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Electronic Supporting Information for:

Thermoreversible poly(ethylene glycol) worm gels for biomedical applications

Nicholas J. W. Penfold,[1,*] Jessica Whatley,[1] and Steven P. Armes[1,*]

[1]Department of Chemistry, Dainton Building, University of Sheffield, Brook Hill, Sheffield, S3 7HF.

EXPERIMENTAL

Materials

PETTC RAFT agent was synthesized in-house. *N*-*N′*-dicyclohexylcarbodiimide (DCC; > 99%), 4-dimethylaminopyridine (DMAP; >99%), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AIBA, >99%) poly(ethylene glycol) monomethyl ether (PEG_{45}, M_n= 2.0 kg mol^{-1} and PEG_{113}, M_n= 5.0 kg mol^{-1}) were purchased from Sigma-Aldrich. 2-Hyxdroxypropyl methacrylate (HPMA) was purchased from Alfa Aesar. 2,2'-Azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044; >99%) was purchased from Wako Chemicals Ltd. Anhydrous dichloromethane was obtained from an in-house Grubbs purification solvent system. Deionized water was obtained from an Elgastat Option 3A water purification unit with a resistivity of 15 MΩ cm. All other chemicals or solvents (HPLC grade) were purchased from either VWR, Sigma-Aldrich or Fischer and used as received.

Synthesis of PETTC RAFT Agent

PETTC was synthesized according to a previously reported protocol.[1] A 1 L conical flask was charged with magnetic stirrer bar, sodium hydride (60% in oil, 7.0 g, 175 mmol) and 400 mL diethyl ether. 2-Phenylethanethiol (21.6 g, 156 mmol) was added dropwise to the stirred grey suspension, which turned white after 2 h. Carbon disulfide (13.5 g, 177 mmol) was added dropwise and a yellow precipitate of 2-phenylethanetrithiocarbonate was formed over 2 h, collected *via* vacuum filtration and dried overnight in a vacuum oven set at 30 °C. Solid iodine (23.0 g, 90.6 mmol) was added to the suspension of the 2-phenylethanetrithiocarbonate (35.7 g, 151 mmol) in 400 mL diethyl ether. After 1.5 h stirring at room temperature the resulting white precipitate of sodium iodide was removed via filtration. The brown filtrate was washed with saturated sodium thiosulfate solution (4 x 150 mL), dried over magnesium sulfate and the solvent was removed under reduced pressure to give bis-(2-phenyl ethane sulfanylthiocarbonyl)disulphide (32.0 g, 75 mmol). A 1 L two-neck round-bottom flask equipped with magnetic stirrer was charged with bis-(2-phenyl ethane sulfanylthiocarbonyl)disulphide (32.0 g, 75 mmol), ACVA (32.0 g, 114 mmol) and ethyl acetate (500 mL). The mixture was purged with nitrogen for 45 min then refluxed under nitrogen atmosphere overnight. The resulting orange solution was washed water (4 x 200 mL), dried over magnesium sulfate and all volatiles were removed under reduced pressure. The crude product was purified by a column chromatography using silica gel as the stationary phase and a mobile phase of 100% dichloromethane and then changing to dichloromethane:ethanol (95:5 v/v) after the first fraction has been removed to yield PETTC as an orange oil (yield 71.6%). ^{1}H NMR (400 MHz, CD_2Cl_2, 25 °C): δ 1.91 (s, 3H, $–(CN)C\mathbf{H_3}$), 2.40–2.62 (m, 2H, $–(CH_3)(CN)\text{-}C\mathbf{H_2}CH_2C(=O)OH$), 2.64-2.87 m, 2H, $–(CH_3)(CN)\text{-}CH_2C\mathbf{H_2}C(=O)OH$), 3.02–3.06 (t, 2H, $–PhC\mathbf{H_2}CH_2S(C=S)S$), 3.60–3.66 (t, 2H, $–PhCH_2C\mathbf{H_2}S(C=S)S$), 7.25–7.40 (m, 5H, $–\mathbf{Ph}CH_2CH_2S(C=S)S$). ^{13}C NMR (400 MHz, $CDCl_3$, 25 °C): δ 24.9 ($\mathbf{C}H_3$), 29.5 ($CH_2\mathbf{C}H_2C(=O)OH$), 33.5 ($PhCH_2\mathbf{C}H_2S$), 34.0 ($\mathbf{C}H_2CH_2C(=O)OH$), 38.0 ($Ph\mathbf{C}H_2CH_2S$), 46.3 ($S\mathbf{C}(CH_3)(CN)CH_2$), 118.9 ($SC(CH_3)(\mathbf{C}N)CH_2$), 126.9-128.6, 139.2 ($\mathbf{Ph}CH_2$), 177.1 ($\mathbf{C}=O$), 216.4 ($\mathbf{C}=S$). MS (ES$^+$) m/z calcd: 339.0 Found: 339.0 Anal. Calcd for $C_{15}H_{17}NO_2S_3$: C, 53.07; H, 5.05; N, 4.13; S, 28.33 Found: C, 53.02; H, 5.72; N, 3.88; S, 27.21.

