

Super-helices self-assembled from a binary system of amphiphilic polypeptide block copolymers and polypeptide homopolymers†

Chunhua Cai,^a Jiaping Lin,^{*a} Tao Chen,^a Xiao-Song Wang^{*b} and Shaoliang Lin^a

Received (in Cambridge, UK) 5th January 2009, Accepted 6th March 2009

First published as an Advance Article on the web 27th March 2009

DOI: 10.1039/b823367e

We report here a discovery that amphiphilic polypeptide block copolymers and polypeptide homopolymers are able to aggregate together into super-helical structures of rods and rings, in which polypeptide chains form the core and PEG chains form the shell.

Self-assembly of multi-components into specific functional structures is ubiquitous and pivotal in nature. For example, tobacco mosaic virus has a rod-like helical structure with coated proteins aggregated around a nucleic acid. As nature provides many inspirations for material science,¹ developing strategies for virus-mimetic hierarchical polymer assemblies have been attempted for novel biological materials.²

Block copolymer self-assembly has been studied extensively and provides many opportunities for various nanostructures.³ Several recent researches indicated that, by introducing a second component, hierarchical well-defined superstructures could be achieved.⁴ This progress offered many hopes for the construction of complicated biological analogues in the future. To this end, understanding subtle interplays between two polymers for structure determination is especially important and is becoming the focus of the research in the area.

Herein, we report for the first time that poly(γ -benzyl-L-glutamate)-*block*-poly(ethylene glycol) (PBLG-*b*-PEG) and homo-poly(γ -benzyl-L-glutamate) (homo-PBLG) can co-operatively self-assemble into hybrid helical rods and rings in water. The superstructures possess a PBLG axis coated by PEG chains. We also discovered that, unlike many other helix systems,⁵ interfacial tension is a driving force responsible for the formation of the helices.

The aggregates were prepared by a selective precipitation method. First, PBLG_{31 000}-*b*-PEG₂₀₀₀ and PBLG_{520 000} homopolymers (subscripts denote the molecular weight for each segment) were dispersed in a mixed solvent of THF–DMF (3 : 7 by volume), the weight ratio of PBLG-*b*-PEG and PBLG was 100 : 15 and the concentration of the polymers was 0.35 g L⁻¹. To the solution, deionized water, a selective solvent for PEG, was added at a rate of *ca.* 1 mL min⁻¹ with vigorous stirring. When water content reached *ca.* 25% (by volume) of original polymer solution, dialysis process was applied to

remove organic solvents, leading to superstructures of PBLG-*b*-PEG/PBLG in water. ¹H NMR spectra revealed that the characteristic signals due to PBLG block relative to those due to PEG are reduced markedly when the PBLG-*b*-PEG and PBLG self-assemble into these superstructures (see ESI†). This indicates that the aggregates have PEG shells, which maintain the colloidal stability of the assemblies.

The morphology of the hybrid aggregates was examined using SEM (see Fig. 1a). As shown in the figure, helices including rods and rings were formed. The rings probably were formed through a head-to-tail connection of the helical rods.^{3,d} AFM measurement also verified the helical structures (see Fig. 2). To confirm the structures, we performed HRTEM, in which right-handed helical sense is clearly revealed (see Fig. 3). As shown in the figure, the diameter of the helices is *ca.* 140 nm with a screw pitch of *ca.* 80 nm. In a control experiment, we self-assembled PBLG-*b*-PEG under the same conditions with the absence of PBLG homopolymers. In this case, spheres were observed exclusively (see Fig. 1b), which suggests that interplay between the block copolymers and PBLG homopolymers played an important role in the construction of the helices.

We, therefore, examined the effect of molecular weight of PBLG homopolymers on the aggregation. PBLG with molecular weights of 40 000, 110 000, and 520 000 were used to

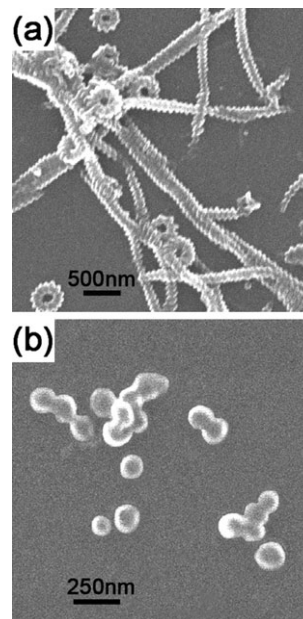


Fig. 1 SEM images of (a) super-helical rods and rings self-assembled from PBLG_{31 000}-*b*-PEG₂₀₀₀ and PBLG_{520 000}; (b) pure PBLG_{31 000}-*b*-PEG₂₀₀₀ micelles.

