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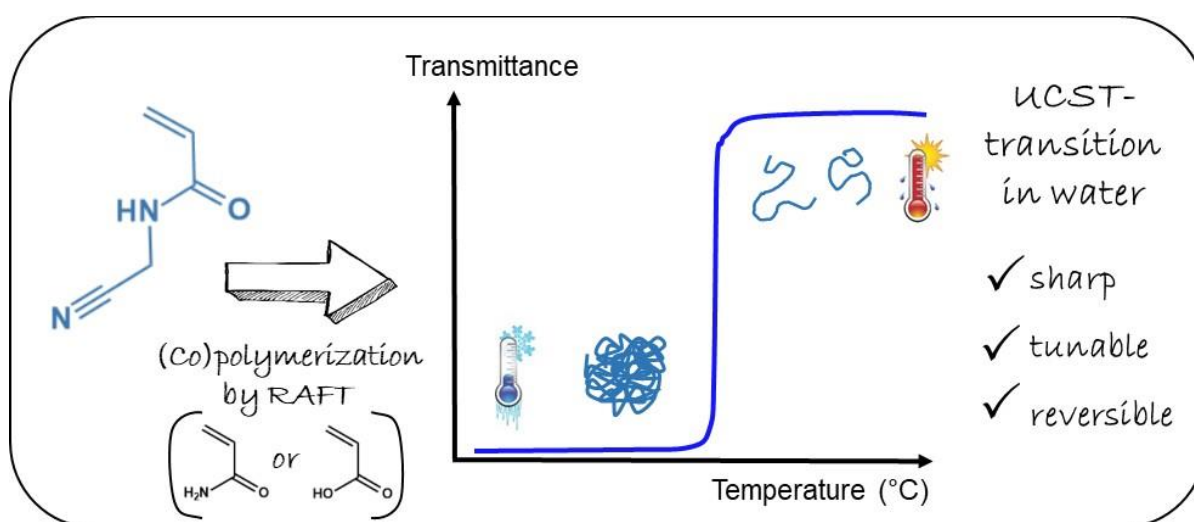
Abstract

In this present work, we report the synthesis of a new family of UCST-thermoresponsive polymers based on *N*-cyanomethylacrylamide (CMAm). We demonstrate that the thermally-initiated reversible addition fragmentation chain transfer (RAFT) polymerization of CMAm conducted in DMF is well controlled. The homopolymer presents a sharp and reversible UCST-type phase transition in pure water with a very small hysteresis between cooling and heating cycles. We demonstrate that the cloud point (T_{CP}) of PCMAm is strongly molar mass dependent and shifts towards lower temperatures in saline water. Moreover, the transition temperature can be tuned over a large temperature range by copolymerization of CMAm with acrylamide or acrylic acid. The latter copolymers are both thermo-and pH-responsive. Interestingly, by this strategy sharp and reversible UCST-type transitions close to physiological temperature can be reached, which makes the copolymers extremely interesting candidates for biomedical applications.

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Over the last decades, water-soluble thermosensitive polymers, presenting an upper critical solution temperature (UCST) in water have been the subject of extensive research in polymer science because they can be used for various applications, especially in the biomedical field.[1,2] They present a globule-to-coil conformation transition upon temperature increase in aqueous solution, meaning that they are insoluble below and soluble above a critical temperature. There are two classes of polymers exhibiting a UCST-type transition in water: ionic polymers, such as some polysulfobetaines[3,4,5,6] or polycarboxybetaines[7], and neutral polymers. Among the latter family, only few homopolymers present an UCST in pure water suitable for applications: poly(*N*-acryloylglycinamide)[8,9] (PNAGA) and its derivatives such as poly(*N*-acryloylasparaginamide) (PNAAAm)[10,11], poly(6-acryloyloxymethyluracil)[12], poly(*N*-acryloyl-nipecotamide) (PNANAm)[13] and poly(methacrylamide) (PMAm)[14]. In contrast, polyacrylamide (PAm) has a theoretical UCST below 0 °C in water[14,15], which can be increased by copolymerization of acrylamide (Am) with a hydrophobic comonomer such as styrene[16] or acrylonitrile (AN).[14,17,18] The resulting statistical copolymer P(Am-*co*-AN) is one of the most studied neutral UCST copolymers and used in many applications, in particular for drug delivery.[19,20] It is generally obtained by

