Supporting Information

Peptide Amphiphiles with Distinct Supramolecular Nanostructures for Controlled Antibacterial Activities

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Experimental Section

Materials

Gemini-type peptide amphiphiles N^{α}-dodecanoyl-lysine-lysine-O^{α}-dodecylamide 12-$(Lys)_2$-12, N^{α}-dodecanoyl-lysine-lysine-lysine-lysine-O^{α}-dodecylamide 12-$(Lys)_4$-12, and N^{α}-dodecanoyl-lysine-lysine-lysine-lysine-lysine-lysine-O^{α}-dodecylamide 12-$(Lys)_6$-12 were synthesized through solid phase method and purified by high-performance liquid chromatography. Their structures were confirmed by ^{1}H NMR and mass spectra, and the purities are higher than 95% as checked by high performance liquid chromatography. Phosphate buffered saline (1× PBS, pH 7.4) was used throughout the work.

Methods

Critical aggregate concentration (CAC) determination: The CAC of each PA was determined from surface tension measurements on a DCAT 11 surface tensiometer (DataPhysics). Stock Solution of PA solution was progressively adding into PBS and examined by a Pt/Ir plate method at 25.00 ± 0.01 °C. The CAC value was calculated by plotting the surface tension against the log of PA concentration. The measurements were repeated at least twice.

Dynamic light scattering (DLS) measurements: Size distributions of the aggregates formed by these PAs in PBS were measured by a Malvern Zetasizer Nano-ZS instrument equipped with a 4 mW He−Ne laser (λ = 632.8 nm). The temperature was controlled at 25.0 ± 0.1 °C using a thermostated chamber.

Cryogenic transmission electron microscopy (Cryo-TEM): Samples for Cryo-TEM were prepared by pipetting 5 μL of PA solutions onto a carbon-coated holey TEM grid, blotted and plunged into liquid ethane. The vitrified samples were kept in -180 °C and imaged with a JEM-2011 TEM (120 kV). Digital images were recorded in the minimal electron dose mode by a Gatan multi-scan cooled charge-coupled device (CCD) camera.

Preparation of *E. coli* solutions: A single colony of Amp^r *E. coli* on a solid Luria Broth (LB) agar plate was transferred to 10 mL liquid LB culture medium with 50 μg/mL ampicillin and then was grown for 6 − 8 h at 37 °C under constant shaking of 180 rpm. *E. coli* was harvested by centrifuging (7100 rpm for 2 min) and was washed with PBS twice. After removal of the supernatant, the remaining *E. coli* was suspended in PBS and diluted to the optical density of 1.0 at 600 nm (OD_{600} = 1.0).

Assessment of antibacterial activity: The antimicrobial activity of PAs to *E. coli* was evaluated using a traditional surface plating method.[1] Certain concentrations of PAs were separately added into *E. coli* solution and the mixtures were incubated for 30 min at 37 °C. Next, the *E. coli* suspensions were serially diluted 10^4-fold with PBS. 100 μL of the diluted *E. coli* was spread on the solid agar plate (LB) with 50 μg/mL ampicillin, and *E. coli* colonies were counted after incubation for 14 − 16 h at 37 °C. The inhibition ratio (IR) was calculated according to the equation[2]

$$\text{IR}\ (\%) = \frac{(\text{A} - \text{B})}{\text{B}} \times 100$$

Where A is the mean number of *E. coli* colonies in the control sample (without PAs), and B is the mean number of *E. coli* after treated with the PAs. The diameter of the solid agar plates was 90 mm. The results were repeated three times.

Scanning electron microscopy (SEM): The morphological changes of *E. coli* before and after the addition of different PAs were observed by SEM. After incubation of *E. coli* with PAs for 30 min, the mixture was centrifuged (7100 rpm for 5 min). The supernatant was removed, and the *E. coli* pellets were resuspended in sterile water. 2 μL of *E. coli* suspensions were dropped onto clean silicon slices, followed by naturally drying in the super clean bench. After the specimens became dried, 0.1% glutaraldehyde was added to fixate for 1 h and then 0.5% glutaraldehyde for another 1 h. Next, the specimens were washed with sterile water three times, dehydrated by adding ethanol in a graded series (50% for 6 min, 70% for 6 min, 90% for 6 min, and 100% for 6 min), and then dried in a vacuum drying oven. Finally, the specimens were coated with platinum before SEM observation (Hitachi S4800).

