

Supplementary Materials for:

**High-Molecular Weight Bottlebrushes via Continuous Flow Photoiniferter
Polymerization of Macromonomers**

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1. Characterization Techniques

General Methods

¹ H NMR spectra were recorded at 25 °C in CDCl₃ or DMSO-d₆ on an Agilent 400 MHz DD2 spectrometer. The values of dn/dc for copolymers were determined using a BI-DNDC differential refractometer (Brookhaven Instr. Corp., Holtsville, NY, USA) at 27-30 °C in the concentration range of 1–15 mg/mL. The concentrations of monomers in reaction mixtures were measured by HPLC using a Shimadzu Prominence chromatographic system equipped with refractometric and matrix UV detectors, a thermostat and a Kromasil 100–5-C18 4.6 × 250 mm column. Acetonitrile was used as an eluent, the flow rate was 0.9 mL/min, and the thermostat temperature was 55 °C.

Molecular weights and molecular weight distributions of polymers were determined by GPC using a Chromos LC-301 instrument with an Alpha-10 isocratic pump, a Waters 410 refractometric detector and two exclusive columns, Phenogel 5 μm 500A and Phenogel 5 μm 10E5A, from Phenomenex (with a measurement range from 1 k to 1000 k); tetrahydrofuran was used as an eluent. Polystyrene standards were used for calibration.

Differential scanning calorimetry (DSC) was performed for polymer samples (ca. 10–15 mg in an aluminum pan) under dry argon flow on a DSC 204F1 Phoenix calorimeter (Netzsch, Selb, Germany) equipped with CC 200 controller for liquid nitrogen cooling. The heating and cooling rates were 10 °C/min and –10 °C/min, respectively, between –80 °C and 80 °C.

Dynamic (DLS) and Static (SLS) Light Scattering

Laser light scattering (LLS) experiments were performed using a Photocor Complex multi-angle light scattering instrument (Photocor Ltd., Russia) equipped with a thermostabilized diode laser ($\lambda = 659$ nm, 35 mW) and a thermo-electric Peltier temperature controller (temperature range from 5 to 100 °C, accuracy of 0.1 °C). LLS was used to determine hydrodynamic radii (R_h) of polymer molecules and micelles (DLS), weight average molecular weights (M_w), second virial coefficients (A_2), and aggregation numbers (N_{agg}) of micelles (SLS).

After preparation, polymer solutions were kept at room temperature for 24 h to reach equilibrium and were filtered through CHROMAFIL PET syringe filters (0.20 μ m) before starting measurements. At least three measurements were taken for each sample, resulting in an average hydrodynamic radius R_h (nm). M_w and A_2 were determined using the single-angle Debye plot method.

The scattering geometry of the instrument used was as follows: a vertically polarized incident light and detection without a polarizer (VU geometry, R_v). The Rayleigh ratio for toluene at the incident wavelength of 659 nm and measurement temperature was calculated according to [1].

Turbidimetry

Turbidimetry was used to determine solution cloud points (C_p), i.e., phase transition temperatures. Aqueous polymer solutions with concentrations of 1% (wt.) were used for the experiments; the rate of heating was approximately 0.3 °C/min. The C_p values were determined as a position of the maximum of the first derivative of the s-shaped turbidity curve [34]. Optical transmittance was measured using a KFC-2MP colorimeter (Zagorsk Optical and Mechanical Plant, Sergiev Posad, Russia) at a wavelength of 540 nm.