Synthesis of poly(ethylene glycol)$_{45}$ (PEG$_{45}$) macromolecular chain transfer agent

All glassware was dried in a 200 °C oven for 24 h prior to use. A 1 L round-bottom flask was charged with poly(ethylene glycol) monomethyl ether (M_n = 2000 g mol^{-1}, 40.4 g, 20.2 mmol) and toluene (800 mL). Approximately 500 mL of toluene was distilled off under a dry nitrogen atmosphere, cooled to room temperature and then dichloromethane (ca. 200 mL was added until dissolution occurred). A second 25 mL round-bottom flask was charged with DCC (12.5 g, 60.6 mmol), DMAP (0.247 g, 2.02 mmol) and DCM (10 mL) and then added to the PEG solution dropwise *via* cannula transfer under a nitrogen atmosphere. A third 25 mL round-bottom flask was charged with PETTC (10.28 g, 30.3 mmol) and DCM (10 mL) and then slowly added to PEG solution, which was then stirred at room temperature. After 16 h the reaction solution was filtered and then concentrated under vacuum. Isolation was achieved *via* precipitation into 2 L of cold diethyl ether (-78 °C). This precipitation protocol was repeated twice more to isolate PEG$_{45}$-PETTC as a fine, yellow powder which was dried in a vacuum oven set at 40 °C. ^{1}H NMR end-group analysis indicated a mean degree of esterification of 94%. THF GPC analysis indicated an M_n of 2,400 g mol^{-1} and M_w / M_n of 1.04 relative to PEG standards.

Synthesis of poly(ethylene glycol)$_{113}$ (PEG$_{113}$) macromolecular chain transfer agent

All glassware was dried in a 200 °C oven for 24 h prior to use. A 1 L round-bottom flask was charged with poly(ethylene glycol) monomethyl ether (M_n = 5000 g mol^{-1}, 39.9 g, 7.98 mmol) and toluene (800 mL). Approximately 500 mL of toluene was distilled off under a dry nitrogen atmosphere, cooled to room temperature and then dichloromethane (ca. 200 mL was added until dissolution occurred). A second 25 mL round-bottom flask was charged with DCC (4.94 g, 23.9 mmol), DMAP (0.0975 g, 0.798 mmol) and DCM (10 mL) and then added to the PEG solution dropwise *via* cannula transfer under a nitrogen atmosphere. A third

25 mL round-bottom flask was charged with PETTC (4.07 g, 12.0 mmol) and DCM (10 mL) and then slowly added to PEG solution, which was then stirred at room temperature. After 16 h the reaction solution was filtered and then concentrated under vacuum. Isolation was achieved *via* precipitation into 2 L of diethyl ether at room temperature. This precipitation protocol was repeated twice more to isolate PEG$_{113}$-PETTC as a fine, yellow powder which was dried in a vacuum oven set at 40 °C. ^{1}H NMR end-group analysis indicated a mean degree of esterification of 95%. THF GPC analysis indicated an M_n of 5,800 g mol^{-1} and M_w / M_n of 1.03 relative to PEG standards.

