

Supporting Information

Superhelices with Designed Helical Structures and Temperature-Stimulated Chirality Transitions

Chunhua Cai,[†] Jiaping Lin,^{,†} Xingyu Zhu,[†] Shuting Gong,[†] Xiao-Song Wang,^{*,‡} and Liquan Wang[†]*

[†] Shanghai Key Laboratory of Advanced Polymeric Materials, State Key Laboratory of Bioreactor Engineering, 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)

[‡] Department of Chemistry, Waterloo Institute for Nanotechnology (WIN), University of Waterloo, Waterloo N2L 3G1 (Canada)

*These authors jointly supervised this work.

Correspondence and requests for materials should be addressed to J. L. (E-mail: jlin@ecust.edu.cn).

1. Polymer synthesis

Synthesis of PBLG homopolymer. Poly(γ -benzyl-L-glutamate) (PBLG) homopolymer was synthesized in anhydrous 1,4-dioxane solution using ring-opening polymerization of BLG-NCA initiated by anhydrous triethylamine.^{S1,S2} The monomer concentration is *ca.* 3.0 wt%. The reaction was performed in flame-dried reaction bottle under a dry nitrogen atmosphere for 3 days at 15 °C. At the end of the polymerization, the reaction mixture was poured into a large volume of anhydrous ethanol. The precipitates were collected and dried under vacuum. The resulting products were purified twice by repeated precipitation from a chloroform solution into a large volume of anhydrous methanol.


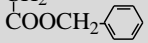
The molecular weight of PBLG was obtained from a multi-detector gel permeation chromatography (GPC/SLS) performed at 35 °C (waters 515) with DMF as mobile phase. The absolute number-average molecular weight (M_n) was estimated to be 528000 by SLS, and the polydispersity index (PDI) is determined to be 1.15 by GPC.

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 BLG-NCA in anhydrous 1,4-dioxane initiated by mPEG-NH₂ macroinitiator according to our previous work.^{S3,S4} Before use, mPEG-NH₂ was dried by dissolving in toluene and then removing toluene in high vacuum. The reaction was performed in flame-dried reaction bottle under a dry nitrogen atmosphere for 3 days at 15 °C. The purification procedure of PBLG-*b*-PEG block copolymer is same with that of PBLG homopolymer.

The block copolymer molecular weight was estimated using ¹H NMR measurement (Avance 550, Bruker, CDCl₃ as solvent). Since the degree of polymerization (DP) of the PEG block is known (112), the molecular weight of PBLG block can be 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.^{S3,S4} According to the NMR analysis, the DP of PBLG block is calculated to be 150, and the M_n value of the PBLG block and PBLG-*b*-PEG block copolymer were calculated to be 33000 and 38000, respectively. And from SLS measurement (multi-detector GPC/SLS instrument), the absolute M_n of PBLG-*b*-PEG block copolymer is 37000, and the

PDI value is 1.21. The M_n values from GPC and ^1H NMR testing are well comparable. The details for the characterization of block copolymers can be found in our previous work.^{S3,S4} The structure information of PBLG homopolymer and PBLG-*b*-PEG block copolymer is provided in Table S1.

Table S1. Characterization of PBLG Homopolymer and PBLG-*b*-PEG Block Copolymer.

Polymer	Molecular structure	M_n^a	DP ^a	PDI ^b
PBLG	$\begin{array}{c} \text{--[NH-CH-CO]}_n \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{COOCH}_2\text{-} \end{array}$ 	528000	2411	1.15
PBLG- <i>b</i> -PEG	$\begin{array}{c} \text{--[NH-CH-CO]}_m\text{--[CH}_2\text{-CH}_2\text{-O]}_n \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{COOCH}_2\text{-} \end{array}$ 	PEG: 5000 PBLG: 33000	PEG: 112 PBLG: 150	1.21

^a For PBLG homopolymer, the absolute M_n is obtained by SLS testing from multi-detector gel permeation chromatography (GPC/SLS); for PBLG-*b*-PEG block copolymer, the M_n value and the degree of polymerization (DP) of PEG segment is known, the M_n and DP of PBLG block are derived according to ^1H NMR spectra.

