Thioester-functional Polyacrylamides: Rapid Selective Backbone Degradation Triggers Solubility Switch Based on Aqueous LCST/UCST

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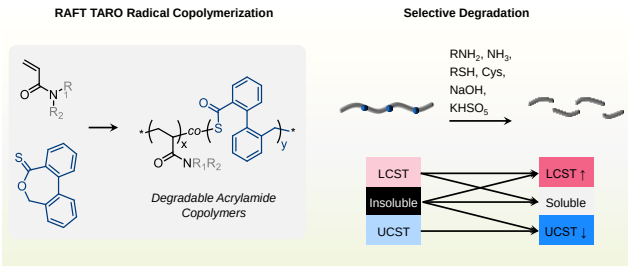


Abstract. Radical ring-opening polymerization is a clever strategy to incorporate cleavable linkages into otherwise non-degradable vinyl polymers. But conventional systems suffer from slow copolymerization, harsh non-selective degradation conditions, and limited application potential because the degradation products (often oligomers or polymers themselves) have properties like the intact species. This work presents fast selective degradation accompanied by a drastic change in a key property, aqueous solubility. The thionolactone dibenzo[c,e]oxepane-5-thione was found to copolymerise radically with a range of primary, secondary, and tertiary neutral and zwitterionic acrylamides with rapid incorporation of degradable biphenyl thiocarboxylate repeat units. Intact copolymers displayed temperature-responsive (LCST or UCST-type) aqueous solubility behaviour, tuneable through the molar composition and (exploiting the non-azeotropic copolymerization behaviour) comonomer sequence. Various conditions led to selective and complete degradation of the backbone thioesters through hydrolysis, aminolysis, transthioesterification (including under physiological conditions), and oxidative hydrolysis which drastically increased aqueous solubility. Polymers containing as little as 8 mol-% of thioester repeat units underwent a temperature-independent insoluble–soluble transition upon degradation with cysteine or potassium persulfate. Insoluble polymers were used to block syringe filters which allowed flow of degradant solutions only, relevant relevant to lab-on-a-chip, sensing, and embolic biomedical applications.

Keywords. degradable polymers, radical ring-opening polymerization, stimulus-responsive polymers, UCST in water, TARO polymerization, redox-sensitive polymers, copolymerization

Introduction

The growing interest in degradable polymers ranges from an environmental view to reduce plastic pollution[1-3] to the need for bespoke biomaterials suited to tissue engineering and nanomaterials able to deliver drugs into diseased cells.[4-6] Radical polymerization, a key industrial method, delivers commodity vinyl plastics on a multi-ton scale. At the same time, precision synthesis enabled by modern reversible deactivation radical polymerization (RDRP) methods[7-8] has revolutionised the availability of functional vinyl polymers for biomedical applications.[9-14] Having an all-carbon backbone, however, vinyl polymers are not degradable.

The radical ring-opening polymerization (RROP) of cyclic monomers enables the introduction of functional groups into the backbone of vinyl copolymers.[6, 15] Mostly used are cyclic ketene acetals (CKAs)[16] and allyl sulfide lactones[17-19] which install esters into the backbone and therefore provide degradability. However, this methodology has drawbacks. First, CKAs show low reactivity with the major (meth)acrylic families of monomers,[6] while allyl sulfide lactones do not copolymerise with acrylic comonomers.[19] Second, and specific to these types of RROP-suited monomers, the complete cleavage of backbone esters requires harsh conditions that are not tolerated by many other functional groups and that are impractical for many applications (0.1–1 M NaOH or KOH is common). But there is another drawback that is inherent to the fundamental concept of RROP. Homopolymerization or alternating copolymerization[20-22] of RROP-suited monomers is rare, difficult, or, in some cases, even impossible.[19, 22] Instead, the potential of RROP lies in the creation of vinyl copolymers that share desirable properties with the vinyl base material. The cleavage of the added functional backbone groups therefore leads to the degradation of (long) polymer chains into shorter polymer chains or oligomers. This degradation typically involves a measurable reduction of the average hydrodynamic size of chains. Indeed, size exclusion

chromatography (SEC) remains the main analytical method to monitor the degradation of RROP-made copolymers. Unfortunately, however, the degradation of polymers into oligomers is usually not accompanied by a drastic change in material properties required for applications—in stark contrast to polycondensation-derived materials which feature ester groups in every repeat unit and degrade into monomers.

Very recently our group[23] and, independently, the Gutekunst group[24] developed thiocarbonyl addition–ring-opening (TARO) polymerization. It exploits the addition of a radical onto the C=S double bond of a thionolactone (cyclic –C(=S)O–), followed by ring-opening (though β scission), re-initiation, and formation of a backbone thioester (–C(=O)S–). Details of the homopolymerization[22] and copolymerization with acrylates[23-24] and maleimides[22] with the thionolactone dibenzo[c,e]oxepane-5-thione (DOT) have been published.

Herein, we present the first detailed investigation of the copolymerization of DOT with a series of acrylamides. Taking advantage of the hydrophobic nature of DOT, temperature responsive acrylamide copolymers were prepared that have a lower critical solution temperature (LCST) in water, *i.e.*, they are water-soluble only below a critical temperature.[25] Adding to a very small subset of polymers with a more unusual thermal behaviour,[26] we also present the first examples of backbone degradable polymers with a UCST (upper critical solution temperature; soluble above a critical temperature) in water. DOT–acrylamide systems did not show ideal (azeotropic) copolymerization behaviour but faster incorporation of the degradable units into copolymers—in contrast to typically sluggish CKAs. However, this behaviour could be exploited to tune the thermal behaviour[27-28] of the resulting copolymers. In fact, we show that water-insoluble copolymers could be designed to become fully water-soluble upon cleavage of the backbone thioesters, providing a drastic property change. In addition to hydrolysis, polymer degradation was

achieved through aminolysis, thiolysis, and oxidative cleavage, taking advantage of thioester-selective chemistries. Finally, the application potential of these materials is demonstrated on syringe filters that were obstructed with an insoluble polymer and that were selectively unblocked through degradant solutions.

Experimental Section

Instrumentation. NMR spectroscopic measurements were performed on 400 or 500 MHz Bruker instruments in 5 mm NMR tubes. Residual solvent signals of $CHCl_3$ (δ_H = 7.26 ppm, δ_C = 77.2 ppm), DMSO-d_5 (δ_H = 2.50 ppm), and HDO (δ_H = 4.70 ppm) were used as references. Fourier transform infrared spectroscopy (FT-IR) was performed on a Perkin Elmer FT-IR spectrometer under attenuated total reflection (ATR). Size exclusion chromatography (SEC) was performed on a Viscotek GPCMax VE 2001 setup with three linear 7.5×300 mm PLgel mixed-D columns connected to a Viscotek VE3580 refractive index (RI) detector and a Malvern 270 dual detector (viscometer and light scattering). The instrument operated at 35 °C with tetrahydrofuran (THF) containing 250 ppm BHT as mobile phase at a flow rate of 1.0 mL×min^{-1}. The system was calibrated using a series of narrow molecular weight distribution PMMA standards with molecular weights ranging from 5 kg×mol^{-1} to 298 kg×mol^{-1}. LCST/UCST cloud points were determined by temperature-dependant turbidity measurements on a Thermo Scientific Evolution 201 UV-Visible Spectrometer equipped with a Peltier control and cooling unit (PCCU1) in plastic cuvettes of 10 mm path length at a wavelength of 550 nm with heating/cooling rates of 1 °C/min. Polymer concentrations were 5 g/L. For clear solutions the baseline was corrected to zero absorbance, A.

Transmittance, $T = 10^{-A}$, was plotted against temperature, and cloud points were determined at T = 95%.

