# SUPPORTING INFORMATION

## Biocompatible Cationic polypeptoids with antibacterial selectivity depending on hydrophobic carbon chain length

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## Experimental Procedures

### 1. Materials

Iodomethane ($CH_3I$, ≥AR, 99.0%), bromoethane ($C_2H_5Br$, ≥AR, 99.5%, without stabilizers), n-butylamine ($C_4H_{11}N$, ≥AR, 99.5%), glyoxylic acid ($C_2H_2O_3$, ≥AR, 99.0%), sodium hydroxide (NaOH, ≥AR, 97%), sodium hydride (NaH 60% dispersion in mineral oil), sodium chloride (NaCl, ≥AR, 99.5%) and agar powder were purchased from Shanghai Aladdin Biochemical Technology. Boc-glycine ($C_7H_{13}NO_4$ ≥AR, 99.5%), tetrahydrofuran ($C_4H_8O$ ≥AR, 99.5%), phosphorus trichloride ($PCl_3$, ≥AR, 99.0%), ethyl ether ($Et_2O$ ≥AR, 98%), Benzophenone ($C_{13}H_{10}O$, ≥AR, 99.5%) and magnesium sulfate ($MgSO_4$, ≥AR, 99.0%) were purchased from Shanghai Maclean Biochemicals. Dichloromethane ($CH_2Cl_2$ HPLC, 99.9%), ethyl acetate ($C_4H_8O_2$ HPLC, ≥99.9%) and benzylamine ($BnNH_2$ HPLC, ≥99.9%) were purchased from J&K Scientific. Yeast extract was purchased from Energy Chemical. Nutrient broth was purchased from G-CLONE. Cell Counting Kit-8 (CCK-8 kit) was purchased from Beyotime Biotechnology. GIBCO RPMI 1640 cell culture medium, fetal bovine serum (FBS) and tryptone were purchased from Thermo Fisher Scientific. High purity

nitrogen was obtained from Shanghai Wugang Gas. The bacterial strains used were *E. coli* MG1655 (ATCC700926), *E. coli* DH5α, and *Staphylococcus aureus* (ATCC-6538) from Biofeng. They were stored in an ultra-low temperature refrigerator at -80°C. The cells were mouse epithelioid fibroblasts (L929) from the Typical Culture Collection Committee Cell Bank of the Chinese Academy of Sciences. Human blood was obtained from the authors. Female BALB/c mice (aged 6-8 weeks, 16-20 g weight) were purchased from Zhejiang Vital River Laboratory Animal Technology Co., Ltd. (China). All mouse studies were performed according to the protocol approved by the Soochow University Laboratory Animal Center. The mice used in the experiments were randomly grouped. All *in vivo* animal experiments were conducted in accordance with relevant ethical regulations.

**2. Characterizations**

$^{1}$H NMR spectra was recorded on Bruker 400Hz spectrometer. Total internal reflection Fourier transform infrared spectra of PNBs polymers were acquired on a Nicolet 6700FT-IR spectrometer (Thermo Fisher Scientific). The Waters 1525μ gel permeation chromatography (GPC) was used. DMF/LiBr (0.05 M) was used as the mobile solution with a flow rate of 1 mL/min. The column temperature was 50℃ and the sample cell was 40℃. The test time was 40 min. Cell viability was assessed by the CCK-8 method using a multifunctional microplate reader (Varioskan Flash, Thermo Scientific). an Olympus IX71 fluorescence microscope was used to observe cell growth. Scanning electron microscopy (SEM, S4700, Hitachi) was used to observe and characterize the morphological features of bacteria after interacting with modified surfaces. Zeta potential measurements were conducted using the Zetasizer nano ZS instrument. All samples were prepared at a concentration of 1 mg/mL. All of the measurements were repeated three times to obtain the average values.