free radical polymerization (FRP) or reversible deactivation radical polymerization (RDRP) - in particular through reversible addition fragmentation chain transfer (RAFT) - of Am and AN in organic solvents[14] or in water[21]. Uchiyama and Otsuka *et al.*[18] reported that - in addition to hydrogen bonds between the Am units[14,22] - dipole−dipole interactions between the cyano groups in the AN units are a major driving force for the UCST behavior. Due to a difference in reactivity of both comonomers[23], the Am/AN composition and distribution in the polymer chains depend however greatly on the polymerization technique, and therefore the cloud point (T_{CP}) is sensitive to the polymerization conditions.[18] It was also reported that the content of AN in the polymer P(Am-*co*-AN) was inferior to the content of AN in the feed, because part of the AN monomer can be easily lost by evaporation during the removal of dissolved oxygen before radical polymerization.[18] To overcome these issues, we propose herein a much more robust and simple strategy relying on one single monomer, namely *N*-cyanomethyacrylamide (CMAm). CMAm combines the functional groups of P(Am-*co*-AN), and we postulated that PCMAm might be a promising candidate to prepare a novel family of UCST-type (co)polymers. Importantly, the synthesis of the monomer is very straightforward. Moreover, it has the advantage of being solid at room temperature, therefore being handled more easily compared to AN. To the best of our knowledge, only one publication[24] mentions the use of this monomer for the preparation of submicron-sized *N*-isopropylacrylamide/CMAm copolymer gel particles. The homopolymer itself has never been studied.

Here, we report for the first time the homopolymerization of CMAm by FRP and RAFT-RDRP. Furthermore, we study systematically the thermoresponsive behavior of the resulting homopolymers by turbidimetry. In particular, we evaluate the impact of the number-average degree of polymerization (DP_n) and demonstrate the possibility to fine-tune the transition temperature by the addition of hydrophilic comonomers within the polymer chains.

The CMAm monomer was readily obtained in one-step, by amidification of acryloyl chloride with aminoacetonitrile in the presence of NaOH following a reported procedure (see S.I. and **Figure S1**).[24] We firstly investigated its ability to be radically homopolymerized in a controlled fashion by RAFT in DMF solution, using ethyl 2-(butylthiocarbonothioylthio)propanoate (see S.I. and **Figure S2**), a trithiocarbonate-type chain transfer agent (CTA) well-known to control the polymerization of acrylamides (**Scheme 1**). Not only the transfer agent but also the initiator (2,2'-azobis(2-methylproprionitrile), AIBN) was chosen nonionic in order to avoid any possible effect of the chain end on the thermoresponsive behavior.[9,13] **Figure S3** shows the monomer conversion versus time for the homopolymerization of CMAm under RAFT conditions (Experiment P4 in Table 1).
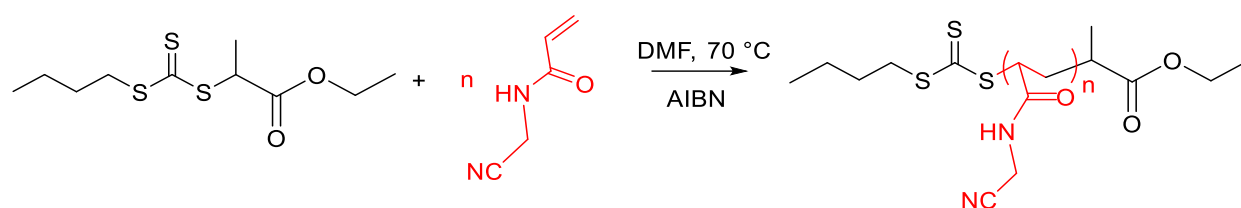
Scheme 1. Synthesis route for PCMAm polymers via RAFT polymerization.