Materials, Synthesis, and Methods. All reagents were purchased from Sigma-Aldrich and used as received, unless noted otherwise. 2,2'-Azobis(isobutyronitrile) (AIBN) was recrystallized from methanol and stored in a freezer. *N*-isopropylacrylamide (NIPAm) was recrystallized from toluene–petroleum ether (50:50) and stored in a freezer. Liquid monomers were deinhibited by passing through a column of basic alumina immediately before use. Dibenzo[c,e]oxepane-5-thione (DOT) was prepared as previously described.[22]

The sulfobutyl betaine acrylamide monomer ***N*-3-(*N*-4-sulfobutyl-*N*,*N*-dimethylammonium)propylacrylamide (SBBAm)** was prepared in analogy to a literature procedure.[29] Briefly, *N*-(3-(dimethylamino)propyl)acrylamide (4 g, 25.6 mmol, 1.11 eq), 1,4-butanesultone (3.13 g, 23.0 mmol, 1 eq) and BHT (55 mg) were refluxed in acetonitrile for 48 h. The precipitate was filtered off, washed with acetonitrile, and dried in vacuum. 6.3 g (93%) of white solid were obtained. ^{1}H NMR (400 MHz, D_2O), δ/ppm = 6.16 (m, 2 H, H_2C=), 5.72 (dd, 1 H, ^3J = 2 Hz, 9 Hz, =C*H*–), 3.29 (m. 6 H, NHCH_2, CH_2NCH_2), 3.00 (s, 6 H, (CH_3)$_2$N), 2.89 (t, 2 H, ^3J = 7 Hz, C$H_2SO_3^-$), 1.96, 1.84, 1.72 (3 m, 3× 2 H, CH_2).

General polymerization procedure. DOT and comonomer (in varying molar ratios as described in the main text), *S*-benzyl-*S*'-propyl trithiocarbonate (1 equiv), AIBN (0.25 equiv) and DMSO (propylene carbonate–(2,2,2-trifluoroethanol 1:1 was used for SBBAm) (total monomer conc. = 3.3 M) were added into a ground-glass joint tube. The reaction was sealed with a rubber septum and degassed with nitrogen for 30 min through a needle, with a shorter needle fitted for gas release. The tube was placed in a pre-heated oil bath set at 80 °C and left for a predetermined amount of time. After cooling and exposing to air, the monomer conversion was determined by ^{1}H

NMR spectroscopy of the crude mixture diluted with $CDCl_3$ or D_2O. Copolymers based on *N*,*N*-dimethylacrylamide, *N*,*N*-diethylacrylamide, and NIPAm were isolated by three precipitations into a 20-fold excess of diethyl ether followed by centrifugation, decanting, and drying in vacuum. Copolymers based on poly(ethylene glycol) methylether acrylate), acrylamide, and SBBAm were purified by dialysis against methanol, then water in regenerated cellulose membranes (3500 $gmol^{-1}$ molecular weight cut-off), followed by freeze-drying.

Copolymerization reactivity ratios were determined as described elsewhere.[22]

RAFT copolymerization kinetics. RAFT copolymerization kinetics of DOT and DMAm, as an example acrylamide monomer, were determined through AIBN-initiated RAFT copolymerization. A stock solution was prepared containing DOT (39 mg, 0.17 mmol, 10 equiv), DMAm (158 µL, 1.53 mmol, 90 equiv), naphthalene (as internal standard, 106 mg, 0.83 mmol, 50 equiv), *S*-benzyl-*S*′-propyl trithiocarbonate (4 mg, 17 µmol, 1 equiv), AIBN (0.7 mg, 4 µmol, 0.25 equiv), and DMSO-d_6 (2.0 mL, 0.83 M). A sample of the stock solution was analysed by ^{1}H NMR spectroscopy. Aliquots of 0.4 mL each were taken from the stock solution, sealed with a septum, and degassed by bubbling nitrogen for 30 min. Each aliquot was heated to 80 °C for a predetermined amount of time from 5 to 360 min. After cooling down to RT, the mixtures were analysed by ^{1}H NMR spectroscopy. Monomer conversions were determined from the comparison of signals of residual monomers with the internal standard.

Aminolysis of copolymers. Typically, copolymer (2–5 mg) was dissolved in solvent (methanol, THF, or DCM, 1 mL) containing degradant (ammonia, isopropylamine, or dimethylamine at a predetermined concentration) and stirred at RT overnight. The mixture was evaporated to dryness by blowing in a stream of nitrogen gas. The residue was dissolved in THF and analysed by SEC. Thiolysis using propane–triethylamine (1:1) followed the same procedure.

Hydrolysis of copolymers. Aqueous NaOH (2 M, 1 mL) was added to copolymer (5 mg) and the mixture was stirred at RT for 48 h. The mixture was neutralised to pH 7 with aqueous HCl and evaporated to dryness in a stream of nitrogen gas. The residue was extracted with THF and analysed by SEC.

Cysteinolysis of copolymers. The pH of an aqueous solution containing cysteine (10 mM) and tri(carboxyethyl)phosphine (as reducing agent, 10 mM) was adjusted to 9 with aq. NaOH. Copolymer was dissolved in this mixture (final concentration = 5 g/L) with stirring at RT and the cloud point of the mixture was determined.

Oxidative hydrolysis of copolymers. Copolymer (5 mg) was dissolved in water and oxone ($KHSO_5 \cdot ½KHSO_4 \cdot ½K_2SO_4$, 3.1 mg, final conc. 10 mM, persulfate–thioester approx 5:1) was added under stirring and cloud points were determined of the mixture. The water was then removed by lyophilisation, the residue was extracted with THF and analysed by SEC.

Filter Unblocking Experiments. Syringe filters (Phenex, 4 mm regenerated cellulose syringe filter, 0.2 μm) were blocked with p($DMAm_{63}$-DOT_{15}) by suspending the polymer in water (5 g/L) and pushing the suspension through the filter with an attached syringe until no more liquid would pass through (typically after 100–150 μL). The filter was dried before attaching a new syringe (without plunger), filled with PBS buffer (50 mL) and left for 24 h to confirm the filter was blocked. The solution was removed, the filter dried and exposed to PBS buffer containing degradant (10 mM cysteine–TCEP (1:1) at pH 9, 10 mM oxone, or 100 mM *N*-acetylcysteine at pH 7.4) and the flow rate was determined by measuring the volume of solution passed through the filter in the first 23 minutes after exposing the filter to the degradant solution, with the exception of the *N*-acetylcysteine experiment in which flow through the filter occurred only after several hours.

