Electronic Supplementary Material (ESI) for Polymer Chemistry.

Electronic Supplementary Information

Strategies from Nature: Polycaprolactone-based Mimetic Antimicrobial Peptide Block Copolymers with Low Cytotoxicity and Excellent Antibacterial Efficiency

*Xinyu Zhou, †[a] Jing He †[b] and Chuncai Zhou *[a]*

a School of Materials Science and Engineering, Tongji University, Shanghai, 201804, China

b Department of Anatomy and Neurobiology, Tongji University School of Medicine, Shanghai, 201804, China

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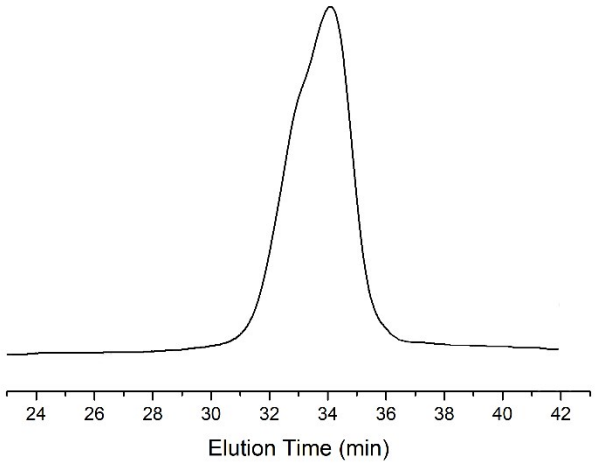

Fig. S1 GPC trace of PCL_{16}-NH_2 in THF as the eluent at 25 ℃.

As shown in **Fig. S1**, the molecular weight and molecular distribution of PCL was 2872 and 1.13, respectivily. This confirmed the successful synthesis of narrow diatribution PCL. In additon, the molecular weight of PCL are higher than the results calculated by ^{1}H NMR **(Fig. S2)**. This may be possible because GPC tests are calibrated with polystyrene standard which is different from our polymers and this will cause some errors in final results.

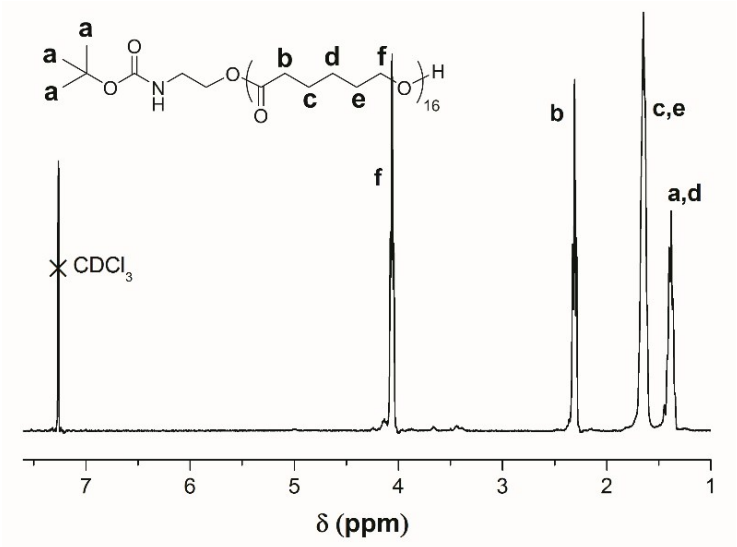

Fig. S2 ^{1}H NMR spectrum of PCL_{16}-NH(Boc) in $CDCl_3$.

Determination of the composition of PCL_{16}-NH(Boc) :

Table S1. The integrals of different peaks and the degree of polymerization of PCL_{16}-NH(Boc).

Spectrum	$A_{3.4\ ppm}$	A_b	A_f	A_{c+e}	A_{a+d}
Fig. S2	2.0	31.53	32.05	63.34	41.02

In **Table S1**, $A_{3.4\ ppm}$, A_b, A_f, A_{c+e}, and A_{a+d} were the integral areas of separated or overlapped peaks. First of all, the peak at 3.4 ppm corresponded to the -CH_2 beside the amide bond of Boc group and the integral area of this peak was set to be 2 (H) as the internal reference. Based on this, other peaks' areas were listed as Table S1. The degree of polymerization (DP) of PCL_x could be calculated by A_b and the results could be verified by A_f, A_{c+e}, or A_{a+d}.

Based on A_b of peak b, the DP of PCL_x was determined as following :

$$DP = \frac{A_b}{2} = 15.765 \approx 16$$

Based on A_f of peak f, the DP of PCL_x was determined as following:

$$DP = \frac{A_f}{2} = 16.025 \approx 16$$

Based on A_{c+e} of peak c and e, the DP of PCL_x was determined as following:

$$DP = \frac{A_{c+e}}{2+2} = 15.835 \approx 16$$

Based on A_{a+d} of peak a and d, the DP of PCL_x was determined as following:

$$DP = \frac{A_{a+d} - A_a}{2} = \frac{41.02 - 9}{2} = 16.01 \approx 16$$

wherein, the A_a corresponded to the 9 (H) of peak a.