Synthesis of [x PEG$_{45}$ + z PEG$_{113}$] – PHPMA$_n$ block copolymer nanoparticles *via* RAFT aqueous dispersion polymerization of HPMA

A typical RAFT dispersion polymerization of [0.70 PEG$_{45}$ + 0.30 PEG$_{113}$] – PHPMA$_{200}$ at 10% w/w was conducted as follows. A sample vial was charged with PEG$_{45}$ macro-CTA (0.020 g, 8.12 μmol), PEG$_{113}$ macro-CTA (0.020 g, 3.54 μmol), HPMA (0.335 g, 2.32 mmol), VA-044 initiator (7.47 x 10^{-4} g, 2.31 μmol) and deionized water (3.377 g). This was sealed, cooled in ice and purged with nitrogen for 25 minutes. The resultant de-oxygenated solution was submerged in an oil bath at 50 °C for 4 hours. Polymerization was quenched by exposing to air and cooling to room temperature. A monomer conversion of > 99% was determined *via* ^{1}H NMR studies. THF GPC studies indicate an M_n of 34.7g mol^{-1} and an M_w / M_n of 1.15. A series of [x PEG$_{45}$ + z PEG$_{113}$] – PHPMA$_y$ block copolymers were prepared at 10% w/w by systematically varying *x, z,* and n. Post-mortem TEM analysis was conducted to assign each block copolymer composition a particle morphology. A summary table of all the block copolymer composition, monomer conversion and molecular weight data for these syntheses is included in the supporting information, Table S1.

Redispersion protocol for [0.70 PEG_{45} + 0.30 PEG_{113}]-$PHPMA_{75}$ block copolymer in Nutristem

The resuspension of PEG-functionalised worms in Nutristem was achieved using a modified protocol from Kocik *et al.*[22] As synthesized aqueous block copolymer nanoparticles were cooled to 2 °C for 1 hour, frozen in liquid nitrogen and freeze-dried to obtain a fine, pale yellow powder and Nutristem was added to obtain the desired concentration and was stirred in at 4 °C for 3 hours.

^1H NMR Spectroscopy

All ^1H NMR spectra were recorded on a 400 MHz Bruker Avance-400 spectrometer operating at 298 K, with 64 scans averaged per spectra. RAFT agent, PEG_{45} and PEG_{113} macro-CTAs were recorded in CD_2Cl_2. Spectra for all block copolymers were recorded in CD_3OD.

Transmission Electron Microscopy (TEM)

