Supporting Information

Polypseudorotaxane Constructed from Cationic Polymer with Cucurbit[7]uril for Controlled Antibacterial Activity

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Catalogue

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SI-1 Materials and methods

Materials. All the materials as following were of analytical purity grade and used without further purification and all of them were obtained from commercial suppliers: ε-poly-L-lysine hydrochlorides, mono potassium phosphate, hydrochloric acid, sodium hydroxide, amantadine and deuterium oxide (D, 99.8%). Cucurbit[7]uril (CB[7]) was purchased from Dr. Anthony Day. Water was obtained from a Milli-Q Integral Water Purification System (18.2 MΩ cm). The phosphorous buffer was prepared by mixing mono potassium phosphate and water then adjusted to expected pH by hydrochloric acid or sodium hydroxide.

Isothermal Titration Calorimetry (ITC). ITC experiments were carried out with a Microcal VP-ITC apparatus at 298.15 K. All the sample solutions for titration were prepared in 20 mM phosphorous buffer. In a typical titration experiment, the host molecule (CB[7]) was in the injection syringe at a concentration of 1.0 mM, while the ε-poly-L-lysine hydrochlorides (L₃) was in the sample cell at a concentration in the range of 0.10 mM. All the titration schedules consisted of 1 injection of 5 µL for eliminating initial errors and 28 consecutive injections of 10 µL with a 300 s interval between injections. All solutions were degassed prior to titration. The titration curves were fitted by Origin 7.0 using the one set of site binding model in order to determine the macroscopic enthalpy change, entropy change and stepwise association constant.

Nuclear Magnetic Resonance (NMR) Spectroscopy. $^1$H NMR spectra were acquired in deuterium oxide at 25.0 °C and recorded on a JOEL JNM-ECA400 apparatus (400 MHz). The concentrations of all solutions were prepared to be 1.0 - 5.0 mM by PBS D$_2$O solution in different cases.

Antibacterial experiments on agar plates. A single colony of E. coli on a solid Luria-Bertani(LB) agar plate was transferred to 10 mL of liquid LB culture medium and was grown at 37.0 °C for 6 hours. Bacteria were harvested by centrifuging at the speed of 7100 rpm for 2 min, and then were washed with phosphate buffer (PBS, 20 mM, pH = 6.0) for two times. The obtained E. coli were resuspended in PBS, and then diluted to an optical density of 1.0 at 600 nm by PBS (OD600 = 1.0). The concentration of E. coli is equal to 5.0 x 10$^8$ colony-forming unit per milliliter (CFU/ml). For tuning the antibacterial activity, E. coli in the concentration of 1.0 x 10$^6$ CFU/ml obtained by dilution of the previous solution was incubated with 50 µM L₃ complexed with different ratios of CB[7] at 37.0 °C for 30 min, respectively. Then, the E. coli suspensions were all diluted 1 x10$^2$ fold by PBS. A 100 µL portion of each solution was spread on the solid LB agar plate. Every suspension was spread on three same solid LB agar plates as repeated trials. After 16 h incubation, the bacterial colonies were formed at 37.0 °C. The bacterial colony-forming units (N) were counted and the inhibition rates (IR) were calculated according to the following equation: IR = (N$_{control}$ - N)/N$_{control}$ x 100%. All experiments were repeated for two times.

Dynamic Light Scattering (DLS). DLS experiments were carried out by Malvern 3000HS Zetasizer with a monochromatic coherent He–Ne laser (633 nm). The concentrations of all solutions were prepared to be 3.0 mM by PBS D$_2$O solution in different cases. Before usage, all solutions were filtered by 450 nm PES filter membrane.
**Asymmetric Flow Field Flow Fractionation (As4F)**. As4F experiments were performed by Wyatt Technology Eclipse 3+ with multi-angle light scattering detector (DAWN HELEOS-II), Ultraviolet and Differential Refraction detector (Optilab rEX). The details of As4F experimental conditions are described as following: the detection flow was 0.75 mL/min; the cross flow was 1.0 mL/min; the inject flow was 0.2 mL/min. The separation membrane was made of polyether sulfone (5kDa) and the carrier was the PBS buffer (20 mM, pH = 6.0) filtered by a water membrane (220 nm).

**SI-2 Characterization of ε-poly-L-lysine hydrochlorides by $^1$H NMR**

![1H NMR spectrum](image)

**Figure S1.** $^1$H NMR spectrum of 5.0 mM ε-poly-L-lysine hydrochlorides in PBS D$_2$O buffer of pH = 6.0 at 25.0 °C.

By end group analysis, the polymerization degree of ε-poly-L-lysine hydrochlorides was calculated to be around 30. There was few ethanol (EtOH) in this polymer. The ratio of EtOH in the polymer sample was 0.0093 wt.%, which was quite low and would not influence the following experiments.
SI-3 Concentration effect to influence the association extent

**Figure S2.** Relationship between association extents of $L_3$-$n$CB[7] and concentration in different ratios ($n$) calculated by equilibrium equation in PBS buffer of pH = 6.0 at 25.0 °C. ([$L_3$] = 50.0 µM in all cases)

Equilibrium:


\[ c \quad nc \]

\[ c-x \quad nc-x \quad x \]

\[ K_a = \frac{x}{(c-x)(nc-x)} = 2.51 \times 10^6 \text{ M}^{-1} \]

\[ c = 5 \times 10^{-5} \text{ M}^{-1} \]

Then, x can be obtained by inputting different n.

Association extent = $x/c \times 100\%$

As shown in Figure S2, association extents of $L_3$-$n$CB[7] could be obtained. By inputting n as 0.7, the association extent was calculated to be 58.65 %. The complexed component was about 18 repeating units while the free component was about 12 repeating units. Therefore, the minimum length for high antibacterial activity is around 12 repeating units.
SI-4 Characterization of competitive complexations by $^1$H NMR

**Figure S3.** $^1$H NMR spectra of competitive complexations of L$_3$-CB[7] with amantadine in PBS D$_2$O buffer of pH 6.0 at 25.0 °C. ([L$_3$] = 50.0 µM in all cases)

As shown as above, amantadine (ADA) can act as a competing agent to replace L$_3$ from L$_3$-CB[7]. By gradually adding ADA into L$_3$-CB[7], the peaks of protons on the L$_3$ returned to its original form (right), indicating that L$_3$ were released. Besides, the peaks of protons on the CB[7] were clearly changed to be much sharper peaks indicating the formation of the host-guest complex of ADA-CB[7]. Therefore, these results confirmed that the binding between L$_3$ and CB[7] is dynamic and reversible.

SI-5 pH effect to influence the binding affinity of L$_3$-CB[7] characterized by ITC

**Figure S4.** Stoichiometric numbers and binding constants of the host-guest complexation between L$_3$ and CB[7] in different pH conditions in PBS buffer at 25.0 °C.

As shown as above, pH condition might influence on the complexation of L$_3$-CB[7]. When
pH was lower than 6.0, the stoichiometric numbers and binding constants were almost the same. When pH was higher than 6.0, the stoichiometric numbers and binding constants were decreased. Because higher pH conditions may lead to the partial deprotonation of $L_3$. Since positive charges can enhance the binding, the binding affinity would decrease in higher pH conditions. Therefore, in order to guarantee the complexation, pH 6.0 is the appropriate condition in all experiments.