Electronic Supplementary Information

Silkworm Cocoons by Cylinders Self-Assembled from H-Shaped Alternating Polymer Brushes

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Scheme S1, Table S1, Figures S1-S7, Calculations, Synthesis and Characterization.

Contents

1. Table, Scheme and Figures
Scheme S1. Synthesis of disulfide-bridged H-shaped (PEG ₁₆ -alt-PCL ₂₁) ₄ -S-S-(PEG ₁₆ -alt-
PCL ₂₁) ₄ polymer brush (Polymer 1) by RAFT process using S-CPDB as an original RAFT
agent
Table S1. Results for synthesized disulfide-linked Polymer 1 by RAFT polymerization in
dioxane at 70 °C
Fig. S1 ¹ H NMR spectrum of MI-PCL ($l \approx 21$) macroinitiator in CDCl ₃ 4
Fig. S2 GPC trace of MI-PCL ($M_{n,GPC} = 4080, M_w/M_n = 1.12$)
Fig. S3 IR spectrum of Polymer 1
Fig. S4 ¹ H NMR spectrum of Polymer 1 in CDCl ₃ 5
Fig. S5 Magnified TEM image of silkworm cocoons from Polymer 1 in Fig. 3B. The dark
areas are related to more phosphotungstic acid stains
Fig. S6 Determination of the diameter of cylinders by Polymer 1. The diameter of a cylinder
is <i>ca</i> . 16.5 nm7
Fig. S7 More TEM image of cylinder rafts from (PEG ₁₆ -alt-PCL ₂₁) ₄ -SH (Polymer 2)7
2. Calculations
2.1 Calculation of the contour length of the main chain and side chains of Polymer 1
2.2 Calculation of the number of disulfide bonds in a cylinder self-assembled from Polymer 1
3. Materials
4. Synthesis
5. Characterization
6. References

1. Table, Scheme and Figures

Scheme S1. Synthesis of disulfide-bridged H-shaped (PEG₁₆-*alt*-PCL₂₁)₄-S-S-(PEG₁₆-*alt*-PCL₂₁)₄ polymer brush (Polymer 1) by RAFT process using S-CPDB as an original RAFT agent



Table S1. Results for synthesized disulfide-linked Polymer 1 by RAFT polymerization in dioxane at 70 $^{\circ}C^{a}$

СТА	М	t (h)	C% ^b	$M_{\rm n,th}^{c}$	$M_{n,LS}^{d}$	$M_{\rm w}/M_{\rm n}^{d}$	$M_{n,NMR}^{e}$
S-CPDB	St-PEG, MI-PCL	20	85.6	30000	30500	1.14	30900

^{*a*} Polymerization conditions: $[St-PEG]_0:[MI-PCL]_0:[S-CPDB]_0:[AIBN]_0 = 10:10:1:0.4$, $[St-PEG]_0 = 0.10$ mol L⁻¹. ^{*b*} Monomer conversion determined by gravimetry. ^{*c*} Theoretically calculated molecular weight. ^{*d*} Number-average molecular weight and polydispersity determined by GPC-MALLS. ^{*e*} Number-average molecular weight determined by ¹H NMR analysis.



Fig. S1 ¹H NMR spectrum of MI-PCL ($l \approx 21$) macroinitiator in CDCl₃.



Fig. S2 GPC trace of MI-PCL ($M_{n,GPC} = 4080, M_w/M_n = 1.12$).



Fig. S3 IR spectrum of Polymer 1.



Fig. S4 ¹H NMR spectrum of Polymer 1 in CDCl₃.



Fig. S5 Magnified TEM image of silkworm cocoons from Polymer 1 in Fig. 3B. The dark areas are related to more phosphotungstic acid stains.



Fig. S6 Determination of the diameter of cylinders by Polymer 1. The diameter of a cylinder is *ca.* 16.5 nm.



Fig. S7 More TEM image of cylinder rafts from (PEG₁₆-*alt*-PCL₂₁)₄-SH (Polymer 2).