The same procedure was used for the FRP in DMF except that no RAFT agent was added (P6 in **Table 1** and **Figure S4**). As shown in **Figure S5** and resumed in **Table 1**, the thermally-initiated RAFT polymerization of CMAm conducted in DMF was well controlled as the polymers displayed a dispersity ($Đ = M_w/M_n$) below 1.5 and the SEC chromatograms evolved toward higher molar masses by targeting higher DP_n. The efficiency of the transfer reaction was also confirmed by ^{1}H NMR (**Figure S6**) and by SEC equipped with an in-line UV detector. Indeed, SEC chromatogram (**Figure S7**) obtained with the UV detector at 309 nm shows the presence of the trithiocarbonate functionality, which strongly absorbs at this wavelength.[25] Thermal analysis by differential scanning calorimetry and thermogravimetry were performed on the homopolymer obtained by FRP (P6, Table 1) showing respectively a glass transition temperature of 200 °C and a thermal degradation at about 250 °C (**Figure S8**).

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Table 1. Experimental conditions and results for the RAFT-mediated polymerizations of CMAm performed at 20 wt% in DMF[#]

Entry	$[CMAm]_0$ / $[CTA]_0$	Time (min)	Conv[a] (%)	$DP_{n,th}$[b]	$DP_{n,exp}$[c]	$M_{n,th}$[b] (kg mol^{-1})	$M_{n,SEC}$[d] (kg mol^{-1})	$Đ$[d]	T_{CP}[e] (°C)
P1	20/1	100	76	15	21	1.9	4.7	1.21	54/54
P2*	41/1	300	69	28	28	3.3	7.6	1.17	61/58
P3	60/1	280	85	51	56	5.9	11.5	1.26	80/79
P4	115/1	243	62	71	73	8.1	14.8	1.27	85/84
P5	230/1	360	82	189	183	21.1	22.2	1.47	>90
P6**	-	76	83	-	-	-	52.1	2.24	>90

[#] Polymerizations were performed at 70 °C in presence of a RAFT agent (CTA) using AIBN as a radical initiator at an initial molar ratio of CTA/AIBN: 1/0.1-0.2. *The polymerization was performed at 35 °C in presence of V-70 as a radical initiator. **Free radical polymerization (without RAFT agent) at an initial molar ratio of CMAm/AIBN: 200/1.

[a] Determined by ^{1}H NMR analysis. [b] Theoretical number-average degree of polymerization, $DP_{n,th}$, and number-average molar mass, $M_{n,th}$, calculated using the experimental conversions. [c] Determined by ^{1}H NMR spectroscopy in DMSO-d_6 using the relative integration of the chain end and the monomer units (an example is shown in Figure S6 for P2). [d] Number-average molar mass, M_n, and dispersity, $Đ$, determined by SEC in DMF (+ LiBr 1g L^{-1}) with a PMMA calibration. [e] Determined in water by turbidimetry at 0.5 wt%, on 1st cooling/2nd heating.

