

Supporting Information for:

RAFT aqueous dispersion polymerization of 4-hydroxybutyl acrylate: effect of end-group ionization on the formation and colloidal stability of sterically-stabilized diblock copolymer nanoparticles

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Experimental

Materials

2-Phenylethanethiol, carbon disulfide (CS₂), 2-bromo-2-methylpropionic acid, *n*-hexane, sodium hydroxide (NaOH), ascorbic acid (AsAc), potassium persulfate (KPS), glutaraldehyde (GA) and KCl were purchased from Sigma Aldrich (UK). Hydrochloric acid (HCl, 35 % aqueous solution), ethyl acetate and DMF (HPLC grade) were purchased from VWR Chemicals (UK). Acetone, diethyl ether and anhydrous magnesium sulfate (MgSO₄) were purchased from Fisher Scientific (UK). Potassium phosphate tribasic (K₃PO₄) was purchased from Alfa Aesar (Heysham, UK) while CD₃OD was purchased from Cambridge Isotope Laboratories (UK). 2-Hydroxyethyl acrylate (HEA) monomer was purchased from Acros Organics (Geel, Belgium) and 4-hydroxybutyl acrylate (HBA) monomer was kindly donated by BASF (Ludwigshafen, Germany). Both these monomers were purified by solvent extraction prior to use, as detailed below. Azobis(2-[2-imidazolin-2-yl]propane) dihydrochloride (VA-044) initiator was purchased from FluoroChem (Glossop, UK) and 2,2'-azobis(2-methylpropionitrile) (AIBN) was purchased from Molekula (UK). Deionized water was obtained from an Elgastat Option 3A water purification unit and used for all experiments. Morpholine-functionalized PETTC RAFT agent (MPETTC) was kindly prepared in-house by Dr N. J. W. Penfold as previously described.¹ All chemicals were used as received unless specified.

Synthesis of 2-(phenylethylthiocarbonothioylthio)-2-methylpropanoic acid (PETMP) RAFT Agent

This RAFT agent synthesis was adapted from a previously reported protocol² using the principles described by Skey and O'Reilly.³ To a vigorously stirred suspension of K₃PO₄ (6.16 g, 29.0 mmol, 1.20 eq.) in acetone (65 mL), 2-phenylethanethiol (3.24 mL, 24.2 mmol) was added dropwise. After 30 min, CS₂ (3.64 mL, 60.5 mmol, 2.50 eq.) was added dropwise over 10 min, which led to the initial milky-white suspension becoming yellow. After stirring for 60 min at room temperature, 2-bromo-2-methylpropionic acid (4.04 g, 24.2 mmol) was added and the reaction mixture was stirred for a further 20 h. The reaction mixture was then filtered to remove any residual insoluble K₃PO₄ and the acetone removed under reduced pressure to yield an orange-yellow solid. This solid was dissolved in diethyl ether (65 mL) and washed with two portions of 3.2 M HCl (65 mL) followed by three washes with water (65 mL). The ether phase was dried over MgSO₄, filtered and the volatiles removed under reduced pressure to yield a yellow-orange solid. This was recrystallized from 1: 10 ethyl acetate: hexane to give bright yellow PETMP crystals (53% yield), which were subsequently ground to yield a fine powder, stirred in excess *n*-hexane overnight, filtered and dried under vacuum (34% final yield). Found C, 52.10; H, 5.40; S, 31.96; C₁₃H₁₆O₂S₃ required C, 51.97; H, 5.37; S, 32.01; ¹H NMR (400 MHz, CD₃OD, 298 K) δ_H (ppm): 1.71 (6H, CH₃), 2.90-3.00 (t, 2H, SCH₂CH₂Ph), 3.50-3.60 (t, 2H, SCH₂CH₂Ph), 7.17-7.35 (m, 5H, Ph); ¹³C NMR (400 MHz, CD₃OD, 298 K) δ_C (ppm) 25.8 (CH₃), 35.3 (CH₂Ph), 38.7 (SCH₂CH₂Ph), 57.2 (C(CH₃)₂), 127.6, 129.6, 141.0 (Ph), 176.2 (C=O), 223.0 (C=S). m/z (+ESI) 339.0 ([M+K]⁺), 323.0 ([M+Na]⁺), 301.0 ([M+H]⁺), 179.1, 163.0, 105.1.

Purification of HEA and HBA Monomers

To remove its diacrylate impurities, HEA monomer was extracted twenty times using an equal volume of *n*-hexane for each extraction. Residual *n*-hexane was removed under reduced pressure to

give purified HEA (89 % yield). The same protocol was used to purify HBA monomer (83 % yield). ¹H NMR (400MHz, CD₃OD, 298K, HEA) δ_H (ppm): 6.44 (1H, CH₂=CH, dd), 6.21 (1H, CH₂=CH, dd), 5.91 (1H, CH₂=CH, dd), 4.24 (2H, OCH₂CH₂OH, t), 3.78 (2H, OCH₂CH₂OH, t). ¹H NMR (400MHz, CD₃OD, 298K, HBA) δ_H (ppm): 6.39 (1H, CH₂=CH, dd), 6.17 (1H, CH₂=CH, dd), 5.89 (1H, CH₂=CH, dd), 4.20 (2H, OCH₂, t), 3.60 (2H, CH₂OH, t), 1.76 (2H, OCH₂CH₂, p), 1.63 (2H, CH₂CH₂OH, p).

One-Pot Synthesis of HOOC-PHEA₇₃-PHBA_y Diblock Copolymer Nano-Objects at 44 °C

Step 1. Preparation of the HOOC-PHEA₇₃ Precursor via RAFT Aqueous Solution Polymerization. HEA (2.51 g, 21.7 mmol), PETMP RAFT agent (0.093 g, 0.31 mmol) and VA-044 (20.0 mg, 0.062 mmol, CTA/VA-044 = 5.0) were dissolved in deionized water (1.7520 g) in a 15 mL vial to produce a 60% w/w solution. The vial was sealed and purged with nitrogen for 30 min before placing in a pre-heated oil bath at 44 °C. After continuous stirring at this temperature for 2.5 h, the HEA polymerization was quenched by exposing the reaction mixture to air while cooling to room temperature. ¹H NMR spectroscopy studies indicated a final HEA conversion of more than 99%. DMF GPC analysis indicated an M_n of 15,400 g mol⁻¹ and a M_w/M_n of 1.13. A small amount of HOOC-PHEA macro-CTA was precipitated three times into a ten-fold excess of diethyl ether, washed three times with diethyl ether and redissolved in the minimum amount of deionized water with volatiles being removed under reduced pressure. The resulting solution was then freeze-dried overnight to yield a film-forming yellow solid. ¹H NMR spectroscopy studies of the precipitate indicated a DP of 73 *via* end-group analysis, suggesting a RAFT agent efficiency of 95 %.

