Supplementary Material (ESI) for Chemical Communications
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Supporting Information

Amphiphilic Block Copolymer Nano-fibers via RAFT-Mediated Polymerization in Aqueous Dispersed System

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Materials

4,4'-azobis-4-cyanopentanoic acid (ACPA, >98%, Fluka), anhydrous acrylic acid (AA, >99%, Fluka), poly(ethylene glycol) methyl ether acrylate (PEGA, M_n = 454 g.mol^{-1}, >99%, Aldrich), 1,3,5-trioxane (>99%, Fluka), sodium hydroxide aqueous solution 1N (pure, Acros organics), sodium hydrogencarbonate ($NaHCO_3$, 99.7%, Aldrich), ethanol (for analysis from VWR Rectapur) and tetrahydrofuran (THF, for analysis from VWR Normapur) were used as received. Deionized water was used for all polymerizations. Styrene (S, 99%, Aldrich) was distilled under reduced pressure before use. The RAFT agent 2-(dodecylthiocarbonothioylthio)-2-methylpropanoic acid (TTCA) was synthesized as described elsewhere [J.T. Lai, D. Filla, R. Shea, *Macromolecules,* 2002, 35, 6754-6756]. The molecular structure of the RAFT agent can be found in the communication (Scheme 1).

Synthesis of the poly(acrylic acid-*co*-PEG acrylate) macromolecular RAFT agents, P(AA-*co*-PEGA)-TTC

In a typical experiment (AA/PEGA: 50/50, samples **3-7** in Table 1), the copolymerization of AA (1.45 g, 2×10^{-2} mol) and PEGA (9.12 g, 2×10^{-2} mol) was carried out in 8.13 g ethanol with ACPA as an initiator (17 mg, 6.1×10^{-5} mol) and TTCA as a RAFT agent (323.2 mg, 8.9×10^{-4} mol). 1,3,5-Trioxane (266.1 mg, 2.96×10^{-3} mol) was added as an internal reference for NMR analysis. The solution was poured into a septum-sealed flask, purged for 30 min with nitrogen and heated to 70 °C in a thermostated oil bath under stirring. For kinetic studies, sampling was performed at regular time intervals, and the polymerization was finally quenched after 3 h 40 min (95% conversion) by immersion of the flask in iced water. The overall monomer conversion was determined by ^{1}H-NMR spectroscopy in D_2O by the relative integration of the internal reference peak at 5.1 ppm and the vinylic proton peaks of AA and PEGA at 5.5 and 6.0 ppm. For the synthesis of the living P(AA-*co*-PEGA)-TTC that was later used as a macroRAFT agent, the polymerization was stopped after 55 min at 71% of monomer conversion. The polymer was recovered by precipitation in cold diethyl ether and then dried under reduced pressure. It was then analysed by size exclusion chromatography (SEC) in THF, after methylation (with trimethylsilydiazomethane; L. Couvreur, C. Lefay, J. Belleney, B. Charleux, O. Guerret, S. Magnet, *Macromolecules* 2003, 36, 8260-8267). Combination of the signals of the refractive index detector and the light scattering (LS) detector allowed us to determine an absolute value of M_n = 13800 g.mol^{-1}. The refractive index increment, *dn/dc,* was determined in-line by injecting the copolymer at four different concentrations. The *dn/dc* in THF at 40°C was 0.059. The composition of the copolymer was determined using quantitative ^{13}C-NMR analysis via the relative integration of the signals at 176 ppm (C=O of AA) and at 174 ppm (C=O of the ester group in PEGA) in $CDCl_3$.

For the homopolymers PAA-TTC and P(PEGA)-TTC, the same procedure was used. The number-average molar mass, M_n, and polydispersity index, M_w/M_n, of PAA-TTC were determined, after methylation, by SEC in THF using a PS calibration curve whereas P(PEGA)-TTC was analyzed by combination of the signals of the refractive index detector and the light scattering (LS) detector.