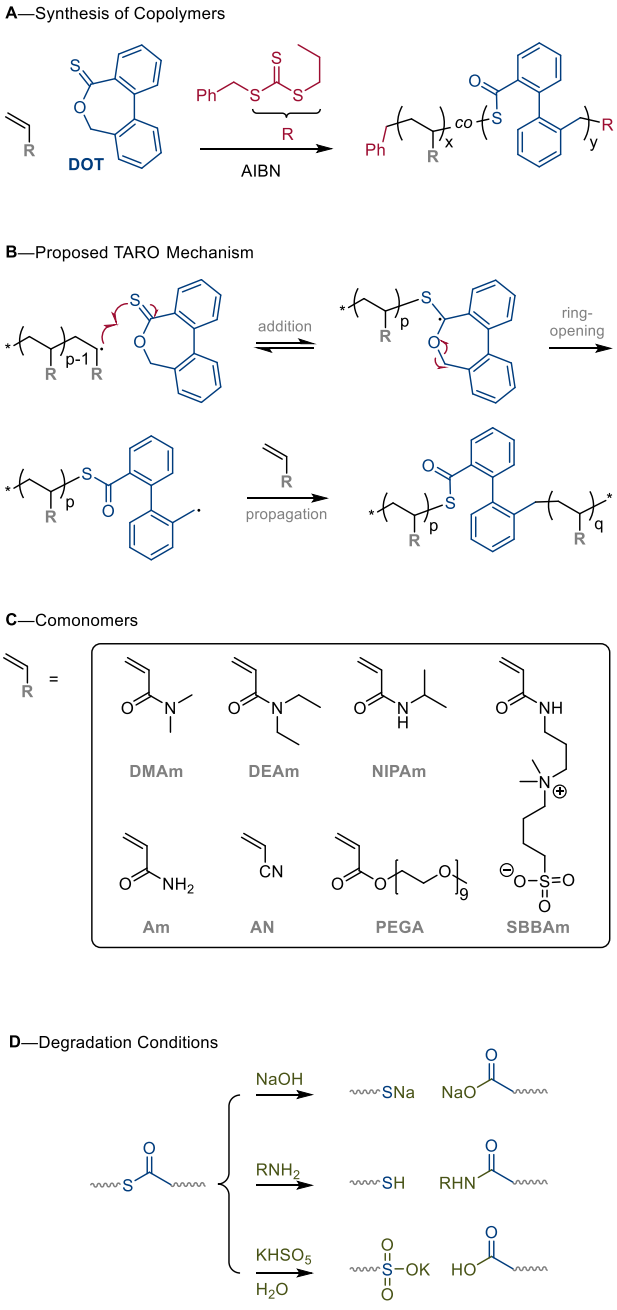
Results and Discussion

Copolymer synthesis

The first stage of this study was investigating the copolymerization behaviour of the thionolactone dibenzo[c,e]oxepane-5-thione (DOT) with acrylamides (Scheme 1AB). *N*,*N*-Dimethylacrylamide (DMAm) was chosen as a representative comonomer for the estimation of reactivity ratios. Free-radical DMAm–DOT copolymerizations with DOT feed mole fractions between 0.1–0.9 were halted at low global monomer conversions and the molar compositions of the formed copolymers were estimated through ^{1}H NMR spectroscopy. The system DMAm–DOT did not copolymerise azeotropically, with DOT being incorporated faster than DMAm for all feed compositions, see Figure 1. This behaviour contrasts with the copolymerization of cyclic ketene acetals with acrylic comonomers and has the advantage that the (arguably more valuable) cyclic comonomer can be incorporated fully. Kinetic studies of the RAFT-controlled[7] DMAm–DOT copolymerization confirmed the faster incorporation of DOT at all tested feed compositions (10, 25, 50, and 75 mol-% DOT) and the expected formation of gradient copolymers with a higher DOT content at the start of the copolymerization, see Figure 2 and Figure S1. For example, with DOT feeds of 10 and 25 mol-%, DOT conversion reached approx. 50% after 60 minutes, while only approx. 20 % of the DMAm feed had been polymerised after this time. A marked reduction of the polymerization rate was observed at 75 mol% DOT feed with both comonomers reaching <2% conversion after 60 minutes, in agreement with the drastic retardation observed for the homopolymerization of DOT.[22] Somewhat surprisingly, it was nonetheless possible to prepare DOT–acrylamide copolymers with a DOT content well above 50 mol-%. Poly(*N*-isopropylacrylamide$_{19}$-*co*-DOT$_{53}$), p(NIPAm$_{19}$-DOT$_{53}$), which contained 74 mol-% of DOT repeat units, was obtained after reaching 42% and 96% conversion respectively of a 45:55 equiv NIPAm–

DOT feed mixture after polymerizing overnight (Table 1, entry 2). NMR spectroscopic analysis of this copolymer through ^{1}H (Figure S2), $^{1}H–^{13}C$ heteronuclear single quantum coherence (HSQC) (Figure S3), ^{13}C and ^{13}C distortionless enhancement of polarisation transfer (DEPT) (Figures S4–S6), $^{1}H–^{1}H$ correlation spectroscopy (COSY) (Figure S7), and $^{1}H–^{13}C$ heteronuclear multiple bond coherence (HMBC) (Figure S8) methods revealed clearly distinguishable DOT–DOT sequences indicating that, in spite of severe difficulties in preparing DOT homopolymer,[22-24] DOT self-propagation is, in fact, possible.

Scheme 1. (A) RAFT radical copolymerization of DOT with vinyl comonomers; (B) proposed mechanism[23] of thiocarbonyl addition–ring-opening (TARO) radical polymerization; (C) structures and abbreviations of the employed comonomers; and (D) schemes for the basic hydrolysis, aminolysis (including cysteinolysis),[30] and oxidative cleavage[31] of backbone thioesters.



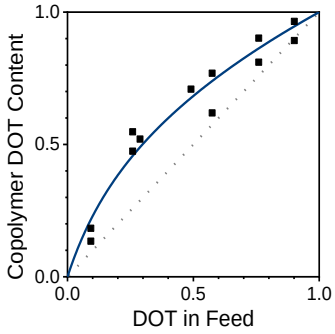


Figure 1. Molar DOT content in copolymer *vs* molar DOT fraction in the monomer feed for the free radical copolymerization of DMAm–DOT in DMSO-d_6 (black squares) with a non-linear least-squares fit for the reactivity ratios $r_{DOT} = 1.89$, $r_{DMAm} = 0.34$ (blue line). The (rare) case of ideal (azeotropic) copolymerization is shown as reference (dotted grey line).

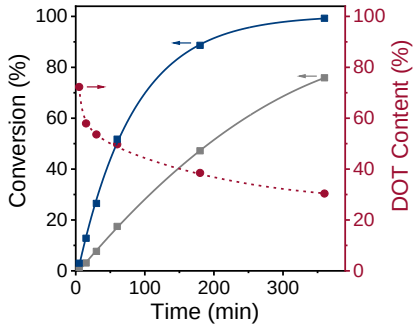


Figure 2. RAFT copolymerization kinetics for DMAm–DOT with an initial [DMAm]:[DOT]:[RAFT agent] = 75:25:1 in DMSO-d_6: DOT conversion (blue squares, left axis), DMAm conversion (grey squares, left axis) and DOT content (from ^{1}H NMR spectroscopy) of the copolymers (red circles, right axis). The data points are connected with fitted curves for clarity. Note that more DOT was incorporated in the beginning of the polymerization and that the overall DOT content remained above the 25 mol-% feed content. After 6 h, DOT was fully consumed.

Table 1. List of thioester backbone-functional copolymers prepared by TARO RAFT radical polymerization (see Scheme 1A–C).

Entry	Code [a]	DOT content (mol-%) [a]	Comonomer Feed (eq)	Polym. time (h)	M_n^{NMR} [a] (kg/mol)	M_n^{SEC} (kg/mol)	$Đ^{SEC}$	water solubility [b]
1	p(NIPAm$_{188}$-DOT$_{10}$) [c]	5	190+10	16	23.5	27.9	1.94 [c]	insoluble
2	p(NIPAm$_{19}$-DOT$_{53}$) [c]	74	45+55	16	14.1	25.8	1.73 [c]	insoluble
3	p(DMAm$_{50}$-NIPAm$_{50}$)	0	50+50	16	5.6	n.d. [d]	n.d.	T_{CP}^{LCST} = 60 °C
4	p(DMAm$_{47}$-NIPAm$_{47}$-DOT$_5$)	5	47.5+47.5+5	16	11.1	14.5	1.29	T_{CP}^{LCST} = 36 °C
5	p(DMAm$_{50}$-DEAm$_{50}$)	0	50+50	16	5.1	4.6	1.23	T_{CP}^{LCST} = 60 °C
6	p(DMAm$_{47}$-DEAm$_{47}$-DOT$_5$)	5	47.5+47.5+5	16	11.2	7.5	1.16	T_{CP}^{LCST} = 36 °C
7	p(DMAm$_{41}$-DEAm$_{27}$-DOT$_{3.5}$)	5	83+55+4	1	10.1	5.3	1.18	T_{CP}^{LCST} = 30 °C
8	p(DMAm$_{123}$-DOT$_{11}$)	8	129+11	2	14.6	2.1	1.61	soluble
9	p(DMAm$_{89}$-DOT$_{10}$)	10	90+10	16	11.1	2.8	1.44	T_{CP}^{LCST} = 68 °C
10	p(DMAm$_{69}$-DOT$_{9.5}$)	12	130+10	2	9.0	3.5	1.31	T_{CP}^{LCST} = 25 °C
11	p(DMAm$_{69}$-DOT$_{9.5}$)	12	69+9.5	16	9.0	3.1	1.32	T_{CP}^{LCST} = 63 °C
12	p(DMAm$_{122}$-DOT$_{18}$)	14	260+20	3	14.7	4.5	1.37	insoluble
13	p(DMAm$_{63}$-DOT$_{15}$)	19	85+15	16	9.6	6.4	1.2	insoluble
14	p(DMAm$_{74}$-DOT$_{25}$)	25	75+25	16	12.9	8.8	1.23	insoluble
15	p(PEGA$_{69}$-DOT$_{30}$)	30	70+30	16	40.0	28.0	1.24	T_{CP}^{LCST} = 78 °C
16	p(SBBAm$_{176}$-DOT$_{17}$)	9	180+20	16	55.2	n.d.	n.d.	T_{CP}^{UCST} = 47 °C
17	p(Am$_{102}$-DOT$_7$)	6	193+7	16	9.1	n.d.	n.d.	soluble
18	p(Am$_{141}$-DOT$_{14}$)	9	186+14	16	13.4	n.d.	n.d.	soluble
19	p(Am$_{172}$-AN$_{10}$-DOT$_8$)	4	182+10+8	16	14.8	n.d.	n.d.	soluble
20	p(Am$_{67}$-AN$_{20}$-DOT$_7$)	8	173+20+7	16	7.7	n.d.	n.d.	insoluble