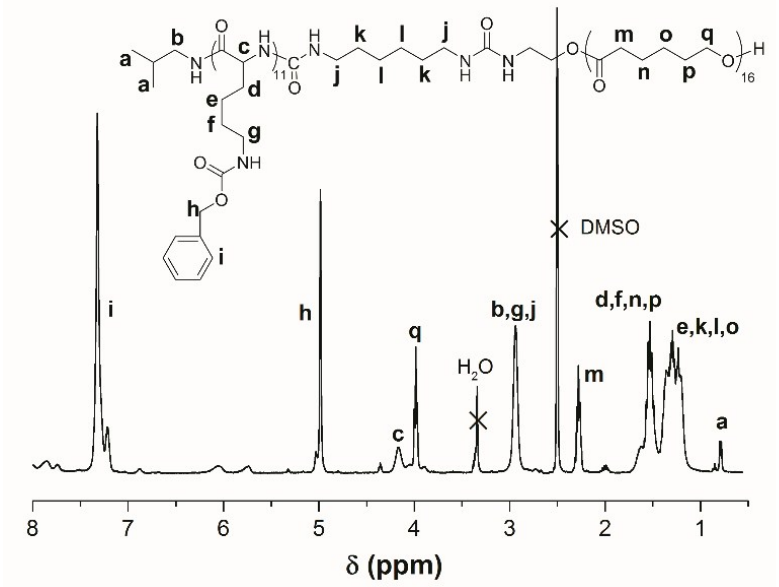

Fig. S3 ^{1}H NMR spectrum of PCL_{16}-*b*-Poly(Z-Lys)$_{11}$ with Cbz protection in d_6-DMSO.

Determination of the composition of PCL_{16}-*b*-Poly(Z-Lys)$_{11}$:

Table S2. The integrals of different peaks and the degree of polymerization of PCL_{16}-*b*-Poly(Z-Lys)$_{11}$.

Spectrum	A_m	A_h	A_i	A_c	A_{b+g+j}	$A_{d+f+n+p}$	$A_{e+k+l+o}$	A_q
Fig. S3	32.0	21.59	54.77	11.44	27.59	107.48	61.32	32.85

In **Table S2**, A_m, A_h, A_i, A_c, A_{b+g+j}, $A_{d+f+n+p}$, $A_{e+k+l+o}$ and A_q were the integral areas of separated or overlapped peaks. First of all, the peak m corresponded to the -CH_2 beside the -C=O of PCL repeated units and A_m was set to be 32 (H) as the internal reference. Based on this, other peaks' areas were listed as Table S2. The degree of polymerization (DP) of Z-lysine units could be calculated by A_h or A_i and the results could be verified by the integral areas of other peaks.

Based on A_h of peak h, the DP of Z-Lysine was determined as following :

$$DP = \frac{A_h}{2} = 10.795 \approx 11$$

Based on A_i of peak i, the DP of Z-Lysine was determined as following :

$$DP = \frac{A_i}{5} = 10.954 \approx 11$$

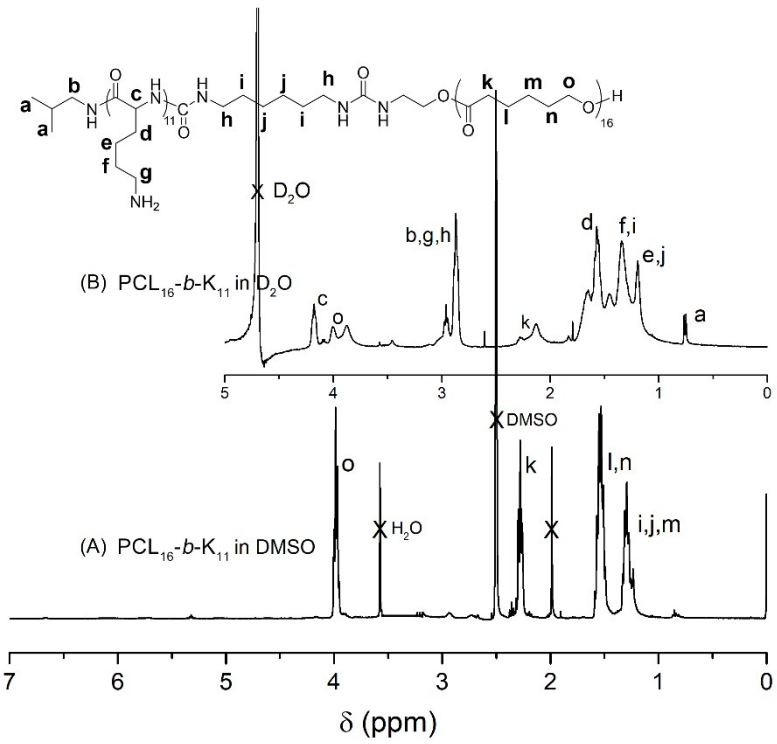

Fig. S4 ^{1}H NMR spectrum of PCL_{16}-*b*-K_{11} in d_6-DMSO and D_2O.