^b The PDI values of the polymers are obtained from GPC testing.

2. The effect of PBLG length in PBLG-*b*-PEG on the morphology phase diagram for the aggregates self-assembled from PBLG-*b*-PEG/PBLG mixtures.

The self-assembly behavior of PBLG-*b*-PEG/PBLG pairs with different PBLG block length were also investigated under same experimental conditions. Morphology phase diagrams were plotted based on abundant SEM observations (Figure S1). As shown in Figure S1a, when the molecular weight of the PBLG block is relatively smaller (PBLG₃₁₀₀₀-*b*-PEG₅₀₀₀/PBLG₅₂₈₀₀₀ pair), there is no left handed superhelix zone, and the boundary for right-handed superhelix and abacus zone shifts to higher DMF volume fractions. When the molecular weight of the PBLG block is relatively larger (PBLG₃₈₀₀₀-*b*-PEG₅₀₀₀/PBLG₅₂₈₀₀₀ pair), all morphology zones are observed. The major difference is that the both boundaries for abacus/left-handed superhelix and abacus/right-handed superhelix move to lower DMF volume fractions.

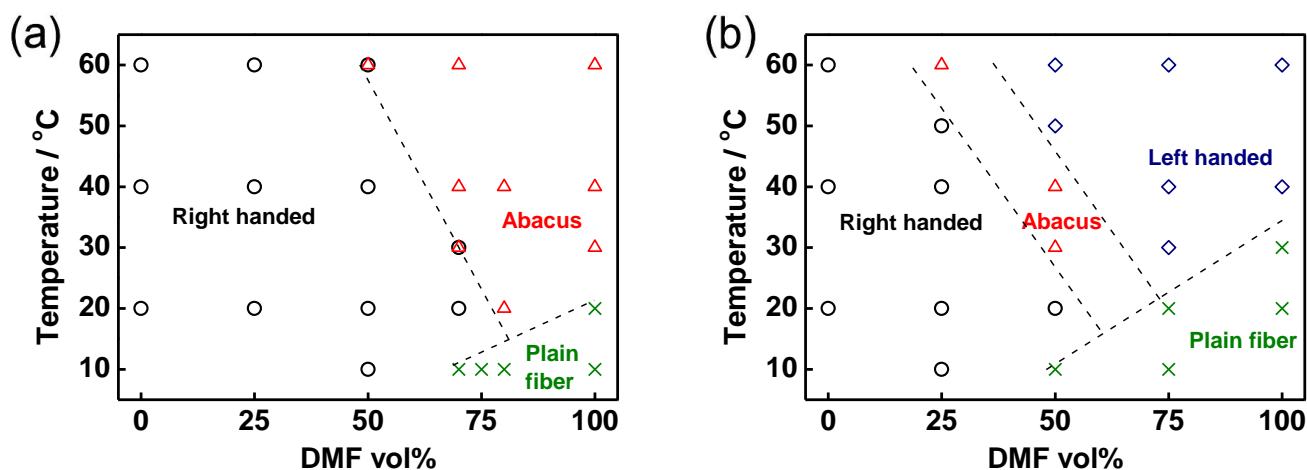


Figure S1. Morphology phase diagram of aggregates self-assembled from (a) PBLG₃₁₀₀₀-*b*-PEG₅₀₀₀/PBLG₅₂₈₀₀₀ mixtures and (b) PBLG₃₈₀₀₀-*b*-PEG₅₀₀₀/PBLG₅₂₈₀₀₀ mixtures as functions of temperature and initial solvent composition.

3. Temperature-induced reversible morphology transitions

When selective solvent, water was added to the solution of PBLG-*b*-PEG/PBLG mixtures (in THF/DMF solvent), the hydrophobic polypeptides become insoluble. At proper added water content (15.0 vol% to the organic solvent), self-assembled aggregates are formed. By changing the solution temperatures, it was found that right-handed superhelices, abacus-like structures, and left-handed superhelices can reversibly transform between each other.

