Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

Ultra-high molecular weight complex coacervates via polymerization-induced electrostatic self-assembly

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Materials

All chemicals were purchased and used as received, unless otherwise stated.

The RAFT agent, 2-(((ethylthio)carbonothioyl)thio)propanoic acid (PAETC), was synthesized according to literature protocol.[1] *N,N'*-Dimethylacrylamide (DMA, Sigma-Aldrich, 99%) was filtered through a basic aluminium oxide (Fisher Scientific) column to remove the radical inhibitor. The following chemicals were obtained from Fisher: dimethyl sulfoxide (DMSO, ACS Grade), potassium bromide (KBr, ACS Grade), toluene, anhydrous, (99.8%, Thermo Scientific™), Iodomethane (99%, stab. with copper, Thermo Scientific™). The following chemicals were obtained from VWR: *N,N*-dimethylformamide (DMF, ACS Grade). The following chemicals were obtained from Sigma-Aldrich: 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS, 99%). The following chemicals were obtained from Cambridge Isotope Laboratories, Inc., deuterated chloroform ($CDCl_3$, 99.8% with 0.05% v/v TMS) and dimethyl sulfoxide (DMSO-d6, 99.9% with 0.05% v/v TMS). Type 1 ultrapure water was obtained using the Millipore Synergy UV-R water purification system.

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. DMF was added as an internal standard. Theoretical molecular weights were calculated using monomer conversion determined via ^1H NMR spectroscopy.

Gel Permeation Chromatography (GPC)

Molar mass distributions were obtained using gel permeation chromatography (GPC). Differential refractive index (DRI) and light scattering of the polymers were measured. Samples were prepared with a concentration of 1-3 mg mL^{-1} and filtered using 0.2 µm PTFE filters before auto-sampler injections. Temperature was set to 50 ºC.

System	Solvent	Flow rate	Columns	Detectors
DMAc	DMAc with 50 mM LiCl	1.0 mL/min	In series, ViscoGel guard column (5 µm), two ViscoGel I-series G3078 mixed bed columns. (Malvern) Range: 0–2.0 × 10^4 Da and 0–1.0 × 10^6 Da)	Wyatt Optilab T-rEX refractive index detector at 658 nm, and a Wyatt miniDAWN TREOS light scattering detector at 656 nm.
Aqueous, UHMW Neutral/ Anionic	80/20 v/v mixture of 0.1 M $NaNO_3$ and MeCN	0.5 mL/min	TSKgel G6000PW_{XL} (Tosoh Biosciences LLC) Range: 4.0 × 10^4 - 8.0 × 10^6 Da (polyethylene glycols and oxides)	Wyatt Optilab rEX refractive index detector operating at 658 nm, and a Wyatt miniDAWN Treos light scattering detector operating at 656 nm.
Aqueous, UHMW Cationic	80/20 v/v mixture of 0.1 M $NaNO_3$ and MeCN	0.5 mL/min	TSKgel G6000PW_{XL} – CP (Tosoh Biosciences LLC) Range (PEO, PEG) 1,000 ~ 1.0 × 10^7 Da	Wyatt Optilab rEX refractive index detector operating at 658 nm, and a Wyatt miniDAWN Treos light scattering detector operating at 656 nm.

Dynamic Light Scattering (DLS) and Zeta potential

DLS and zeta potential was collected using Malvern Zetasizer Nano ZS (Model No. ZEN 3600, Malvern Instruments Ltd.). Polymers were prepared at 1 mg/mL. PDMA homopolymers were dissolved and analysed in deionised water. Charged polymers and coacervates were studied at 0.4 M KBr solution.

Transmission Electron Microscopy (TEM)

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

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 2100 Plus operating at 200 kV, equipped with a Gatan OneView IS camera.

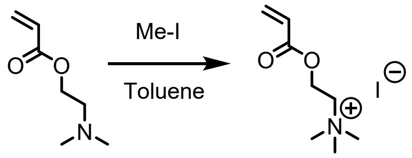
Confocal Laser Scanning Microscopy (CLSM).