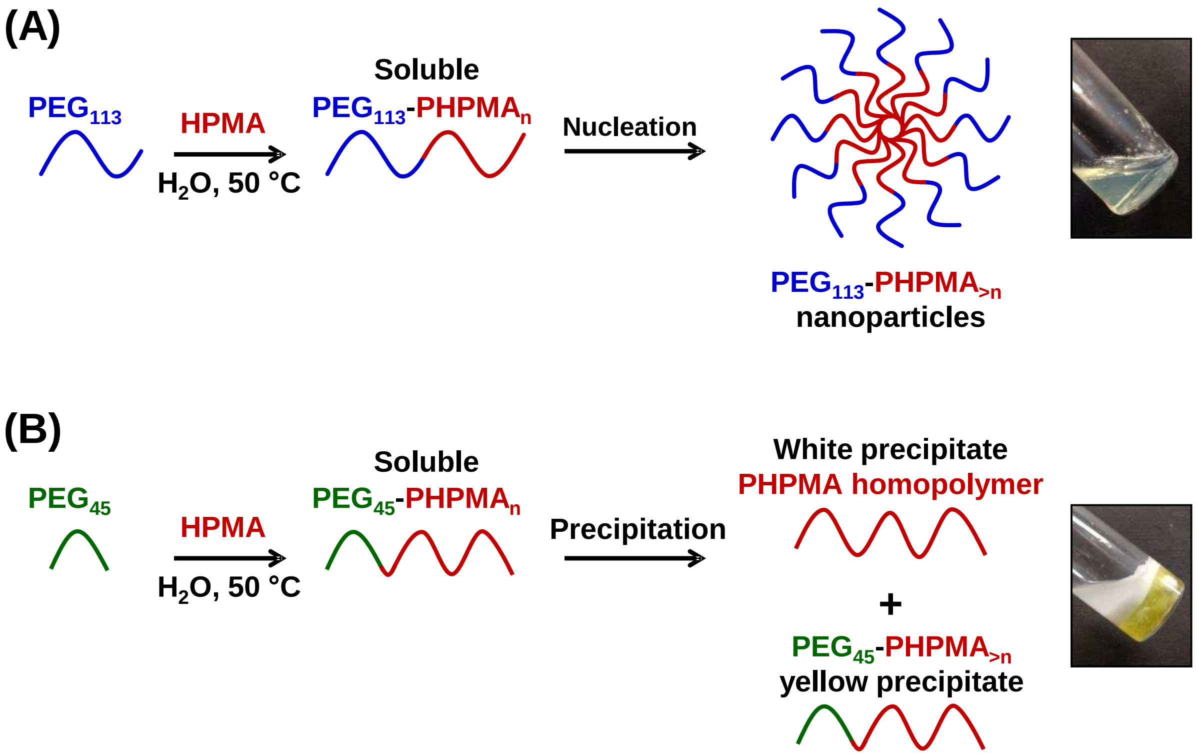
Aqueous block copolymer dispersions were diluted from 10% w/w to 2% w/w with deionized water and gently stirred overnight at room temperature. These dispersions were further diluted to 0.1% w/w and stirred for a further 3 h. Copper/palladium grids were surface-coated in-house to produce a thin film of carbon and plasma glow-discharged for 30 seconds to give a hydrophilic surface. A 10 µL droplet of 0.1% w/w aqueous dispersion was placed on the hydrophilic carbon/palladium grid for 40 seconds, blotted to remove excess sample and then negatively stained with uranyl formate solution (0.75% w/v, 10 µL) for a further 20 seconds. Excess stain was removed by blotting with filter paper and carefully dried using a vacuum house. Imaging was performed using a FEI Tecnai Spirit 2 microscope operating at 80 kV and equipped with an Orius SC1000B camera.

Tetrahydrofuran Gel Permeation Chromatography (THF GPC)

The THF GPC set-up comprised an Agilent 1260 Infinity series degasser and pump, two Agilent PLgel 5 μm MIXED-C columns in series, a variable wavelength detector (VWD) operating at 298 nm and a refractive index (RI) detector. THF eluent contained triethylamine (2.0% w/w), butylhydroxytoluene (0.05% w/v). This set-up was operating at a flow rate of 1.0 mL min^{-1} and 30 °C. Seven poly(ethylene glycol) standards with M_p values ranging from 238 g mol^{-1} to 86,200 g mol^{-1} or twelve near-monodisperse poly(methyl methacrylate) standards with M_p values ranging from 800 g mol^{-1} to 2,200,000 g mol^{-1} were used for column calibration.

Rheology

An AR-G2 rheometer equipped with a variable temperature Peltier plate and a 40 mm 2° aluminium cone was used for all rheological experiments. The storage modulus (G') and loss modulus (G'') were determined at 10% w/w as a function of temperature applied strain of 1.0 % and a frequency of 1.0 rad s^{-1}. Temperature sweeps were performed from 25 °C to 4 °C to 25 °C with 10 min equilibration per 1 °C. The protocol for assessing the thermoresponsive behaviour [0.70 PEG_{45} + 0.30 PEG_{113}]-$PHPMA_{75}$ block copolymer slightly differs. Here the temperature sweep was conducted from 10 °C to 37 °C to 10 °C, with an equilibration time of 2 min per 1 °C.