Cryogenic Transmission Electron Microscopy (Cryo-TEM)

In the first step, supporting copper electron microscopy grids with non-periodic holes in the amorphous carbon film (Lacey, Agar Scientific) were treated in a glow discharge using a PELCO easiGlow set-up for 30 s under standard conditions (0.25 mA, residual pressure in the chamber of 0.26 mBar) to make their surface hydrophilic. Then, 3 μ l of a copolymer solution (1 mg/mL) were applied to the prepared grid installed in the chamber of the Vitrobot Mark IV (Thermo Fisher Scientific, USA), after which the excess solution was blotted with filter paper, and the vitrification procedure (ultrafast freezing of a thin liquid layer on the grid surface in ethane condensed at liquid nitrogen temperature) was performed under the following parameters: blot force – 0, blot time – 2.5 sec, chamber temperature – 10°C or 40°C, chamber humidity – 100%. Before measuring at 40°C, the sample was pre-conditioned in an external thermostat at

45°C for 20 minutes. Frozen grids were transferred to a cryogenic transmission electron microscope in liquid nitrogen.

The vitrified samples were studied using a Tecnai G2 SPIRIT cryogenic transmission electron microscope (Cryo-TEM) (Thermo Fisher Scientific, USA) equipped with an Eagle CCD detector (Thermo Fisher Scientific, USA) with a resolution of 4096×4096 pixels. The study was carried out at an accelerating voltage of 120 kV in the low-dose mode. Images were acquired at 37000x magnification and defocused in the [-3 : -5] μm range; the total electron dose was 80 $\text{e}^-/\text{\AA}^2$. Experimental data for tomography were obtained at a magnification of 18000x (pixel size 3.7 \AA), range of angles of the tilt-series of [-60°: 60°] range with step increment of 2°, defocus - 8.0 μm . The total electron dose was 150 $\text{e}^-/\text{\AA}^2$. Three-dimensional reconstruction was performed by weighted back-projection using IMOD software (<https://bio3d.colorado.edu/imod/>), and patch-tracking was used to align the tilt-series. The obtained tomograms were deconvoluted with contrast transfer function (CTF) and filtered using IsoNet software. UCSF Chimera (<https://www.cgl.ucsf.edu/chimera/>) was used for visualization.

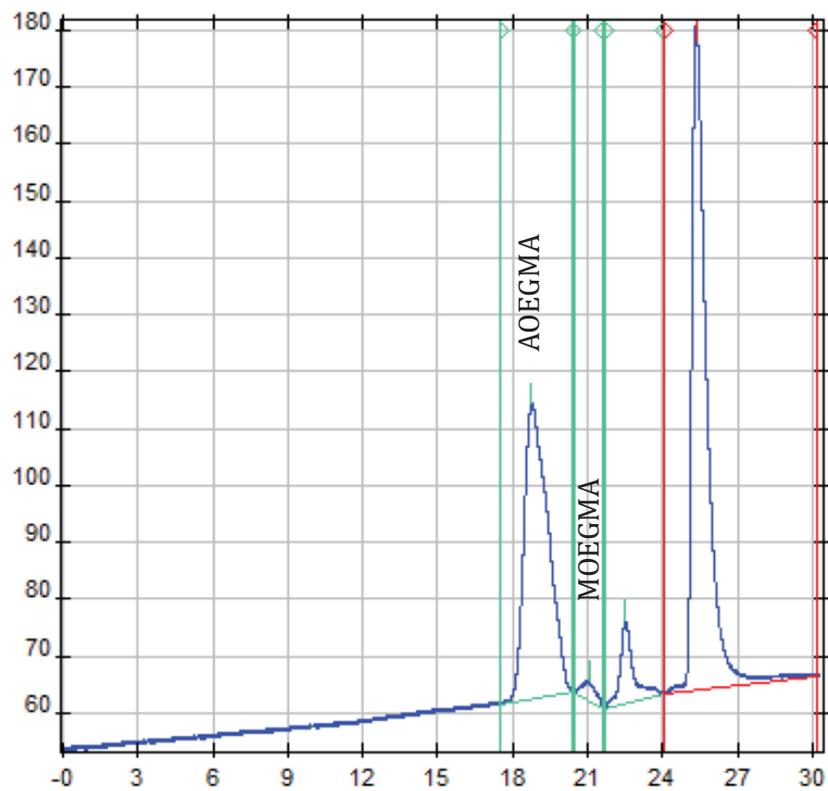
Synthesis of 4-cyano-4-(dodecylsulfanylthiocarbonyl)sulfanyl pentanoic acid