[a] the comonomer content and molar mass were calculated from monomer conversion determined by ^{1}H NMR spectroscopy; [b] cloud point (T_{CP}) determined optically on mixtures of 1 mL mQ water and 5 mg copolymer; [c] sample prepared by free radical copolymerization; [d] not determined

After demonstrating the ability of DOT to copolymerise with acrylamides, a series of novel acrylamide–DOT copolymers was prepared. The selection of acrylamide comonomers is shown in Scheme 1C and included DMAm, *N*,*N*-diethylacrylamide (DEAm), NIPAm, acrylamide (Am), the sulfobutyl betaine acrylamide (SBBAm) *N*-3-(*N*-4-sulfobutyl-*N*,*N*-dimethylammonium)propylacrylamide, in addition to acrylonitrile (AN) (used as a third comonomer) and poly(ethylene glycol) methylether acrylate (PEGA) as an acrylate comparison. Copolymers are listed in Table 1. Copolymerization occurred in all cases and was well-controlled with generally low dispersities confirming the concurrent success of RAFT and TARO polymerization, see Figure S9. The goal was to prepare degradable polymers that are temperature-responsive, *i.e.*, switch between water-soluble and water-insoluble states at a critical temperature—a behaviour well-documented for several members of the polyacrylamide family.[25-26] Specifically, this study aimed to exploit the reported influence of polymer molecular weight[25, 32] and end group functionality[33] on the critical temperature to evoke an isothermal solubility switch[34-36] (and thereby a drastic change in a material property) upon degradation.

Degradable temperature-responsive copolymers with LCSTs in water

The most common type of temperature-responsive polymer has a lower critical solution temperature (LCST) in water. Counterintuitively, LCST-type polymers are insoluble in water above a critical temperature, a result of the dissolved state requiring an entropically unfavourable structuring of water around hydrophobic parts of the polymer chains.[25] The archetypal LCST-type polymer is pNIPAm with an LCST of 32 °C.[37] Degradable copolymers of NIPAm and cyclic ketene acetals have been prepared[38-40] with some reported to show LCST behaviour,[40] but a degradation-triggered shift of the critical temperature has not been detailed. Herein, NIPAm–DOT

copolymers, even at a low DOT content of 5 mol-%, were found to be insoluble in water independent of temperature (Table 1, entries 1–2). This observation was not surprising given the hydrophobic nature of the DOT-derived biphenyl thiocarboxylate repeat units and prompted the inclusion of the hydrophilic comonomer DMAm. The DOT-less control samples p(DMAm$_{50}$-NIPAm$_{50}$) and p(DMAm$_{50}$-DEAm$_{50}$) (Table 1, entries 3 and 5) both had measured LCST-type cloud points at 60 °C (by virtue of pNIPAM and pDEAM homopolymers having very similar LCSTs).[25] Containing 5 mol-% of DOT, however, p(DMAm$_{47}$-NIPAm$_{47}$-DOT$_5$) and p(DMAm$_{47}$-DEAm$_{47}$-DOT$_5$) (entries 4 and 6) both had measured cloud points of 36 °C, demonstrating the large impact of DOT repeat units in reducing the copolymer solubility and a reliable way of preparing polymers with LCST cloud points just below body temperature. Notably, the measured thermal transitions were sharp (drop of measured transmittance from >99% to <2% within 1 °C for heating at 1 °C/min) and were fully reversible, see Figure S10–11. While there are ample literature cases of tuning LCST transition temperatures through the modification of polymer side groups[28] and end groups,[33] these examples represent rare cases of tuning a polymer's smart solution behaviour through hydrophobic modification in the backbone.[40] LCST behaviour was also observed in a series of DMAm–DOT copolymers with DOT contents of 12–14 mol-% (Table 1, entries 8–14). These copolymers did not contain a typical LCST-type monomer (pDMAm homopolymer is not temperature responsive) but a combination of hydrophilic and hydrophobic repeat units. Similarly, p(PEGA$_{69}$-DOT$_{30}$) (entry 15) had a sharp LCST transition at 78 °C, Figure S12, showing that the strategy was also applicable to other hydrophilic comonomers including this acrylate known to form biocompatible water-soluble homopolymers.[41]

While non-ideal copolymerization behaviour (as found for DMAm–DOT, Figure 1–2) is typically considered a nuisance, this behaviour could be exploited to tune the thermal

responsiveness. To demonstrate this, two copolymers, both with the composition p(DMAm$_{69}$-DOT$_{9.5}$) were prepared; one (Table 1, entry 10) by polymerising a mixture of 130:10 equiv of DMAm–DOT for 2 h (after which neither comonomer had fully converted and a copolymer with DOT repeat units throughout the backbone had been formed) and one (entry 11) by polymerizing a mixture of 69:9.5 equiv DMAm–DOT to full conversion which produced a gradient copolymer with a DMAm-rich tail (Figure 3A). The two samples had very similar dispersities and SEC traces (Figure 3B), virtually identical ^{1}H NMR spectra (Figure S13), and differed only in their comonomer sequence. The gradient sample (entry 11), however, had a much higher LCST cloud point (63 °C *vs* 25 °C measured for its sister copolymer), Figure 3C. Presumably, the DMAm-rich tail enhanced the solubility of this sample, while the DOT-rich region may have had a reduced interface with water through a partial single chain collapse. This notion is supported by the observed lower hydrodynamic size (in THF) of the gradient sample (Figure 3B) and the fact that collapsed or self-assembled hydrophobic end groups are known to have a lower impact on the LCST transition temperature than fully solvated hydrophobic end groups.[33] These experiments demonstrate the importance of determining the copolymerization behaviour of comonomer pairs and its implications for the copolymer sequence.

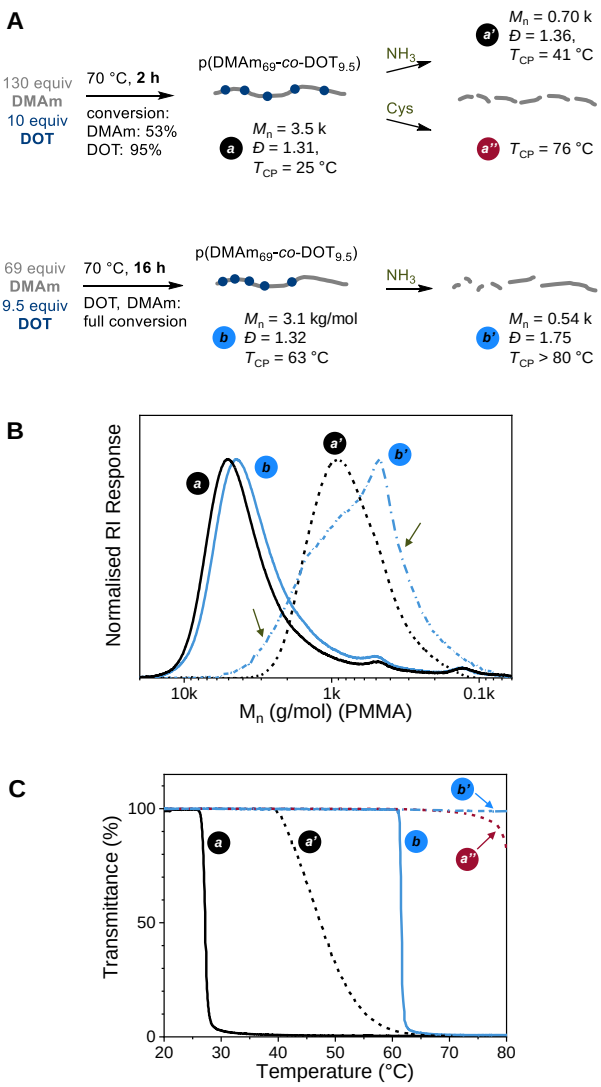


Figure 3. Influence of copolymer microstructure on the thermal responsiveness: (A) Synthesis of two samples with identical composition but different comonomer sequence; (B) SEC traces of intact copolymers (solid lines) and after degradation with ammonia (dashed lines) showing a broader distribution of fragment sizes for the gradient sample (denoted b', see green arrows) in agreement with the assumed irregular distribution of thioesters throughout the backbone; and (C) temperature-dependent optical transmittance demonstrating the different thermal behaviour of the intact copolymers and the increase of LCST transition temperatures upon degradation.