Determination of the composition of PCL_{16}-*b*-Poly(Z-Lys)$_{20}$:

Table S3. The integrals of different peaks and the degree of polymerization of PCL_{16}-*b*-Poly(Z-Lys)$_{20}$.

Spectrum	A_m	A_h	A_i	A_c	A_{b+g+j}	$A_{d+f+n+p}$	$A_{e+k+l+o}$	A_q
Fig. 1A	32.0	39.87	97.39	19.58	46.32	141.80	79.32	32.34

In **Table S3**, A_m, A_h, A_i, A_c, A_{b+g+j}, $A_{d+f+n+p}$, $A_{e+k+l+o}$ and A_q were the integral areas of separated or overlapped peaks. First of all, the peak m corresponded to the -CH_2 beside the -C=O of PCL repeated units and A_m was set to be 32 (H) as the internal reference. Based on this, other peaks' areas were listed as Table S3. The degree of polymerization (DP) of Z-lysine units could be calculated by A_h or A_i and the results could be verified by the integral areas of other peaks.

Based on A_h of peak h, the DP of Z-Lysine was determined as following :

$$DP = \frac{A_h}{2} = 19.935 \approx 20$$

Based on A_i of peak i, the DP of Z-Lysine was determined as following :

$$DP = \frac{A_i}{5} = 19.478 \approx 20$$

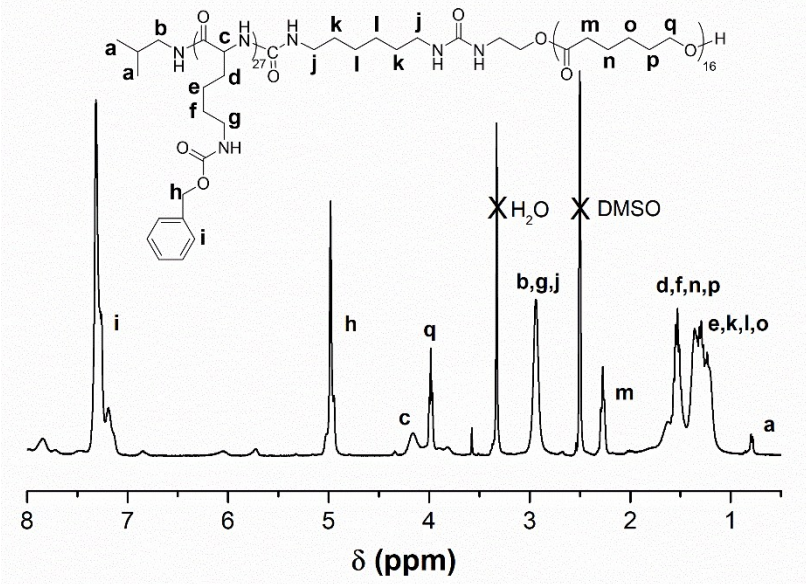

Fig. S5 ^{1}H NMR spectrum of PCL_{16}-*b*-Poly(Z-Lys)$_{27}$ with Cbz protection in d_6-DMSO.

Determination of the composition of PCL_{16}-*b*-Poly(Z-Lys)$_{27}$:

Table S4. The integrals of different peaks and the degree of polymerization of PCL_{16}-*b*-Poly(Z-Lys)$_{27}$.

Spectrum	A_m	A_h	A_i	A_c	A_{b+g+j}	$A_{d+f+n+p}$	$A_{e+k+l+o}$	A_q
Fig. S5	32.0	54.06	132.97	27.24	59.98	170.27	94.53	31.50

In **Table S4**, A_m, A_h, A_i, A_c, A_{b+g+j}, $A_{d+f+n+p}$, $A_{e+k+l+o}$ and A_q were the integral areas of separated or overlapped peaks. First of all, the peak m corresponded to the -CH_2 beside the -C=O of PCL repeated units and A_m was set to be 32 (H) as the internal reference. Based on this, other peaks' areas were listed as Table S4. The degree of polymerization (DP) of Z-lysine units could be calculated by A_h or A_i and the results could be verified by the integral areas of other peaks.

Based on A_h of peak h, the DP of Z-Lysine was determined as following :

$$DP = \frac{A_h}{2} = 27.03 \approx 27$$

Based on A_i of peak i, the DP of Z-Lysine was determined as following :

