

SYNTHESIS AND CHARACTERIZATION OF ULTRATHIN METAL COORDINATION PRUSSIAN BLUE NANORIBBONS

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Experimental section

Materials. Ethylenediamine, methyl acrylate, hydrobromic acid, L-glutamic acid, benzyl alcohol, pyridine, triphosgene, $\text{NH}_4\text{Na}_2[\text{Fe}(\text{CN})_5\text{NH}_3]\cdot 2\text{H}_2\text{O}$ and 4-(aminomethyl)pyridine were purchased from Aldrich (China) and used without further purification unless otherwise indicated.

Determination of thickness and width of PB hybrid nanoribbons: The thickness and width of nanoribbons were measured from height images and corresponding cross-sectional profiles of tapping mode AFM. The average values were obtained by counting 50 values of thickness and width from various locations of height images. The thickness of nanoribbons was determined to be *ca.* 1.0 nm (Fig. S1). Considering that PB monolayers were constructed from bulky cyanoferrate complex coordinated with small Fe^{3+} ions, the thickness of PB monolayers was dominated by the dimension of cyanoferrate complex. Cyanoferrate complex has dimension of 0.49 nm \times 0.62 nm (Scheme S1). So the thickness of PB monolayers is in the range from 0.49 nm to 0.62 nm. It was noticed that the thickness of PB hybrid nanoribbons was nearly double of thickness of PB monolayers. PBLG-Fe exhibited amphiphilic nature. As PBLG-Fe self organizes in water, hydrophobic PBLG layer is sandwiched between two hydrophilic ferrate layers. Subsequent coordination polymerization of cyanoferrate complex with Fe^{3+} led to the formation of tri-layered structures consisting of a PBLG

layer sandwiched between two PB monolayers (see Scheme 1). The thickness of the flexible PBLG layer was neglectable contrasting to the rigid PB layers.

The apparent width of PB ribbons was determined to be 19.7 nm from AFM images. By using a model based on a spherical tip and a rectangular cross-section of the ribbons, the broadening effect was given by equation (1):¹

$$2\Delta = 2\sqrt{h(2R - h)} \quad (1)$$

where the height of the nanoribbons $h = 1.0$ nm and a terminal radius of tapping mode AFM tips $R = 13$ nm, $2\Delta = 10$ nm. The true nanoribbons width was obtained by subtracting 2Δ from the apparent width. So the true width of PB hybrid nanoribbons was 9.7 nm.

The length of fully extended peptide bonds was reported to be 0.38 nm.² The length of each arm of PBLG is $0.38 \times 9 = 3.42$ nm (9 is the number of repeating units of each arm of PBLG used). The length of the fully extended initiator (compound 2) is estimated to be 2.20 nm using ChemBioDraw software (Scheme S2). It was noticed that the width of nanoribbons (9.7 nm) was close to the length of double arms of PBLG-Fe plus initiator ($3.42 \text{ nm} \times 2 + 2.20 \text{ nm} = 9.04 \text{ nm}$). Therefore, we believed that PBLG-Fe macromolecules were aligned perpendicular to the long axes of the ribbons. Indeed some sets of parallel lines perpendicular to the long axis of PB ribbons were observed in high-magnification TEM images (Fig. 1b).

