

Supplementary Information

Aqueous degradability of water-soluble, thioester-
containing polyacrylamides with UCST-type behaviour in
salt solutions obtained by rROP

Thomas J Neal,^a Julien Nicolas^{a,}*

^a Université Paris-Saclay, CNRS, Institut Galien Paris-Saclay, 17 avenue des Sciences
91400 Orsay, France.

*To whom correspondence should be addressed.

Email: julien.nicolas@universite-paris-saclay.fr

Tel.: +33 (0)1 80 00 60 81

Table of content

Experimental	3
Materials	3
Methods	3
<i>Synthesis of P(AAm-co-DOT) statistical copolymers via RAFT copolymerisation – copolymer 4</i>	3
<i>Synthesis of P(AAm-co-DOT) statistical copolymers via FRP – copolymer 10</i>	3
<i>Synthesis of P(NIPAAm-co-DOT) statistical copolymers – copolymer 9</i>	4
<i>Degradation kinetics of the copolymers with isopropylamine</i>	4
<i>1-Point degradation analysis of the copolymers with isopropylamine</i>	4
<i>1-Point degradation analysis of the copolymers with NaOH(aq)</i>	4
<i>1-Point degradation analysis of the copolymers with cysteine</i>	4
<i>1-Point degradation analysis of the copolymers with bleach</i>	5
Size Exclusion Chromatography (SEC)	5
Nuclear Magnetic Resonance (NMR)	6
Supplementary Figures	7
References	13

Experimental

Materials

Acrylamide (AAM, 99%), *N*-isopropylacrylamide (NIPAAm, 99%), 4,4'-azobis(4-cyanovaleric acid) (ACVA, 98%), dioxane (anhydrous, 99.8%), acetic acid (99%), NaOH, and isopropylamine ($\geq 99\%$) were purchased from Sigma-Aldrich. DMSO (HPLC grade) and acetone were obtained from VWR Chemicals. Hydrochloric acid (HCl, 37%) was supplied by Carlo-Erba. DMSO- d_6 was purchased from Eurisotop. 2-(Dodecylthiocarbonothioylthio)-2-methylpropionic acid (DDMAT, 97%) was purchased from BLD Pharmatech Ltd. Dibenzo[*c,e*]oxepane-5-thione (DOT) was prepared as reported previously.¹ AAm and NIPAAm were recrystallised before use in acetone and hexane, respectively. La Croix – Eau de Javel (bleach, 3.7% v/v sodium hypochlorite) can be purchased from any commercial French supermarket.

Methods

Synthesis of P(AAm-co-DOT) statistical copolymers via RAFT copolymerisation – copolymer 4

In a typical procedure, ACVA (1.3 mg, 5 μ mol), DDMAT (4 mg, 9 μ mol), AAm (200 mg, 2.8 mmol), DOT (53 mg, 0.23 mmol), were added to sample vial and dissolved in DMSO (1.5 mL). Once all components were dissolved 0.17 mL of water was added. The reaction was sealed then degassed by bubbling argon through the solution for 20 min. The vial was then placed in an oil bath at 73 °C and left to react for 20 h. The polymerisation was terminated by exposure to air and cooling to ambient temperature. The copolymer was isolated by precipitation in acetone and dried under vacuum. The resultant copolymer was analysed by ¹H NMR in DMSO- d_6 and by SEC in DMSO.

Synthesis of P(AAm-co-DOT) statistical copolymers via FRP – copolymer 10

In a typical procedure, ACVA (2.6 mg, 9 μ mol), AAm (200 mg, 2.8 mmol), DOT (32 mg, 0.14 mmol), were added to sample vial and dissolved in DMSO (1.5 mL). Once all components were dissolved 0.17 mL of water was added. The reaction was sealed then degassed by bubbling argon through the solution for 20 min. The vial was then placed in an oil bath at 73 °C and left to react for 20 h. The polymerisation was terminated by exposure to air and cooling to ambient temperature. The copolymer was isolated by precipitation in acetone and dried under vacuum. The resultant copolymer was analysed by ¹H NMR in DMSO- d_6 and by SEC in DMSO.

Synthesis of P(NIPAAm-co-DOT) statistical copolymers – copolymer 9

In a typical procedure, ACVA (0.8 mg, 3 μ mol), DDMAT (2 mg, 6 μ mol), NIPAAm (200 mg, 1.8 mmol), DOT (20 mg, 0.088 mmol), were added to sample vial and dissolved in dioxane (1 mL). The reaction was sealed then degassed by bubbling argon through the solution for 20 min. The vial was then placed in an oil bath at 73 °C and left to react for 20 h. The polymerisation was terminated by exposure to air and cooling to ambient temperature. The copolymer was isolated by precipitation in diethyl ether and dried under vacuum. The resultant copolymer was analysed by ^1H NMR in $\text{DMSO-}d_6$ and by SEC in DMSO.

