Supporting Information

Self-assembly of NH$_2$-(α,L-lysine)$_5$-COOH and SDS into nanodiscs or nanoribbons regulated by pH

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1. Materials

NH₂-(α,L-lysine)₅-COOH, NH₂-(α,L-lysine)₅-COOH, NH₂-(α,L-lysine)₅-COOH, and NH₂-(α,L-lysine)₅-COOH were purchased from Scilight Biotechnology, LLC, China. The purity of all of them is higher than 98%. SDS, K₂PtCl₄, Pyrene, and glutaraldehyde (GA, 50 wt.% solution in water) were all obtained from solarbio, Shanghai. All polypeptides used in this study are linear polymers where each amino acid residue participates in two peptide bonds and is linked to its neighbours in a head-to-tail fashion rather than forming branched chains.

2. Self-assembly of disc-like structures of NH₂-(α,L-lysine)₅-COOH, and SDS in aqueous solution

500 μL of NH₂-(α,L-lysine)₅-COOH (0.8 mM) was mixed with equal volume of SDS at a NH₂-(α,L-lysine)₅-COOH/SDS molar ratio of 1 to 10, and resulting mixture was allowed to stand for 3 days. The supernatant was collected, followed by analyses by TEM, AFM, and LSCM.

3. Effect of the NH₂-(α,L-lysine)₅-COOH/SDS ratio on the formation of the disc-like structures

Different volumes (48–112 μL) of SDS (50 mM) were mixed with NH₂-(α,L-lysine)₅-COOH solution in ddH₂O to make a NH₂-(α,L-lysine)₅-COOH/SDS ratio of 1/6, 1/10 and 1/14 in eppendorf tubules, respectively. The reaction mixture was allowed to stand for 10 h at room temperature ~25 °C. Then, the supernatant was collected for TEM analyses.

4. Kinetics of the formation of the disc-like structures

4.5 mL of NH₂-(α,L-lysine)₅-COOH (0.8 mM) was mixed with equal volume of SDS at a NH₂-(α,L-lysine)₅-COOH/SDS molar ratio of 1 to 10, and resulting mixture was divided into nine equal
parts. Then, a cultivation time of 0, 1 h, 2 h, 5 h, 10 h, 24 h, and 3 days at 25 °C was, respectively, conducted to observe different morphologies assembled from NH$_2$-(α,L-lysine)$_5$-COOH and SDS. After different cultivation times, the supernatants of these nine parts were observed by TEM.

5. Effect of pH on the morphology of self-assembly of NH$_2$-(α,L-lysine)$_5$-COOH and SDS

1.5 mL of NH$_2$-(α,L-lysine)$_5$-COOH (0.8 mM) was incubated with equal volume of SDS at a NH$_2$-(α,L-lysine)$_5$-COOH/SDS molar ratio of 1/10, and resulting mixture was divided into three equal parts. The pH of the first part was adjusted to pH 5.0 by adding NaOH solution to the mixture, while adjusting the pH of the second and the third to 7.0 and 11.0 with NaOH solution using the same method, respectively. Resulting mixture was allowed to stand for 3 days. The supernatants of these three mixtures were observed by TEM.

6. Transmission Electron Microscopy (TEM) analyses

Transmission electron microscope (TEM) experiments were carried out as following: Liquid samples were directly placed on carbon-coated copper grids and excess solution removed with filter paper, and then were stained using 2% uranyl acetate for 2 min. Transmission electron micrographs were imaged at 80 kV through a Hitachi H-7650 scanning electron microscope.

7. Atomic Force Microscope (AFM) analyses

An aliquot (5 μL) of the sample solution (0.4 mM in ddH$_2$O, prepared as described in the text) was pipetted on to freshly cleaved mica and dried at room temperature before imaging. The AFM images were collected in tapping mode using a Nanoman VS equipped with a 20 μm × 20 μm scanner. The scanning speed was at a line frequency of 1 Hz. The images were saved at a resolution of 512 × 512 points.
8. Laser Scanning Confocal Microscopy

Laser scanning confocal microscopy of the supernatant of a mixture of NH$_2$-(α,L-lysine)$_5$-COOH and SDS in H$_2$O (prepared as described in the text) was performed on a Leica TCS SP5 Confocal Microscope equipped with a 488/340 nm Ar laser line (30 mW) for the dye excitation. The sample (200 μL) was mixed with a cationic DAPI and an anionic FITC hydrophilic fluorescent dyes, respectively. The sample was allowed to stand for 2 hours to allow the dye to absorb into the disc-like assemblies prior to imaging. Imaging of an xy plane with an optical slice of 5 μm clearly showed that the assemblies of NH$_2$-(α,L-lysine)$_5$-COOH and SDS were disc-like.