Step 2. Chain Extension of HOOC-PHEA₇₃ with HBA via RAFT Aqueous Dispersion Polymerization at pH 7. For a typical RAFT aqueous dispersion polymerization of HBA targeting HOOC-PHEA₇₃-PHBA₂₀₉ at 20% w/w solids and pH 7, the following protocol was followed: HBA (0.5364 g, 3.72 mmol), 60% w/w crude PHEA₇₃ macro-CTA solution (0.2602 g, 0.018 mmol) and VA-044 (1.2 mg, 3.56 μmol, macro-CTA/VA-044 = 5.0) were dissolved in deionized water (2.66 g) and adjusted to pH 7.0 using an aqueous solution of 0.1 M NaOH to produce a 20% w/w solution in a 15 mL vial. This vial was sealed and purged with nitrogen for 30 min before immersing in a pre-heated oil-bath at 44 °C. After stirring for 9 h, the HBA polymerization was quenched by exposing the contents of the vial to air while cooling to room temperature to yield a pale yellow, transparent viscous liquid. ¹H NMR spectroscopy studies indicated a final HBA conversion of more than 99% and a final PHBA DP of 217. DMF GPC analysis indicated an M_n of 50,800 g mol⁻¹ and a M_w/M_n of 1.31.

A range of PHBA DPs were targeted, with ¹H NMR spectroscopy studies indicating final HBA conversions of more than 99% in each case and final PHBA DPs of between 109 and 421, as summarized in Table S1.

One-Pot Synthesis of Mo-PHEA₇₆-PHBA₁₆₀ Diblock Copolymer Nanoparticles [Mo denotes 2-N-morpholinoethyl end-group]

Step 1. Preparation of the Mo-PHEA₇₆ Precursor via RAFT Aqueous Solution Polymerization at 70 °C. HEA (1.00 g, 8.61 mmol), MPETTC RAFT agent (0.0556 g, 0.12 mmol) and AIBN (4.0 mg, 0.025 mmol, CTA/AIBN = 5.0) were dissolved together in deionized water (0.7064 g) in a 15 mL vial to produce a 60% w/w solution. The vial was sealed and purged with nitrogen for 30 min before being

placed in a pre-heated oil bath at 70 °C. After continuous stirring at this temperature for 2.5 h, the HEA polymerization was quenched by exposure to air while cooling to room temperature. ¹H NMR spectroscopy studies indicated a final HEA conversion of 99%. DMF GPC analysis indicated an M_n of 15,800 g mol⁻¹ and a M_w/M_n of 1.15. A small amount of this Mo-PHEA macro-CTA was precipitated three times into a ten-fold excess of diethyl ether, washed three times with diethyl ether, redissolved in the minimum amount of deionized water and the volatiles were removed under reduced pressure. The resulting solution was then freeze-dried overnight to yield a film-forming yellow solid. ¹H NMR spectroscopy studies of the precipitate indicated a mean DP of 76 *via* end-group analysis, suggesting a RAFT agent efficiency of 91 %.

Step 2. Chain Extension of Mo-PHEA₇₆ with HBA at pH 3 via RAFT Aqueous Dispersion

Polymerization at 30 °C. HBA (0.5735 g, 3.98 mmol), 60% w/w crude Mo-PHEA₇₆ macro-CTA solution (0.41 g, 0.027 mmol) and AsAc (1.2 mg, 6.63 μmol, macro-CTA/AsAc = 4.0) were dissolved in deionized water (2.2185 g) and this reaction solution was adjusted to pH 3 using an aqueous solution of 0.1 M HCl in a 15 mL vial. This reaction vial was then sealed and purged with nitrogen for 30 min. KPS (1.8 mg, 6.63 μmol, macro-CTA/KPS = 4.0, prepared as a 2.0 g dm⁻³ solution) was degassed with nitrogen separately for 30 min. The reaction vial was immersed in a pre-heated oil bath set at 30 °C then the KPS solution was added to initiate the polymerization, targeting a final solids concentration of 20% w/w. The reaction was quenched after 3.5 h by exposing the contents of the vial to air while cooling to room temperature, yielding a pale yellow, transparent liquid. ¹H NMR spectroscopy studies indicated a final HBA conversion of more than 99% and a mean PHBA DP of 160. DMF GPC analysis indicated an M_n of 42,700 g mol⁻¹ and a M_w/M_n of 1.35.

Post-Polymerization Crosslinking with Glutaraldehyde

1 M NaOH (20 μL) was added to an aqueous solution of glutaraldehyde (GA, 0.50 g, 50% w/w in water) to adjust the solution pH from pH 3 to pH 7.

Crosslinking concentrated copolymer dispersions. A 10% w/w aqueous dispersion of HOOC-PHEA₇₃-PHBA₁₃₆ nanoparticles (pH 7, 0.20 g, 95 μmol HBA) and the aqueous GA solution (50% w/w at pH 7, 17 mg, 63 μmol, GA/HBA molar ratio = 0.66) were combined in a 7 mL glass vial. The ~10% w/w reaction mixture was stirred for 16 h at 20 °C before being diluted with deionized water (pH 7, final copolymer concentration = 0.05% w/w) prior to TEM studies.

A 20% w/w aqueous dispersion of HOOC-PHEA₇₃-PHBA₂₄₃ nanoparticles (equilibrated at 40 °C for 4 h, 0.2 g, 221 μmol HBA) was combined with GA (146 μmol GA, GA/HBA molar ratio = 0.66). This ~20% w/w dispersion was then sequentially diluted (75 mg deionized water, 8 additions, 1 every 15 min) to 5% w/w at 40 °C over a 2 h period. This 5% w/w dispersion was then stirred for 1 h at 40 °C before being diluted with deionized water (pH 7, final copolymer concentration = 0.05% w/w) prior to TEM studies.

A 10% w/w aqueous dispersion of HOOC-PHEA₇₃-PHBA₄₂₁ nanoparticles (0.2 g, 121 μmol HBA) was combined with GA (80 μmol, GA/HBA molar ratio = 0.66) and stirred for 16 h at 20 °C before being diluted with deionized water (pH 7, final copolymer concentration = 0.05% w/w) prior to TEM studies.

Crosslinking dilute copolymer dispersions. To evaluate their morphology after dilution, 20% w/w aqueous dispersions of HOOC-PHEA₇₃-PHBA_x nano-objects (x = 136, 243 or 421; 0.01 g; 10, 11 or 12 μmol HBA, respectively) were diluted with deionized water (pH 7, final copolymer concentration =

0.1% w/w) prior to GA addition (pH 7.0; 6, 7 or 8 μmol GA, respectively; GA/HBA molar ratio = 0.66). Crosslinking of HOOC-PHEA₇₃-PHBA₂₄₃ nanoparticles was conducted at 40 °C (with 4 h being allowed for thermal equilibration) with continuous stirring for 16 h. The HOOC-PHEA₇₃-PHBA₁₃₆ and HOOC-PHEA₇₃-PHBA₄₂₁ dispersions were reacted with GA at 20 °C with continuous stirring for 16 h.

