Electronic Supplementary Information

for

High length-diameter ratio nanotubes self-assembled from facial cyclopeptide

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Experimental Section

1. Materials

N-Fluorenyl-9-methoxycarbonyl protected L-amino acids (L-Fmoc-Gln(Trt)-OH, L-Fmoc-Glu-OAll, L-Fmoc-Lys(BOC)-OH) and D-amino acids (D-Fmoc-Ala-OH, D-Fmoc-Leu-OH), 2-chlorotrityl chloride resin (100-200 mesh, loading: 1.1 mmol/g, 1% DVB), Rink Amide-AM resin (100-200 mesh, loading: 0.59 mmol/g, 1% DVB), O-benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU), 1-hydroxybenzotriazole (HOBt), and piperidine were provided by GL Biochem (Shanghai) Ltd. (China) and used as received. Trifluorocaetic acid (TFA), Triisopropylsilane (TIS), and 1,2-ethanedithiol (EDT) were purchased from Shanghai Reagent Chemical Co. (China). $Pd(PPh_3)_4$ and N-Methylmorpholine (NMM) were purchased from ACROS (USA) and used without further purification. Trifluorocaetic acid (TFA), Diisopropylethylamine (DIEA), dimethylformamide (DMF) and chloroform ($CHCl_3$) were distilled before used. All other reagents and solvents were used without further purification.

2. Synthesis of the linear peptide

The linear peptide NH_2-*D*-Leu-*L*-Lys-*D*-Ala-*L*-Lys-*D*-Leu-*L*-Gln-COOH (named as LP) was synthesized manually following the manual Fmoc solid phase peptide synthesis (SPPS) method.[S1] 1.0 g 2-chlorotrityl chloride resin was placed in a peptide synthesis vessel, swelled with DMF for 30 min and washed with DMF for three times. The *L*-Fmoc-Gln(Trt)-OH was loaded on the resin and then other amino acids were conjugated onto it. The coupling of the first residue was carried out with 2 equiv. of *L*-Fmoc-Gln(Trt)-OH (relative to the resin substitution degree) and 10 equiv. of DIEA in a DMF solution. Coupling cycle of the linear peptide consisted of, a) Fmoc group

deprotection (20% piperidine/DMF, 2 x 15 min), b) DMF wash (4 x 1 min), c) amino acid coupling with 2 equiv. of *D/L* Fmoc-protecting amino acid, 2.4 equiv. of HBTU and HOBt, and 6 equiv. of DIEA for 2 h, and d) DMF wash (3 x 1 min). Kaiser reagent was employed to examine the completion of reaction. After the completion of the peptide synthesis, the peptide-containing resin was washed with DMF, methanol (MeOH), and methylene chloride (DCM) three times, respectively. The resin was finally dried under vacuum overnight. Peptide was generally de-protected and cleaved from the resin by a modified TFA cleavage procedure (TFA/H_2O/TIS/EDT=94/2/2/2, 2 h at rt). After the filtration to remove the solid support, the solution was concentrated by rotary evaporation and the peptide was precipitated by addition of cold diethyl ether (Et_2O). The precipitate was centrifuged and washed with Et_2O.

3. **Synthesis of the cyclopeptides**

The cyclopeptide *c*-[*D*-Leu-*L*-Lys-*D*-Ala-*L*-Lys-*D*-Leu-*L*-Gln] (named as CP) and other two cyclopeptides (c-[*D*-Leu-*L*-Gln-*D*-Leu-*L*-Gln-*D*-Leu-*L*-Gln] and c-[*D*-Leu-*L*-Cys-*D*-Ala-*L*-Asp-*D*-Leu-*L*-Gln]) were also synthesized *via* manual SPPS but based on Rink Amide-AM Resin.[S2] Typically, 1.0 g Rink Amide AM Resin was swelled as above. Different from the 2-chlorotrityl chloride resin, Amide AM resin should be deprotected before the coupling. Fmoc group was removed from the resin with 20% piperidine/DMF (2 x 20 min). After the draining from the resin, the residual piperidine was washed with DMF for four times. Fmoc-Glu-OAll (2 eq) was firstly assembled to the resin with 2.4 equiv. of HBTU and HOBt, and 6 equiv. of DIEA for 2 h, then the resin was washed with DMF (3 x 1 min). Cycle coupling was carried out as above. After the completion of the linear peptide synthesis, the C-terminal protecting group (All) of Glu was removed by 2 equiv. of $Pd(PPh_3)_4$ with