Confocal laser scanning microscopy (CLSM) characterization of membrane damage: After treatment of *E. coli* with PAs for 30 min, the mixtures were centrifuged to obtain the *E. coli* pellets. The *E. coli* pellets ware then stained by two fluorescent nucleic acid stains: SYTO9 and propidium iodide according to the procedure. The samples were then examined by CLSM using a 488 nm laser for SYTO9 and 559 nm for PI.

Zeta potential measurements: *E. coli* was incubated by PAs of different concentrations at 37 ℃ for 30 min. Unbound PAs then were removed by centrifugation (7100 rpm, 5 min) at 4 ℃. The pellets obtained were suspended in water and the suspensions were placed on ice for zeta potential measurements. Untreated *E. coli* (without PAs) was also incubated under the same conditions as the control.

Isothermal titration microcalorimetry (ITC): The calorimetric measurements were conducted on a TAM III microcalorimeter system with a stainless steel sample cell of 1 mL at 25.00 ± 0.01 ℃. The sample cells were initially loaded with 750 μL PBS or *E. coli* PBS solution, and then the PA solution (40 μM 12-$(Lys)_2$-12, 160 μM 12-$(Lys)_4$-12, and 120 μM 12-$(Lys)_6$-12, respectively) was injected consecutively into the stirred sample cell in portions of 10 μL via a 500 μL Hamilton syringe controlled by a 612 Thermometric Lund pump until the interaction progress was completed. The system was stirred at 60 rpm with a gold propeller. Each ITC curve was repeated at least twice with deviation within ± 4%. The dilution enthalpies of the PAs were subtracted from the corresponding observed enthalpy curve of the PAs with *E. coli*.

Cytotoxicity assay: HaCaT cells at a density of 6×10^3 cells/well were seeded into 96-well culture plates and were grown for 12 ~ 24 h until adherence in a humified atmosphere containing 5% CO_2 and at 37 ℃. Three PAs solutions of different concentrations were added into 96-well culture plates. After incubated for 24 h at 37 ℃, the supernatant was removed and 100 μL 0.5 mg/mL MTT was added, and then incubated for 4 h at 37 ℃. Subsequently, after removing the supernatant, 100 μL DMSO was added into well to dissolve the produced formazan. After shaking the plates for 2 min, absorbance values per wells at 520 nm were read by a microplate reader. The cell viability rate (VR) was calculated by the equation of

$$\text{VR}\ (\%) = \frac{\text{A}}{\text{A}_0} \times 100\%$$

Where A is the absorbance of the experimental groups with PAs and A_0 is the absorbance of the control group without PAs. Each assay was repeated six times.

MIC determination: The MIC values of PAs 12-$(Lys)_2$-12, 12-$(Lys)_4$-12 and 12-$(Lys)_6$-12 were evaluated according to their dose-responsive fitting curves. The corresponding function of fitting curves as follows (y is antibacterial activity of PA and x is the concentration of PA).

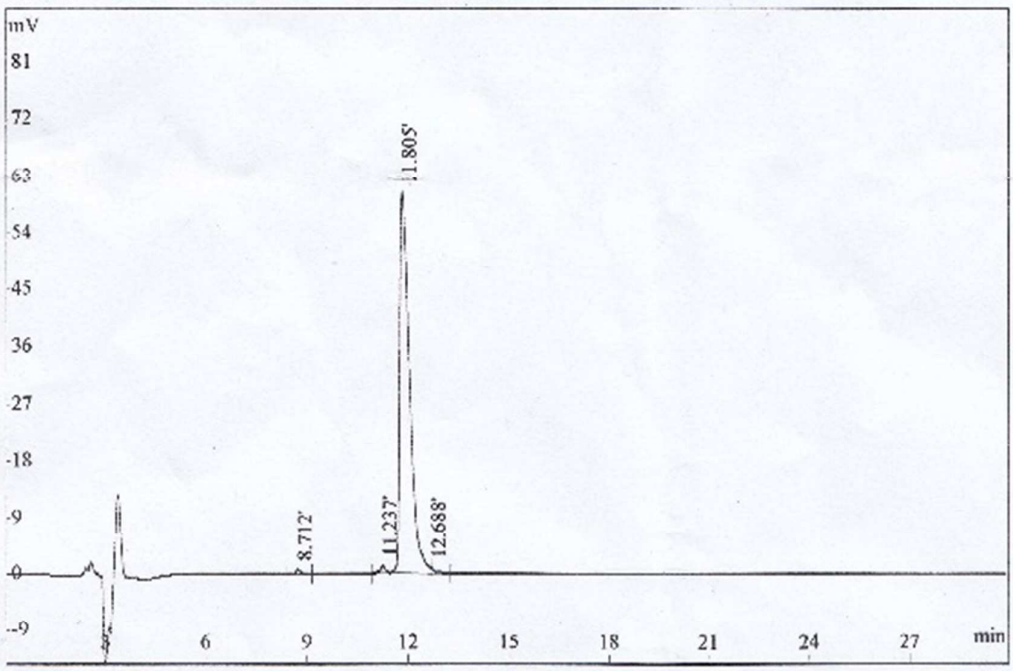
$$y_{12\text{-}(Lys)2\text{-}12} = 5.23584 + \frac{94.74909}{1+10^{6.16406(1.28047-\log x)}} \quad [1]$$

