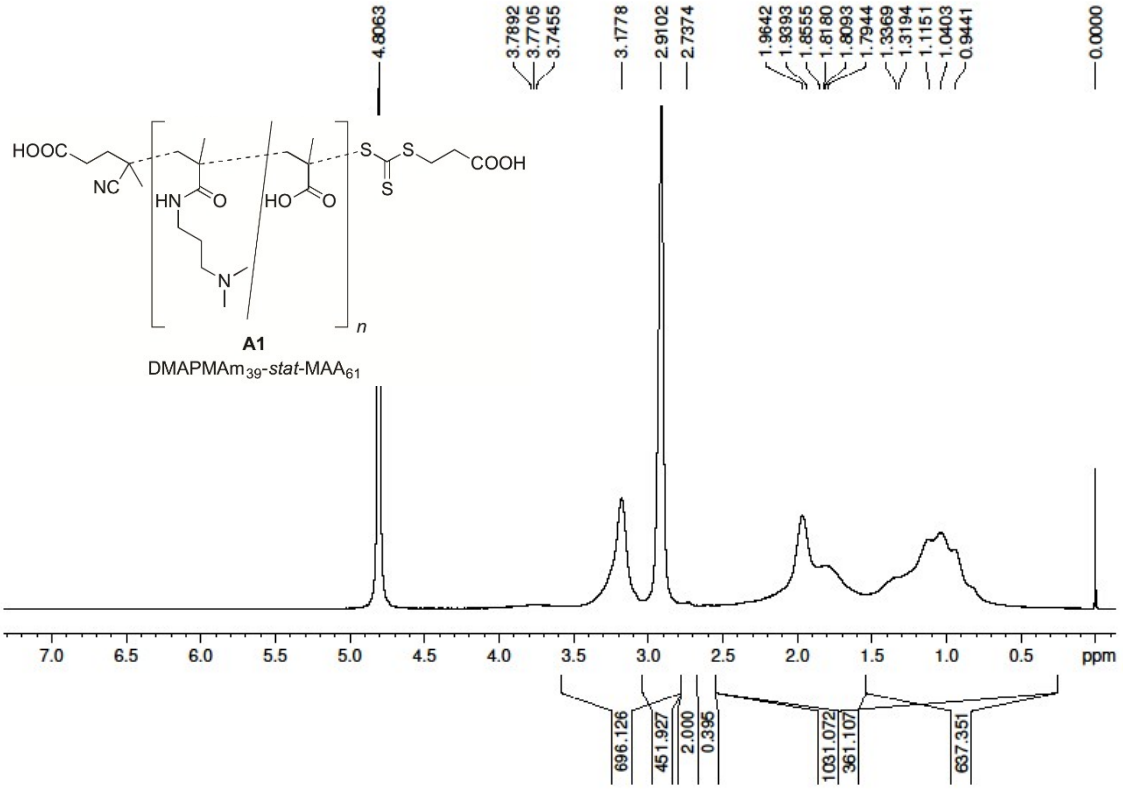

Fig. S1 ^{1}H NMR spectrum of polymer **A1** (D_2O).

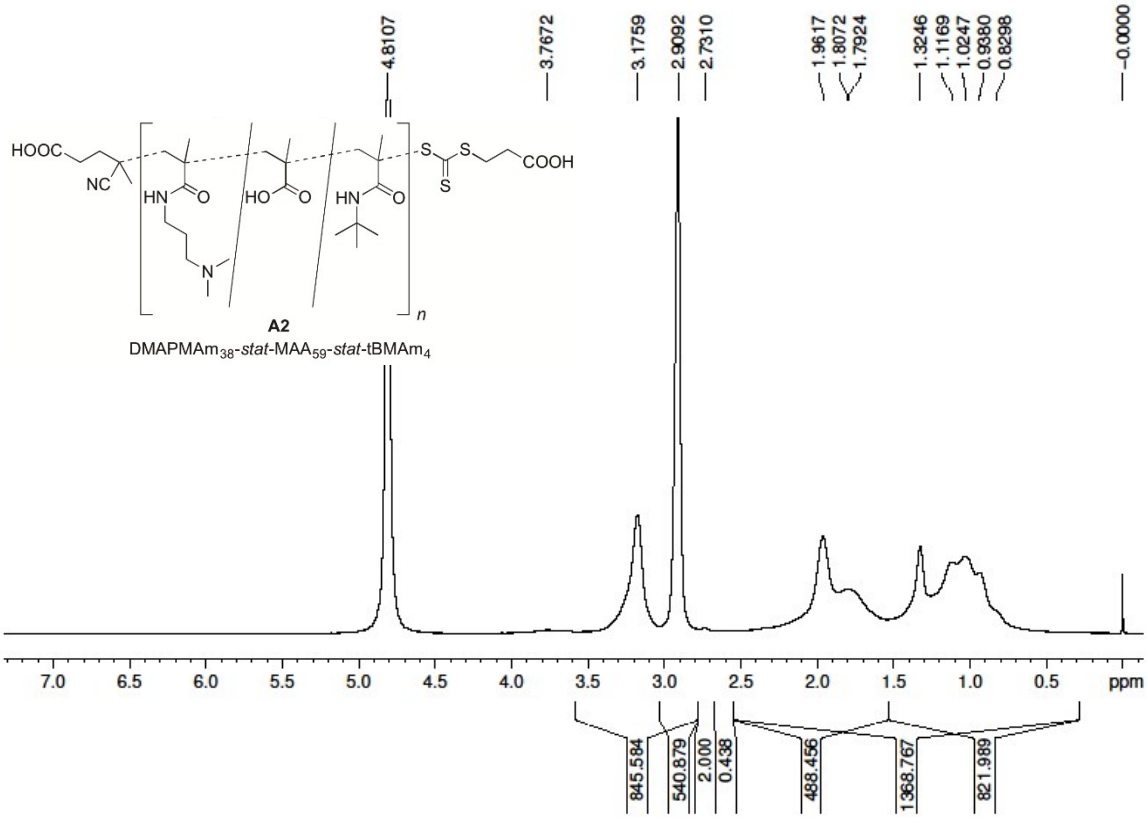

Fig. S2 ^{1}H NMR spectrum of polymer **A2** (D_2O).