Scheme S1. (A) Mechanism for the formation of sterically-stabilized nanoparticles via RAFT aqueous dispersion polymerization. Note that in this case the depicted macro-CTA is PEG_{113} which is capable of providing sufficient steric-stabilisation and thus facilitating particle nucleation during the PISA synthesis. (B) Mechanism for the precipitation polymerization of yellow PEG_{45}-$PHPMA_n$ block copolymer chains during the attempted PISA synthesis. Note that in this latter case the PEG_{45} macro-CTA is not long enough to enable sufficient steric-stabilisation of nanoparticles. Furthermore, free radical precipitating polymerization of remaining HPMA monomer occurs to form a white precipitate.

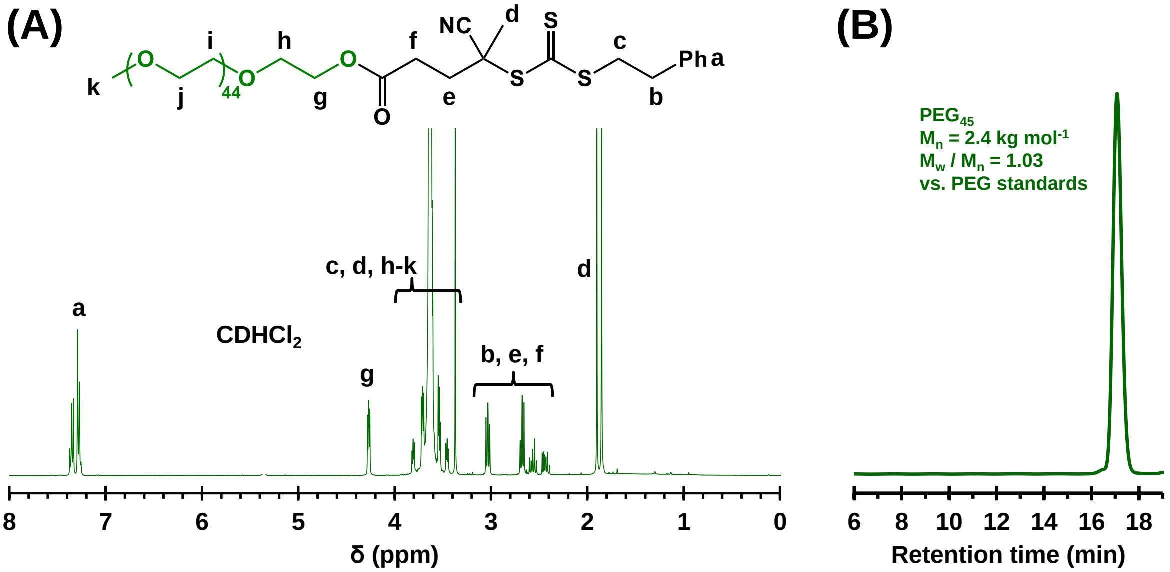


Figure S1. (A) Fully assigned ^{1}H NMR spectra recorded in CD_2Cl_2 and at 298K for a PEG_{45}-PETTC macro-CTA. A mean degree of esterification of 94% was calculated by comparison of the integral values of aromatic proton signals (a) at 7.2 – 7.4 ppm against the PEG backbone (c, d, h-k) at 3.3 - 3.9. (B) THF GPC chromatogram recorded for PEG_{45} macro-CTA. Molecular weight data is expressed relative to PEG standards.

