Supplementary Material (ESI) for Chemical Communications

Supporting Information for:

Self-assembling can make more complex morphologies from chiral amphiphilic peptides

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Materials and Methods

Preparation of Molecular Assemblies. Polypeptide (12 mg) was dissolved in ethanol (120 μL). Then an aliquot (30 μL) of the peptide solution was injected into a buffer (1 mL, 10 mM Tris-HCl, pH 7.4) with stirring at 0 °C. After 30 min, the dispersion was purified by Sephacryl S-100 column (1.5 × 30 cm, GE healthcare Bio-Sciences) using 10 mM Tris-HCl buffer (pH 7.4) as an eluent to remove ethanol. Molecular assemblies of different compositions were prepared similarly.

Circular Dichroism (CD). CD measurements were carried out on a JASCO J600 spectropolarimeter with an optical cell of 0.1 cm optical path length at room temperature. The sample concentration in 10 mM Tris-HCl buffer (pH 7.4) was 0.375 mM (per amino acid residue).

Transmission Electron Microscopy (TEM). TEM images were taken using a JEOL JEM-2000EXII at an accelerating voltage of 100 kV. For the observation, a drop of dispersion was mounted on a carbon-coated Cu grid and stained negatively with 2% uranyl acetate, followed by suction of the excess fluid with a filter paper.

Frozen-Hydrated/Cryogenic-TEM (Cryo-TEM). The dispersions in a buffer were frozen quickly in liquid ethane, which was cooled with liquid nitrogen. The samples were examined at 100 kV accelerating voltage at the liquid nitrogen temperature.

Fourier Transform Infrared Spectroscopy/Attenuated Total Reflection (FT-IR/ATR). Infrared transmission spectroscopy of the assembly dispersion was performed on a Fourier transform infrared spectrometer (Nicolet 6700 FT-IR, Thermo Fisher Scientific, MA) at room temperature with a solution cell. The sample concentration in ultrapure water was ca. 0.33 mM.

Synthesis of SLL and SDL.

The Aib-containing dodecapeptides were synthesized by the conventional liquid phase method. Boc group of the dodecapeptide (600 mg, 0.454 mmol) was removed by treatment with trifluoroacetic acid (TFA, 6 mL) and anisole (0.6 mL). The TFA salt was washed with isopropylether and dried in vacuo for 2 h. The salt was dissolved in chloroform and washed with 4 wt% $NaHCO_3$ and saturated NaCl aqueous solutions. The organic layer was dried over anhydrous $MgSO_4$ and the solvent was removed and dried in vacuo to afford H-(Leu-Aib)$_6$-OMe (546 mg). To a solution of Sar NCA (634 mg, 5.51 mmol) in *N,N*-dimethylformamide (DMF) (12 mL), a solution of H-(Leu-Aib)$_6$-OMe in DMF/$CHCl_3$ (9:1 v/v, 273 mg/10 mL) was added. After complete consumption of the Sar NCA was confirmed, glycolic acid (85 mg, 1.12 mmol, 5.0 eq), 2-(1-H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate methanaminium (HATU, 426 mg, 1.12 mmol, 5 eq.) and diisopropyl ethyl amine (DIEA, 293 mL, 1.68 mmol, 7.5 eq.) were added at 0 °C to react with the *N*-terminal, and the solution was stirred at 0 °C for 10 min and at room temperature for 10 h. Then another portions of glycolic acid (34 mg, 0.45 mmol, 2.0 eq.), HATU (170 mg, 0.45 mmol, 2.0 eq.), and DIEA (118 mL, 0.67 mmol, 3.0 eq.) were added to the solution. After stirring for 12 h, the solution was condensed, and the residue was purified by a Sephadex LH20 column with methanol as an eluent to afford polypeptided **SLL** or **SDL** (504 mg). The degree of polymerization of the poly(Sar) block was determined to be 25 from the relative areas of Sar*N*-CH_3 signal against the OCH_3 signal in the ^{1}H NMR spectra.

^{1}H NMR (400 MHz, MeOH-d) δ (ppm) 8.2–7.7 (m, 11H, amide), 7.4–7.3 (br, 1H, amide), 4.6–3.8 (br, 56H, LeuC$^{\alpha}$*H*, SarCH_2), 3.66 (s, 3H, OCH_3), 3.3–2.8 (m, 75H, Sar N-CH_3), 1.9–1.3 (m, 36H, LeuCH_2, Leu$C^{\gamma}H$, AibCH_3), 1.1–0.8 (m, 36H, Leu(CH_3)$_2$).