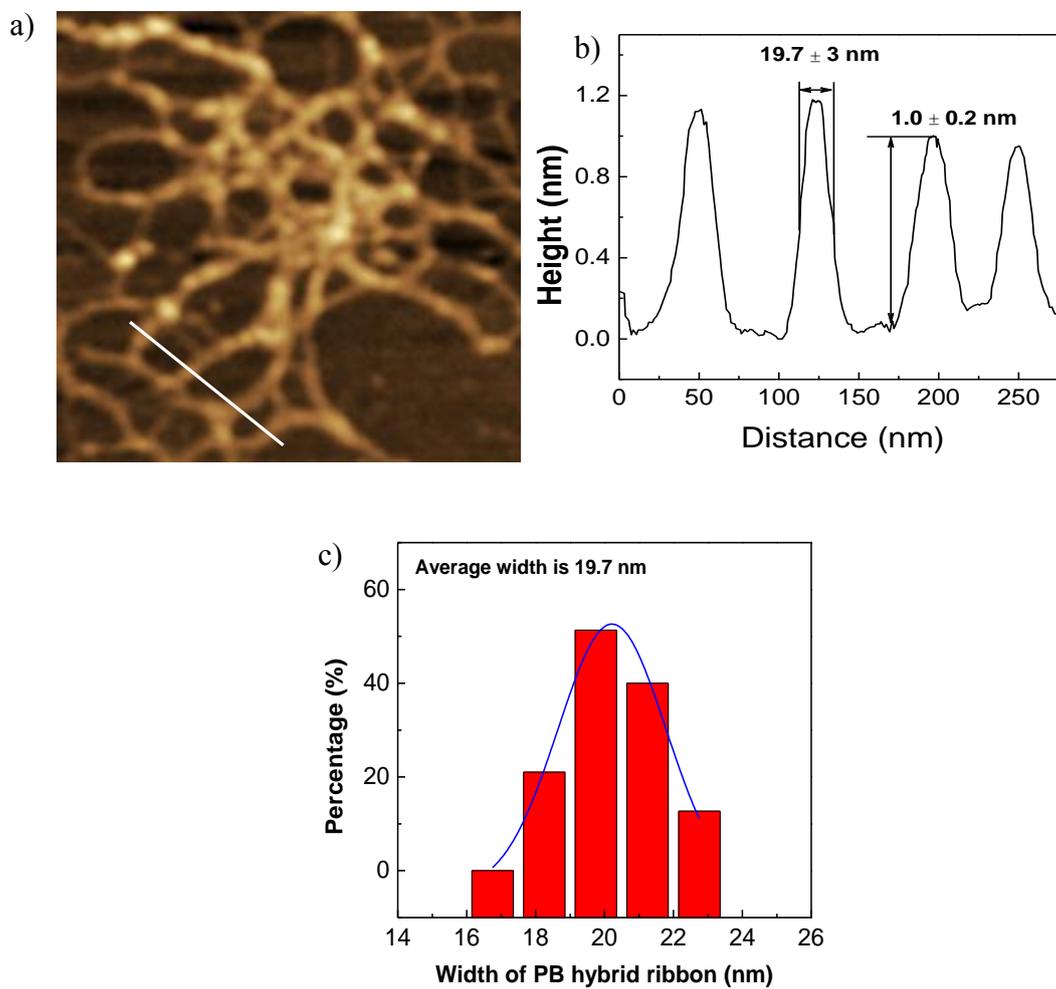
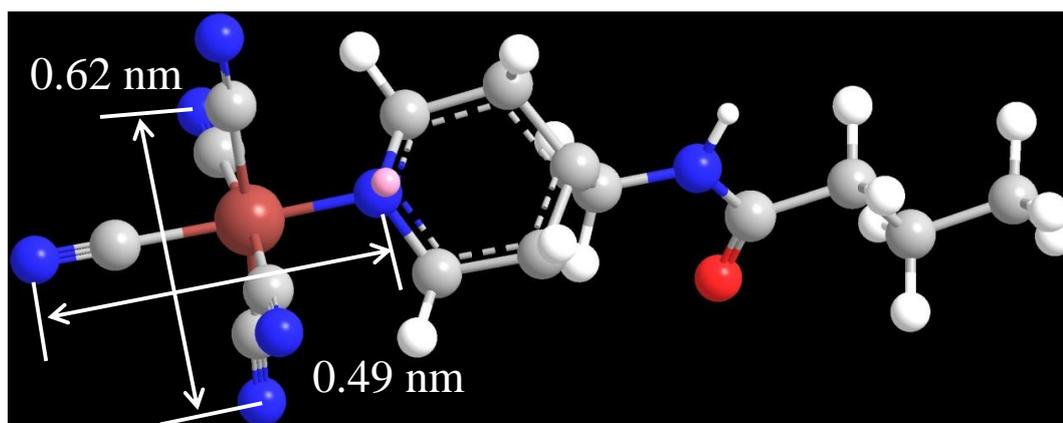
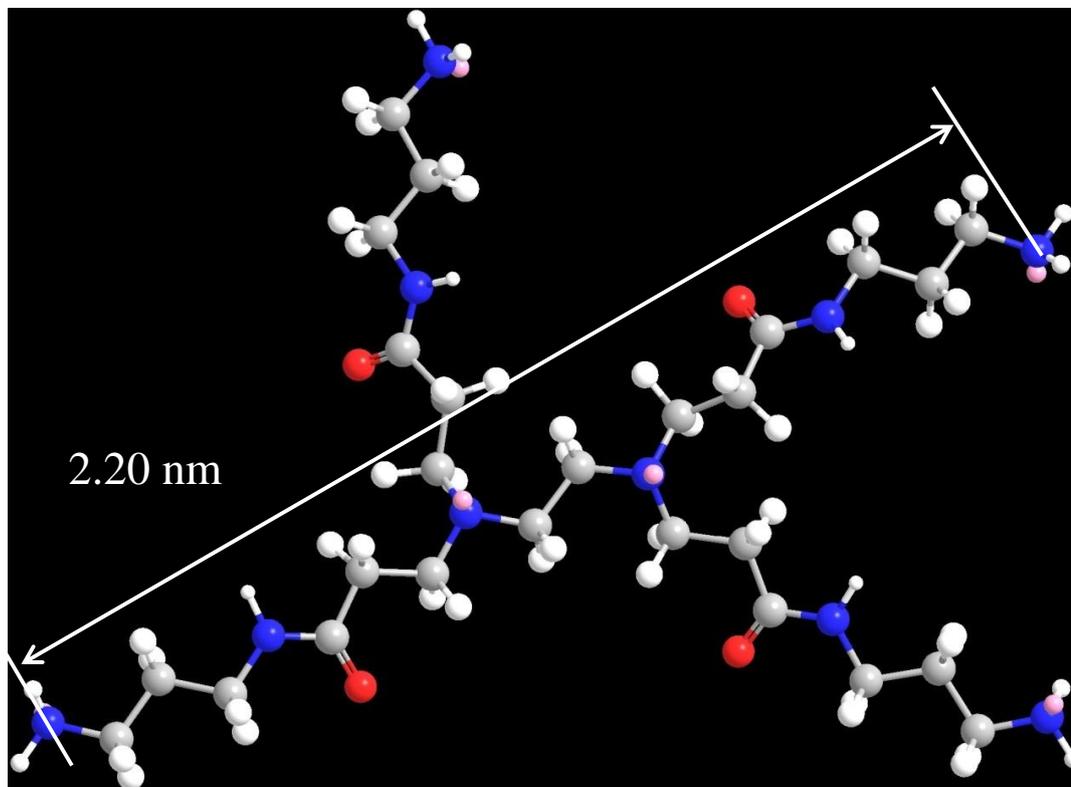