**3. Synthesis.**

**3.1. Synthesis of N-substituted N-carboxyanhydride monomers (NNCAs).**

The detailed synthetic procedures of *N*-substituted *N*-carboxyanhydride monomers (NNCAs), including *N*-methyl NCA (Me-NCA, M1), *N*-Ethyl NCA (Et-NCA, M2) and N-butyl NCA (Bu-NCA, M3) were reported elsewhere[1,2].

**3.2. Synthesis of Poly (N-methyl glycine), P1.**

Monomer M1 (250 mg, 2.17 mmol) was dissolved in 4 mL anhydrous THF. A stock solution of benzylamine in THF as the initiator (0.434 mmol, [M1] / [I] = 50:1) was subsequently added. The polymerization was stirred at 70℃ for 48-72 h (until the characteristic $\nu_{C=O}$ peaks of NCA was completely disappeared) under nitrogen. The reaction mixture was precipitated into cooled diethyl ether to obtain a white solid (87.8% yield). All the other homopolymers are synthesized in a similar way.

**3.3. Modification of P1 with bromoethane (PNBM)**

Add bromoethane in 10:1 equivalent to a solution of P1 polymer dissolved in chloroform. It was heated to 70°C and condensed and refluxed for 12-18 h. After the reaction, the precipitate was precipitated by ether precipitation, then washed with DCM and methanol and dried under vacuum to obtain PNBs.

**3.4. Synthesis of Polyurethane (PU)**

The PU solution was reported elsewhere[3].

**4. Preparation of PU-PNBM on the glass substrate**

PU emulsions were diluted three times with anhydrous ethanol and they were mixed with PNBs polymer aqueous solution and ethanol in the ratio (v/v/v=1/1/2). 2-Hydroxybenzophenone was added and the mixture was irradiated with UV light for 5 min. The mixture (50 μL) was added dropwise to a glass substrate of size 0.7 cm × 0.7 cm. These samples were placed in an oven at 60°C to remove the solvent and then irradiated with UV light for 7 min to cure it on the glass surface to form PU-PNBs films. PU films without PNBs polymer were prepared as controls.

**5. Antibacterial Assays.**

Gram-negative bacterium *E. coli* (MG1655) and Gram-positive bacterium *S. aureus* (ATCC-6538) were used as model bacteria. The antibacterial properties of PNBs polymers in solution and PU-PNBs films were tested by a plate counting method.

**5.1 Treatment of bacteria.**

*E. coli* (MG1655) frozen at -80°C were screened on LB plates. They were incubated at 37°C for 12 hours to achieve activation. The activated individual colonies were added to 2 mL of LB liquid medium and incubated at 37°C and 190 rpm in a shaker for 8-12

hours. *S. aureus* (ATCC-6538) were cultured in broth medium for 12−18 h. After diluting the bacteria with sterilized phosphate buffered saline (PBS) buffer, the OD of the bacterial solutions were tested at 600 nm (*E. coli* MG1655) and 670 nm (*S. aureus* ATCC-6538). The density of the bacterial suspensions were reduced to $10^7$ CFU/mL.

**5.2 Antibacterial activity of PNBs polymers in the solution**

A series of solutions containing PNBs polymers at concentrations of 50-800 μg/mL in PBS buffer were prepared. 50 μL ($10^6$ CFU/mL) suspension was added dropwise to LB or broth agar plates and spread evenly. Incubate in a 37°C incubator for 8-12 hours (*E. coli* MG1655) or 15-18 hours (*S. aureus* ATCC-6538). Samples without PNBs polymers were used as controls. The number of individual colonies was calculated. The inhibition efficiency was estimated by the formula.

$$\%\ bacteriostatic\ efficiency = \frac{the\ number\ of\ colonies^{control} - the\ number\ of\ colonies^{sample}}{the\ number\ of\ colonies^{control}} \times 100$$

Each sample was measured in triplicate.