$$y_{12\text{-}(Lys)4\text{-}12} = 0.45742 + \frac{99.64515}{1+10^{5.00134(0.61743-\log x)}} \quad [2]$$

$$y_{12\text{-}(Lys)6\text{-}12} = -0.72658 + \frac{100.68176}{1+10^{2.58155(0.50435-\log x)}} \quad [3]$$

MIC is defined as the concentration where 90% bacteria were killed. Therefore, when y is equal to 90%, the corresponding x value is MIC of PA ($MIC_{12\text{-}(Lys)2\text{-}12}$ = 27.0 μM, $MIC_{12\text{-}(Lys)4\text{-}12}$ = 6.4 μM and $MIC_{12\text{-}(Lys)6\text{-}12}$ = 7.5 μM).

Supplementary Figures



Rank	Time	Conc.	Area	Height
1	8.712	0.7499	9454	818
2	11.237	1.208	15235	1136
3	11.805	97.03	1223229	60649
4	12.688	1.018	12835	924
Total		100	1260753	63527

Figure S1. High-performance liquid chromatography of 12-$(Lys)_2$-12.

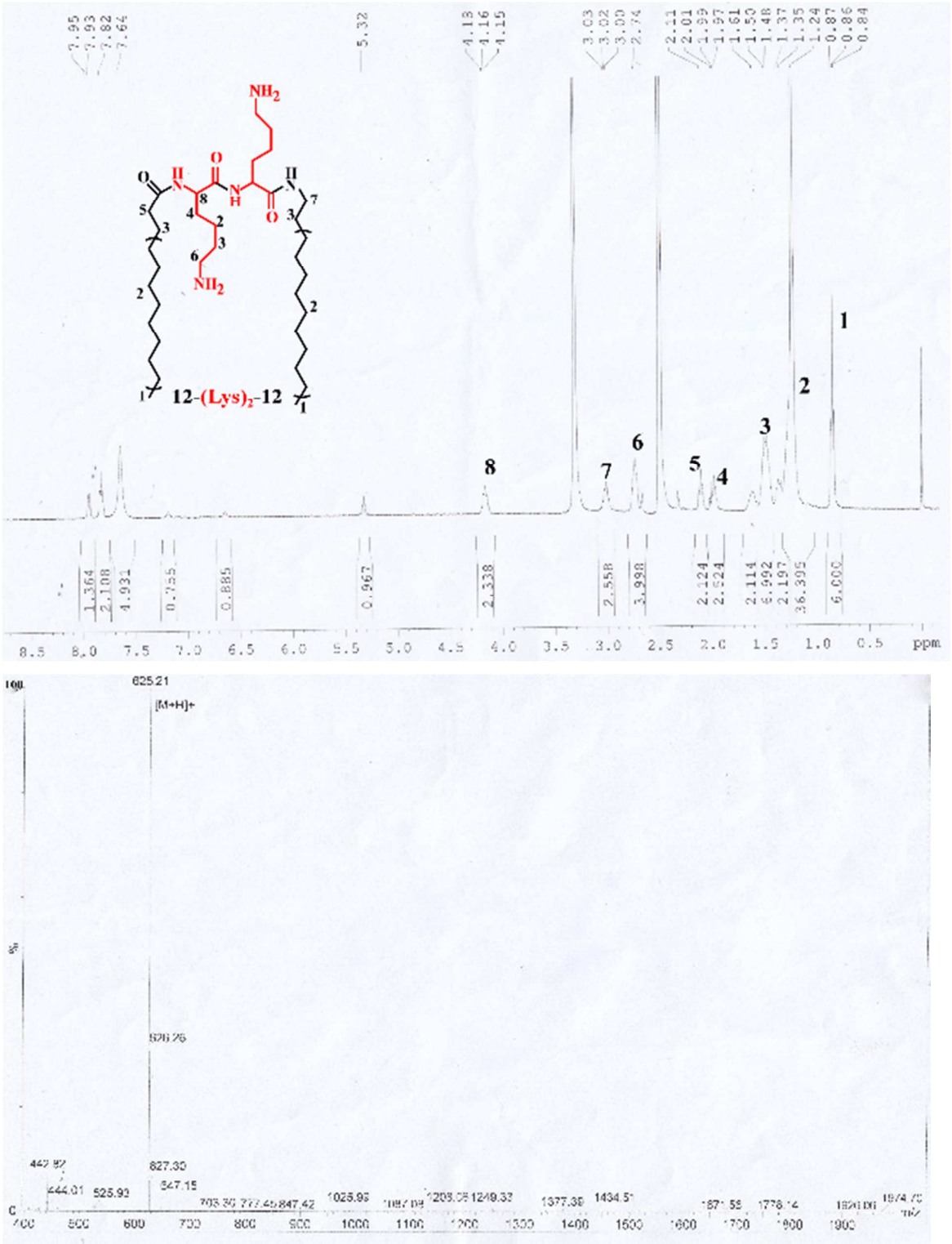
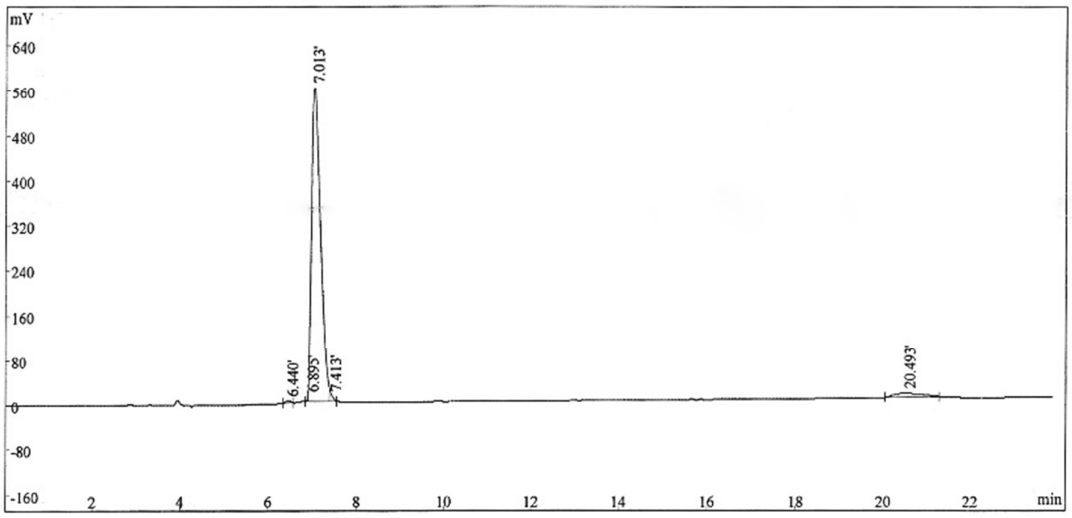


Figure S2. ^{1}H NMR and ESI mass spectrum of 12-(Lys)$_2$-12.



Rank	Time	Conc.	Area
1	6.440	0.2589	19847
2	6.895	0.1097	8411
3	7.013	95.3	7303330
4	7.413	0.6202	47531
5	20.493	3.716	284790
Total		100	7663909

Figure S3. High-performance liquid chromatography of 12-$(Lys)_4$-12.