n-Dodecylthiol (15.4 g, 76 mmol) was added slowly to a stirred suspension of sodium hydride (3.15 g, 79 mmol) in diethyl ether (150 mL) at a temperature <10 °C. The reaction mixture was cooled to 0 °C, and carbon disulfide (6.0 g, 79 mmol) was added to give a yellow precipitate of sodium S-dodecyl trithiocarbonate separated by filtration.

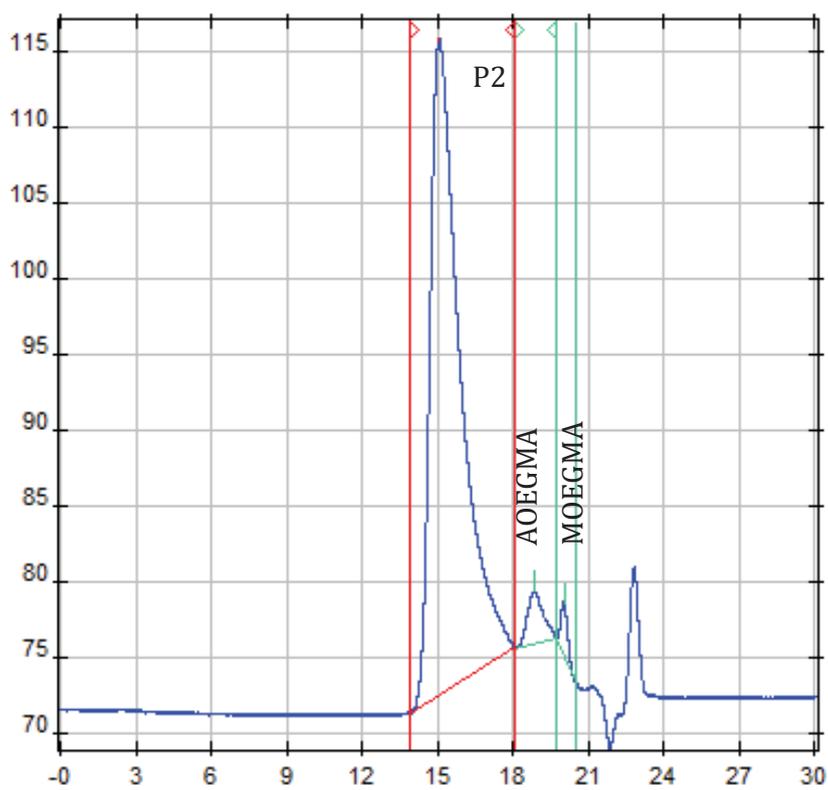
To a suspension of sodium S-dodecyl trithiocarbonate (14.6 g, 0.049 mol) in diethyl ether (100 mL), iodine (6.3 g, 0.025 mol) was added. The reaction mixture was then stirred at room temperature for 1 h, and the precipitated white sodium iodide was removed by filtration. The yellow filtrate was washed with an aqueous solution of sodium thiosulfate to remove excess iodine and dried over sodium sulfate, and evaporated to give a residue of bis-(dodecylsulfanylthiocarbonyl) disulfide. A solution of 4,4'-azobis(4-cyanopentanoic acid) (2.10 g, 0.0075 mol) and bis-(dodecylsulfanylthiocarbonyl) disulfide (2.77 g, 0.005 mol) in ethyl acetate (50 mL) was heated at reflux for 18 h. After removal of the volatiles in vacuo, the crude product was extracted with water (5 ×100 mL) to obtain 4-cyano-4-(dodecylsulfanylthiocarbonyl)sulfanyl pentanoic acid as a pale yellow solid (3.65 g, 87% yield), mp 58–59 °C, after recrystallization from hexane.