Degradation kinetics of the copolymers with isopropylamine

In a typical procedure, P(AAm-co-DOT) (30 mg) was added to a sample vial and dissolved in 3 mL of deionised water. Once dissolved 0.6 mL of isopropylamine was added and the solution was left to stir at ambient temperature. 1 mL aliquots were taken at 2 h, 5 h, and 22 h. Each aliquot was quenched and neutralised by the dropwise addition of acetic acid (10%). The water was removed from each aliquot *via* lyophilization and the aliquots were analysed by SEC in DMSO.

1-Point degradation analysis of the copolymers with isopropylamine

In a typical procedure, P(AAm-co-DOT) (10 mg) was added to a sample vial and dissolved in 1 mL of deionised water. Once dissolved 0.2 mL of isopropylamine was added and the solution was left to stir at ambient temperature for 2 h at which point the solution was quenched and neutralised by the dropwise addition of acetic acid (10%). **During the degradation reaction no colour change observed.** The water was removed from the sample *via* lyophilization and the sample analysed by SEC in DMSO.

1-Point degradation analysis of the copolymers with NaOH(aq)

In a typical procedure, P(AAm-co-DOT) (10 mg) was added to a sample vial and dissolved in 0.5 mL of deionised water. Once dissolved 0.5 mL of 5 % NaOH aqueous solution was added and the solution was left to stir at ambient temperature for 2 h at which point the solution was quenched and neutralised by the dropwise addition of 5% HCl solution. **During the degradation reaction no colour change observed.** The water was removed from the sample *via* lyophilization and the sample was analysed by SEC in DMSO.

1-Point degradation analysis of the copolymers with cysteine

In a typical procedure, P(AAm-co-DOT) (10 mg) was added to a sample vial and dissolved in 0.5 mL of deionised water. Once dissolved 0.5 mL of 20 mM L-cysteine:TCEP (10:1) solution was added and the solution was left to stir at 37 °C for 1 week at which point the solution was frozen in liquid nitrogen. **During the degradation reaction no colour change observed.** The water was removed from the sample *via* lyophilization and the sample was analysed by SEC in DMSO.

1-Point degradation analysis of the copolymers with bleach

In a typical procedure, P(AAm-co-DOT) (10 mg) was added to a sample vial and dissolved in 0.5 mL of deionised water. Once dissolved 0.5 mL of household bleach (details in materials section) was added and the solution was left to stir at ambient temperature for 1 week at which point acetic acid was added dropwise to disrupt any salts formed and the solution was frozen in liquid nitrogen. **During the degradation reaction the colourless solutions turned pale yellow/brown in colour due to the oxidation of the thiol groups to form sulfonic acid moieties on the degradation product.**² The water was removed from the sample *via* lyophilization and the sample was analysed by SEC in DMSO.

Dynamic Light Scattering (DLS)

Intensity-averaged nanoparticle diameter, polydispersity index (PDI) and derived count rate were measured by DLS with a Zetasizer Ultra from Malvern (173° scattering angle) at temperatures between 50 °C and 5 °C. Salt solutions were prepared by first dissolving the copolymers at 0.2 wt% in 1 mL of deionised water. These aqueous solutions were then diluted with 1 mL of 20 mM NaCl aqueous solution producing a 0.1 wt% copolymer solution in 10 mM NaCl.

Size Exclusion Chromatography (SEC)

SEC was performed at 60 °C using two columns in series from Agilent Technologies (PL PolarGel-M, 300 × 7.5 mm; bead diameter 8 µm; molar mass range 1000–5,00,000 g mol⁻¹) preceded by a guard column from Agilent Technologies (PL PolarGel-M, 7.5 × 50 mm; bead diameter 8 µm) and a triple detection system (Viscotek TDA/GPCmax from Malvern) with a differential refractive index detector, low and right-angle light scattering detectors, a differential viscometer detector, and an additional UV detector. The eluent was DMSO with 0.1% w/v LiBr and 0.36 wt.% of 2,6-di-*tert*-butyl-4-methylphenol (BHT) as a marker at a flow rate of 0.7 mL min⁻¹. The system was calibrated using pullulan standards (peak molar masses, $M_p = 504\text{--}739,000\text{ g mol}^{-1}$) from Agilent Technologies. This allowed the determination of the number-average molar mass (M_n), the weight-average molar mass (M_w) and the dispersity

($\bar{D} = M_w/M_n$). All samples were filtered over 0.22 μm PTFE filters prior to injection. Data were collected and processed with OmniSEC 5.12 software. It should be noted that UV SEC chromatograms are used to present the molar mass reduction triggered by the addition of bleach. This is due to the additives contained in the commercial bleach (*i.e.* non-ionic surfactants) causing additional negative peaks in the RI SEC chromatogram (Please see Figure S7). All other chromatograms are presented using the RI SEC chromatograms.