In situ synthesis of amphiphilic block copolymer self-assemblies via emulsion polymerization

In a typical emulsion polymerization (sample **6**, in Table 1), the P(AA-*co*-PEGA)-TTC macroRAFT agent (AA/PEGA: 50/50 mol/mol, 427.4 mg, 3.1×10^{-5} mol) was solubilized in 3.0 mL of deionized water and the pH was adjusted to 8 with 1M NaOH solution. 2 mL of a 3.2 mM stock solution of ACPA containing 10 mM of $NaHCO_3$, to help dissolution, were then added to the macroRAFT agent solution. $NaHCO_3$ (14.7 mg, 1.7×10^{-4} mol), to reach 0.038 M in the solution, and the monomer, styrene (654.9 mg, 6.3×10^{-3} mol) was finally added. After deoxygenation by nitrogen bubbling for 30 min, the septum sealed flask was immersed in an oil bath thermostated at 80 °C. Samples were periodically withdrawn to monitor the monomer conversion by gravimetry and to characterize the formed objects. The polymerization was finally quenched after 7 h (75% conversion) by immersion of the flask in iced water. After drying, the number-average molar masses, M_n, and polydispersity indices, M_w/M_n, of the polymer were determined by SEC in THF using a PS calibration curve. Detailed characteristics of the block copolymers are summarized in Table 1 and in Figure SI-1. The morphological analysis of the aqueous dispersions of diblock copolymers nano-objects was then performed by TEM.

Self-assembling of the amphiphilic diblock copolymer P(AA-*co*-PEGA)-*b*-PS in water

The self-assembly process and turbidity measurements were performed according to published procedures [L. Zhang, A. Eisenberg, *Macromol. Symp.*, 1997, 113, 221-232; A. Choucair, A. Eisenberg, *Eur. Phys. J. E,* 2003, 10, 37-44]. In detail, the dried diblock copolymer (either the final copolymer of an experiment corresponding to experiment **6** in Table 1 without purification, or the same copolymer after removal of the residual P(AA-co-PEGA)-TTC macroRAFT agent, via two successive precipitations in cold water) was first dissolved at a concentration of 1.0 wt % in 1,4-dioxane, which is a good solvent for both polymer blocks. After an equilibration period of 15 h, deionized water was continuously added to 1.5 mL of the solution under gentle shaking. After every addition of a water droplet, the solution was left to equilibrate for 10 min or more until the optical density was stable. The optical density was measured at a wavelength of 650 nm using a quartz cell (path length = 2 cm) with a Unicam UV-visible spectrophotometer. The cycles of water addition, equilibration, and turbidity measurement were continued until the increase in turbidity upon water addition remained very small. The turbid solution was then dialyzed against water for 3 days to remove 1,4-dioxane using a Spectra/Por regenerated cellulose membrane with a molar mass cutoff of 3500 Da. The final concentration of polymer in the solution was 0.65 wt% % (Figure SI-2).