Examples of SEC traces for the starting macromonomers and isolated copolymers

a



b



c

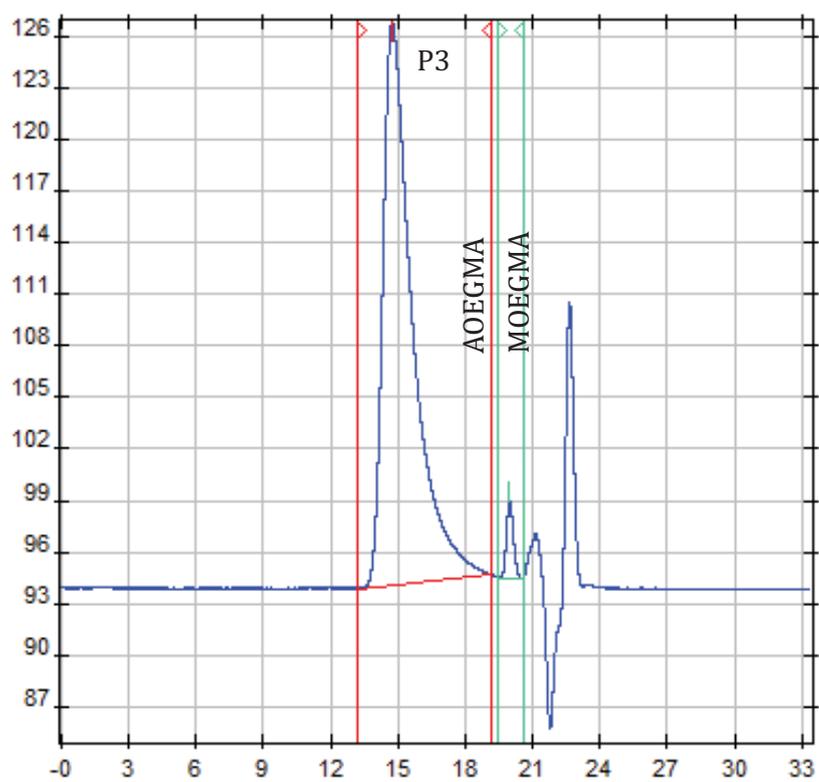
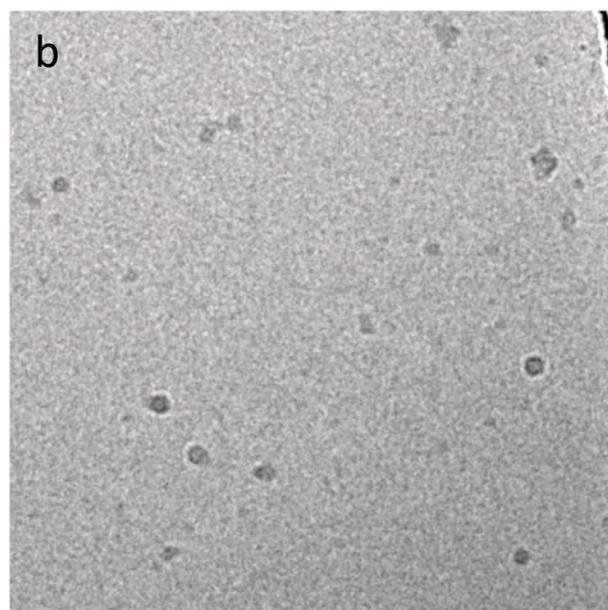
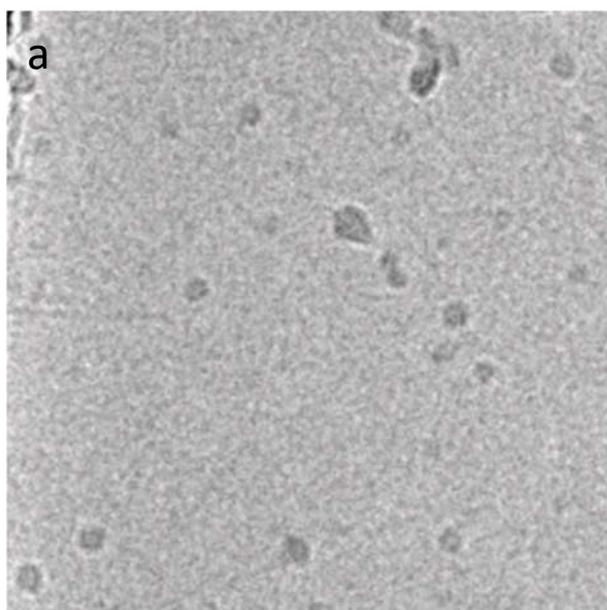


