Supporting Information

Construction， mechanism and antibacterial resistance insight of the polypeptide-based nanoparticles

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Characterization

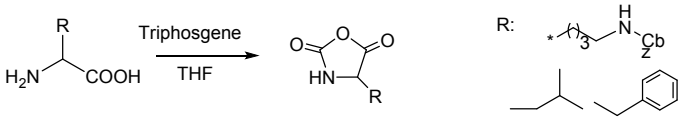
Proton Nuclear Magnetic Resonance (^{1}H NMR). The NMR spectra were recorded on a Bruker AV 400 MHz spectrometer, using tetramethylsilane as an internal standard and DMSO-d_6, $CDCl_3$, or D_2O as solvent.

DLS Study. Measurement of particle size Distribution Polymer Micellar solution was carried out by dynamic light scattering apparatus (DLS) on a Malvern Zetasizer Nano-ZS at a fixed scattering angle of 90°. The micellar solution was centrifuged for 10 min at 5000 rpm to remove the insoluble particles and measured with polymer concentration of 1mg/mL for sample preparation.

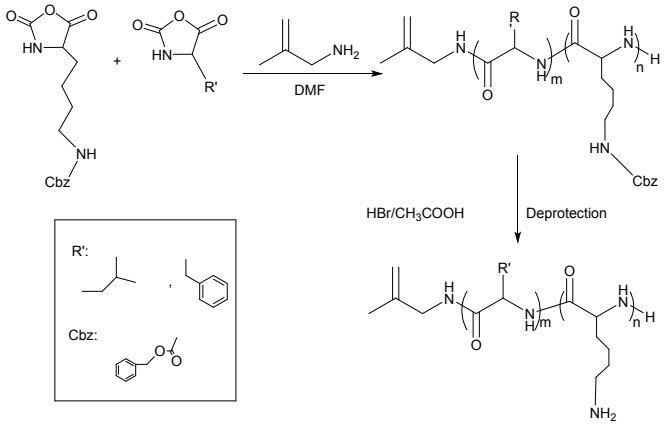
Scanning Electron Microscopy (SEM). The morphologies of micelle and bacteria were observed utilizing scanning electronic microscope (SEM). To obtain SEM images of p**2**, a drop of solution was spread on a silicon wafer and dried. For bacterial cells were incubated at 37℃ to the mid log growth phase (OD_{600}=0.4~0.6), and then treated with p**2** at 2×MIC for 3 h. The treated bacteria was collected, washed with PBS and fixed with 2.5% (v/v) glutaraldehyde for 2-4 h, then washed again with PBS and distilled water, respectively. After dehydrating by 30, 50, 70, 90 and 100% ethanol and then replacing with tertiary butyl alcohol, which was dripped on the silicon wafer and freeze-dried. Samples were treated with gold before observation.

GPC. The molecular weight and polydispersity index of polymers were determined on a Water 1515 gel permeation chromatography (GPC) instrument equipped with two Plgel 5 mm Mixed-D column. THF or DMF was used the eluent with a flow rate of 1 mL/min, and the polystyrene as standard was used for column calibration. The polymers was centrifuged and filtered prior to experiments.

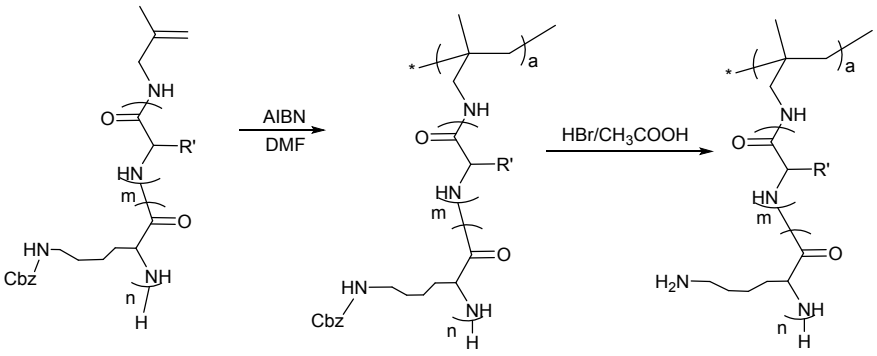
Zeta potential. Zeta potential studies was measured on a Water Nano-ZS 90 Nanosizer (Malvern Instrument) at a fixed scattering angle of 90° at room temperature.



Scheme S1. Synthetic routes of the monomer NCAs.



Scheme S2. Synthetic route of the liner polymers.



