

Supporting Information for

Reverse Sequence Polymerization-Induced Self-Assembly in Aqueous Media: An Efficient New Route to Block Copolymer Nano-Objects

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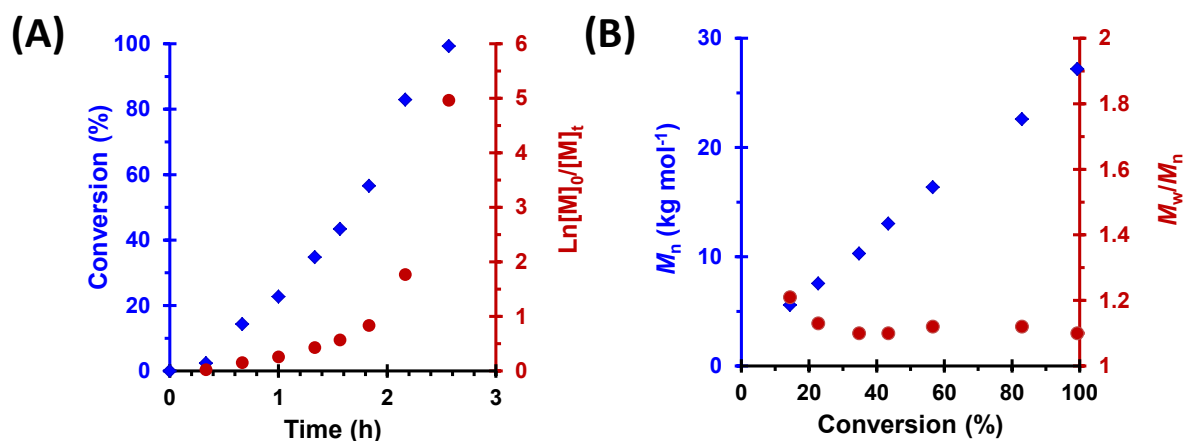


Figure S1. Kinetic data obtained for the surfactant-free RAFT aqueous dispersion polymerization of HPMA using a cationic MPETTC RAFT agent at 56 °C: (A) monomer conversion vs. time curve and corresponding semi-logarithmic plot; (B) evolution in number-average molecular weight (M_n) and dispersity (M_w/M_n) with monomer conversion. Polymerization conditions: $[HPMA]/[MPETTC] = 140$, $[MPETTC]/[AIBA] = 5.0$, target solids concentration = 10% w/w, pH 3.

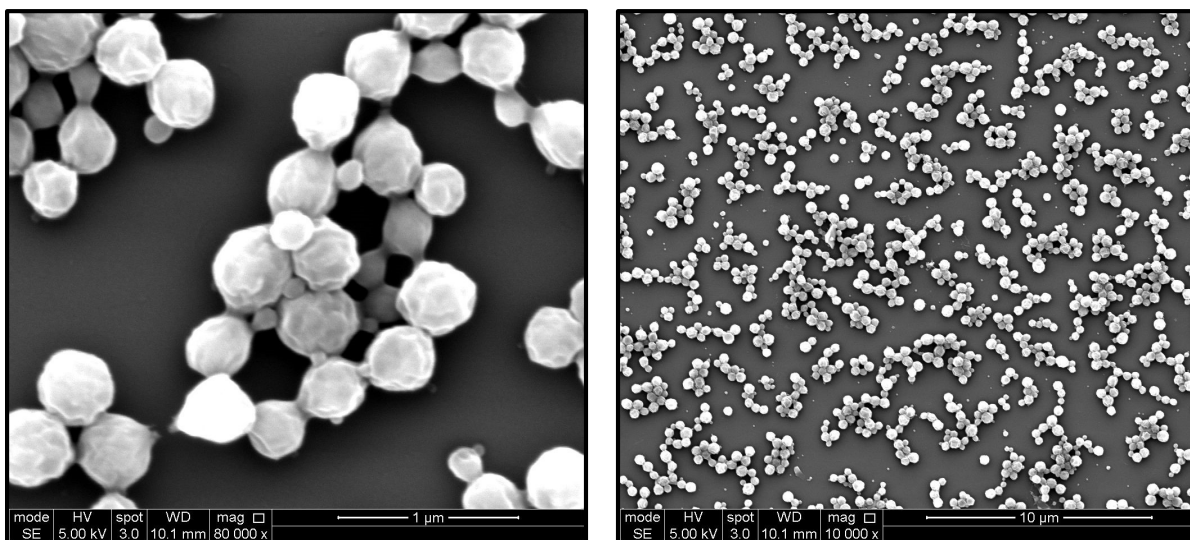


Figure S2. SEM images recorded for various dried aqueous dispersions of PHPMA₁₄₀ latexes prepared at pH 3. The particle buckling observed in the left-hand image suggests that the original latex is relatively hydrated in its wet state. This is consistent with the weakly hydrophobic nature of this homopolymer (see main manuscript).

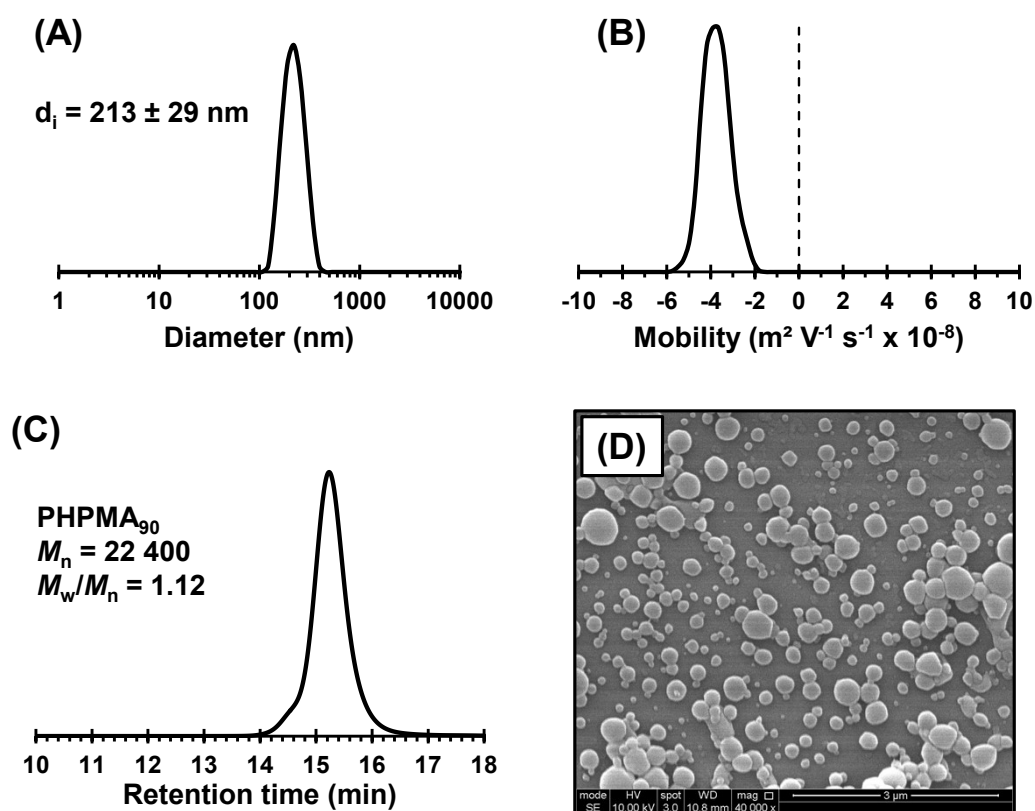


Figure S3. (A) DLS particle size distribution and (B) electrophoretic mobility distribution obtained for a 0.1% w/w dispersion of an anionic PHPMA₉₀ latex prepared using the carboxylic acid functionalized PETTC RAFT agent (zeta potential = -52 mV at pH 7). (C) DMF GPC curve (refractive index detector; calibrated against a series of poly(methyl methacrylate) standards) recorded for PHPMA₉₀. (D) SEM image recorded for this latex dispersion after drying at pH 7.