Figure S1. Examples of SEC traces for the (a) starting macromonomer mixture, P3, and (b, c) isolated copolymers, P2 and P3, with different initial comonomer ratio.

Cryo-TEM images



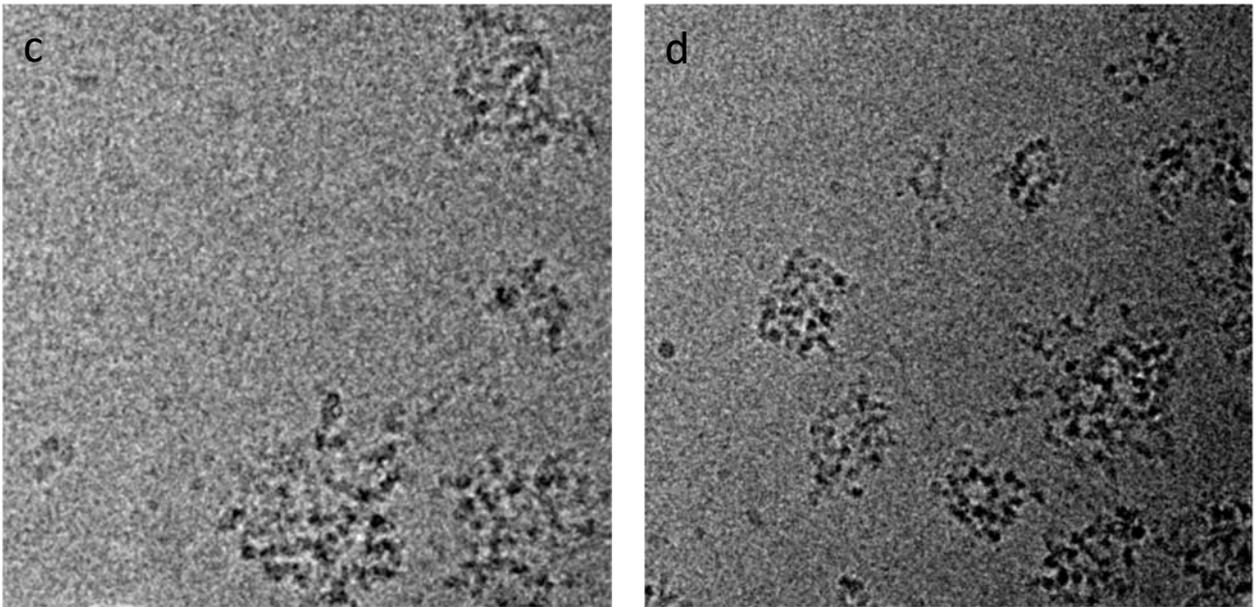
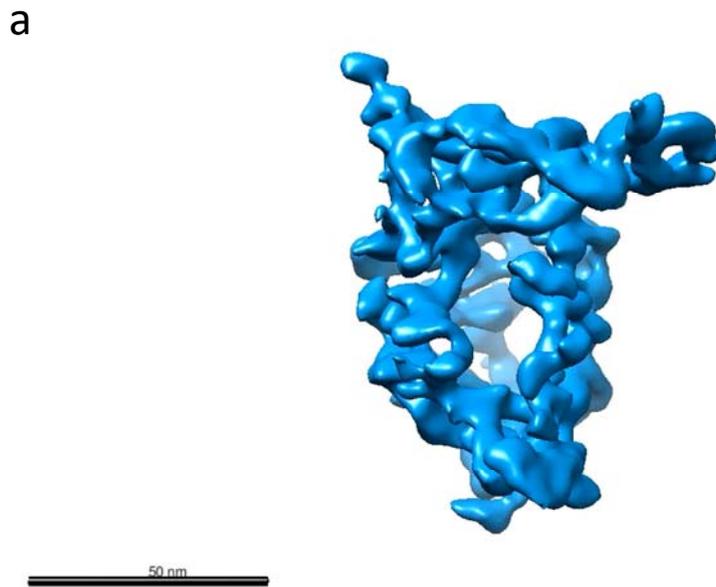


