Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

In situ monitoring of PISA morphologies

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Materials

The RAFT agent, 4-((((1-ethoxy-1-oxopropan-2-yl)thio)carbonothioyl)thio)butanoic acid (CTA), was synthesized according to a literature procedure.[1] The initiator Dimethyl-2,2'-azobisisobutyrate (V-601) was purchased from Fujifilm Wako Pure Chemical Corporation. *N,N*-Dimethylacrylamide (DMA, Sigma-Aldrich, 99%) was filtered through a basic aluminium oxide (Fisher Scientific) column to remove the radical inhibitor. Diacetone acrylamide (DAAm, 99%) was purchased from Alfa Aesar.

Solvents were purchased from several suppliers—Honeywell, Fisher and Sigma-Aldrich. All other chemicals were purchased from Sigma-Aldrich now Merck (Gillingham, UK) and used as received, unless otherwise stated.

Methods

Nuclear Magnetic Resonance (NMR) spectroscopy

NMR spectra were measured using the Bruker DPX-300, Bruker DPX-400, and Bruker Avance 111 HD NMR spectrometers, which operated at 300, 400, and 500 MHz, respectively. The residual solvent peaks were used as internal references.

Size-Exclusion Chromatography (SEC)

Molar mass distributions were obtained using size-exclusion chromatography (SEC). The polymers were measured using the Agilent 390-LC MDS instrument which measured differential refractive index (DRI), viscosity, light scattering, UV absorption, and fluorescence emission. Samples were prepared with a concentration of 1-3 mg mL^{-1} and filtered using 0.2 μm PTFE filters before auto-sampler injections.

SEC	**Agilent 1260 Infinity II**	**Agilent 1260 Infinity II**
Eluent	DMAc with 50 nM LiCl at 50 °C	DMF with 5 mM NH_4BF_4 at 50 °C
Detectors	RI, Viscometer, LS	RI, Viscometer, VWD, LS, UV, Fluorescence
Columns	Viscogel I-series 5 μm guard + two ViscoGel I-series G3078 mixed bed columns	2 x PLgel Mixed-D

(http://www.chem.agilent.com/Library/brochures/5990-7994-GPCorganics-Apr11-9lo.pdf)

Deconvolution of SEC chromatograms was carried out with the 'Fit Peak (Pro)' function using the software 'Origin 2019b'. The fitting results can be found with the deconvoluted SEC traces under 'Peak Analysis'.

Fluorescence Emission Spectroscopy

Fluorescence emission spectra were measured using an Agilent Technologies Cary Eclipse Fluorescence spectrometer. The *in situ* fluorescence measurements were measured at 70 °C and stirring was applied throughout the experiment.

Transmission Electron Microscopy (TEM)

Carbon coated grids, carbon film on copper 300 mesh, were purchased from EM Resolutions.

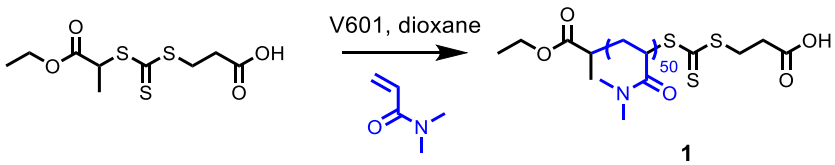
The PISA solutions were diluted by a factor of 100 or 300. In a typical procedure, polymer solution (5 µL) was transferred to a new Eppendorf tube (2 mL) to which a 1:1 vol ratio of methanol to water (495 µL) was added. This solution (10 µL) was drop-casted on freshly glow-discharged carbon-coated grids placed on filter paper. Bright-field TEM micrographs were obtained with a Jeol 2100Plus operating at 200 kV, equipped with a Gatan OneView IS camera.

Electrospray Ionization Time-of-Flight (ESI-TOF) Mass Spectrometer

Mass spectra were measured using Agilent 6130B single Quad (ESI) mass spectrometer. Samples were prepared at 1 mg/mL in 1:1 vol ratio methanol to water.

Synthesis and Characterization

DMA polymerization



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PDMA (**1**): For the synthesis of the PDMA homopolymer, CTA (50.8 mg, 0.180 mmol), DMA (0.892 g, 9.00 mmol), V-601 azo-initiator (0.830 mg, 3.60 nmol) and 1,4-dioxane (3 mL) were all weighed into a vial with a magnetic stirrer and sealed with a rubber septum. The solution was mixed thoroughly and deoxygenated by bubbling nitrogen

for 10 min. The vial was then placed in an oil bath set at 70 °C for 5 h. Reaction aliquots to determine conversion *via* ^{1}H NMR spectroscopy were taken using a degassed syringe. After the polymerization, the mixture was cooled and opened to air. The crude polymer was precipitated three times in diethyl ether to remove any residue monomer and dried *in vacuo*. The product was a yellow solid powder. Yield: 0.88 g, 93%

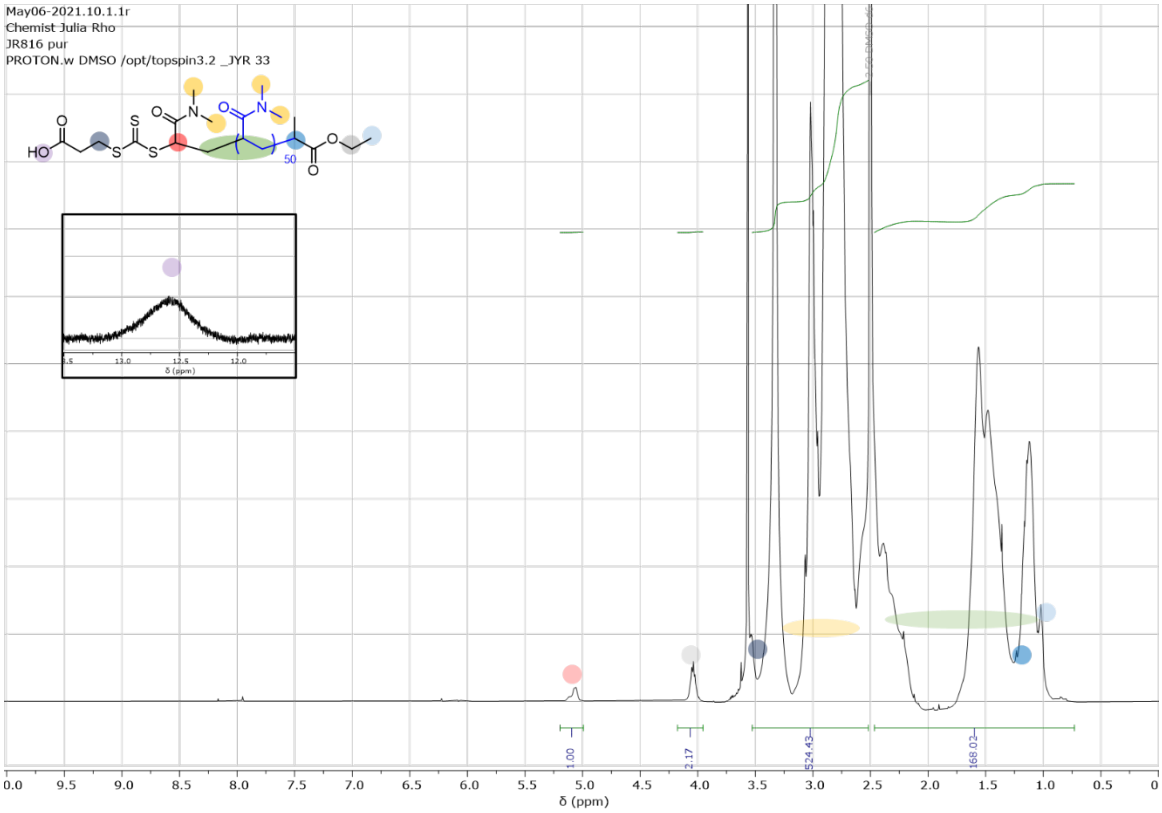


Figure S1. ^{1}H NMR spectrum of PDMA (**1**) in deuterated DMSO (500 MHz).