$CHCl_3$:AcOH:NMM=37:2:1 (15 mL/g of resin),[S3] and the mixture was stirred under Ar at room temperature for 24 h. After removing the solution, the resin was washed with $CHCl_3$ (6 x 1 min), 0.5% DIEA in DMF (6 x 1 min), 5% HoBt in DMF (6 x 1 min), DMF (6 x 1 min), respectively. The N-terminal protecting group (Fmoc) was removed with 20% piperidine/DMF, and then washed with DMF (6 x 1 min). The peptide was cyclized by adding 3 equiv. of PyBOP and HoBt (3.0 eq.) and 8 equiv. of DIEA in DMF (coupling reagent concentration at 0.5 M), followed by stirring for 3 days. Kaiser reagent was employed to examine the completion of reaction. After filtration and washing with DMF (3 x 1 min), MeOH (3 x 1 min), CH_2Cl_2 (3 x 1 min), the resin was dried under high vacuum. Deprotection and cleavage of cyclopeptides from the resin were carried out as above.

4. **Characterization of the peptides**

The molecular weight of peptides was analyzed by electrospray ionization mass spectrometry (ESI-MS, LCQ Advantage, Finigan, USA) in a component solvent of Acetonitrile and MeOH at a concentration of 0.5 mg/mL. The theoretical molecular weights of LP and CP are 699.5 and 681.5, which found in ESI-MS were $[M-H]^-$ at m/z 699.1, $[M-H+M_{(TFA)}]^-$ at m/z 812.9, $[M-H+2M_{(TFA)}]^-$ at m/z 928.7 and a series of double molecular weight peaks in LP (Fig. S2), and m/z at 682.4 corresponding to $[M+H]^+$ in CP (Fig. S3). The theoretical molecular weights of c-[*D*-Leu-*L*-Gln-*D*-Leu-*L*-Gln-*D*-Leu-*L*-Gln] and c-[*D*-Leu-*L*-Cys-*D*-Ala-*L*-Asp-*D*-Leu-*L*-Gln] are 723.4 and 643.3, which found in ESI-MS are $[M-H+M(_{TFA})]^-$ at m/z 836.4 for c-[*D*-Leu-*L*-Gln-*D*-Leu-*L*-Gln-*D*-Leu-*L*-Gln] (Fig. S4), and $[M-H]^-$ at m/z 642.4, $[M-H+M(_{TFA})]^-$ at m/z 756.0 and $[2M-H]^-$ at m/z 1285.2 for c-[*D*-Leu-*L*-Cys-*D*-Ala-*L*-Asp-*D*-Leu-*L*-Gln] (Fig. S5).

5. **Transmission electron microscopy (TEM) observation of the peptide self-assemblies**

The morphology of peptide self-assemblies was observed by transmission electron microscopy (TEM, JEM-2010, Japan) with an accelerating voltage of 100 kV. 2 mg of cyclopeptide was dissolved in 2 mL of de-ionized water and 0.1 M NaOH or HCl was added to adjust the pH value to ~6.5, the solution was stood for one day for the self-assembly. The samples were prepared by dipping a copper grid with carbon film into the solution. After the deposition for 1 min, the remaining solution was removed and the samples were dried under an infrared lamp for the observation under TEM.

6. **Circular dichroism (CD) and fourier transform infrared (FT-IR) spectroscopy investigation of the peptide self-assemblies**

CD spectra of the peptide self-assemblies were carried out on a J-810 spectropolarimeter (Jasco, Japan) with 1 mg/mL peptide solution. FT-IR spectra of the self-assemblies were performed on an AVATAR 360 spectrometer. The freeze-dried peptide self-assemblies were pressed into pellets with potassium bromide (KBr) for the measurements.