Nuclear Magnetic Resonance (NMR)

NMR spectroscopy was performed in 5 mm diameter tubes in DMSO- d_6 at 25 °C. ^1H -NMR spectroscopy was performed on a Bruker Avance 300 spectrometer at 300 MHz. The chemical shift scale was calibrated based on the internal solvent signals ($\delta = 2.50$ ppm for DMSO- d_6). Data were processed with MestReNova 11.0.4 software.

Supplementary Figures

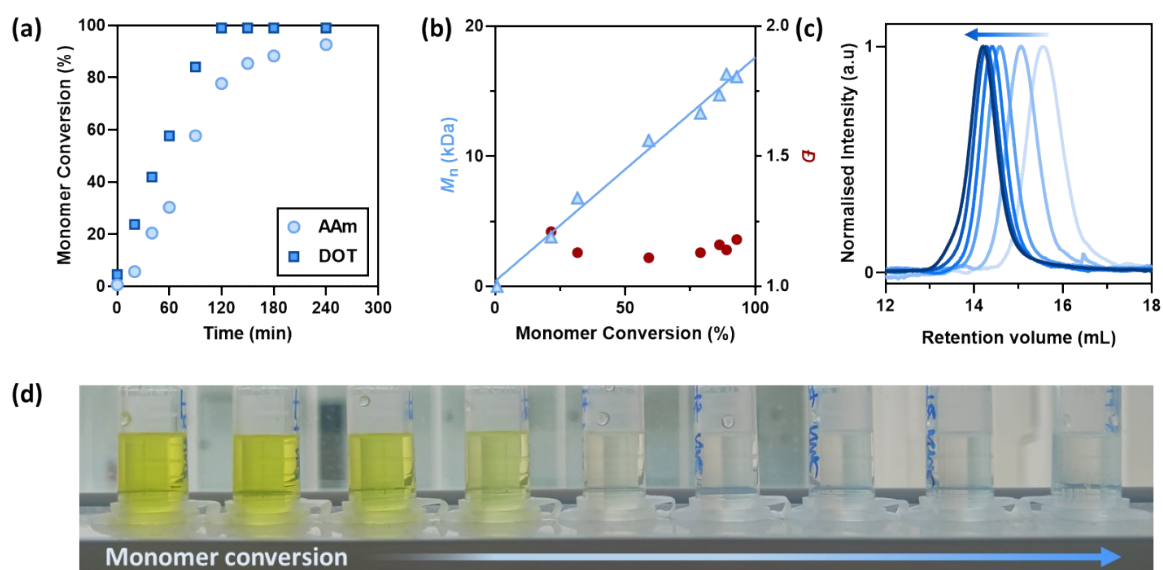


Figure S1. Kinetic data obtained for the RAFT solution copolymerization between DOT and AAm: (a) evolution of the monomer conversion vs. time curves; (b) evolution of the number average molecular weight (M_n) and dispersity with monomer conversion; (c) evolution of SEC chromatograms during the copolymerisation, and (d) photos of the aliquots taken during the copolymerisation (diluted for NMR) showing the loss of colour as the DOT is inserted into the copolymer chain.

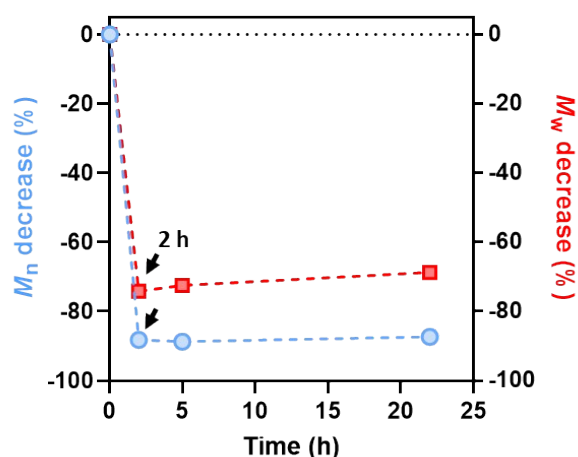


Figure S2. Number- and weight-average molar mass (M_n and M_w , respectively) decrease over time for P(AAm-co-DOT) copolymer **6** during degradation under aminolytic ($i\text{PrNH}_2$) conditions.