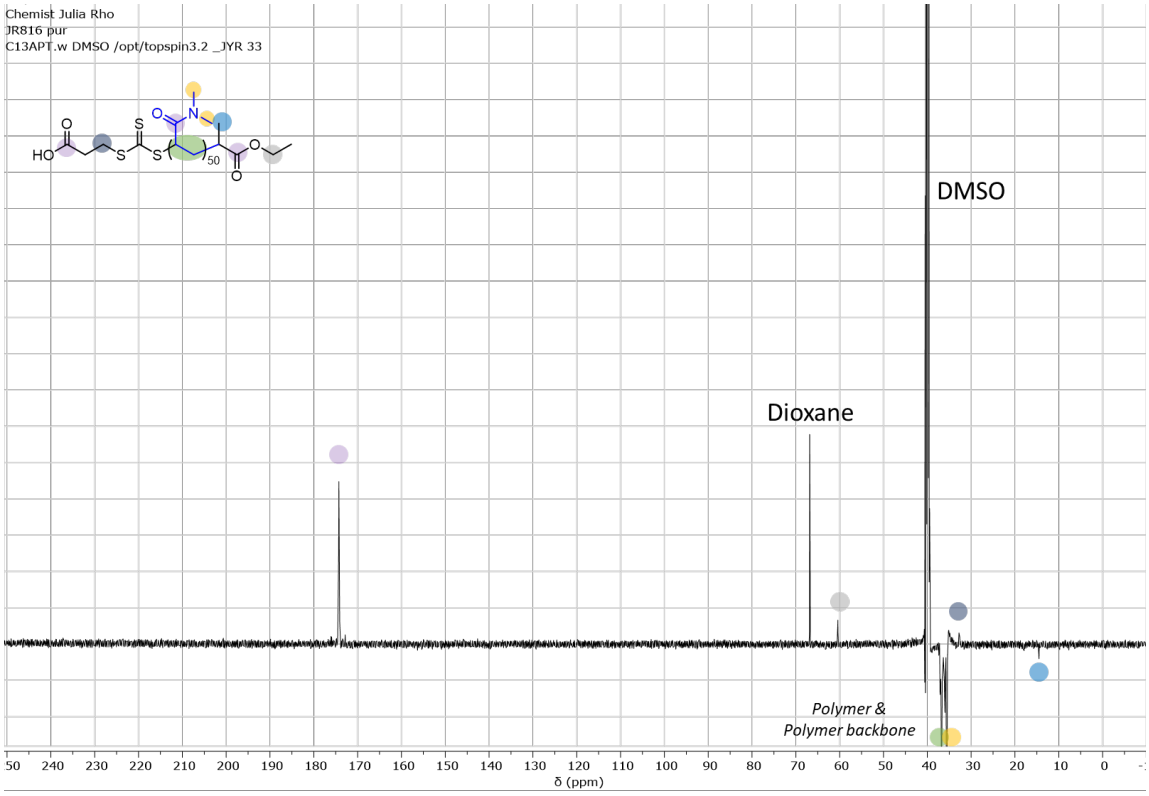


Figure S2. ^{13}C NMR spectrum of PDMA (**1**) in deuterated DMSO (500 MHz).

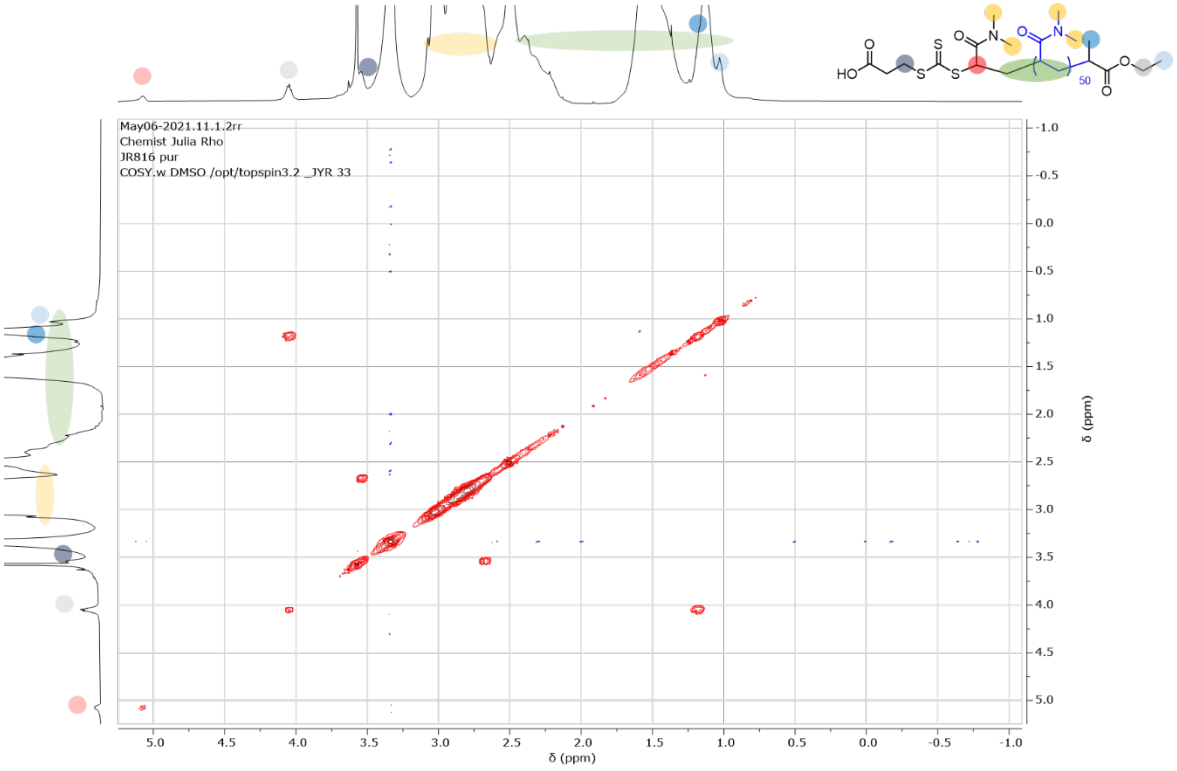


Figure S3. COSY NMR spectrum of PDMA (**1**) in deuterated DMSO (500 MHz).