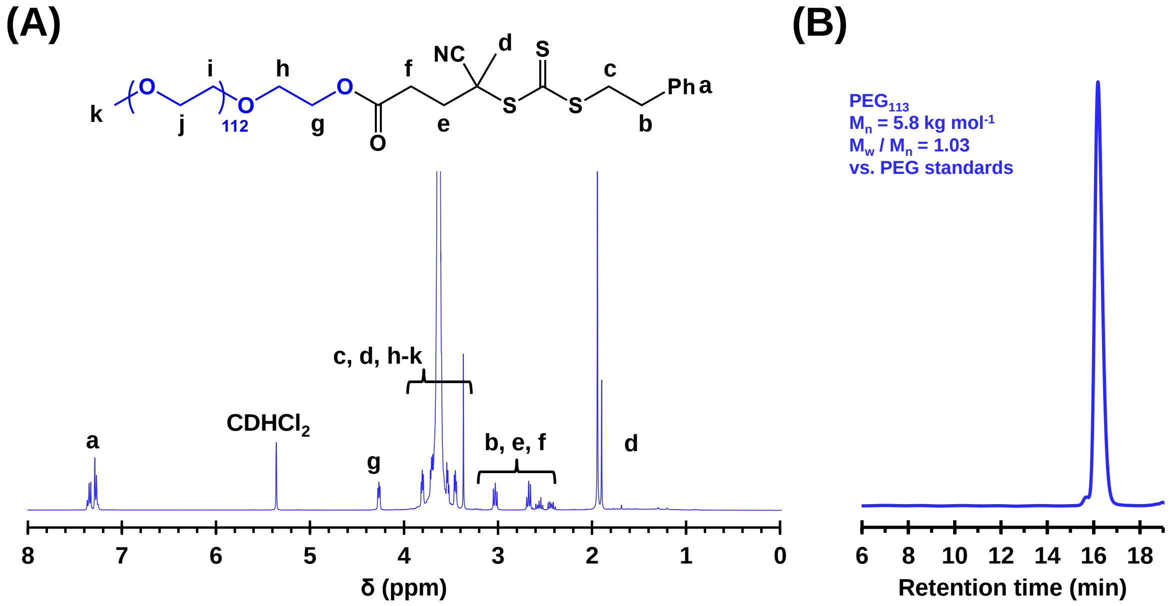


Figure S2. (A) Fully assigned ^{1}H NMR spectra recorded in CD_2Cl_2 and at 298K for a PEG_{113}-PETTC macro-CTA. A mean degree of esterification of 95% was calculated by comparison of the integral values of aromatic proton signals (a) at 7.2 – 7.4 ppm against the PEG backbone (c, d, h-k) at 3.3 - 3.9 ppm. (B) THF GPC chromatogram recorded for PEG_{113} macro-CTA. Molecular weight data is expressed relative to PEG standards.