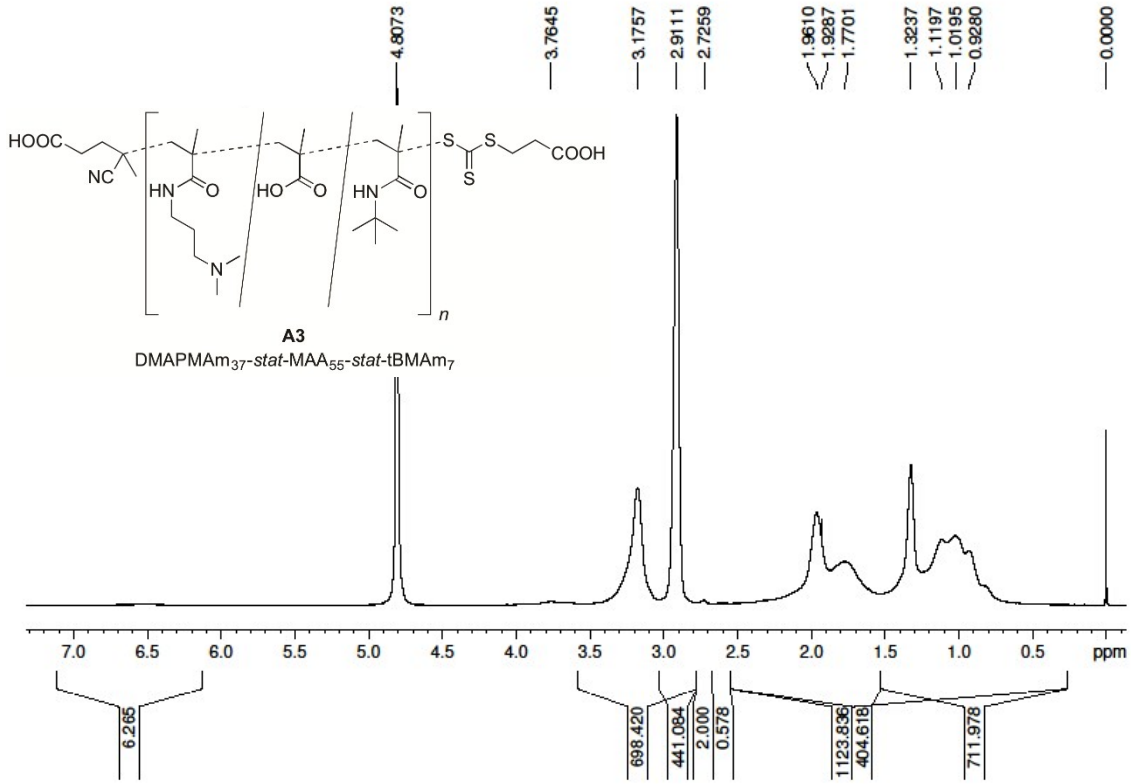

Fig. S3 ^{1}H NMR spectrum of polymer **A3** (D_2O).

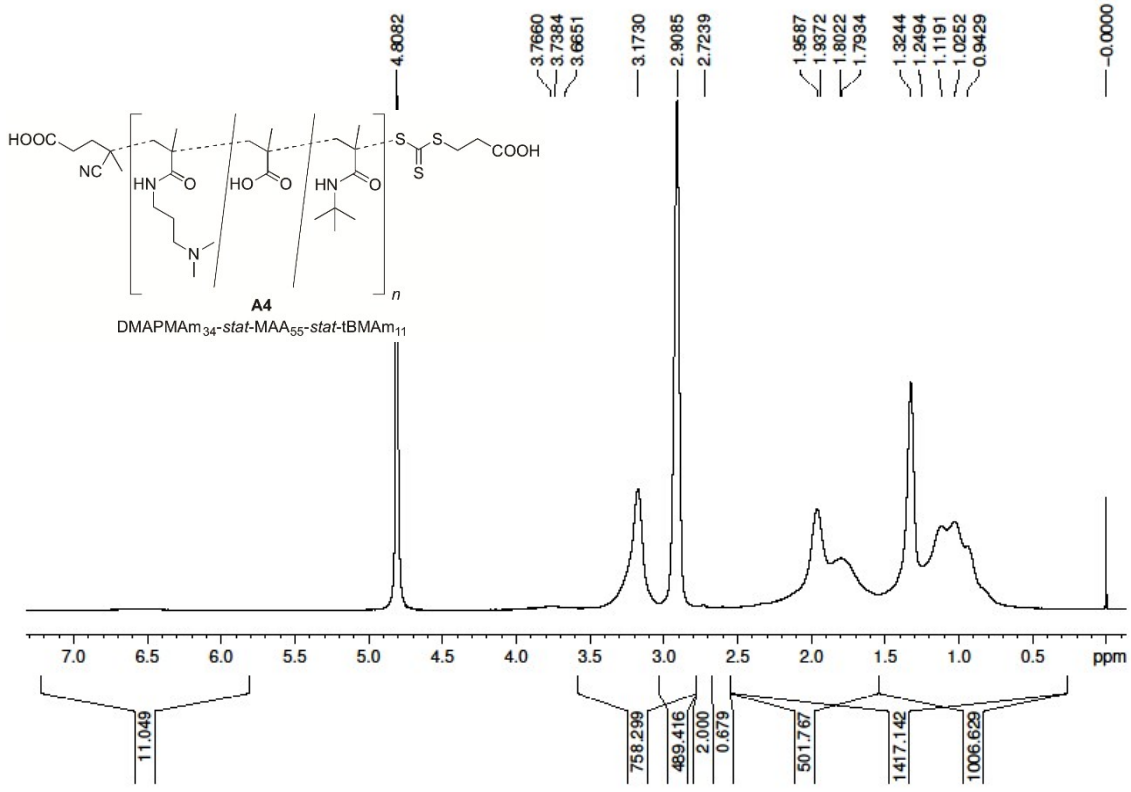

Fig. S4 ^{1}H NMR spectrum of polymer **A4** (D_2O).