Shown in the main text Figure 4 in the main text is the SEM image for the temperature-induced reversible morphology transitions. However, to gain clear images, solvent was removed via dialysis after annealing at certain temperature. In order to rule out the possibility that the morphologies were altered during the process of dialysis, we examined the samples right after annealing using TEM. With each temperature variation, the solution is aged for at least 10 hrs. A similar reversible morphology transformation as a function of annealing temperature is observed. The detail results are presented in Figure S2. As shown in the Figure S2a, right-handed superhelices are obtained at 20 °C. Increasing the solution temperature to 40 °C, the right-handed superhelices were observed to transform to abacus-like structures (Figure S2b). Figure S2c show that by heating the self-assembly solution further to 60 °C, left-handed superhelices were obtained. Within the cooling process, a morphological transition from left-handed superhelices to abacus-like structures and then to right-handed superhelices was observed (Figure S2c-e). These results are well consistent with the aggregate morphologies observed after dialysis process. However, as can be seen, after dialysis process the aggregate morphologies are obviously much clear.

These results indicated that under proper water content in the solution (it is 15.0 vol% in the present work), hierarchical assemblies can be obtained from PBLG-*b*-PEG/PBLG mixtures. Changing the self-assembling temperature can induce the morphology transitions from right-handed superhelices to left-handed superhelices. And the dialysis process is essential to freeze the assembled structures, and thus high quality microscopic images can be obtained for the samples.