^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.

E-mail: jplinlab@online.sh.cn; Fax: +86 21-6425-3539

^b School of Chemistry, University of Leeds, Leeds, UK LS2 9JT.

E-mail: X.S.Wang@Leeds.ac.uk; Fax: +44 113-343-2947

† Electronic supplementary information (ESI) available: Polymer synthesis and characterization, aggregate characterization, ¹H NMR spectra for PBLG-*b*-PEG block copolymers in CDCl₃ and PBLG-*b*-PEG/PBLG helices in D₂O, MD simulations. See DOI: 10.1039/b823367e

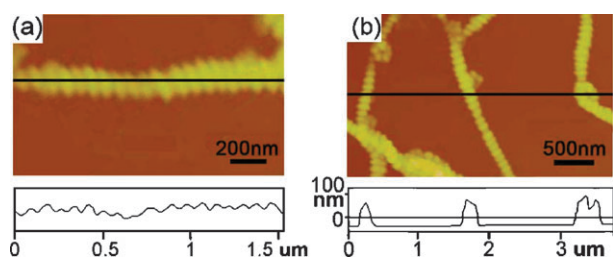


Fig. 2 AFM images of super-helical rods self-assembled from PBLG₃₁₀₀₀-*b*-PEG₂₀₀₀ and PBLG₅₂₀₀₀₀. (a) Length profile; (b) cross section profile.

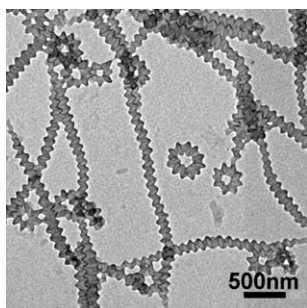


Fig. 3 HRTEM images of super-helical rods and rings self-assembled from PBLG₃₁₀₀₀-*b*-PEG₂₀₀₀ and PBLG₅₂₀₀₀₀.

aggregate with PBLG-*b*-PEG, respectively. The resulting aggregates were characterized using TEM. As shown in Fig. 4, one can see that the molecular weight of PBLG influenced morphologies. For the system containing lower molecular weight of PBLG, spheres were observed (Fig. 4a). Helices start to appear when PBLG with higher molecular weight was used for the aggregation (Fig. 4b and c). For the sample with PBLG molecular weight of 110 000, shorter helices were observed. Further increase in molecular weight of PBLG to 520 000 generated long helical rods. The long

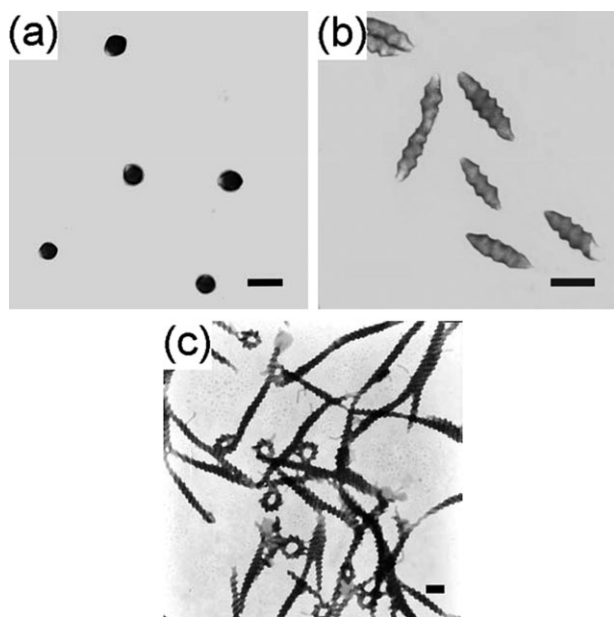


Fig. 4 TEM images of the aggregates self-assembled from PBLG₃₁₀₀₀-*b*-PEG₂₀₀₀ and PBLG with various molecular weights of (a) 40 000; (b) 110 000; (c) 520 000. The scale bars represent 200 nm.

helical rods are relatively flexible and, as a result, some helical rings were created by connecting two ends of the rods. Since pure PBLG-*b*-PEG aggregates into spheres in water (see Fig. 1b), the observed morphological variation as a function of PBLG molecular weight suggested that PBLG acted as a scaffold determining the structures of the hybrid aggregates.