Characterisation Methods

¹H NMR Spectroscopy. ¹H NMR spectra were recorded in CD₃OD at 298 K using a 400 MHz Bruker Avance-400 spectrometer (64 scans averaged per spectrum).

Gel Permeation Chromatography (GPC). GPC analysis was used to assess the copolymer molecular weight distributions. This was conducted at 60 °C using HPLC-grade DMF eluent containing 10 mM LiBr at a flow rate of 1.0 mL min⁻¹. The GPC instrument comprised an Agilent 1260 Infinity GPC system with a PL guard column and two Agilent PL gel 5 μm MIXED-C columns connected in series, a RI detector and a variable wavelength detector set to 298 nm. Calibration was performed using ten near-monodisperse PMMA standards with M_p values ranging between 625 and 618,000 g mol⁻¹.

Variable Temperature UV-Visible Spectroscopy. Spectra were recorded for 1.0% w/w aqueous solutions of PHEA macro-CTAs with DPs of 49, 72 and 100 at pH \sim 2.5 and pH \sim 7. The absorbance was recorded between 200 and 800 nm at a range of temperatures between 25 and 90 °C (the latter temperature corresponds to the upper limit for the instrument set-up). A PC-controlled UV-1800 spectrophotometer was used in conjunction with a twin Peltier controller unit and a quartz cuvette sample holder. Transmittance was calculated from the normalized absorbance using a path length of 1.0 cm.

Dynamic Light Scattering (DLS). Measurements were performed on 0.10% w/w aqueous dispersions diluted with deionized water (ultrafiltered with a 0.20 μm filter and adjusted to the desired pH using either dilute HCl or NaOH) using a Malvern Zetasizer NanoZS instrument equipped with a 4 mW He-Ne laser ($\lambda = 633$ nm) and an avalanche photodiode detector. Measurements were recorded using disposable plastic cuvettes at 25 °C using a fixed scattering angle of 173°. The Stokes-Einstein equation was used to calculate z-average hydrodynamic diameters, which were averaged over three consecutive runs comprising ten measurements per run.

Aqueous Electrophoresis. Zeta potentials were determined at 25 °C using the same Malvern Zetasizer NanoZS instrument. These measurements were performed on 0.10% w/w aqueous dispersions containing 1 mM KCl as a background electrolyte, with the dispersion pH being adjusted using either dilute HCl or NaOH as required. Zeta potentials were calculated from the Henry equation using the Smoluchowski approximation.

Small Angle X-ray Scattering (SAXS). SAXS patterns were recorded using a laboratory-based Xeus 2.0 beamline (Xenocs, Grenoble, France) equipped with a liquid gallium MetalJet X-ray source (Excillum, Kista, Sweden; $\lambda = 0.134$ nm) and a Pilatus 1M pixel detector (Dectris, Baden, Switzerland). The instrument was set up to resolve a q range of 0.003 to 0.11 \AA^{-1} , where q is the length of the scattering vector (i.e. $q = 4\pi \cdot \sin\theta/\lambda$ and θ is one-half of the scattering angle). Measurements were conducted on 1.0% w/w aqueous copolymer dispersions diluted from 20% w/w using deionized water and then placed within 2.0 mm diameter capillary cells. The resulting two-dimensional

scattering patterns were calibrated and reduced to one-dimensional curves using Irena SAS macro for Igor Pro.⁴

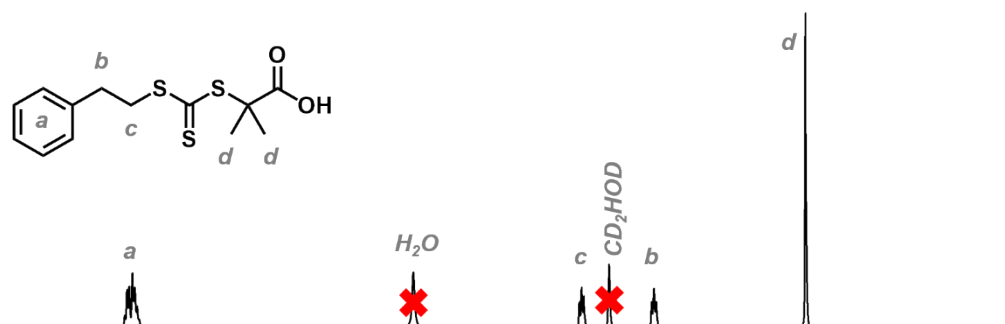
Transmission Electron Microscopy (TEM). Dispersions crosslinked with GA at low concentration were dried at 0.1% w/w while more concentrated dispersions crosslinked with GA were sequentially diluted to 0.05% w/w using deionized water (adjusted to pH 7). Copper-palladium TEM grids were surface-coated with a thin film of carbon before undergoing plasma glow-discharge for 30 s to produce a hydrophilic surface. Dilute aqueous dispersions of crosslinked HOOC-PHEA₇₃-PHBA_y nanoparticles were deposited onto each grid for 40 s before blotting to remove excess sample. 0.75 % w/v aqueous uranyl formate solution was deposited as a negative stain for 20 s prior to careful blotting and drying with a vacuum hose. Imaging was performed at 100 kV using a Philips CM100 instrument equipped with a Gatan 1k CCD camera.

Shear-Induced Polarized Light Imaging (SIPLI). SIPLI experiments were conducted on as-synthesized 20% w/w aqueous dispersions of diblock copolymer nano-objects at 25 °C using an Anton Paar Physica MCR301 mechano-optical rheometer equipped with a SIPLI attachment and variable temperature Peltier heaters incorporated in both the bottom plate and the hood. An Edmund Optics 150W MI-150 high-intensity fibre-optic white light source was used for sample illumination. The polarizer was oriented at a 90° angle to the analyzer to produce polarized light images, which were recorded using a Lumenera Lu165c color CCD camera. All measurements were conducted using a plate-plate geometry consisting of a fused quartz bottom plate and a 25 mm polished steel disk fixture with a fixed sample thickness of 1.0 mm. Once loaded, samples were allowed to equilibrate for 5 min. Angular speed (shear rate at the sample edge) sweeps were conducted from low to high speed in stages: (1) 0.0008-0.08 rad s⁻¹ (0.01-1 s⁻¹), (2) 0.08-0.4 rad s⁻¹ (1-5 s⁻¹), (3) 0.4-1.6 rad s⁻¹ (5-20 s⁻¹) and (4) 1.6-8.0 rad s⁻¹ (20-100 s⁻¹), with 5 min intervals between each sweep to allow the sample to relax while stationary. Each angular speed experiment was conducted over 500 s and involved a linear ramp with a fixed 5 s measure point duration, with a polarized light image being recorded at selected points.