**5.3 Antibacterial mechanism**

BCA (Bicinchoninic acid) Protein Test[4]: The BCA protein concentration test is an experiment in which the protein concentration can be deduced from the absorbance value. Under alkaline conditions, the protein reduces $Cu^{2+}$ to $Cu^{+}$, and $Cu^{+}$ forms a violet colored complex with BCA reagent, and two molecules of BCA chelate a $Cu^{+}$. The absorption value of the water-soluble complex at 562 nm was compared with the standard curve to calculate the concentration of the protein to be measured. When bacterial cells are disrupted, the proteins that leak out from the inside are tested. We configured the working solution in a 96-well plate by adding 200 μL of working solution and 25 μL of a certain concentration of polymer solution to each well. The plate was incubated at 37℃ for 30 min, and after cooling to room temperature, the OD value was tested at 562 nm using an enzyme marker.

**5.4 Antibacterial activity of PU-PNBs films**

The bacterial inhibition efficiency of PU-PNBs films on the glass substrates was studied by *E. coli* MG1655 and *S. aureus* ATCC-6538. The samples were sterilized under UV

light for 20 min. The bacterial suspension (50 μL, $10^6$ CFU/mL) was dropped onto the films. After adding PBS buffer (950 μL), they were intermittently sonicated for 5 min to ensure bacterial separation from the film. The suspension (50 μL) was dropped onto LB or broth agar plates and spread evenly. Nutrient agar plates were incubated at 37°C. PU films without PNBs polymer were used as controls. Each sample was measured in triplicate. The number of individual colonies was calculated and the efficiency of bacterial inhibition was estimated.

**6. Live/dead staining assay.**

To determine the bactericidal efficiency of the solution proper and the surface, we used the standard live/dead fluorescent staining method. 1mL bacterial suspension ($1 \times 10^7$ CFU/mL) was injected into each well of a 48-well plate and incubated for 3 hours. The PNBB solution or the PU-PNBB film was then stained with live/dead stain solution (30 μL, 1:1 mixture Syto9 (3.34 mM) and propidium iodide (20 mM)) in a light-free environment for 15 min. A fluorescence microscope (IX71, Olympus, 40× lens) was used to observe live (green) and dead (red) bacteria. Images were processed with image-J software.

**7. In vitro cytotoxicity assays**

The CCK-8 method was employed to assess the cytotoxicity of the PNBs polymer and PU-PNBs films using mouse fibroblast (L929) cells. Then 200 μL of fresh 1640 medium containing 100 μg/mL PGB polymer was added. They were incubated for 1, 3 and 5 days, respectively. Finally, 20 μL of CCK-8 dye was added to each well and incubated for 2 hours at 37°C. The absorbance was measured at 450 nm using a multifunctional microplate reader. Wells with untreated cells were used as controls. Cell viability (%) was calculated by the formula.

$$\%\ cell\ viability = \frac{OD^{sample} - OD^{blank}}{OD^{postive\ control} - OD^{blank}} \times 100$$

**8. Hemolysis assay**

A series of solutions containing PNBB polymers at concentrations of 1000, 900, 800, 700, 500, 300, 200, 100, and 50 μg/mL were prepared in saline. Then, fresh human whole blood (containing sodium citrate anticoagulant) was mixed with normal saline in

the ratio (v/v=4/5) and gently shaken to dilute. The polymer solution is mixed with the diluted fresh human whole blood in the ratio (v/v = 50/1) and shaken gently. The mixture was incubated at 37°C for 1 hour. The mixture was centrifuged at 800 rpm for 5 min. 200 μL of supernatant was taken and the absorbance value was measured at 545 nm. Normal saline was used as a negative control and purified water was used as a positive control. Hemolytic activity was calculated by the formula.

$$\%\ hemolysis = \frac{OD^{sample} - OD^{negative\ control}}{OD^{postive\ control} - OD^{negative\ control}} \times 100$$

**9. Erythrocyte morphology assay**

Erythrocytes were collected from human blood and washed three times with PBS buffer. The erythrocytes were resuspended to 5% (v/v) in PBS buffer and 500 μL of erythrocyte suspension was added to a 24-well plate. Polymer aqueous solution (500 μL) was added to achieve the desired concentration, and then incubated for 1 h at 37°C. After centrifugation, wash the RBCs with PBS buffer three times. Fix RBCs with 4% glutaraldehyde in PBS buffer overnight at 4°C. After washing three times with PBS buffer, RBCs were dehydrated with gradients of ethanol (30, 50, 70, 80, 90, 95 and 100%). After drying in air and spraying with gold, the samples were used for SEM characterization.

