Electronic Supplementary Information for:

**Tubulation onto peptide vesicle by phase-separation of a binary mixture of amphiphilic right-handed and left-handed helical 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.
**Synthesis of sSLL.** sSLL was prepared by the same method\(^1\) as SLL but the capping reagent was lipoic acid instead of glycolic acid. After complete consumption of Sar NCA was confirmed, lipoic acid (50 mg, 5 eq.), HATU (90 mg, 5 eq.) and triethylamine (64 μL, 7.5 eq.) were added to the solution. After stirring for 12 h, another lipoic acid (25 mg, 2.5 eq.), HATU (45 mg, 2.5 eq.) and triethylamine (32 μL, 3.8 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 sSLL.

\(^1\)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, SarC\(^\alpha\)H), 3.66 (s, 3H, OCH\(_3\)), 3.55 (quin, 1H, SSCH), 3.3–2.8 (m, 79H, Sar N-CH\(_3\), SSCH\(_2\), CH\(_2\)CH\(_2\)CO), 2.5–2.4 (br, 2H, SSCH\(_2\)CH\(_2\)), 2.0–1.7 (br, 8H, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_2\)CO, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_2\)CO, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_2\)CO), 1.7–1.3 (m, 36H, LeuCH\(_2\), LeuC\(^\gamma\)H, AibCH\(_3\)), 1.1–0.8 (m, 36H, Leu(CH\(_3\))\(_2\)).
Mixtures of SLL and SDL in the molecular assemblies were checked by CD whether the helix contents were affected by mixing in the assemblies.

**Fig. S1.** CD spectra with changing the mixing ratio of SLL and SDL in the molecular assemblies. The molecular assemblies were formed from varying mole ratios of amphiphilic polypeptides, SLL and SDL, in 10 mM Tris-HCl buffer (0.18 mg / 1 mL).
We can improve the purity of the nano round-bottom flask assembly by modifying the preparation process. At first, two types of molecular assemblies, the nanotube of SLL (50 wt%) and the nano planar sheet composed of an equimolar mixture of SLL and SDL (25 and 25 wt%), were prepared separately. Then, both molecular assemblies were mixed together and heated at 90 °C for 1 h to obtain the round-bottom flask assembly with a high yield of 38%. The dispersion was purified by a size exclusion chromatography of Sephacryl S-100 to obtain nearly pure round-bottom flask assembly. In this case, the planar sheet has a chance to fuse with the nanotube to yield the nano round-bottom flask or is just transformed by itself to vesicle. However, the yield of the round-bottom flask was found to be 38% (the number of the round-bottom flask/(the number of the round-bottom flask + the number of the nanotube) = 168/443 in six TEM images), which coincides with the calculated value for quantitative production without the vesicle transformation (Fig. S2). Priority of the round-bottom flask assembly over the vesicle under the present preparation conditions remains to be solved.

![TEM images](image1.png)

**Fig. S2.** TEM images (negative staining with uranyl acetate) of molecular assemblies prepared from a mixture of the nanotube prepared from SLL and the planar sheet prepared from an equimolar mixture of SLL and SDL after heat treatment (90 °C, 1 h). The nanotube and the planar sheet assemblies were
prepared in 10 mM Tris-HCl buffer (pH 7.4) (3 mg/1 mL) by the ethanol injection method with and without heat treatment, respectively.

The calculation method is as follows:
The occupied surface area of a molecule, **SLL**, is same to that of **SDL** in assembly. So, the percentage of constituent molecules, **SLL** or **SDL**, of the morphology can be calculated by the surface area. Now, **SLL/FDL** = 75/25 in this system, because the nanotube of **SLL** (50 wt%) and the planar sheet composed of an equimolar mixture of **SLL** and **SDL** (25 and 25 wt%), were prepared, and then, both molecular assemblies were mixed. The surface area of the spherical part (diameter = 180 nm) of round-bottom flask type is 101736 nm². This part is composed of a mixture of **SLL** (25 wt%) and **SDL** (25 wt%). On the other hand, the surface area of the neck part (diameter 60 nm and length 200 nm) is 37680 nm², suggesting that this part is composed of **SLL** (19 wt%), by (25 + 25)*(37680/101736). Therefore, It is considered that the rest **SLL** (31 wt%) formed the free nanotube in this system, indicating that the ratio of the round-bottom flask (the round-bottom flask/(the round-bottom flask + the free nanotube)) is 19/(19 + 31).