2. Calculations

2.1 Calculation of the contour length of the main chain and side chains of Polymer 1

The contour length of PEG side chains and PCL side chains were calculated by accumulating the length of carbon-carbon and carbon-oxygen covalent bonds at a specific angle:

The angle of carbon-carbon covalent bonds is 109.28°.

The angle of carbon-oxygen covalent bonds is 120°.

$$l_{PEG} = \left(l_{c-c} \times \cos\left(\frac{180^{\circ} - \theta_{c-c}}{2}\right) \times n_{c-c} + l_{c-o} \times \cos\left(\frac{180^{\circ} - \theta_{c-o}}{2}\right) \times n_{c-c} + l_{c-o} \times \cos\left(\frac{180^{\circ} - \theta_{c-o}}{2}\right)$$

$$l_{PCL} = \left(l_{c-c} \times \cos\left(\frac{180^\circ - \theta_{c-c}}{2}\right) \times n_{c-c} + l_{c-o} \times \cos\left(\frac{180^\circ - \theta_{c-o}}{2}\right) \times n_{c-c} \times \cos\left(\frac{180^\circ - \theta_{c-o}}{2}\right)$$

The contour length of the main chain was calculated by accumulating the length of carbon-carbon, carbon-oxygen and carbon-sulfur covalent bonds at a specific angle:

The angle of carbon-sulfur covalent bonds is 120°.

 $l_{main \ chain} = l_{spacer} + 2 \times l_{half \ brush} + 2 \times l_{end \ group}$

l_{spacer}

$$= \left(l_{c-c} \times \cos\left(\frac{180^{\circ} - \theta_{c-c}}{2}\right) \times n_{c-c} + l_{c-o} \times \cos\left(\frac{180^{\circ} - \theta_{c-o}}{2}\right) \times n_{c-c}\right)$$
$$\times 2 + l_{s-s} \times \cos\left(\frac{180^{\circ} - \theta_{s-s}}{2}\right) \times n_{s-s}$$
$$= \left(0.154 \ nm \times \cos\left(\frac{180^{\circ} - 109.28^{\circ}}{2}\right) \times 4 + 0.143 \ nm \times \cos\left(\frac{180^{\circ} - 120^{\circ}}{2}\right) \times 2 + 0.207 \ nm \times \cos\left(\frac{180^{\circ} - 120^{\circ}}{2}\right) \times 1 \approx 2.0 \ nm$$

$$l_{half \ brush} = l_{c-c} \times \cos\left(\frac{180^{\circ} - \theta_{c-c}}{2}\right) \times n_{c-c} \times DP = 0.154 \ nm \times \cos\left(\frac{180^{\circ} - \theta_{c-c}}{2}\right) \times 4 \approx 2.0 \ nm$$

l_{end group}

$$= l_{c-c} \times \cos\left(\frac{180^{\circ} - \theta_{c-c}}{2}\right) \times n_{c-c} + l_{c-s} \times \cos\left(\frac{180^{\circ} - \theta_{c-s}}{2}\right) \times n_{c-c}$$

=
$$0.154 nm \times \cos\left(\frac{180^{\circ} - 109.28^{\circ}}{2}\right) \times 1 + 0.182 nm \times \cos\left(\frac{180^{\circ} - 12}{2}\right) \times 1 + 0.266 nm \approx 0.7 nm$$

 $l_{main chain} = l_{spacer} + 2 \times l_{half brush} + 2 \times l_{end group}$

 $= 2.0 nm + 2 \times 2.0 nm + 2 \times 0.7 nm = 7.4 nm$

2.2 Calculation of the number of disulfide bonds in a cylinder self-assembled from Polymer 1

The calculation is based on our previous method.¹

In the TEM images (Fig. 1 and Fig. 3), only the hydrophobic domain (mainly PCL) is visible. The molecular weight of Polymer 1 is 30900 by ¹H NMR. The molecular weight of the hydrophilic PEG is 6000 (750 \times 8 = 6000), which forms invisible coronas in TEM images. Therefore, the molecular weight of the cylinder core-forming

part is 24900 (30900 - 6000 = 24900). Please note that in the following calculations, PCL stands for the cylinder core-forming part in Polymer 1.

