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A Facile Method for Generating Worm-like Micelles with Controlled Lengths and Narrow Polydispersity

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1. Materials

All reagents were purchased from Sigma-Aldrich (U.K.) and were used as received unless stated otherwise. 4,4'-azobis-4-cyanopentanoic acid (ACVA, 98%) was used as the initiator of the radical polymerizations. Benzyl methacrylate (BzMA, 96%) and methacrylic acid (MAA, 99%) monomers were used for the synthesis of poly(methacrylic acid)-poly(benzyl methacrylate) diblock copolymer. 4-cyano-4-(phenylcarbonothioylthio)pentanoic (CPCP) acid was employed as the chain-transfer agent (CTA) of the RAFT polymerisation. Glycerol monomethacrylate (GMA, 99.8%; Geo Special Chemicals), 2-hydroxypropyl methacrylate (HPMA, 97%) monomers were used for the synthesis of poly(glycerol monomethacrylate)-poly(2-hydroxypropyl methacrylate). The block copolymer was cross-linked using ethylene glycol dimethacrylate (> 98%). 2-cyano-2-propyl dithiobenzoate (CPDB) was used as the CTA of the polymerisation. Trimethylsilyldiazomethane was used for the methylation of the methacrylic acid residues and deuterated dimethyl sulfoxide (C_2D_6OS), chloroform ($CDCl_3$) and methanol (CD_3OD) were used for the ^1H NMR analyses of the macro-CTA and the block copolymers. Polycarbonate films (ipPore Track Etched Membrane, pore size 5 nm) were used to disperse the nanoparticles for the SEM imaging.

2. Synthesis and Characterization of the Block Copolymers

2.1 Synthesis of poly(methacrylic acid) (PMAA) Macro-CTA Agent

A round-bottomed flask was charged with MAA (5.00 g; 58 mmol), 4-cyano-4-(phenylcarbonothioylthio)pentanoic CTA (279 mg, 0.998 mmol), ACVA (50.0 mg; 0.179 mmol; [CTA]/[ACVA] = 5.6) and ethanol (5.00 g). The reaction mixture was sealed and purged with nitrogen gas for 45 min and then placed in an oil bath heated at 70 °C for 3 h. The obtained PMAA macro-CTA ($M_n \approx 13{,}500$ g mol^{-1}, $M_w \approx 14{,}700$ g mol^{-1}, $M_w/M_n \approx 1.09$) was purified by dialysis, first against 1:1 water/ methanol and then against pure deionized water. The PMAA macro-RAFT agent was

subsequently isolated by lyophilization. A mean degree of polymerisation (DP) of 70 ($PMAA_{70}$) was estimated using ^1H NMR spectroscopy performed in $CDCl_3$ by comparison of the integrated signal intensity of the aromatic protons of the CTA structure at 7.2-7.4 ppm with that of the methacrylic polymer backbone protons at 0.4-2.5 ppm.

2.2 Synthesis of poly(methacrylic acid)-poly(benzyl methacrylate) Diblock Copolymer

BzMA (1.00 g; 5.70 mmol), $PMAA_{70}$ macro-CTA (170 mg; 0.030 mmol) and ACVA (1.60 mg; 0.006 mmol; [macro-CTA]/[ACVA] = 5.00) were dissolved in a round-bottomed flask containing ethanol (1.871 g). The sealed reaction mixture was purged with nitrogen gas for 45 min and then placed in an oil bath heated at 70 °C for 24 hours. The ^1H NMR spectroscopy performed in C_2D_6OS showed full conversion of the benzyl methacrylate (BzMA) monomer and a mean DP of PBzMA block of 100 was calculated. GPC analysis performed in THF eluent showed a near-monodisperse molecular weight distribution (Mn ≈ 29,600 g mol^{-1}, Mw ≈ 33,300 g mol^{-1}, Mw/Mn ≈ 1.12).