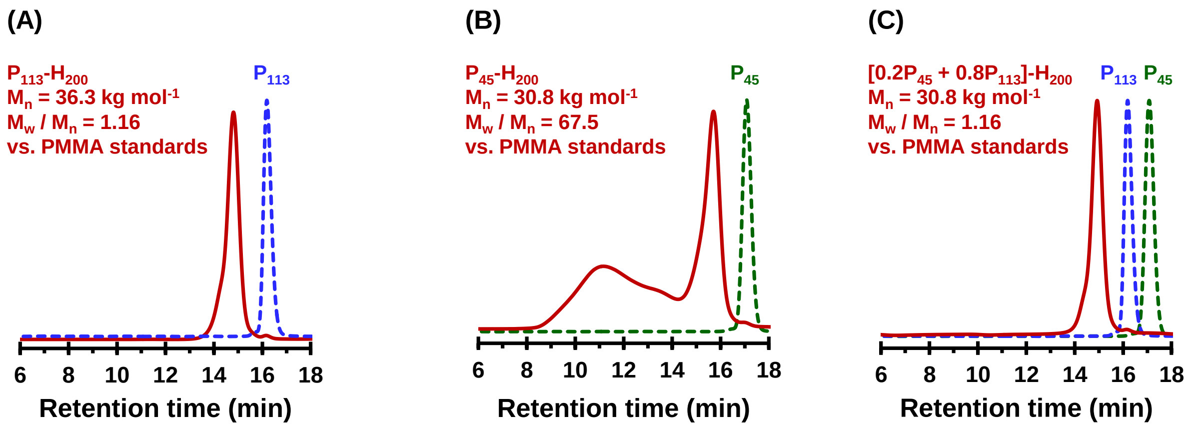


Figure S3. THF GPC chromatograms obtained for (a) PEG_{113} and PEG_{113}-$PHPMA_{200}$, (b) PEG_{45} and PEG_{45}-$PHPMA_{200}$ and (c) [0.20 PEG_{45} + 0.80 PEG_{113}]-$PHPMA_{200}$ block copolymers. Molecular weight data for the PEG_{45} and PEG_{113} macro-CTAs is expressed relative to PEG standards. Molecular weight data for all block copolymers is expressed relative to PMMA standards.

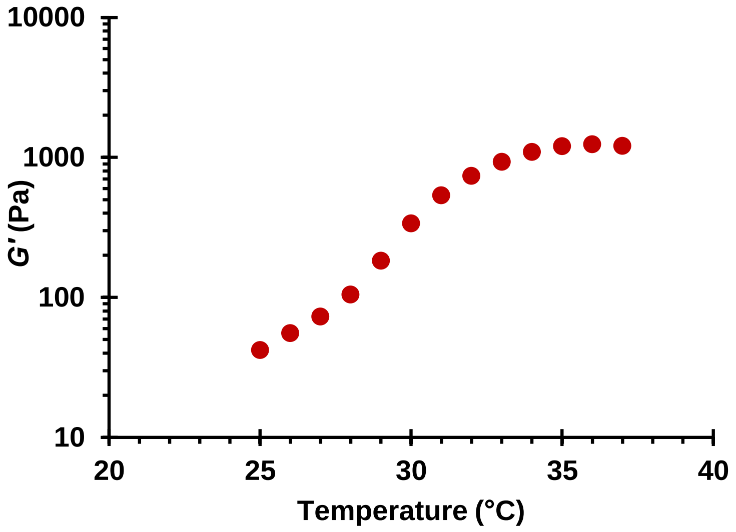


Figure S4 Variation in G' for heating a 10% w/w aqueous dispersion of [0.70 PEG_{45} + 0.30 PEG_{113}]-$PHPMA_{115}$ block copolymer worm gels from 25 °C to 37 °C. Measurements were conducted at an angular frequency of 1.0 rad s^{-1} at an applied strain of 1.0%. An equilibration time of 10 minutes was performed per 1 °C.