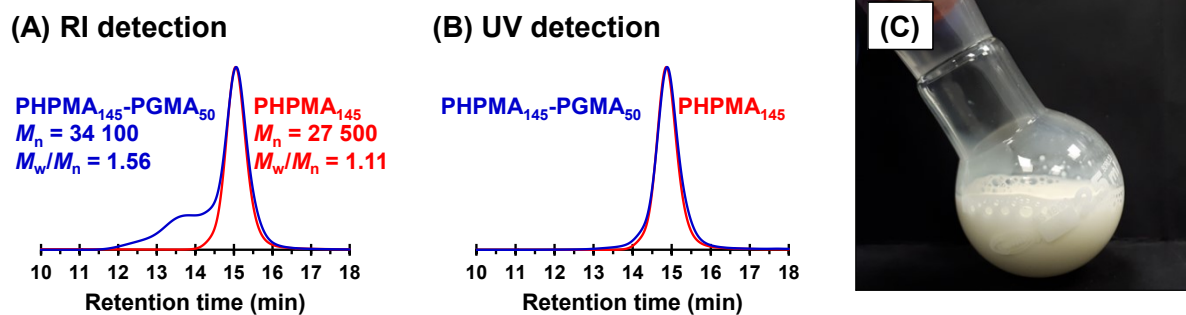


Figure S4. DMF GPC curves recorded using (A) refractive index and (B) UV ($\lambda = 298$ nm) detectors for a PHPMA₁₄₅ precursor latex (red traces) and the corresponding ill-defined product resulting from the attempted RAFT seeded aqueous dispersion polymerization of GMA when targeting PHPMA₁₄₅-PGMA₅₀ – see main manuscript for further details). Molecular weight data are expressed relative to near-monodisperse poly(methyl methacrylate) standards. (C) Digital photograph of the resulting aqueous colloidal dispersion, which is contaminated by polydisperse water-soluble PGMA chains.

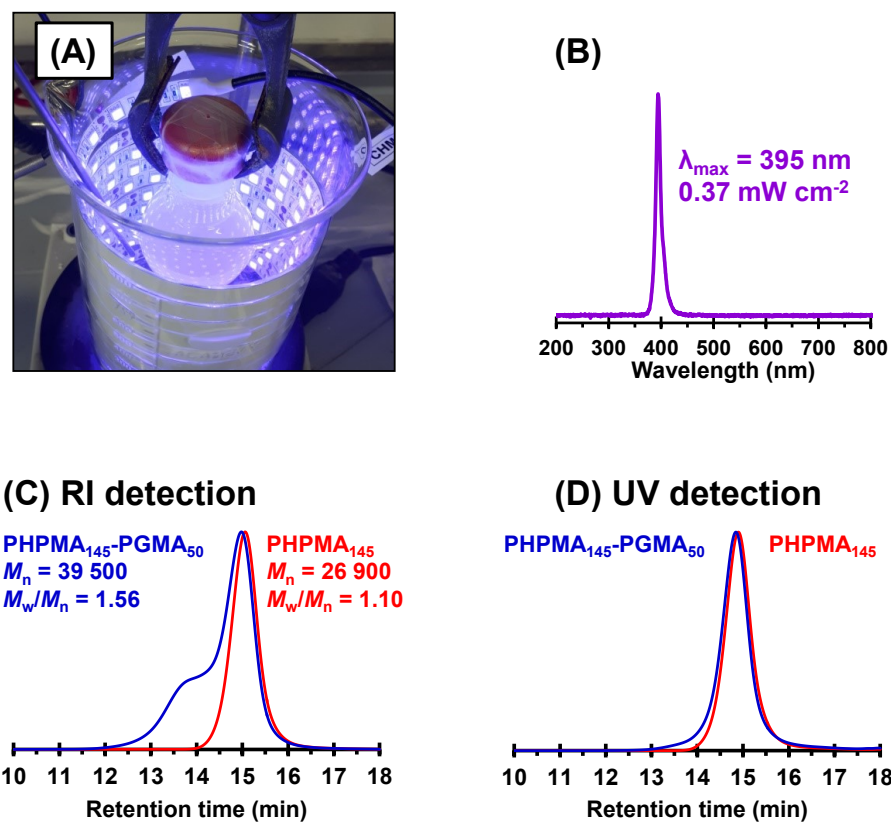
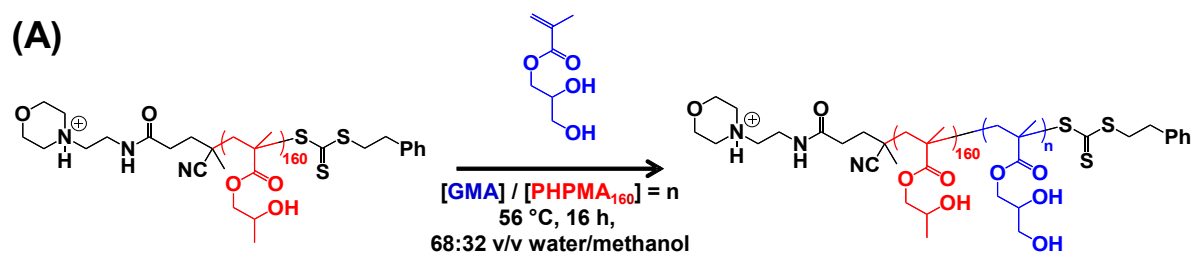
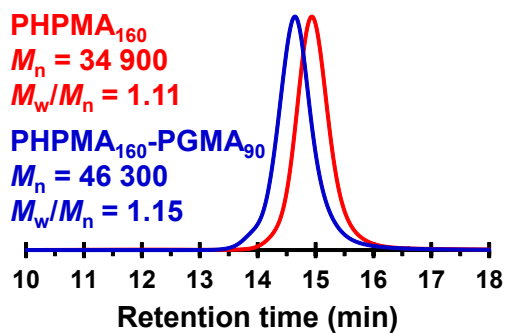


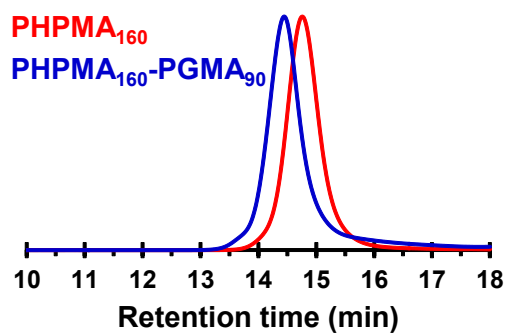
Figure S5. (A) Digital photograph and (B) emission spectrum recorded for our custom-built photo-reactor ($\lambda_{\max} = 395$ nm, light intensity = 0.37 mW cm⁻²). DMF GPC curves recorded using (C) refractive index and (D) UV ($\lambda = 298$ nm) detectors for a PHPMA₁₄₅ precursor latex and the corresponding ill-defined product resulting from the attempted RAFT seeded aqueous dispersion polymerization of GMA when targeting PHPMA₁₄₅-PGMA₅₀. Molecular weight data are expressed relative to near-monodisperse poly(methyl methacrylate) standards.