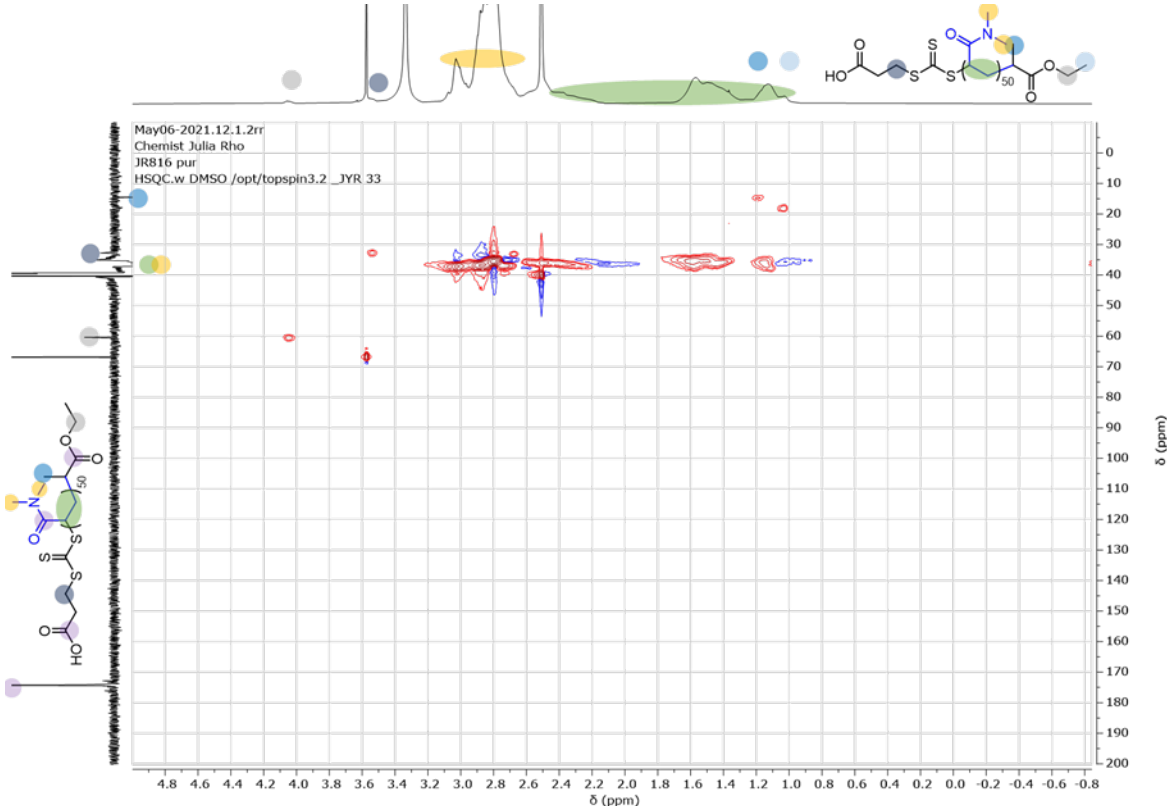


Figure S4. HSQC NMR spectrum of PDMA (**1**) in deuterated DMSO (500 MHz).

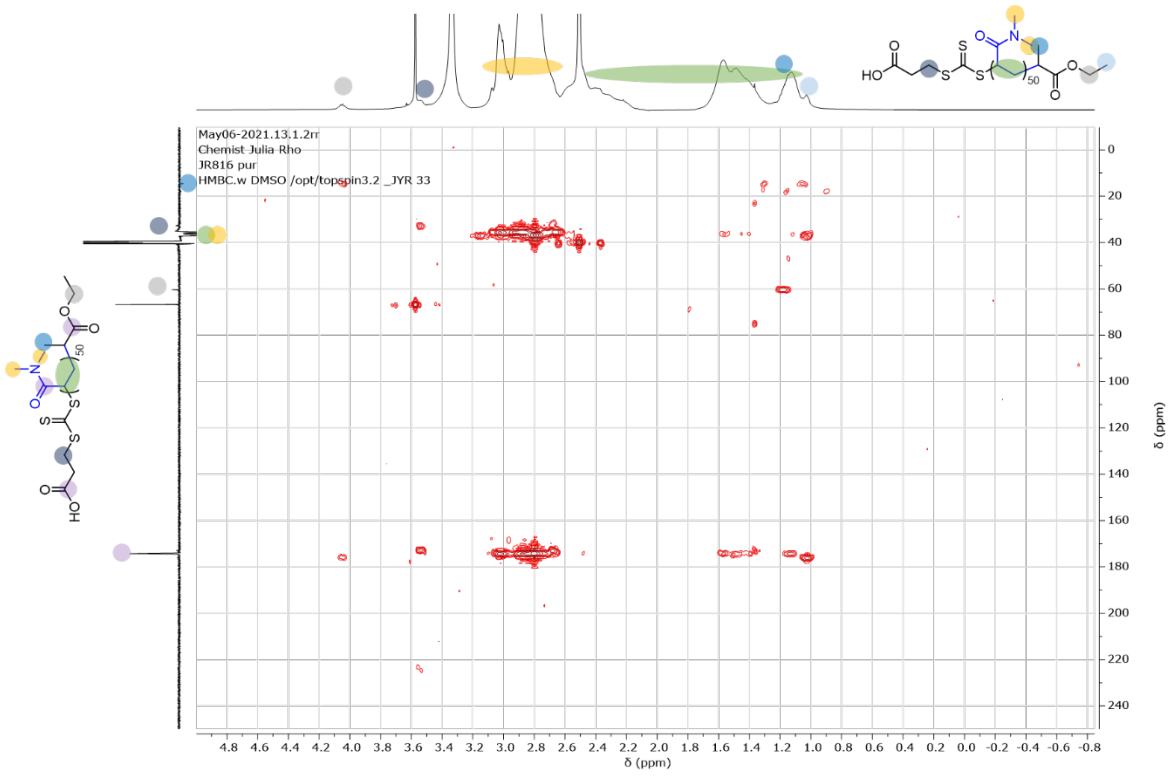


Figure S5. HMBC NMR spectrum of PDMA (**1**) in deuterated DMSO (500 MHz).

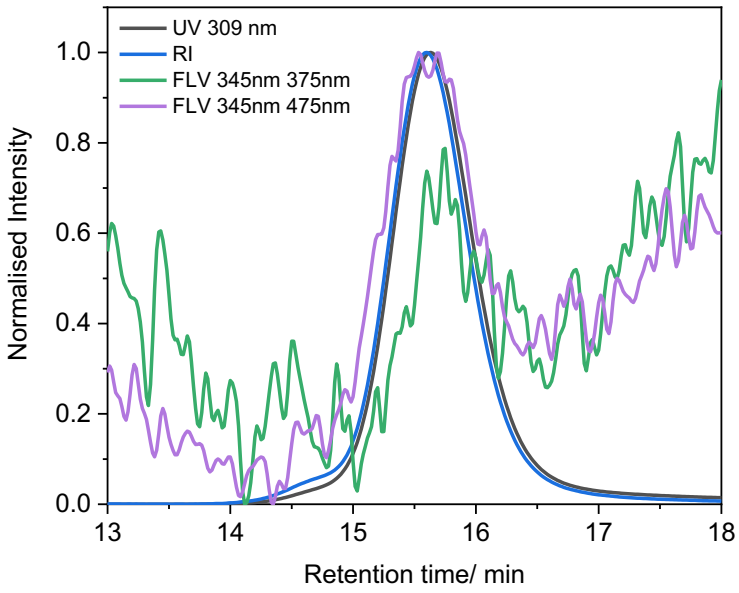
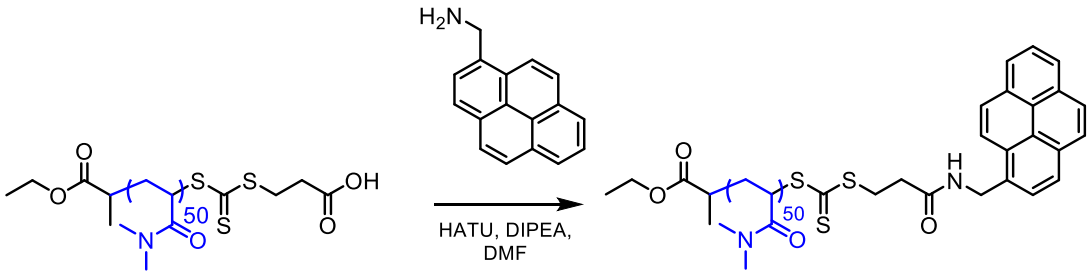


Figure S6. SEC chromatogram of PDMA (**1**) in DMF with 5 mM NH_4BF_4. Detectors set to read UV absorption at 309 nm to detect the presence of the RAFT agent, refractive index (RI), fluorescence emission associated with the homo pyrene (excitation at 345 nm and emission at 375 nm) and pyrene excimer (excitation at 345 nm and emission at 475 nm).