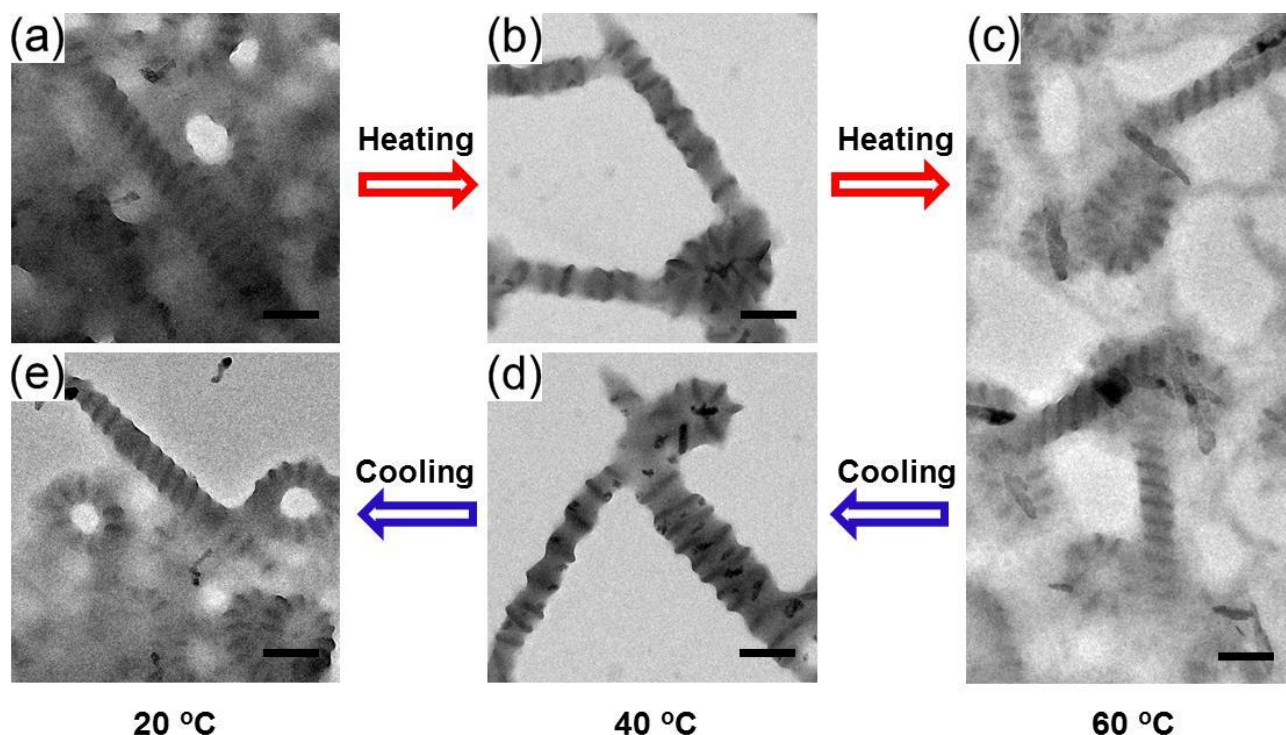


Figure S2. TEM observation for the temperature-induced reversible chirality transformation among right-handed, abacus-like, and left-handed superhelices. (a) Right-handed superhelices observed at 20 °C; (b) abacus-like structures obtained by heating the 20 °C solutions to 40 °C; (c) left-handed superhelices obtained by heating the 40 °C solutions to 60 °C; (d) abacus-like structures obtained by cooling the 60 °C solutions to 40 °C; (e) right-handed superhelices obtained by cooling the 40 °C solutions to 20 °C. With each temperature variation, the solution is aged for at least 10 hrs. The initial solvent is THF/DMF = 1/1 in volume, and the volume ratio of added water to organic solvent is 15.0 vol%. Scale bars: 200 nm. Samples were directly applied to observation, no dialysis process was conducted.

4. Morphology of the aggregates formed from a system with THF/DMF = 1/3 as initial solvent at various temperature

Figure S3 shows the morphology transition of the aggregates as a function of temperature. The sample was prepared in a system with THF/DMF of 1/3. As can be seen, the fibers with no helical features are obtained at 10 °C (Figure S3a), abacus-like structures are observed 20 °C, (Figure S3b). and left-handed superhelices are formed at 40 and 60 °C (Figure S3c and d). Correspondingly, the helical angel (θ) changes gradually from zero at 20 °C (Figure S3b, abacus-like structure) to -14.6 degrees at 40 °C (Figure S3c, left-handed superhelices) and then to -23.2 degrees at 60 °C (Figure S3d, left-handed superhelices). This transition is also reversible. Such observed morphological transitions as a function of temperature match the data discussed in the manuscript (Figure 2).

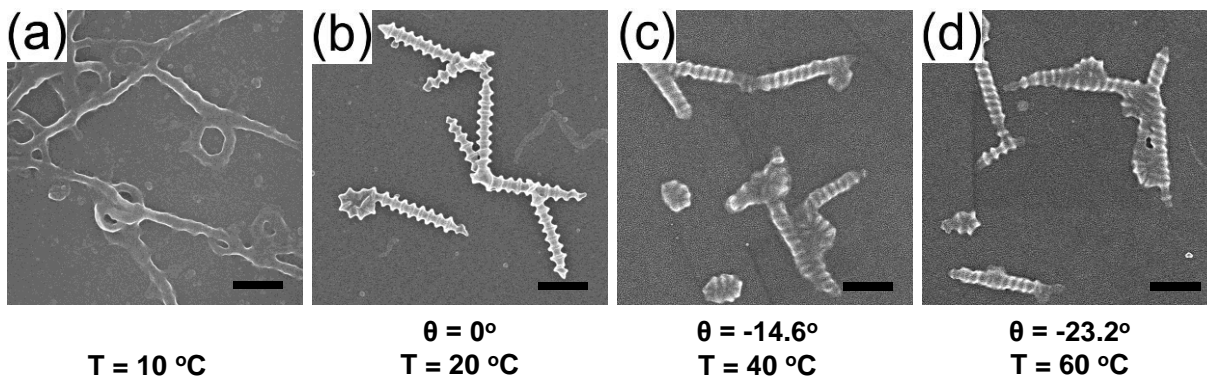


Figure S3. SEM image of the aggregates prepared from THF/DMF = 1/3 (in volume) as initial solvent under various temperature ranging from 10 °C to 60 °C. (a) 10 °C, plain fibers; (b) 20 °C, abacus-like structures; (c) 40 °C, left-handed superhelices; (d) 60 °C, left-handed superhelices.