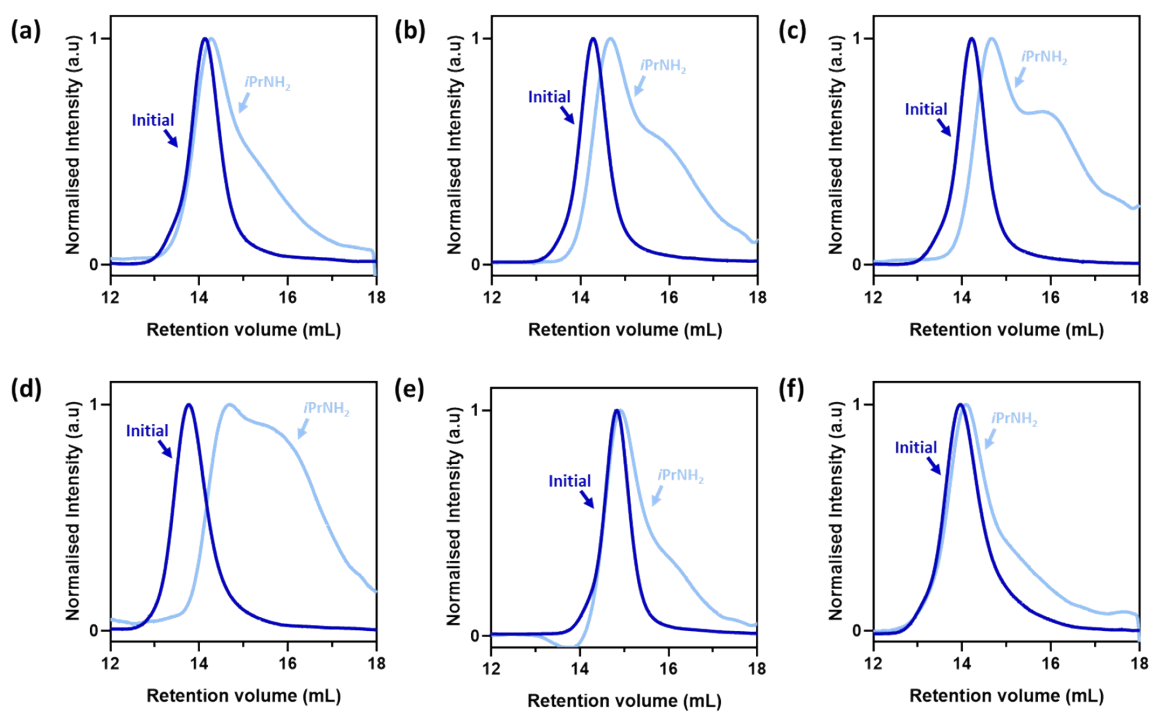


Figure S3. Evolution of the SEC chromatograms of P(AAm-co-DOT) copolymers: (a) **2**, (b) **3**, (c) **4**, (d) **6**, (e) **7**, and (f) **9**, after degradation under aminolytic (*i*PrNH₂) conditions.

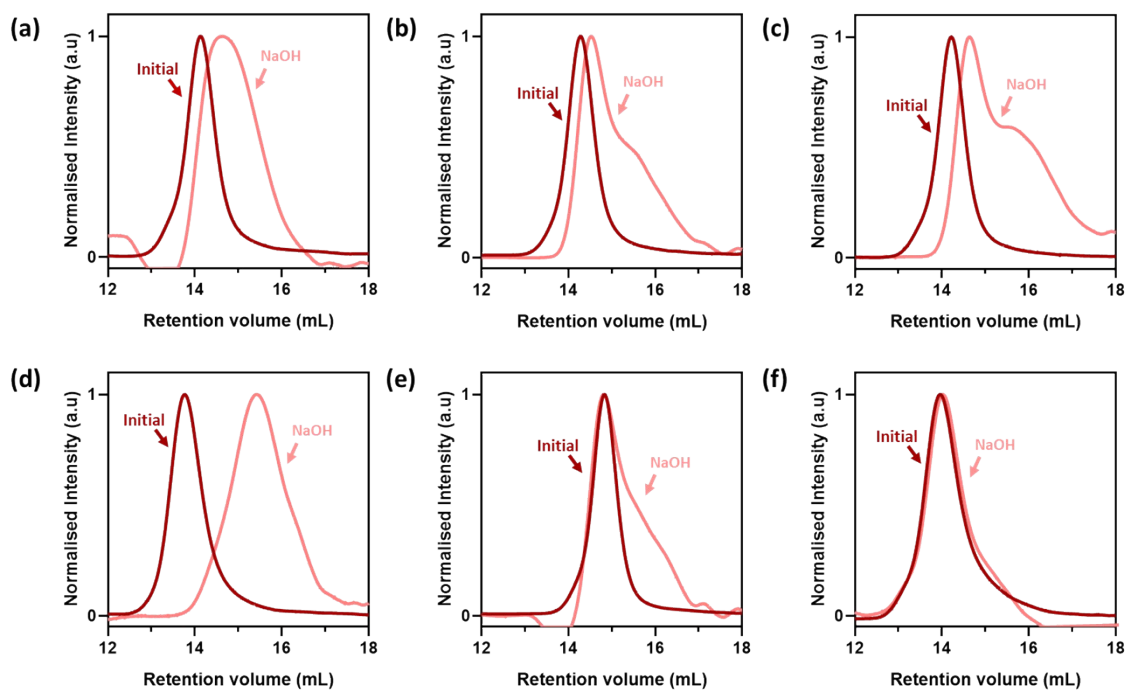
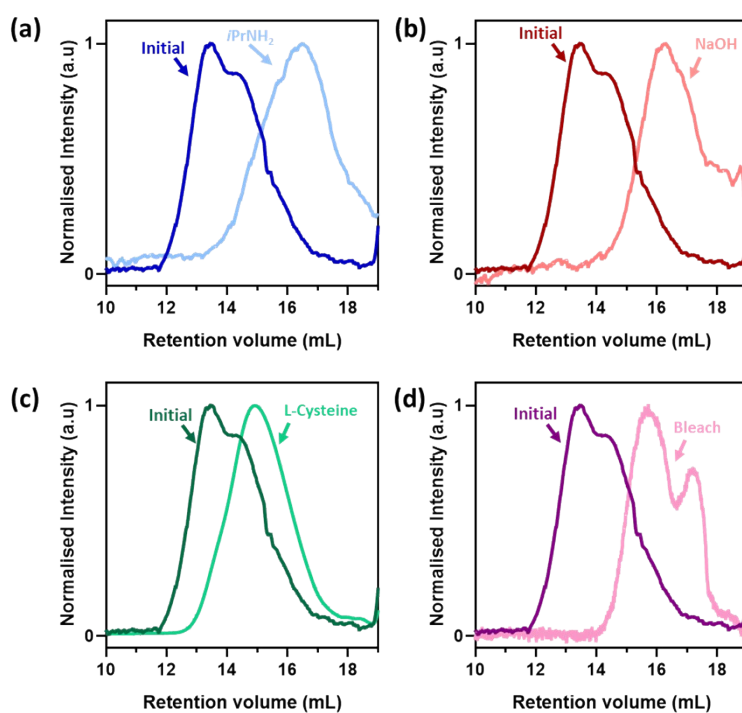