9. Circular Dichroism (CD)

Measurements were performed on a Pistor π-180 CD spectropolarimeter using a quartz cell with the 1 mm path length at 25 °C. After poly(alpha, L-lysine) with polymerization degree of 5 (NH$_2$-(α,L-lysine)$_5$-COOH) was mixed with SDS at a ratio of 1 to 10 at different pH values (5.0, 7.0, and 11.0), respectively, resulting solutions were allowed to stand for 3 days. Then precipitates were removed by centrifugation, and resulting supernatant was used for CD measurements with NH$_2$-(α,L-lysine)$_5$-COOH in H$_2$O as control. Each spectrum was an average of ten scans from 190 nm to 260 nm at a bandwidth of 1 nm.

10. Isothermal Titration Calorimetry (ITC)

ITC experiments were carried out at 25 °C on the Nano-ITC II Instruments (TA Instrument). Prior to analyses, NH$_2$-(α,L-lysine)$_5$-COOH and acetyl NH$_2$-(α,L-lysine)$_5$-COOH were dissolved in 50 mM Mops buffer at pH 7.03, respectively. The SDS was also dissolved in the same above buffer. Before each titration, all solutions were degassed thoroughly under vacuum with stirring. The solution in the sample cell was stirred at 150 rpm to ensure rapid mixing of the titrant upon injection. Titrations were
performed by an automated sequence of 24 injections, each of 2 μL NH₂-(α,L-lysine)₅-COOH/acetyl NH₂-(α,L-lysine)₅-COOH (4.5 mM) titrate into the sample cell containing 190 μL of SDS (3 mM), spaced at 300 s intervals to ensure complete equilibration. A background titration, consisting of the same titrant solution but only the buffer solution in the sample cell, was subtracted from each experimental titration to account for heat of dilution. Three titrations were carried out for each measurement, and the reported experimental values are the average of individual best-fit values, fitting with independent binding model in the Nano Analyze software provided by TA Instruments. Raw data were processed using the Origin 8.0 software.

11. X-ray powder diffraction experiments

X-ray powder diffraction was performed using a D/Max 2500 VB2+/PC. The X-ray generator was set to an acceleration voltage of 40 kV and a filament emission of 50 mA. Samples were scanned between 1° (2θ) and 10° (2θ) using a step size of 0.002° (2θ) and a count time of 0.12 s. The compact powdered samples (the disc-like assemblies formed by NH₂-(α,L-lysine)₅-COOH plus SDS and SDS alone powders) were placed directly on a flat vitreous sample holder and measured directly within this apparatus.

12. Preparation of platinum nanodiscs by using NH₂-(α,L-lysine)₅-COOH/SDS nanodiscs as templates

In a typical synthesis, 10 μL of 20 mM K₂PtCl₄ (pH 5.0) which was aged for one day was added to 1 mL of a solution containing NH₂-(α,L-lysine)₅-COOH (0.4 mM) and SDS (4.0 mM) in distilled water. Resultant solution was adjusted to pH 5.0, followed by standing at room temperature (25 °C) for 3 days. After precipitate was removed, all supernatant was collected, to which 20 μL of 50% glutaraldehyde (pH 5.0) was added under stirring. Resultant solution was allowed to stand for 12 h. To this solution
was added 50 μL of L-ascorbic acid solution (13.2 mg) in distilled water (pH 5.0) at 25 °C. After 2 h, 0.5 mL of the same K₂PtCl₄ aqueous solution (pH 5.0) was mixed with the above resultant solution, followed by standing 2 h. Finally, the pH value of resultant solution was adjusted to 5.0 or 11.0 for TEM analyses.

13. Preparation of platinum wires by using NH₂-(α, L-lysine)₅-COOH/SDS nanodiscs as templatees

Typically, 10 μL of aged K₂PtCl₄ (20 mM, pH 11.0) was added to 1 mL of a solution containing NH₂-(α, L-lysine)₅-COOH (0.4 mM) and SDS (4.0 mM) in distilled water. Resultant solution was adjusted to pH 11.0, followed by standing at room temperature (25 °C) for 3 days. To this solution was added 50 μL of L-ascorbic acid solution (13.2 mg) in distilled water (pH 11.0) at 25 °C. After 2 h, 0.5 mL of the same K₂PtCl₄ aqueous solution (pH 11.0) was added to the above resultant solution. After 2 h, the pH value of resultant solution was adjusted to 11.0 for TEM analyses.