5. CD spectra of polypeptide aggregate solutions

It is well known that PBLG forms α -helix conformation, and the pending phenyl groups extend out of the main chain axis. In addition to the ordered packing tendency of α -helical polypeptide segments, the strong π - π interactions between pending phenyl groups of PBLG chains is believed to display important roles for the formation of ordered structures.^{S5,S6} As learned from literatures, the π - π interaction between pending phenyl groups is a vector, which has direction as well as intensity.^{S7,S8} The direction of such π - π interactions is related to the extension and direction of the plain surface of the phenyl groups, which is influenced by solution temperature and solvent nature.

CD testing was performed on the water dispersed samples prepared with different initial solvent and temperature (there is no organic solvent in sample solution after dialysis, which prevents the disturbance of the organic solvent on the CD testing). The handedness of PBLG backbone and arrangement of pending phenyl group in initial assemble solution can be preserved in the formed structures through dialysis. Figure S4 shows the CD spectra of the polypeptide aggregates in aqueous solutions. All samples under different conditions show a negative minimum around 220-230 nm due to PBLG chiral molecular backbone, suggesting that the initial solvents and assembling temperature have no effect on the chirality of PBLG backbone, that is, it keeps the right-handed α -helix conformation.

However, as indicated by the negative or positive bands in the 240-280 nm regions of CD spectra, the chiral arrangement of pending phenyl group changes with initial solvent and assembling temperature accompanying with the variation of superhelix chirality. A positive band indicates left-handed arrangement of the pending phenyl group; while a negative band corresponds to right-handed form.^{S9} When phenyl groups are highly solvated, they possess a larger dimension and prefer to arrange in right-handed form.^{S10-S12} In addition, the absolute intensity of the band is associated with the chirality degree of the arrangement of pending phenyl group, which further determines the chirality degree of the formed superhelices.

As shown in Figure S4a, it was found that with THF as initial solvent, positive bands were observed whenever the preparation temperature is 20 °C, 40 °C or 60 °C, which indicates a left-handed form of the pending phenyl group with THF as initial solvent. Accordingly, as indicated by the black arrow in Figure S4d, right-handed superhelices are always obtained with THF as initial solvent in the temperature range of 10 °C - 60 °C. The absolute intensity of the band decreases with increasing preparation temperature, which corresponds to the decrease of chirality degree of the superhelices (the absolute value of helical angle θ) with increasing temperature.

When using THF/DMF mixture (1/1 in volume) as initial solvent, since the self-assembled aggregates are less chiral, all the ellipticity of the bands in different temperatures approaches zero (Figure S4b). However, we can still evidently view that the band are changed from positive at 20 °C and 40 °C to negative at 60 °C, indicating the variation of chirality of phenyl group packing. Such phenomena are in accordance with the chirality transition of superhelices from right-handed to abacus and then to left-handed, which is indicated by the red arrow in Figure S4d.

When pure DMF was applied as initial solvent, as shown in Figure S4c, marked negative bands appeared at 40 and 60 °C, indicating the phenyl groups are packed in right-handed form. The results well agree with the morphology of left-handed superhelices (see the blue arrow in Figure S4d). At room temperature a smaller negative band was still observed, however the corresponding aggregate morphology is plain fiber. The reason could be the increased hydrophilicity of PEG blocks in PBLG-*b*-PBLG block copolymers, which results in weaker attractions between PBLG segments and thus hinders the ordered packing of the PBLG blocks.^{S13}

These observations resemble the essential feature of chirality transitions of polypeptide liquid crystals (LC).^{S13} In concentrated organic solutions PBLG can form cholesteric LCs, when temperature or solvent nature changes, the chirality of the LCs can be transformed between right-handed and left-handed forms. With such a LC chirality transition, a change of handedness of the chiral arrangement of pending phenyl groups can be observed. The above results indicated that the transition between right-handed and left-

handed superhelices is accompanied with the handedness change of the pending phenyl group, which depends on temperature and solvent nature.