**10. Bacterial morphology assay**

Bacteria in the logarithmic phase of growth were washed three times with PBS and diluted to $3 \times 10^7$ CFU/mL. Polymer aqueous solution was added to achieve the desired concentration (800 μg/mL) and then incubated at 37°C for 2 h. Fix the bacteria with 4% glutaraldehyde in PBS buffer at 4°C overnight. After washing three times with PBS buffer, the bacteria were dehydrated with gradients of ethanol (30, 50, 70, 80, 90, 95 and 100%). After drying in air and spraying with gold, the samples were used for SEM characterization.

**11. Inhibition of *S. aureus* biofilm formation**

*S. aureus* in logarithmic growth phase was diluted to $3 \times 10^7$ CFU/mL in broth medium as a working suspension. In a 96-well plate, PNBB solution and PU-PNBB sheets were

co-cultured with them for 24 h, 48 h, 72 h. Equal amounts of bacterial suspension (200 μL) were added to each well, and the substrate was replaced into new wells every 24 h. 200 μL of crystalline violet dye (diluted with water at a concentration of 2.5%) was added to the wells and stained for 20 minutes, and then rinsed three times with PBS buffer solution. Finally, ethanol was added and transferred to 96-well plates for dehydration, and OD values were measured at 570 nm with an enzyme marker.

**12. *In vivo* anti-infective studies:**

A mouse model of Staphylococcus aureus biofilm infection was established using female BALB/c mice (aged 6-8 weeks, 16-20 g weight). The dorsal hair of the mice was shaved, and a wound of approximately 6mm in diameter was created (cut away the full thickness of the skin). 100μL suspension of S. aureus (1 x $10^7$ CFU/mL) was applied to the surface of a 1cm × 1cm sterile gauze and the wound was secured with sterile tape. The progression of the wound infection was observed 12 hours post-infection. PU-PNBB film dressing were applied to the wound site. Pure PU films dressing and no treatment wounds were used as control. The wound was observed for healing every two days. On the fifth day of healing, three mice from each group were cut off their full-thickness skin around the wound. The whole skin tissue was ground and sonicated for 5 minutes to disperse in 5 ml of saline. Take 100μL of the solution and spread it evenly on the Petri dish, and calculate the bacterial survival rate by plate counting method. The wound size was calculated as:

$$S = \frac{\pi * L * W}{4}$$

where L and W are the length and width in the diameter direction, respectively.