Fig. S1 Height image of tapping mode AFM of PB hybrid nanoribbons (a), cross-sectional profile along the line in (a) and distribution of width of PB hybrid nanoribbons (c). Image size: 600 nm × 600 nm.



Scheme S1 Rod-stick model of [Fe(CN)₅(Py)] showing dimensions of cyanoferrate complex of 0.49 nm × 0.62 nm. The modeling was carried out using Chemoffice software (ChemBioDraw Ultra 12.0).



Scheme S2 Rod-stick Model of compound 2 illustrating fully extended length of 2.20 nm. The modeling was carried out using Chemoffice software (ChemBioDraw Ultra 12.0).

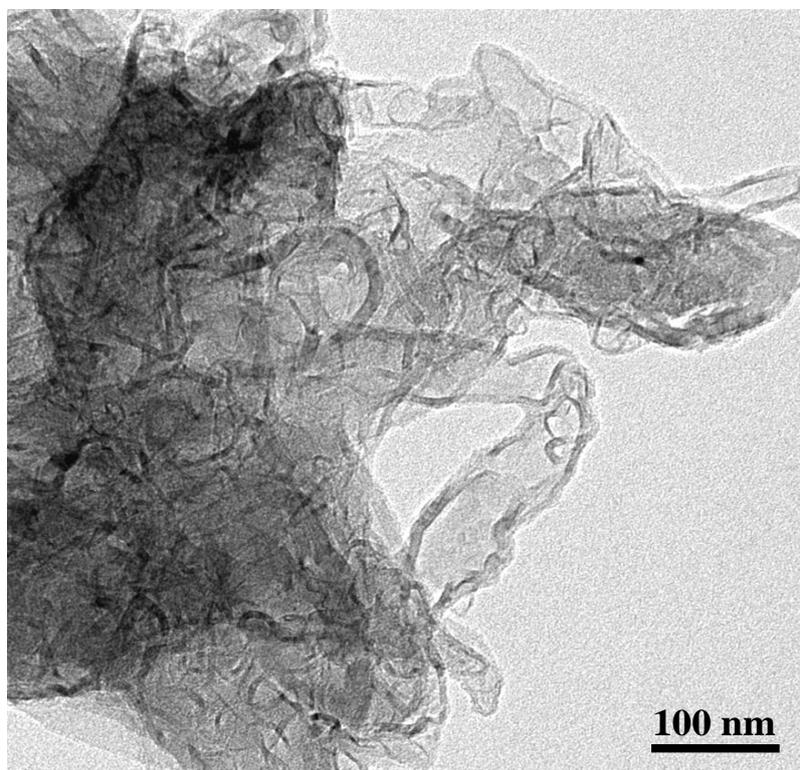


Fig. S2 TEM image of PBLG-Fe suspended in water with the assistance of sonication.