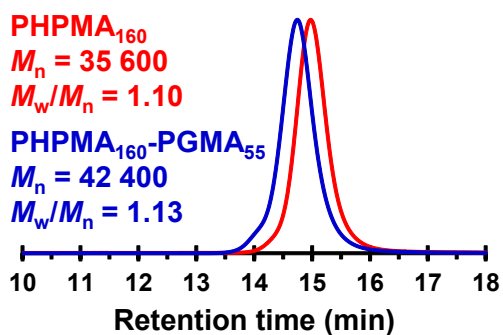
(B) Refractive index



(C) UV



(D) Refractive index



(E) UV

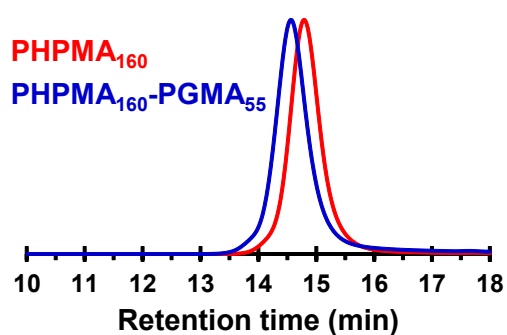


Figure S6. (A) Reaction scheme for the synthesis of PHPMA₁₆₀-PGMA_n diblock copolymer nanoparticles *via* RAFT solution polymerization in a 68:32 v/v water/methanol mixture. DMF GPC curves recorded for the PHPMA₁₆₀ precursor, PHPMA₁₆₀-PGMA₉₀ diblock copolymer and PHPMA₁₆₀-PGMA₅₅ diblock copolymer using (B, D) refractive index and (C, E) UV detectors. Molecular weight data are expressed relative to a series of near-monodisperse poly(methyl methacrylate) calibration standards.

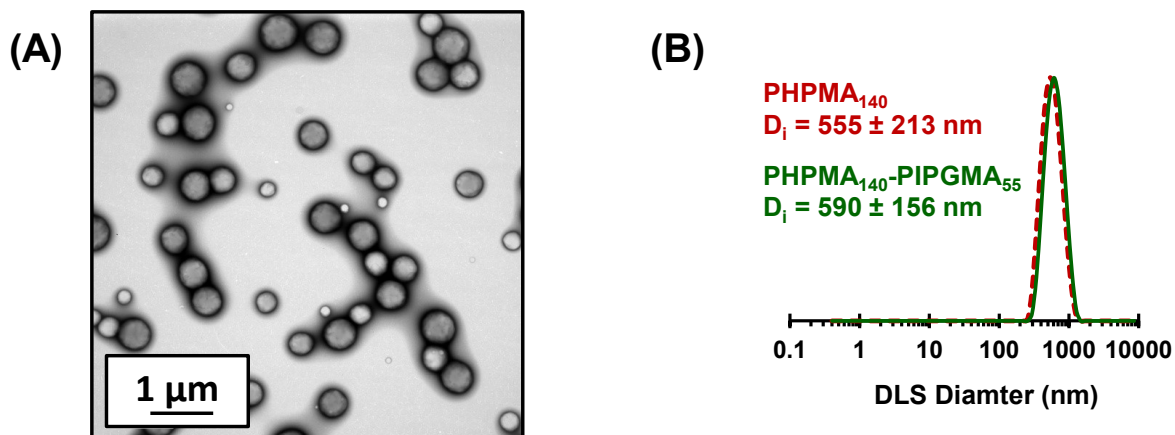


Figure S7. (A) TEM image recorded after drying an 0.1% w/w aqueous dispersion of PHPMA₁₄₀-PIPGMA₅₅ latex at pH 3. (B) Intensity-average particle size distributions recorded for 0.1% w/w aqueous dispersions of the precursor PHPMA₁₄₀ latex and the corresponding PHPMA₁₄₀-PIPGMA₅₅ diblock copolymer latex at pH 3 and 20 °C.

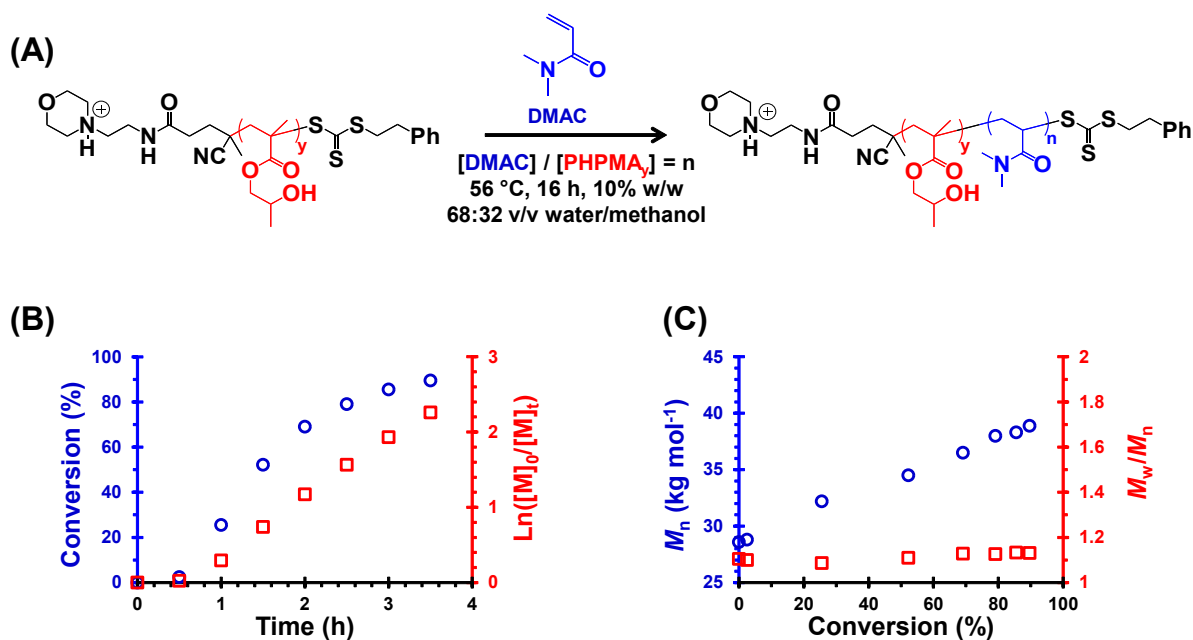


Figure S8. Reaction scheme and kinetic data for RAFT solution polymerization of DMAC using a PHPMA₁₄₀ precursor in a 68:32 v/v water/methanol mixture at 56 °C. (B) Monomer conversion vs. time curve and corresponding semi-logarithmic plot obtained for this polymerization. (C) Evolution in the number-average molecular weight (M_n) and dispersity (M_w/M_n) with monomer conversion for this polymerization. Polymerization conditions: $[\text{DMAC}]/[\text{PHPMA}_{140}] = 160$, $[\text{MPETTC}]/[\text{AIBA}] = 5.0$, 10% w/w solids, pH 3.

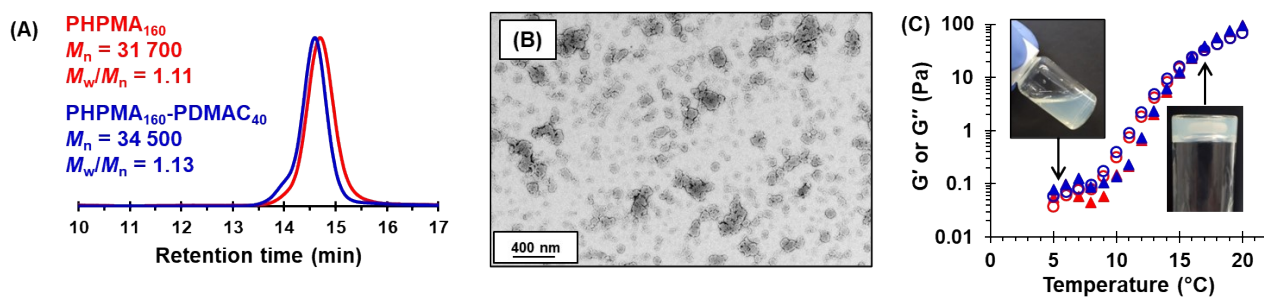


Figure S9. (A) DMF GPC curves recorded for the PHPMA₁₆₀ precursor and the final PHPMA₁₆₀-PDPMAC₄₀ diblock copolymer. Molecular weight data are expressed relative to a series of near-monodisperse poly(methyl methacrylate) standards. (B) TEM image recorded after drying a 0.1% w/w dispersion of PHPMA₁₄₀-PDPMAC₄₀ nanoparticles at pH 4.7. (C) Variation in storage (G' , filled triangles) and loss (G'' open circles) moduli with temperature for a 10% w/w aqueous dispersion of PHPMA₁₆₀-PDPMAC₄₀ at pH 4.7. The heating sweep (red data) was performed prior to the cooling sweep (blue data). A thermal equilibration time of 5 min was allowed for each 1 °C increment and data were recorded at 1% applied strain and 1 rad s⁻¹.