It is well known that the rigid α -helix PBLG usually aggregates into rods in many solvents, this ordered packing of the PBLG chains are stabilized by the strong dipolar π - π interactions between the phenyl groups in PBLG. As a matter of fact, we indeed observed that PBLG homopolymers existed as aggregates in THF-DMF. The structures of the aggregates also related to the molecular weight of PBLG (see ESI[†]). Long and short rods were observed for PBLG₅₂₀₀₀₀ and PBLG₁₁₀₀₀₀, respectively. PBLG₄₀₀₀₀ formed irregular structures (see ESI[†]). Therefore, in the presence of PBLG-*b*-PEG, PBLG segments in the block copolymers tend to be involved with the rods formed from PBLG homopolymers, while PEG segments remain soluble and prevent the fusion of the fibres (see Fig. 5a). In this way, PBLG homopolymers determined the overall structures of the hybrid systems.

To further understand what is the force driving the aggregation of PBLG and PBLG-*b*-PEG co-operatively into helices, we studied the effect of water content on the aggregation behavior of the mixed system. As shown in Fig. 5, the polymer mixture formed rod-like structures with a diameter of about 50 nm in the original organic solvent (see Fig. 5a). Once water was added to the solution of the mixed system, a helical structure was induced. Fig. 5b shows that, with the water content up to 15 wt%, the helical structure become visible. The diameter and pitch distance are *ca.* 80 nm and *ca.* 120 nm, respectively. Increasing the water content up to 23 wt% increased the diameter of the helix to *ca.* 120 nm, as shown

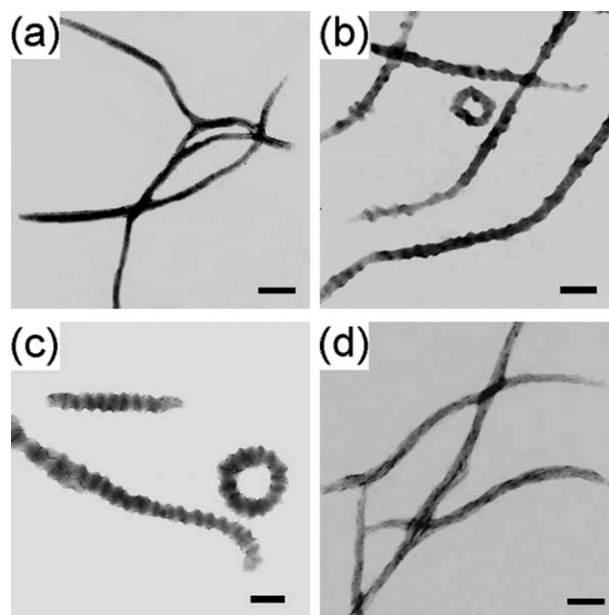
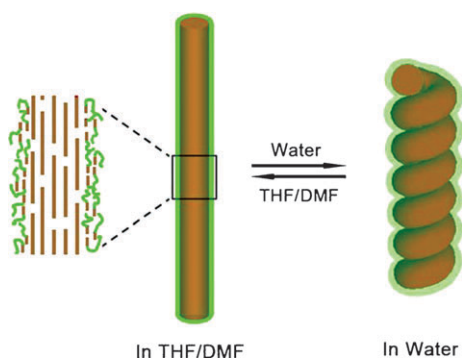


Fig. 5 TEM images of aggregates of PBLG₃₁₀₀₀-*b*-PEG₂₀₀₀ and PBLG₅₂₀₀₀₀ with various added water contents: (a) 0 wt%; (b) 15 wt%; (c) 23 wt%; (d) 5 wt% after adding organic solvents to water solution of the aggregates. The scale bars represent 200 nm.