2.3 Synthesis of poly(glycerol monomethacrylate) (PGMA) Macro-CTA Agent

GMA (1.00 g; 6.25 mmol), 2-cyano-2-propyl dithiobenzoate CTA (21.2 mg; 0.096 mmol) and ACVA (5.38 mg; 0.019 mmol; [CTA]/[ACVA] = 5.00) were dissolved in a round-bottomed flask containing ethanol (1.075 g). The reaction mixture was sealed and purged with nitrogen gas for 45 min and then placed in an oil bath heated at 70 °C for 2 h. The macro-chain transfer agent was purified with an excess dichloromethane (Sigma Aldrich). The precipitate was then isolated and dialyzed against methanol and subsequently freeze dried. ^1H NMR in CD_3OD indicated a monomer conversion of 60% and a mean degree of polymerisation of 53. GPC in DMF eluent (Sigma Aldrich) showed a near-monodisperse molecular weight distribution (Mn ≈ 13,100 g mol^{-1}, Mw ≈ 15,600 g mol^{-1}, Mw/Mn ≈ 1.19) (Fig. S5, ESI†).

2.4 Synthesis of poly(glycerol monomethacrylate)-poly(2-hydroxypropyl methacrylate) Diblock Copolymer

$PGMA_{53}$ macro-CTA (0.55 g; 0.063 mmol), HPMA (1.00 g; 6.90 mmol), and ACVA (3.65 mg; 0.013 mmol; [macro-CTA]/[ACVA] = 5.00) were dissolved in a round-bottomed flask containing deionized water (4.661 g). The reaction mixture was purged with nitrogen gas for 45 min and then placed in an oil bath heated at 70 °C for 3 hours. Ethylene glycol dimethacrylate monomer (20 equivalents vs. PGMA macro-CTA) was introduced under nitrogen into the reaction mixture to ensure the cross-linking of the block copolymer. After stirring overnight to ensure complete monomer conversion, the reaction mixture was quenched by exposure to air. ^{1}H NMR in CD_3OD indicated a full conversion of the monomers and a mean degree of polymerisation of 110 for the PHPMA block. GPC in DMF eluent of the linear worms (not cross-linked) shows a near-monodisperse molecular weight distribution (Mn ≈ 30,200 g mol^{-1}, Mw ≈ 33,000 g mol^{-1}, Mw/Mn ≈ 1.09) (Fig. S5, ESI†). A high molecular weight shoulder was observed, which is most likely due to the small amount of dimethacrylate impurity present in HPMA monomer, resulting in light branching of the PHPMA chains.[1]

3. Characterization of the Worm Nanoparticles

3.1 ^{1}H NMR Spectroscopy

^{1}H NMR spectra were acquired in deuterated dimethyl sulfoxide (C_2D_6OS), chloroform ($CDCl_3$) and methanol (CD_3OD) solvents using a Bruker 400 MHz spectrometer.

3.2 Gel Permeation Chromatography (GPC)

The GPC set-up comprised an Agilent 1260 Infinity system fitted with two 5 µm Mixed-C columns and a guard column, a refractive index detector and an UV/Vis detector operating at 309 nm. For the characterization of PGMA-PHPMA block copolymer, grade DMF eluent containing 10 mM LiBr was used with a flow rate of 10 mL min^{-1}. For PMAA-PBzMA copolymer, THF eluent containing 2.0 vol.%

triethylamine and 0.05 vol.% butylhydroxytoluene (BHT) was used. A series of near-monodisperse poly(methyl methacrylate) standards were used for calibration using the refractive index and the UV/ Vis detectors for determining the molecular weights and molar mass dispersities of the standards.

For GPC analysis, the methacrylic acid residues present in both the PMAA macro-CTA and the PMAA-PBzMA block copolymer were methylated using trimethylsilyldiazomethane to prevent adsorption onto the GPC column. Briefly, $PMAA_{70}$ and $PMAA_{70}$-$PBzMA_{100}$ were first dissolved in THF and then trimethylsilyldiazomethane was added dropwise at room temperature. On addition, the solution became yellow and bubbles appeared. After a few seconds, the solution became colorless and a few more drops of trimethylsilyldiazomethane were added until the yellow color of the solution withstood and the solution stopped bubbling. The solution was then stirred overnight at room temperature.