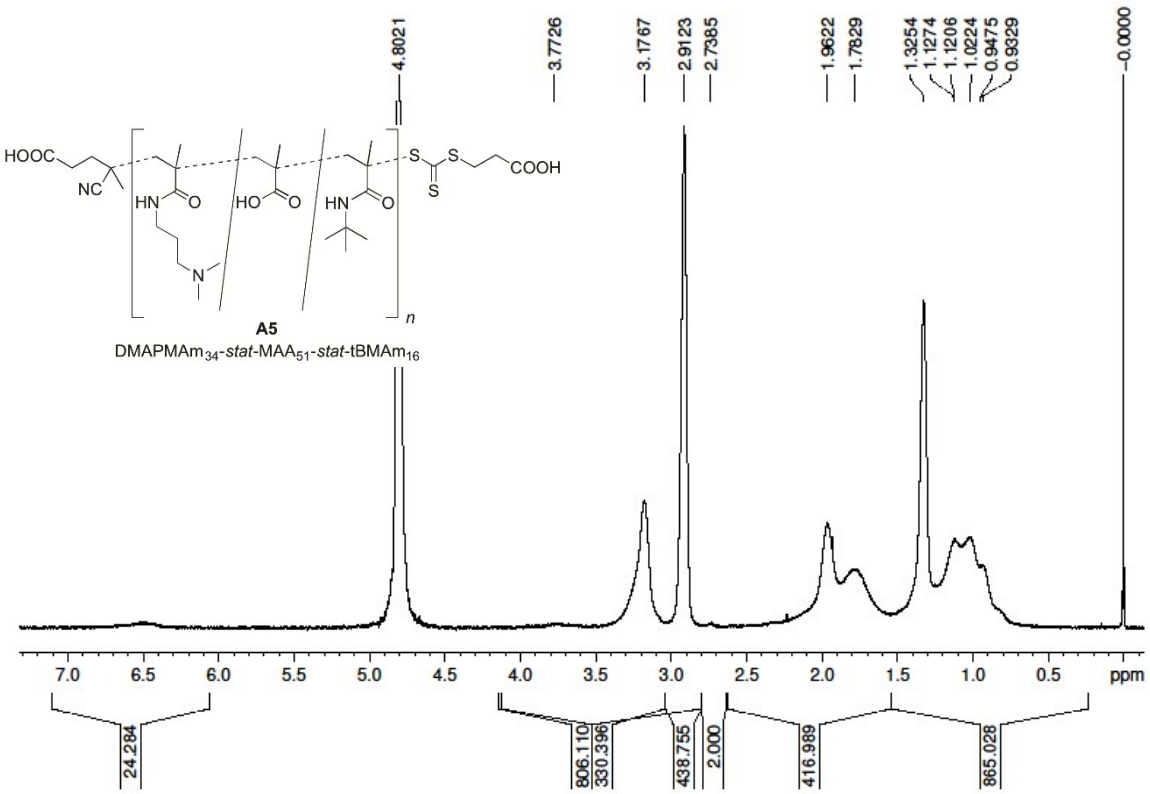

Fig. S5 ^{1}H NMR spectrum of polymer **A5** (D_2O).