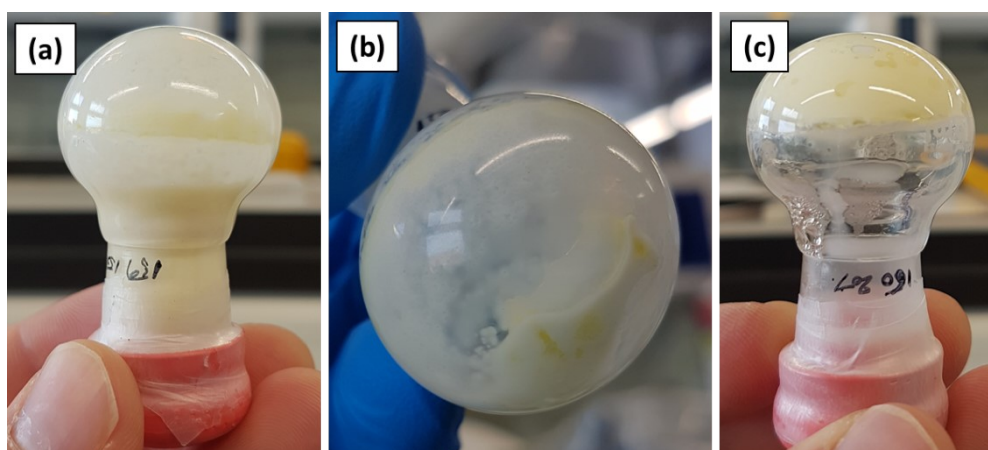


Figure S10. Digital photographs illustrating how performing the emulsion polymerisation at higher solids results in large amounts of precipitation, where (a) and (b) were performed at 15% w/w and (c) was performed at 20% w/w.

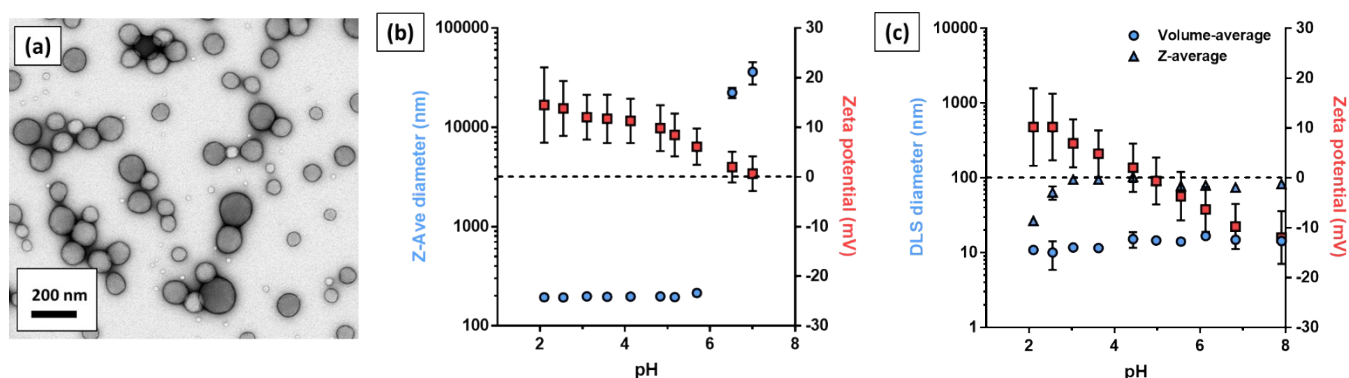


Figure S11. (a) TEM image recorded after drying the PMOEMA₄₀-PIPGMA₂₇ latex, which forms a free-flowing turbid dispersion at 8.2% w/w solids. (b) Variation in z-average diameter and zeta potential with pH for this charge-stabilized PMOEMA₄₀-PIPGMA₂₇ latex [a 0.1% w/w aqueous dispersion in 1 mM KCl was initially prepared at pH 3 and the solution pH was adjusted using NaOH or HCl]. (c) Variation in DLS diameter and zeta potential with pH for the sterically-stabilized PMOEMA₄₀-PGMA₂₇ nanoparticles obtained after deprotection of the PIPGMA chains [a 0.1% w/w aqueous dispersion in 1 mM KCl was initially prepared at pH 3 and the solution pH was adjusted using NaOH or HCl].

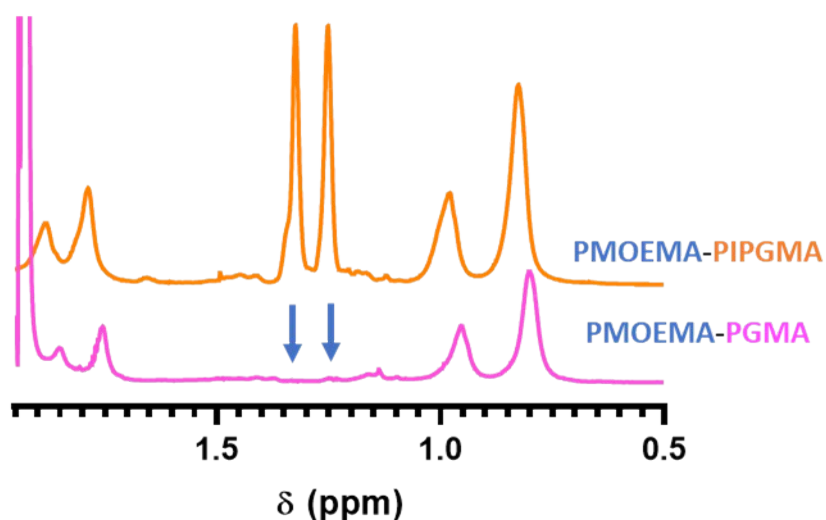


Figure S12. ¹H NMR spectra recorded for PMOEMA₄₀-PIPGMA₂₇ and PMOEMA₄₀-PGMA₂₇ diblock copolymers in d₆-acetone and d₄-methanol, respectively. Hydrolysis of PMOEMA₄₀-PIPGMA₂₇ to form PMOEMA₄₀-PGMA₂₇ is confirmed by the disappearance of the six methyl protons (blue arrows) at 1.36 ppm and 1.44 ppm associated with the acetone protecting group.

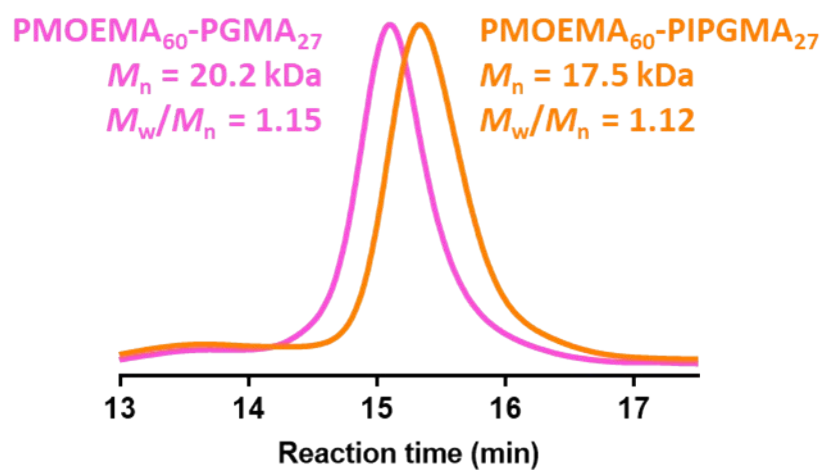
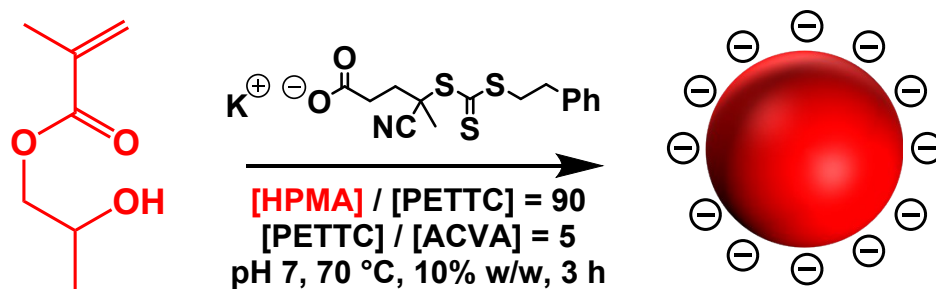