Figure S4. Evolution of the SEC chromatograms of P(AAm-co-DOT) copolymers: (a) **2**, (b) **3**, (c) **4**, (d)



6, (e) **7**, and (f) **9**, after degradation under basic (NaOH) conditions.

Figure S5. Evolution of the SEC chromatograms of P(AAm-co-DOT) copolymer **10** synthesised via FRP after degradation in the presence of (a) $i\text{PrNH}_2$, (b) NaOH, (c) L-cysteine and (d) household bleach

(using the UV chromatogram for the bleach degradation product to avoid additional negative peaks, see figure S7).

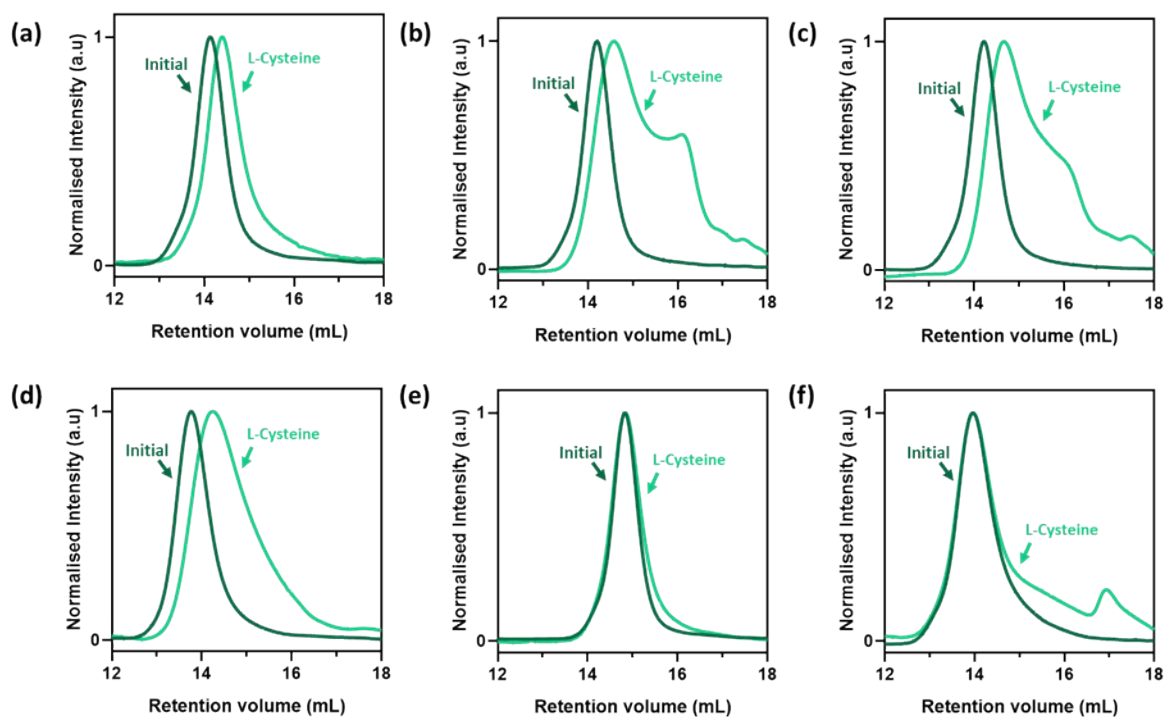


Figure S6. Evolution of the SEC chromatograms of P(AAm-co-DOT) copolymers: (a) **2**, (b) **3**, (c) **4**, (d) **6**, (e) **7**, and (f) **9**, after degradation in the presence of 10 mM cysteine.