FV3000 (Olympus) confocal microscope with an IX-81 inverted base (Olympus) and the 20X and 60X oil lens (Olympus) was used for imaging. The FV3000 system was driven with the FV31S-SW Viewer software platform (Olympus) with scan rates of 1 µs pixel^{-1} at 515 by 512 pixel^{-1} to 1024 by 1024 pixel^{-1}. The solution (10 µL) was drop-casted onto a glass slide. Images were taken using the brightfield and fluorescence detector set to 405 nm.

For particle analysis, a minimum of 100 particles were traced manually using ImageJ software. The resulting measurements are represented in a histogram to better understand the distribution of particle sizes between samples.

Synthesis and Characterisation

The cationic monomer 2-(acryloyloxy)-*N,N,N*-trimethylethan-1-aminium (TMAEA) was synthesis from a protocol adapted from literature.[2]



First, 2-(dimethylamino)ethyl acrylate (DMAEA, 8.5 mL, 55 mM) was filtered through basic alumina column and then added to a 100 mL round bottom flask and diluted with 30 mL of dry toluene. The solution was then purged with argon for 15 min. Iodomethane (4 mL, 66 mM) was slowly added dropwise using a syringe pump with constant stirring. The reaction was left stirring at room temperature overnight. Afterwards, a solid white product was collected via filtration. The solution was then purified by washing (x3) with hexanes. The product was then isolated and dried under vacuum. Yield: 13 g, 85%. Appearance: White solid powder. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ(ppm): 6.40 (dd, 1H, J_{AB} (trans) = 17.6 Hz, J_{AC} (gem) = 1.45 Hz) , 6.24 (dd, 1H, J_{BA} (trans) = 17.4 Hz, J_{BC} (cis) = 10.3 Hz), 6.0 (dd, 1H, J_{AC} (cis) = 10.8 Hz, J_{CA} (gem) = 1.45 Hz), 4.6 (t, 2H), 3.75 (t, 2H), 3.15 (s, 9H).^{13}C NMR (400 MHz, $(CD_3)_2SO$) δ(ppm): 164.78, 132.46 127.84, 63.74, 58.04, 52.04, 52.96.

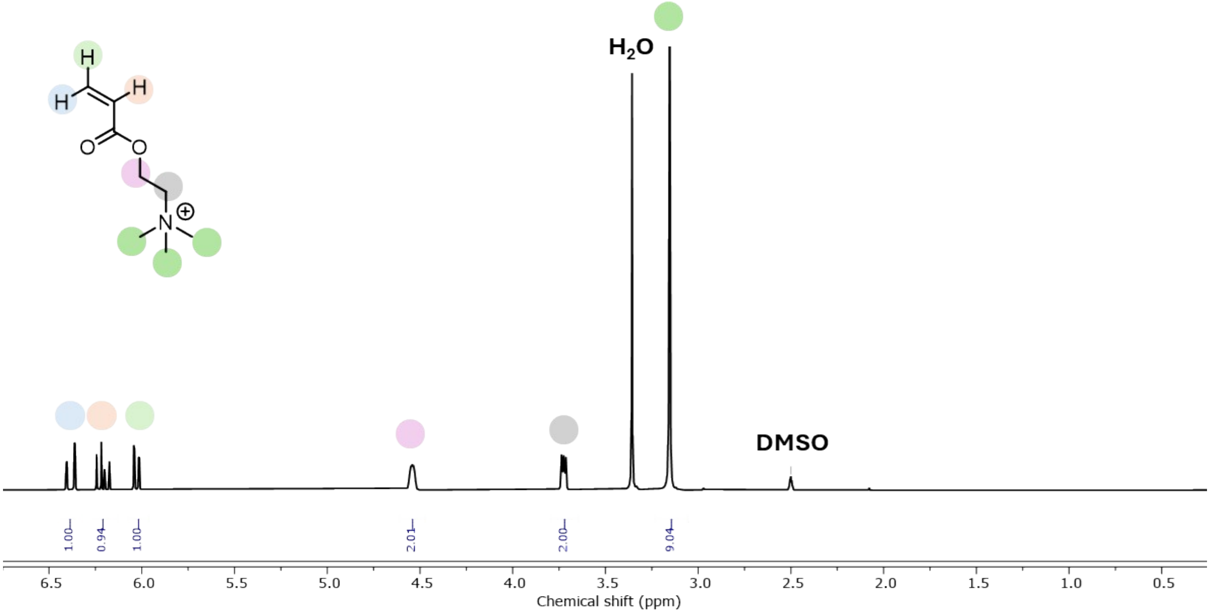


Figure S1. ^{1}H NMR of TMAEA monomer.