Copolymer degradation

Thioester-backbone functional polymers (which are also available through non-radical methods)[42-43] have been receiving increasing attention.[44] Their degradation through thioester cleavage has been described with a limited number of reagents (including cysteine methyl ester,[24] sodium methanolate,[24] and isopropylamine.)[22-23] but has not been systematically assessed. In a comparison of aminolytic conditions (Figure S14AB) we found that thioester cleavage was favoured by a higher solvent polarity (with degradation efficiency increasing in the order of solvents dichloromethane < tetrahydrofuran << methanol) and that ammonia was a better nucleophile than isopropylamine, *i.e.* could be used in lower concentration to effect complete degradation at RT overnight, while triethylammonium propanethiolate was more effective still than ammonia. Surprisingly, an attempted degradation of a DMAm–DOT copolymer in 1 M aq. HCl gave no degradation, while basic hydrolysis (using 2 M aq. NaOH, RT, overnight) led to complete cleavage of the backbone thioesters (Figure S14C). In addition to this range of common nucleophiles, it was found that potassium persulfate (as oxone) led to fast and selective oxidative hydrolysis of backbone thioesters[31] and full copolymer degradation, see Scheme 1D and below. These results demonstrated the impressive variety of degradation conditions available to thioester-functional polymers—in contrast to their oxoester analogues for which degradation is typically limited to hydrolysis.

Shift of LCST transition temperature upon degradation

With a series of novel degradable and temperature-responsive copolymers in hand, the impact of backbone degradation on their smart solution behaviour was assessed next. Recall that the less soluble p($DMAm_{69}$-$DOT_{9.5}$) sample (Table 1, entry 10) had an LCST-type cloud point at 25 °C

(Figure 3C). After degradation with ammonia the sample had a cloud point of 41 °C, confirming the expected increase of LCST with decreasing molar mass.[25] Likewise, the transition temperature of the sister species increased from 63 °C to above 80 °C upon degradation with ammonia. When the former sample was treated with cysteine in water, the critical temperature increased from 25 °C to 76 °C. In this case the fragments carried anionic C-terminal cysteine end groups which contributed to better solubility compared to the uncharged primary amides produced by ammonolysis. These examples demonstrated a drastic change of a material property (water solubility) upon degradation but only within a temperature window (between 25 °C and 41 or 76 °C). Gratifyingly, it was simple to tune the size of this window by varying the DOT content of copolymers. Consider copolymer p($DMAm_{41}$-$DEAm_{27}$-$DOT_{3.5}$) (Table 1, entry 7), which had a DP of 71.5, a DOT content of just 5 mol-% and an LCST cloud point of 30 °C (by virtue of its more hydrophobic DEAm comonomer units and a short polymerization time of 1 h) (Figure 4A). After treatment with 5 equiv (per thioester) of potassium persulfate (as oxone) at RT, the mixture had a cloud point of 50 °C (increase of 20 °C) and the isolated polymer had an SEC-measured (PMMA-equivalent) molecular weight of 2.9 kg/mol which confirmed degradation (Figure 4C). In this context it is worth noting that a DOT-less control sample, p($DMAm_{50}$-$DEAm_{50}$) (Table 1, entry 5) showed no reduction of SEC-measured molecular weight after identical treatment with oxone (Figure S15), confirming the selective oxidation of thioesters. The LCST cloud point of the p($DMAm_{50}$-$DEAm_{50}$)–oxone mixture, however, was some 8 °C higher than before the addition of oxone (Figure S16), indicating that the sensitivity of the LCST transition toward changes in the ionic strength and possible end group effects associated with the RAFT functionality make the observation of cloud points an unreliable method to confirm the absence of degradation in control samples. Conversely, sample p($DMAm_{63}$-DOT_{15}) (Table 1, entry 13) with a comparable DP of 78

was water insoluble due to its high DOT content of 19 mol-% (Figure 4B). Upon treatment with oxone (same total quantity as above, approx. 1.3-fold excess with regards to thioesters), this sample became fully water soluble within minutes, implying a shift of a theoretical LCST from below 0 °C to above 100 °C. The isolated material had an SEC-measured PMMA-equivalent molecular weight of 0.74 kg/mol, confirming degradation into comparatively small fragments. The DOT content thus influenced the degradation-induced LCST shift for two reasons. Due to the hydrophobicity of the unique biphenyl thiocarboxylate repeat groups, an increasing DOT content led to lower water solubility (lower LCST cloud points) of the intact samples. Degradation through hydrolysis, cysteinolysis, or oxidative hydrolysis, however, created anionic water-soluble end groups on the fragments. Secondly, an increasing DOT content in the copolymers led to a decreasing average degree of polymerization of the fragments and thus a larger influence of the hydrophilic end groups and thus a higher LCST cloud point of the fragments, Figure 4. This correlation was also observed in a series of copolymers degraded with cysteine. Containing 5 mol-% of DOT, the LCST cloud point of p(DMAm$_{47}$-DEAm$_{47}$-DOT$_5$) increased by 20 °C (from 36 °C to 56 °C) upon cysteinolysis (Figure S17); at 12 mol-% DOT, the LCST cloud point of p(DMAm$_{69}$-DOT$_{9.5}$) increased by 51 °C (Figure 3A); containing 14 mol-% DOT, sample p(DMAm$_{122}$-DOT$_{18}$) was insoluble but had a cloud point of 74 °C after cysteinolysis (increase > 74 °C) (Figure S18), while p(DMAm$_{63}$-DOT$_{15}$) (19 mol-% DOT) underwent a temperature-independent insoluble–solution transition (increase > 100 °C). An overview of cloud points before and after degradation with various reagents is shown in Table S1.

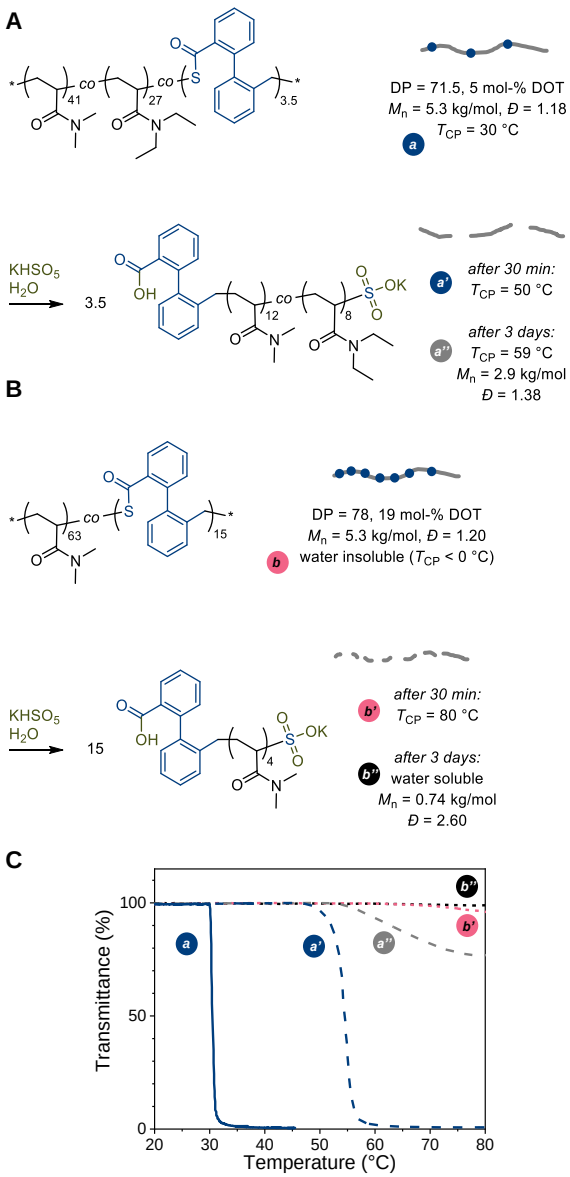


Figure 4. Degradation of thioester backbone-functional copolymers through oxidative hydrolysis using persulfate and influence of the thioester content on the degradation-induced shift of the LCST transition temperature: (A and B) reaction schemes; indices in fragments are average degrees of polymerization after degradation when ignoring the alpha end group fragment; molar masses were measured by SEC and are PMMA-equivalent values and (C) measured turbidity curves for the above samples.

Shift of UCST transition temperature upon degradation

Aqueous UCST behaviour, solubility above a critical temperature, is only known for a very small subset of temperature-responsive polymers, with zwitterionic sulfobetaines[45] and certain non-ionic amide-functional species[46] making up the main types of these smart materials.[26] This behaviour relies on a balance of mixing entropy (which favours dissolution) and uncommonly strong inter-polymer electrostatic or H-bonding attractions (which favour phase separation). Herein, we present a first description of backbone degradable polymers with an aqueous UCST and a degradation-induced shift of the UCST transition temperature.