Conjugation of pyrene to PDMA



PDMA-Pyrene (**2**): PDMA (**1**) (0.843 g, 0.160 mmol, 1.00 eq.), (1- [Bis(dimethylamino) methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) (73.2 mg, 0.193 mmol, 1.20 equiv.) and *N,N*-Diisopropylethylamine (DIPEA) (41.4 mg, 0.320 mmol, 2.00 equiv.) were dissolved in DMF (0.5 mL). The solution was agitated on a roller for 30 min. 1-Pyrenemethylamine hydrochloride (51.5 mg, 0.193 mmol, 1.20 equiv.) was then added, and the solution was stirred for 12 h at room temperature. The excess dye was removed by precipitation in diethyl ether (3 times) followed by

dialysis using a 3.5 – 5 kDa cut off Float-A-Lyzer against a 2:1 (v/v) ratio of 1,4-dioxane: water (twice), then against deionized water (twice). The resulting polymer was dried *in vacuo*. The product was a yellow solid. Yield after precipitation: 380 mg, 73%. Yield after dialysis: 66 mg, 13%.

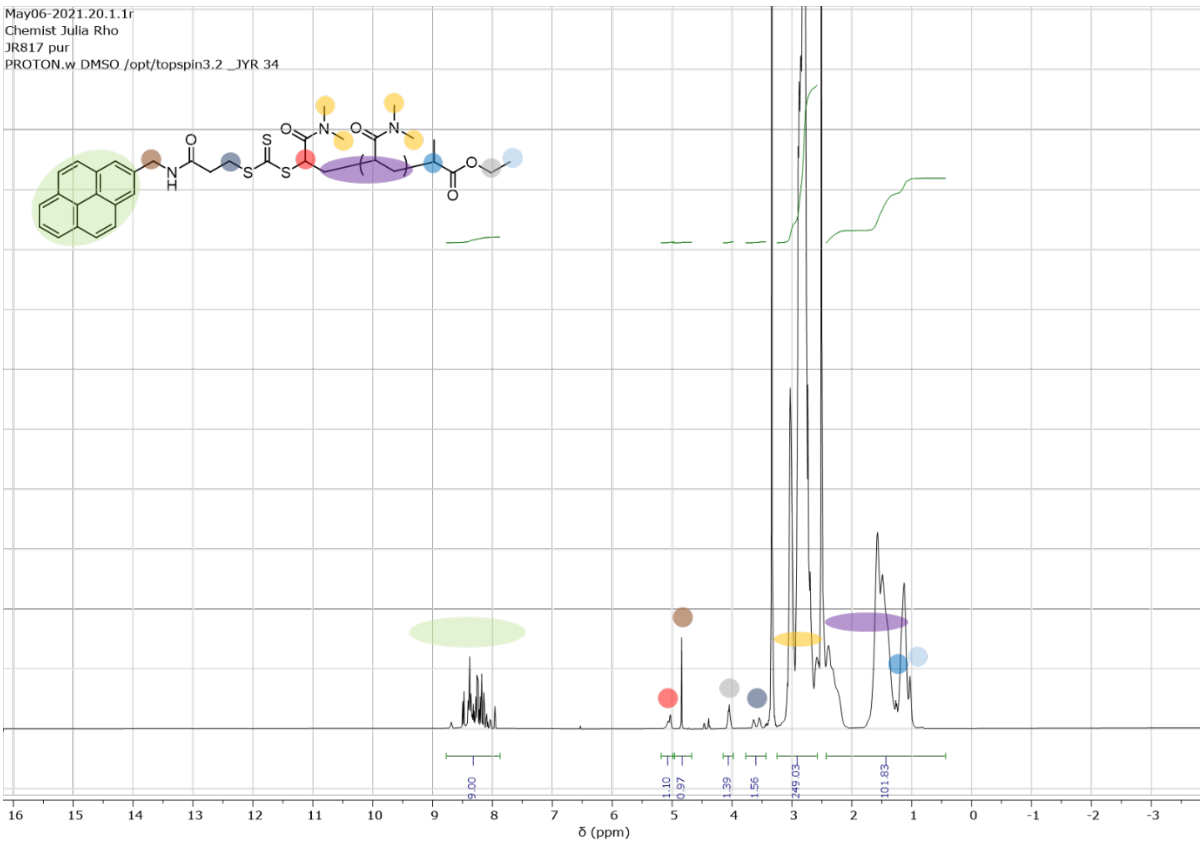


Figure S7. ^{1}H NMR spectrum of PDMA-Pyrene (**2**) in deuterated DMSO (500 MHz).

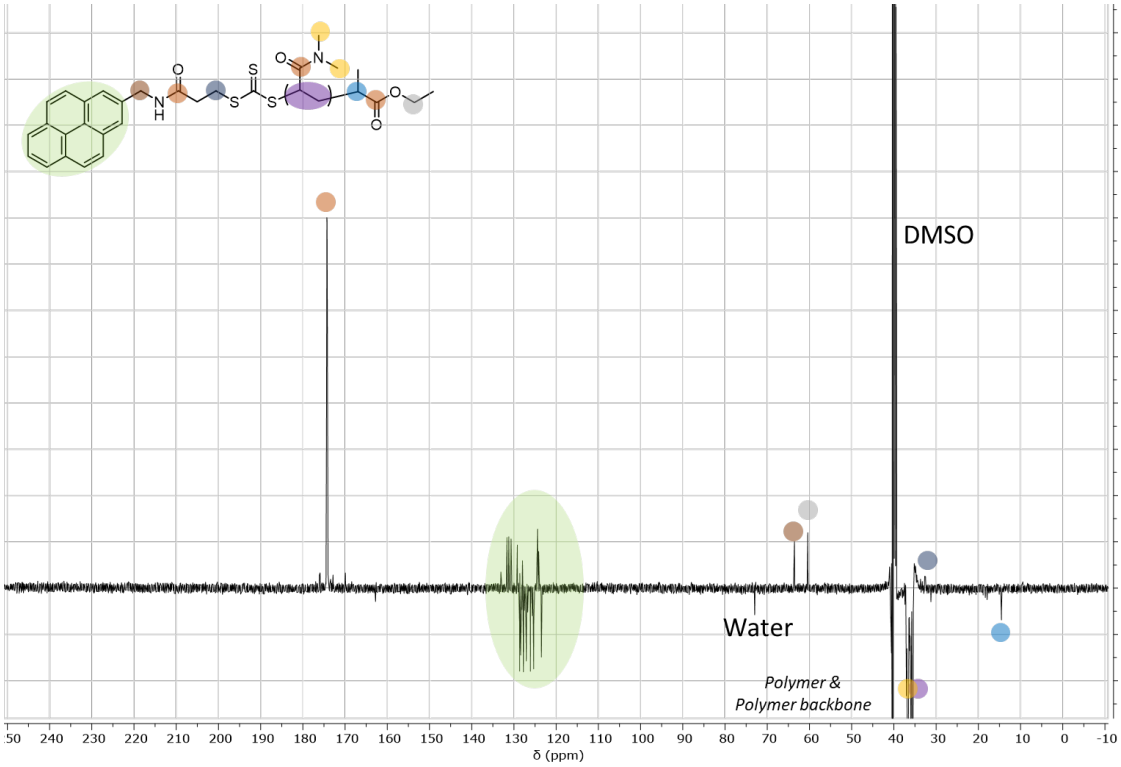


Figure S8. ^{13}C NMR spectrum of PDMA-Pyrene (**2**) in deuterated DMSO (500 MHz).