7. **Wide-angle x-ray diffraction (WXRD) of the peptide nanotubes**

The X-ray diffraction pattern of CP tubes was obtained by a Shimadzu XRD-6000 diffractometer with a Ni filter and Cu Kα1 (current =40 mA, voltage=40 kV, λ=1.54056 Å).

8. **Thermogravimetric analysis (TGA) of the peptide nanotubes**

The thermal stability of CP tubes was investigated by a thermogravimetric analyzer (NETZSCH Jupiter® STA 449C, USA). The TG measurement was carried out under a stream of nitrogen with a heating rate of 20 ^{o}C min^{-1} (purging rate: 40 mL min^{-1}). The curve was collected from 40 ^{o}C to 600 ^{o}C.

References

[S1] S. Y. Qin, X. D. Xu, C. S. Chen, J. X. Chen, Z. Y. Li, X. Z. Zhang and R. X. Zhuo, *Macromol. Rapid Commun.* 2011, **32**, 758.

[S2] C. Qin, C. Xu, R. Zhang, W. Niu and X.Shang, *Tetrahedron Lett.* 2010, **51**, 1257.

[S3] L. R. Lampariello, D. Piras, M. Rodriquez and M. Taddei, *J. Org. Chem.* 2003, **68**, 7893.

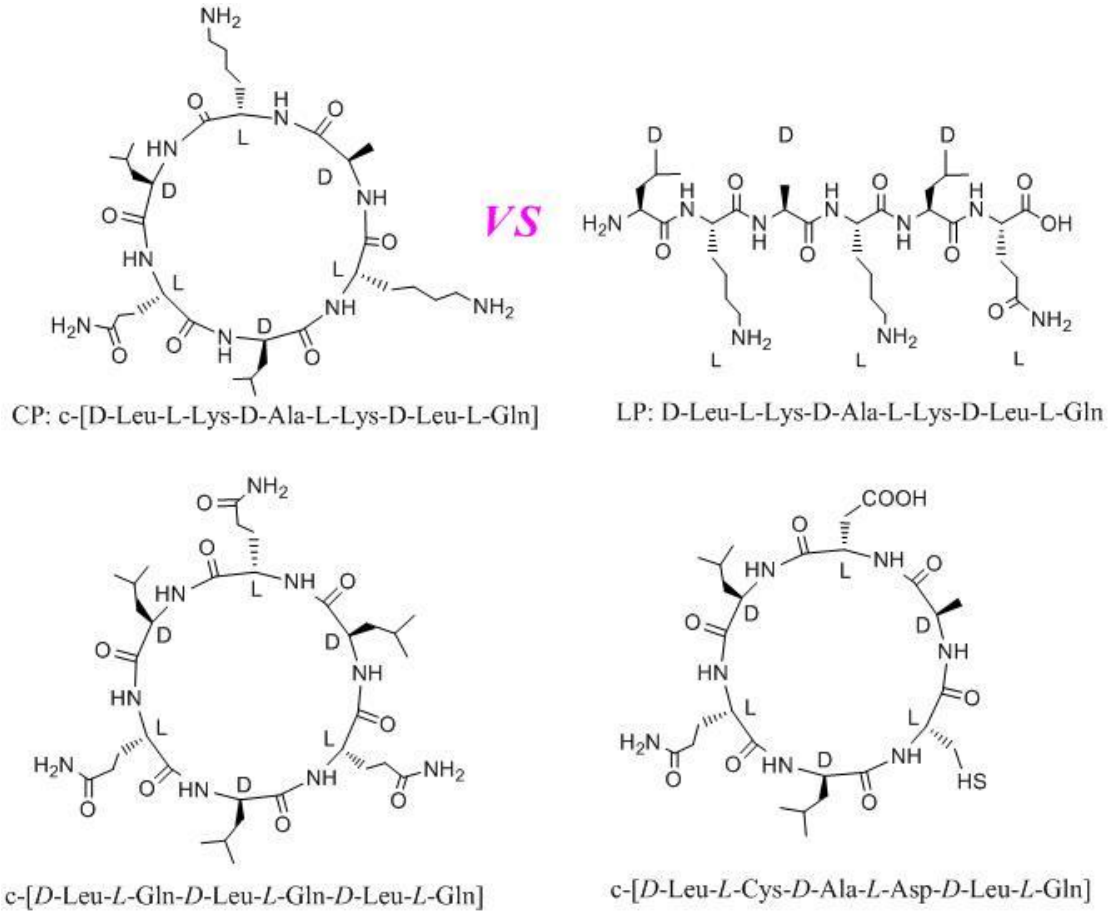

Fig. S1 Chemical structures of cyclopeptide (CP), linear peptide (LP), and two analogous cyclopeptides (c-[*D*-Leu-*L*-Gln-*D*-Leu-*L*-Gln-*D*-Leu-*L*-Gln] and c-[*D*-Leu-*L*-Cys-*D*-Ala-*L*-Asp-*D*- Leu-*L*-Gln]).

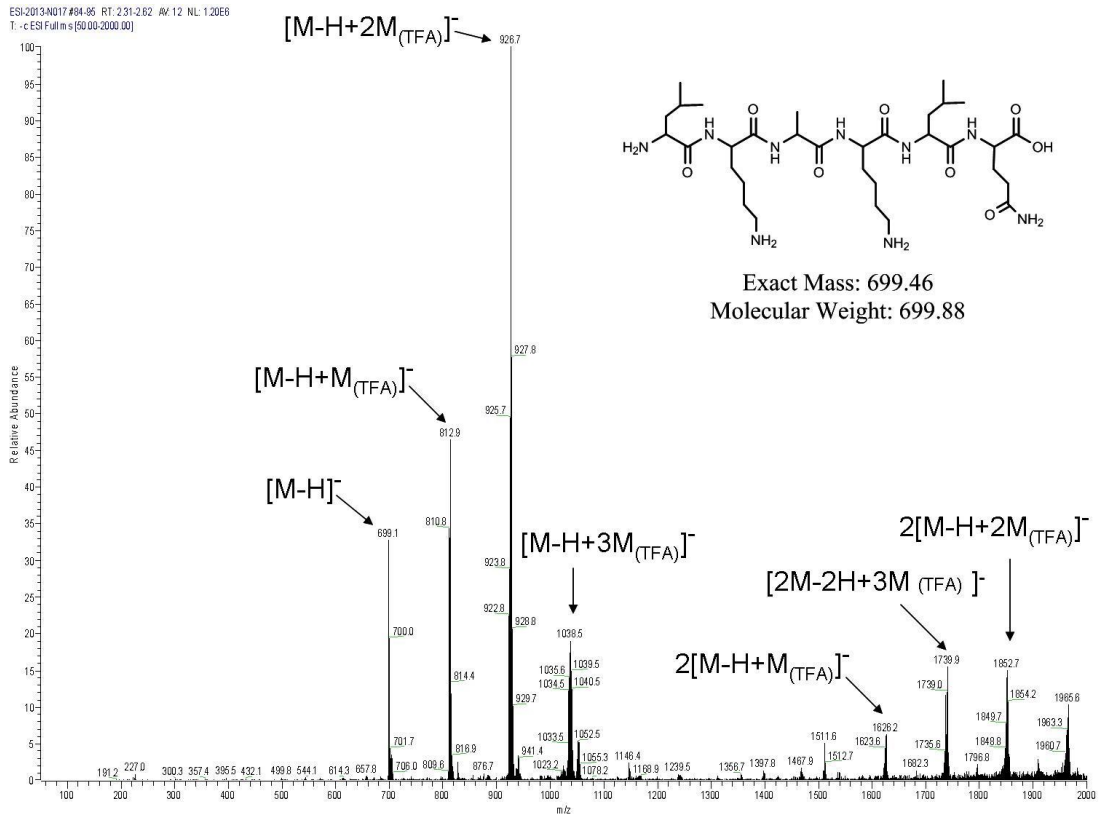

Fig. S2 ESI-MS of the synthesized LP. $[M-H]^-$ at m/z 699.1, $[M-H+M_{(TFA)}]^-$ at m/z 812.9, $[M-H+2M_{(TFA)}]^-$ at m/z 928.7 and a series of double molecular weight peaks were observed.