MALDI-TOF MS analysis also supported the degree of polymerization to be 25.

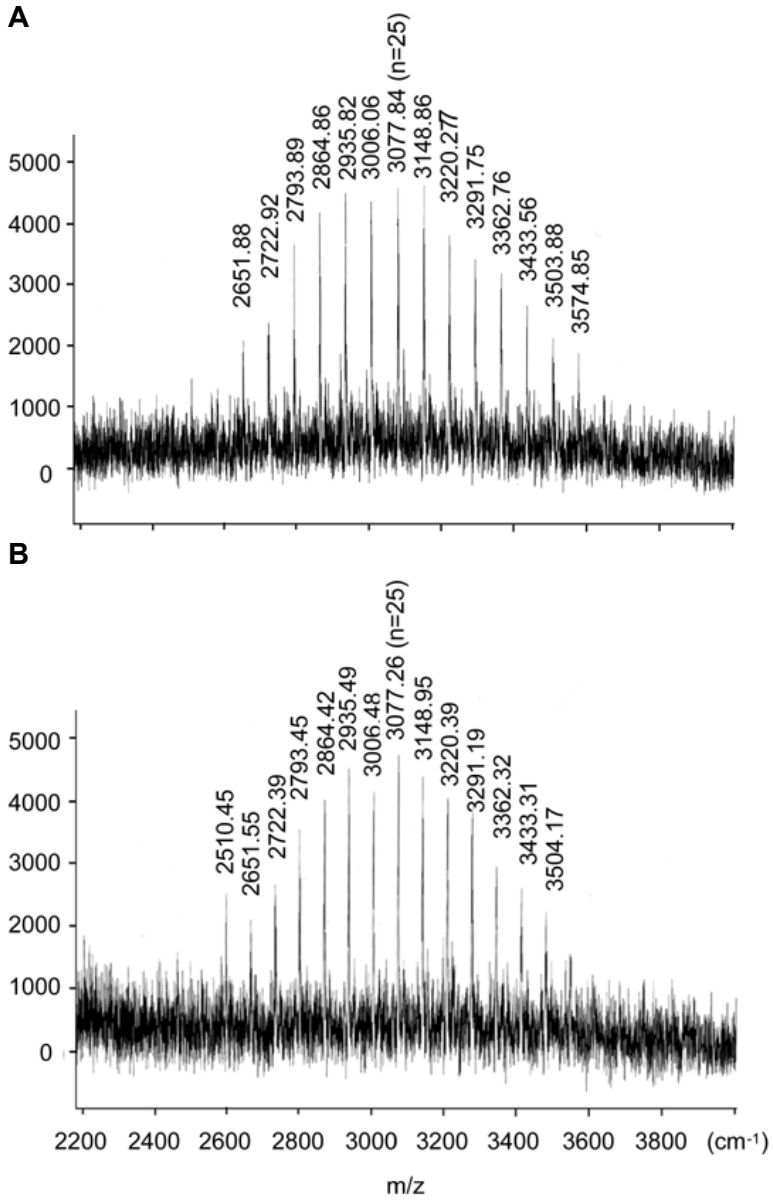

Fig. S1. MALDI-TOF MS spectra of (a) **SLL** and (b) **SDL.** Poly(sarcosine) of 25 mers were attached to the *N*-terminal of hydrophobic blocks of **SLL** and **SDL**. ($[M+Na]^+$ calcd: 3077)

Conformation of the hydrophobic segment of **SLL** and **SDL** was analyzed by CD spectroscopy (Fig. S2) to show α-helical structure with bundle formation.

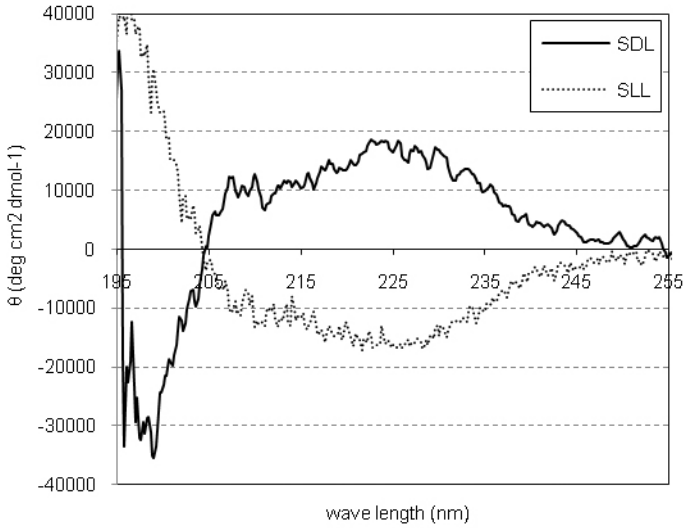

Fig. S2. CD spectra of **SLL** and **SDL** in buffer. The amphiphilic polypeptides were injected into 10 mM Tris-HCl buffer (pH 7.4) (0.18 mg / 1 mL) and then were heated at 90 ^{o}C for 10 min. These spectra suggest that the hydrophobic blocks of **SLL** and **SDL** formed right-handed and left-handed helices, and the helices were tightly packed as bundle in buffer.