The thermoresponsive solution properties of the new homopolymer were examined by turbidimetry. A typical UCST-type thermoresponsive behavior with a sharp transition was observed for PCMAm with DP_n between 21 and 73: polymers were insoluble and precipitated in pure water at low temperatures, whereas they were soluble at high temperatures. **Figure 1** shows that the UCST-type transition temperature increases with the DP_n in the range 21-56 from 54 to 80 °C (measured at the first cooling at 0.5 wt%). For higher DP_ns, the transition temperatures change only slightly. The highest molar mass polymer studied, with $DP_n = 183$ (PCMAm$_{183}$, P5 in Table 1), stayed turbid up to 90 °C, but demonstrated the beginning of a transition > 90 °C. This decrease in solubility with increasing chain length is thermodynamically expected and was previously described for other UCST-type homopolymers such as PNAAAm[11] or PNANAm[13]. As expected, the PCMAm obtained by FRP (P6 in Table 1) with the highest number-average molar mass (M_n) was insoluble between 20 to 90 °C at 0.5 wt% in pure water (**Figure 1A**) and did not show any thermoresponsive behavior.
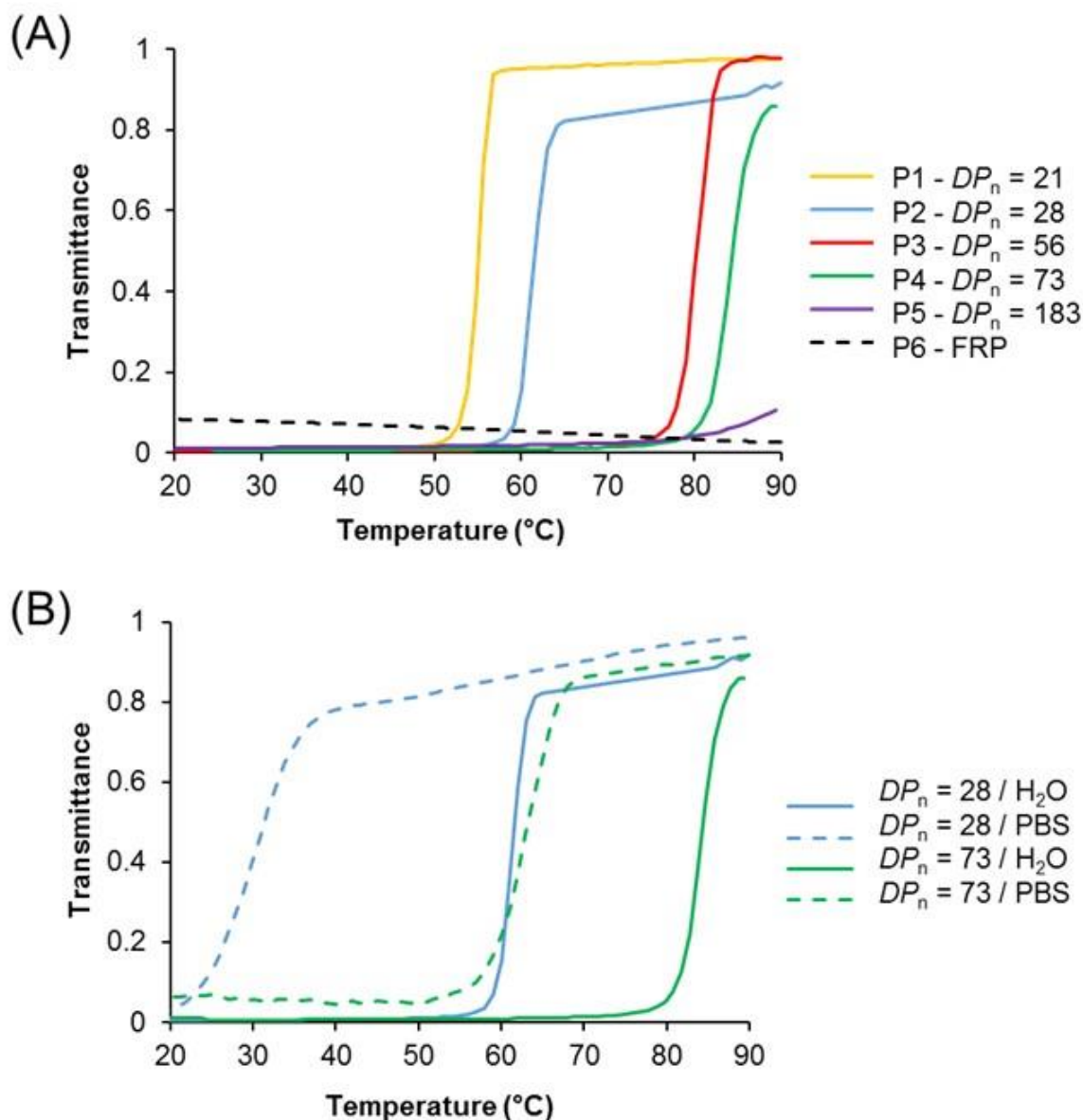
Figure 1. Turbidity curves (first cooling) of PCMAm polymers with various DP_n at 0.5 wt% in water (A) and of PCMAm$_{28}$ (P2, Table 1) and PCMAm$_{73}$ (P4, Table 1) (solid lines) in water and in PBS (dashed lines) (B) at 0.5 wt%.