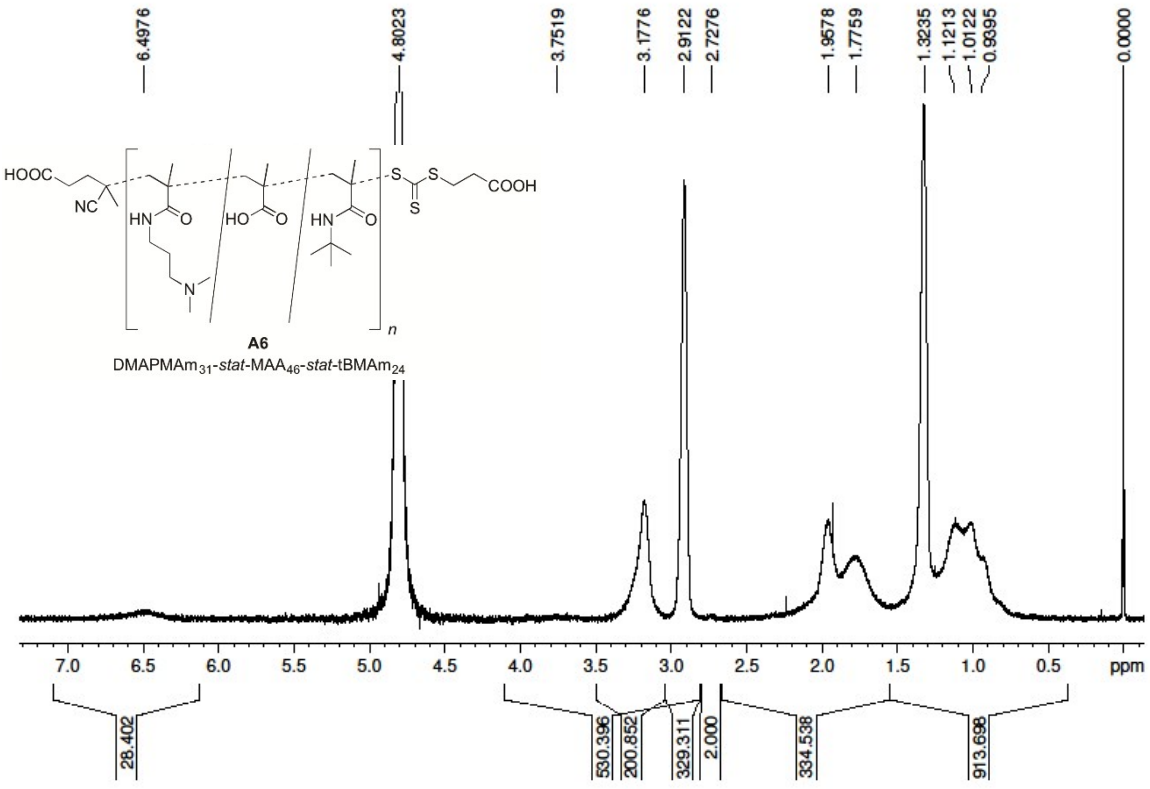

Fig. S6 ^{1}H NMR spectrum of polymer **A6** (D_2O).

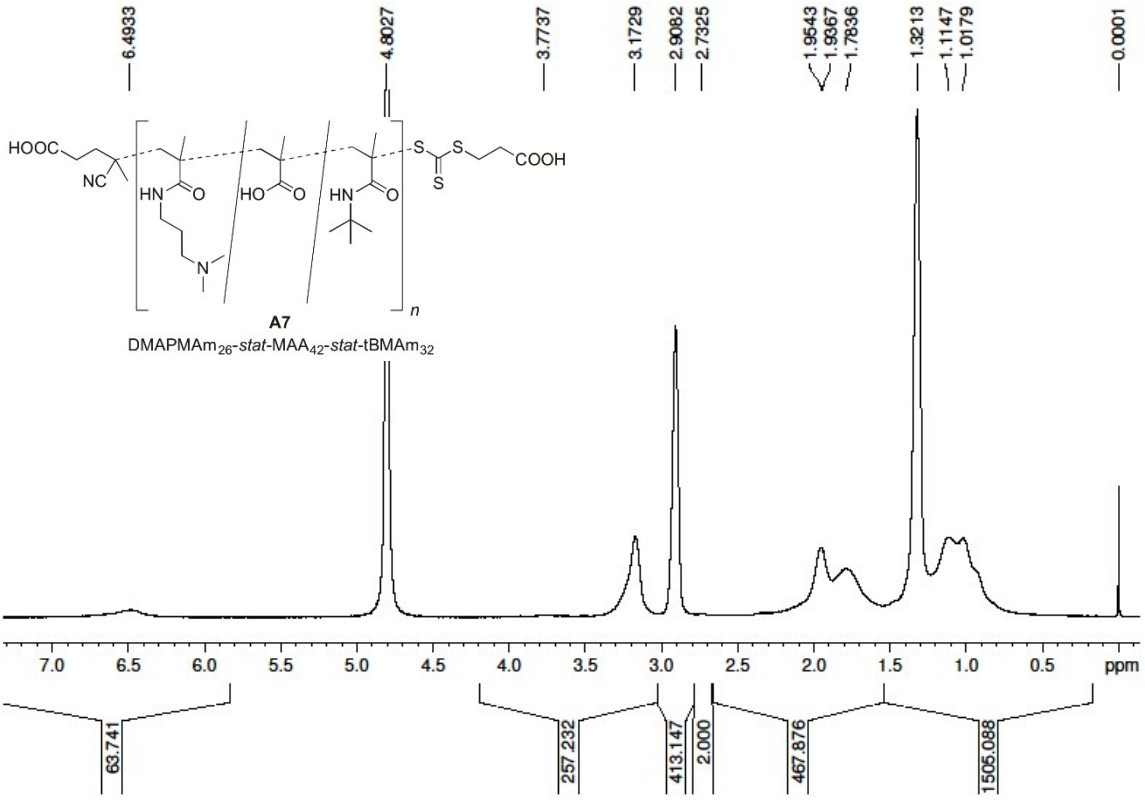

Fig. S7 ^{1}H NMR spectrum of polymer **A7** (D_2O).

