Electronic Supplementary Information (ESI)

The Effect of Hydration and Dehydration on the Conformation,

Assembling Behavior and Photoluminescence of PBLG

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1. Experimental Procedures

1.1 Materials.

All chemicals were used as received unless otherwise indicated. L-glutamate was purchased from Sigma-Aldrich Co., Inc. Tetrahydrofuran (THF), Dimethyl Formamide (DMF) and Ethyl acetate were all distilled over CaH₂ under a reduced pressure to remove the water before use. CuCl was purified by stirring in glacial acetic overnight, filtered, washed with ethanol and dried under vacuum before use. 1-azide-3-aminopropane was purchased from Sigma-Aldrich Co., Inc. and used as the initiator. Dialysis bag (Membra-cell, 12,000 molecular weight cut-off) was provided by Green bird Co., Inc. Triphosgene was purchased from Meryer (Shanghai) Chemical Technology Co., Ltd. All other chemicals were purified according to conventional methods or used as received.

1.2 Instrumentation

NMR spectroscopy: Bruker Avance 500 (¹H: 500 MHz; ³¹P: 162 MHz;) spectrometer was used for ¹H and ³¹P spectra in CDCl₃ at ambient temperature with tetramethylsilicane (TMS) as an internal reference.

Gel permeation chromatography (GPC) spectroscopy: The molecular weight and PDI value were recorded by gel permeation chromatograph (Perkin-Elmer Series 200) which equipped with three Waters Styragel columns (10^3 , 10^4 , and 10^5 Å) and a refractive index detector (set at 35 °C), using DMF (0.01mol/L LiBr) as eluent at 40 ^oC with a flow rate of 1 mL•min⁻¹. The column system was calibrated by a series of monodispersed polydispersity PMMA standards.

ATR-FTIR spectroscopy: Nicolet iS50 spectrophotometer with a resolution of 2 cm⁻¹ in attenuated total reflectance (ATR) mode was used for FT-IR spectra. The samples were prepared by adding various amount of water into the solutions of PBLG (1.0 mg/mL) in dry THF (100 μ L). The FT-IR spectra were recorded quickly on the ATR crystal upon the water addition.

LF-NMR spectroscopy: VTMR20-analyst NMR spectrometer (Ni-umag Electric Corporation, Suzhou, China) at 21MHz was used for the Low Field Nuclear Magnetic Resonance (LF-NMR) spectra. The working temperature was set at 35 °C to ensure the constant rate of molecular motion for probing the system. The relaxation time (T_2) measured by using the Carr-Purcell-Meiboom-Gill sequence (CPMG) with a τ -value (time between 90 pulse and 180 pulse) of 800 µs and 8 scan repetitions. The LF-NMR relaxation curve was fitted to a multi-exponential curve with the MultiExp Inv Analysis software (Niumag Electric Corporation, Suzhou, China) using the inverse La-place transform algorithm.

Scanning electron microscopy (SEM): The morphologies of the aggregates were observed from SEM (Zeiss Gemini 450) operating at an accelerating voltage of 5.0 kV. Before the observations by the electron microscope, the samples were coated by a thin layer of sputtered Au on silicon slice.

Transmission electron microscopy (TEM):TEM images were obtained on a JEOL1200EX II microscope operating at 120 kV and equipped with an SIS MegaViewIII digital camera. No staining of the samples was necessary.

2. Synthetic routes



Figure S1. Synthetic rotes of PBLG homopolymer

Figure S1 illustrates the synthetic rotes of poly (γ -Benzyl-L-glutamate) (PBLG) homopolymer. The PBLG homopolymer was synthesized by the NCA ring-opening polymerization (ROP) of BLG-NCA, and the details as follows.

2.1 Synthesis of γ-benzyl L-glutamate (BLG)

The γ -benzyl L-glutamate (BLG) was synthesized from BLG. 30.0 g BLG (0.204 mol) and 30.0 ml benzyl alcohol (0.28 mol) were transferred into a dry pear-shaped bottle,

and then 20 mL of 60% concentrated sulfuric acid was injected at the rate of one drop per second under ice water bath. Then, the mixed solution was stirred for 3h at 60°C, followed by reducing the pressure for the esterification reaction. After 1.5h, NaHCO₃ solution was used to adjust the pH to neutral level. Large amounts of solid production was precipitated out from the mixed solution. The production was purified twice by recrystallization and dried under vacuum, in a yield of 86.3%.

2.2 Synthesis of γ-benzyl L-glutamate-N-carboxyanhydride (BLG-NCA)

The BLG-NCA was synthesized from γ -benzyl BLG. 7.67 g of γ -benzyl BLG (34.5 mmol) and 200 ml dry-processed Ethyl acetate were transferred into a dry Schlenk flask under a nitrogen atmosphere. The mixed solution was stirred at 80°C, and 4.3 g of triphosgene (14.5 mmol) was added upon the occurrence of obvious refluxes in the condensing pipe. After clarification, the mixed solution was successively washed by NaHCO₃ solvent (content of 2.0%), saturated NaCl solution and ultrapure water, respectively, followed by half hour dry-process by MgSO₄. The filtered solution was poured into a large volume of anhydrous methanol for precipitation, and then the precipitates were collected and dried under vacuum, in a yield of 83.6%.