Sulfobetaine-functional monomers and polymers are notorious for their poor solubility in organic solvents, whereas DOT is insoluble in water. Gratifyingly, the solvent mixture propylene carbonate–2,2,2-trifluoroethanol (1:1) allowed for a homogeneous RAFT copolymerization of DOT and the sulfobutyl betaine acrylamide SBBAm (Scheme 1C). The copolymer p(SBBAm$_{176}$-DOT$_{17}$) had a measured UCST transition temperature of 47 °C (Figure 5A,C). This value was lower than that reported of a pSBBAm homopolymer of 59 °C,[29] which was surprising since the hydrophobic modification of sulfobetaine copolymers (with aromatic side groups) is known to increase UCST transition temperatures.[47] The UCST behaviour of zwitterionic (co)polymers is highly sensitive to the presence of ionic species (with small amounts of added salt drastically increasing solubility).[48] Isopropylamine was therefore used as a volatile degradant to prevent the introduction of ionic species difficult to separate from the degraded polymer fragments. After treatment of p(SBBAm$_{176}$-DOT$_{17}$) with an excess of isopropylamine and evaporation to dryness, the re-dissolved sample had a UCST cloud point of 31 °C, confirming the expected (although small) shift of the UCST transition temperature, attributed to the decrease in molar mass upon

degradation.[29] Unfortunately, due to the poor copolymer solubility, the extent of the degradation could not be confirmed through SEC analysis.

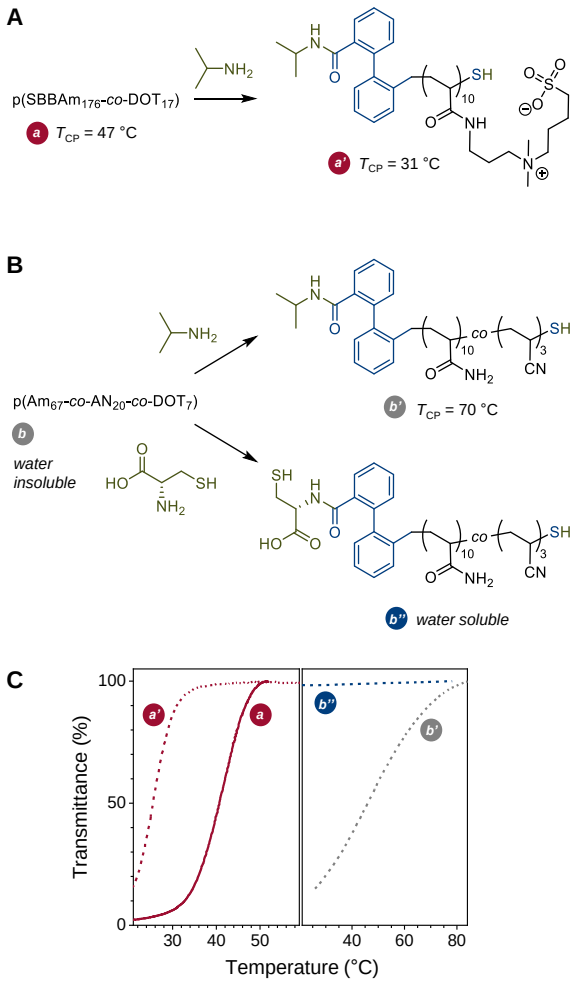


Figure 5. Degradable polymers with an aqueous UCST and shift of the transition temperature through selective degradation for (A) a zwitterionic sulfobutylbetaine copolymer and (B) an acrylamide–acrylonitrile-based terpolymer with (C) measured temperature-dependant optical transmittance.

Acrylamide (Am)–acrylonitrile (AN) copolymers have been shown to have a tuneable UCST in water which, advantageously, is largely independent of the ionic strength.[46] We found copolymers of acrylamide and up to 9 mol-% of DOT and the terpolymer p(Am_{172}-AN_{10}-DOT_8) containing 4 mol-% of DOT to be water-soluble (Table 1, entries 17–19). Conversely, at 8 mol-% DOT and a higher acrylonitrile content, p(Am_{67}-AN_{20}-DOT_7) (entry 20) was insoluble even at high temperatures, suggesting that UCST behaviour for intact terpolymers may be found at an intermediate molar composition. After p(Am_{67}-AN_{20}-DOT_7), the insoluble sample, was treated with isopropylamine, it was soluble above 80 °C and became gradually cloudy upon cooling, displaying UCST behaviour (Figure 5B and C). Treatment of the intact terpolymer with cysteine, on the other hand, resulted in temperature independent solubility, demonstrating that the desired insoluble–soluble transitions could also be realised by exploiting the less common UCST behaviour in combination with a charged degradant.

Of the small number of applied studies of polymers with an aqueous UCST, the recent work by Zhang et al.[49] is noteworthy. The authors presented an amide-functional methacrylamide-based homopolymer with carbonate side groups and an aqueous UCST. Upon hydrolysis of the carbonate groups, the UCST behaviour disappeared and the polymers became water-soluble. This "transient" UCST behaviour was explored in the context of next-generation biomaterials for sustained release applications. The DOT copolymers presented here have similar application potential, with the added benefits that the cleavage of thioesters is a routine intracellular process[50] and that cleavage in the backbone (as opposed to the side groups) may reduce a polymer's hydrodynamic size below the renal threshold and aid in clearing the material from the body.[51] Studies of the cytotoxicity, biodegradation, and drug release potential of DOT copolymers are underway and beyond the scope of the current work.

Herein, we demonstrate the application potential of degradable temperature-responsive polymers by measuring flow rates through syringe filters blocked with a water-insoluble polymer. An aqueous suspension of p($DMAm_{63}$-DOT_{15}) (Table 1, entry 13, water-insoluble), was forced through syringe filters (0.2 μm pore size) until blocked. Filters were dried and attached to columns containing equal volumes and heights of various aqueous solutions, with only gravity acting on the liquids. Flow rates were determined by measuring the volume of eluted solutions after predetermined time intervals, Figure 6AB. No flow of water or PBS buffer was observed through blocked filters over three days. A basic solution of cysteine, however, passed through the filter immediately with flow rates comparable to that of un-treated filters. This observation was attributed to cysteine degrading the insoluble polymer into soluble fragments. Indeed, a sample of a cloudy aqueous p($DMAm_{63}$-DOT_{15}) suspension became clear as soon as a drop of cysteine solution was added (Figure 6C). SEC analysis of the cysteine-treated copolymer confirmed the expected degradation. A blocked filter presented with a 10 mM persulfate solution remained blocked for about 50 min before flow occurred. Degradation of the copolymer through a 100 mM *N*-acetylcysteine (NAC) solution (in PBS buffer at pH 7.4), on the other hand, was slower with no flow within the first hour, little flow during the first day, and sustained flow starting after two days of exposure. Under these physiological conditions, only about 0.8% of NAC-SH groups (pK_a = 9.52)[52] are in their reactive thiolate form which was likely the reason for the slow reaction. While SEC analysis is relatively slow and poorly suited to determine degradation kinetics, these filter tests are a simple way of comparing relative degradation and dissolution speeds. The experiments indicate application potential of the copolymers in lab-on-a-chip applications for "smart" valves that open only when certain analytes are present, with the possibility to distinguish between solutions through the time it takes the valve to open. Temperature-responsive polymers have also

been investigated as embolic materials, *i.e.*, their precipitation inside the body exploited to cause an intentional blocking of blood vessels, *e.g.*, to starve tumour cells or prevent bleeding during surgery.[53-55] However, embolization with common temperature-responsive polymers is irreversible. The polymers presented herein have the potential to enable a *reversible* blocking of blood vessels for applications where a post-treatment blood flow is desirable. As demonstrated crudely with syringe filters, the unblocking could potentially be achieved through doses of NAC, a non-toxic dietary supplement.[56]