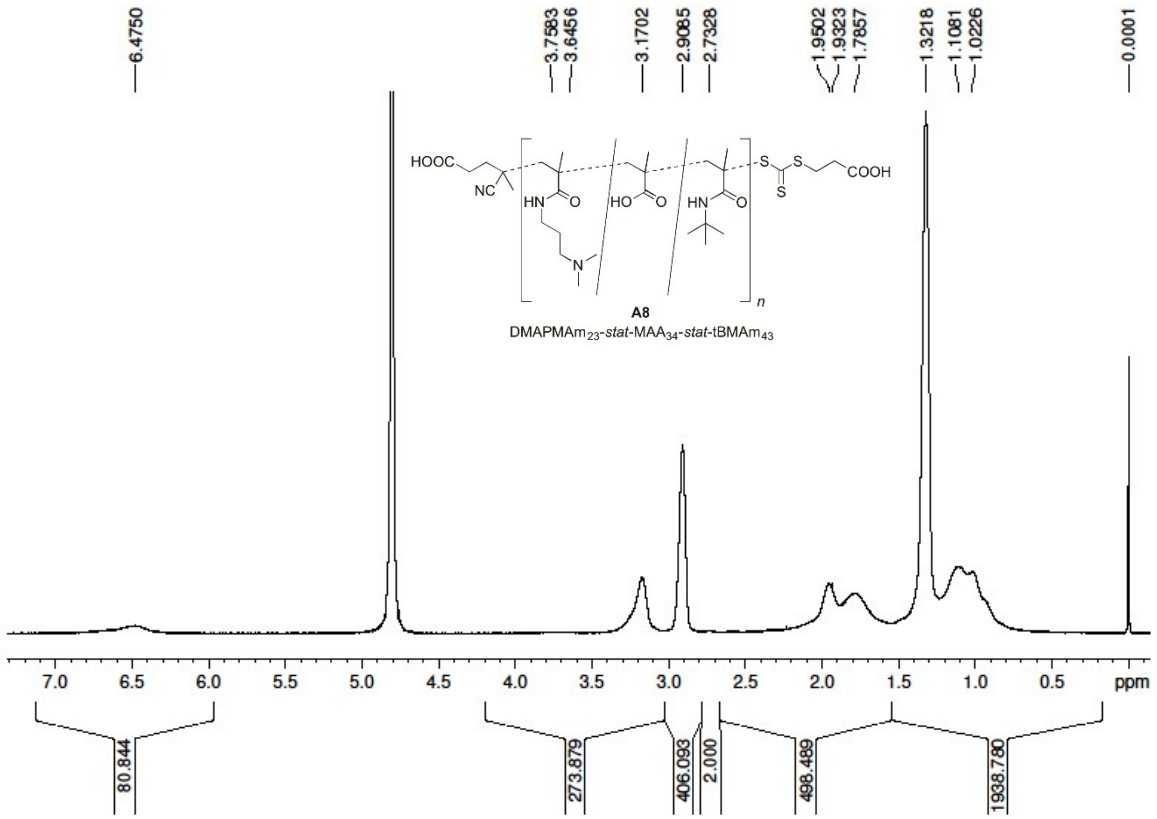

Fig. S8 ^{1}H NMR spectrum of polymer **A8** (D_2O).

4. Mole fraction of monomer *vs.* conversion

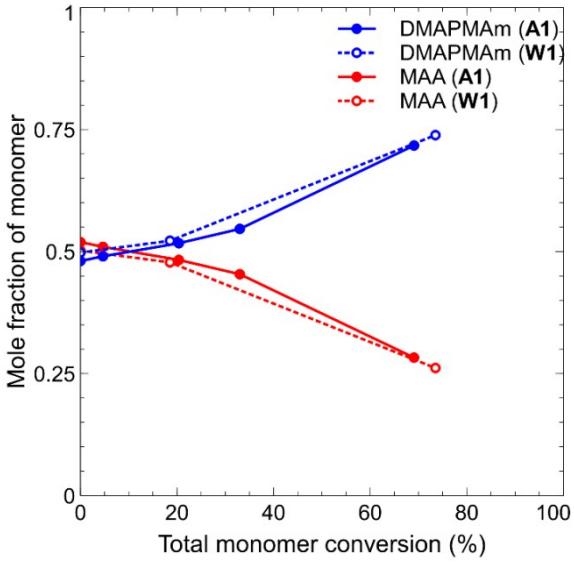

Fig. S9 Mole fraction of monomer as a function of total monomer conversion (%) for polymers **A1** and **W1**, as determined by ^{1}H NMR analysis of reaction aliquots. Data points are coloured according to monomer: red-MAA; blue-DMAPMAm; black-tBMAm and are connected *via* solid lines (acetic acid-synthesised polymers) or dotted lines (water-synthesised polymers).

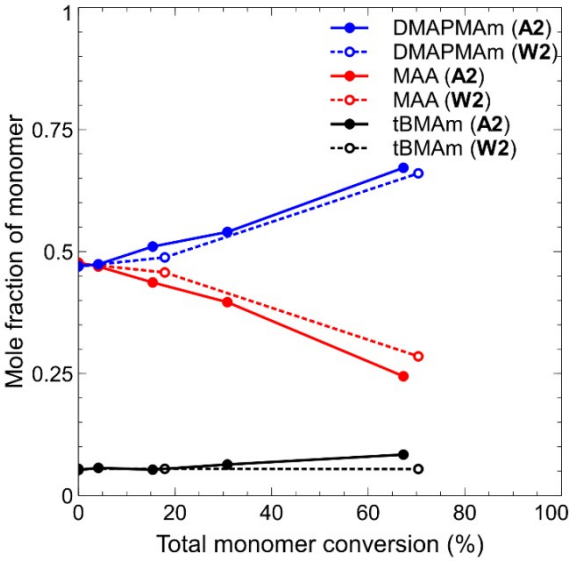

Fig. S10 Mole fraction of monomer as a function of total monomer conversion (%) for polymers **A2** and **W2**, as determined by ^{1}H NMR analysis of reaction aliquots. Data points are coloured according to monomer: red-MAA; blue-DMAPMAm; black-tBMAm and are connected *via* solid lines (acetic acid-synthesised polymers) or dotted lines (water-synthesised polymers).

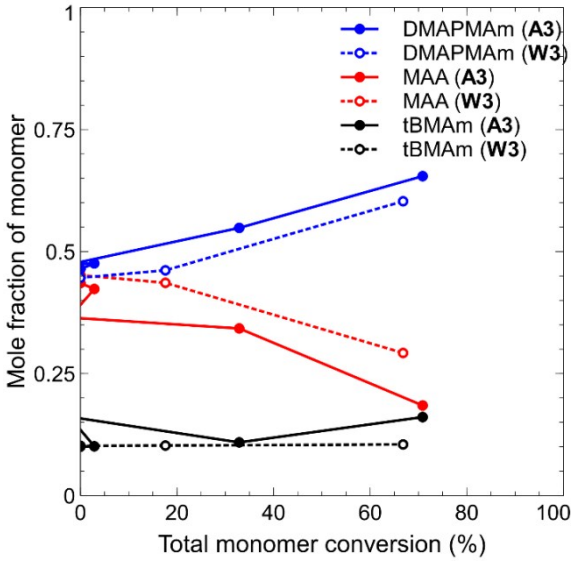

Fig. S11 Mole fraction of monomer as a function of total monomer conversion (%) for polymers **A3** and **W3**, as determined by ^{1}H NMR analysis of reaction aliquots. Data points are coloured according to monomer: red-MAA; blue-DMAPMAm; black-tBMAm and are connected *via* solid lines (acetic acid-synthesised polymers) or dotted lines (water-synthesised polymers).