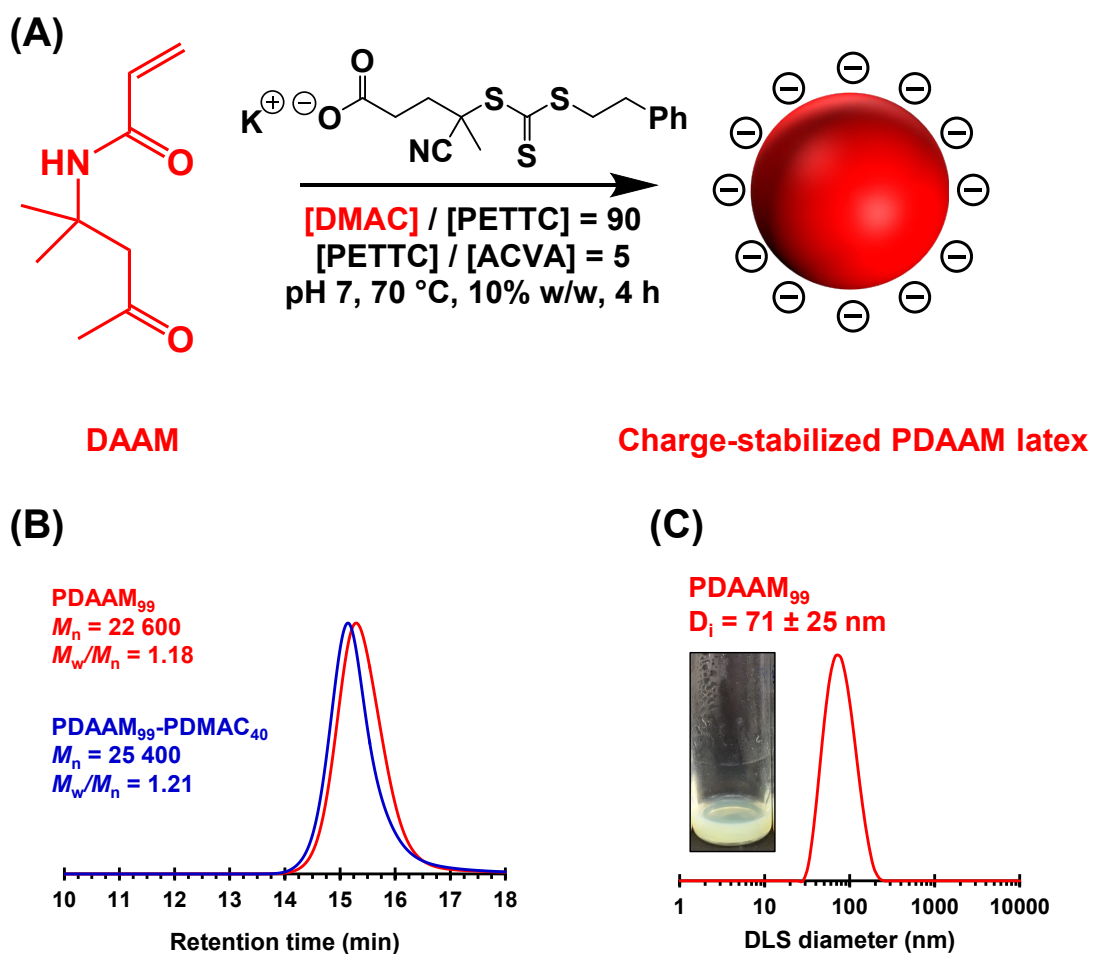
Figure S13. DMF GPC curves recorded for PMOEMA₆₀-PIPGMA₂₇ and PMOEMA₆₀-PGMA₂₇. Molecular weight data are expressed relative to a series of near-monodisperse poly(methyl methacrylate) standards.



HPMA

Charge-stabilized PHPMA latex

Scheme S1. Synthesis scheme for the RAFT aqueous dispersion polymerization of HPMA using a carboxylic acid-functionalized RAFT agent (PETTC) at pH 7 to yield anionic charge-stabilized PHPMA latex particles.



Scheme S2. (A) Synthesis scheme for the RAFT aqueous dispersion polymerization of DAAM using a carboxylic acid-functionalized RAFT agent (PETTC) at pH 7 to yield an anionic charge-stabilized PHPMA latex. (B) DMF GPC curves recorded for both the PDAAM₉₉ precursor and a PDAAM₉₉-PDMAC₄₀ diblock copolymer. Molecular weight data are expressed relative to a series of near-monodisperse poly(methyl methacrylate) calibration standards. (C) DLS particle size distribution recorded for a 0.1% w/w dispersion of PDAAM₉₉ latex particles at pH 7.

Table S1. Summary of the target PHPMA DP, final monomer conversions, GPC data, DLS data and zeta potential data obtained for the synthesis of charge-stabilized PHPMA_x latexes at 56 °C and 10% w/w solids, where x ranges from 60 to 200.

| Target PHPMA DP | Conversion (%) | DMF GPC | | DLS Analysis | | | Comments |
|-----------------|----------------|-------------------------------|-------------|--------------|-------------------------|---------------------|----------------------------|
| | | M_n (kg mol ⁻¹) | M_w / M_n | d_i^a (nm) | DCR ^b (kcps) | Zeta potential (mV) | |
| 60 | >99 | 13.7 | 1.07 | 63 ± 47 | 5400 | - | Unstable on dilution |
| 80 | >99 | 17.3 | 1.08 | 434 ± 185 | 38100 | +52 ± 7 | Unimodal size distribution |
| 100 | >99 | 21.1 | 1.09 | 397 ± 47 | 35200 | +50 ± 5 | Unimodal size distribution |
| 120 | >99 | 25.0 | 1.10 | 505 ± 126 | 24100 | +51 ± 7 | Unimodal size distribution |
| 140 | >99 | 27.6 | 1.11 | 512 ± 172 | 20700 | +51 ± 7 | Unimodal size distribution |
| 160 | >99 | 31.4 | 1.11 | 558 ± 157 | 16000 | +50 ± 8 | Unimodal size distribution |
| 180 | >99 | 36.4 | 1.13 | 2653 ± 2474 | 8200 | +52 ± 9 | Fine precipitate |
| 200 | >99 | 38.9 | 1.13 | 965 ± 648 | 5900 | +50 ± 9 | Fine precipitate |

a. d_i denotes the intensity-average diameter as determined by DLS.

b. DCR is the derived count rate (which is a measure of the scattered light intensity)

Table S2. Summary of the target compositions, monomer conversions and GPC data obtained for two PHPMA₁₆₀ latex precursors and the final PHPMA₁₆₀-PGMA₉₀ and PHPMA₁₆₀-PGMA₅₅ diblock copolymers.

| Entry No. | Precursor PHPMA latex | | | | PHPMA-PGMA diblock copolymer | | | |
|-----------|-----------------------|----------------|-------------------------------|-------------|------------------------------|----------------|-------------------------------|-------------|
| | PHPMA DP | Conversion (%) | M_n (kg mol ⁻¹) | M_w / M_n | PGMA DP | Conversion (%) | M_n (kg mol ⁻¹) | M_w / M_n |
| 1 | 160 | >99 | 34.9 | 1.11 | 90 | >98 | 46.3 | 1.15 |
| 2 | 160 | >99 | 35.6 | 1.10 | 55 | >99 | 42.4 | 1.13 |

Experimental

Materials. MPETTC RAFT agent was synthesized according to a previous literature report.¹ 2-Hydroxypropyl methacrylate (HPMA; 97%) and 2-methoxyethyl methacrylate (MOEMA, 99%) was purchased from Alfa Aesar (UK) and was used as received. 2,2'-Azobis(2-methylpropionamide) dihydrochloride (AIBA; 97%), *N,N'*-dimethylacrylamide (DMAC), and oligo(ethylene glycol) methyl ether methacrylate (OEGMA, mean degree of oligomerization = 4-5; $M_n \sim 300 \text{ g mol}^{-1}$) were purchased from Sigma Aldrich (UK). Glycerol monomethacrylate (GMA; >99%) was kindly donated by GEO Specialty Chemicals (Hythe, UK). Deuterated methanol (CD_3OD ; D, 99.8%) was purchased from Cambridge Isotopes Laboratories Ltd. All other solvents were purchased from either VWR International or Sigma Aldrich and were HPLC-grade quality. Deionized water was obtained from an Elgastat Option 3A water purification unit with a resistivity of 15 $\text{M}\Omega \text{ cm}$.