Assuming the length of one cylinder is 500 nm, then

$$N_{bonds} = N_{agg} = n_{PCL} \bullet N_A = \frac{M_{core}N_A}{M_{n,PCL}} = \frac{\rho_{PCL}V_{core}N_A}{M_{n,PCL}} = \frac{\frac{\pi}{4}D_{core}^2L_{cylinder}\rho_{PCL}N_A}{M_{n,PCL}} = 3100$$

 $ρ_{PCL}$: density of PCL block (1.2×10⁻²¹ g/nm³); $M_{n,PCL}$: number-average molecular weight of hydrophobic cylinder axis-forming part in Polymer 1 (¹H NMR; 24900); D_{core} : the diameter of the cylinder (TEM; 16.5 nm); $L_{cylinder}$: assuming the length of one cylinder (500 nm); V_{core} : volume of the cylinder core; M_{core} : mass of one cylinder core; n_{PCL} : Number of moles of Polymer 1 chains in one cylinder; N_A : Avogadro's constant (6.02×10²³); N_{agg} : mean aggregation number of Polymer 1 in one cylinder; N_{bonds} : the number of the S-S δ bonds in one cylinder

The following is a Table created by Microsoft Excel. The formulas will be appeared when clicking the corresponding cells.

Density of PCL, ρ_{PCL}	1.20E-21	g/nm ³
Molecular weight of PCL, $M_{n,PCL}$	24900	
Cylinder core diameter, D_{core}	16.5	nm
Cylinder length, L Cylinder	500	nm
Volume of one cylinder core, $V_{\rm core}$	106912	nm ³
Mass of one cylinder core, $M_{\rm core}$	1.28E-16	g
Mole number of one cylinder core, n_{PCL}	5.15E-21	mol
N _A constant	6.02E+23	
Aggregation number, N_{agg}	3102	
Number of S-S δ bonds, N_{bonds}	3102	

3. Materials

All solvents, monomers, and other chemicals were purchased from Sigma-Aldrich unless otherwise stated. ε -Caprolactone (CL, 99%) was distilled from calcium hydride under reduced pressure. 4-Vinylbenzyl chloride (VBC, 90%) was passed through a basic alumina column to remove the inhibitor. 2,2'-Azobis(isobutyronitrile) (AIBN) was recrystallized twice from ethanol.

Monomethoxy poly(ethylene glycol) (MPEG, $M_n = 750$ g mol⁻¹, Fluka) was dried by azeotropic distillation in the presence of toluene. *N*,*N'*-Dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) were purchased from Sinopharm Chemical Reagent Co., Ltd. and used as received. Dichloromethane (DCM) and dioxane were dried and distilled over CaH₂. Tetrahydrofuran (THF) and toluene were distilled over sodium and benzophenone and stored under nitrogen. α -Methoxy- ω -vinylbenzyl poly(ethylene glycol) (St-PEG),² maleimidic poly(ε -caprolactone) (MI-PCL, $M_{n,NMR} = 2560$ g mol⁻¹, PDI = 1.12),³ 4-cyanopentanoic acid dithiobenzoate (4-CPDB),⁴ and disulfide-linked RAFT agent S-CPDB⁵ were synthesized and purified according to literature procedures.

4. Synthesis

Synthesis of Polymer 1 by RAFT copolymerization. To a Schlenk tube were added S-CPDB (31.2 mg, 0.046 mmol), St-PEG (400 mg, 0.46 mmol), MI-PCL (1.18 g, 0.46 mmol), AIBN (3.0 mg, 18.3 μ mol), and dry dioxane was added until the total volume was 4.6 mL. The contents were degassed with bubbled nitrogen for 15 min, and then the polymerization was performed at 70 oC for 20 h. The polymerization solution was precipitated into diethyl ether, and 1.38 g (85.6% total monomer conversion) of polymer bush was obtained.