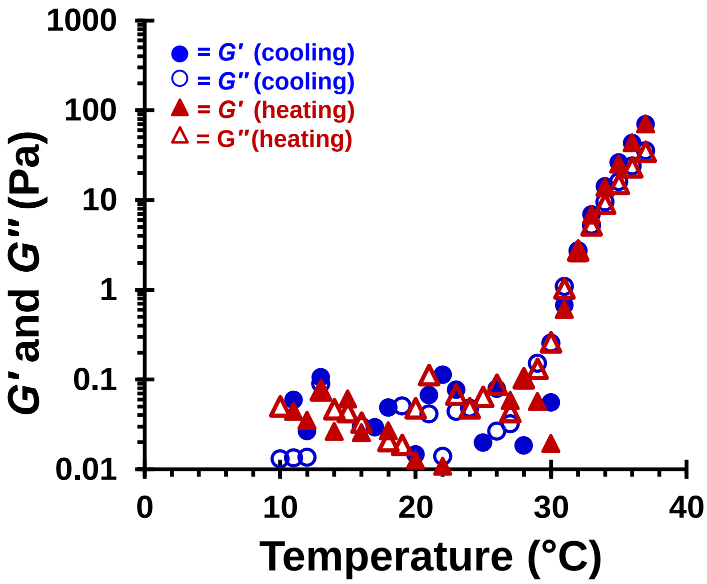


Figure S5. Oscillatory rheology data showing the variation in storage (*G′*, filled symbols) and loss (*G″,* open symbols) moduli with temperature recorded for a 10% w/w aqueous dispersion of [0.70 PEG$_{45}$ + 0.30 PEG$_{113}$] - PHPMA$_{75}$ copolymer nano-objects. Blue circles represent cooling from 37 °C to 10 °C, while red triangles represent heating from 10 °C to 37 °C. Measurements were conducted at an angular frequency of 1.0 rad s^{-1} at an applied strain of 1.0%. An equilibration time of 2 min was allowed before acquiring each data point.

Table S1: Summary of the target composition, determined molecular weight and particle morphology for [x PEG$_{45}$ + z PEG$_{113}$]-PHPMA$_n$. [a]Calculated by ^{1}H NMR spectroscopy. [b]Molecular weight data is determined by THF GPC and relative to PMMA standards. [c]Determined by post mortem TEM analysis at room temperature. Abbreviations: S = spheres, W = worms, R = roses, V = vesicles, P = precipitate

Mol fraction of PEG$_{45}$ (x)	**Target PHPMA DP (n)**	**Conversion[a] (%)**	**M_n (kg mol^{-1})[b]**	**M_w / M_n**	**Morphology[c]**
0.00	100	> 99	25.9	1.10	S
0.20	100	> 99	24.8	1.11	S
0.40	100	> 99	22.9	1.14	S
0.60	100	> 99	22.0	1.12	S
0.70	100	> 99	20.7	1.12	S
0.80	100	> 99	20.2	1.14	S
0.90	100	> 99	19.2	1.18	S&W
1.00	100	> 99	19.0	11.50	P
0.70	115	> 99	21.9	1.13	W
0.80	115	> 99	21.5	1.13	W&V
0.90	115	> 99	20.4	1.24	W&V
0.00	150	> 99	30.3	1.12	S
0.10	150	> 99	29.0	1.11	S
0.20	150	> 99	29.3	1.11	S
0.30	150	> 99	27.9	1.11	S
0.40	150	> 99	27.8	1.15	W
0.50	150	> 99	27.2	1.11	W
0.60	150	> 99	27.2	1.13	W

0.70	150	> 99	26.7	1.10	W&V
0.80	150	> 99	26.0	1.19	R
0.90	150	> 99	25.7	1.28	R
1.00	150	> 99	24.7	1.21	P
0.00	200	> 99	42.1	1.16	W
0.10	200	> 99	39.8	1.16	W
0.20	200	> 99	38.6	1.18	W
0.30	200	> 99	38.1	1.16	W
0.40	200	> 99	36.1	1.15	W
0.50	200	> 99	36.7	1.17	W&V
0.60	200	> 99	36.3	1.15	V
0.70	200	> 99	34.7	1.35	V
0.80	200	> 99	32.6	1.25	R
0.90	200	> 99	37.5	1.35	R
1.00	200	98	37.0	82.30	P
0.00	250	> 99	49.8	1.20	V
0.20	250	> 99	47.0	1.18	V
0.40	250	> 99	44.2	1.18	V
0.60	250	> 99	42.1	1.15	V
0.80	250	> 99	42.9	1.42	R
1.00	250	> 99	255.3	19.50	P
0.00	300	> 99	54.5	1.26	V
0.20	300	> 99	51.9	1.23	V
0.40	300	> 99	50.4	1.25	V
0.60	300	> 99	50.5	1.69	R
0.80	300	> 99	47.0	1.87	P
1.00	300	> 99	64.0	30.60	P
0.00	350	> 99	59.6	1.20	V
0.20	350	> 99	59.1	1.20	V
0.40	350	> 99	58.8	1.21	V
0.60	350	> 99	64.2	1.56	P
0.80	350	98	59.8	1.84	P
1.00	350	98	162.3	24.00	P

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