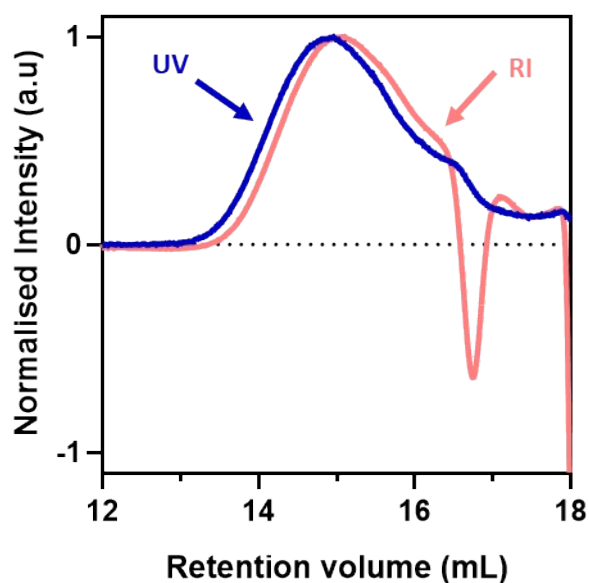


Figure S7. Comparison between the RI and UV SEC chromatogram for P(AAm-co-DOT) copolymer **6**.

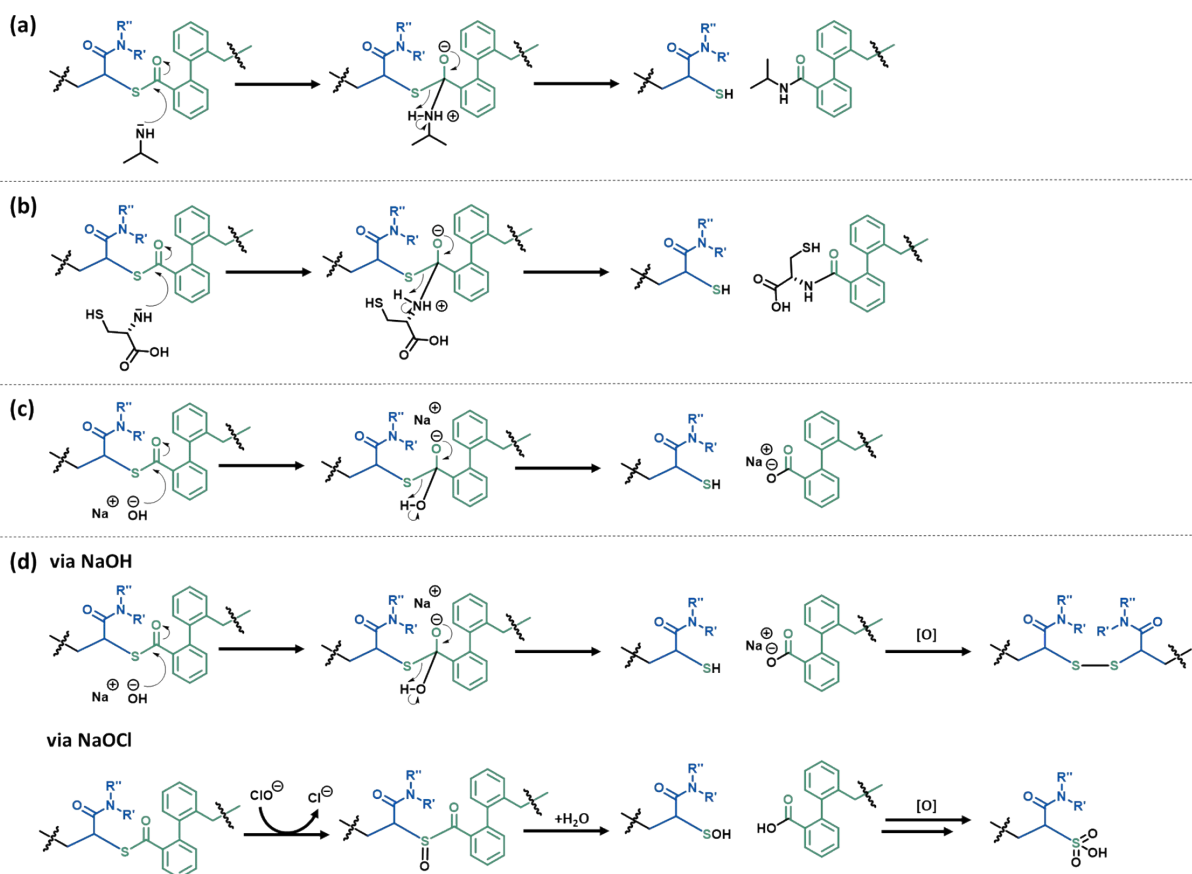


Figure S8. Proposed mechanisms for the degradation of the PDOT moieties using (a) $i\text{PrNH}_2$ ^{2,3} (b) L-cysteine^{2,3} (c) NaOH³ and (d) bleach via both NaOH and NaOCl.²