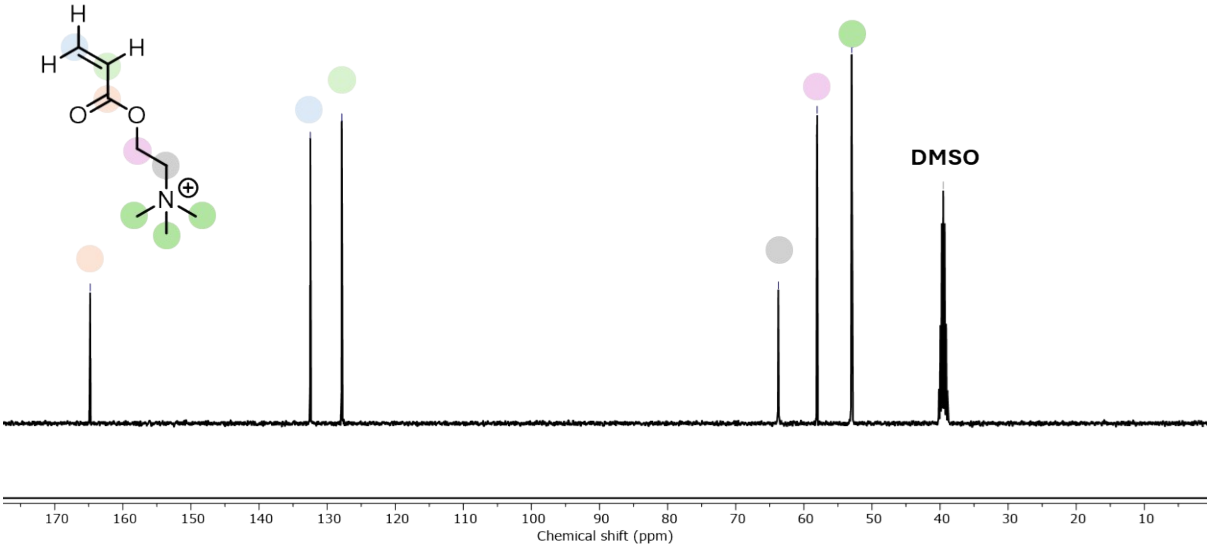
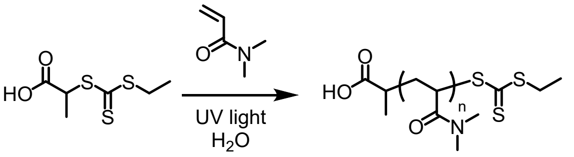


Figure S2. ^{13}C NMR of TMAEA monomer.

DMA polymerization



n = 10,100

DMA (991 mg, 10.0 mmol) was dissolved in water (4.79 mL, 2 M $[DMA]_0$). PAETC (0.210 mg, 0.001 mmol) was added from a stock solution (2 mg mL^{-1}) in DMSO. DMF was added as an NMR standard (100 µL). The solution was transferred to a 10 mL Schlenk flask and sparged with Argon for 15 min while stirring. The reaction was then placed in a UV reactor. The reaction was irradiated for 8 h and quenched upon exposure to oxygen in the atmosphere. The product was used directly in the next step upon confirmation that the reaction reacted full conversion via NMR spectroscopy and reached ~1 MDa via GPC. For characterization, the polymer was purified via dialysis in deionized water (SpectraPor 3.5 kDa MWCO) and subsequently freeze-dried.