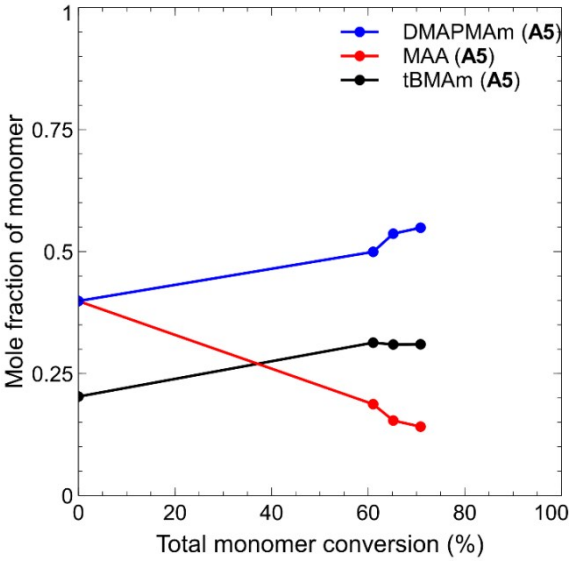

Fig. S12 Mole fraction of monomer as a function of total monomer conversion (%) for polymers **A5**, as determined by ^{1}H NMR analysis of reaction aliquots. Data points are coloured according to monomer: red-MAA; blue-DMAPMAm; black-tBMAm.

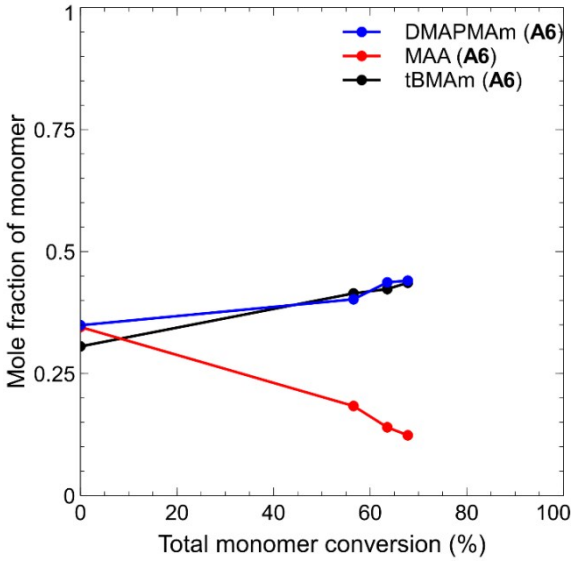

Fig. S13 Mole fraction of monomer as a function of total monomer conversion (%) for polymers **A6**, as determined by ^{1}H NMR analysis of reaction aliquots. Data points are coloured according to monomer: red-MAA; blue-DMAPMAm; black-tBMAm.

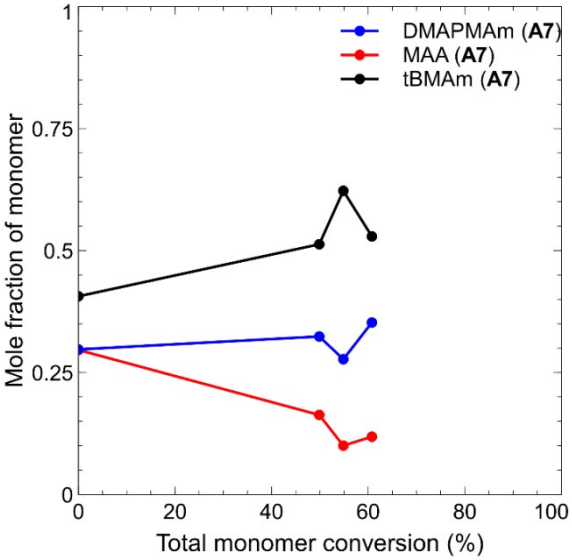

Fig. S14 Mole fraction of monomer as a function of total monomer conversion (%) for polymers **A7**, as determined by ^{1}H NMR analysis of reaction aliquots. Data points are coloured according to monomer: red-MAA; blue-DMAPMAm; black-tBMAm.

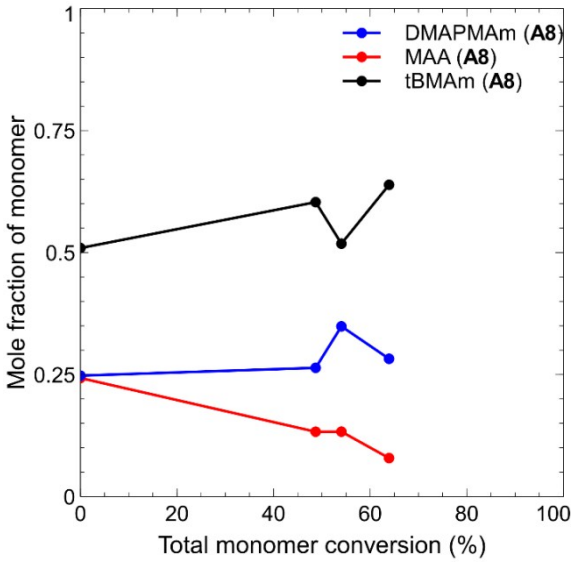

Fig. S15 Mole fraction of monomer as a function of total monomer conversion (%) for polymers **A8**, as determined by ^{1}H NMR analysis of reaction aliquots. Data points are coloured according to monomer: red-MAA; blue-DMAPMAm; black-tBMAm.