Attempt to tune the width of PB hybrid nanoribbons

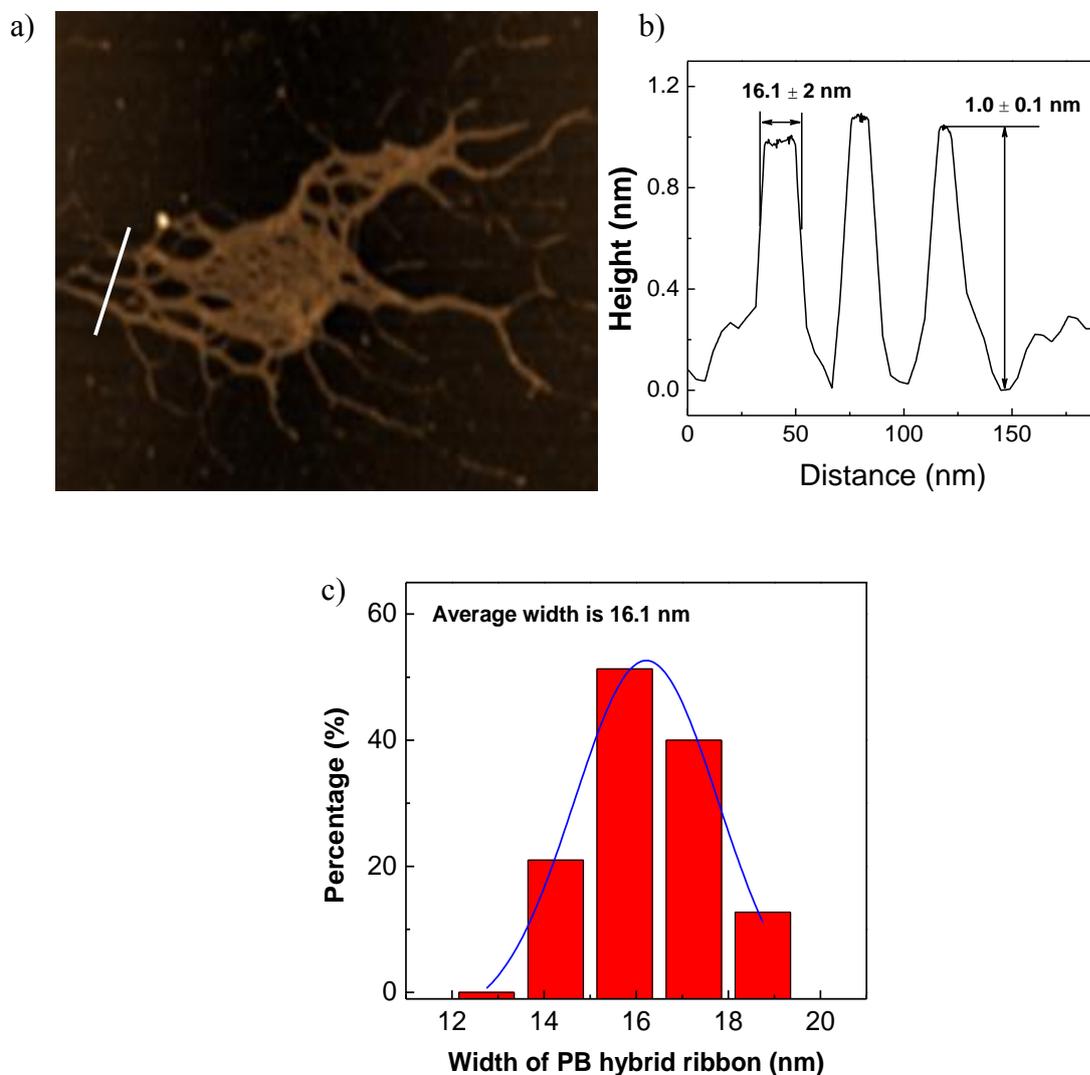
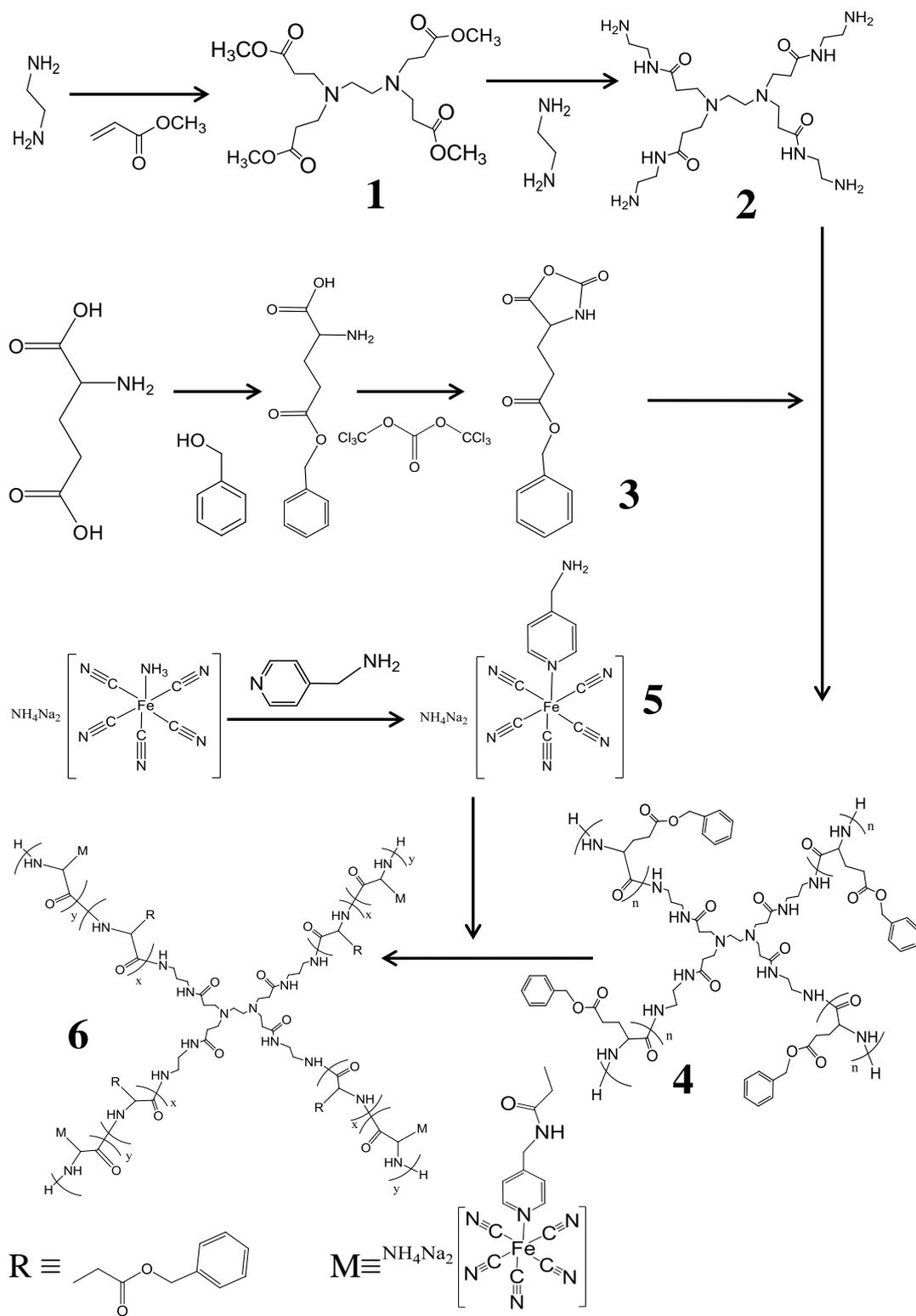