All polymers showed an excellent reversibility of phase transition as shown in **Figure S9** for sample P3. Interestingly, the turbidity measurements showed a very small hysteresis, unlike other UCST homopolymers, such as PNAGA[9], PNANAm[13] or PMAm[26]. We also investigated the impact of polymer concentration on the phase transition behavior of PCMAm. The turbidity curves of sample P3 at 0.25, 0.5 and 1 wt% in **Figure S10**

show actually that the T_{CP} of PCMAm varies only very slightly in the studied concentration range. Moreover, PCMAm retained its thermoresponsiveness in phosphate buffered saline (PBS), but the T_{CP} shifted towards significantly lower temperatures (**Figure 1B**), in accordance with previous results observed for PNAGA[27]. For instance, the T_{CP} for sample P4 with $DP_n = 73$ decreased from 85 °C in pure water towards 63 °C in PBS. Finally, to investigate the influence of the NaCl concentration on the thermoresponsiveness of PCMAm, turbidimetry measurements of PCMAm$_{51}$ aqueous solutions at 0.5 wt% were done at increasing concentrations of NaCl. As shown in **Figure S11**, the T_{CP} is gradually decreased from ~80 to ~67 °C for increasing concentrations of NaCl (up to 0.5 M).[*] This tendency was also observed for PNAGA by Agarwal *et al.*[27] and for P(Am-*co*-AN) by Uchiyama and Otsuka *et al.*[18].

Despite the sharp and reversible transition, the application of PCMAm in the biomedical field might be limited by the relatively high T_{CP}, generally above 50 °C. Therefore, we decided to introduce hydrophilic comonomers, namely acrylamide (Am), and acrylic acid (AA), into the polymer in order to fine-tune the transition temperature. The controlled radical copolymerization of CMAm with Am or AA was performed in DMF at 35 °C in presence of the same RAFT agent and V-70 as a non-ionic radical initiator (**Scheme S1**). First, two series of statistical copolymers with CMAm and Am were synthesized with various feed ratios (percentage of Am ranging from 9 to 30 mol%, **Table 2**) keeping constant the total monomer concentration at 25 wt%, and targeting either $DP_n = 50$ or 100. Kinetic monitoring during the polymerizations carried out by ^{1}H NMR showed that the copolymerizations proceeded without significant variation of the copolymer composition (**Figure S12**). Indeed, the calculated molar percentages of Am in the copolymers determined by ^{1}H NMR at any conversion remained close to the initial ones (final values given in Table 2). This suggests a very similar reactivity of both monomers and reactivity ratios close to one. After purification by precipitation in chloroform, the copolymers were

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[*] It should be noted that the observed slight increase of transmittance at low temperature might be attributed to a macroscopic phase separation, as the polymer precipitates out of the solution leading to a higher transmittance compared to temperatures close to T_{CP} where the polymer gradually dissolves.

characterized by SEC and ^{1}H NMR (Table 2 and **Figures S13A and S14**). Generally, polymerization control was reached for all compositions; lower molar mass dispersities ($Đ < 1.4$) were obtained when shorter polymers were targeted, presumably because they were synthesized in shorter reaction times. In addition, the molar fraction of Am in the copolymers determined by ^{1}H NMR (F_x, Table 2) was very close to the initial molar fraction of Am in the feed, hinting at a random copolymerization mechanism.

Table 2. Experimental conditions for the synthesis of P(CMAm-*co*-X) copolymers (with X = Am or AA) in the presence of CTA and their molecular characteristics[#]