Scheme 1 Schematic representation of the formation of a super-helix. The green lines represent the PEG blocks, and the brown lines represent PBLG blocks.

in Fig. 5c. For the final helices in pure water after dialysis, a diameter of *ca.* 140 nm and a pitch distance of *ca.* 80 nm are observed (see Fig. 3). Interestingly, the helices can return to the original starting morphologies upon the addition of organic solvents to the solution to lower the overall water content to 5 wt% (see Fig. 5d).

Based on these studies, we proposed a possible mechanism to illustrate how PBLG-*b*-PEG and PBLG interact each other for the helices (see Scheme 1). It is known that the rodlike chains of PBLG in solution favour ordered packing with their long axes aligned due to the volume exclusion effect and intermolecular interaction when its concentration is increased.^{6,7} In THF–DMF, the rigid PBLG blocks from both copolymers and homopolymers aggregate to form the core of the hybrid aggregates. The local PBLG concentration in the core could be relative higher and therefore PBLG chains tend to align with characteristics of liquid crystal structure.^{3a} The PEG chains, which have relatively good solubility, stay soluble in solution and provide colloidal stability for the aggregates. This packing mode of rigid rod homopolymer and rod–coil block copolymer was further confirmed by a molecular dynamics (MD) simulation (see ESI†).

When water, a selective solvent for PEG, was added, the interfacial tension of the surface areas of the PBLG rod core increases.⁸ To depress this effect, the PBLG rodlike cores, which act as a scaffold for the supramolecular aggregates, would rearrange themselves to minimize the surface area between the cores and solvents (solvents can enter the PEG shell). Due to the rigid nature of PBLG chains, they can not shrink to a globular form. However, twisting into a helix could be a preferred manner, since the helical structure can reduce the interfacial area effectively.⁹ As a result, we found in our experiments that PBLG rods twisted into helices to compensate the increment of interfacial tension. Such kinds of super-helix structures are also supported by a recent theoretical work. It predicts that helical arrangement is a free energy favorable manner for biomacromolecules such as polypeptides.⁹ In addition to the twist of the rods, end-to-tail connection of the long rods also helps to lower the free energy of the system,^{3d} therefore, rings were observed. Based on this discussion, we illustrate a possible mechanism in Scheme 1 for the formation of the helices upon the addition of water.

In summary, we discovered that PBLG-*b*-PEG/PBLG could self-assemble co-operatively into super-helices in water, while PBLG-*b*-PEG alone forms spherical aggregates. The helices have a PBLG axis covered by PEG chains, which is reminiscent of the structure of tobacco mosaic virus. We also identified that, unlike many other systems, interfacial tension is a major reason responsible for the helical structures. This report enriched our knowledge in the supramolecular chemistry of multi-component systems and will have potential applications in virus-mimetic materials.²

This work was supported by National Natural Science Foundation of China (50673026). Doctoral Foundation of Education Ministry of China (Grant No. 20050251008) and Projects of Shanghai Municipality (06SU07002, 082231, and B502) are also appreciated. XSW thanks EPSRC for a Roberts Academic Award.