5. Temperature dependence of the transmittance (%) curves of polymers A1-A8 at 2.5, 5.0 and 10.0 mg mL^{-1}

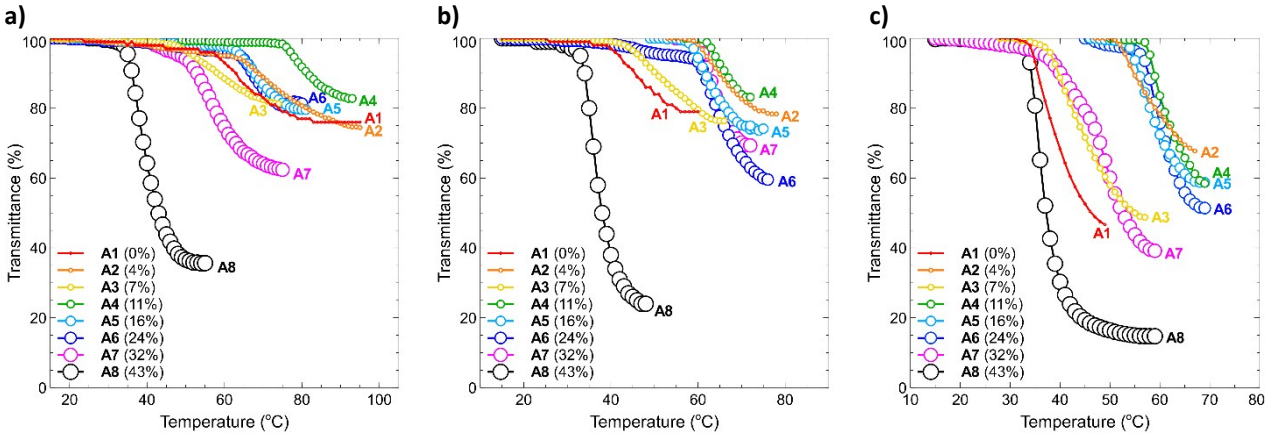

Fig. S16 Temperature dependence of the transmittance (%) of 2.5 mg mL^{-1} (**a**), 5 mg mL^{-1} (**b**), and 10 mg mL^{-1} (**c**) solutions of polymers **A1-A8** in Milli-Q grade water. Data point sizes and colours are set according to the relevant incorporation of tBMAm into each polymer, as indicated in the brackets (%).