## Results and Discussion

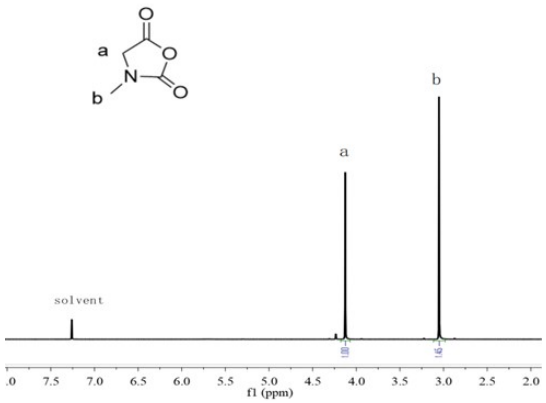


**Figure S1**. $^{1}H$ NMR spectra of Me-NCA (400MHz, solvent: $CDCl_3$)

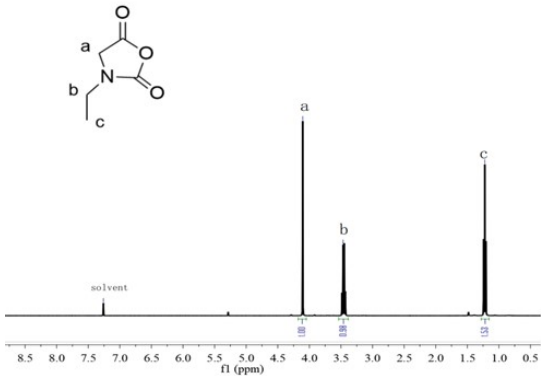


**Figure S2**. $^{1}H$ NMR spectra of Et-NCA (400MHz, solvent: $CDCl_3$)

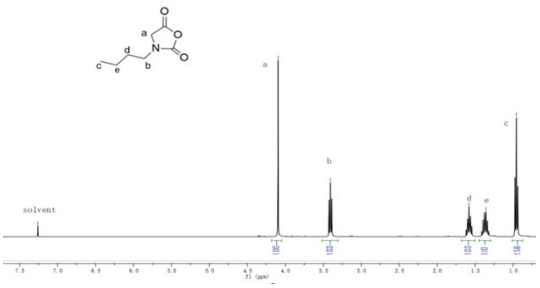


**Figure S3**. $^{1}H$ NMR spectra of Bu-NCA (400MHz, solvent: $CDCl_3$)

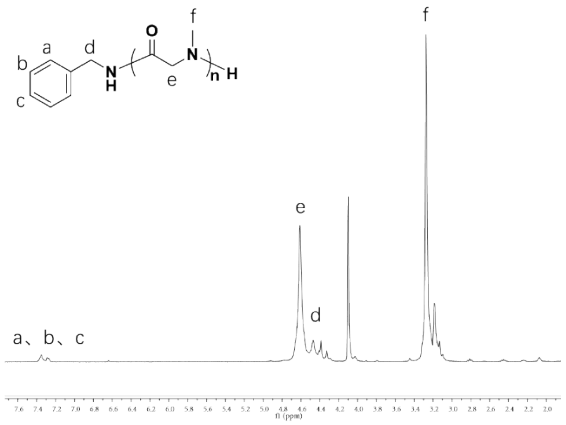


**Figure S4**. $^{1}H$ NMR spectra of PNBM (400MHz, solvent: $CDCl_3$)

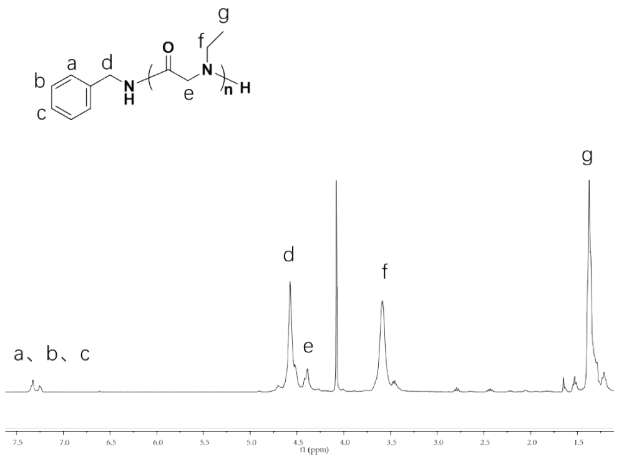