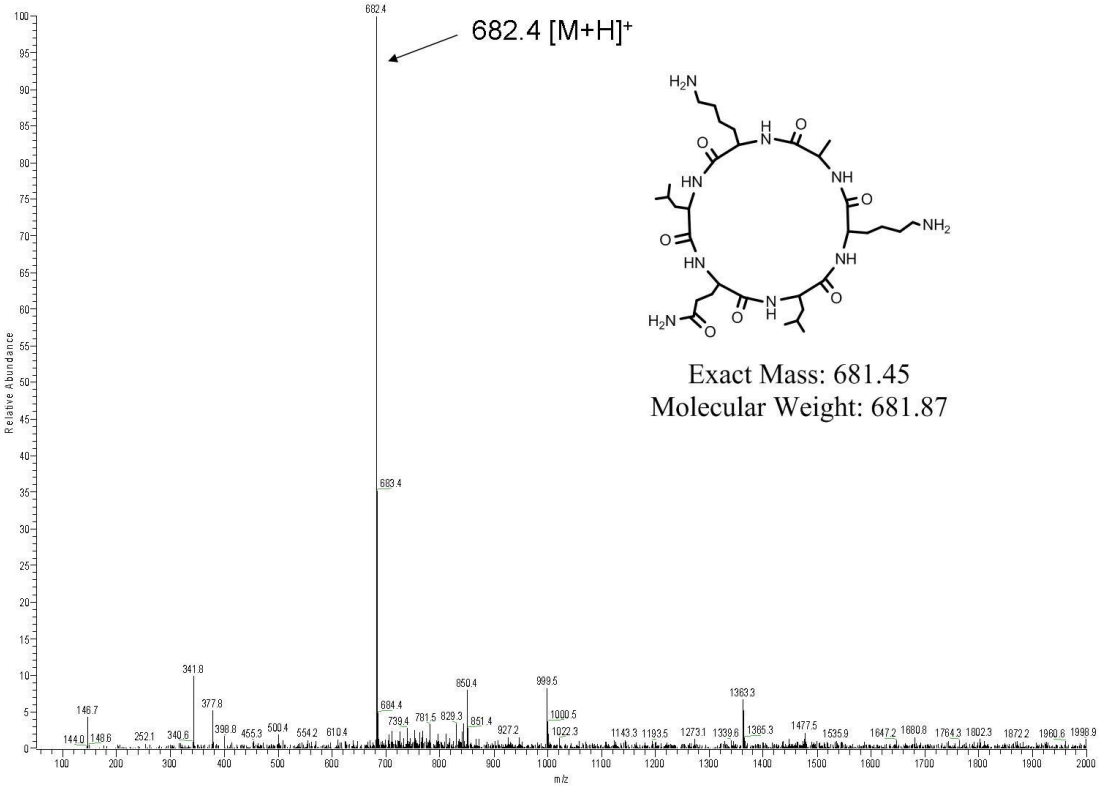

Fig. S3 ESI-MS of the synthesized CP. $[M+H]^+$ at m/z 682.4 was observed.