Fig. S3 Height image of tapping mode AFM of PB hybrid nanoribbons (a), cross-sectional profile (b) along the line in (a) and distribution of width of PB hybrid nanoribbons (c). Image size: 600 nm × 600 nm. PBLG-S-Fe (see synthesis) was used for synthesis of PB hybrid nanoribbons with narrow width. The true nanoribbons width was 16.1 nm – 10 nm = 6.1 nm.

Synthesis.



Scheme S3 Synthetic route for 4-armed star poly(γ -benzyl-L-glutamate) modified with $\text{NH}_4\text{Na}_2[\text{Fe}(\text{II})(\text{CN})_5(4\text{-aminomethylpyridine})]$ (PBLG-Fe).

Synthesis of amidoamine initiator (2): The synthesis protocol for amidoamine initiator was adapted from previous literature procedures.^{3,4} A typical run was shown as follows. A methanol solution (10 mL) of ethylenediamine (0.6 g, 10 mmol) was added dropwise into a methyl acrylate (5.16 g, 60 mmol) in methanol (20 mL) at 0 °C. The resulting reaction mixture was allowed to warm to room temperature and stirred for 2 days under N₂. Then the volatiles were removed under reduced pressure using a rotary evaporator and then in vacuum at 40 °C to give 3.8 g product (1) as slightly yellow oil (yield: 94%). ¹H NMR (300 MHz, CDCl₃, 298 K): δ 2.42 (m, 12 H, NCH₂), 2.72 (m, 8H, CH₂COOCH₃), 3.65 (m, 12H, COOCH₃). The ¹H NMR spectrum agreed with the literature data.^{3,4}

A methanol solution (3 mL) of 1 (0.808 g, 2 mmol) was added dropwise into a round-bottomed flask containing ethylenediamine (1.202 g, 20mmol) and anhydrous methanol (7 mL) at 0 °C using an ice/water bath. The reaction mixture was allowed to warm to room temperature and stirred for 7 days under N₂ until complete disappearance of terminal methyl ester groups of 2, monitored by ¹H NMR. Then the volatiles were removed using a rotary evaporator to get crude product. To the crude product was added 20 mL anhydrous methanol and then removed the solvent using a rotary evaporator. Repeat this cycle three times to remove un-reacted ethylenediamine, and finally residual volatiles were removed in vacuum at 40 °C overnight to give 0.986 g product (2) as viscous slightly yellow oil (yield: 95%). ¹H NMR (300 MHz, CDCl₃, 298 K): δ 2.34–2.44 (m, 12H, COCH₂ and NCH₂CH₂N), 2.64 (m, 8H, CH₂NH₂), 2.82 (m, 8H, NCH₂CH₂CO), 3.30 (m, 8H, CONHCH₂), 7.95 (br, 4H, CONH). The signal at 3.65 ppm derived from OCH₃ is neglectable. The ¹H NMR spectrum agreed with the literature data.^{3,4}