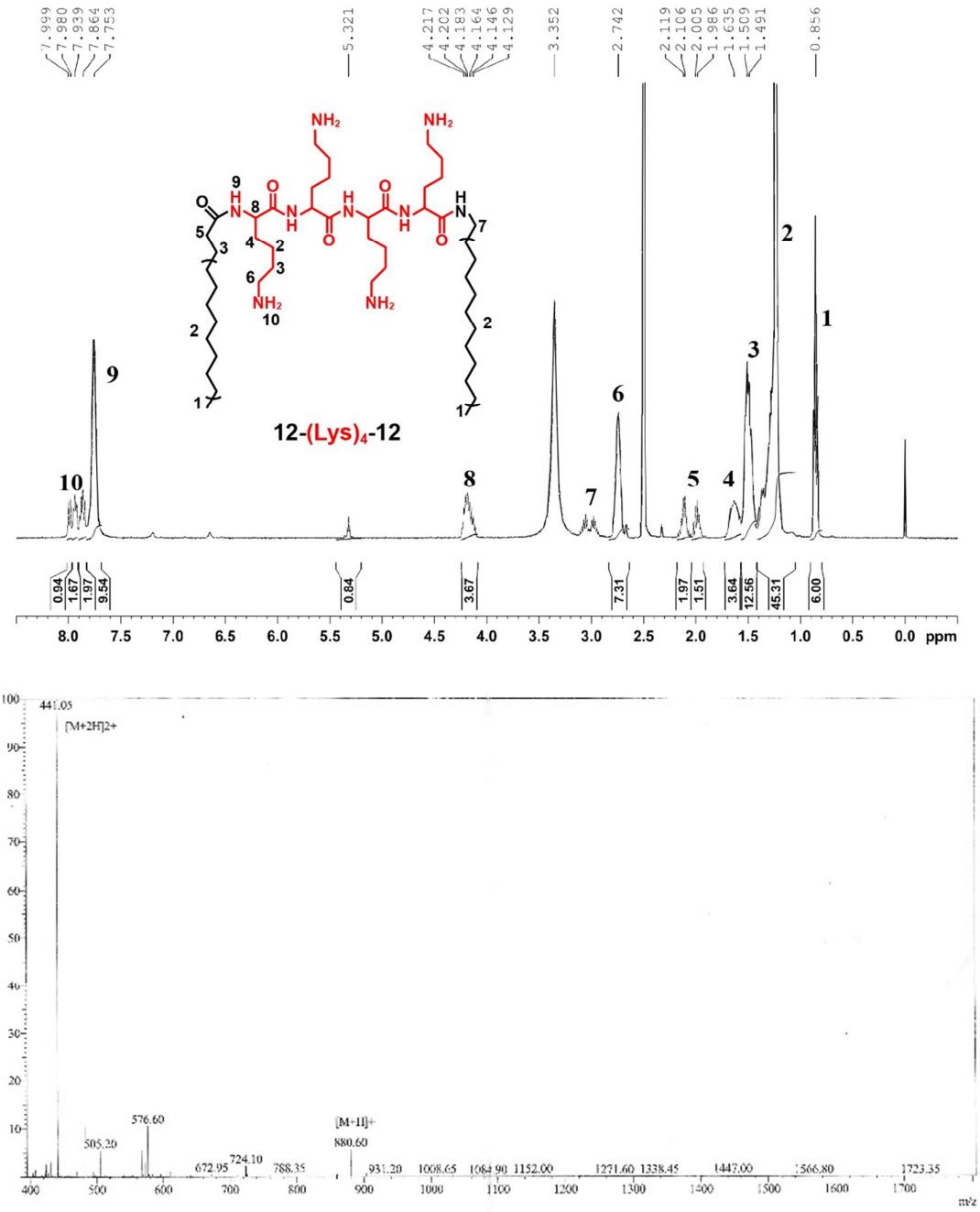
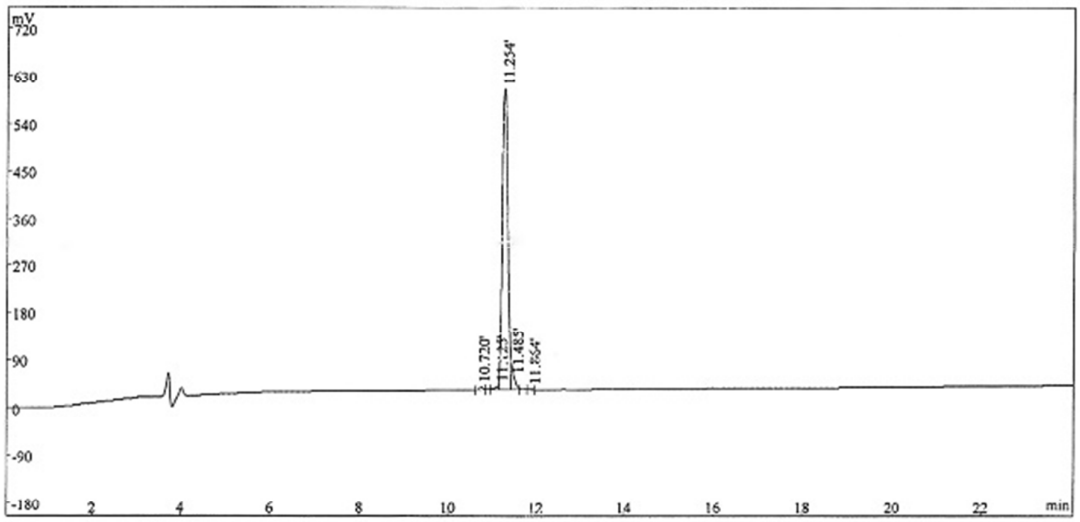