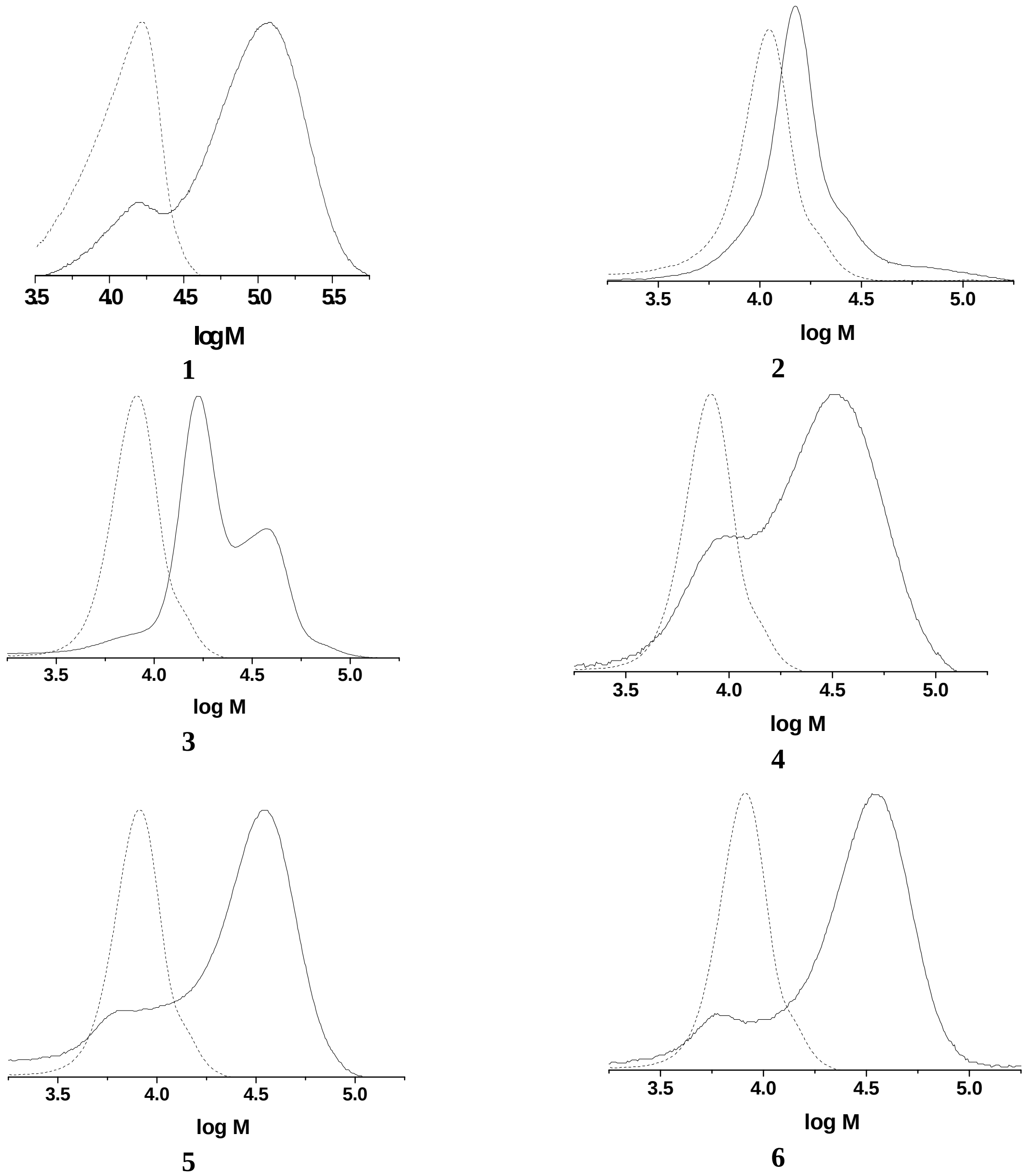
Characterization techniques

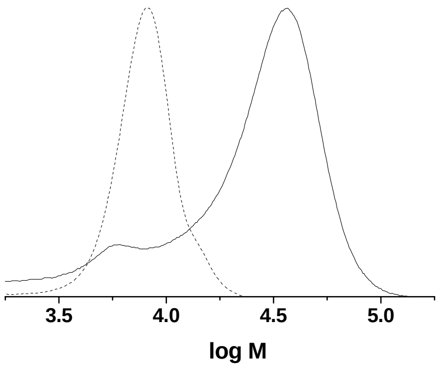
^{1}H-NMR spectra for determination of the monomer conversion and purity of the macroRAFT agents were recorded in D_2O solution at room temperature using a 250 MHz Bruker spectrometer in 5-mm diameter tubes. ^{13}C-NMR spectroscopy was performed at room temperature with a 500 MHz Bruker spectrometer in 10-mm diameter tubes.

The number-average molar mass (M_n), the weight-average molar mass (M_w) and the molar mass distribution (polydispersity index: PDI = M_w/M_n) were determined by size exclusion chromatography (SEC) using THF as an eluent at a flow rate of 1 mL.min^{-1}. Two types of SEC apparatus were used in this study: (a) a routine SEC apparatus equipped with a Viscotek VE 5200 automatic injector and two columns thermostated at 40°C (PSS SDV, linear M, 8 mm × 300 mm, bead diameter: 5 µm). Detection was made with a differential refractive index detector (LDC analytical Refracto Monitor IV). The Viscotek OmniSEC software was used for data analysis and the relative M_n and M_w/M_n were calculated with a calibration curve based on polystyrene (PS) standards (from Polymer Laboratories). (b) A more sophisticated apparatus with a triple detector array (TDA, model 302 from Viscotek) equipped with a two angle light scattering (LS) detector (LALS, $\theta = 7°$, RALS, $\theta = 90°$, laser $\lambda = 670$ nm), a refractive index detector, and two Polymer Laboratories Mixed C columns (5 µm) thermostated at 40°C (the inline viscosimeter was not used in this work). The average molar masses were calculated from the LS signal with the OmniSec software, using the average refractive index increment (*dn/dc*) measured for each sample with the inline refractometer, after injecting the polymer at four different concentrations. *dn/dc* = 0.062 for the P(PEGA)-TTC macroRAFT agent; 0.062 for the P(AA-*co*-PEGA)-TTC macroRAFT agent with AA/PEGA molar ratio = 25/75; 0.059 for the 50/50 composition and 0.060 for the 75/25 composition.