Figure S2. Additional cryo-TEM images of micelles and aggregates formed by P3 in 1 mg/mL aqueous solutions: (a, b) unimolecular micelles formed at 10 °C; (c, d) ensembles of macromolecule aggregates at a temperature above LCST (40 °C)



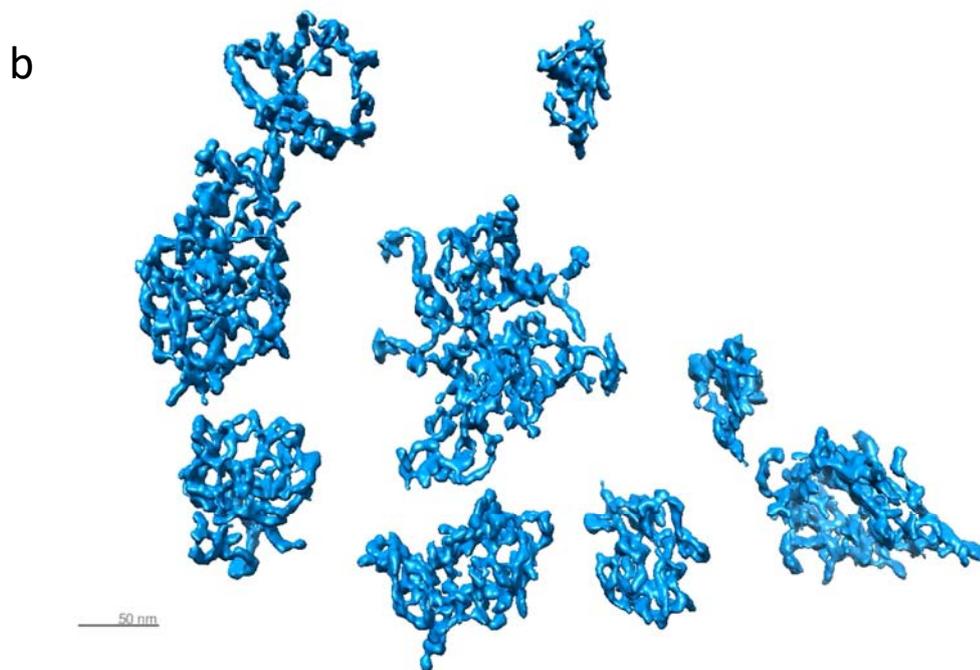


Figure S3. 3D volume rendering of (a) a single particle (aggregate) and (b) an ensemble of aggregates obtained using Cryo-TEM.

References

1. Sivokhin, A.P.; Kazantsev, O.A. Temperature Dependence of the Rayleigh Ratio for Toluene: Thermoresponsive Polymers Characterization. *ChemistrySelect* **2021**, *6*, 9499-9502, doi:<https://doi.org/10.1002/slct.202102196>.