Figure S4. ^{1}H NMR and ESI mass spectrum of 12-(Lys)$_4$-12.



Rank	Time	Conc.	Area
1	10.720	0.6113	29469
2	11.125	0.9384	45235
3	11.254	95.32	4594829
4	11.485	2.981	143708
5	11.864	0.1503	7243
Total		100	4820484

Figure S5. High-performance liquid chromatography of 12-$(Lys)_6$-12.

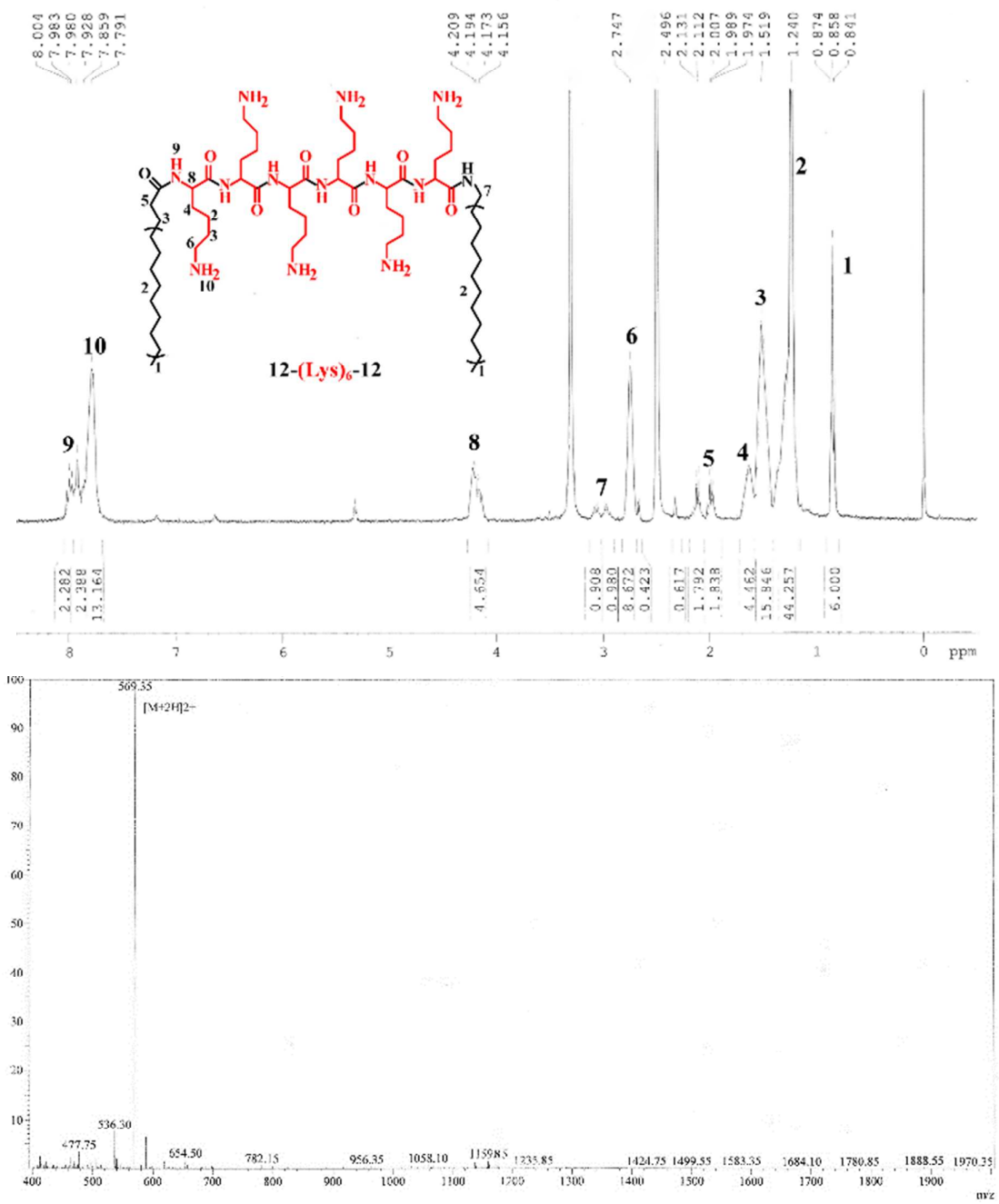


Figure S6. ^{1}H NMR of and ESI mass spectrum of 12-(Lys)$_6$-12.

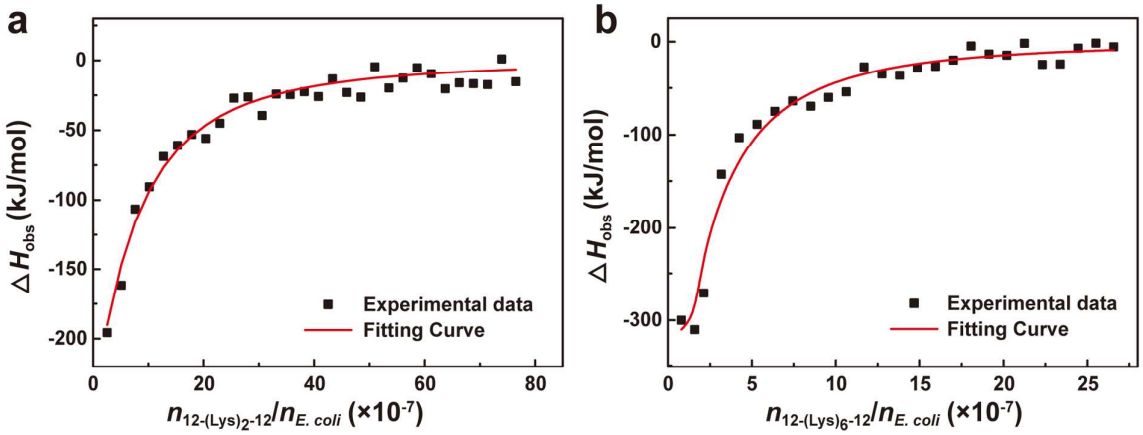


Figure S7. Observed enthalpy changes (ΔH_{obs}) against the PAs/*E. coli* molar ratios by (a) titrating 40 μM 12-$(Lys)_2$-12 into