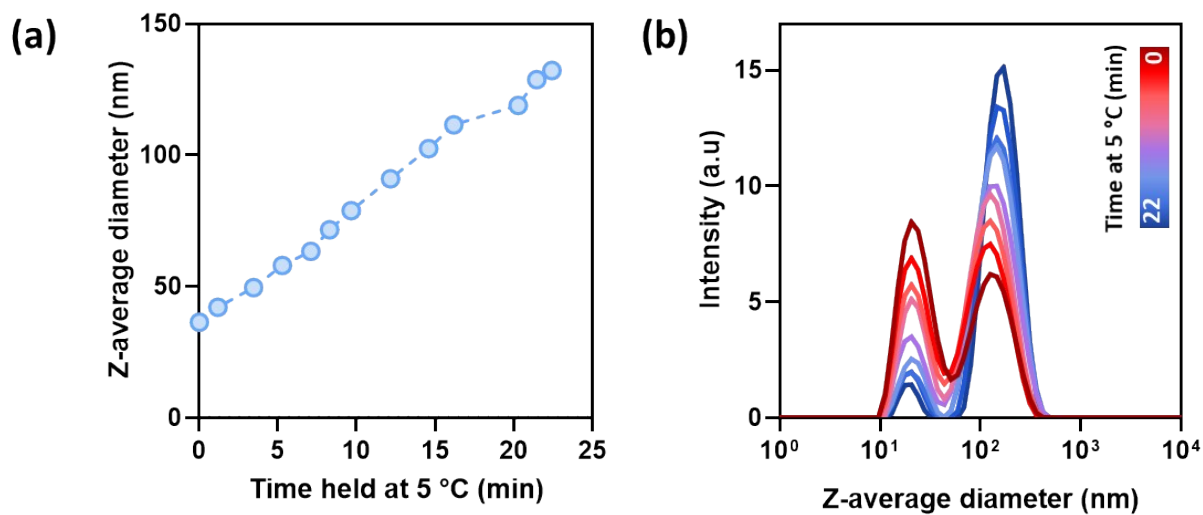


Figure S9. (a) Plot showing the increase in Z-average diameter with time at 5 °C, and (b) the equivalent change in size distribution for 0.1 % w/w 10 mM NaCl aqueous dispersions of P(AAm-co-DOT) copolymer **4**.

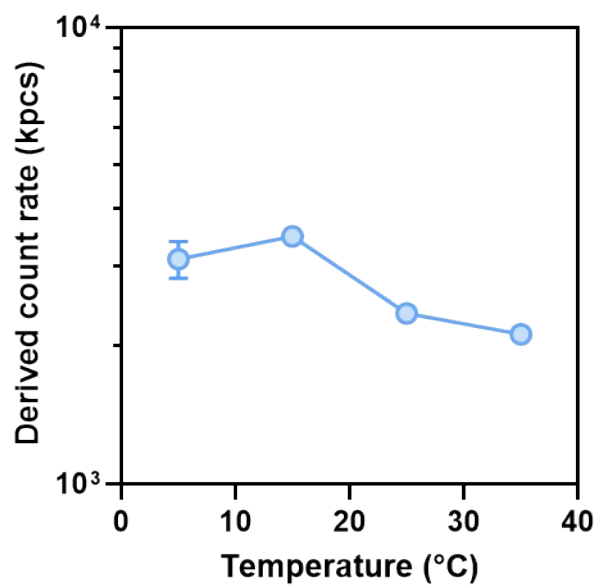


Figure S10. Plot of the derived count rate against temperature for 0.1 % w/w aqueous solution of copolymer **4** (no salt).