The elongation of nanotubes prepared from **SLL** was observed after heating at 50 °C for 14 h and then at 90 °C for 1 h. The diameter of nanotube was 70 nm, which was the same as that before heating. On the other hand, the length of nanotube became 200-900 nm.

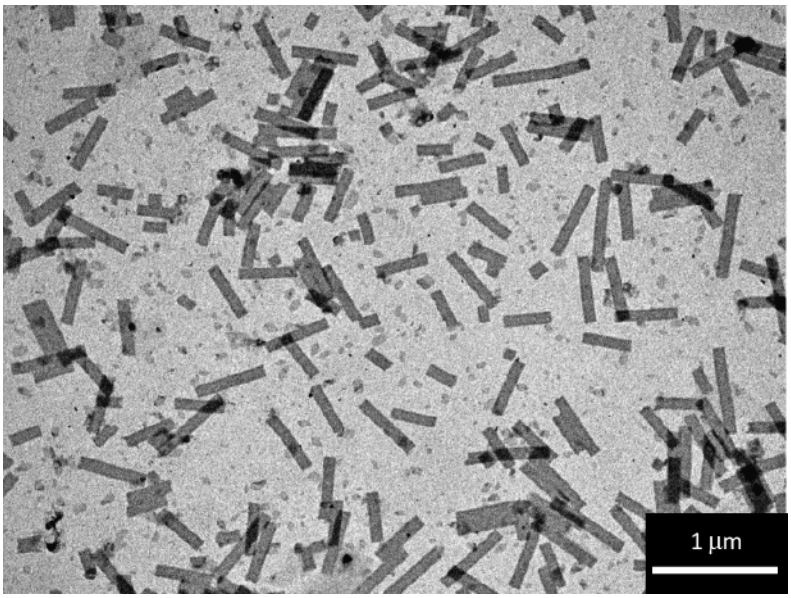

Fig. S3. TEM images (negative staining with uranyl acetate) of nanotube suspension prepared from **SLL** upon heat treatment at 50 °C for 14 h and then at 90 °C for 1 h. The scale bar is 1 μm.