**Figure S5**. $^{1}H$ NMR spectra of PNBE (400MHz, solvent: $CDCl_3$)

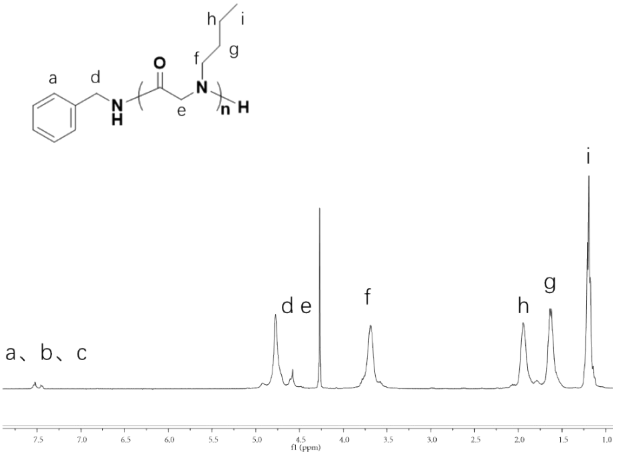


**Figure S6**. $^{1}H$ NMR spectra of PNBB (400MHz, solvent: $CDCl_3$)

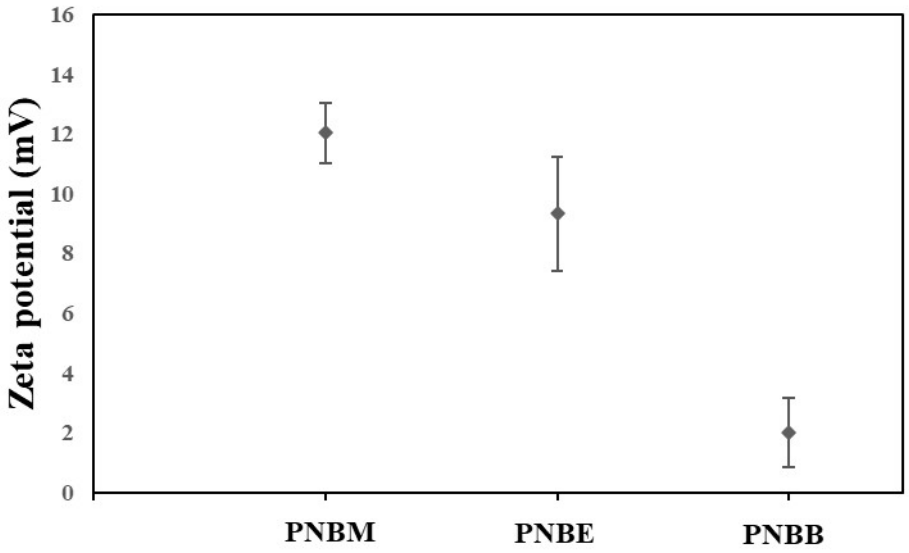


**Figure S7.** Zeta potentials of polypeptoids containing cationic sulfonium ions at 25°C in $H_2O$ at a concentration of 1 mg/mL.

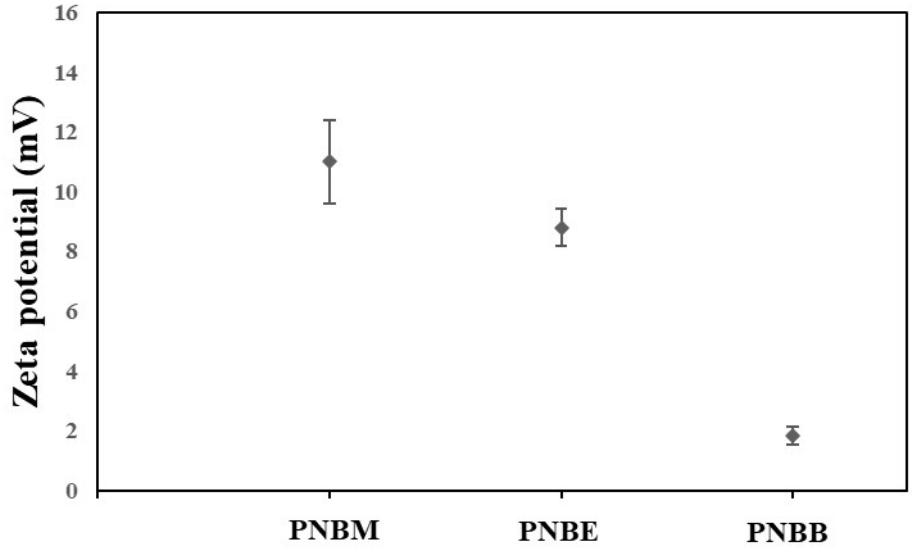


**Figure S8**. Zeta potentials of polypeptoids containing cationic sulfonium ions at 25°C in PBS buffer at a concentration of 1 mg/mL.