Notes and references

- (a) T. Douglas, *Science*, 2003, **299**, 1192; (b) X. Gao and L. Jiang, *Nature*, 2004, **432**, 36.
- (a) E. S. Lee, D. Kim, Y. S. Youn, K. T. Oh and Y. H. Bae, *Angew. Chem., Int. Ed.*, 2008, **47**, 2418; (b) Y.-B. Lim, E. Lee, Y.-R. Yoon, M. S. Lee and M. Lee, *Angew. Chem., Int. Ed.*, 2008, **47**, 4525; (c) Y. Xu, J. Ye, H. Liu, E. Cheng, Y. Yang, W. Wang, M. Zhao, D. Zhou, D. Liu and R. Fang, *Chem. Commun.*, 2008, 49.
- (a) S. Lin, N. Numasawa, T. Nose and J. Lin, *Macromolecules*, 2007, **40**, 1684; (b) J. Rodriguez-Hernandez and S. Lecommandoux, *J. Am. Chem. Soc.*, 2005, **127**, 2026; (c) Z. Li, E. Kesselman, Y. Talmon, M. A. Hillmyer and T. P. Lodge, *Science*, 2004, **306**, 98; (d) D. J. Pochan, Z. Chen, H. Cui, K. Hales, K. Qi and K. L. Wooley, *Science*, 2004, **306**, 94; (e) S. Jain and F. S. Bates, *Science*, 2003, **300**, 460; (f) D. E. Discher and A. Eisenberg, *Science*, 2002, **297**, 967; (g) Y.-R. Yoon, Y. Lim, E. Lee and M. Lee, *Chem. Commun.*, 2008, 1892; (h) P. Bhargava, Y. Tu, J. X. Zheng, H. Xiong, R. P. Quirk and S. Z. D. Cheng, *J. Am. Chem. Soc.*, 2007, **129**, 1113.
- (a) R. M. Capito, H. S. Azevedo, Y. S. Velichko, A. Mata and S. I. Stupp, *Science*, 2008, **319**, 1812; (b) T. Maeda, Y. Furusho, S.-I. Sakurai, J. Kumaki, K. Okoshi and E. Yashima, *J. Am. Chem. Soc.*, 2008, **130**, 7938; (c) J. Hu, G. Liu and G. Nijkang, *J. Am. Chem. Soc.*, 2008, **130**, 3236; (d) X. Wang, G. Guerin, H. Wang, Y. Wang, I. Manners and M. A. Winnik, *Science*, 2007, **317**, 644; (e) R. Zheng, G. Liu and X. Yan, *J. Am. Chem. Soc.*, 2005, **127**, 15358; (f) L. Zhang and A. Eisenberg, *J. Am. Chem. Soc.*, 1996, **118**, 3168.
- (a) S. Zhong, H. Cui, Z. Chen, K. L. Wooley and D. J. Pochan, *Soft Matter*, 2008, **4**, 90; (b) Y. Yan, Z. Yu, Y. Huang, W. Yuan and Z. Wei, *Adv. Mater.*, 2007, **19**, 3353; (c) L. Li, H. Jiang, B. W. Messmore, S. R. Bull and S. I. Stupp, *Angew. Chem., Int. Ed.*, 2007, **46**, 5873; (d) P. Jonkheijm, P. Schoot, A. P. H. J. Schenning and E. W. Meijer, *Science*, 2006, **313**, 80; (e) J. J. L. M. Cornelissen, M. Fischer, N. A. J. M. Sommerdijk and R. J. M. Nolte, *Science*, 1998, **280**, 1427; (f) Z. Yang, G. Liang, M. Ma, Y. Gao and B. Xu, *J. Mater. Chem.*, 2007, **17**, 850.
- (a) J. Bae, J.-H. Choi, Y.-S. Yoo, N.-K. Oh, B.-S. Kim and M. Lee, *J. Am. Chem. Soc.*, 2005, **127**, 9668; (b) K. T. Kim, C. Park, G. W. M. Vandermeulen, D. A. Rider, C. Kim, M. A. Winnik and I. Manners, *Angew. Chem., Int. Ed.*, 2005, **44**, 7964; (c) J. C. Nelson, J. G. Saven, J. S. Moore and P. G. Wolynes, *Science*, 1997, **277**, 1793.
- (a) P. J. Flory, *Adv. Polym. Sci.*, 1984, **59**, 1; (b) A. Abe and M. Ballauff, in *Liquid Crystallinity in Polymer*, ed. A. Ciferri, VCH, New York, 1991, pp. 131–167; (c) A. Abe and P. J. Flory, *Macromolecules*, 1978, **11**, 1122; (d) J. Lin, S. Lin, T. Hiejima, H. Furuya and A. Abe, *Macromolecules*, 2003, **36**, 626; (e) P. Parras, V. Castelletto, I. W. Hamley and H.-A. Klok, *Soft Matter*, 2005, **1**, 284.
- (a) H. Shen and A. Eisenberg, *J. Phys. Chem. B*, 1999, **103**, 9473; (b) Z. Ou-Yang and J. Liu, *Phys. Rev. Lett.*, 1990, **65**, 1679.
- (a) Y. Snir and R. Kamien, *Science*, 2005, **307**, 1067; (b) J. R. Banavar, T. X. Hoang, J. H. Maddocks, A. Maritan, C. Poletto, A. Stasiak and A. Trovato, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 17283.