2.3 Synthesis of poly (y-Benzyl-L-glutamate) (PBLG) homopolymer

PBLG homopolymer was synthesized in dimethyl formamide (DMF) solution using ring-opening polymerization of BLG-NCA initiated by 1-azide-3-aminopropane.¹ 1.5 g BLG-NCA, 60 μ L initiators and 15 mL DMF were transferred into a dry Schlenk flask under a nitrogen atmosphere. After 72 hours, a large volume of methanol was added into the mixed solution, and then the solid polymer can be achieved by centrifuge. The production was collected and dried under vacuum, in a yield of 88.6%.

3. Preparation of self-assemblies

The main operations of self-assembly were divided into the following steps. PBLG homopolymer was first dissolved in THF, and then various amounts of water were dropped into the solution at the rate of 0.85 μ L/s. The self-assembly solutions were quenched by a large amount of water addition (~10-fold) to prevent any morphological changes. THF was removed through a dialysis process against 2.0 L deionized water for 24 h or *via* N₂ bubbling for 1 h. The cut-off molecular weight of cellulose membrane for the dialysis is ca. 12,000. It should be noted that the dialysis process has negligible effect on the morphologies of the assemblies. For example, no morphological transformation was observed before and after dialysis.

4. Characterization

As shown in Figure S2, ¹H NMR (500 MHz, CDCl₃) of **BLG-NCA**: δ (ppm) =7.45 - 7.32 (-CH₂Ar*H*), 6.62 (-N*H*CO), 5.15 (-C*H*₂Ar*H*), 4.38 (-C*H*NH), 2.62 - 2.07 (COC*H*₂C*H*₂). ¹H NMR (500 MHz, CDCl₃) of **PBLG homopolymer**: δ (ppm) = 7.54-7.19 (-CH₂Ar*H*), 5.07 (-C*H*₂Ar*H*), 3.94 (C*H*NH), 2.86-1.94 (COC*H*₂C*H*₂). GPC: Elution time = 15.2 min (PBLG₁₀₀) and 17.5 min (PBLG₁₂).



Figure S2. ¹H NMR spectra of a) BLG-NCA, b) PBLG homopolymer and c) the GPC spectra of PBLG₁₀₀ and PBLG₁₂ homopolymers

5. Morphological transition from helical tapes to toroids

Figure S3 illustrates the SEM images of PBLG₁₂ self-assemblies in THF/water (100 μ L, 1.0 mg/mL) with various water contents (50 - 70 μ L). The intermediate morphologies (60 μ L water content) and the ununiform toroids (65 μ L water content) are successively observed. It indicates that there is no other self-assembled morphology between the helical ribbons and toroids.



Figure S3. SEM images for the self-assemblies of PBLG₁₂ in THF/water (100 μ L, 1.0 mg/mL) with the water contents of a) 50 μ L, b) 60 μ L, c) 65 μ L and d) 70 μ L, respectively.

6. Effects of hydration and dehydration on PBLG conformation

To study the conformation change of PBLG₁₂ and PBLG₁₀₀ at low water contents (0-20 μ L), CD analysis have been introduced for the solutions of PBLG₁₂ and PBLG₁₀₀ in THF/water, respectively. As manifested by the two negative Cotton effects at 211 nm and 222 nm in Figure S4a and S4b, the hydrated PBLG₁₂ and PBLG₁₀₀ render α -helical conformation²⁻⁴ at low water contents (< 20 μ L).



Figure S4. CD spectra of PBLG₁₂ in THF (100 μ L) in the present of the water contents of 0-20 μ L.



Figure S5. CD spectra of PBLG₁₀₀ in THF (100 μ L) in the present of the water contents of 0-20 μ L.

7. Morphological transition of PBLG₁₀₀ from fibers to particles

PBLG₁₀₀ macromolecules undergo a self-assembly in the THF/water solution and the resultant assemblies are stable in water upon dialysis to remove THF. The SEM images of the resultant colloids for PBLG₁₀₀ in water are displayed in Figure S6, which illustrates the morphologies of fibers, coexistences and particles for the colloids prepared in THF/water solutions with 30 μ L, 50 μ L and 70 μ L water contents,

respectively. Unlike the self-assembling behaviors of $PBLG_{12}$, the particles rather than toroids are formed at high water contents due to the helical rigidity of $PBLG_{100}$ at high water contents.



Figure S6. SEM images of the morpholigies of PBLG₁₀₀ self-assemblies in THF/water with the water contents of a) 30 μ L, b) 50 μ L and c) 70 μ L.

8. Dialysis has no effect on the FL emission

As shown in Figure S7, the FL spectra of the self-assemblies after dialysis is in line with that in THF/water. It suggests that the self-assembling behaviors of PBLG rather than the solvent of THF should be responsible for this FL emission. It supports that the FL emission is caused by the packing of β sheets because of forming through-space conjugation of phenyl groups.



Figure S7. The FL spectra for the self-assemblies of PBLG₁₂ after dialysis

9. References

X. Lin; X. He; C. Hu; Y. Chen; Y. Mai; S. Lin, *Polym. Chem.*, 2016, 7, 2815-2820.
B. M. Bulheller; A. Rodger; J. D. Hirst, *Phys. Chem. Chem. Phys.*, 2007, 9, 2020-2035.

3. J. T. Pelton; L. R. McLean, Anal. Biochem., 2000, 277, 167-176.

4. Z. Geng; B. Xiong, L. Wang, K. Wang, M. Ren, L. Zhang, J. Zhu, Z. Yang, *Nat. Commun.*, 2019, *10*, 4090.