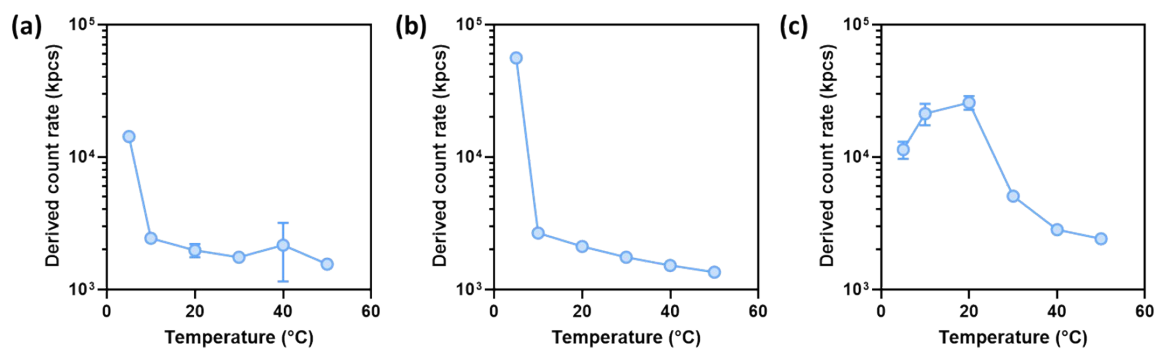


Figure S11. Plot of the derived count rate against temperature for 0.1 % w/w 10 mM NaCl aqueous dispersions of P(AAm-co-DOT) (a) copolymer **3**, (b) copolymer **4** and (c) copolymer **6**.

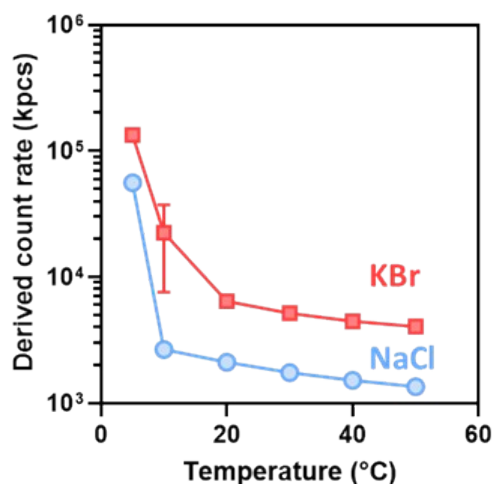


Figure S12. Plot of the derived count rate against temperature for 0.1 % w/w dispersions of P(AAm-co-DOT) copolymer 4 in 10 mM NaCl (blue circles) and 10mM KBr (red squares).

Table S1. Degradation efficiency (described by equations S1 – 4) of P(AAm/NIPAAm-co-DOT) copolymers obtained by RAFT-mediated rROP

Sample	F_{DOT}	Initial M_w (kDa)	$iPrNH_2$ (aq) M_w [%deg. efficiency] (kDa)	$NaOH$ (aq) M_w [%deg. efficiency] (kDa)	L -Cysteine (aq) M_w [%deg. efficiency] (kDa)	Bleach M_w [%deg. efficiency] (kDa)
1	0.00	21.4	n/a	n/a	n/a	n/a
2	0.01	20.4	13.3[54%]	10.9[72%]	14.1[48%]	14.5[45%]
3	0.10	17.0	7.8[57%]	9.2[48%]	8.8[51%]	10.9[38%]
4	0.11	18.7	7.5[63%]	7.7[61%]	8.1[59%]	6.2[70%]
5	n/a	6.5	n/a	n/a	n/a	n/a
6	0.08	26.9	6.9[77%]	6.0[81%]	14.9[46%]	6.5[79%]
7	0.07	10.3	6.9[37%]	7.3[33%]	9.3[11%]	6.2[45%]
8	0.00	20.1	n/a	n/a	n/a	n/a
9	0.06	22.0	17.8[20%]	20.1[9%]	19.6[12%]	13.3[42%]
10	0.17	31.2	4.2[52%]	3.0[56%]	11.4[28%]	3.5[54%]

Degradation efficiency calculation⁴

$$n = \frac{1 - F_{DOT}}{F_{DOT}} \quad (S1)$$

$$\Delta M_{MAX} = M_I - (n \times MW_M + MW_{DOT}) \quad (S2)$$

$$\Delta M_{Deg} = M_I - M_{Deg} \quad (S3)$$

$$\% \text{ deg. efficiency} = \frac{\Delta M_{Deg}}{\Delta M_{MAX}} \quad (S4)$$

Where n is the average consecutive non-degradable monomer units (M), ΔM_{MAX} is theoretical maximum change in the molar mass during degradation, ΔM_{Deg} is the observed change in molar mass during degradation, and M_I and M_{Deg} are the molar masses before and after degradation, respectively.

References

- 1 N. M. Bingham and P. J. Roth, *Chem. Commun.*, 2019, **55**, 55–58.
- 2 O. Ivanchenko, S. Mazières, S. Harrisson and M. D. O. Degra-, *Macromolecules*, 2023, **56**, 4163–4171.
- 3 N. M. Bingham, Q. un Nisa, S. H. L. Chua, L. Fontugne, M. P. Spick and P. J. Roth, *ACS Appl. Polym. Mater.*, 2020, **2**, 3440–3449.
- 4 J. Tran, E. Guégain, N. Ibrahim, S. Harrisson and J. Nicolas, *Polym. Chem.*, 2016, **7**, 4427–4435.