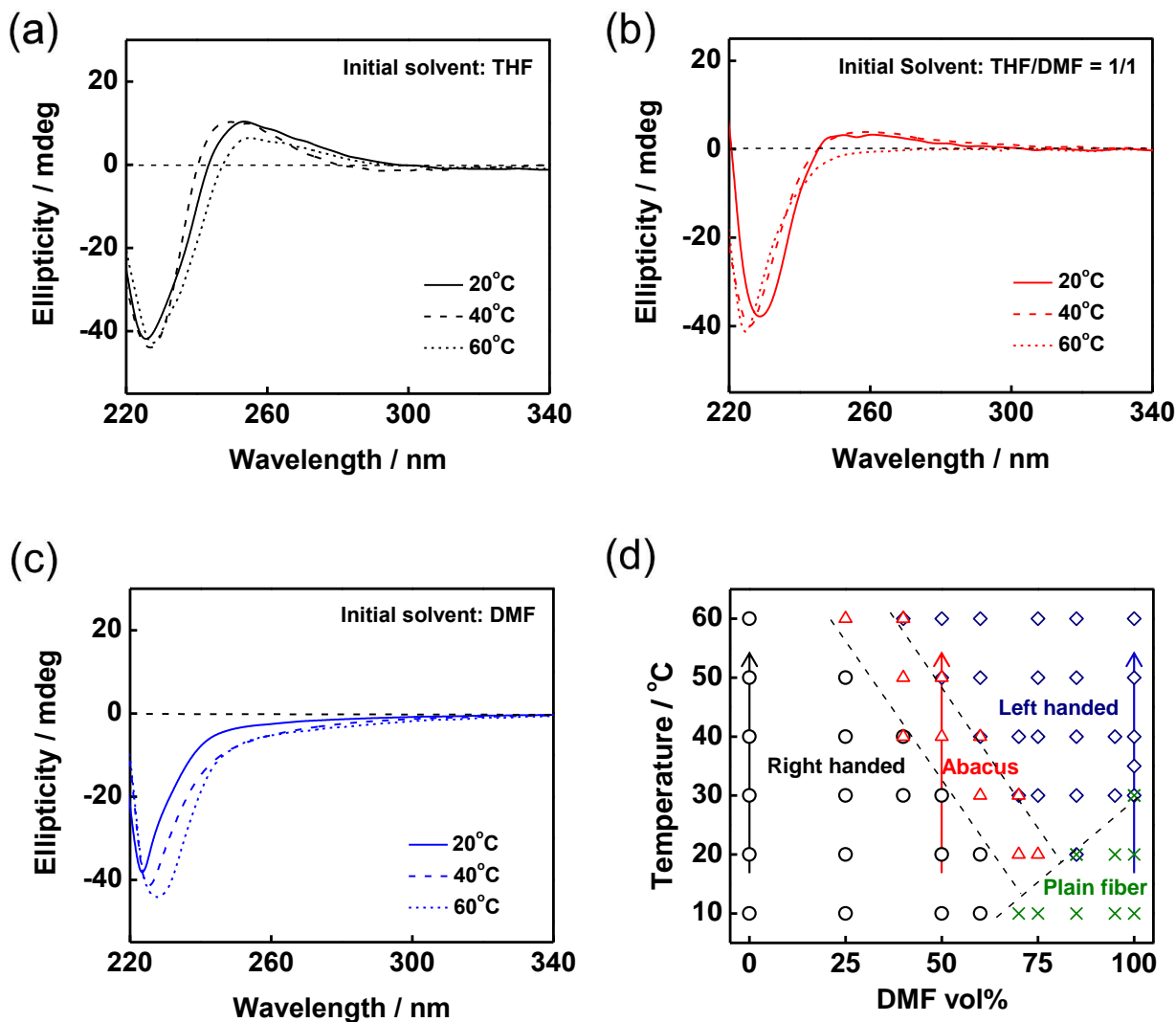


Figure S4. The chiral arrangement of pending phenyl groups. CD spectra were recorded for the PBLG-*b*-PEG/PBLG assemblies prepared at various organic solvents: (a) THF; (b) THF/DMF = 1/1; and (c) DMF. Experimental temperature ranged from 20 to 60 °C. (d) Morphology diagrams of aggregates self-assembled from PBLG-*b*-PEG/PBLG mixtures as functions of temperature and initial solvent composition. The black, red and blue arrows indicate the temperature-induced morphology transition of the aggregates prepared at various initial solvent compositions.

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