3.3 Small-Angle X-ray Scattering (SAXS)

SAXS patterns were collected at a synchrotron source (Diamond Light Source, Beamline I22, Didcot, U.K.), using a monochromatic X- ray radiation (wavelength $\lambda = 0.124$ nm). A q-range from 0.02 nm^{-1} to 1 nm^{-1} was used, where $q = 4\pi\sin(\theta)/\lambda$ is the length of the scattering vector and θ is half of the scattering angle. A 2D Pilatus P3-2M pixel detector was used for measurements. Glass capillaries of 2 mm diameter were used as sample holders. Ethanolic dispersions of 10 mg mL^{-1} of $PMAA_{70}$-$PBzMA_{100}$ worm particles were sonicated using an ultrasonic processor (Fisherbrand™ Q125 Sonicator – 120 Watts) operating at 10% amplitude. The temperature was kept as close to 0 °C as possible using an ice bath. Ethanol was used for the absolute intensity calibration. SAXS patterns were collected every minute for an hour with 3 min equilibration before data collection. The samples remained static in the holder during the *in situ* measurements. Scattering data were reduced using standard routines from the beamline (integration, normalization and absolute intensity calibration), using Dawn Software. SAXS patterns were fitted to structural models previously reported for molecularly dissolved chains (unimers)[2] and/ or worms[3] within PISA systems using Irena SAS macros for Igor Pro.[4]

3.4 Dynamic Light Scattering (DLS)

Size distributions were obtained by DLS using a Malvern Zetasizer NanoZS apparatus at a fixed scattering angle of 173°. Ethanolic dispersions of 0.10 wt.% of PMAA-PBzMA worm micelles were analyzed prior to sonication, and at different time-points of sonication. The results were averaged over three consecutive measurements. It is noted that the hydrodynamic diameters reported by DLS do not correspond to the mean contour length of the worms, since this technique assumes a sphere-equivalent model that is not suitable for the accurate size measurement of worm micelles. Nevertheless, the DLS results provide qualitative information about the evolution of the relative size and dispersity of the worms during the sonication process.

Dynamic light scattering (DLS) supports the gradual splitting of the worms, where a dramatic decrease of the hydrodynamic diameter (from 2.5 μm to 550 nm) and size dispersity (from 0.42 to 0.15) after only five minutes of sonication can be observed, followed by a decrease of the rate of the worm splitting events (Fig. S4, ESI†). Additionally, the DLS analyses show that the sonication of the worms generates narrower size distribution compared to the initial micelles (from 2.5 μm; PDI = 0.42 to 310 nm; PDI = 0.084), which corroborates the results obtained from the analyses of the SEM micrographs.

3.5 Electron Microscopy

Samples for transmission electron microscopy (TEM) were prepared by placing a 15 μL droplet of an aqueous suspension of 0.10 wt.% copolymer on a TEM grid for 1 min. Copper TEM grids coated with a carbon film were employed, and these were treated with a plasma glow discharge for 30 sec to create a hydrophilic surface prior to addition of the aqueous copolymer dispersion. Excess solution was removed via blotting and the grids were stained with uranyl acetate (0.75 wt.%) for 1 min. Excess stain was removed via blotting and the grids were carefully air dried. TEM was conducted using a FEI Tecnai TF20 FEGTEM operating at 200 kV.

Scanning Electron Microscopy (SEM) was carried out using a FEI NanoSEM Nova 450 to image the worm block copolymers. The PMAA-PBzMA and the cross-linked PGMA-PHPMA worms were dispersed on polycarbonate films backed with adhesive conducting pads. The samples were mounted on SEM stubs and coated with 4 nm iridium to enable imaging of the non-conductive worm particles.

3.6 Analysis of SEM Images

Estimation of the mean contour length of the worms from the SEM micrographs was conducted using ImageJ software. Over 200 worms were processed in order to obtain the number-average length (L_n), the weight-average length (L_w) and the polydispersity index (PDI) of the worms (L = length and N = number of worms), which were calculated from Equations (1) and (2). The PDI and the standard deviation (σ) of the measured cylindrical micelles lengths are related by Equation (3).

$$L_n = \frac{\sum_{k=1}^{n} N_k L_k}{\sum_{k=1}^{n} N_k} \quad (1)$$

$$L_w = \frac{\sum_{k=1}^{n} N_k L_k^2}{\sum_{k=1}^{n} N_k L_k} \quad (2)$$

$$PDI = \frac{L_w}{L_n} = \left(\frac{\sigma}{L_n}\right)^2 + 1 \quad (3)$$

3.7 Size Reduction of Worms Using Sonication

Sonication experiments were carried out by immersing a glass vial, containing various concentrations of worm particles, into an ice bath to keep a temperature as close as possible to 0 °C. A sonotrode (Fisherbrand™ Probe Microtips – 2 mm diameter) was placed inside the glass vial at half of the solution

height to enable uniform sonication of the worms. The power delivered by the sonotrode was controlled by an external ultrasonic processor (Fisherbrand™ Q125 Sonicator – 120 Watts) operating at 10% amplitude. Aliquots of the solution were collected at various sonication times for subsequent characterization.