$$DP = \frac{A_i}{5} = 26.594 \approx 27$$

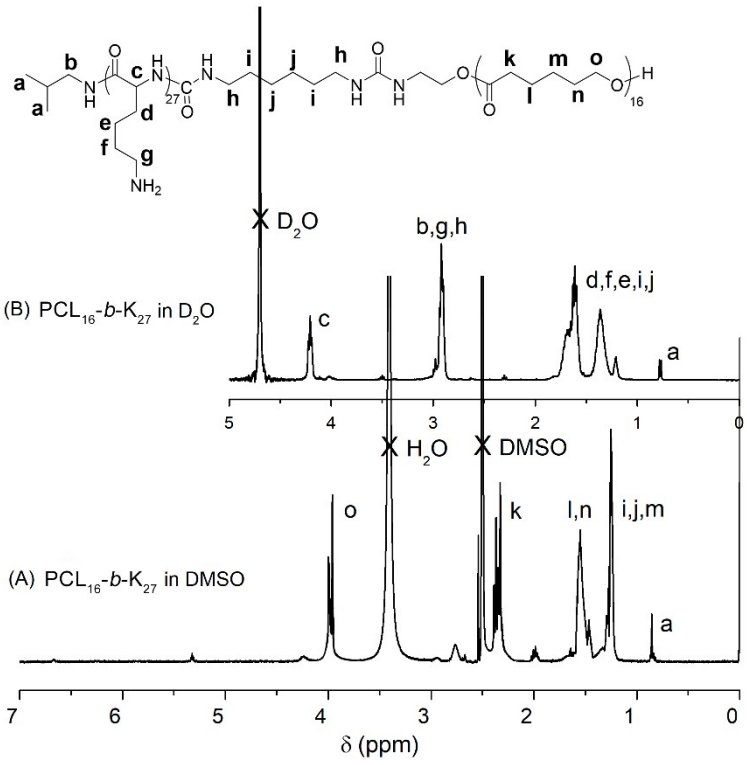

Fig. S6 ^{1}H NMR spectrum of PCL_{16}-*b*-K_{27} in d_6-DMSO and D_2O.

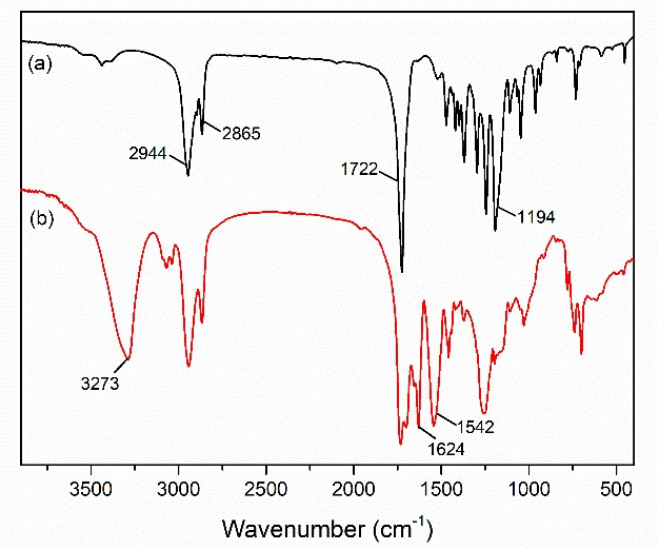

Fig. S7 The FT-IR spectra of (a) PCL_{16}-NH_2, (b) PCL_{16}-*b*-K_{20}

The FT-IR spectroscopy of PCL_{16}-NH_2 and PCL_{16}-*b*-K_{20} were shown in **Fig. S7**. The characteristic peaks of PCL are displayed at 2944 cm^{-1} (-C-H asymmetric stretching), 2865 cm^{-1} (-C-H symmetric stretching), 1722 cm^{-1} (-C=O stretching) and 1194 cm^{-1} (-C-O-C stretching) which appear in both curves (a) and (b). After coupling with polylysine, a strong peak at 3273 cm^{-1} representing the -NH groups of polylysine was shown in the curve (b). Besides, the characteristic peaks at 1542 cm^{-1} and 1583 cm^{-1} owing to the occurrence of polylysine were also observed. It could be confirmed that the diblock copolymers have been synthesized successfully.

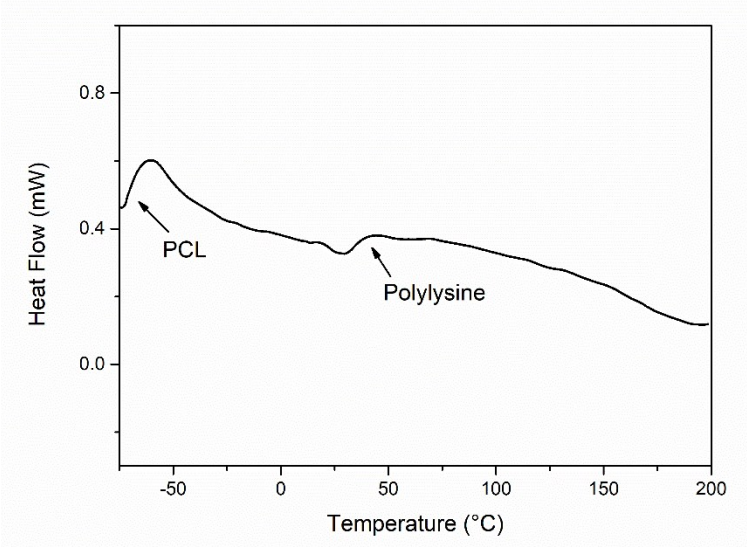

Fig. S8 DSC curve of the PCL_{16}-*b*-K_{20} at a heating rate of 10 °C min^{-1}.