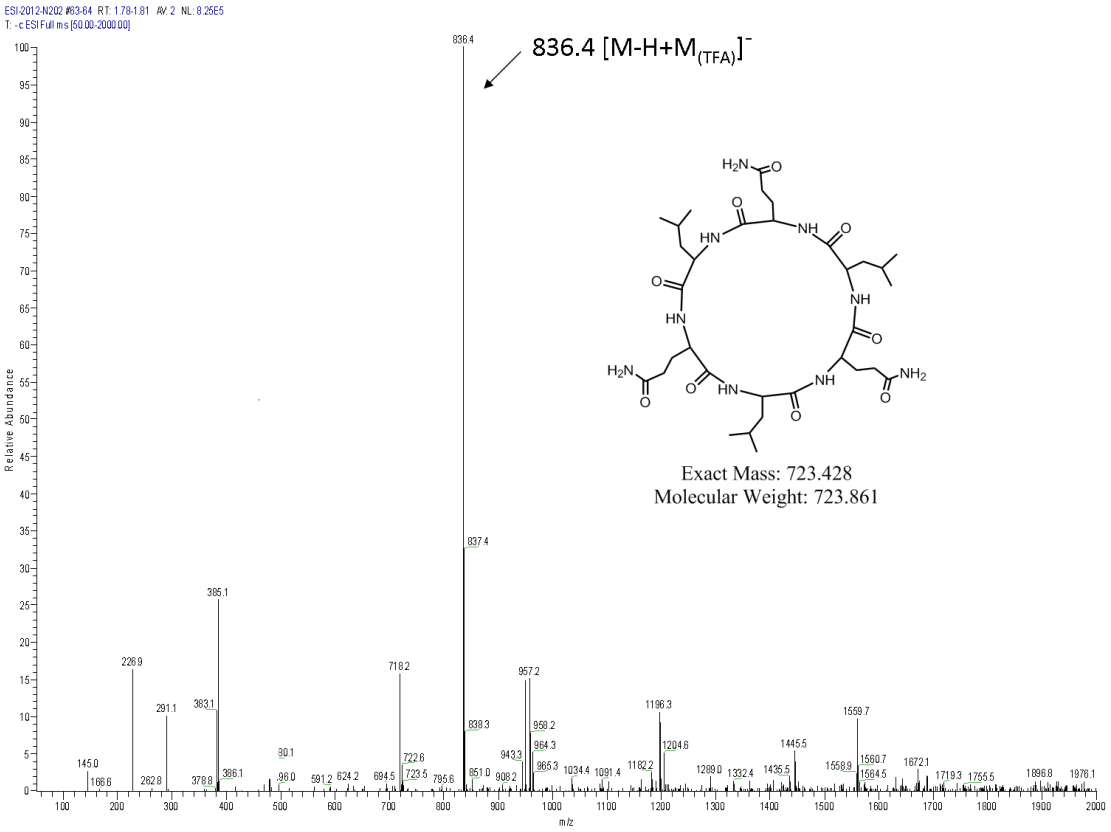

Fig. S4. ESI-MS of the cycloeptide: c-[*D*-Leu-*L*-Gln-*D*-Leu-*L*-Gln-*D*-Leu-*L*-Gln]. $[M-H+M(_{TFA})]^-$ at m/z 836.4 was observed.