4. Supporting Figures

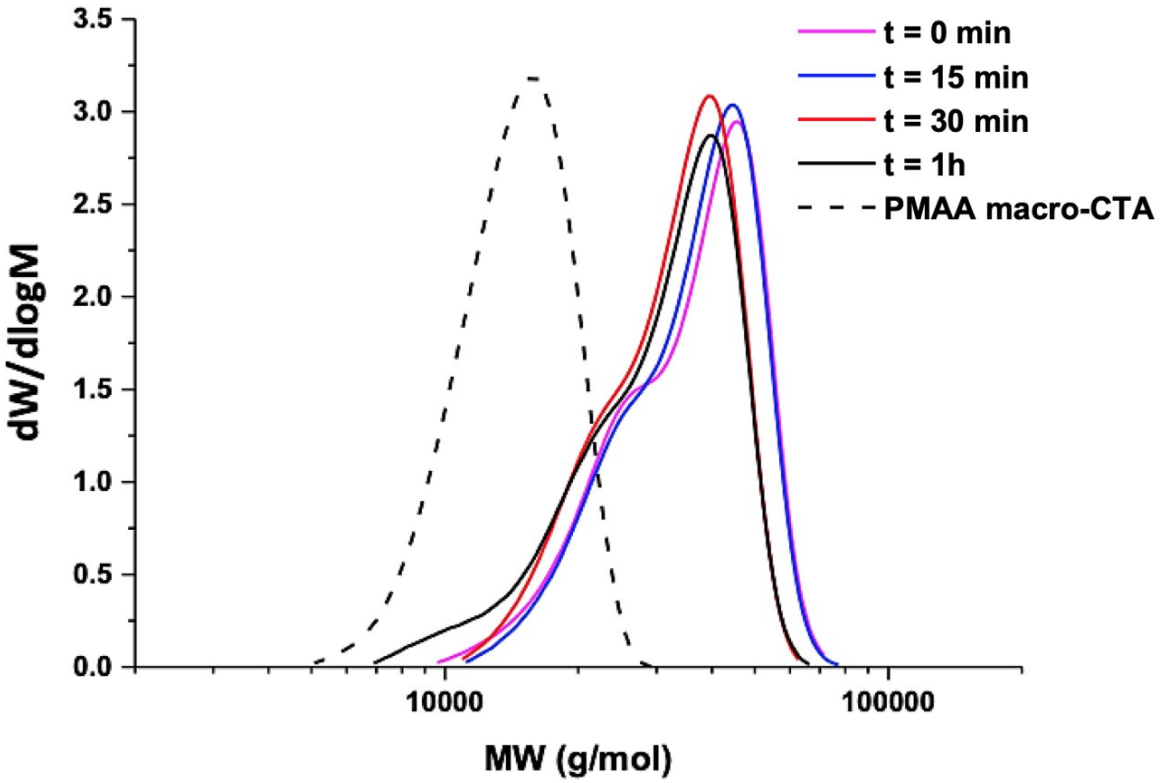

Fig. S1 Gel permeation chromatography traces of $PMAA_{70}$ macro-CTA (dashed lines) and $PMAA_{70}$-$PBzMA_{100}$ (solid lines) upon sonication. Exhaustive methylation of methacrylic acid residues using an excess of trimethylsilyldiazomethane was necessarily to prevent adsorption of the polymers to the GPC column.