14. SDS cmc measurements in the absence and presence of NH₂-(α, L-lysine)₅-COOH by spectroscopic analysis

Pyrene-saturated aqueous solutions were prepared as previously described. Typically, 2.5 mg of pyrene was added into 500 mL of ddH₂O. After this solution was stirred for more than 24 h at room temperature in the dark, insoluble pyrene was removed by filtration. Increasing amounts of SDS/pyrene-saturated solution were mixed with 15 mM NH₂-(α, L-lysine)₅-COOH/pyrene-saturated solutions (53.4 μL) in eppendorf tubules. Resultant solution was adjusted to pH 5.0 and 11.0, respectively, followed by standing at room temperature in the dark for 10 min before measurements. The pyrene concentration was ca. 1.0×10⁻⁶ M in all samples for analysis. The fluorescence measurements were carried out on a Cary Eclipse spectrofluorimeter (Varian Palo Alto, USA). For
experiments with pyrene, slit widths were set at 10 nm (excitation) and 10 nm (emission), and the excitation wavelength was 332 nm. The $I_1/I_3$ ratio was calculated as the ratio of peak I (374 nm) and peak III (385 nm) in the vibration fine structure of pyrene monomer emission.

15. The supposed formation process of disc-like assemblies

The growth of the nanobelt structures (Fig. 4B), which were formed at the early phases of disc-like assemblies, along each dimension is obviously regulated by different forces. One dimensionality is clearly linked to fast growth along the direction of the hydrophobic interactions with respect to a much slower lateral adhesion among them. A single nucleation event followed by uniform growth in the fast and slow directions, in analogy to living polymerization, is a possible explanation. Lateral growth of the nanobelt should depend mainly on electrostatic interactions between different molecules because one NH$_2$-(α,L-lysine)$_5$-COOH-SDS$_5$ complex has two hydrophilic heads, α-COO$^-$ and α-NH$_3^+$. Agreeing with this view, ITC results show that, similar to NH$_2$-(α,L-lysine)$_5$-COOH, acetyl NH$_2$-(α,L-lysine)$_5$-COOH (where α-NH$_3^+$ of NH$_2$-(α,L-lysine)$_5$-COOH was acetylated) also can bind up to five of SDS molecules with a comparable binding constant as (2.76 ± 0.12) × 10$^4$ M$^{-1}$ (Fig. S4C and D), but no disc-like nanostructure was observed by TEM under the same conditions. Therefore, such acetylation could eliminate the electrostatic interactions between different NH$_2$-(α,L-lysine)$_5$-COOH -SDS$_5$ complexes, thereby preventing lateral growth of the nanobelt. Further support for this idea comes from ITC results showing that the value of $\Delta H^\circ$ (-12.95 ± 0.08 KJ/mol) from the reaction of acetyl NH$_2$-(α,L-lysine)$_5$-COOH and SDS is only one fourth of that from NH$_2$-(α,L-lysine)$_5$-COOH and SDS (-46.38 ± 1.22 KJ/mol) (Table S1, and Fig. S4), suggesting that there is no polymerization reaction occurring in the solution except for the electrostatic interaction between SDS and the side chains of acetyl NH$_2$-(α,L-lysine)$_5$-COOH.
Supplementary Figures (Figs. S1-8) and Table S1