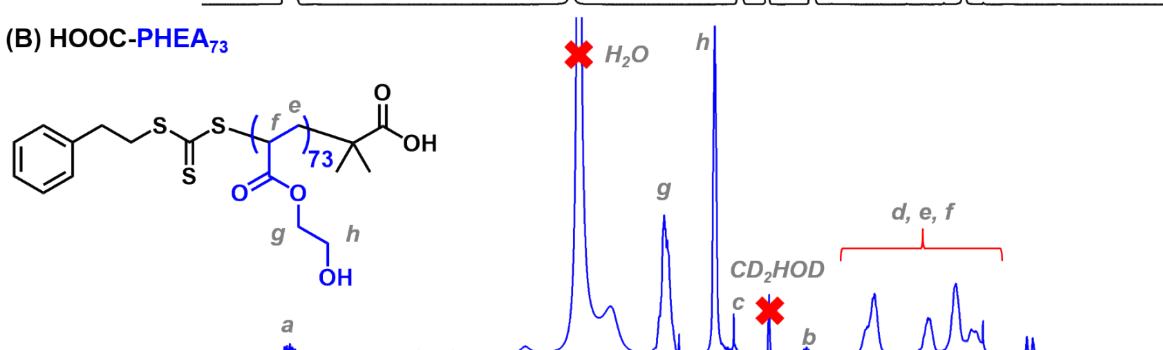
Variable Temperature Oscillatory Rheology. Measurements were conducted on as-synthesized 20% w/w aqueous dispersions of nanoparticles using an AR-G2 rheometer equipped with a bottom plate including variable temperature Peltier heater and a 40 mm 2° aluminum cone fixture. The rheometer was operated in oscillatory mode to record G' , G'' and $|\eta^*|$ as a function of temperature at an applied strain amplitude of 1.0 % and an angular frequency of 1.0 rad s⁻¹. Thermal cycles were conducted at 2 °C intervals between 2 and 50 °C (or between 2 and 70 °C if appropriate), allowing 3 min for thermal equilibration at each temperature. Each sample was equilibrated at 2 °C for 20 min prior to the initial heating cycle.

Supporting Figures, Schemes and Tables

(A) PETMP



(B) HOOC-PHEA₇₃



(C) HOOC-PHEA₇₃-PHBA₂₁₇

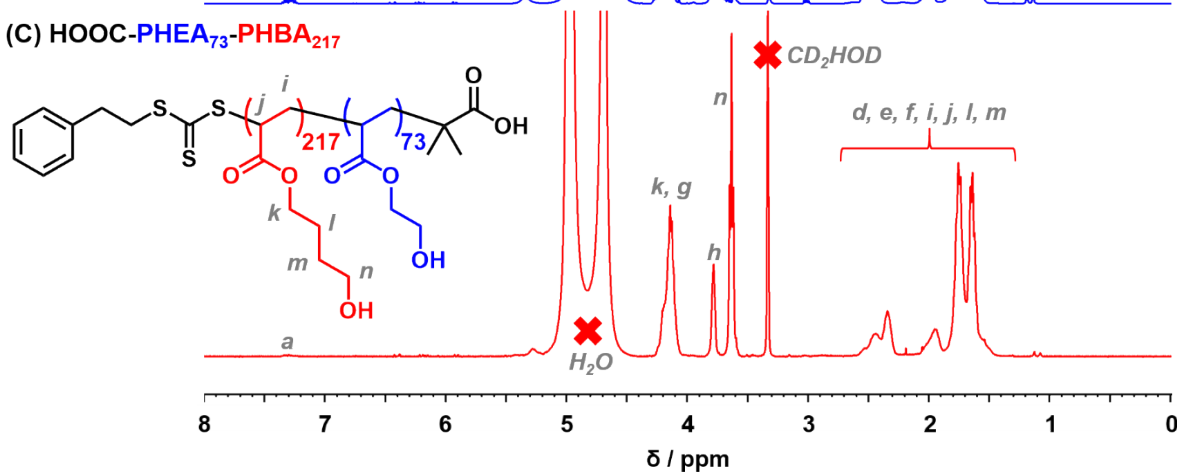


Fig. S1 Assigned ^1H NMR spectra recorded in CD_3OD for (A) the PETMP RAFT agent (B) the HOOC-PHEA₇₃ precursor and (C) the HOOC-PHEA₇₃-PHBA₂₁₇ diblock copolymer prepared by the one-pot protocol outlined in Scheme 1, indicating high monomer conversions for both blocks.

Table S1 Summary of the DMF GPC data (refractive index detector; expressed relative to PMMA calibration standards) and DLS z-average diameters, D_h (and polydispersities, PDI) obtained for a series of HOOC-PHEA₇₃-PHBA_y diblock copolymers prepared at pH 7 using the ‘one-pot’ protocol outlined in Scheme 1. Final PHBA DPs were determined by ¹H NMR analysis in CD₃OD. DLS studies were conducted on 0.1% w/w aqueous dispersions after dilution from 20% w/w dispersions using ultrafiltered deionized water adjusted to pH 7.

PHBA DP	$M_n / \text{g mol}^{-1}$	$M_w / \text{g mol}^{-1}$	M_w/M_n	D_h / nm	PDI
0 (HOOC-PHEA ₇₃)	15,400	17,300	1.13	-	-
109	34,500	42,000	1.22	14	0.15
136	37,900	46,800	1.23	18	0.12
163	43,300	54,500	1.26	24	0.08
189	47,000	60,100	1.28	25	0.06
217	50,800	66,600	1.31	28	0.04
243	56,000	74,900	1.34	31	0.03
265	60,900	82,400	1.35	33	0.04
289	65,800	90,500	1.38	35	0.03
316	67,200	93,400	1.39	38	0.04
340	69,700	100,400	1.44	42	0.02
365	75,300	111,300	1.48	45	0.02
421	78,400	122,400	1.56	52	0.01