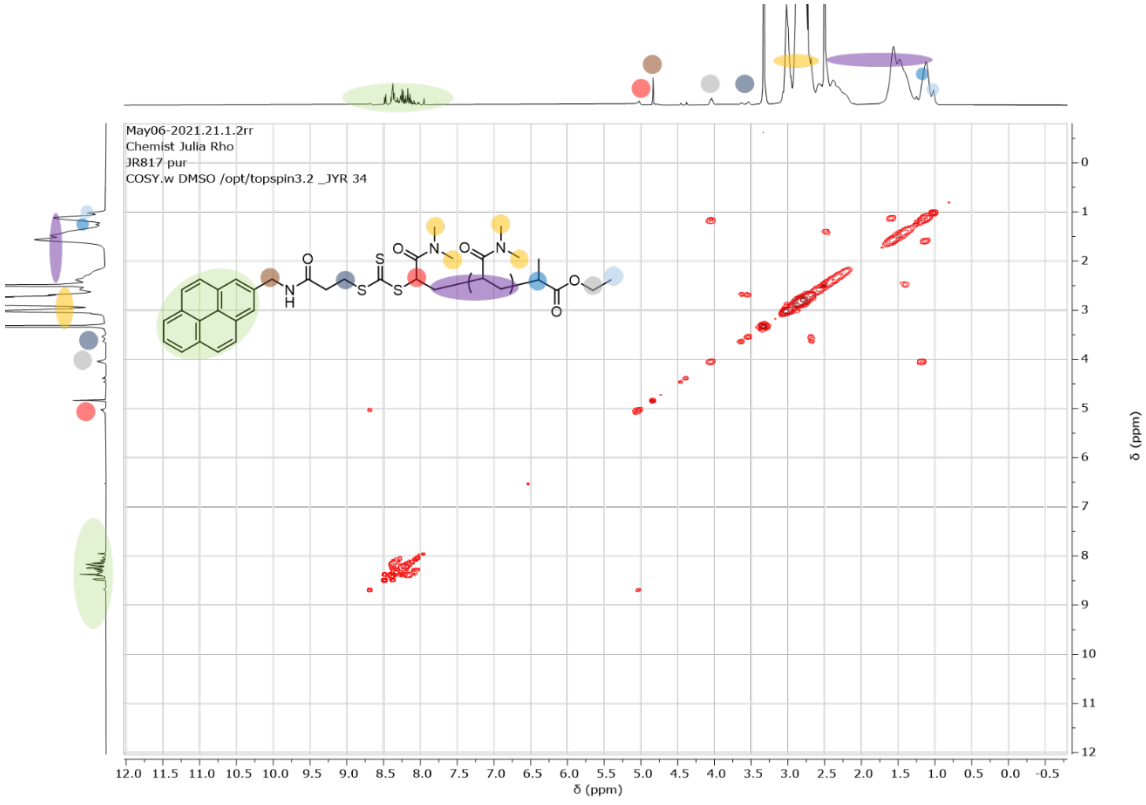


Figure S9. COSY NMR spectrum of PDMA-Pyrene (**2**) in deuterated DMSO (500 MHz).

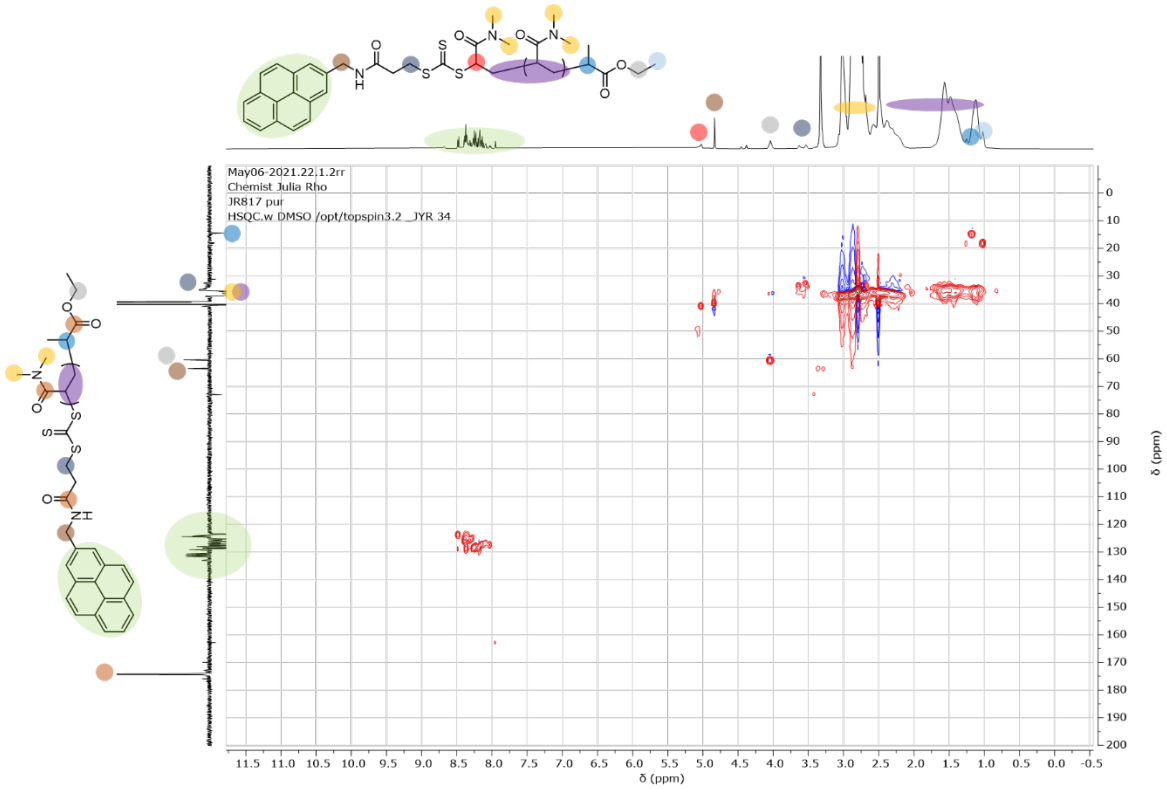


Figure S10. HSQC NMR spectrum of PDMA-Pyrene (**2**) in deuterated DMSO (500 MHz).

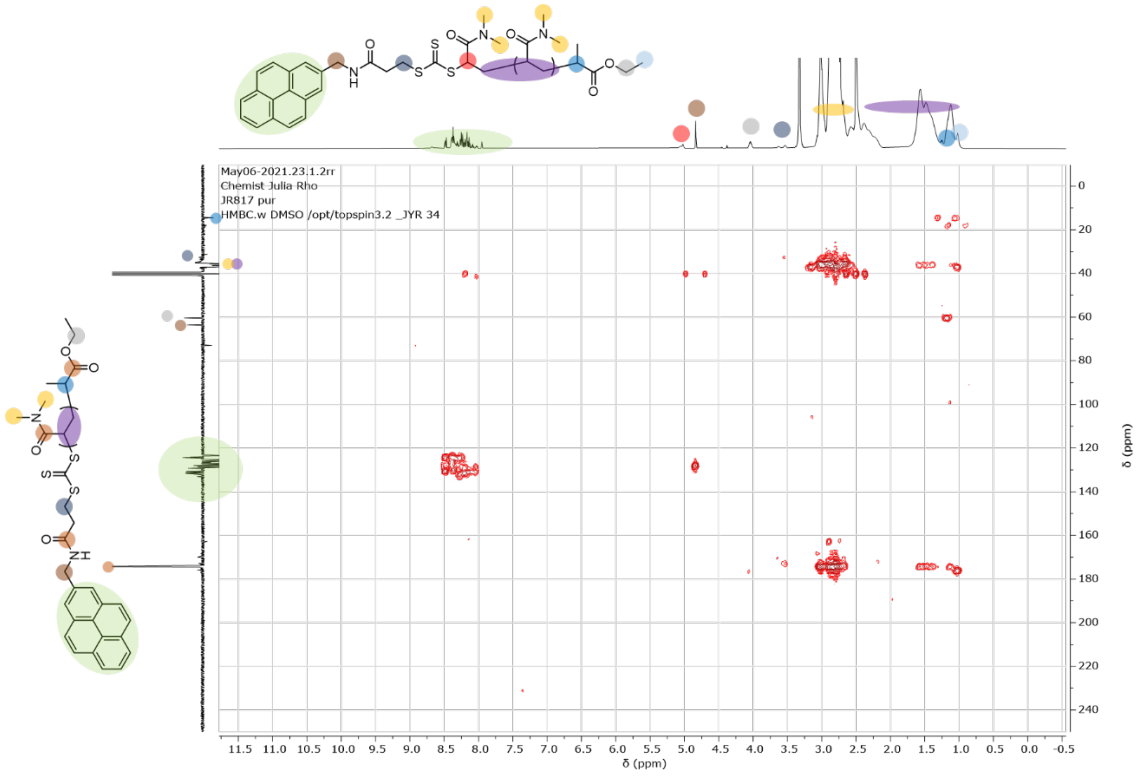


Figure S11. HMBC NMR spectrum of PDMA-Pyrene (**2**) in deuterated DMSO (500 MHz).