PDMA: Appearance: White solid powder. GPC (DMAc): $M_{w\,GPC\,DMAc}$ = 1.18 Mg mol^{-1}, $Đ$ = 1.14). GPC (Aqueous): $M_{w\,GPC\,Aq}$ = 1.29 Mg mol^{-1}, $Đ$ = 1.23. ^{1}H NMR: Conversion: 99%, $M_{n,\,theo}$ = 1.00 Mg mol^{-1}. DLS: 37.2 nm, PDI = 0.279.

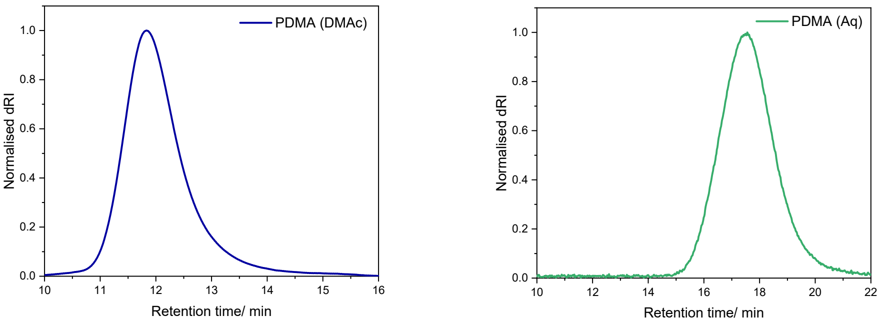
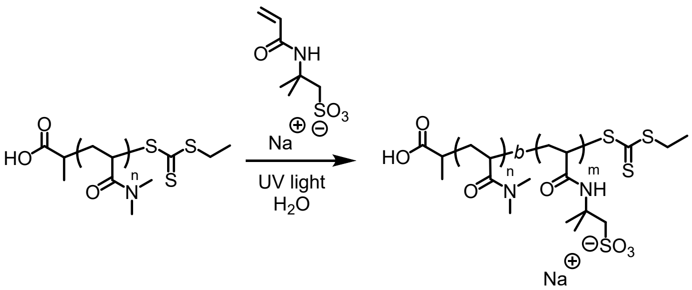


Figure S3. Gel permeation chromatography of PDMA homopolymer in A) DMAc (with 50 mM LiCl) and B) Aqueous conditions (80/20 v/v mixture of 0.1 M $NaNO_3$ and MeCN).

Photoiniferter-mediated chain extension

PDMA-*b*-PAMPS:



n= 10,100, m= 200

AMPS (41.0 mg, 0.198 mmol, 200 equiv) was dissolved in 5 mL of deionised water. The monomer solution was added to the Schlenk flask containing the PDMA macroCTA (0.990 µmol, 1 equiv). DMF was added as an NMR standard (100 µL). The reaction was left on a shaker plate overnight to ensure a homogeneous reaction solution before polymerization. The reaction was sparged for 15 min before being placed in the UV reactor and irradiated for 8 h. The resulting polymer was purified via dialysis in deionized water (SpectraPor 3.5 kD MWCO) and subsequently freeze-dried. Yield: 0.98 g, 95%. Appearance: White solid powder. ^{1}H NMR:

Conversion: 97%, $M_{\mathrm{w,\ theo}}$ = 1.04 Mg mol^{-1}. GPC (Aqueous, UHMW Neutral/Anionic): $M_{\mathrm{w\ GPC}}$ = 1.41 Mg mol^{-1}, $Đ$ = 1.23). DLS: 48.9 nm, PDI = 0.335.