Scheme S3. Synthetic route of the pectinate polymers

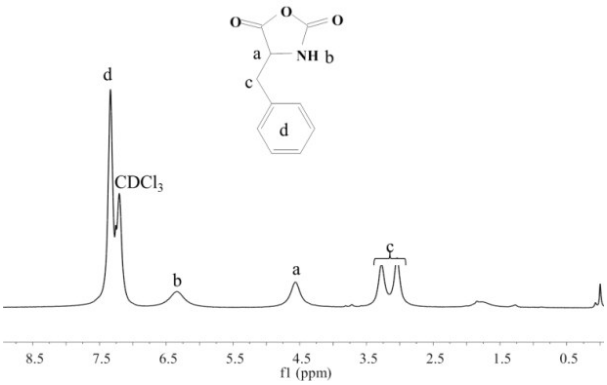


Figure S1. ^{1}H NMR spectrum of Phe-NCA in $CDCl_3$.

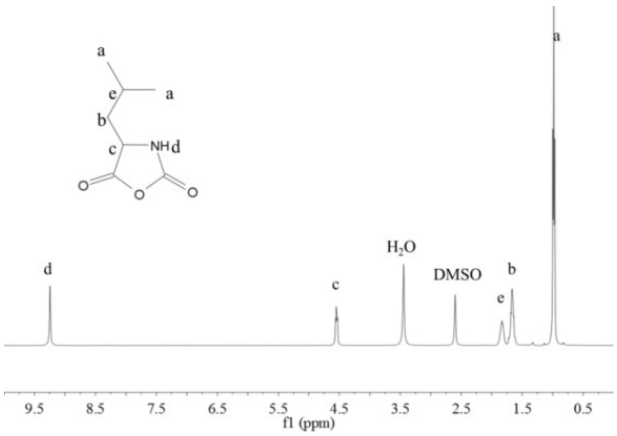


Figure S2. ^{1}H NMR spectrum of Leu-NCA in DMSO-d_6

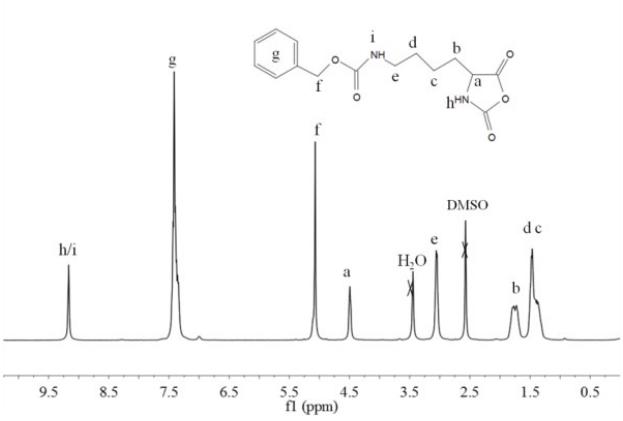


Figure S3. ^{1}H NMR spectrum of Z-Lys-NCA in DMSO-d_6.

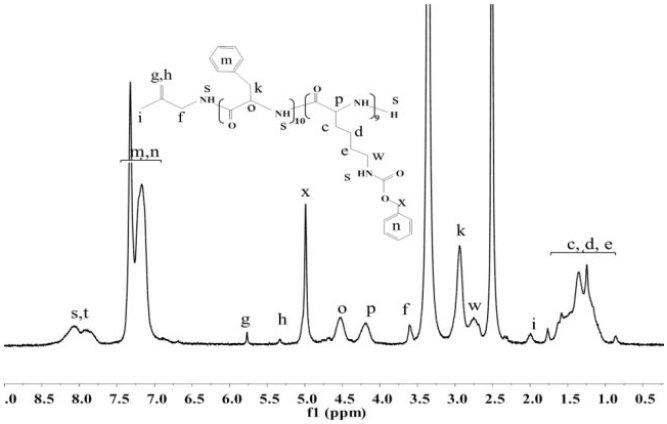


Figure S4. ^{1}H NMR spectrum of polymer 1 in DMSO-d_6

Determination of the composition of polymer 1:

Table S1. The integrals of different characteristic peaks

Spectrum	A(m+n)	A(g+h)	Ax
Figure S4	94.78	2	17.43

The integral area of peak was set to be 2 as the internal reference, corresponding to the amount of the alkenyl hydrogen of initiator. The DP of polymer was determined as the following:

$$\text{DP}=\frac{Am+n}{Ag+h}\times\frac{2}{5}=18.9\approx19$$

$$\text{DP of lysine}=\frac{Ax}{Ag+h}=8.71\approx9$$

DP of phenyalanine = 19-9=10

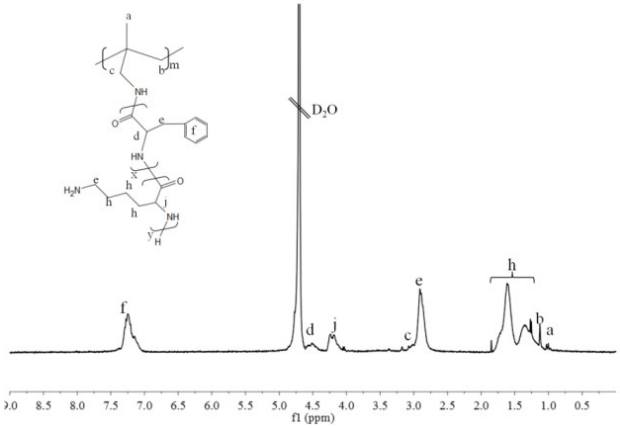


Figure S5. ^{1}H NMR spectrum of polymer 2 in D_2O

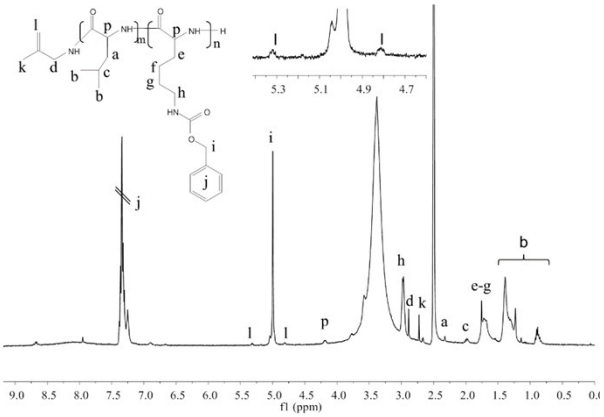


Figure S6. ^{1}H NMR spectrum of polymer 3 in DMSO-d_6

Determination of the composition of polymer 3

Table S2. The integrals of different Characteristic peaks

Spectrum	Ai	Al	Ab
Figure S6	18.02	2	49.25

The integral area of peak was set to be 2 as the internal reference, corresponding to the amount of the alkenyl hydrogen of initiator. The DP of polymer was determined as the following:

$$\text{DP of leucine} = \frac{Ai}{Al} \times \frac{2}{2} = 9.01 \approx 9$$

$$\text{DP of phenyalanine} = \frac{Ab}{Al} \times \frac{2}{6} = 8.2 \approx 8$$

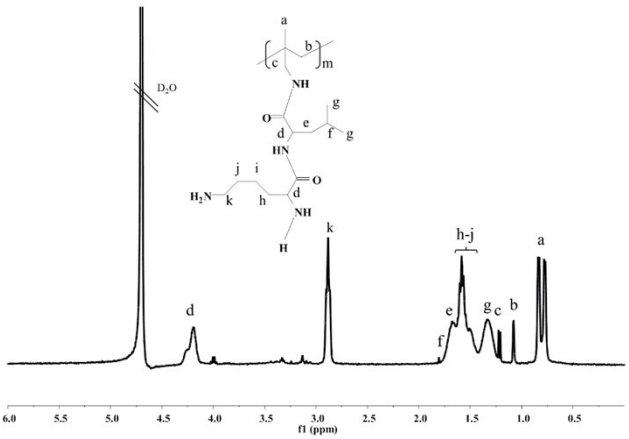


Figure S7. ^{1}H NMR spectrum of p**4** in D_2O

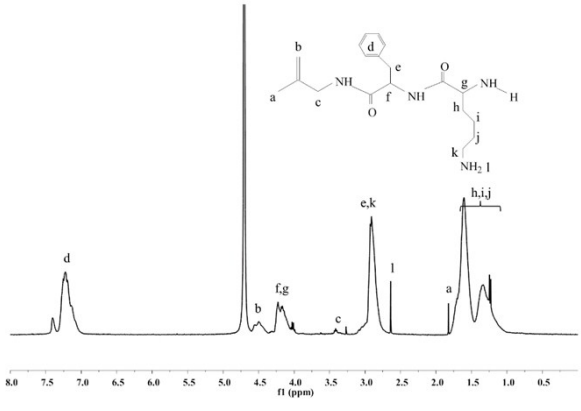


Figure S8. ^{1}H NMR spectrum of p**5** in D_2O

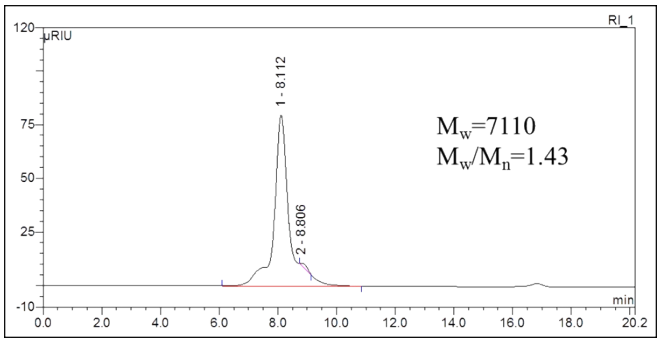


Figure S9. The GPC trace of p**2**.

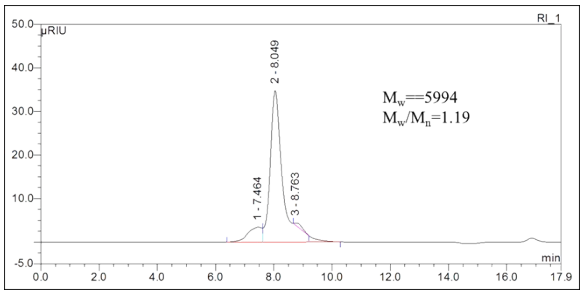


Figure S10. The GPC trace of p**4**.

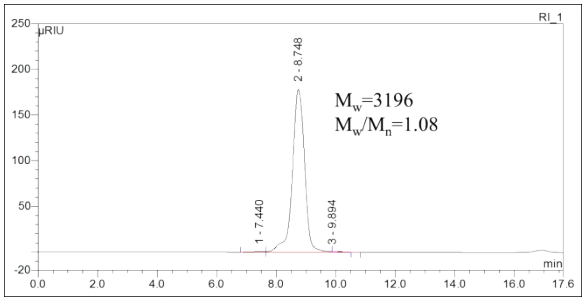


Figure S11. The GPC trace of p**5**.

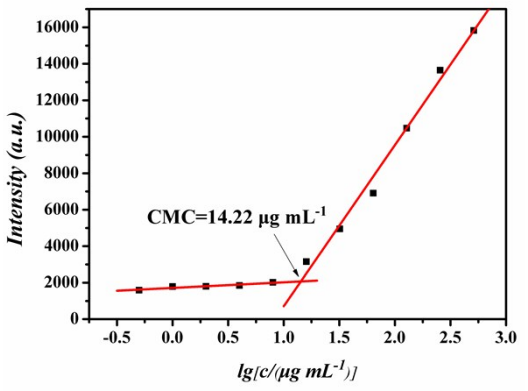


Figure S12. Determination of the critical micellization concentration of p**4**

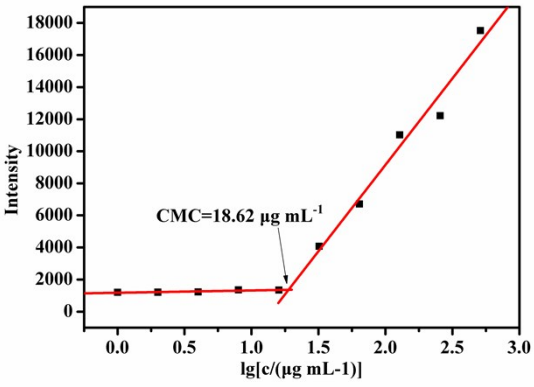


Figure S13. Determination of the critical micellization concentration of p**5**

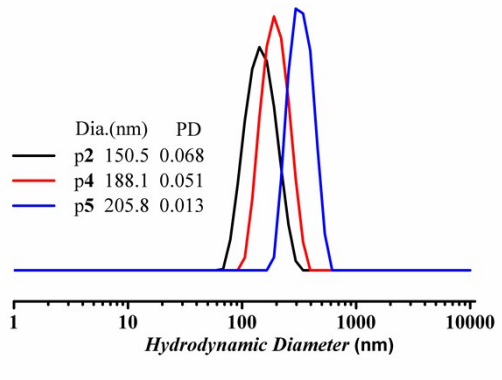


Figure S14. DLS studies of different copolymers nanoparticles (by intensity).