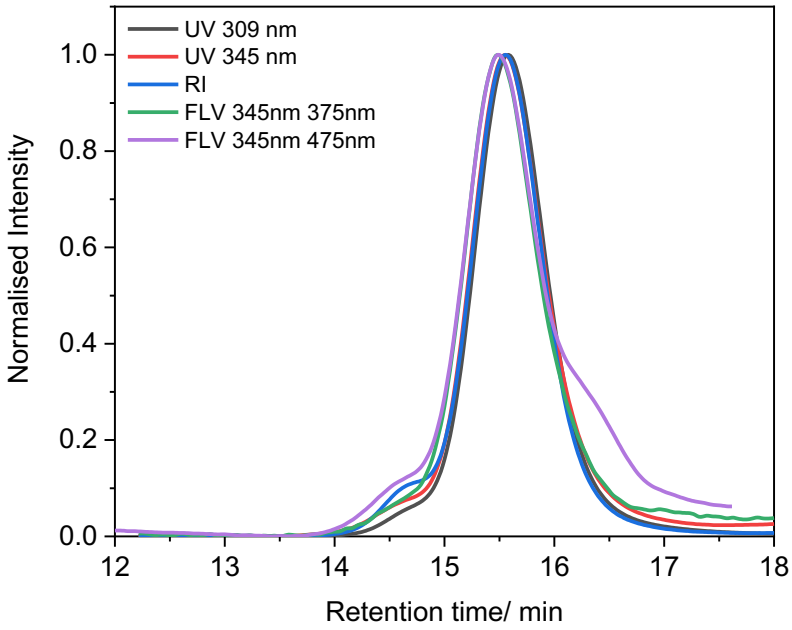


Figure S12. SEC chromatogram of PDMA-Pyrene (**2**) in DMF with 5 mM NH_4BF_4. Detectors set to read UV absorption at 309 nm and 345 nm to detect the presence of the RAFT agent and pyrene dye respectively, refractive index (RI), fluorescence emission associated with the homo pyrene (excitation at 345 nm and emission at 375 nm), and pyrene excimer (excitation at 345 nm and emission at 475 nm).

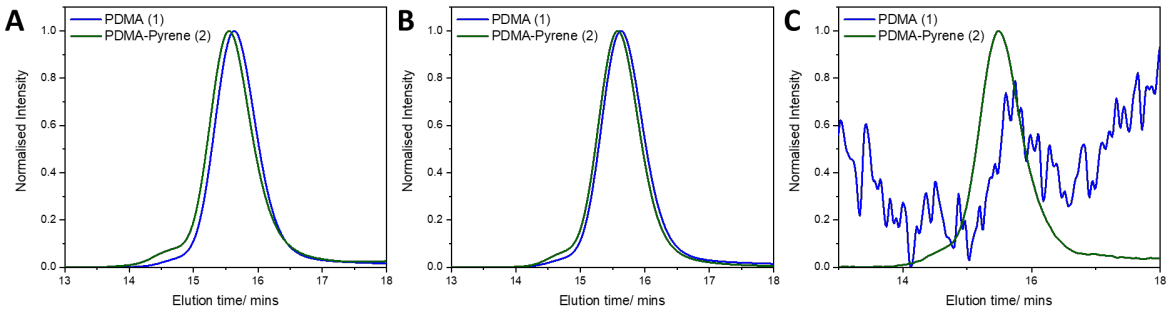


Figure S13. SEC chromatograms of PDMA (**1**) and PDMA-Pyrene (**2**) in DMF with 5 mM NH_4BF_4 overlaid by A) refractive index, B) UV absorption at 309 nm, and C) Fluorescence emission at 375 nm upon excitation at 345 nm.

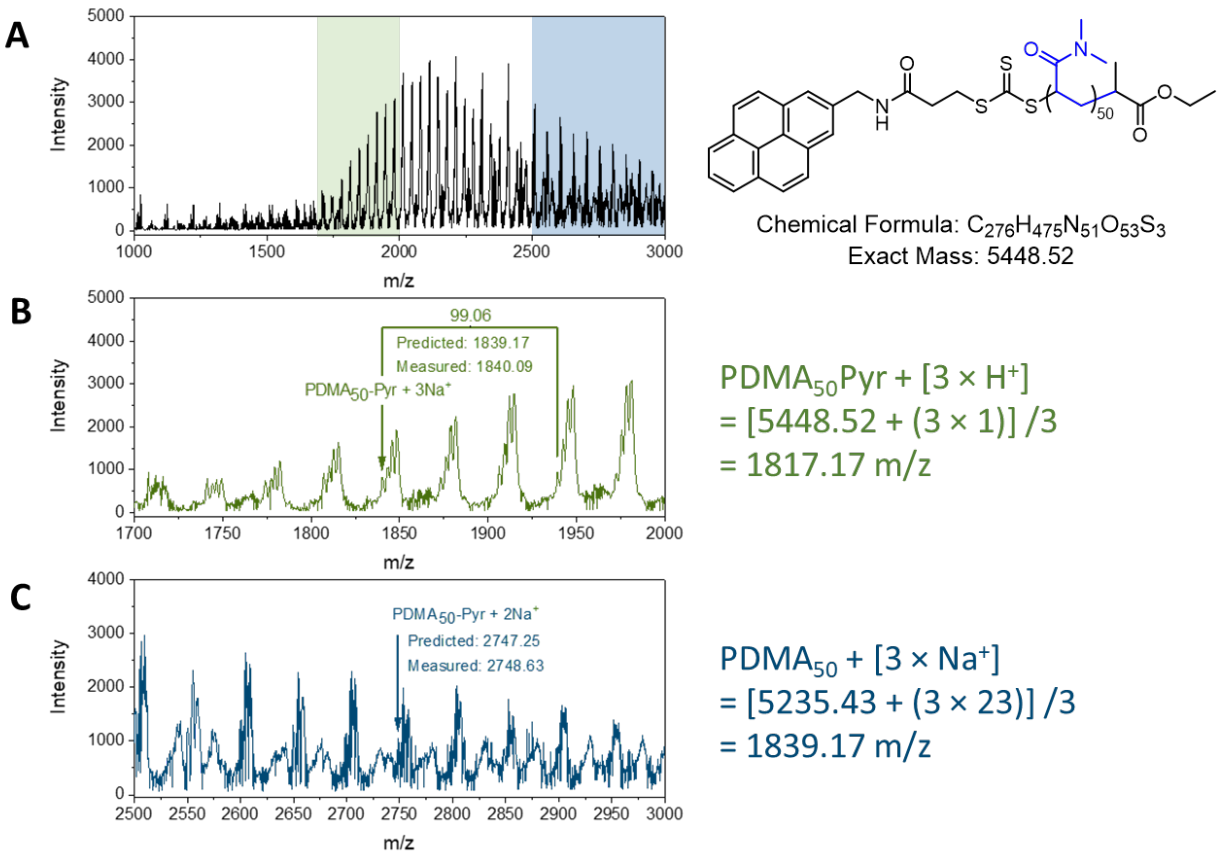


Figure S14. Electrospray Ionisation- Time of Flight (ESI-ToF) of PDMA-Pyrene (**2**).