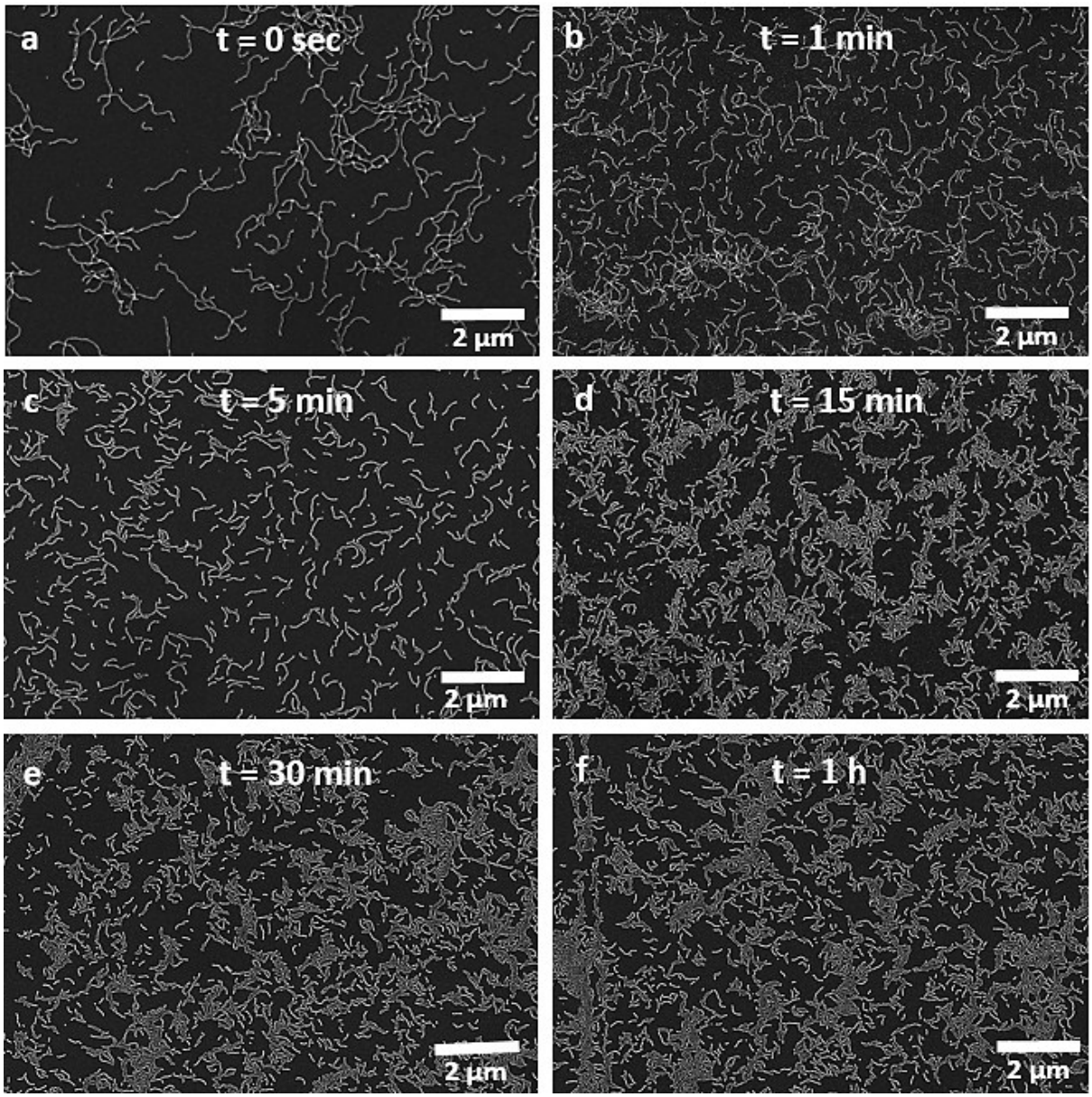

Fig. S2 SEM micrographs of the PMAA-PBzMA worms (10 mg mL^{-1}) sonicated over time in ethanol and in an ice bath, using a sonicator operating at 10% amplitude.

