Supplementary information

Super-Helices Self-Assembled from a Binary System of Amphiphilic Polypeptide Block Copolymers and Polypeptide Homopolymers

Chunhua Cai, Jiaping Lin, Tao Chen, Xiao-Song Wang and Shaoliang Lin

a Key Laboratory for Ultrafine Materials of Ministry of Education, School of Materials Science and Engineering, East China University of Science and Technology, Shanghai 200237, China.

b School of Chemistry, University of Leeds, Leeds, LS2 9JT, UK.
1. Polymer synthesis

**Materials.** \(\alpha\)-Methoxy-\(\omega\)-amino poly(ethylene glycol) (CH\(_3\)O-PEG-NH\(_2\), \(M_w = 2000\)) was purchased from Sigma-Aldrich Co., Inc., and dissolved in toluene in a flame-dried reaction bottle, followed by removing toluene in high vacuum. The resulting dried CH\(_3\)O-PEG-NH\(_2\) was used for the synthesis of block copolymers. Tetrahydrofuran (THF), hexane, 1,4-dioxane and triethylamine were refluxed with sodium and distilled immediately before use. All other solvents are of analytical grade and used without further purification. Dialysis bag (Membra-cel, 3500 molecular weight cut-off) was provided by Serva Electrophoresis GmbH.

**Synthesis of PBLG homopolymer.** Poly(\(\gamma\)-benzyl-L-glutamate) was prepared by a standard N-carboxyanhydride (NCA) method\(^{51-53}\). Briefly, PBLG was synthesized in 1,4-dioxane solution using ring-opening polymerization of N-carboxyl-benzyl-L-glutamate anhydride (\(\gamma\)-BLG-NCA) initiated by triethylamine. The monomer concentration is 3.0 wt % and the polymerization lasted for 72 h at 15 °C under a dry nitrogen atmosphere. At the end of the polymerization, the reaction mixture was poured into a large volume of anhydrous ethanol. The precipitates were dried under vacuum. The resulting products were purified twice by repeated precipitation from a chloroform solution into a large volume of anhydrous methanol. Three PBLG samples were prepared with their molecular weights of 40,000, 110,000 and 520,000, respectively. The molecular weights were estimated from the \([\eta]\) value measured in dichloroacetic acid (DCA) according to the Doty et al. relation\(^{54}\).
Synthesis of PBLG-b-PEG block copolymer. Poly(γ-benzyl-L-glutamate)-block-poly(ethylene glycol) (PBLG-b-PEG) block copolymer was synthesized by ring-opening polymerization of γ-BLG-NCA in 1,4-dioxane initiated by CH₃O-PEG-NH₂ according to our previous work. The reaction was performed in flame-dried reaction bottle under a dry nitrogen atmosphere for 72 hours at 15 °C. The reaction mixture was poured into a large volume of anhydrous ethanol. The precipitated products were dried under vacuum and then purified twice by repeated precipitation from a chloroform solution into a large volume of anhydrous methanol. The block copolymer molecular weight was estimated using ¹H NMR measurement (Avance 550, Bruker). It was calculated by the peak intensities of the methylene proton signal (5.1 ppm) of polypeptide and the ethylene proton signal (3.6 ppm) of PEG in the ¹H NMR spectrum. The molecular weight of the PBLG block was calculated to be 31,000.

2. Characterizations

¹H NMR. ¹H NMR spectra were recorded using Avance 550, Bruker. For PBLG-b-PEG, CDCl₃ was used as solvent. Super-helices was freeze-dried from water and redissolved in D₂O for the measurements.

Gel Permeation Chromatography (GPC). The polydispersities of the homopolymers and block copolymer were determined by gel permeation chromatography (GPC) in DMF at room temperature, performed on a Waters 1515 instrument.

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Transmission Electron Microscopy (TEM). The morphologies of the aggregates were examined by TEM (JEM-2000EXII, JEOL) operated at an accelerating voltage of 60 kV. Drops of solution were placed on a copper grid coated with carbon film and then were dried at room temperature. Before the observations, the sample was stained by phosphotungstic acid aqueous solution (0.5 wt %).

High Resolution Transmission Electron Microscopy (HRTEM). The morphologies of the aggregates were also examined by HRTEM (JEM-2100F, JEOL) operated at an accelerating voltage of 200 kV. Drops of solution were placed on a copper grid coated with carbon film and then were dried at room temperature. Before the observations, the sample was stained by phosphotungstic acid aqueous solution (0.5 wt %).

Scanning Electron Microscopy (SEM). The surface profile of the aggregates was obtained from SEM (JSM-6460, JEOL) operated at an accelerating voltage of 20 kV. The sample was prepared by placing drops of solution on a copper grid coated with carbon film and then were dried at room temperature. Before the observations, the samples were sputtered by carbon.