Synthesis of γ -Benzyl-L-glutamate carboxyanhydrides (BLG-NCA) (3): The preparation for BLG-NCA was adapted from literature procedures.⁵⁻⁷ A typical run was shown as follows. 60 mL 48% hydrobromic acid and 33 g L-glutamic acid were added to 220 mL benzyl alcohol. This mixture was heated at 70 °C with violently stirring until all glutamic acid was dissolved (ca. 1.5 hours). The

reaction mixture was cooled to 30 – 40 °C, and then added to a solution of 33 mL pyridine in 220 mL 95% ethanol under stirring. Precipitation occurred upon cooling to 20 °C and the precipitation was allowed to continue at 3 °C for 12 h. The precipitate was then collected by filtration, washed with ethanol, then with ethyl ether and air-dried. The product was re-crystallized from 500 mL of 5% ethanol aqueous solution, followed by adding sufficient sodium bicarbonate to keep the pH at 7. After filtration, the solution was cooled as rapidly as possible to 3 °C, and left for 12 h. The precipitate was collected by filtration, washed with water and adjusted to pH 7 with sodium bicarbonate, washed with distilled water, slurried with ethanol, filtered, washed with ethyl ether and air-dried, to yield 12.8 g white plates of γ -benzyl-L-glutamate (yield: 25.2%). ^1H NMR (300 MHz, CDCl_3 , 298K): δ 2.41 (m, 2H, COCH_2CH_2), 2.84 (t, 2H, COCH_2CH_2), 4.37 (t, 1H, COCHNH_2), 5.18 (s, 2H, COOCH_2Ph), 7.30–7.39 (m, 5H, COOCH_2Ph).

γ -Benzyl-L-glutamate (10 g) was suspended in 150 mL anhydrous THF and then triphosgene (4.5 g) was added under nitrogen. The mixture was stirred at 50 °C under N_2 until it turned into a transparent solution within 3 h. The product was precipitated by pouring the solution into 500 mL hexane, isolated by filtration, and purified by re-crystallizing three times from the THF/hexane mixed solution. The yield was 49%. ^1H NMR (300 MHz, CDCl_3 , 298K): δ 2.14 (m, 2H, COCH_2CH_2), 2.61 (t, 2H, COCH_2CH_2), 4.39 (t, 1H, COCHNH), 5.12 (br, 2H, COOCH_2Ph), 6.6 (s, 1H, CONH), 7.30–7.38 (m, 5H, COOCH_2Ph). The ^1H NMR spectrum agreed with the literature data.⁷

Synthesis of 4-armed poly(γ -benzyl-L-glutamate) (PBLG) (4): 0.02 g compound **2** (0.035 mmol) and 0.4 g BLG-NCA (1.52 mmol) were dissolved in 15 mL anhydrous THF and the solution was stirred for 72 h at room temperature under N_2 . The resulting mixture was added dropwise to ethanol. The precipitate was isolated by filtration and dried under vacuum at 40 °C overnight to yield 0.315 g 4-armed poly(γ -benzyl-L-glutamate) as white solids. To improve the solubility of PBLG in CDCl_3 , ca. 15 vol% trifluoroacetic acid (TFA) was added for ^1H NMR measurement. ^1H NMR (300 MHz,

CDCl_3 , 298 K): δ 1.90–2.24 (br, 84H, COCH_2CH_2 from PBLG, COCH_2 and $\text{NCH}_2\text{CH}_2\text{N}$ from initiator **2**), 2.41–2.69 (br, 80H, COCH_2CH_2 from PBLG, NH_2CH_2 from initiator **2**), 4.58 (br, 36H, COCHNH), 5.12 (m, 72H, COOCH_2Ph), 7.28–7.42 (br, 180H, COOCH_2Ph), 8.01 (m, 40H, CONH). ^{13}C NMR (300 MHz, $\text{CDCl}_3+15\text{vol}\%$ TFA, 298 K): δ 31.6 (CH_2), 40.5 (β -sheet), 66.3 (CH_2Ph), 128.7 (Ph), 172.2 ($\text{C}=\text{O}$). The ^1H NMR and ^{13}C NMR spectra agreed with the literature data.⁷ The number of repeating units was determined to be 36, and the molecular weight was $516+219*36=8.4$ kg/mol. Molecular weight and molecular weight distribution of PBLG were determined to be 11.8 kg/mol and 1.40 by using GPC calibrated with PEO standard, respectively. FT-IR (KBr): ν (cm^{-1}) 3291 (N-H stretching), 3037 (C-H stretching of phenyl rings), 1735 (C=O stretching), 1628 (C=O stretching of β -sheet), 1529 (C-N stretching of β -sheet).