6. T_{CP} (°C) *vs.* molar ratio of tBMAm (%) of polymers A5-A8 at 2.5, 5.0 and 10.0 mg mL^{-1}

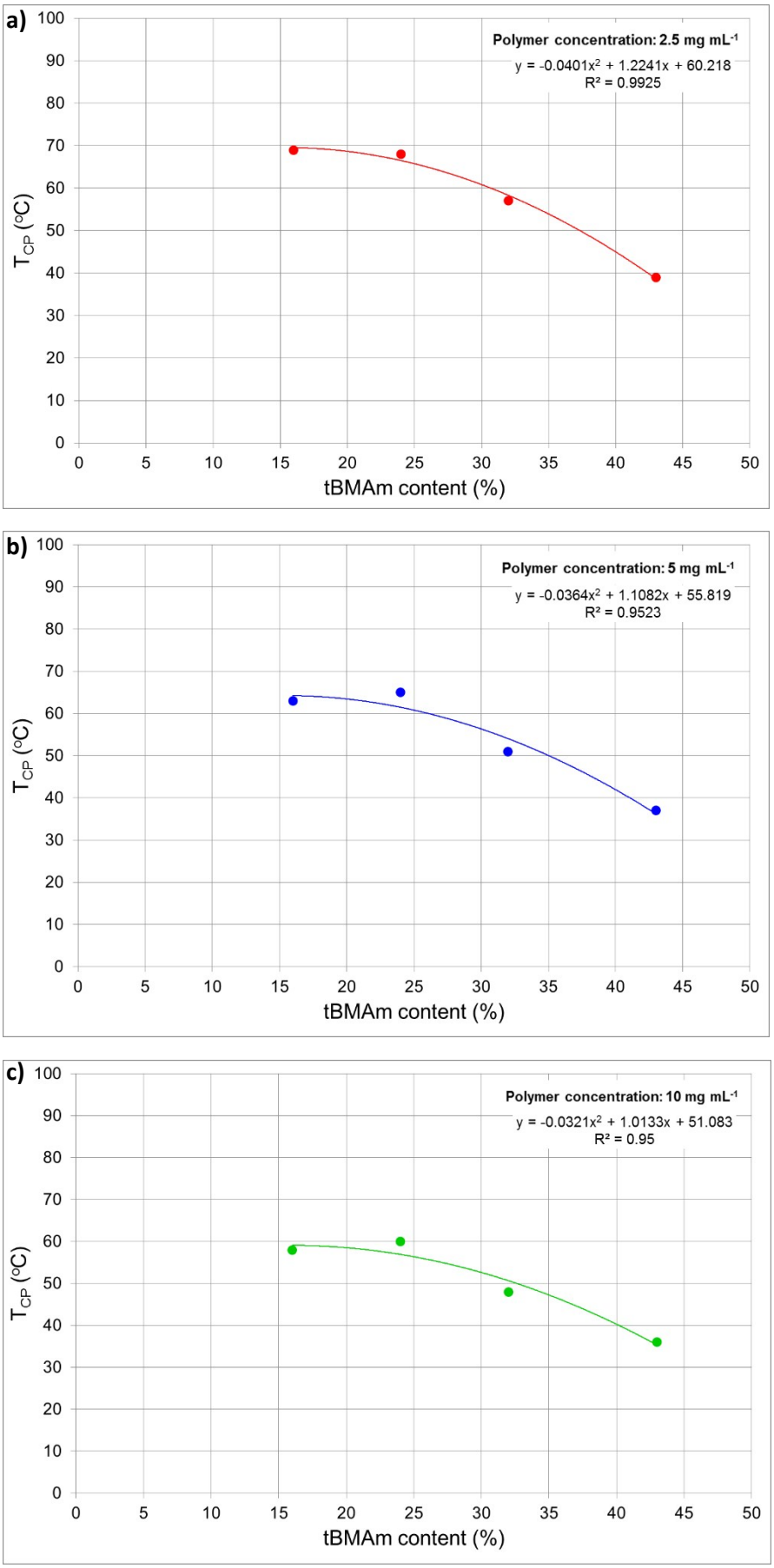

Fig. S17 T_{CP} (°C) *vs.* molar ratio of tBMAm content (%) in polymers **A5-A8** at (a) 2.5 mg mL^{-1}, (b) 5.0 mg mL^{-1}, (c) 10.0 mg mL^{-1}. A clear correlation is observed between decreasing T_{CP} and increasing tBMAm content.

7. The ^{1}H NMR spectra of polymer A8 in the presence and absence of salt at various temperatures

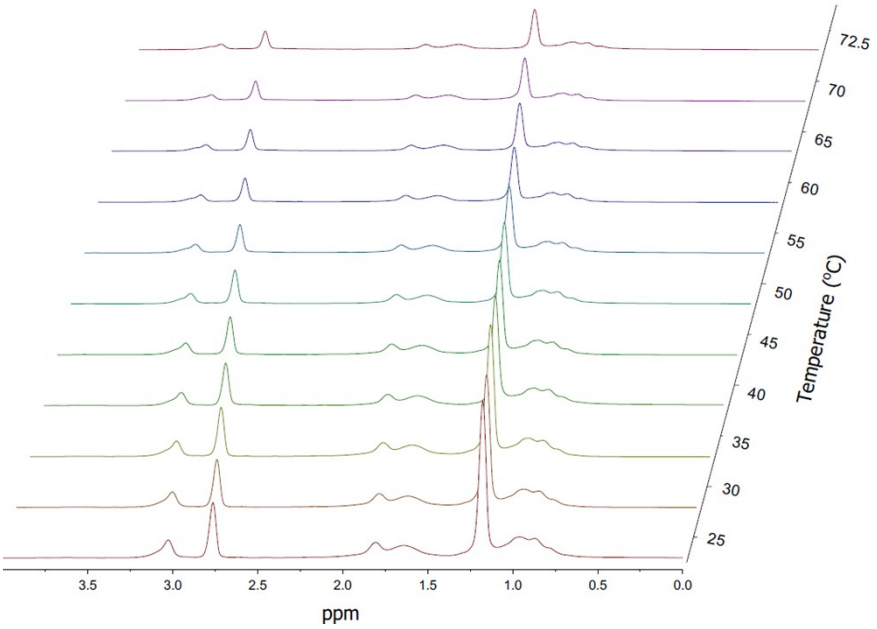

Fig. S18 ^{1}H NMR spectra of polymer **A8** (10 mg mL^{-1} solution in D_2O) at 5 °C* temperature intervals between 25 °C to 72.5 °C. (*final interval is 2.5 °C)

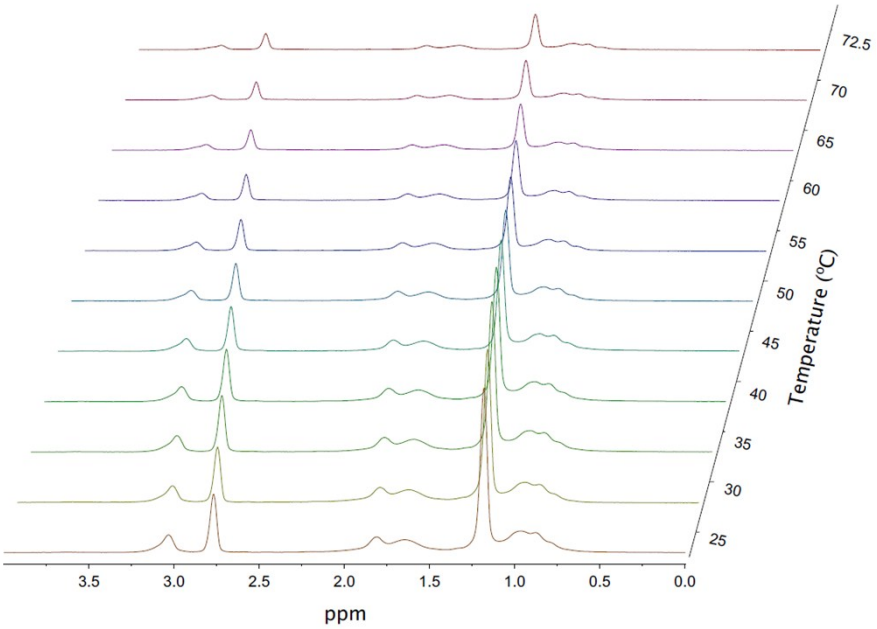

Fig. S19 ^{1}H NMR spectra of polymer **A8** (10 mg mL^{-1} solution in D_2O, 10 mM NaCl) at 5 °C* temperature intervals between 25 °C to 72.5 °C. (*final interval is 2.5 °C).