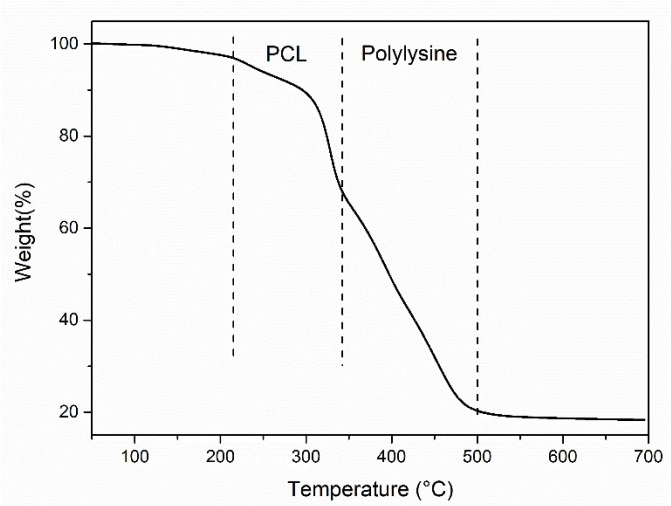

Fig. S9 TGA curve of the PCL_{16}-*b*-K_{20} at a heating rate of 10 °C min^{-1}.

As shown in **Figure S8**, there were two significant endothermic processes around -70 °C and 34 °C in the DSC trace, which corresponded to the glass transition temperatures of PCL and polylysine, respectively. In additon, the TGA trace **(Figure S9)** can also observe two weightless platform which revealed the existence of PCL and polylysine. Overall, both DSC and TGA results confirmed the formation of diblock copolymers.

Antibacterial results

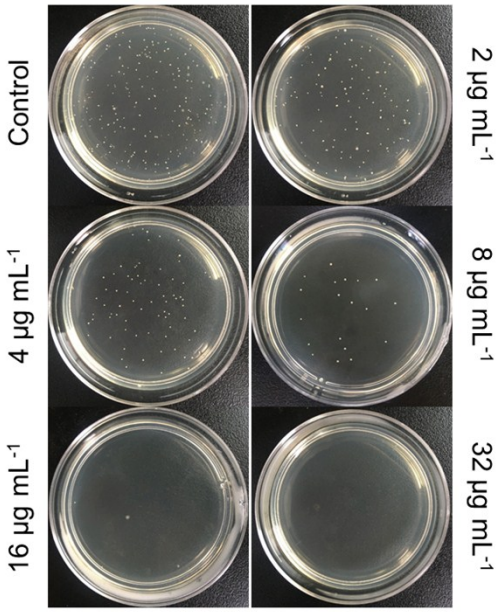

Fig. S10 Digital photographs of *E. coli* culture plates treated with different concentrations of PCL_{16}-*b*-K_{11} diblock copolymers. All the plates were diluted 10^5 times.

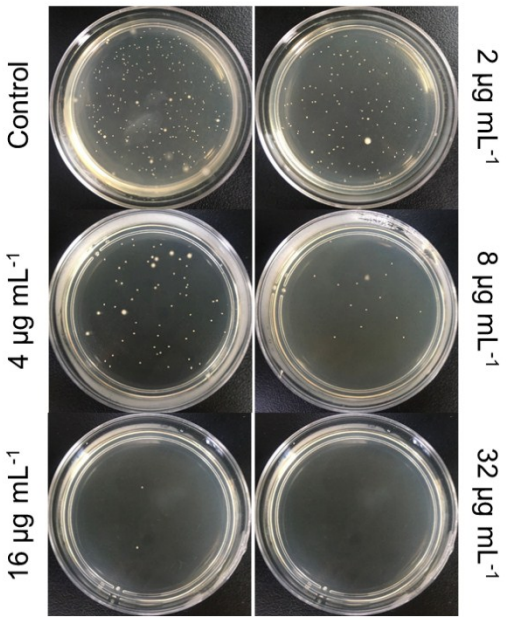

Fig. S11 Digital photographs of *S. aureus* culture plates treated with different concentrations of PCL_{16}-*b*-K_{11} diblock copolymers. All the plates were diluted 10^5 times.

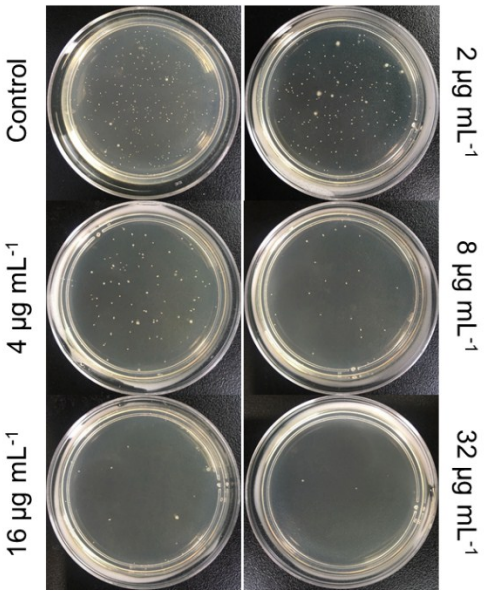

Fig. S12 Digital photographs of *E. coli* culture plates treated with different concentrations of PCL_{16}-*b*-K_{27} diblock copolymers. All the plates were diluted 10^5 times.