Characterization Techniques

¹H Nuclear Magnetic Resonance (NMR) Spectroscopy

All ¹H NMR spectra were recorded in CD_3OD using a 400 MHz Bruker Avance-400 spectrometer operating at 298 K with 16 scans being averaged per spectrum.

DMF Gel Permeation Chromatography (GPC)

Aqueous polymer solutions were diluted to 0.50% w/w using HPLC-grade DMF eluent containing 10 mM LiBr and DMSO (1.0 % v/v) was used as a flow rate marker. GPC studies were conducted at 60 °C using a flow rate of 1.0 mL min^{-1} . The GPC set-up comprised an Agilent 1260 Infinity series degasser and pump, an Agilent PL-gel guard column, two Agilent PL-gel Mixed-C columns, a refractive index detector and a UV detector set at a wavelength of 298 nm. Eleven near-monodisperse poly(methyl methacrylate) standards with M_p values ranging from 2,380 g mol^{-1} to 2,200,000 g mol^{-1} were used for calibration.

Dynamic Light Scattering (DLS) and Aqueous Electrophoresis

DLS and aqueous electrophoresis measurements were conducted at 25 °C using a Malvern Instruments Zetasizer Nano ZS series instrument equipped with a 4 mW He–Ne laser ($\lambda = 633$ nm) and an avalanche photodiode detector. Scattered light was detected at 173°. Aqueous dispersions were diluted to a final concentration of 0.1% w/w solids using an acidic aqueous solution of 1 mM KCl (adjusted to pH 3 using HCl). Intensity-average hydrodynamic diameters were calculated via the Stokes-Einstein equation. Zeta potentials were calculated from the Henry equation using the Smoluchowski approximation. The aqueous dispersion pH was adjusted using either 0.1-1.0 M HCl or 0.1-1.0 M KOH as required.

Scanning Electron Microscopy (SEM)

Aqueous dispersions of PHPMA latexes were diluted to 1.0% w/w at pH 3 and 2 cm x 2 cm silicon wafers were cleaned according to a previously reported protocol.² The anionic silicon wafers were simply dipped into the dilute aqueous cationic latex dispersions for 20 seconds at room temperature, extensively washed with pH 3 water to remove excess latex particles and dried with compressed air. Wafers were mounted onto SEM stubs using electrically conductive adhesive pads. The stubs were gold-coated for 2 min and then imaged using an Inspect F microscope operating at 5 kV. Number-average particle diameters were determined using ImageJ software (~ 100 particles counted per sample).

Transmission Electron Microscopy (TEM)

Copper/palladium grids were surface-coated in-house to produce a thin film of amorphous carbon and then plasma glow-discharged for 20 seconds to afford a hydrophilic surface. A 10 μ L droplet of the freshly-prepared 0.1% w/v aqueous copolymer dispersion was placed on the hydrophilic grid for 15 seconds, blotted to remove excess sample and then negatively-stained with uranyl formate solution (0.75% w/v; 10 μ L) for a further 15 seconds. Excess stain was removed by blotting and each grid was carefully dried with a vacuum house. TEM studies were performed using a FEI Tecnai Spirit 2 microscope equipped with an Orius SC1000B camera operating at 80 kV.

Oscillatory Rheology

An AR-G2 rheometer equipped with a variable temperature Peltier plate and a 40 mm 2° aluminum cone was used for all rheological experiments. The storage modulus (G') and loss modulus (G'') were determined as a function of temperature at an applied strain of 1.0 % and an angular frequency of 1.0 rad s⁻¹. An equilibration time of 5.0 min was allowed prior to starting each experiment at 5 °C. For 10% w/w aqueous dispersions of PHPMA₁₆₀-PDMAC₄₀ nanoparticles, the thermal cycle was performed from 5 °C to 20 °C to 5 °C at 1 °C intervals, with 5.0 min being allowed for thermal equilibration before each measurement.

Synthesis Protocols

Latex syntheses via RAFT aqueous dispersion polymerization of HPMA at pH 3 using MPETTC RAFT agent

A typical protocol is described for the synthesis of an aqueous dispersion of PHPMA₁₄₀ latex via RAFT aqueous dispersion polymerization of HPMA. MPETTC was synthesized and purified according to a previously reported protocol.¹ A 25 mL round-bottomed flask was charged with HPMA monomer (1.22 g, 8.51 mmol), MPETTC RAFT agent (27.4 mg, 60.7 μmol, [HPMA] / [MPETTC] = 140), AIBA (3.30 mg, 12.2 μmol, [MPETTC]/[AIBA] = 5.0) and deionized water (11.3 g). The resulting aqueous solution was carefully adjusted to pH 3 using 1 M HCl (~ 50 μL) to afford a transparent orange 10% w/w solution. The reaction flask was sealed with a rubber septum and the aqueous solution was sparged with N₂ gas for 30 min. The flask was then placed in a pre-heated oil bath set at 56 °C for 3 h. The initially clear pale-yellow solution became increasingly turbid, eventually affording a milky-white latex dispersion. The HPMA polymerization was quenched by exposure to air and cooling to room temperature. The resulting PHPMA₁₄₀ latex was characterized by ¹H NMR spectroscopy, DMF GPC, DLS, aqueous electrophoresis, SEM and TEM. A series of PHPMA_n latexes were prepared (where the degree of polymerization, n, was varied between 60 and 200) simply by adjusting the initial [HPMA]/[MPETTC] molar ratio and water content to maintain a constant solids content of 10% w/w.

One-pot synthesis of a PHPMA_n-PGMA_x diblock copolymer via RAFT solution polymerization of GMA in a methanol/water mixture

A typical experimental protocol is described for the two-step synthesis of a PHPMA₁₄₀-PGMA₅₅ diblock copolymer using a one-pot protocol. A 25 mL round-bottomed flask was charged with HPMA monomer (1.22 g, 8.51 mmol), MPETTC RAFT agent (27.4 mg, 60.7 μ mol, [HPMA]/[MPETTC] = 140), AIBA (3.30 mg, 12.2 μ mol, [MPETTC]/[AIBA] = 5.0) and deionized water (11.3 g) and carefully adjusted to pH 3 with 1 M HCl (~ 50 μ L) to afford a transparent orange 10% w/w solution. The reaction flask was sealed with a rubber septum and the reaction mixture was sparged with N₂ gas for 30 min. The flask was then placed in a pre-heated oil bath set at 56 °C for 3 h. A small aliquot was removed under N₂ for analysis by ¹H NMR spectroscopy and DMF GPC. Separately, a 10 mL round-bottomed flask was charged with GMA monomer (0.55 g, 3.43 mmol, [GMA]/[PHPMA] = 55) and methanol (5.0 g, 6.3 mL), sealed with a rubber septum and sparged with N₂ gas for 30 min. This alcoholic GMA solution was injected into the aqueous PHPMA latex mixture under N₂. This addition resulted in immediate latex dissolution, and the ensuing RAFT solution polymerization of GMA proceeded at 56 °C for 16 h. The polymerization was quenched by exposure to air and the resulting PHPMA₁₄₀-PGMA₅₅ diblock copolymer was analyzed by ¹H NMR spectroscopy and DMF GPC to determine the final monomer conversion and assess the molecular weight distribution, respectively. This protocol was adjusted to prepare a series of PHPMA_n-PGMA_y diblock copolymers by varying the [GMA]/[PHPMA] molar ratio with appropriate adjustment of the volume of added methanol to ensure full latex dissolution.