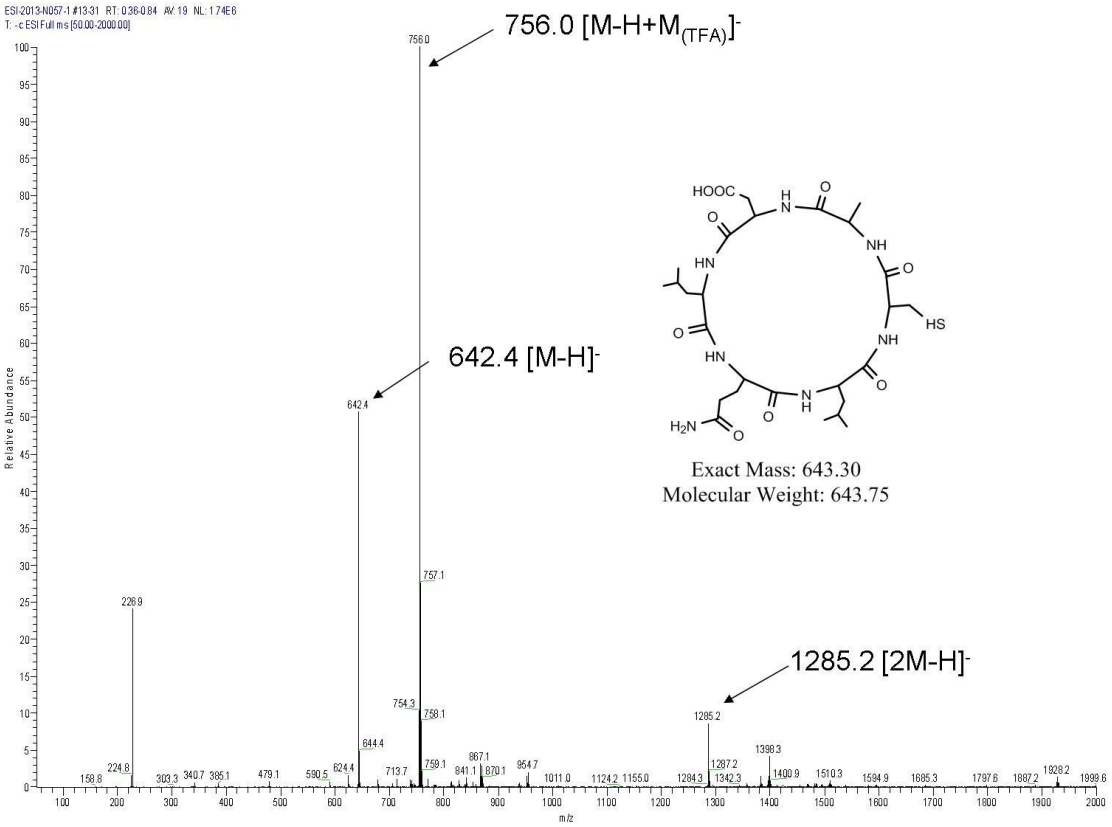

Fig. S5 ESI-MS of the cycloeptide: c-[*D*-Leu-*L*-Cys-*D*-Ala-*L*-Asp-*D*-Leu-*L*-Gln]. $[M-H]^-$ at m/z 642.4, $[M-H+M(_{TFA})]^-$ at m/z 756.0 and $[2M-H]^-$ at m/z 1285.2 were observed.

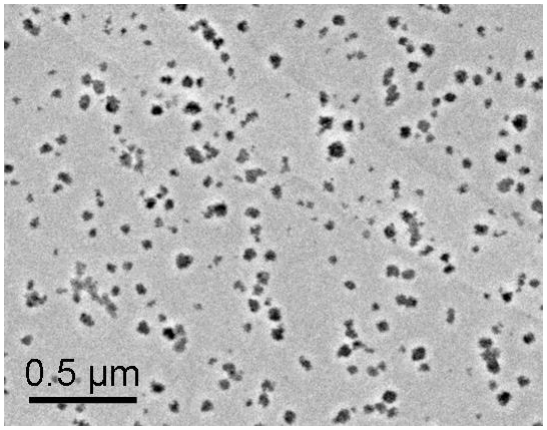

Fig. S6 TEM image of the self-assembled LP in aqueous solution (1.0 mg/mL) at pH around 6.

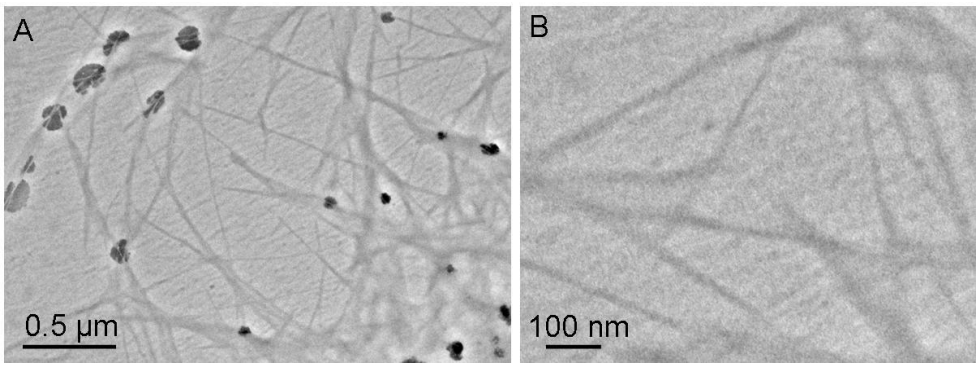

Fig. S7 TEM images of the self-assembled nanofibers obtained by c-[*D*-Leu-*L*-Cys-*D*-Ala-*L*-Asp-*D*-Leu-*L*-Gln].