For synthesis of PBLG with lower molar mass (PBLG-S), exactly same protocol was adopted except that 0.285 g BLG-NCA (1.08 mmol) was used. 0.228 g white polymer product was collected. Molecular weight was 6.5 kg/mol theoretically. ^1H NMR (300 MHz, CDCl_3 , 298 K): δ 1.90–2.24 (br, 60H, COCH_2CH_2 from PBLG, COCH_2 and $\text{NCH}_2\text{CH}_2\text{N}$ from initiator **2**), 2.41–2.69 (br, 56H, COCH_2CH_2 from PBLG and NH_2CH_2 from initiator **2**), 4.58 (br, 24H, COCHNH), 5.12 (m, 48H, COOCH_2Ph), 7.28–7.42 (br, 120H, COOCH_2Ph), 8.01 (m, 28H, CONH). ^{13}C NMR (300 MHz, $\text{CDCl}_3+15\text{vol}\%$ TFA, 298 K): δ 31.6 (CH_2), 40.5 (β -sheet), 66.3 (CH_2Ph), 128.7 (Ph), 172.4 and 172.2 ($\text{C}=\text{O}$). The ^1H NMR and ^{13}C NMR spectra agreed with the literature data.⁷ The number of repeating units was determined to be $6 \times 4 = 24$. Molecular weight was calculated to be $516+219*24=5.8$ kg/mol. Molecular weight and molecular weight distribution of PBLG were determined to be 7.8 kg/mol and 1.30 using GPC calibrated with PEO standard, respectively. FT-IR (KBr): ν (cm^{-1}) 3291 (N-H stretching), 3037 (C-H stretching of phenyl rings), 1735 (C=O stretching), 1628 (C=O stretching of β -sheet), 1529 (C-N stretching of β -sheet).

Determination of molecular weight, polydispersity (PDI) and repeating units of PBLG: Molecular weight, polydispersity index and repeating units for 4-armed star PBLG were summarized in Table S1.

Table S1 Number-average molecular weight, polydispersity index and the number of repeating units of the PBLG synthesized.

Polymer	^a <i>M</i> _n	^b <i>M</i> _n	^c <i>M</i> _n	^c PDI	^d DP
	(kg/mol)	(kg/mol)	(kg/mol)		
PBLG	9.0	8.4	11.8	1.40	9×4=36
PBLG-S	6.5	5.8	7.8	1.30	6×4=24

^aCalculated theoretically. ^bDetermined by ¹H NMR. ^cDetermined by GPC using DMF as an eluent at flow rate of 1 mL/min calibrated with PEO standard. ^dDP denoted the number of repeating units of PBLG synthesized.

Synthesis of ferrate-containing PBLG (PBLG-Fe) (6): The protocol for cyanoferrate complex was adapted from literature procedures.⁸ A typical run was shown as follows. NH₄Na₂[Fe(CN)₅NH₃]·2H₂O (1.00 g, 3.3 mmol) was mixed with a tenfold excess of 4-(aminomethyl)pyridine (3.56 g, 33 mmol) in 10 mL distilled water, and the mixture magnetically stirred at room temperature under nitrogen overnight. The product was precipitated by the addition of 100 mL ethanol, and was isolated through filtration. The crude product was washed with cold ethanol (100 mL × 3) and dried under vacuum to yield 0.900 g NH₄Na₂[Fe(II)(CN)₅Py] (**5**) as yellow powder. The yield is 66%. ¹H NMR (300 MHz, D₂O, 298 K): δ 4.08 (s, 2H, NH₂CH₂Py), 7.18 (d, 2H, Py), 8.95 (d, 2H, Py). The ¹H NMR spectrum

agreed with the literature data.⁸ ¹H NMR of 4-(aminomethyl)-pyridine was also carried out for purpose of comparison. ¹H NMR (300 MHz, D₂O, 298 K): δ 3.76 (s, 2H, NH₂CH₂Py), 7.27 (d, 2H, Py), 8.35 (d, 2H, Py). The product **5** showed a redox wave on a glassy carbon working electrode with $E_{1/2}$ of 0.20 V vs. Ag/AgCl in 0.1 M KCl solution under N₂.