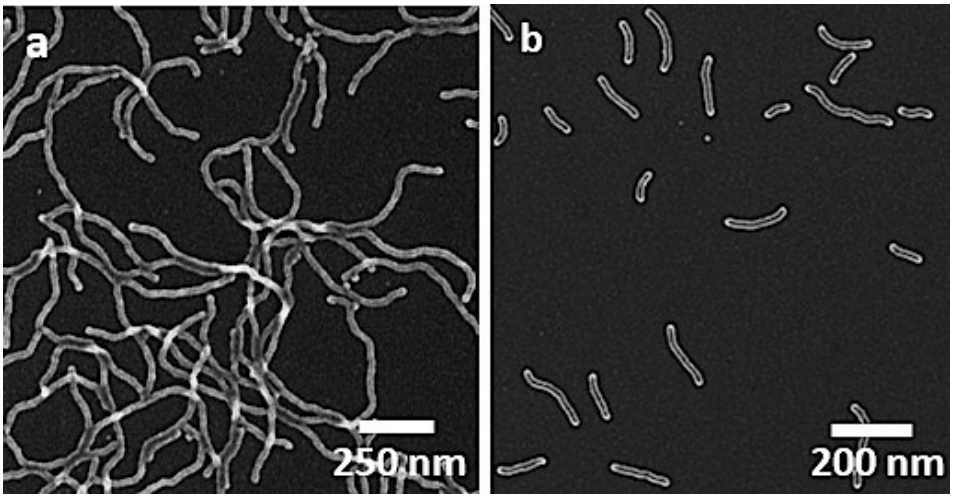

Fig. S3 (a) SEM micrographs of the PMAA-PBzMA worms (10 mg mL^{-1}) prior to sonication, and (b) after 30 min of sonication in ethanol and in an ice bath, using a sonicator operating at 10% amplitude.

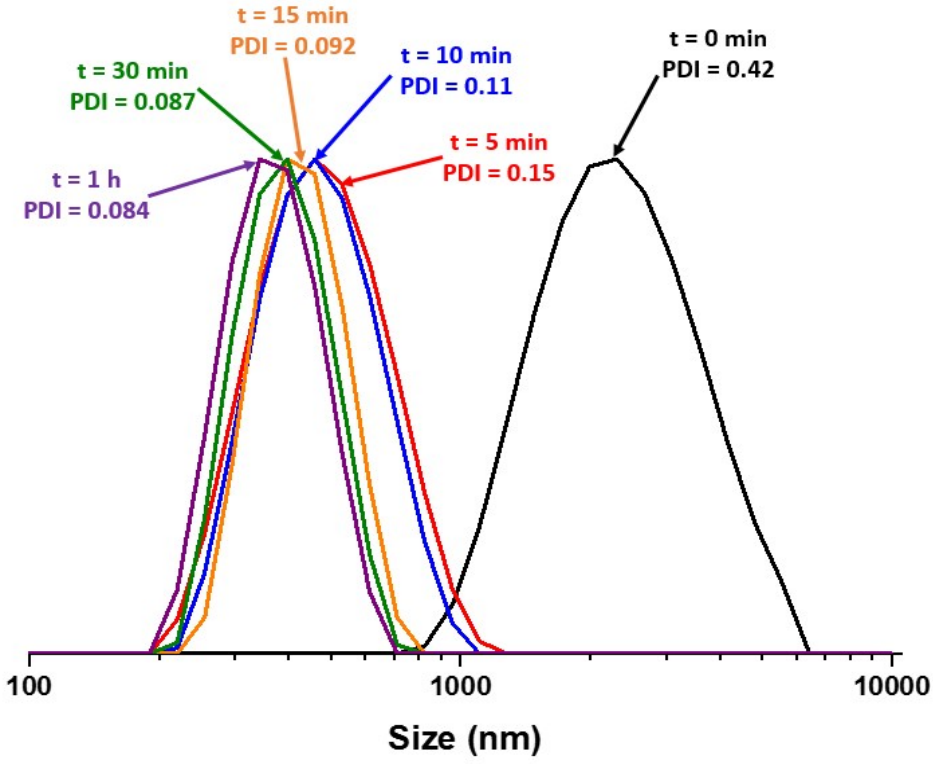

Fig. S4 Dynamic light scattering of normalized intensity of the PMAA-PBzMA worms (0.10 wt.%) upon sonication in ethanol. A significant decrease of the worm hydrodynamic diameter and narrower size distributions can be observed upon sonication. The hydrodynamic diameters were obtained by assuming a sphere-equivalent model. The values should therefore be taken with caution, considering the anisotropic nature of the worms.