Fig. S1 Effect of the ratio of SDS to NH$_2$-(α,L-lysine)$_5$-COOH on the shape of assemblies of NH$_2$-(α,L-lysine)$_5$-COOH and SDS as revealed by TEM. (A) The NH$_2$-(α,L-lysine)$_5$-COOH/SDS ratio of 1/6. (B) The NH$_2$-(α,L-lysine)$_5$-COOH/SDS ratio of 1/10. (C) The NH$_2$-(α,L-lysine)$_5$-COOH/SDS ratio of 1/14. Upon incubation of NH$_2$-(α,L-lysine)$_5$-COOH with SDS at different ratios for 10 h, respectively, all the samples were negatively stained with 2% (w/v) uranyl acetate aqueous solution, followed by TEM analyses. Conditions: [NH$_2$-(α,L-lysine)$_5$-COOH] = 0.4 mM, in H$_2$O, 25 °C.
Fig. S2 TEM images of disc-like structures formed upon mixing NH$_2$-(α-L-lysine)$_5$-COOH with SDS at a ratio of 1 to 10 in different absolute concentrations. (A) The concentration of NH$_2$-(α,L-lysine)$_5$-COOH and SDS were 0.1 and 1.0 mM, respectively. (B) The concentration of NH$_2$-(α,L-lysine)$_5$-COOH and SDS were 0.2 and 2.0 mM, respectively.
Fig. S3 Plots of $I_1/I_3$ of pyrene (ca. 1 µM) solubilized in water (■), and 0.4 mM NH$_2$-(α,L-lysine)$_5$-COOH (● pH 5.0, ▲ pH 11.0) solutions as a function of SDS concentration at 25 °C.
Fig. S4 Calorimetric titration of SDS with NH$_2$-(a,L-lysine)$_5$-COOH or acetyl NH$_2$-(a,L-lysine)$_5$-COOH. (A) Raw data of SDS and NH$_2$-(a,L-lysine)$_5$-COOH. (B) A titration plot of the integrated heat versus the NH$_2$-(a,L-lysine)$_5$-COOH /SDS molar ratio. (C) Raw data of SDS and acetyl NH$_2$-(a,L-lysine)$_5$-COOH. (D) A titration plot of the integrated heat versus the acetyl NH$_2$-(a,L-lysine)$_5$-COOH /SDS molar ratio. (E) Schematic representation of the structure of NH$_2$-(a,L-lysine)$_5$-COOH-SDS$_5$ complex.
**Fig. S5** Characterization of the secondary structure of self-assemblies of NH$_2$-(a,L-lysine)$_5$-COOH and SDS at different pH values.
**Fig. S6** Small-angle X-ray diffractometer traces of both the disc-like assemblies formed by NH$_2$-($\alpha$-L-lysine)$_5$-COOH plus SDS (curve b) and SDS alone (curve a) powders. The powder of the disc-like assemblies were prepared by mixing NH$_2$-($\alpha$-L-lysine)$_5$-COOH with SDS at a ratio of 1 to 10 in H$_2$O for 3 days, and resulting supernatant was collected, followed by lyophilization.
Fig. S7 Schematic representation of the structure of nanobelts assembled from NH$_2$-(a$_5$L-lysine)$_5$-COOH and SDS as shown in Figure 4B. Nanobelts are intermediates for the formation of the disc-like assemblies.
Fig. S8 TEM images of the platinum discs (A, B) and nanowires (E, F). (C) Energy dispersive X-ray detection of the platinum disc as marked in (a) and (b) of Fig. 6B. (D) No platinum disc was found with either aged $\text{K}_2\text{PtCl}_4$ + ascorbic acid or the $\text{NH}_2$-($\alpha,\text{L-lysine})_5$-COOH/SDS solution + aged $\text{K}_2\text{PtCl}_4$ + ascorbic acid in the absence of glutaraldehyde at final pH 5.0 or 11.0. The platinum discs and nanowires were observed by TEM without uranyl.
Table S1. Best fit parameters for ITC measurements of SDS binding to NH$_2$-(α,L-lysine)$_5$-COOH or acetyl NH$_2$-(α,L-lysine)$_5$-COOH in 50 mM Mops buffer, pH 7.0, 25 °C.\textsuperscript{a}

<table>
<thead>
<tr>
<th>sample</th>
<th>$K$ (M\textsuperscript{-1})</th>
<th>$\Delta H$ (kJ/mol)</th>
<th>$\Delta G$ (kJ/mol)</th>
<th>$\Delta S$ (J/mol K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH$_2$-(α,L-lysine)$_5$-COOH</td>
<td>(7.09±0.15) $\times 10^4$</td>
<td>-46.38±1.22</td>
<td>-27.69±0.05</td>
<td>-62.69±4.26</td>
</tr>
<tr>
<td>Acetyl NH$_2$-(α,L-lysine)$_5$-COOH</td>
<td>(2.76±0.12) $\times 10^4$</td>
<td>-12.95±0.78</td>
<td>-25.35±0.10</td>
<td>41.58±2.95</td>
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\textsuperscript{a}The reported thermodynamic quantities are apparent values. Standards errors from replicate determinations are indicated.