OD = 0.15 *E. coli* solutions and (b) titrating 120 μM 12-$(Lys)_6$-12 into OD = 1 *E. coli* solutions. The dilution enthalpy of the PAs has been subtracted.

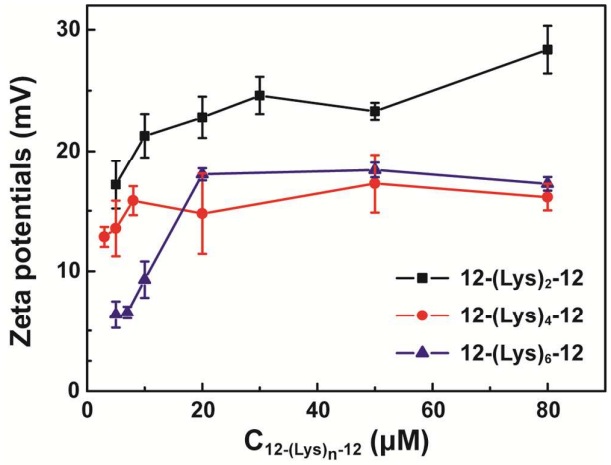


Figure S8. Zeta potential results of 12-$(Lys)_2$-12, 12-$(Lys)_4$-12, and 12-$(Lys)_6$-12 in PBS solutions at different concentrations.

References

[1] C. Xing, Q. Xu, H. Tang, L. Liu, S. Wang, *J. Am. Chem. Soc.* **2009**, *131*, 13117-13124.

[2] S. Chen, S. Chen, S. Jiang, M. Xiong, J. Luo, J. Tang, Z. Ge, *ACS Appl. Mater. Interfaces* **2011**, *3*, 1154-1162.