Atomic Force Microscopy (AFM). AFM images were obtained with a multimode atomic force microscopy (Nanoscope IIIa, Veeco), employing the tapping mode. The samples were prepared by placing one drop of solution on a fresh-cleaved mica surface. The excess of the solution was gently removed by a filter paper and the remaining wet film was allowed to dry in air.
3. GPC analysis of PEG-b-PBLG block copolymer and PBLG homopolymers

GPC was employed to evaluate the molecular weight distributions of the PEG-b-PBLG block copolymer and PBLG homopolymers. The results are given in Figure S1. All the samples showed a monomodal distribution, indicating a well controlled polymerization process. All the polymers show narrow molecular weight distributions. According to GPC analyses, The polydispersities of the PBLG homopolymers are 1.13, 1.19 and 1.15 for PBLG\textsubscript{40,000}, PBLG\textsubscript{110,000}, and PBLG\textsubscript{520,000}, while the polydispersity of the PBLG-b-PEG block copolymer is 1.07.

Figure S1. GPC traces of the PEG-b-PBLG block copolymer and PBLG homopolymers.
4. Self-assembled structures confirmed by NMR analysis

Figure S2 shows the $^1$H NMR spectra of PBLG-$b$-PEG block copolymers in CDCl$_3$ (Figure S2a), and freeze-dried self-assembled aggregates in D$_2$O (Figure S2b). By the comparison of Figure S2a and b, one can see the intensity of the signals due to PBLG blocks (2.1, 2.2, 2.7, 4.1, 5.1 and 8.4 ppm) relative to those due to PEG (3.6 ppm) is reduced remarkably (Figure S2b), indicating that the PBLG blocks aggregated into the inner cores of the super-helices and their chain mobility was restricted.

**Figure S2.** $^1$H NMR spectra of (a) PBLG-$b$-PEG block copolymer in CDCl$_3$, and (b) PBLG-$b$-PEG/PBLG aggregates in D$_2$O.
5. TEM images of homo-PBLG in initial solvent

Figure S3 shows the TEM images of the homo-PBLG aggregated in the organic solvents of THF/DMF (3 : 7 by volume). PBLG with low molecular weight of 40,000 formed irregular structures (Figure S3a). Increasing the molecular weight to 110,000, short cylinders were observed, as shown in Figure S3b. Figure S3c shows that further increasing the molecular weight to 520,000, long rods were produced. These results indicated that the molecular weight of homo-PBLG is one important factor determining the structures of homo-PBLG in THF/DMF.

![Figure S3](image)

**Figure S3.** TEM images for the samples aggregated from PBLG homopolymers with molecular weights of (a) 40,000, (b) 110,000 and (c) 520,000 in the organic solvent of THF/DMF (3 : 7 by volume). The concentration of the original PBLG solutions is 0.1g/L. The scale bars represent 400 nm.

6. Effects of drying process and initial polymer concentration

The supramolecular structures are frozen and in a swollen state when they are in aqueous solution or water/solvent mixed solution. The drying process in the TEM, SEM and AFM

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measurements may have influence on the diameter and screw-pitch of the super-helices. But the chain packing mode and aggregate morphology could not be affected. This could be same situation as those reported in literatures, i.e. the drying process can influence the size of the aggregates, however, the morphology is not affected.\textsuperscript{S8}

We have also performed experiments regarding the influence of the initial polymer concentration on the morphologies of formed hybrid aggregate. The preliminary results revealed that the effect of the initial polymer concentration is less marked.

7. MD simulations of supramolecular structures

The molecular dynamics (MD) simulations were carried out by using the simulator, coarse-grained molecular dynamics program (COGNAC) of OCTA. The simulator was developed by Doi’s group, which is public on a web-site \textsuperscript{S9}. COGNAC uses the reduced unit system for setting data. To convert it to the real unit, a set of unit parameters, such as reduced length, reduced energy and reduced mass, is given in ref. S10.

To construct an AB-type rod-coil diblock copolymer and an A-type rodlike homopolymer capturing the essential features of the PBLG-\textit{b}-PEG diblock copolymer molecule and PBLG homopolymer molecule, respectively, potentials that should be given are bonding potential, $U_{\text{mol}}$, and non-bonding potential, $U_{ij}$. That is, the potentials that act on the beads (atoms) are divided into two parts. The former can construct a desired molecule from atoms, while the latter describes intermolecular interactions.
Figure S4. The layouts of AB rod-coil diblock copolymer (a) and A-type rodlike homopolymer (b).

The homopolymer and copolymer molecules are represented by a linear chain consisting of several beads connecting by bond stretching potential. In this work, for AB-type diblock, A-block with 9 beads and B-block with 3 beads, coded as A₉B₃, is constructed. And for the A-type homopolymer, chain with 40 beads, coded as A₄₀, is constructed. The illustration of these molecule models are shown in Figure S4. In Figure S4a the beads colored by blue and green are A-block and flexible B-block, respectively. Shown in Figure S4b is the A-type rigid homopolymer model colored by red. To realize the A-block (also A homopolymer) with rigid form, angle bending potential is combined to keep the rigidity of the molecule. Without angle bending potential constrain, the B-block is represented by a flexible chain as shown in Figure S4a. Therefore, $U_{\text{mol}}$ is a combination of $U_{\text{bond}}(r)$ and $U_{\text{angle}}(\phi)$. $U_{\text{bond}}(r)$ is a function of distance between the chemically bonded beads $r$. A harmonic type is applied and defined by