Entry	X	mol% of X[a]	$[M]_0/[CTA]_0$	Time (h)	Conv.[b] (mol%)	mol% of X[c]	F_X[d]	$DP_{n,th}$[e]	$DP_{n,exp}$[f]	$M_{n,th}$[e] (kg mol^{-1})	$M_{n,SEC}$[g] (kg mol^{-1})	$Đ$[g]	T_{CP}[h] (°C)
P7	-	0	161/1	3.0	68	0	0	109	113	12.4	18.9	1.37	85/-[†]
P8		9	100/1	4.0	56	9	0.08	56	60	6.2	10.8	1.27	67/67
P9		19	100/1	3.7	57	18	0.15	58	61	6.2	10.7	1.26	37/40
P10	Am	30	100/1	5.3	53	28	0.25	53	57	5.5	9.1	1.37	22/27
P11		9	140/1	14.8	64	8	0.06	90	102	9.9	17.9	1.47	77/80
P12		20	152/1	6.8	66	20	0.18	101	111	10.6	17.7	1.55	65/68
P13		30	147/1	6.8	68	28	0.27	100	106	10.2	16.3	1.38	40/44
P14		14	100/1	5.0	56	14	0.13	56	59	6.1	13.8	1.15	55/59
P15		21	97/1	6.2	54	22	0.20	52	56	5.6	11.3	1.27	53/55
P16	AA	26	88/1	2.3	48	28	0.28	43	47	4.4	10.0	1.14	35/40
P17		41	103/1	2.0	49	43	0.40	50	52	5.0	10.1	1.17	18/24
P18		24	153/1	5.2	65	26	0.23	100	96	10.3	19.9	1.26	55/57
P19		37	141/1	5.0	66	39	0.38	93	97	9.1	18.6	1.08	28/32

[#]Polymerizations were performed in DMF at 35 °C in presence of a RAFT agent (CTA) and V-70 as a radical initiator at 25 wt%. X represents the comonomer (Am or AA) and M represents both comonomers. [a] Initial mol% of X in the mixture of monomers. [b] Overall monomer conversion determined by ^{1}H NMR analysis. [c] mol% of X in the copolymer deduced from ^{1}H NMR analysis from the individual molar conversions of X (see SI). [d] Molar fraction of X in the purified copolymer deduced from ^{1}H NMR analysis (see SI). [e] Theoretical number-average degree of polymerization, $DP_{n,th}$, and number-average molar mass, $M_{n,th}$, calculated using the experimental conversions. [f] Determined by ^{1}H NMR spectroscopy in DMSO-d$_6$ using the relative integration of the chain end and the monomer units. [g] Number-average molar mass, M_n, and dispersity, $Đ$, determined by SEC in DMF (+ LiBr 1g L^{-1}) with a PMMA calibration. [h] Determined in water by turbidimetry at 0.5 wt%, on 1st cooling/2nd heating.

The thermoresponsiveness of the P(CMAm-*co*-Am) copolymers in water at 0.5 wt% was then investigated by turbidimetry (Table 2 and **Figure 2A**). In all cases, the thermal response of the copolymer solutions was sharp and reversible with a very small hysteresis ($\leq$ 5 °C, see Table 2) during the cooling and the heating cycle. As summarized in **Table 2** and presented in **Figure 2A**, the T_{CP} decreased with increasing the hydrophilic monomer content in the copolymer, as expected.[8,28]
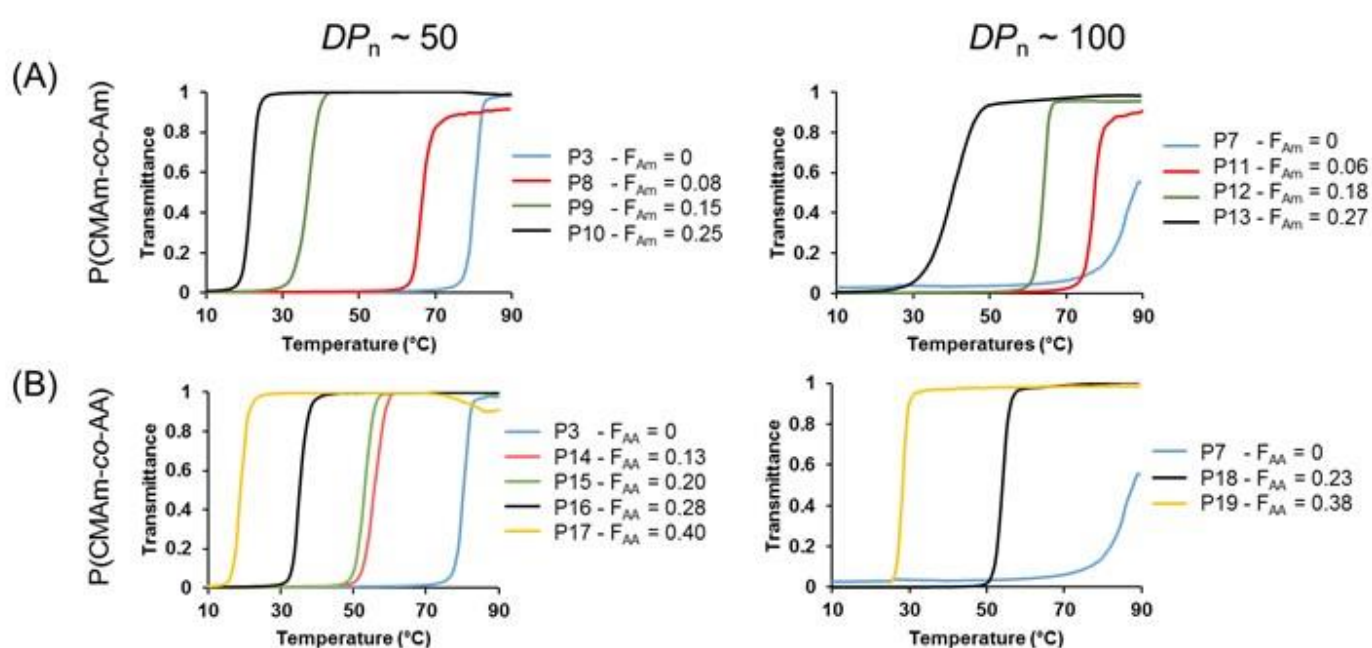