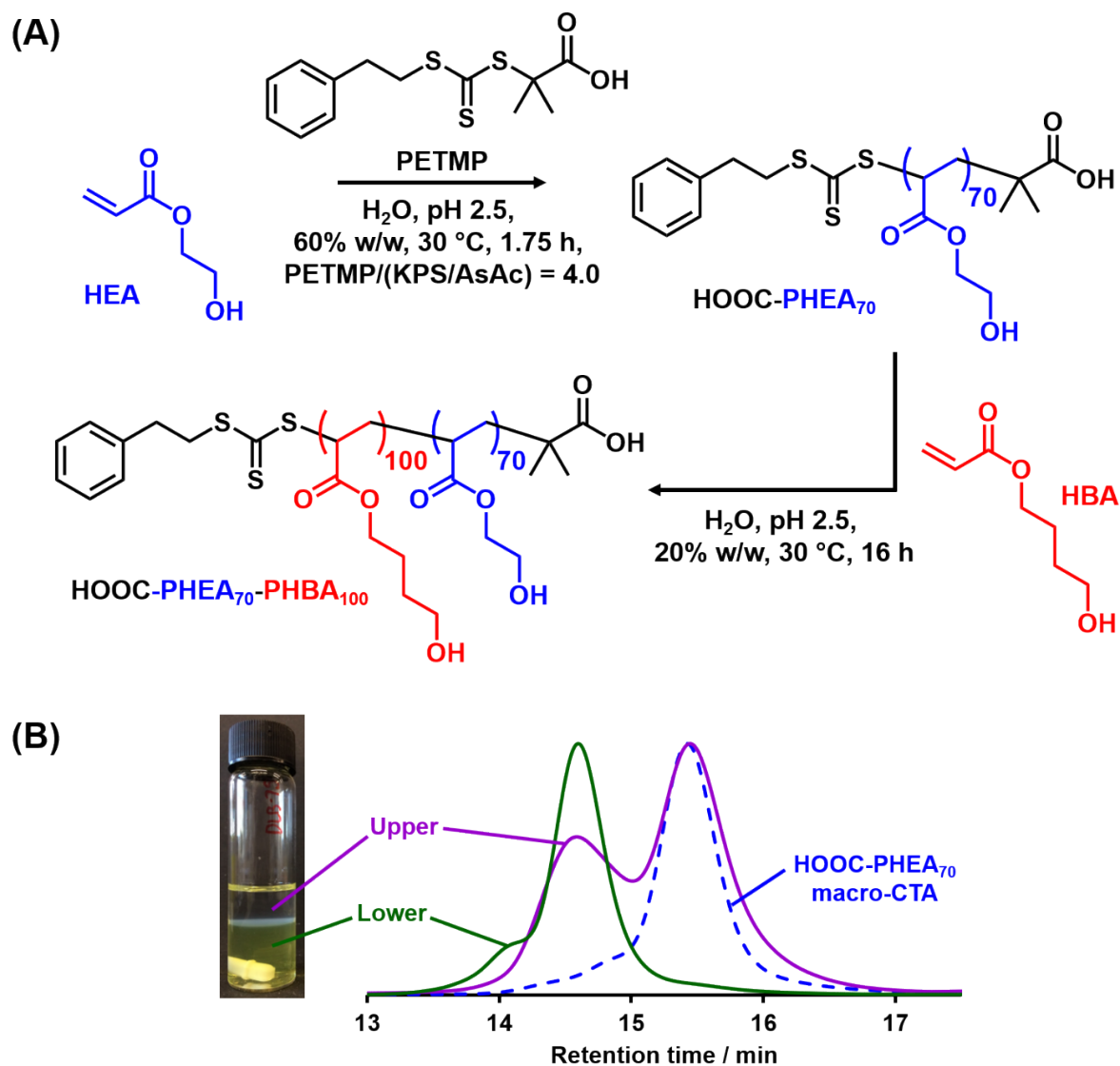


Fig. S2 (A) Reaction scheme outlining an attempted one-pot PISA synthesis in aqueous media targeting HOOC-PHEA₇₀-PHBA₁₀₀ at pH 2.5. First, the HOOC-PHEA₇₀ precursor was prepared *via* RAFT aqueous *solution* polymerization of HEA at 30 °C using an KPS/AsAc redox initiator and PETMP RAFT agent at 60% w/w solids. Subsequent chain extension of the HOOC-PHEA₇₀ macro-CTA was conducted *via* RAFT aqueous *dispersion* polymerization of HBA targeting 20% w/w solids. (B) Corresponding DMF GPC traces recorded for the HOOC-PHEA₇₀ precursor (blue dotted trace), and the upper (purple trace) and lower (green trace) phase-separated fractions of the aqueous dispersion of diblock copolymer nano-objects prepared by route (A). The digital photograph illustrates the visual appearance of the resulting colloidally unstable aqueous copolymer dispersion.

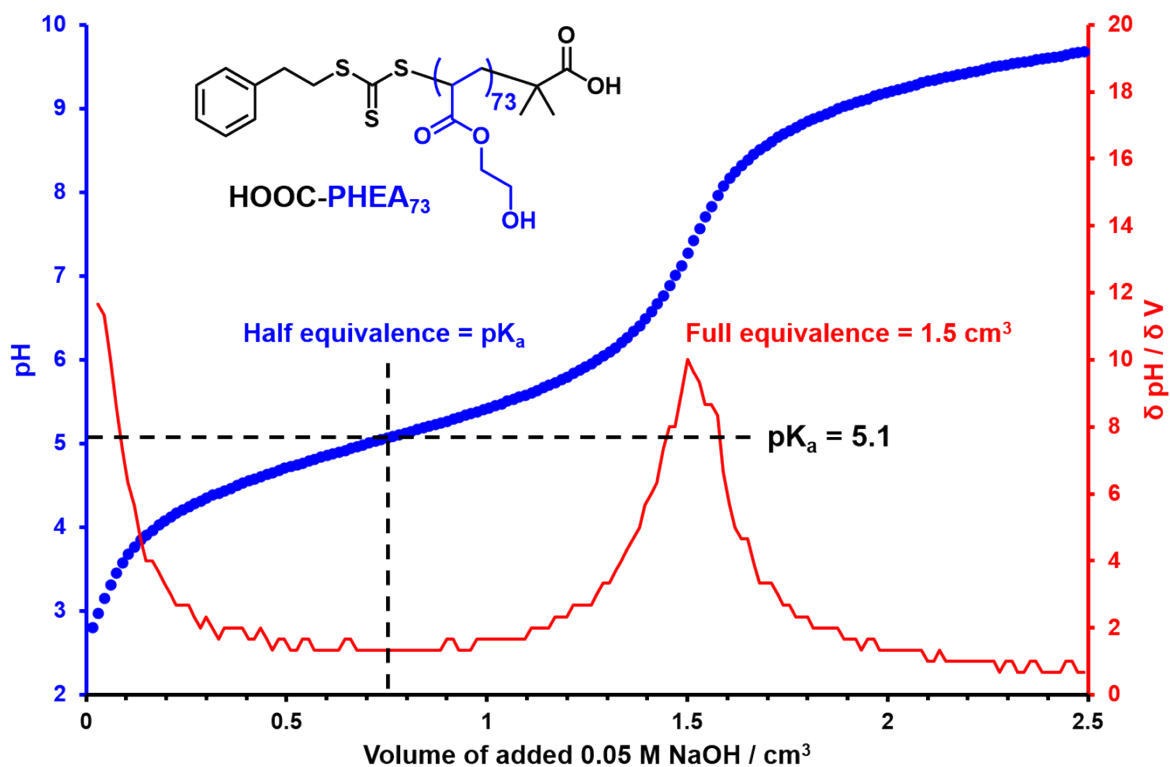


Fig. S3 Representative acid titration curve recorded for a 30% w/w aqueous solution of HOOC-PHEA₇₃. A mean pK_a of 5.1 was calculated based on three acid titrations. In each case, the solution pH was adjusted using an aqueous solution of 0.05 M NaOH added in 15 μ L increments using a micropipette.