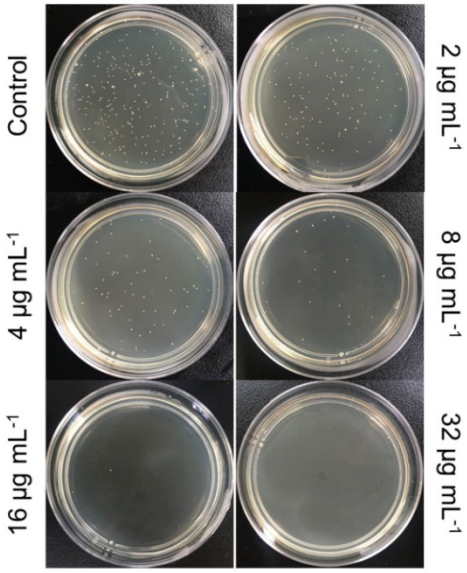

Fig. S13 Digital photographs of *S. aureus* culture plates treated with different concentrations of PCL_{16}-*b*-K_{27} diblock copolymers. All the plates were diluted 10^5 times.

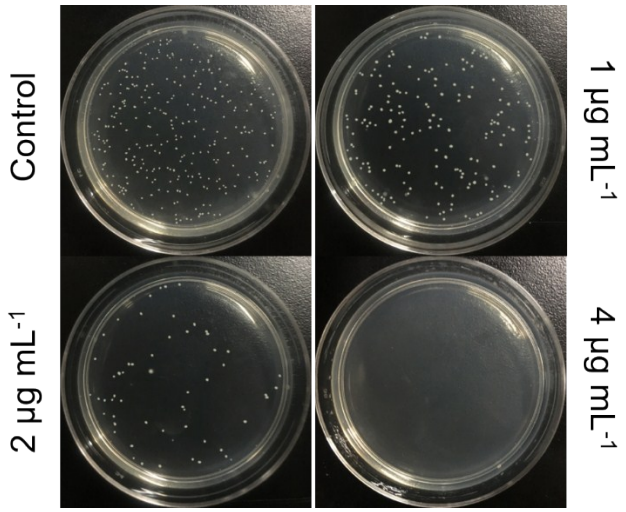

Fig. S14 Digital photographs of *E. coli* culture plates treated with different concentrations of the antibiotic Gentamycin Sulfate. All the plates were diluted 10^5 times.

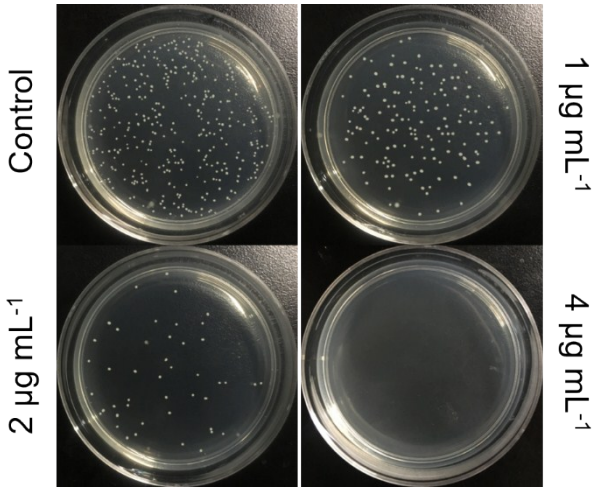

Fig. S15 Digital photographs of *S. aureus* culture plates treated with different concentrations of the antibiotic Gentamycin Sulfate. All the plates were diluted 10^5 times.

Table S5 Antibacterial efficacy of PCL_{16}-*b*-K_n diblock copolymers against *E. coli*: antibacterial rate and log reduction

Copolymers	$C_{copolymers}$ (μg mL^{-1})	Antibacterial rate (%)	Log(CFU/mL) reduction
PCL_{16}-*b*-K_{11}	2	45.2	0.3
	4	69.4	0.5
	8	92.4	1.1
	16	99.6	2.4
	32	99.9	3.6
PCL_{16}-*b*-K_{20}	2	61.9	0.4
	4	91.3	1.1
	8	99.7	2.5
	16	99.9	3.9
PCL_{16}-*b*-K_{27}	2	48.8	0.3
	4	70.3	0.5
	8	92.2	1.1
	16	97.3	1.6
	32	99.3	2.5
	64	99.9	3.7

Table S6 Antibacterial efficacy of PCL_{16}-*b*-K_n diblock copolymers against *S. aureus*: antibacterial rate and log reduction

Copolymers	$C_{copolymers}$ (μg mL^{-1})	Antibacterial rate (%)	Log(CFU/mL) reduction
PCL_{16}-*b*-K_{11}	2	50.8	0.3
	4	75.6	0.6
	8	93.9	1.2
	16	99.7	2.6
	32	99.9	3.9
PCL_{16}-*b*-K_{20}	2	61.9	0.4
	4	91.3	1.1
	8	99.7	2.5
	16	99.9	3.9
PCL_{16}-*b*-K_{27}	2	54.8	0.3
	4	75.9	0.6
	8	95.3	1.3
	16	99.6	2.5
	32	99.9	3.6