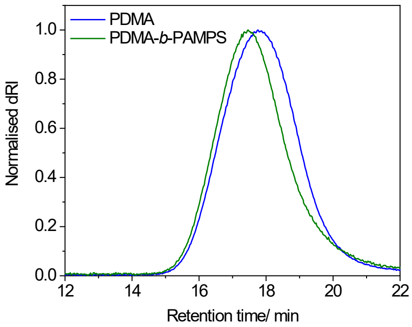
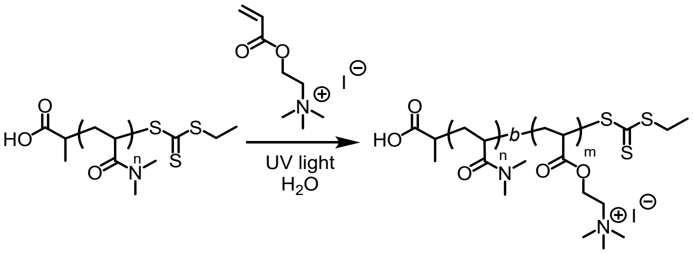


Figure S4. Size exclusion chromatography of PDMA and PDMA-b-PAMPS measured in aqueous conditions.

PDMA-*b*-PTMAEA:



n= 10,100, m= 200

TMAEA (56.5 mg, 0.198 mmol, 200 equiv) was dissolved in 5 mL of deionised water. The monomer solution was added to the Schlenk flask containing the PDMA macroCTA (0.990 µmol, 1 equiv). DMF was added as an NMR standard (100 µL). The reaction was left on a shaker plate overnight to ensure a homogeneous reaction solution before polymerization. The reaction was sparged for 15 min before being placed in the UV reactor and irradiated for 8 h. The resulting polymer was purified via dialysis in deionized water (SpectraPor 3.5 kD MWCO) and subsequently freeze-dried. Yield: 0.96 g, 94%. Appearance: White solid powder. GPC (Aqueous, UHMW Cationic): $M_{\mathrm{w\ GPC}}$ = 1.24 Mg mol^{-1}, $Đ$ = 1.36). ^{1}H NMR: Conversion = >99%, $M_{\mathrm{w,\ theo}}$ = 1.03 Mg mol^{-1}. DLS: 52.3 nm, PDI = 0.372.

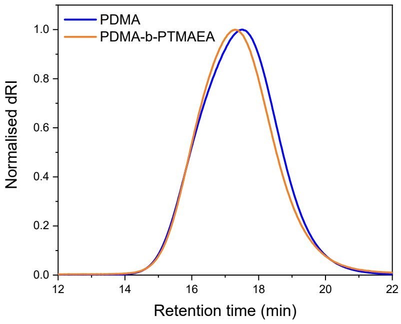


Figure S5. Size exclusion chromatography of PDMA and PDMA-b-PTMAEA measured in aqueous conditions.

Self-assembly of UHMW charged diblock copolymers

Freeze dried diblock copolymers PDMA-*b*-PAMPS (1.00 mg) and PDMA-*b*-PTMAEA (1.00 mg) were independently dissolved in 0.5 M KBr solution (1 mL). The solutions were then added in a separate vial in a 1:1 vol ratio (0.5 mL: 0.5 mL).

For fluorescent CLSM images, the self-assembled coacervates solution was stained using fluorescein dye. This was prepared by mixing 100 µL of Fluorescein stock solution (1 mg/mL, Fluorescein Sodium, Pure (Fisher Scientific)) with 100 µL of the coacervate solution.

General procedure for polymerization-induced electrostatic self-assembly

PDMA-*b*-PAMPS macroCTA (1.00 g, 0.100 µmol, 1 equiv) and TMAEA (56.5 mg, 0.198 mmol, 2000 equiv) were added to a vial. KBr (5 mL, 0.5 M) in deionised water was used as the solvent. DMF was added as an NMR standard (100 µL). The solution was transferred into a Schlenk flask and left on a shaker plate overnight. The reaction was sparged for 15 min before being placed in the UV reactor and irradiated for 8 h. The resulting polymer was analysed via NMR spectroscopy, GPC, and DLS to determine conversion, molecular weight, and hydrodynamic volume, respectively.

PDMA-*b*-PAMPS-*b*-TMAEA: NMR (d.DMSO): Conversion = 39%, $M_{\mathrm{w,\ theoretical}}$= 1.54×10^{6} g mol^{-1}. DLS: 2.33 µm, PDI = 0.426.