$$U_{\text{bond}}(r) = \frac{1}{2}k_b (r - r_0)^2$$

(1)
where $k_b$ is the bond spring constant, and $r_0$ is the equilibrium bond length. The value of $k_b$ is set to be 10 000, for both A- and B-blocks. $U_{\text{bond}}(r)$ consists of the potentials of A-block, B-block and the junction bond of these two blocks. Setting values of bond length $r_0$ are 0.75, 1.0 and 1.0 for them, respectively. In this simulation, two types of molecules with $A_9B_3$ and $A_{40}$ were constructed. Such constructions roughly correspond to the compositions of PBLG-$b$-PEG and PBLG samples studied in the present work, respectively. According to the NMR analysis, the repeat unit ratio of PBLG/PEG in PBLG$_{31000}$-$b$-PEG$_{2000}$ is about 142:45, which is close to the ratio 9:3 of A-block: B-block for $A_9B_3$ copolymer. While the repeat unit number of A homopolymer is set to be 40. Such constructions of polymer length and atom numbers result in a repeat unit ratio 12:40 of $A_9B_3$ copolymer to $A_{40}$ homopolymer, close to the ratio 187:502 of PBLG$_{31000}$-$b$-PEG$_{2000}$ to PBLG$_{110000}$. It is noted that longer length of model molecule need larger simulation box in the simulations, which could cause longer time to simulate. Therefore, for simplicity, we used the molecular model for PBLG$_{110000}$.

For the A-block (also A homopolymer), the angle bending potential, $U_{\text{angle}}(\phi)$, is a cosine harmonic function of the angle $\phi$ defined by the three chemically connected beads.

$$U_{\text{angle}}(\phi) = \frac{1}{2} k_a (\cos \phi - \cos \phi_0)^2$$

where $k_a$ is the angle spring constant, and $\phi_0$ is the equilibrium angle. To realize A-block with rigid form, the equilibrium angle $\phi_0$ is set as a value of 0.1° (substantially zero). The constant $k_a$ is set to be 10 000.

The interaction energy $U_{ij}$ is given by the standard Lennard-Jones 12:6 potential $U_{ij}$ acting between any pair of beads $i$ and $j$:
where \( r_y^c \) is the cut-off distance, \( r_y = |\vec{r}_i - \vec{r}_j| \), with \( \vec{r}_i \) and \( \vec{r}_j \) being the locations of the \( i \)th and \( j \)th beads, respectively. The different solubility in this model is realized using a method mentioned by Bhattacharya et al.\textsuperscript{S11, S12} It is introduced by a repulsive cut-off distance for the A-B and B-B (\( r_{AB}^c = 2^{1/6} \), \( r_{BB}^c = 2^{1/6} \)), and an attractive cut-off for the A-A interaction (\( r_{AA}^c = 2.5 \)). Such selections of \( r_y^c \) make the A-block form the core of aggregates. The diameter \( \sigma \) of LJ bead is kept to be unity for any pairs of species. All of the pairwise interactions are set to be unity, i.e., \( \varepsilon_{AA} = \varepsilon_{AB} = \varepsilon_{BB} = 1.0 \).

All the simulations are carried out on a cubic cell (60 × 60 × 60) using a dynamic algorithm with temperature controlling method (NVT ensemble), which can be found in detail in ref. S10. In order to minimize the effect of finite system size, periodic boundary conditions were imposed. A randomly distribution mode for 400 A\(_9\)B\(_3\) copolymers and 30 A\(_{40}\) homopolymers is applied to generate the initial structures of molecules and the structure relaxation is done by stochastic dynamic simulation. The integration time step \( \Delta t = 0.004 \) was selected. The length of simulation runs were 3 \( \times \) 10\(^6\) time steps, i.e., 12 000 time units, which insured that the simulated system reached the equilibrium. All calculations were performed at a temperature \( T_0 = 3.0 \).

Typical snapshot of aggregates self-assembled from mixtures of AB copolymers and A homopolymers are shown in Figure S5a, where the A- and B-blocks of copolymer are colored by blue and green, respectively, while the A homopolymer is colored by red. As can be seen, rodlike
aggregates are formed. Shown in Figure S5b is a typical structure of a single hybrid rodlike aggregate formed by the mixture. Due to the poor solubility, the rigid A homopolymers and A-blocks of the copolymers aggregate to form the cores of the rodlike aggregates. The cores are surrounded by soluble B-blocks as shell. Within the aggregate core, the homopolymers remain in the interior, while the A-blocks of the copolymers are located close to the outside shell. Both rigid homopolymers and A-blocks are arranged with their long axes parallel to each other and perpendicular to the radial direction of the rodlike aggregates.

**Figure S5.** The snapshot of hybrid aggregates formed by AB rod-coil diblock copolymers and A-type homopolymers (a). The snapshot of a single hybrid rodlike aggregate (b).
References

S9  http://octa.jp