Biodegradation of the PCL_{16}-*b*-K_{20} copolymers

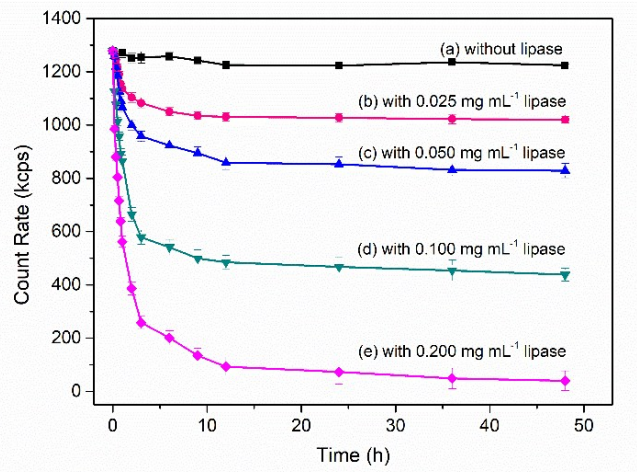

Fig. S16 Count rate variation of PCL_{16}-*b*-K_{20} copolymer vesicles during enzymatically catalyzed degradation. (a) 0.05 mg mL^{-1} vesicle solution without lipase; (b) 0.05 mg mL^{-1} vesicle solution with 0.025 mg mL^{-1} lipase solution; (c) 0.05 mg mL^{-1} vesicle solution with 0.050 mg mL^{-1} lipase solution; (d) 0.05 mg mL^{-1} vesicle solution with 0.100 mg mL^{-1} lipase solution; (e) 0.05 mg mL^{-1} vesicle solution with 0.200 mg mL^{-1} lipase solution.

The biodegradation properties of PCL_{16}-*b*-K_{20} copolymer were explored in the presence of lipase at different concentrations (0.025 mg mL^{-1}, 0.050 mg mL^{-1}, 0.100 mg mL^{-1} and 0.200 mg mL^{-1}). The results was shown in **Fig. S16**. As control, curve **a** showed the count rate variation of PCL_{16}-*b*-K_{20} vesicles without any lipase, indicating the stability of the vesicles without enzyme. While as shown in the curves **b, c, d** and **e**, the vesicles could be degraded in the presence of lipase. Moreover, the degradation speed was accelerated with the increase of the lipase concentration. Up to 0.200 mg mL^{-1} lipase concentration, nearly all of the copolymer vesicles were degraded during 48 h. Besides, the diblock copolymers haven't been degraded entirely in 30 min while they can reach up to 99% antibacterial rate in 30 min. Thus, the diblock copolymers can still realize rapid antibacterial effect before its degradation in the presence of lipase in vivo.

The critical vesiculation concentration (CVC) of the PCL_{16}-*b*-K_{20} copolymers

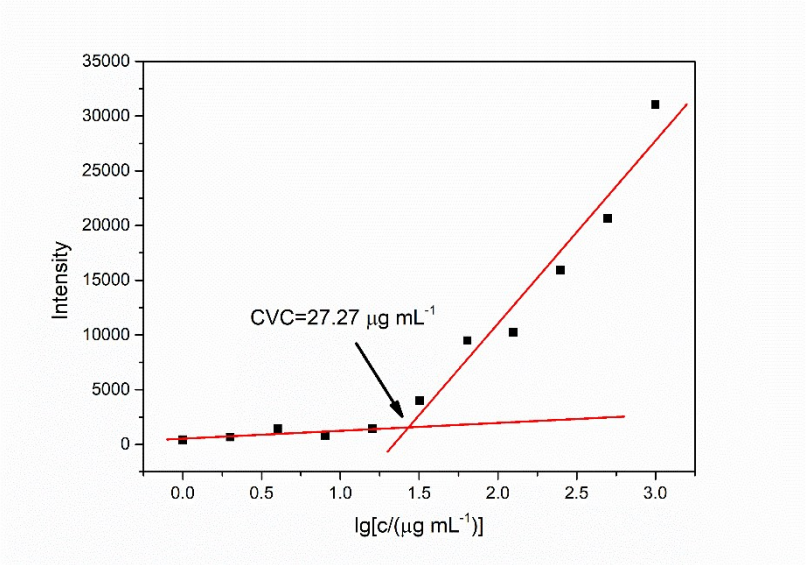

Fig. S17 The CVC values of PCL_{16}-*b*-K_{20}

The CVC of PCL_{16}-*b*-K_{20} were 27.27μg mL^{-1} as shown in **Fig. S17**. The PCL_{16}-*b*-K_{20} diblock copolymers could self-assemble into nanoparticles at a low concentration. Besides, compared with the MICs of the PCL_{16}-*b*-K_{20} diblock copolymers, they possessed higher CVC values than the MICs, indicating that copolymers may be individual chains when acting on the bacterial membrane at low concentrations.