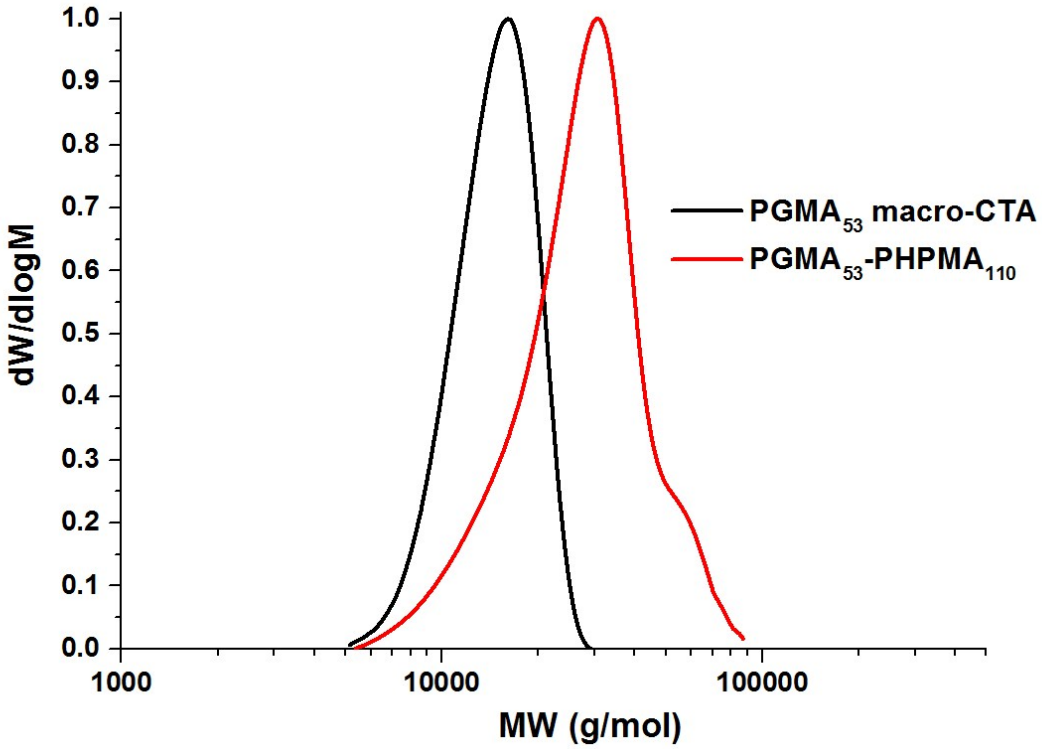

Fig. S5 Gel permeation chromatography traces of $PGMA_{53}$ macro-CTA (black line) and $PGMA_{53}$-$PHPMA_{110}$ (red line). A high molecular weight shoulder is observed for $PGMA_{53}$-$PHPMA_{110}$, which is most likely resulting from light branching of the PHPMA chains