Figure 2. Turbidity curves (cooling step) of P(CMAm-*co*-Am)$_x$ (A) and P(CMAm-*co*-AA)$_x$ (B) (Table 2) at 0.5 wt% in water.

Interestingly, P(CMAm-*co*-Am) copolymers with a DP_n, ~ 50 exhibited a thermoresponsive transition which was tunable by the Am content over a huge temperature range, between 22 and 80 °C. When the DP of the copolymer was doubled keeping constant the comonomer content, a significant increase in the T_{CP} was observed, similar to the PCMAm homopolymer. For instance, comparing the copolymers P10 and P13 (with Am content ~ 26 mol%) we can observe an increase in the T_{CP} of 18 °C.

In the second series, AA was tested as a comonomer. Acrylic acid contains a carboxylic acid moiety, which could provide a pH-dependent thermosensitive behavior to AA-containing PCMAm-based copolymers. A series of statistical copolymers were synthesized varying the comonomer feed ratio (**Table 2**), while keeping constant the total monomer concentration at 25 wt%. The mol% of AA in the monomer feed was varied from 14 to 41% in order to tune the copolymer composition. As reported in **Table 2**, the molar percentages of AA in the copolymers determined by ^{1}H NMR remained close to the initial ones indicating again a very similar reactivity of both monomers. After purification by precipitation and successful methylation[29] of the carboxylic acid groups (yielding methyl acrylate units), the molar masses of the final samples were determined by SEC (Table 2 and **Figure S13B**). Symmetric and narrow molar mass distributions were obtained ($Đ$ < 1.3) in all cases. The thermoresponsiveness of the P(CMAm-*co*-AA) copolymers in water at 0.5 wt% was then investigated by turbidimetry (**Figure 2B**). For all copolymers, the thermal response of the solutions was sharp and reversible with a slight hysteresis ($\leq$ 6 °C, **Table 2**) between the cooling curve and the heating curve. Again, the T_{CP} decreased with increasing AA content, and at constant AA content, it was higher for longer copolymer lengths. For instance, by comparing the copolymers P16 and P18 in **Table 2** with the same AA content (~25 mol%), we can observe an increase of the T_{CP} by 20 °C. Interestingly, for both comonomers, the T_{CP} decreases almost linearly with the comonomer content as shown in **Figure 3** and **Figure S15**. Strong interactions between the hydrophilic comonomer and water should explain why the solubilization is favored and occurs at lower temperatures as the proportion of comonomer increases.
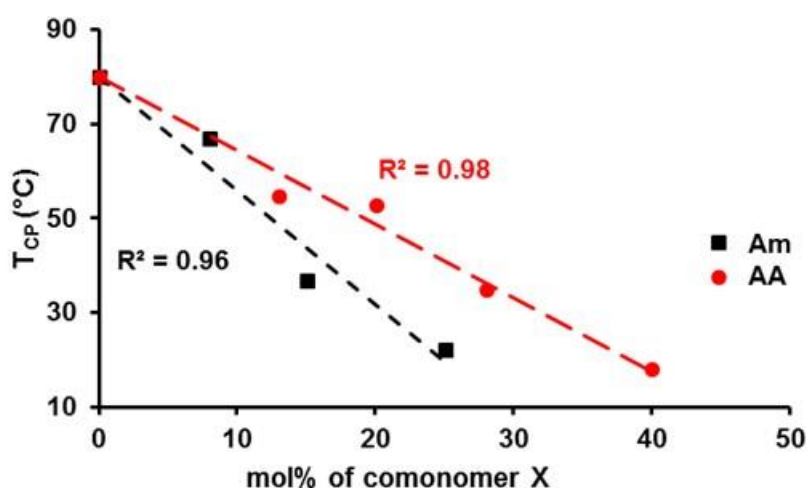
Figure 3. Evolution of T_{CP} (cooling step) versus the mol% of comonomer in the P(CMAm-*co*-X)s of DP_n ~ 50. Black squares: X = Am (P3 in Table 1 and P8-P10 in Table 2). Red circles: X = AA (P3 in Table 1 and P14-P17 in Table 2).