Synthesis of Polymer 2 by cleavage of Polymer 1. Polymer 2 was obtained by treatment of Polymer 1 in the presence of 10 mM DTT for 90 min and purified by dialysis.

Self-assembly of Polymer 1 into silkworm cocoons. The self-assembly of Polymer 1 was based on a solvent-switch method. Typically, the polymer brush was dissolved in DMF to form a homogenous solution and double volume of deionized water was added dropwise with a syringe at a rate of about one drop every 3 seconds under magnetic stirring. The blue solution was stirred for 30 minutes after all the water was added. Subsequently, the solution was dialyzed against pure water for 24 h to remove DMF in a dialysis tubing with a molecular weight cut off from 8000 to 14000. The final polymer concentration was 0.286 mg/mL.

Self-assembly of Polymer 2 into cylinder rafts. The self-assembly of the (PEG16-alt-PCL21)4-SH (Polymer 2) was carried out according to a similar procedures to the self-assembly of Polymer 1. The final polymer concentration was 0.200 mg/mL.

5. Characterization

Proton nuclear magnetic resonance (¹H NMR). ¹H NMR (400 MHz) spectra were recorded on a Varian spectrometer at 25 °C using CDCl₃ as a solvent.

Gel Permeation Chromatography (GPC). Apparent molecular weight $(M_{n,GPC})$ and polydispersity (PDI) of linear polymers were measured on a Waters 150-C GPC using three Ultrastyragel columns (pore size 50, 100, and 1000 nm, with molecular weight ranges of 100-10000, 500-30000, and 5000-600000 g mol⁻¹, respectively) with 10 μ m bead size at 35 °C. THF was used as an elution at a flow rate of 1.0 mL min⁻¹, and the samples were calibrated with PMMA standard samples. GPC with multiple angle laser scattering detection (GPC-MALLS) was used to determine the absolute number-average molecular weight $(M_{n,LS})$ of nonlinear polymers, in which GPC was conducted in DMF at 40 °C with a flow rate of 1.0 mL min⁻¹, and three MZ-Gel SDplus columns (pore size 10³, 10⁴ and 10⁵ Å, with molecular weight ranges of 1000-40000, 4000-500000, and 10000-2000000, respectively) with 10 µm bead size were used. Detection systems consisted of a RI detector (Optilab rEX) and a multiangle (14°-145°) laser light scattering detector (DAWN HELLOS) with the He-Ne light wavelength at 658.0 nm. The refractive index increment dn/dc for samples were measured off-line by Optilab rEX refractive index detector ($\lambda =$ 658 nm) at 25 °C using a series of different concentration solutions. Data were collected and processed by use of ASTRA software from Wyatt Technology, and molecular weights were determined by the triple detection method.

Fourier Transform Infrared (FT-IR) spectroscopy. FT-IR spectra were recorded on a Perkin-Elmer 2000 spectrometer using KBr discs.⁶

Dynamic Light Scattering (DLS). DLS measurements were carried out at 25 °C and a fixed scattering angle of 90° with Zetasizer Nano series instrument (Malvern Instruments ZS 90) equipped with a multipurpose autotitrator (MPT-2). Each reported measurement was conducted three runs.⁷

Transmission Electron Microscopy (TEM). TEM images of self-assembly solutions without dilution were obtained using a JEM-2100F electron microscope operating at an acceleration voltage of 200 kV equipped with a Gatan 894 Ultrascan 1k CCD camera. 7.5 µL of self-assembly

solutions was dropped onto a carbon-coated copper grid disposed for 30 s with a plasma cleaner to make it more hydrophilic and dried at ambient temperature. The copper grid was then stained with 1% aqueous phosphotungstic acid solution at pH 7.0.⁸

6. References

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