Experimental Section

Antibacterial test

Antibacterial properties of PCL_{16}-*b*-K_n diblock copolymers against Gram-negative *E. coli* (ATCC35218) and Gram-positive *S. aureus* (ATCC29213) were evaluated by the plate counting method. *E. coli* and *S. aureus* were cultured to a mid-log phase at 37 °C for 18 h in LB broth medium. 1mL of bacteria suspension was centrifuged at 9000 r/min to remove LB broth and then diluted to 10^6 colony forming unit (CFU) mL^{-1} using saline. Different concentrations (from 1000 μg mL^{-1} to 2 μg mL^{-1}) of the diblock copolymer solution were prepared by semi-dilution with deionized water. 100 μL of the diblock copolymer solution at each concentration was mixed with 100 μL bacterial-saline suspension. Bacterial-saline suspension without the diblock copolymer solution was served as the control. After incubation at 37 °C for 2 h, each mixed solution was diluted to different concentrations (10-fold). Each diluted solution (100 μL) was mixed with 100 μL bacteria-saline suspension at 37 ℃ for 48 h. Three parallel samples were conducted for each concentration. The antibacterial efficacy could be observed intuitively from the photographs taken of the agar plates. Also, the antibacterial rate and log reduction were calculated to quantify the antibacterial activity.

Hemolysis test

The blood of goat was centrifuged and washed by phosphate buffer saline (PBS, pH=7.4) solution for several times to achieve pure red blood cells, and then erythrocyte/PBS solution was prepared in which the erythrocyte concentration was 5% (v/v). Different concentrations (from 2000 μg mL^{-1} to 16 μg mL^{-1}) of PCL_{16}-*b*-K_n copolymer solutions were prepared in the method of semi-dilution with PBS. 600 μL of copolymer solutions at each concentration were added into several centrifuge tubes and treated with 600 μL of erythrocyte/PBS solution at 37 °C. The erythrocyte solution with 0.1% Triton X-100 was set as positive control. The erythrocyte solution without any treatment was set as negative control. After treating for 1 h, all samples were centrifuged and 100 μL supernatant of each sample was added into a 96-well

plate. Hemoglobin release was detected by measuring the absorbance of samples at 540 nm. Percentage of hemolysis was calculated as the equation (1):

$$\text{Hemolysis}(\%) = \frac{O_s - O_n}{O_p - O_n} \times 100\% \quad (1)$$

Wherein O_s represents the supernatant absorbance of copolymer samples for a certain concentration; O_n represents the supernatant absorbance of erythrocyte solution without treatment (negative control); O_p represents the supernatant absorbance of a completely hemolysis solution (positive control).

Self-assemble of the PCL_{16}-*b*-K_{20} copolymers

The diblock copolymers (5.000 mg) were dissolved in 2.0 mL THF/H_2O (1:1, v:v), and then 4.0 mL deionized water was added dropwise to the solution during 10 min with continuous stirring. The solution was stirred for another 12 h. After that, the solution was dialyzed in a dialysis tube (3500 MWCO) against deionized water for 24 h (renewing the deionized water every 2 h).

The critical vesiculation concentration (CVC) was evaluated, which is defined as the lowest concentration that copolymers can self-assemble into vesicles in water. Pyrene (3.0 mg), the probe detecting the vesicle formation, was dissolved in 25.0 mL of acetone. Each of eleven centrifuge tubes was filled with 10 μL of pyrene/acetone solution and kept open overnight to allow acetone to evaporate. Different concentrations (1000, 500, 250, 125, 64, 32, 16, 8, 4, 2 and 1 μg mL^{-1}) of PCL_{16}-*b*-K_{20} vesicle solutions were prepared by 2-fold dilution with deionized water. Then, 4.0 mL of vesicle solution at each concentration was added into glass bottles with continuous stirring for 12 h. Fluorescence intensities were recorded by exciting samples at 334 nm and both slit width for excitation and emission were 5 nm. The emission wavelengths were from 350-500 nm and the intensity at 371.9 nm was chosen as the evaluate indicators. Then, the scattergram (the intensity *vs.* the log of each concentration) was drawn with two linear fitting lines. The CVC value was determined by calculating the intersection of two linear fitting lines.

Biodegradation of the PCL_{16}-*b*-K_{20} copolymers

The degradation process was reflected through the changes of derived count rate of PCL_{16}-*b*-K_{20} vesicles by DLS. The lipase (5.0 mg) was dissolved in 1.0 mL of deionized water to form 5.0 mg mL^{-1} lipase solution. Then, the aqueous lipase solution (0.020 mL, 0.040 mL, 0.082 mL and 0.167 mL) was mixed with 4.0 mL of PCL_{16}-*b*-K_{20} vesicle solution (50 μg mL^{-1}). And the mixture was incubated at 37 ℃ to characterize the changes of the count rate at certain intervals by DLS. A control experiment was also carried out using the PCL_{16}-*b*-K_{20} vesicle solution without lipase.