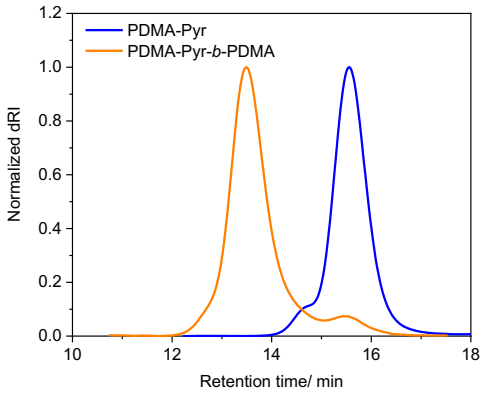


Figure S15. SEC chromatogram in DMF with 5 mM NH_4BF_4 of PDMA-Pyrene (**2**) chain extended with dimethyl acrylamide (DMA).

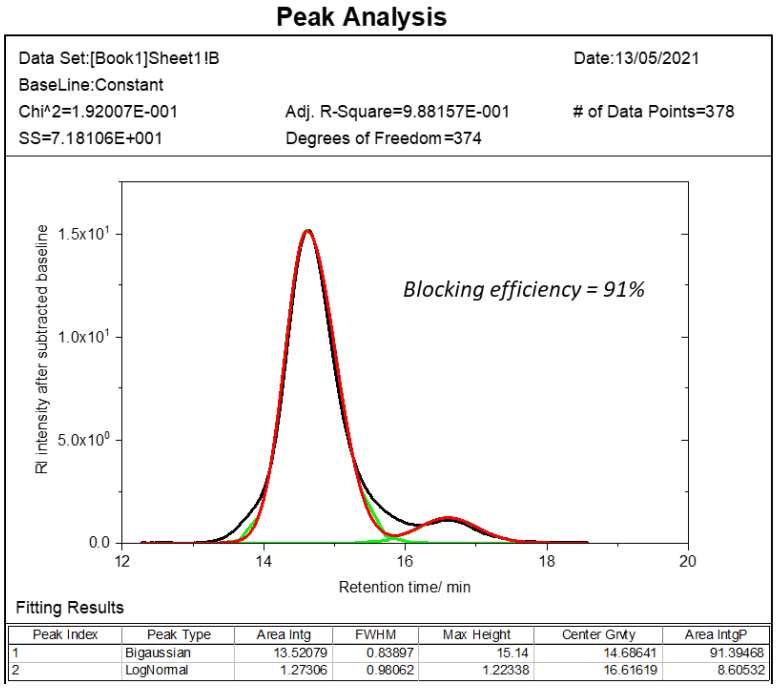
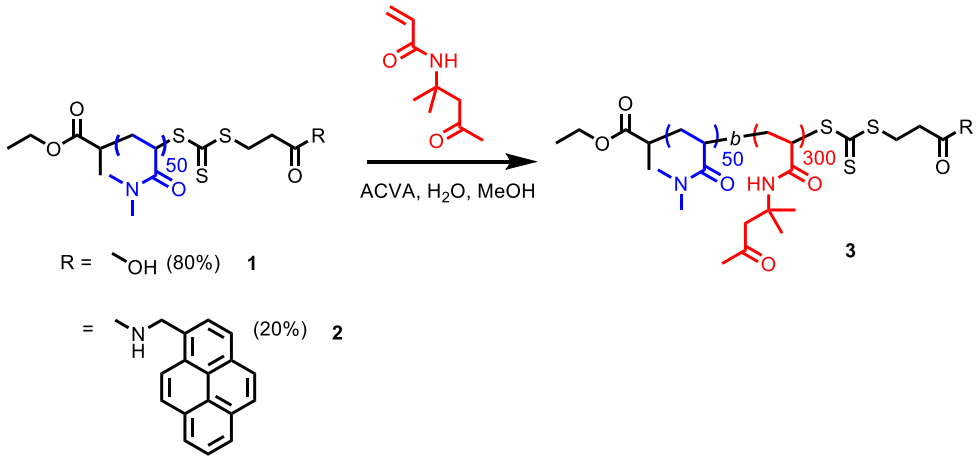


Figure S16. Deconvoluted SEC chromatogram of PDMA-Pyr-*b*-PDMA.

General PISA protocol:



PDMA macroCTA (10.0 mg, 1.91 mmol, 1.00 eq.), diacetone acrylamide (DAAm) (96.9 mg, 0.572 mmol; target DP = 300), ACVA initiator (0.267 mg, 0.954 µmol, 0.500 eq.) were dissolved in a vial with a 1:1 (v/v) ratio of methanol and water (1 g, corresponding to 10% w/v total solid content at full conversion). The solution was mixed thoroughly and deoxygenated by bubbling nitrogen for 10 min. The vial was then placed in an oil bath set at 70 °C for 5 h with stirring. The polymerizations were quenched by removing the vial from the oil bath and opening to air. To minimize any change in morphology before TEM analysis, small aliquots were quickly taken for NMR spectroscopy and

SEC followed by the addition of *O,O´*-1,3-propanediylbishydroxylamine dihydrochloride (O-alkyl hydroxylamine crosslinker). A respective volume of the stock solution of the crosslinker (20 mg/mL) made with water was added to the solution using a micropipette and left to stir for 1 min at the elevated temperatures matching that of the polymerization (i.e., 70 °C). The crosslinker content was adjusted to 10 wt% with respect to DAAm monomer.

Different degrees of polymerization (50, 100, 200, and 300) were targeted to observe a range of morphologies (sphere, worms, and vesicles). See table S1 for details.

Table S1. Summary of target DPs, monomer conversions, molar mass data, and morphologies observed *via* TEM after crosslinking. All chain extension polymerizations were done at 10% w/w solids content.

Polymer	**DP**	**Conversion[a] (%)**	**$M_{n,\,NMR}$[a] (g mol^{-1})**	**$M_{n,\,SEC}$[b] (g mol^{-1})**	**$Đ_{SEC}$[b]**	**Morphology[c]**
$PDMA_{50}$-$PDAAm_{50}$	50	>99	13,700	15,600	1.34	n.a.
$PDMA_{50}$-$PDAAm_{100}$	100	>99	22,200	22,700	1.49	S
$PDMA_{50}$-$PDAAm_{200}$	200	>99	39,100	33,700	1.77	W
$PDMA_{50}$-$PDAAm_{300}$	300	>99	56,000	55,300	1.63	V
$PDMA_{50}$*-$PDAAm_{50}$	50	>99	13,800	16,000	1.35	n.a.
$PDMA_{50}$*-$PDAAm_{100}$	100	>99	22,200	23,200	1.53	S
$PDMA_{50}$*-$PDAAm_{200}$	200	>99	37,100	34,000	1.83	W
$PDMA_{50}$*-$PDAAm_{300}$	300	>99	56,000	44,600	2.00	V

[a]Determined by ^{1}H NMR spectroscopy in DMSO-d_6. [b]Determined by size-exclusion chromatography (SEC) in DMF with 5 mM NH_4BF_4 as eluent and poly(methyl methacrylate) standards. [c]Determined by transmission electron microscopy (TEM). All PISA reactions for TEM were taken to full conversion and crosslinked at 70 ℃ using 10 wt% O-alkyl bishydroxylamine crosslinker relative to DAAm monomer to preserve the morphology for TEM analysis. *Polymerization were conducted using 20% pyrene-functionalized macroCTA and 80% non-pyrene-functionalised macroCTA.

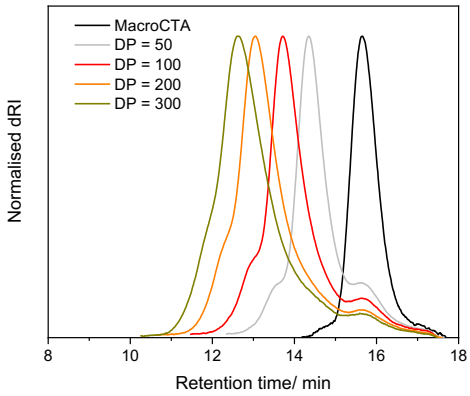


Figure S17. SEC chromatogram of PISA control experiments using 100% non-pyrene-functionalized macroCTA (**1**).