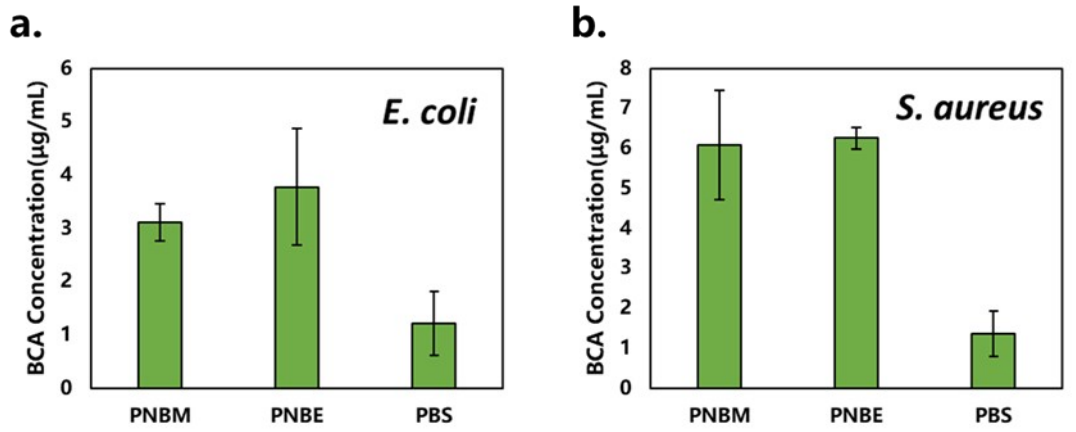


Figure S9. (a) Protein leakage concentrations after co-incubation of PNBM and PNBE (1 mg/mL) with *E. coli* ($10^7$ CFU/mL) under the BCA assay. (b) Protein leakage concentrations after co-incubation of PNBM and PNBE (1 mg/mL) with *S. aureus* ($10^5$ CFU/mL) under the BCA assay. Data are shown as mean ± SD (n = 3 per group).

Table S1. The bacteriostatic efficiency of PNBs with different molecular weights.

| Sample | $M_w$ (g/mol) | concentration of 80% antibacterial efficiency (*S.aureus*) |
|---|---|---|
| PNBM | 3660 | >90 μg/mL |
| PNBM | 23840 | >90 μg/mL |
| PNBE | 3150 | >90 μg/mL |
| PNBE | 18820 | >90 μg/mL |
| PNBB | 5190 | >80 μg/mL |
| PNBB | 21920 | >80 μg/mL |

*Mw (g/mol) (weight-average molecular weight) was determined by GPC analysis and $^1$H NMR analysis.*

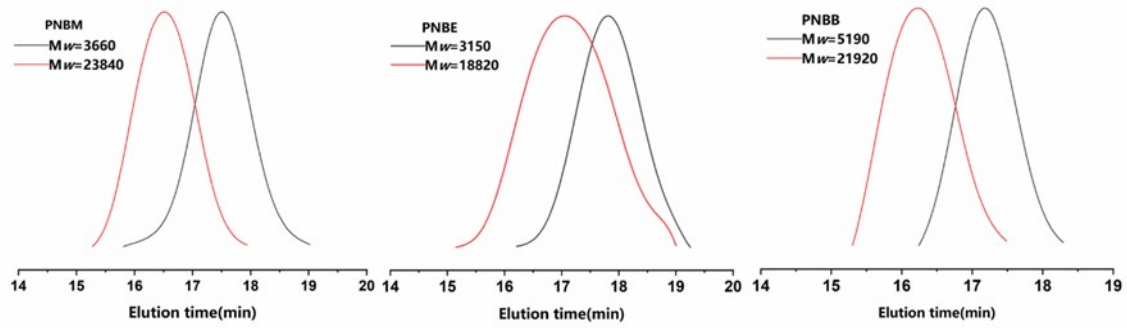


**Figure S10.** Representative GPC chromatograms of PNBM, PNBE and PNBB.

## Reference

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