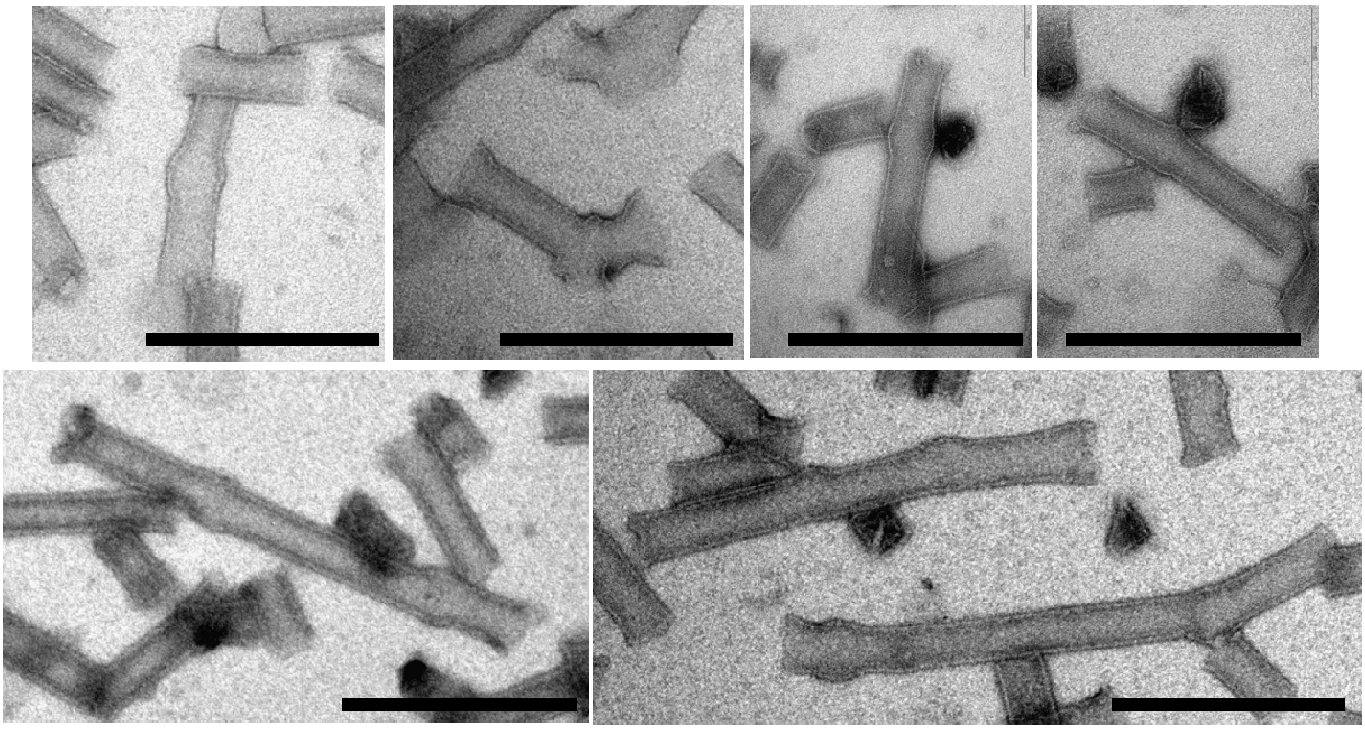

Fig. S4. TEM images of some bunchy connecting regions of two and more nanotubes observed upon heating a mixture of **SLL** and **SDL** nanotube dispersion at 50 °C for 3h. The scale bar is 500 nm.

The difference of molecular packing between assembly from stereo-complex component and single component was checked by electron diffraction analysis. The electron diffraction pattern obtained from the planar sheet assembly suggest the face-centred rectangular lattice (a = 2.65, b = 3.00, α = 90º) of **SLL** and **SDL**. On the other hand, that from the nanotube assembly shows the square lattice of **SDL**. These results indicate that the molecular packing pattern of mixture assembly is different from that of single component assembly.

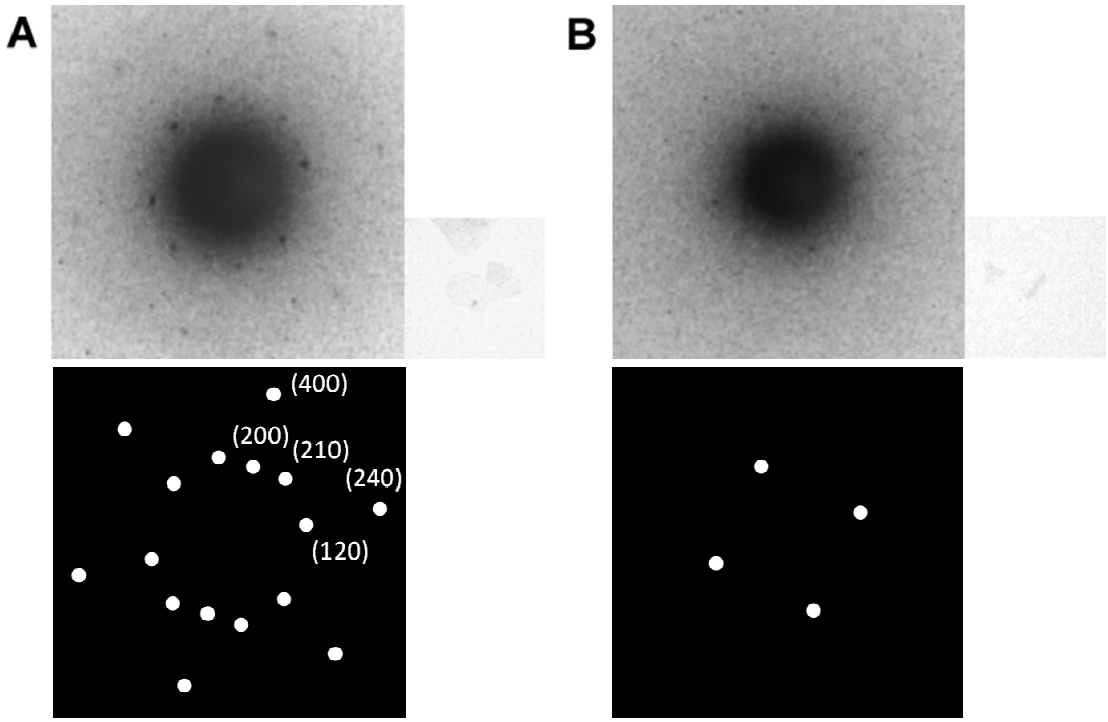

Fig. S5. TEM images and electron diffraction patterns of (**A**) the planar sheet assembly from an equimolar mixture of **SLL** and **SDL** and (**B**) the nanotube assembly from a single component of **SDL** in 10 mM Tris-HCl buffer (pH 7.4).