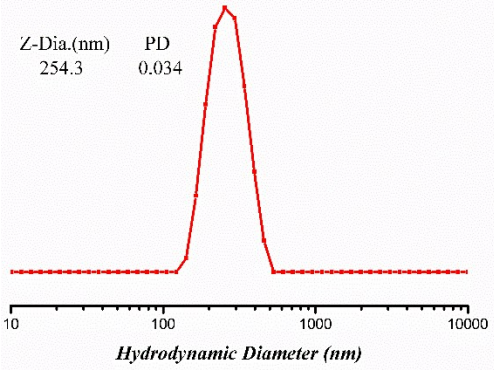


Figure S15. DLS studies of different copolymers nanoparticles (by intensity) in MH.

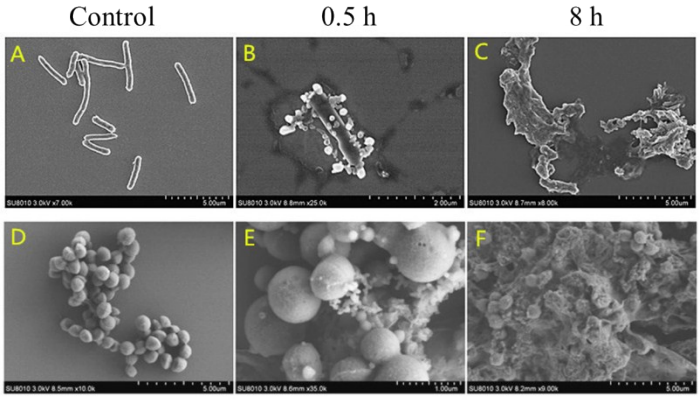


Figure S16. SEM images of *P. aeruginosa* (A-C) and MRSA (ATCC43300) (D-F) before and after treatment with assembled p**2**.

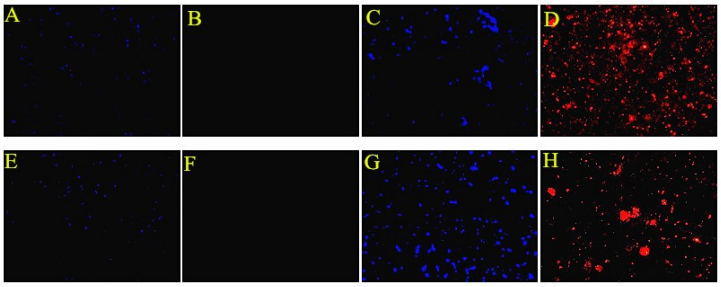


Figure S17. Fluorescence micrographs of MRSA (ATCC43300) (A-C) and *P. aeruginosa* (A-C).

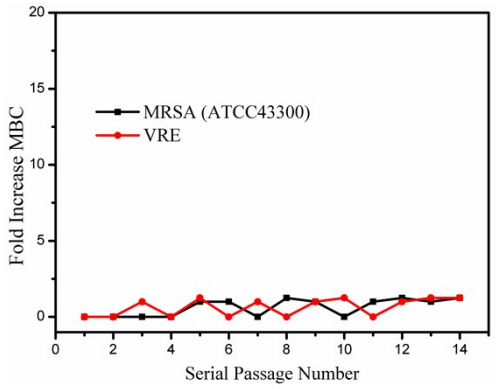


Figure S18. Resistance acquisition of MRSA (ATCC43300) and VRE in the presence of sub-MIC levels of assembled p**2**.

Table S3. Zeta potentials of three copolymer nanoparticles in aqueous solution at room temperature.

Polymer micelles	p**5**	p**4**	p**2**
Zeta potential(mV)	+13.5	+21.6	+29.7

Table S4. Numerical ΔμRIU values of p2 with different concentrations on different bacterial cells.

Concentrations (mg/mL)	ΔμRIU±5	
	S.aureus (ATCC29213)	*E.coli* (BL21)
	p **2**	
20	3180.52	2183.71
5	1675.37	1595.99
1	927.82	621.32
0.2	463.84	457.98
0.1	286.32	271.60
0.05	219.83	207.14

Table S5. Numerical ΔμRIU values of p2, p4 and Imipenem on different bacterial cells

Compound(0.2 mg/mL)	ΔμRIU±5	
	S. aureus (ATCC29213)	*E. coli* (BL21)
p**4**	320.33	97.83
p**2**	463.84	457.98
Imipenem	7.95	4.31