Latex syntheses via RAFT aqueous dispersion polymerization of HPMA at pH 7 using the PETTC RAFT agent

A 12 mL vial was charged with a magnetic stir bar, HPMA (0.4434 g, 3.07 mmol), PETTC (0.0116 g, 34.2 μ mol, target DP = 90), ACVA (0.0021 g, 6.49 μ mol, [PETTC]/[ACVA] = 5.0), H₂O (4.11 g) and carefully adjusted to pH 7 using either 1 M or 0.1 M KOH. The resulting transparent orange solution was sealed, sparged with N₂ gas for 30 min and placed in a pre-heated 70 °C oil bath for 3 h. The HPMA polymerization was quenched by cooling the flask to room temperature with simultaneous exposure of the reaction mixture to air, affording a free-flowing, white dispersion of charge-stabilized PHPMA latex particles. ¹H NMR spectroscopy and DMF GPC were used to determine the final monomer conversion and assess the molecular weight distribution, respectively.

Latex syntheses via the photoiniferter aqueous dispersion polymerization of HPMA at pH 3 using MPETTC RAFT agent and attempted chain extension using GMA

A custom-built photo-reactor was constructed by wrapping approximately 1.3 m of a SMD5050 LED strip (~ 80 LEDs) around the inside of a 1 L Pyrex beaker. Emission spectra indicated a λ_{max} of 395 nm at a maximum

intensity of 0.37 mW cm^{-2} (see Figure S5). Under constant illumination, the temperature inside the photo-reactor was determined to be $33 \text{ }^{\circ}\text{C}$. The synthesis of a charge-stabilized PHPMA₁₄₅ latex via photoiniferter polymerization was conducted as follows. A 50 mL round-bottomed flask was charged with a magnetic stir bar, HPMA (1.57 g, 10.9 mmol), MPETTC (0.0338 g, 75 μmol , target DP = 145) and water (14.43 g). The resulting aqueous solution was adjusted to pH 3 using 1 M HCl to afford a transparent orange solution. The flask was sealed with a rubber septum and the reaction solution was sparged using N₂ gas for 30 min. The flask was then placed inside the photo-reactor and irradiated for 3 h at $33 \text{ }^{\circ}\text{C}$. A small aliquot was removed and ¹H NMR spectroscopy and DMF GPC were used to determine the final monomer conversion and assess the molecular weight distribution, respectively. Separately, a 10 mL round-bottomed flask was charged with GMA monomer (0.61 g, 3.81 mmol, [GMA]/[PHPMA₁₄₅] = 50) and deionized water (5.50 g), sealed with a rubber septum, adjusted to pH 3 with 1M or 0.1M HCl and sparged with N₂ gas for 30 min. This aqueous GMA solution was injected into the aqueous PHPMA latex mixture under N₂ and heated at $56 \text{ }^{\circ}\text{C}$ for 16 h. Polymerization was quenched by exposing the reaction mixture to air and removing the flask from the photo-reactor. ¹H NMR and DMF GPC studies were conducted to assess the final monomer conversion and molecular weight distribution, respectively.

PHPMA-PIPGMA latex synthesis and the subsequent hydrolysis of the IPGMA residues

A 25 mL round bottom flask was charged with MPETTC (22.4 mg, 49.5 μmol), HPMA (1.0 g, 6.94 mmol, [HPMA]/[MPETTC] molar ratio = 140) and a magnetic stirrer. Once the MPETTC had fully dissolved in the HPMA, deionized water (8.23 g) was added. The resulting aqueous solution was adjusted to pH 3 using 0.1 M HCl to afford a yellow solution. 1 mL of a pH 3 aqueous solution of AIBA initiator (2.69 mg, 9.90 μmol , [MPETTC]/[AIBA] molar ratio = 5.0) was added to the reaction solution. The flask was sealed with a rubber septum and the reaction mixture was sparged with N₂ gas for 15 min before placing the flask in a pre-heated oil bath set at $56 \text{ }^{\circ}\text{C}$. The initial pale-yellow solution became increasingly turbid, eventually affording a milky-white latex dispersion. In a separate vial, IPGMA was sparged with N₂ for 15 min. After 4 h, the degassed IPGMA (0.53 g, 2.67 mmol, [IPGMA]/[PHPMA₁₄₀] molar ratio = 55) was added to the PHPMA₁₃₅ latex. The reaction was then left overnight (16 h) at $56 \text{ }^{\circ}\text{C}$. The polymerization was quenched by exposure to air and cooling to $20 \text{ }^{\circ}\text{C}$.

To deprotect the PIPGMA moieties to form hydrophilic PGMA the pH of an aqueous dispersion of PHPMA₁₄₀-PIPGMA₅₅ latex particles was adjusted to pH 1 using 1M HCl. The initial milky-white dispersion was then heated to $70 \text{ }^{\circ}\text{C}$ and slowly transformed into a slightly turbid dispersion over 3 h. ¹H NMR spectroscopy studies confirmed that essentially all the acetone protecting groups were removed under these conditions, thus converting the

hydrophobic PIPGMA block into a hydrophilic PGMA block. The resulting aqueous dispersion of PHPMA₁₄₀-PGMA₅₅ nanoparticles was dialyzed against deionized water overnight to remove excess acid, salt and traces of acetone.

Wholly aqueous one-pot reverse-sequence PISA synthesis of PHPMA₁₃₅-POEGMA₃₀ diblock copolymer nano-objects via RAFT aqueous dispersion polymerization

A 14 mL glass vial was charged with HPMA (0.200 g, 1.38 mmol, MPETTC (4.6 mg, 10.3 μ mol; [HPMA]/[MPETTC] molar ratio = 135) and a magnetic flea. Once the MPETTC had fully dissolved in the HPMA monomer, deionized water (1.36 g) was added and the resulting aqueous solution was adjusted to pH 3 using 0.1 M HCl to afford a yellow solution. A 1.0 mL aqueous solution of VA-44 initiator (0.7 mg, 2.06 μ mol; [MPETTC]/[VA-44] molar ratio = 5.0; adjusted to pH 3) was added to the reaction solution. The vial was sealed with a rubber septum and the reaction mixture was sparged with N₂ gas for 15 min. Then the vial was placed in a pre-heated oil bath set at 40 °C. The initial pale-yellow solution became increasingly turbid, eventually affording a milky-white latex dispersion. In a separate vial, OEGMA was sparged with N₂ for 15 min. After 4 h, degassed OEGMA (0.090 g, 0.300 mmol, [OEGMA]/[PHPMA₁₃₅] molar ratio = 30) was added to the PHPMA₁₃₅ latex. The OEGMA polymerization was allowed to proceed for 16 h at 40 °C and then quenched by exposing the reaction mixture to air and cooling the vial to 20 °C.