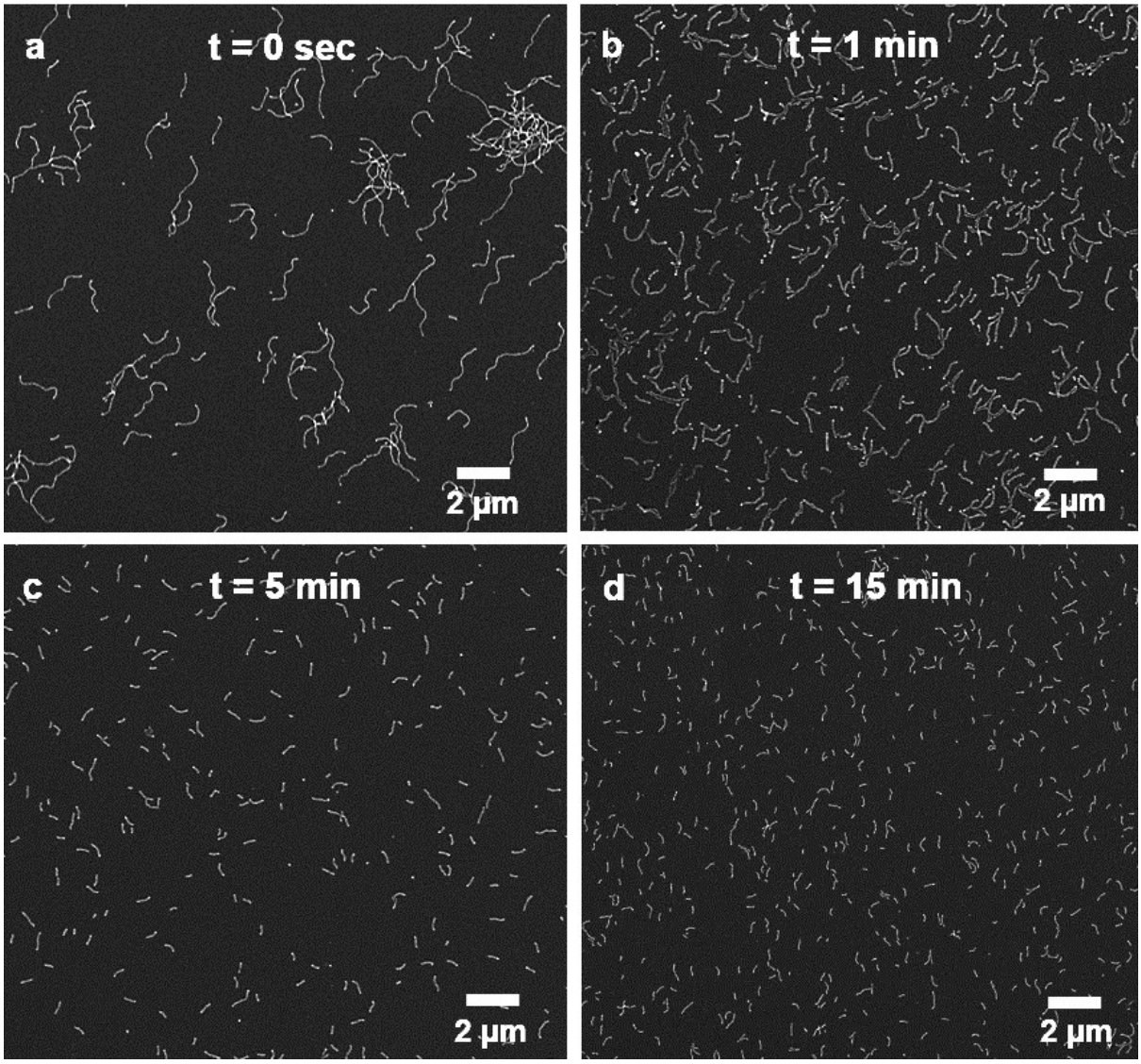

Fig. S6 SEM micrographs of the cross-linked $PGMA_{53}$-$PHPMA_{110}$ worms (10 mg mL^{-1}) sonicated over time in water and in an ice bath, using a sonicator operating at 10% amplitude.

5. Supporting Tables

Table S1 Values of the number-average length (L_n), weight-average length (L_w), the polydispersity index (PDI), the standard deviation over the number-average length (σ/L_n) and aspect ratio of the PMAA-PBzMA worm micelles (10 mg mL^{-1} and 100 mg mL^{-1}) after mild sonication in ethanol and in an ice bath.

	Worm concentration: 10 mg mL^{-1}					**Worm concentration: 100 mg mL^{-1}**				
Sonication time (sec)	**L_n (nm)**	**L_w (nm)**	**PDI**	**σ/L_n**	**Aspect ratio**	**L_n (nm)**	**L_w (nm)**	**PDI**	**σ/L_n**	**Aspect ratio**
0	1450	3125	2.15	1.074	58	1450	3125	2.15	1.074	58
30	440	617	1.40	0.634	17.6	420	480	1.14	0.379	16.8
60	316	398	1.30	0.509	12.6	398	429	1.08	0.279	15.9
300	238	310	1.26	0.550	9.5	351	369	1.05	0.228	14.8
600	218	266	1.22	0.468	8.7	266	275	1.03	0.180	10.6
900	215	261	1.21	0.460	8.6	255	263	1.04	0.193	10.2
1800	198	236	1.19	0.439	7.9	254	266	1.04	0.204	10.2
3600	179	211	1.18	0.425	7.2	247	256	1.03	0.186	9.9

Table S2 Values of the mean contour length of the worms (L_c), the radius and dispersity of the core-forming block (R_{core}) and the radius of gyration of the worm corona (R_g) of the PMAA-PBzMA worm micelles (10 mg mL^{-1}) after sonication in ethanol and in an ice bath.

Sonication time (sec)	L_c (nm)	R_{core} (nm)	R_{core} dispersity (%)	R_g (nm)
0	517.2	10.4	10.2	2.8
30	154	10.4	11.6	2.6
60	134	10.4	12	2.6
300	122	10.5	12.3	2.5
600	109	10.4	12.8	2.4
900	102	10.5	12.8	2.5
1800	68	10.5	13.7	2.2
3600	53	10.6	14.1	2.1

6. References

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