The difference of molecular packing between assembly from stereo-complex component and single component was checked by the Fourier transform infrared spectroscopy/attenuated total reflection (FT-IR/ATR) analysis. On the assembly from single component of **SLL** and mixture of **SLL** and **SDL**, the positive peaks at 2850 cm^{-1} show the C-H asymmetrical stretching vibration modes. On the other hand, the peaks of the C-H symmetrical stretching vibration modes of each assemblies are observed at 2920 and 2927 cm^{-1}, respectively. One possible interpretation for the high-frequency shift of C-H symmetrical stretching vibration band is the decreasing of the mobility of side chain of amino acids in the stereo-complex state. These results indicate that the intermolecular entanglement of mixture assembly is more tight than that of single component assembly.

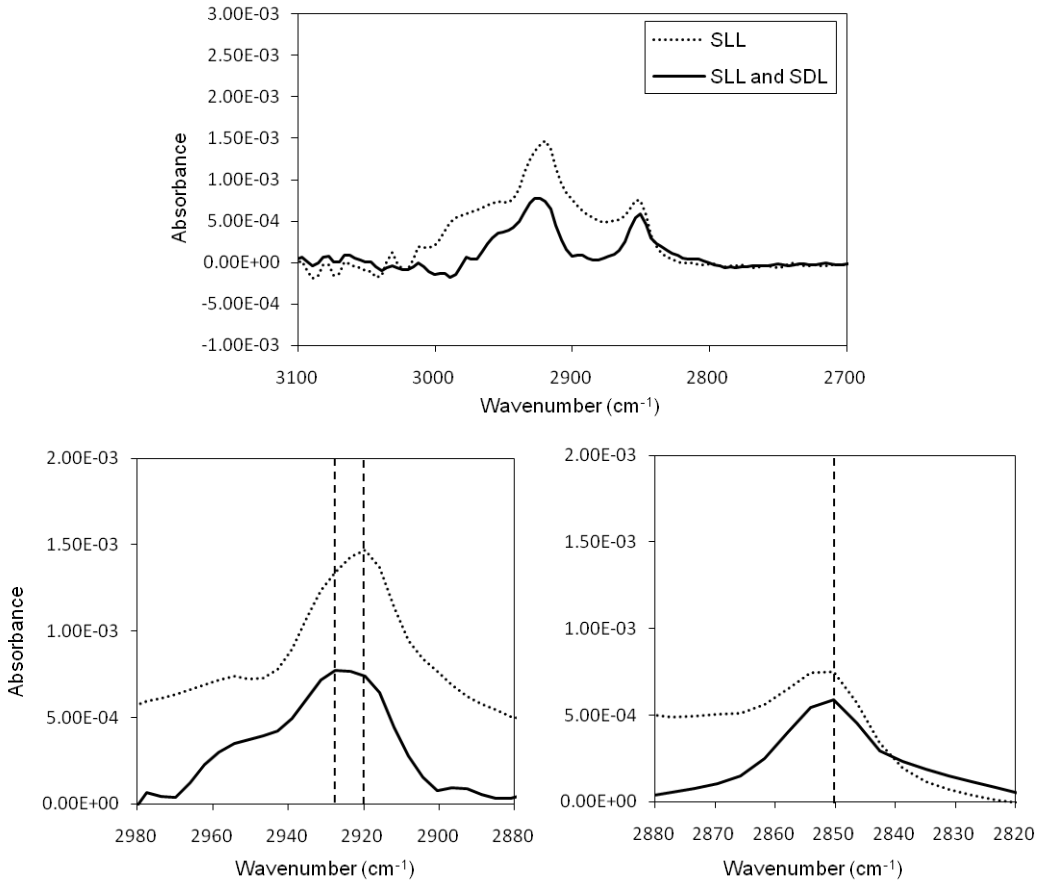

Fig. S6. IR spectra in the C-H stretching region of amino acid of assembly from a mixture of **SLL** and **SDL** (solid line) and a single component of **SLL** (dash line).