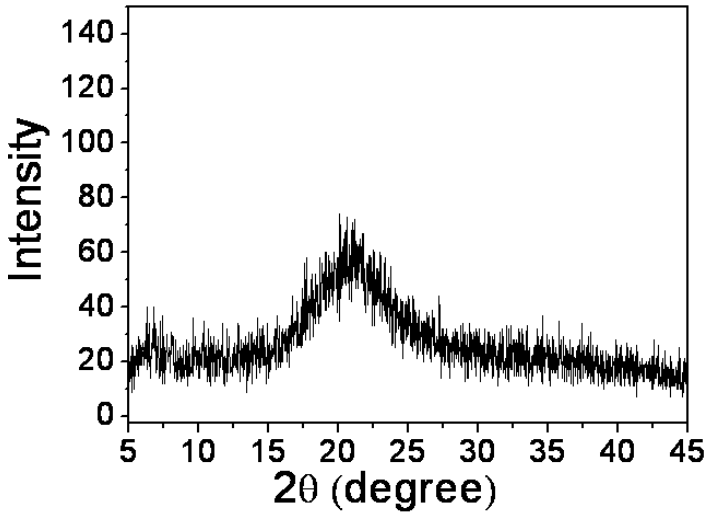

Fig. S8 WXRD curve of the self-assembled CP nanotubes.

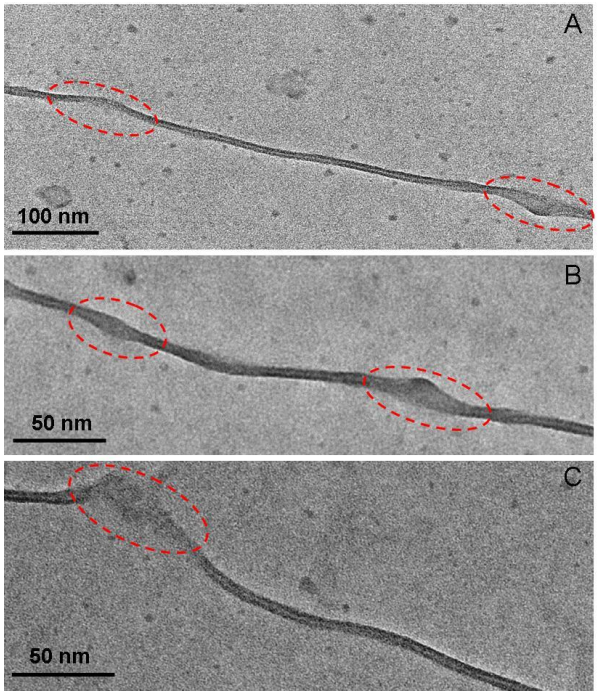

Fig. S9 TEM images of cyclopeptide tubes formed via pathway I, in which the cyclopeptide units firstly self-assembled into nano-ring seeds and further spontaneously organize into nanotubes. Some defects and bulges (marked by red cycles) were found in the self-assembled tubes.

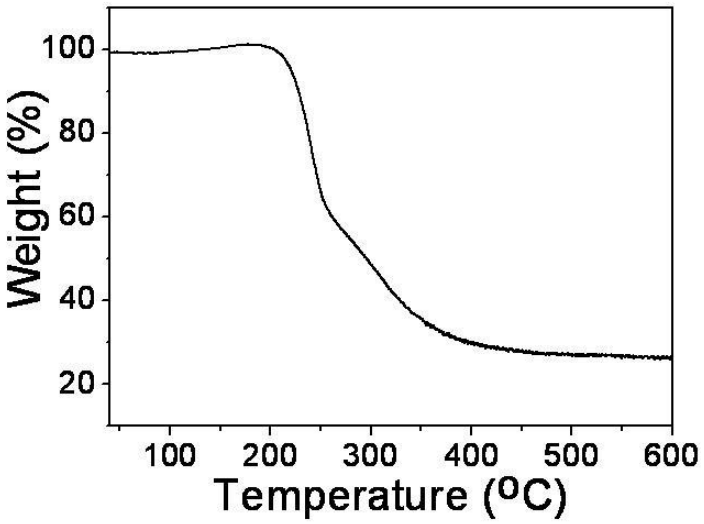

Fig. S10 TG curve of the self-assembled CP nanotubes.