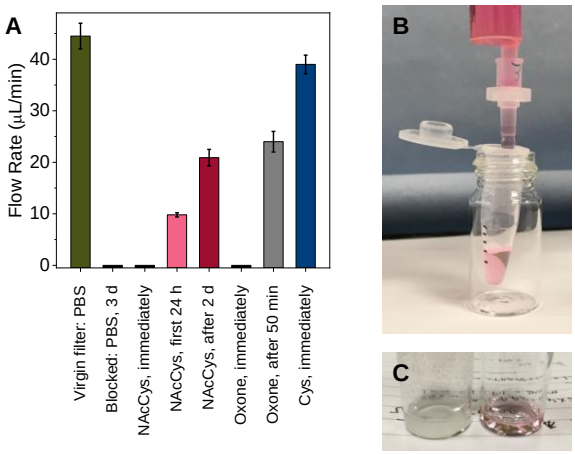


Figure 6. (A) Measured flow rates of aqueous solutions through syringe filters: non-treated filter as reference (PBS buffer, pH 7.4, left bar) and filters previously blocked with an aqueous p(DMAm$_{63}$-DOT$_{15}$) suspension (remaining bars) for PBS buffer (pH 7.4), *N*-acetylcysteine (100 mM, pH 7.4), persulfate (10 mM), and cysteine (10 mM, pH 9); (B) photograph of a typical experimental setup. The syringe was open at the top and no pressure was applied. A small amount of Rhodamine B dye was added to this cysteine solution for better visibility on the photograph; (C) photographs of cloudy suspension of p(DMAm$_{63}$-DOT$_{15}$) in water (5 g/L) (left) and of a sample of the same suspension presenting as clear solution immediately after addition of a drop of (dye-labelled) cysteine solution.

Conclusion

By exploiting the rapid copolymerization of the thionolactone DOT with acrylamides, the hydrophobic nature of the resultant biphenyl thiocarboxylate repeat units, and the selective reactivity of these thioesters in combination with smart (LCST or UCST-type) solution behaviour, we created a series of polymers with unique properties. These materials could be tuned to be water-insoluble above or below a critical temperature or independent of temperature. Upon exposure to a range of very specific conditions such as high pH, nucleophiles (including a drug under physiological conditions), or an oxidant, the polymers degraded into shorter polymers with enhanced aqueous solubility, including temperature-independent solubility. The materials could be applied for the selective opening of a blocked passage. This study significantly expands the horizon of the smart polymer arena by enabling the placement of stimulus responsive functionality into the backbone of polymers. Especially the demonstrated degradability under physiological conditions makes the family of thioester functional polymers promising as advanced materials with potential in lab-on-a-chip or biomedical sensing applications.

Supporting Information. Polymerization kinetics, copolymer NMR spectra, SEC results, turbidity curves, comparison of degradation conditions, overview of cloud point shifts.

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References

1. Albertsson, A.-C.; Hakkarainen, M., Designed to Degrade. *Science* **2017,** *358* (6365), 872.

2. Haider, T. P.; Völker, C.; Kramm, J.; Landfester, K.; Wurm, F. R., Plastics of the Future? The Impact of Biodegradable Polymers on the Environment and on Society. *Angew. Chem., Int. Ed.* **2019,** *58* (1), 50-62.

3. Zumstein, M. T.; Narayan, R.; Kohler, H.-P. E.; McNeill, K.; Sander, M., Dos and Do Nots When Assessing the Biodegradation of Plastics. *Environ. Sci. Technol.* **2019,** *53* (17), 9967-9969.

4. Brannigan, R. P.; Dove, A. P., Synthesis, Properties and Biomedical Applications of Hydrolytically Degradable Materials Based on Aliphatic Polyesters and Polycarbonates. *Biomater. Sci.* **2017,** *5* (1), 9-21.

5. Delplace; Nicolas, Degradable Vinyl Polymers for Biomedical Applications. *Nat. Chem.* **2015,** *7* (10), 771.

6. Tardy; Nicolas; Gigmes; Lefay; Guillaneuf, Radical Ring-Opening Polymerization: Scope, Limitations, and Application to (Bio)Degradable Materials. *Chem. Rev.* **2017,** *117* (3), 1319.

7. Moad, G.; Rizzardo, E.; Thang, S. H., Living Radical Polymerization by the RAFT Process – A Third Update. *Aust. J. Chem.* **2012,** *65* (8), 985-1076.

8. Ribelli, T. G.; Lorandi, F.; Fantin, M.; Matyjaszewski, K., Atom Transfer Radical Polymerization: Billion Times More Active Catalysts and New Initiation Systems. *Macromol. Rapid Commun.* **2019,** *40* (1), 1800616.

9. An, Z.; Qiu, Q.; Liu, G., Synthesis of Architecturally Well-defined Nanogels via RAFT Polymerization for Potential Bioapplications. *Chem. Commun.* **2011,** *47* (46), 12424-12440.

10. Boyer, C.; Bulmus, V.; Davis, T. P.; Ladmiral, V.; Liu, J.; Perrier, S., Bioapplications of RAFT Polymerization. *Chem. Rev.* **2009,** *109* (11), 5402-5436.

11. Chu, D. S. H.; Schellinger, J. G.; Shi, J.; Convertine, A. J.; Stayton, P. S.; Pun, S. H., Application of Living Free Radical Polymerization for Nucleic Acid Delivery. *Acc. Chem. Res.* **2012,** *45* (7), 1089-1099.

12. Gregory, A.; Stenzel, M. H., The Use of Reversible Addition Fragmentation Chain Transfer Polymerization for Drug Delivery Systems. *Expert Opin. Drug Delivery* **2011,** *8* (2), 237-269.

13. Quinn, J. F.; Whittaker, M. R.; Davis, T. P., Glutathione Responsive Polymers and Their Application in Drug Delivery Systems. *Polym. Chem.* **2017,** *8* (1), 97-126.

14. Smith, D.; Holley, A. C.; McCormick, C. L., RAFT-synthesized Copolymers and Conjugates Designed for Therapeutic Delivery of siRNA. *Polym. Chem.* **2011,** *2* (7), 1428-1441.

15. Bailey, J.; Chou, J. L.; Feng, P. Z.; Kuruganti, V.; Zhou, L. L., Recent Advances in Free Radical Ring-opening Polymerization. *Acta Polym.* **1988,** *39* (7), 335-341.

16. Agarwal, S., Chemistry, Chances and Limitations of the Radical Ring-opening Polymerization of Cyclic Ketene Acetals for the Synthesis of Degradable Polyesters. *Polym. Chem.* **2010,** *1* (7), 953-964.

17. Paulusse; Amir; Evans; Hawker., Free Radical Polymers with Tunable and Selective Bio- and Chemical Degradability. *J. Am. Chem. Soc.* **2009,** *131* (28), 9805.

18. Phelan, M.; Aldabbagh, F.; Zetterlund, P. B.; Yamada, B., Mechanism and Kinetics of the Free Radical Ring-opening Polymerization of Cyclic Allylic Sulfide Lactones. *Polym.* **2005,** *46* (26), 12046-12056.

19. Evans; Moad; Rizzardo; Thang, New Free-Radical Ring-Opening Acrylate Monomers. *Macromolecules* **1994,** *27* (26), 7935.

20. Hill, M. R.; Kubo, T.; Goodrich, S. L.; Figg, C. A.; Sumerlin, B. S., Alternating Radical Ring-Opening Polymerization of Cyclic Ketene Acetals: Access to Tunable and Functional Polyester Copolymers. *Macromolecules* **2018,** *51* (14), 5079-5084.

21. Shi, Y.; Agarwal, S., Thermally Stable Optically Transparent Copolymers of 2-Methylene-1,3-Dioxepane and N-Phenyl Maleimide with Degradable Ester Linkages. In *e-Polym.*, 2015; Vol. 15, p 217.

22. Spick, M. P.; Bingham, N. M.; Li, Y.; de Jesus, J.; Costa, C.; Bailey, M. J.; Roth, P. J., Fully Degradable Thioester-Functional Homo- and Alternating Copolymers Prepared through Thiocarbonyl Addition–Ring-Opening RAFT Radical Polymerization. *Macromolecules* **2020,** *53* (2), 539-547.

23. Bingham, N. M.; Roth, P. J., Degradable Vinyl Copolymers Through Thiocarbonyl Addition–Ring-opening (TARO) Polymerization. *Chem. Commun.* **2019,** *55* (1), 55-58.

24. Smith, R. A.; Fu, G.; McAteer, O.; Xu, M.; Gutekunst, W. R., Radical Approach to Thioester-Containing Polymers. *J. Am. Chem. Soc.* **2019,** *141* (4), 1446-1451.

25. Aseyev, V.; Tenhu, H.; Winnik, F., Non-ionic Thermoresponsive Polymers in Water. In *Self Organized Nanostructures of Amphiphilic Block Copolymers II*, Müller, A. H. E.; Borisov, O., Eds. Springer Berlin Heidelberg: 2011; Vol. 242, pp 29-89.