As a control the PDMA-*b*-PAMPS was also chain extended with AMPS (41.4 mg, 0.198 mmol, 2000 equiv) using the same conditions as stated above.

PDMA-*b*-PAMPS-*b*-PAMPS: NMR (d.DMSO): Conversion = 25%, $M_{\mathrm{w,\ theoretical}}$= 1.54×10^{6} Mg mol^{-1}. DLS: 63.1 nm, PDI = 0.389.

Supplemental Figures

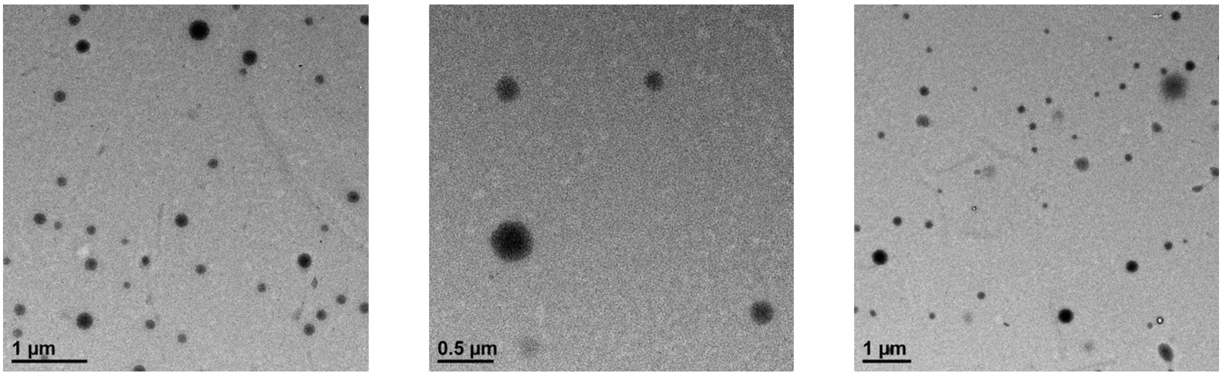


Figure 6. Additional TEM images of UHMW complex coacervates (PDMA-*b*-PAMPS and PDMA-*b*-PTMAEA).

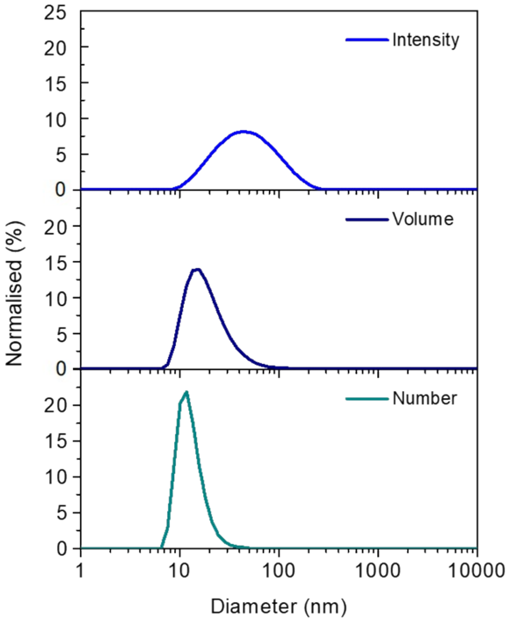


Figure S7. DLS of PDMA macroCTA in deionised water. Z-average diameter and PDI were 37 nm and 0.279 respectively.

Table S1. Zeta potentials of the charged polymers before and after mixing

Polymer(s)	**Zeta Potential (mV)**
PDMA-*b*-PAMPS	-5.2
PDMA-*b*-PTMAEA	1.8
PDMA-*b*-PAMPS + PDMA-*b*-PTMAEA	-2.4

References

1. R. A. Olson, M. E. Lott, J. B. Garrison, C. L. G. Davidson, L. Trachsel, D. I. Pedro, W. G. Sawyer and B. S. Sumerlin, *Macromolecules*, 2022, **55**, 8451-8460.
2. M. Semsarilar, V. Ladmiral, A. Blanazs and S. P. Armes, *Langmuir*, 2013, **29**, 7416-7424.