As acrylic acid monomer units in the P(CMAm-*co*-AA) copolymers are pH sensitive, the impact of the degree of ionization of the acid units was studied on copolymer P16 by varying the pH of the medium at which the turbidity measurements were performed. While this copolymer was soluble at pH = 5.7 (no thermosensitive behavior was observed), a T_{CP} of 35 °C was observed at natural pH (3.7) (cooling step) which increased to 40 °C at pH = 2.0 (**Figure S16**). It is therefore possible to adjust the solubility and transition temperature of P(CMAm-*co*-AA) by modifying the pH of the aqueous solution, in which it is dissolved.

Finally, we also investigated the impact of polymer concentration on the phase transition behavior of P(CMAm-*co*-X) copolymers (with X = Am or AA). The turbidity curves of samples P10 (P(CMAm$_{0.75}$-*co*-Am$_{0.25}$)$_{57}$) and P16 (P(CMAm$_{0.72}$-*co*-AA$_{0.28}$)$_{47}$) at 0.25, 0.5 and 1 wt% in **Figure S17** show actually that the T_{CP} of the copolymers is more sensitive to the concentration than that of the homopolymer (**Figure S10**), at least in the studied concentration range. Indeed, for both copolymers P10 and P16 (with F_X ~0.26), the T_{CP} decreased by around 25 °C when the copolymer concentration was decreased from 1 to 0.25 %wt, whereas the T_{CP} of the homopolymer decreased in the same conditions by less than 5 °C.

In conclusion, new UCST-type thermosensitive PCMAm homopolymers and P(CMAm-*co*-X) statistical copolymers (with X = Am or AA) of various compositions have been synthesized for the first time, in a

controlled fashion by RAFT polymerization. PCMAm homopolymers exhibit a sharp and reversible UCST-type thermal transition in pure water between 54 °C and 85 °C depending on the degree of polymerization, with a very small hysteresis between the cloud point upon cooling and heating. The transition temperature was tuned by the insertion of hydrophilic comonomer units, notably Am and AA. For instances, with a low amount of acrylamide ($\leq$ 27 mol%) the T_{CP} could be tuned over a huge temperature range, from ~20 to 85 °C. Compared to well-studied P(Am-*co*-AN) copolymers, for which the T_{CP} can also be tuned by the copolymer composition, CMAm is much easier to handle than AN, because CMAm is a solid monomer at room temperature. In addition, its copolymerization with Am (or AA) in DMF appears random avoiding thus a gradual change of composition over the polymer chain during RDRP, or the formation of a mixture of polymer chains with different compositions during FRP. Furthermore, the copolymer comporting AA units was not only responsive to temperature but also to pH. We believe that these new (co)polymers pave the way towards a new class of responsive materials, and their sharp and reversible transition temperatures adjustable to physiological temperature makes the copolymers extremely interesting candidates for biomedical applications.

Supporting Information

Experimental details and supplementary data.

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