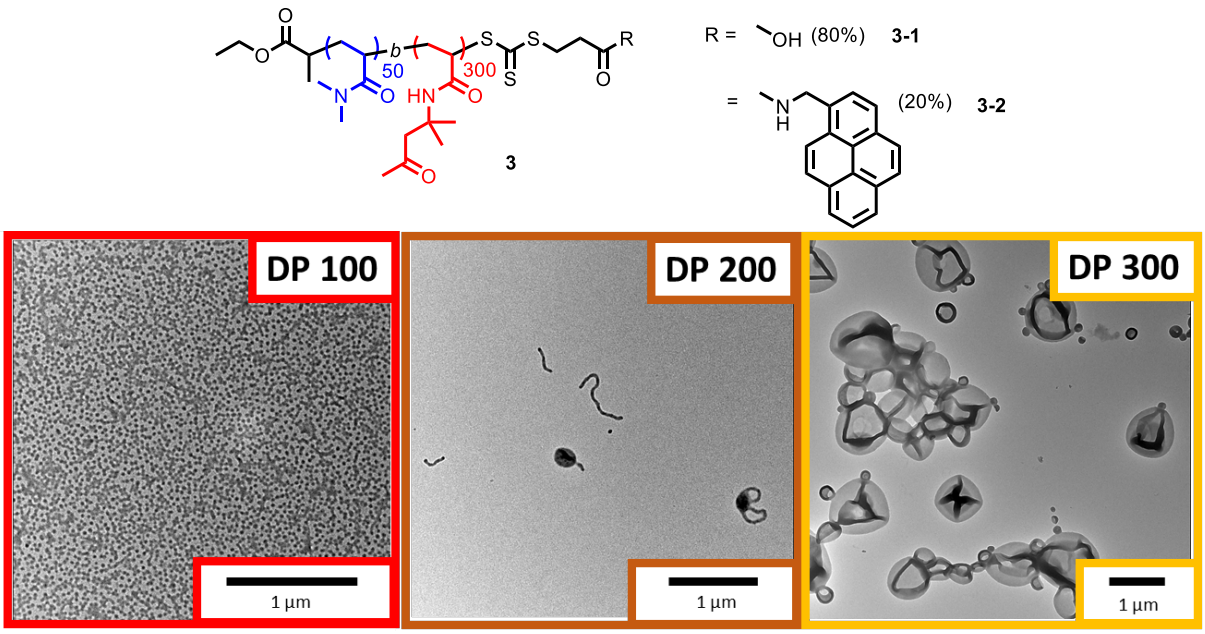


Figure S18. TEM images of PISA control experiments using 100% non-pyrene-functionalized macroCTA (**1**).

In situ polymerization:

To measure the kinetics of the polymerization, the reaction detailed in the 'General PISA protocol', *vide supra,* was scaled by a factor of 10 to yield a total stock solution with a mass of 10 g. An aliquot of each solution (1 mL) was transferred to separate vials. All the vials were sealed, degassed, and placed in a 70 °C oil bath with constant stirring. At each time point a vial was removed, opened to air, and two small aliquots

were taken to measure monomer conversion and molar mass, after which *O*-alkyl bishydroxylamine crosslinker (10 wt% relative to DAAm monomer) was added. An aliquot of the same stock solution (1 mL) was taken to perform the polymerization inside a 4-window 3500 µL quartz cuvette with an airtight stopper which is compatible with the fluorometer. Using an appropriate rubber septum, this solution was also degassed before starting the polymerization.

Table S2. Kinetics monitored by ^{1}H NMR spectroscopy and size-exclusion chromatography. Polymerization were conducted using 20% pyrene-functionalized macroCTA and 80% non-pyrene-functionalized macroCTA.

Time (min)	NMR[a]		SEC[b]	
	Conversion (%)	$M_{n,\,NMR}$ (g mol^{-1})	$M_{n,\,SEC}$ (g mol^{-1})	$Đ_{SEC}$
15	21	11,800	22,400	1.32
30	36	20,200	27,800	1.53
45	51	28,600	22,300	1.59
60	60	33,600	37,900	2.22
120	84	47,100	54,800	1.94
180	93	52,100	40,000[d]	2.74
180[c]	>99	56,100	51,000	2.00

[a]Determined by ^{1}H NMR spectroscopy in DMSO-d_6. [b]Determined by SEC in DMF with 5 mM NH_4BF_4 as eluent and poly(methyl methacrylate) standards. [c]The polymerization was conducted in the fluorometer at 70 °C with stirring. [d]Due to low molecular weight tailing observed in the SEC at high DP, at 180 min the M_n determined is lower than expected; however, from ^{1}H NMR spectroscopy we can confirm the increase in conversion.

Fluorescence spectroscopy:

Before the polymerization, a Varian Cary-block temperature controller was used to set the temperature to 70 °C. This temperature was left to stabilize for 15 min. Upon inserting the cuvette containing the polymerization solution into the fluorometer, the fluorescence spectrum of the reaction was collected at 5 min intervals over a period of 3 h. During the PISA reaction the solution was stirred constantly.

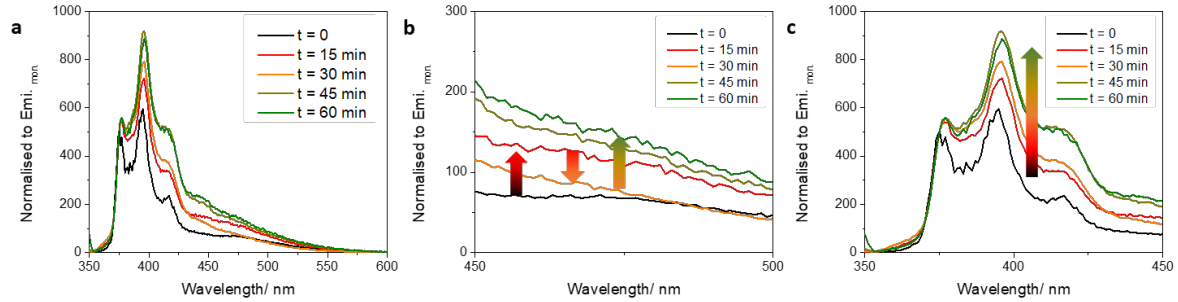


Figure S19. a) Fluorescence emission spectra of the *in situ* PISA reaction over 60 min. The emission spectra were normalized to the first monomeric pyrene emission band (I_1). b) Enlarged emission spectra between 450 and 500 nm to observe the increases and decreases in I_{exi}/I_{mon} ratio. c) Enlarged emission spectra between 350 and 450 nm to observe a decrease in I_1/I_3 ratio.

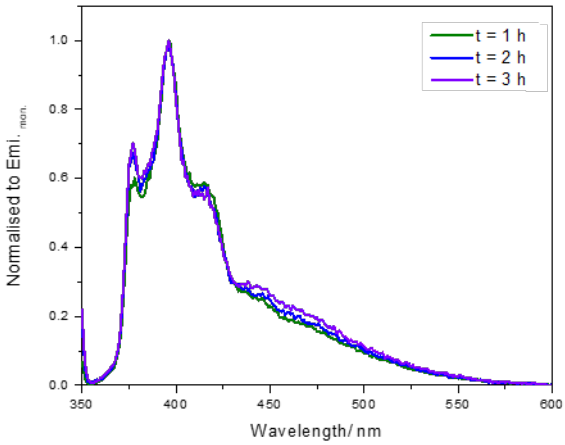


Figure S20. Fluorescence emission spectra of the *in situ* PISA reaction as a function of time. The emission spectra have been normalized to the first monomeric pyrene emission band (I_1). No changes in the I_{exi}/I_{mon} and I_1/I_3 ratio were observed during this period (1-3 h).

Reference

1. M. Gaëlle, B. Patricia, G. Jean-Michel, B. Laurent, R. Jutta and S. François, *Macromol. Rapid Comm.*, 2019, **40**, 1800315.