5 0.170 g (0.02 mmol, 0.88 mmol COOCH₂Ph group) PBLG in 3 mL dried DMF was mixed with 0.17 g (0.47 mmol) NH₄Na₂[Fe(II)(CN)₅Py] (**5**) in 10 mL methanol. The mixture was stirred for 72 h at 40 °C under N₂. The product was precipitated by adding the reaction mixture dropwise into cold methanol, and washed with methanol (100 mL × 3) until filtrate is colorless. The solid was dried in vacuum at 40 °C overnight to yield 0.210 g ferrate-containing PBLG (PBLG-Fe, **6**). FT-IR (KBr): ν (cm⁻¹) 3291 (N-
10 H stretching), 3037 (C-H stretching of phenyl rings), 2060 (C≡N stretching), 1735 (C=O stretching), 1628 (C=O stretching of β-sheet), 1529 (C-N stretching of β-sheet). PBLG-Fe showed a redox wave on a glassy carbon working electrode with $E_{1/2}$ of 0.20 V vs. Ag/AgCl in 0.1 M KCl solution under N₂.

For synthesis of PBLG-Fe with lower molar mass (PBLG-S-Fe), the same procedure was adopted except that 0.17 g (0.029 mmol, 0.7 mmol COOCH₂Ar group) PBLG-S was used. 0.21 g solid
15 products (PBLG-S-Fe) was collected. FT-IR (KBr): ν (cm⁻¹) 3291 (N-H stretching), 3037 (C-H stretching of phenyl rings), 2060 (C≡N stretching), 1735 (C=O stretching), 1628 (C=O stretching of β-sheet), 1529 (C-N stretching of β-sheet). PBLG-S-Fe showed a redox wave on a glassy carbon working electrode with $E_{1/2}$ of 0.20 V vs. Ag/AgCl in 0.1 M KCl solution under N₂. The weight percentage of ferrate complex and iron of PBLG-S-Fe was calculated to be 23.6% and 3.7%,
20 respectively.

Determination of iron percentage of PBLG-Fe: Conversion of PBLG into PBLG-Fe was calculated from mass change during the reaction of PBLG with ferrate complex using the following formula:

$$\begin{aligned} \text{conv} &= \frac{(w_{\text{PBLG-Fe}} - w_{\text{PBLG}})/(M_c - M_{\text{benzyl alcohol}})}{w_{\text{PBLG}} \times \frac{DP}{M_{\text{PBLG}}}} \times 100\% \\ &= \frac{(0.21 \text{ g} - 0.17 \text{ g})/(358 \text{ g/mol} - 108 \text{ g/mol})}{(0.17 \text{ g}) \times 36/(8500 \text{ g/mol})} \times 100\% = 22.2\% \end{aligned} \quad (2)$$

where $w_{\text{PBLG-Fe}}$ and w_{PBLG} denoted the weight of PBLG-Fe and PBLG, respectively. M_c , $M_{\text{benzyl alcohol}}$, M_{PBLG} denoted molecular weight of ferrate complex, benzyl alcohol and PBLG, respectively.

The weight percentage of ferrate complex of PBLG-Fe, w_c was calculated using the following formula:

$$\begin{aligned} w_c &= \frac{DP \times \text{conv} \times M_c}{M_{\text{PBLG}} + DP \times \text{conv} \times (M_c - M_{\text{benzyl alcohol}})} \times 100\% \\ &= \frac{39 \cdot \text{conv} \cdot (358 \text{ g/mol})}{8500 \text{ g/mol} + 39 \cdot \text{conv} \cdot (250 \text{ g/mol})} \times 100\% \end{aligned} \quad (3)$$

The weight percentage of iron of PBLG-Fe w_{Fe} was calculated using the following formula:

$$w_{\text{Fe}} = \frac{56 \text{ g/mol}}{358 \text{ g/mol}} w_c \times 100\% = 0.156 w_c \times 100\% \quad (4)$$

The weight percentage of ferrate complex and iron element of PBLG-Fe was summarized in Table S2.

Table S2 The weight percentage of ferrate complex and iron element of PBLG-Fe synthesized.

sample	W_c	W_{Fe}
	(wt%)	(wt%)
PBLG-Fe	26.7	4.2
PBLG-S-Fe	22.7	3.5

Notes: Calculated from the weight change. W_c and W_{Fe} denoted the weight percentage of ferrate complex and iron element in PBLG-Fe and PBLG-S-Fe, respectively.

5 **Synthesis of conventional PB particles:** Conventional PB particles were prepared by slowly adding $FeCl_3$ aqueous solution (10 mL, 0.2 M) to pentacyano(4-(aminomethyl)-pyridine)ferrate ($NH_4Na_2[Fe(II)(CN)_5py]$, **5**) aqueous solution (10 mL, 0.2 M). The resulting suspension was stirred for 30 min at room temperature, followed by centrifugation and washing with distilled water (20 mL \times 3). The resulting dark blue solid was isolated by filtration and dried at 40 °C under reduced pressure
10 overnight.

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