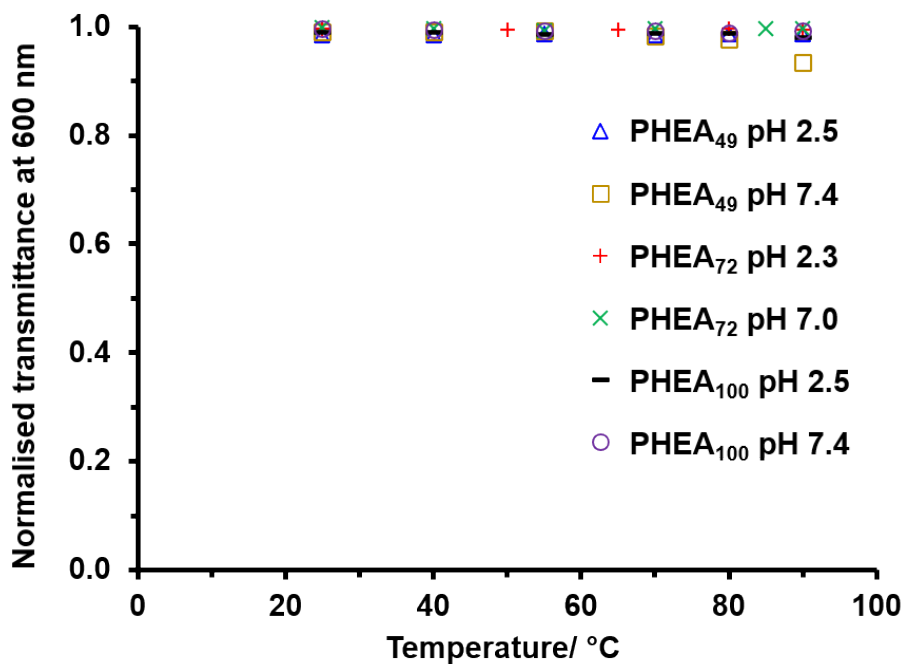


Fig. S4 Temperature dependence of the normalized transmittance recorded at 600 nm for 1.0% w/w aqueous solutions of HOOC-PHEA_x precursors ($x = 49, 72$ or 100) at either pH 2.3-2.5 or pH 7.0-7.4.

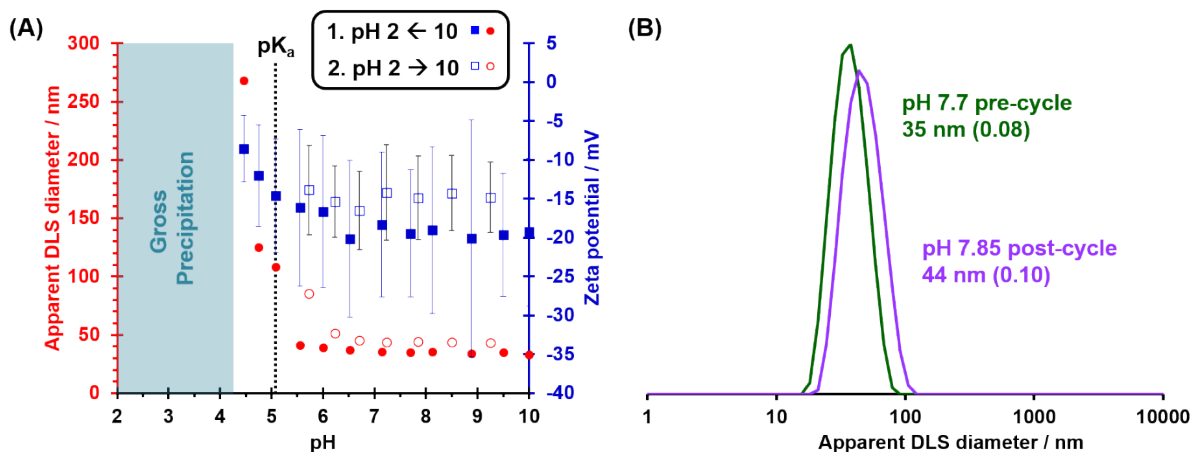


Fig. S5 (A) Variation in apparent z-average diameter (red circles) and zeta potential (blue squares) with solution pH determined for a 0.1% w/w aqueous dispersion of HOOC-PHEA₇₃-PHBA₂₁₇ nanoparticles at 25 °C in the presence of 1 mM KCl as background salt. Measurements were performed by first titrating from pH 10 to pH 2 (filled symbols) using aqueous HCl (0.01-0.1 M as required), before titrating back up to pH 10 (empty symbols) using aqueous NaOH (0.01-0.1 M as required). The data indicate reasonably good reversibility regarding the flocculation and subsequent redispersion of such nanoparticles. (B) DLS particle size distributions recorded for HOOC-PHEA₇₃-PHBA₂₁₇ nanoparticles at pH 7.7 prior to flocculation (green trace) and at pH 7.85 after redispersion (purple trace), indicating comparable DLS diameters and polydispersities.

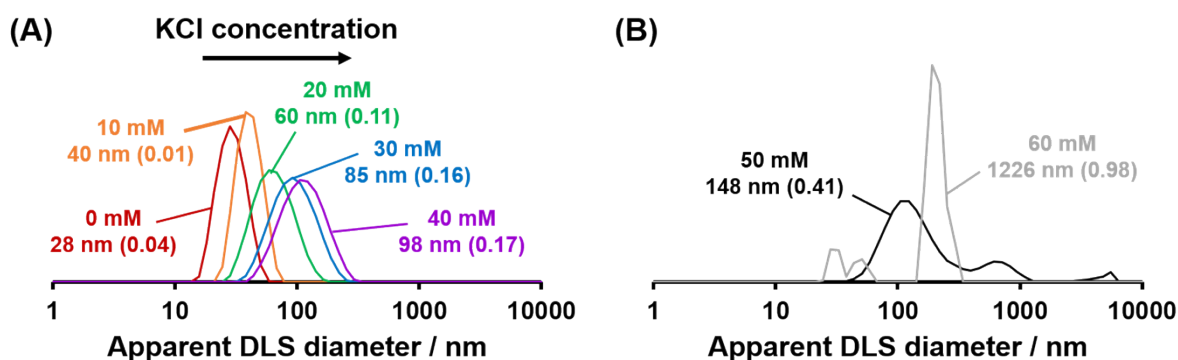


Fig. S6 (A) DLS particle size distributions recorded for a 0.1% w/w aqueous dispersion of HOOC-PHEA₇₃-PHBA₂₁₇ nanoparticles at pH 7 and various KCl concentrations; these data indicate that incipient flocculation can occur in the presence of just 10 mM KCl. (B) Multimodal DLS particle size distributions observed in the presence of 50 mM and 60 mM KCl.