26. Seuring, J.; Agarwal, S., Polymers with Upper Critical Solution Temperature in Aqueous Solution. *Macromol. Rapid Commun.* **2012,** *33* (22), 1898-1920.

27. Zhao, C.; Ma, Z.; Zhu, X. X., Rational Design of Thermoresponsive Polymers in Aqueous Solutions: A Thermodynamics Map. *Prog. Polym. Sci.* **2019,** *90*, 269-291.

28. Roth, P. J., Composing Well-Defined Stimulus-Responsive Materials Through Postpolymerization Modification Reactions. *Macromol. Chem. Phys.* **2014,** *215* (9), 825-838.

29. Zhu, Y.; Noy, J.-M.; Lowe, A. B.; Roth, P. J., The Synthesis and Aqueous Solution Properties of Sulfobutylbetaine (Co)polymers: Comparison of Synthetic Routes and Tuneable Upper Critical Solution Temperatures. *Polym. Chem.* **2015,** *6* (31), 5705-5718.

30. Wang, C.; Guo, Q.-X.; Fu, Y., Theoretical Analysis of the Detailed Mechanism of Native Chemical Ligation Reactions. *Chem. - Asian J.* **2011,** *6* (5), 1241-1251.

31. Bunton, C. A.; Foroudian, H. J.; Kumar, A., Sulfide Oxidation and Oxidative Hydrolysis of Thioesters by Peroxymonosulfate ion. *J. Chem. Soc., Perkin Trans. 2* **1995,** (1), 33-39.

32. Ieong, N. S.; Hasan, M.; Phillips, D. J.; Saaka, Y.; O'Reilly, R. K.; Gibson, M. I., Polymers with Molecular Weight Dependent LCSTs Are Essential for Cooperative behaviour. *Polym. Chem.* **2012,** *3* (3), 794-799.

33. Roth, P. J.; Jochum, F. D.; Forst, F. R.; Zentel, R.; Theato, P., Influence of End Groups on the Stimulus-Responsive Behavior of Poly[oligo(ethylene glycol) methacrylate] in Water. *Macromolecules* **2010,** *43* (10), 4638-4645.

34. Phillips, D. J.; Gibson, M. I., Towards Being Genuinely Smart: 'Isothermally-responsive' Polymers as Versatile, Programmable Scaffolds for Biologically-adaptable Materials. *Polym. Chem.* **2015,** *6* (7), 1033-1043.

35. Phillips, D. J.; Gibson, M. I., Degradable Thermoresponsive Polymers which Display Redox-responsive LCST Behaviour. *Chem. Commun.* **2012,** *48* (7), 1054-1056.

36. Theato, P., One is Enough: Influencing Polymer Properties with a Single Chromophoric Unit. *Angew. Chem., Int. Ed.* **2011,** *50* (26), 5804-5806.

37. Taylor, L. D.; Cerankowski, L. D., Preparation of Films Exhibiting a Balanced Temperature Dependence to Permeation by Aqueous Solutions—a Study of Lower Consolute Behavior. *J. Polym. Sci. Pol. Chem.* **1975,** *13* (11), 2551-2570.

38. Galperin, A.; Long, T. J.; Ratner, B. D., Degradable, Thermo-Sensitive Poly(N-isopropyl acrylamide)-Based Scaffolds with Controlled Porosity for Tissue Engineering Applications. *Biomacromolecules* **2010,** *11* (10), 2583-2592.

39. Sun; Zhuo; Liu, Synthesis and Enzymatic Degradation of 2-Methylene-1,3-Dioxepane and Methyl Acrylate Copolymers. *J. Polym. Sci. Pol. Chem.* **2003,** *41* (18), 2898.

40. Siegwart, D. J.; Bencherif, S. A.; Srinivasan, A.; Hollinger, J. O.; Matyjaszewski, K., Synthesis, Characterization, and In Vitro Cell Culture Viability of Degradable Poly(N-isopropylacrylamide-co-5,6-benzo-2-methylene-1,3-dioxepane)-based Polymers and Crosslinked Gels. *J. Biomed. Mater. Res., Part A* **2008,** *87A* (2), 345-358.

41. Lutz, J.-F., Polymerization of Oligo(ethylene glycol) (Meth)acrylates: Toward New Generations of Smart Biocompatible Materials. *J. Polym. Sci. Pol. Chem.* **2008,** *46* (11), 3459-3470.

42. Bannin, T. J.; Kiesewetter, M. K., Poly(thioester) by Organocatalytic Ring-Opening Polymerization. *Macromolecules* **2015,** *48* (16), 5481-5486.

43. Kato, M.; Toshima, K.; Matsumura, S., Enzymatic Synthesis of Polythioester by the Ring-Opening Polymerization of Cyclic Thioester. *Biomacromolecules* **2007,** *8* (11), 3590-3596.

44. Aksakal, S.; Aksakal, R.; Becer, C. R., Thioester Functional Polymers. *Polym. Chem.* **2018,** *9* (36), 4507-4516.

45. Laschewsky, A., Structures and Synthesis of Zwitterionic Polymers. *Polymers* **2014,** *6* (5), 1544-1601.

46. Seuring, J.; Agarwal, S., First Example of a Universal and Cost-Effective Approach: Polymers with Tunable Upper Critical Solution Temperature in Water and Electrolyte Solution. *Macromolecules* **2012,** *45* (9), 3910-3918.

47. Woodfield, P. A.; Zhu, Y. C.; Pei, Y. W.; Roth, P. J., Hydrophobically Modified Sulfobetaine Copolymers with Tunable Aqueous UCST through Postpolymerization Modification of Poly(pentafluorophenyl acrylate). *Macromolecules* **2014,** *47* (2), 750-762.

48. Mary, P.; Bendejacq, D. D.; Labeau, M.-P.; Dupuis, P., Reconciling Low- and High-Salt Solution Behavior of Sulfobetaine Polyzwitterions. *J. Phys. Chem. B* **2007,** *111* (27), 7767-7777.

49. Zhang, Z.; Li, H.; Kasmi, S.; Van Herck, S.; Deswarte, K.; Lambrecht, B. N.; Hoogenboom, R.; Nuhn, L.; De Geest, B. G., A Synthetic, Transiently Thermoresponsive Homopolymer with UCST Behaviour within a Physiologically Relevant Window. *Angew. Chem., Int. Ed.* **2019,** *58* (23), 7866-7872.

50. Grillo, M. P., Drug-S-Acyl-Glutathione Thioesters: Synthesis, Bioanalytical Properties, Chemical Reactivity, Biological Formation and Degradation. *Curr. Drug Metab.* **2011,** *12* (3), 229-244.

51. Fox, M. E.; Szoka, F. C.; Fréchet, J. M. J., Soluble Polymer Carriers for the Treatment of Cancer: The Importance of Molecular Architecture. *Acc. Chem. Res.* **2009,** *42* (8), 1141-1151.

52. Aldini, G.; Altomare, A.; Baron, G.; Vistoli, G.; Carini, M.; Borsani, L.; Sergio, F., N-Acetylcysteine as an Antioxidant and Disulphide Breaking Agent: The Reasons Why. *Free Radical Res.* **2018,** *52* (7), 751-762.

53. Matsumaru, Y.; Hyodo, A.; Nose, T.; Ito, S.; Hirano, T.; Ohashi, S., Application of Thermosensitive Polymers As a New Embolic Material for Intravascular Neurosurgery. *J. Biomater. Sci. Polym. Ed.* **1996,** *7* (9), 795-804.

54. Bearat, H. H.; Preul, M. C.; Vernon, B. L., Cytotoxicity, in Vitro Models and Preliminary In Vivo Study of Dual Physical and Chemical Gels for Endovascular Embolization of Cerebral Aneurysms. *J. Biomed. Mater. Res. A* **2013,** *101* (9), 2515-25.

55. Shi, X.; Gao, H.; Dai, F.; Feng, X.; Liu, W., A Thermoresponsive Supramolecular Copolymer Hydrogel for the Embolization of Kidney Arteries. *Biomater. Sci.* **2016,** *4* (11), 1673-1681.

56. Cotgreave, N-Acetylcysteine: Pharmacological Considerations and Experimental and Clinical Applications. In *Adv. Pharm.*, Sies, H., Ed. Academic Press: 1996; Vol. 38, p 205.