One-pot synthesis of a PHPMA₁₄₀-PDMAC_x diblock copolymer (x = 40 or 160) via RAFT solution polymerization of DMAC in a methanol/water mixture

A typical experimental protocol is described for the two-step synthesis of a PHPMA₁₄₀-PDMAC₄₀ diblock copolymer using a one-pot protocol. A 25 mL round-bottomed flask was charged with HPMA monomer (2.33 g, 15.5 mmol), MPETTC RAFT agent (50.0 mg, 111 μ mol, [HPMA]/[MPETTC] = 140), AIBA (6.00 mg, 22.1 μ mol, [MPETTC]/[AIBA] = 5.0) and deionized water (20.7 g). This aqueous solution was then carefully adjusted to pH 3 using 1 M HCl to afford a transparent orange 10% w/w solution. The reaction flask was sealed with a rubber septum and the reaction mixture was sparged with N₂ gas for 30 min. The flask was then placed in a pre-heated oil bath set at 56 °C for 3 h. A small aliquot was removed under N₂ for analysis by ¹H NMR spectroscopy and DMF GPC. Separately, a 25 mL round-bottomed flask was charged with DMAC monomer (0.44 g, 4.42

mmol, $[\text{DMAC}]/[\text{PHPMA}_{140}] = 40$) and methanol (11.0 g, 13.8 mL), sealed with a rubber septum and sparged with N_2 gas for 30 min. This alcoholic DMAC solution was injected into the aqueous PHPMA latex under N_2 . This addition resulted in immediate latex dissolution, and the ensuing RAFT solution polymerization of DMAC proceeded at 56 °C for 16 h. The polymerization was quenched by exposure to air, and the resulting PHPMA_{140} - PDMAC_{40} diblock copolymer was analyzed by ^1H NMR spectroscopy and DMF GPC to determine the final monomer conversion and assess the molecular weight distribution, respectively. This protocol was also used to prepare a PHPMA_{140} - PDMAC_{160} diblock copolymer by adjusting the $[\text{DMAC}]/[\text{PHPMA}_{140}]$ molar ratio by increasing the DMAC concentration whilst keeping the PHPMA_{140} concentration constant (see figure S8).

PDAAM latex synthesis via RAFT aqueous dispersion polymerization of DAAM at pH 7 using PETTC RAFT agent and subsequent chain extension using DMAC in a 66:34 v/v methanol/water mixture

A 12 mL sample vial was charged with a magnetic stir bar, DAAM (0.345 g, 2.04 mmol), PETTC (7.0 mg, 20.6 μmol , target DP = 99), ACVA (1.2 mg, 4.1 μmol , $[\text{PETTC}]/[\text{ACVA}] = 4.0$) and H_2O (3.18 g). The resulting aqueous solution adjusted to pH 7 using either 1 M or 0.1 M NaOH. The sealed vial was sparged with N_2 for 30 min and placed in a pre-heated oil bath set at 70 °C for 4 h. A small aliquot was removed under N_2 for analysis by ^1H NMR spectroscopy and DMF GPC. Separately, a 10 mL round-bottomed flask was charged with DMAC monomer (81.8 mg, 0.825 mmol, $[\text{DMAC}]/[\text{PDAAM}_{99}] = 40$) and methanol (5.0 g, 6.3 mL), sealed with a rubber septum and sparged with N_2 gas for 30 min. This alcoholic DMAC solution was injected into the aqueous PDAAM_{99} latex dispersion under N_2 . This addition resulted in immediate latex dissolution, and the ensuing RAFT solution polymerization of DMAC proceeded at 70 °C for 4 h. This polymerization was quenched by exposing the reaction mixture to air and cooling the flask to ambient temperature. The resulting PDMAC_{99} - PDAMC_{40} diblock copolymer was analyzed by ^1H NMR spectroscopy and DMF GPC to determine the final monomer conversion and assess its molecular weight distribution, respectively.

Protocol for the resuspension of dry diblock copolymer powder in water

Near-monodisperse diblock copolymers were synthesized via solution polymerization in various water/methanol mixtures (36-66 % methanol by volume). Dry diblock copolymers were obtained by removing the methanol under vacuum, followed by freeze-drying overnight from water. A previously reported protocol was utilized to redisperse dry copolymer powder to form an aqueous dispersion of

diblock copolymer nanoparticles.³ Thus, an 8 mL sample vial was charged with a magnetic stirrer bar, 0.20 copolymer powder and 1.80 g ice-cold deionized water. The resulting 10% w/w cold suspension was gently stirred in an ice/water mixture at 0 °C until full dissolution had occurred, then allowed to warm up to room temperature.

RAFT aqueous emulsion polymerization of MOEMA at pH 3 to produce an aqueous dispersion of PMOEMA latex particles

A typical protocol is described for the synthesis of an aqueous dispersion of PMOEMA₄₀ latex particles *via* RAFT aqueous emulsion polymerization of MOEMA using an MPETTC RAFT agent. A 25 mL round-bottomed flask was charged with MPETTC (78.2 mg, 173 μmol) and deionized water (14.4 g). The resulting aqueous solution was carefully adjusted to pH 3 using 1 M HCl (50 μL) to afford a 7.0% w/w orange solution. MOEMA (1.00 g, 693 μmol, [MOEMA]/[MPETTC] molar ratio = 40) and AIBA initiator (4.70 mg, 17.3 μmol, [MPETTC]/[AIBA] molar ratio = 10) was added to the reaction mixture. The flask was sealed with a rubber septum and the resulting aqueous emulsion was sparged with N₂ gas for 30 min before placing the flask in a pre-heated oil bath set at 56 °C. The initial pale-yellow reaction mixture became increasingly turbid, eventually affording a milky-white latex dispersion. After 4 h, the MOEMA polymerization was quenched by exposing the reaction mixture to air and cooling the flask to 20 °C. The MOEMA conversion was determined to be >99% by ¹H NMR spectroscopy and DMF GPC was employed to assess the molecular weight distribution of the PMOEMA chains. The latex particles were characterized using DLS, aqueous electrophoresis and TEM. Similar polymerizations were performed at 5.0, 7.5, 10, 15 and 20% w/w.

One-pot synthesis of a PMOEMA-PIPGMA latex via RAFT aqueous emulsion polymerization at pH 3

A typical protocol for the synthesis of an aqueous dispersion of PMOEMA₄₀-PIPGMA₂₇ latex particles *via* RAFT aqueous emulsion polymerization is described. A 25 mL round-bottomed flask was charged with MPETTC (78.2 mg, 173 μmol) and deionized water (14.4 g). The resulting aqueous solution was

adjusted to pH 3 using 1 M HCl (50 μ L) to afford a 7.0% w/w orange solution. MOEMA (1.00 g, 693 μ mol, [MOEMA]/[MPETTC] molar ratio = 40) and AIBA initiator (4.70 mg, 17.3 μ mol, [MPETTC]/[AIBA] molar ratio = 10.0) was added to produce an aqueous emulsion. The flask was sealed with a rubber septum and the reaction mixture was sparged with N₂ gas for 30 min before placing the flask in a pre-heated oil bath set at 56 °C. The initial pale-yellow solution became increasingly turbid, eventually affording a milky-white latex dispersion. In a separate vial, IPGMA was sparged with N₂ for 15 min. After 4 h, the degassed IPGMA (0.84 g, 422 μ mol, [IPGMA]/[PMOEMA₄₀] molar ratio = 27) was added to the PMOEMA₄₀ latex. After a further 4 h at 56 °C, the polymerization was quenched by exposure to air and cooling to 20 °C. The IPGMA conversion was determined to be 87% by ¹H NMR spectroscopy and DMF GPC was employed to assess the molecular weight distribution of the target PMOEMA₄₀-PIPGMA₂₇ diblock copolymer chains. The latex particles were characterized using DLS, aqueous electrophoresis and TEM.

Conversion of PMOEMA-PIPGMA latex into PMOEMA-PGMA nanoparticles via acid hydrolysis

The pH of an aqueous dispersion of PMOEMA₄₀-PIPGMA₂₇ latex particles (2.0 mL, 11% w/w) in a sample vial was adjusted to pH 1 using conc HCl. The initial milky-white dispersion was then heated to 70 °C and slowly transformed into a slightly turbid dispersion over 3 h. ¹H NMR spectroscopy studies confirmed that essentially all the acetone protecting groups were removed under these conditions, thus converting the hydrophobic PIPGMA block into a hydrophilic PGMA block. The resulting aqueous dispersion of PMOEMA₄₀-PIPGMA₂₇ nanoparticles was dialyzed against deionized water overnight to remove excess acid, salt and traces of acetone.

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