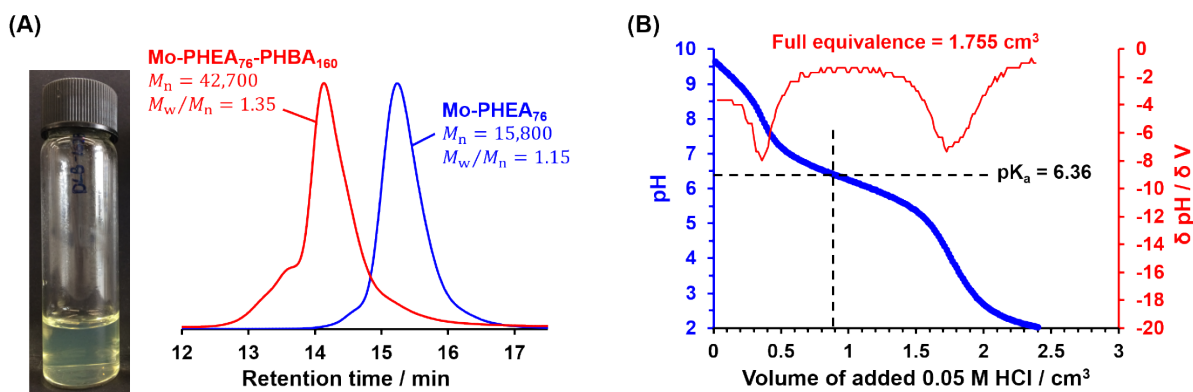


Fig. S7 (A) DMF GPC traces recorded for the Mo-PHEA₇₆ precursor (blue trace) and Mo-PHEA₇₆-PHBA₁₆₀ diblock copolymer chains (red trace) prepared using the one-pot protocol outlined in Fig. 5A, indicating high blocking efficiency and reasonably low final dispersities. The digital photograph illustrating the physical appearance of the as-synthesized 20% w/w aqueous dispersion of Mo-PHEA₇₆-PHBA₁₆₀ nanoparticles at pH 3. (B) Acid titration curve recorded for a 30% w/w aqueous solution of Mo-PHEA₇₆ macro-CTA, indicating a pK_a of 6.36 (average of two experiments). The aqueous solution pH was adjusted by adding 0.05 M HCl in 15 μL increments.

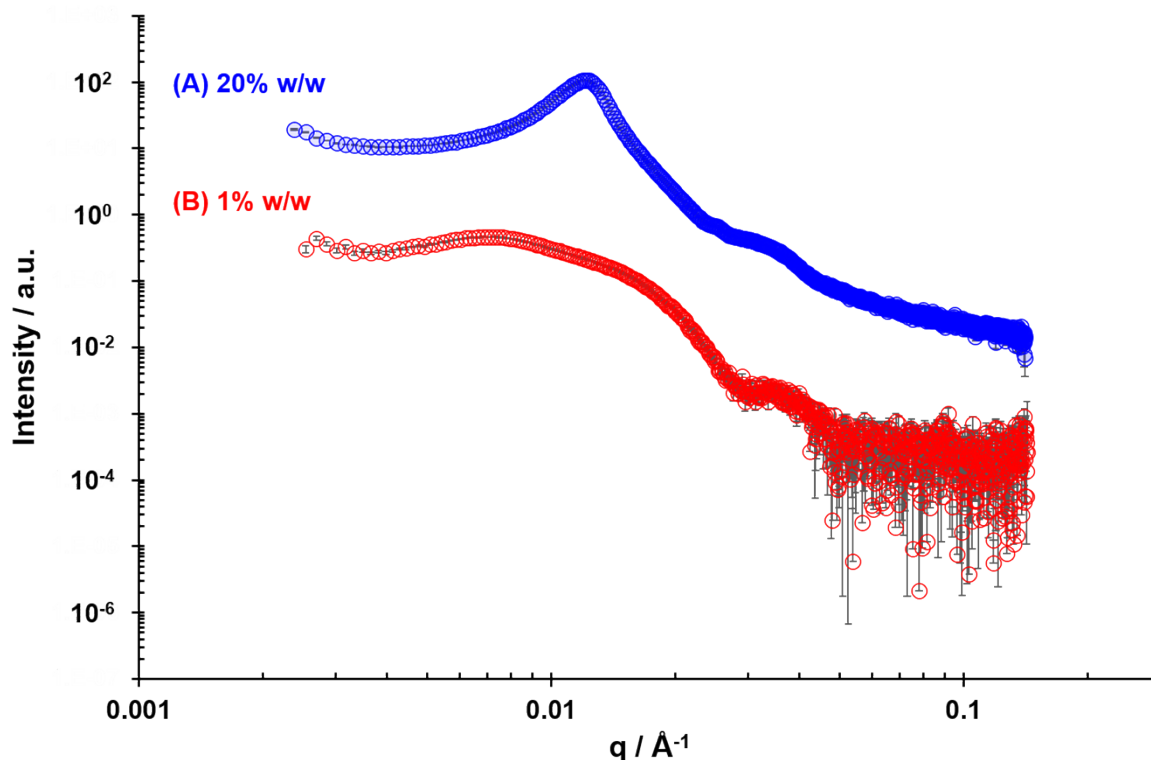
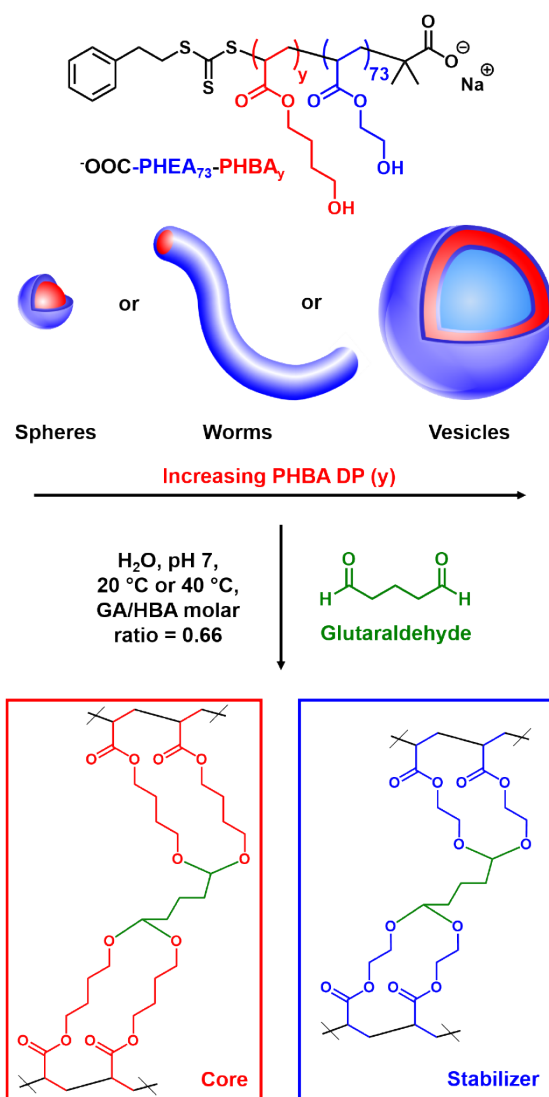


Fig. S8 Small-angle X-ray scattering patterns recorded for an aqueous dispersion of HOOC-PHEA₇₀-PHBA₃₀₀ nano-objects at pH 7.0: (A) 20% w/w solids (as synthesized, blue symbols) and (B) 1% w/w solids (red symbols). The pronounced structure factor is attributed to strong interparticle interactions (A and B) and the relatively high copolymer concentration (A). This feature complicates the data analysis.