The morphological analysis of the aqueous dispersions of diblock copolymers nano-objects was performed by Transmission Electron Microscopy (TEM) on samples dropped on a carbon-coated copper grid and dried under air. The TEM images were recorded without staining using a JEOL JEM 100CX II electron microscope at an accelerating voltage of 100 kV equipped with a 1376 X 1032 pixels CCD camera (Olympus, KeenView). TEM micrographs in Figure 1-2 and Figure 2-5 were performed by TEM on samples stained by uranyl acetate. They were recorded using a Philips CM120 electron microscope equipped with a Gatan SSC 1K_1K CCD camera. The dimensions of the nano-objects reported in the article are all based on TEM images of dried samples.

Figure SI-1. Size exclusion chromatograms of the P(AA-*co*-PEGA)-TTC macroRAFT agents (dotted line) and of the diblock copolymers P(AA-*co*-PEGA)-*b*-PS (plain line) in THF (see Table 1 for the characteristics)





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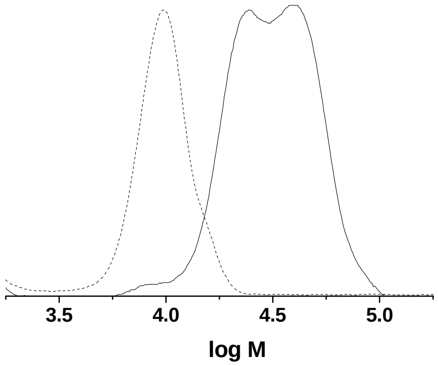

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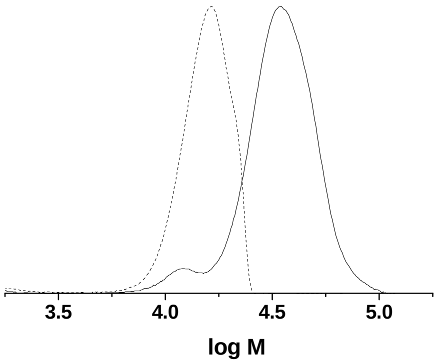

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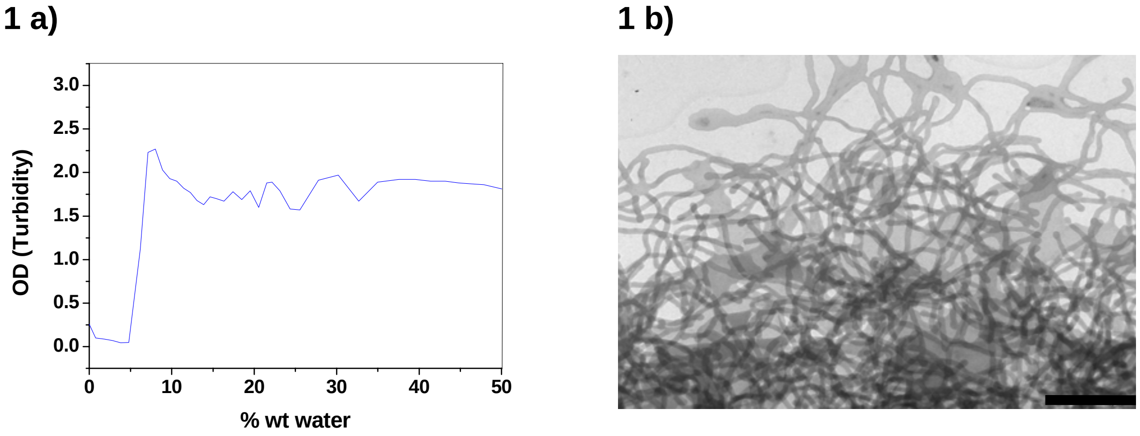
Figure SI-2. **1a)** and **2a)** Turbidity curves (optical density measured at 650 nm) for the successive addition of **1a)** distilled water to 1 wt% 1,4-dioxane solution of dried P(AA-*co*-PEGA)-*b*-PS (experiment **6**) without purification and **2a)** 0.038 M aqueous solution of $NaHCO_3$ at pH 8 to 1 wt% 1,4-dioxane solution of P(AA-*co*-PEGA)-*b*-PS (experiment **6**) after removal of the residual P(AA-*co*-PEGA)-TTC. **1b)** and **2b)** TEM images of the corresponding self-assemblies after dialysis: **1b)** for the non-purified copolymer and **2b)** for the purified one; scale bar: **1b)** 1 µm and **2b)** 500 nm.

Non-purified P(AA-*co*-PEGA)-*b*-PS copolymer



Purified P(AA-*co*-PEGA)-*b*-PS copolymer