Scheme S1 Reaction scheme for the covalent stabilization of HOOC-PHEA₇₃-PHBA_y nano-objects at pH 7 and various copolymer concentrations (0.1, 10 or 20% w/w) using excess glutaraldehyde (GA; GA/HBA molar ratio = 0.66). In principle, GA can react with the pendent hydroxyl groups present in both the PHEA and PHBA blocks, resulting in both core and shell crosslinking under such conditions.

Table S2 Summary of the number-average TEM diameters and TEM morphology assignments for three aqueous dispersions of HOOC-PHEA₇₃-PHBA_y diblock copolymer nanoparticles crosslinked using GA at pH 7 at various copolymer concentrations.

PHBA DP (y)	Copolymer concentration / % w/w	Number-average TEM diameter / nm	TEM morphology
136	0.1	28 ± 6	Spheres
	10	24 ± 9	Spheres
243	0.1	41 ± 8	Spheres
	20	480 ± 285 ^a	Worms
421	0.1	76 ± 19	Spheres
	10	1103 ± 1054	Vesicles

^a corresponds to the mean worm contour length, with an associated worm width of 55 ± 17 nm.

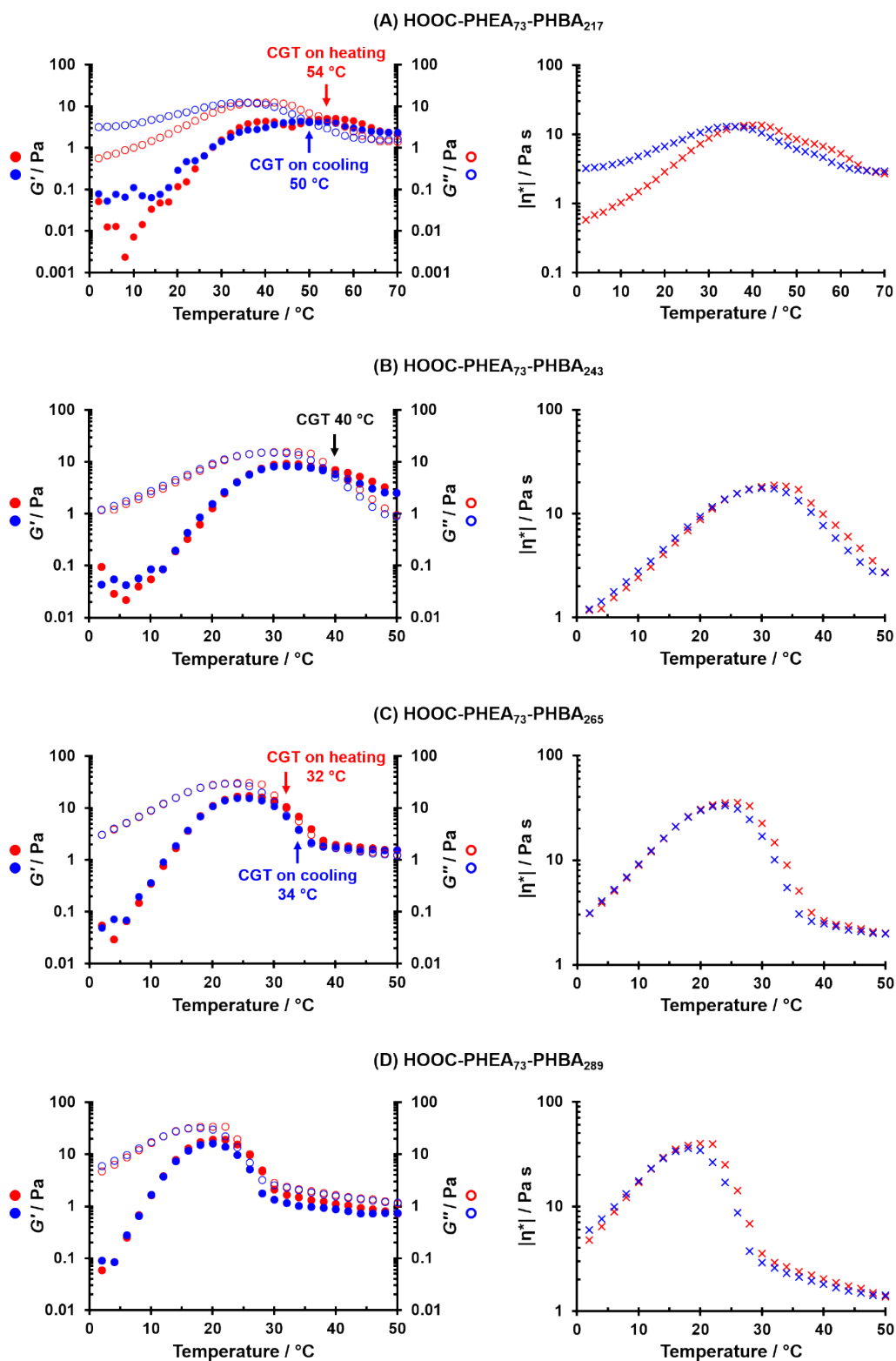


Fig. S9 Variable temperature oscillatory rheology studies obtained on first heating (red symbols) and then cooling (blue symbols) 20% w/w aqueous dispersions of (A) HOOC-PHEA₇₃-PHBA₂₁₇, (B) HOOC-PHEA₇₃-PHBA₂₄₃, (C) HOOC-PHEA₇₃-PHBA₂₆₅ (data replicated from Fig. 10F) and (D) HOOC-PHEA₇₃-PHBA₂₈₉ nanoparticles. Dispersions were equilibrated for 20 min at 2 °C prior to heating, with G' (closed circles), G'' (open circles) and $|\eta^*|$ (crosses) recorded at 2 °C intervals with 3 min being allowed for thermal equilibration at each temperature. These heating and cooling cycles were conducted at an applied strain amplitude of 1.0% and an angular frequency of 1.0 rad s⁻¹.

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