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Supplementary Materials for

A Peptide Selectively Recognizes Gram-Negative Bacteria and Forms Bacteria Extracellular Trap (BET) through Interfacial Self-Assembly

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Methods

Synthesis and characterization of LPS targeting peptides (LTP)

According to patent CN01104591.4, six LPS-neutralizing peptides were selected as LTPs for evaluation in experiments. Then, six LTPs were synthesized by solid phase peptide synthesis (SPPS). Afterwards, these six LTPs were characterized by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS).

Recognition of Gram-negative bacteria by LTPs

In order to verify the selective recognition of Gram-negative bacteria by LTPs, we labeled LTPs and Polymyxin B (PMB), a peptide antibiotic with high affinity to LPS with fluorescein isothiocyanate (FITC). The molar ratio of FITC to peptide is 1:10. The FITC-labeled peptides were dissolved in DMSO to obtain a mother liquor (3 mM). The mother liquor was diluted with 0.9% normal saline to acquire a solution (30 μM, DMSO = 1%). The *Escherichia coli* (*E. coli,* ATCC 25922) and *Staphylococcus aureus* (*S. aureus,* ATCC 25923) from freezer stocks were shaken (37 °C, 16 h) to grow to saturation in 5 mL of Tryptic Soy Broth (TSB) medium, respectively. The overnight culture was diluted 1:100 into 5 mL fresh TSB and incubated at 37 °C for 4-6 h to reach the logarithmic growth period of the bacteria. Then, bacterial concentration was adjusted to 10^8 CFU/mL (OD_{600} = 0.1) by ultraviolet (UV) spectrophotometer, and serially diluted to 10^5 CFU/mL. Next, the peptide solution and *E. coli* or *S. aureus* were co-incubated in a constant temperature incubator at 37 °C for 4 hours, and the co-localization of each peptide with two bacteria were observed by confocal laser scanning microscope (CLSM).

Synthesis and characterization of BET and C-BET peptides

BET and C-BET peptides were synthesized by SPPS and then characterized by matrix-assisted laser analysis and ionization time-of-flight mass spectrometry (MALDI-TOF-MS).

Preparation and characterization of BET and C-BET NPs

BET and C-BET were dissolved in DMSO to form a mother liquor with concentration of 3 mM. The water/DMSO mixed solution with water fraction of 0-90% were prepared, then take 20 μL of mother liquor into water/DMSO mixed solution to dilute, and then detect the formation process of nanoparticles under UV spectrophotometer and fluorescence spectrophotometer.

CD characterization of BET and C-BET

BET and C-BET peptides were dissolved in hexafluoroisopropanol to obtain the mother liquor with a concentration of 30 mM, and then a concentration of 30 μM nanoparticles were prepared by gradient dilution and detected their secondary structure using Circular Dichrograph (CD). Subsequently, LPS solution was added as an inducer, and after 72 h, the secondary structure of the peptide induced by LPS was detected using circular dichrograph (CD).

Transformation of BET NPs to NFs induced by LPS

First, prepare BET nanoparticles according to the aforementioned method, and then add LPS solution (1%, w/w) to induce morphological transformation. At the time points of 0, 24, 48, and 72 h, respectively, 10 μL of the prepared solution was dropped onto the carbon support membrane and precipitated for 10 min. The residual solution was absorbed with filter paper, and then 10 μL of

phosphotungstic acid dye was added dropwise. After 5 min, absorb the dye solution with filter paper, wash the carbon support membrane 1-2 times with deionized water. Finally air-dried naturally and scanned with a biological transmission electron microscope (TEM). BET and C-BET peptides were dissolved in hexafluoroisopropanol to obtain the mother liquor with concentration of 30 mM, and then nanoparticles of the same concentration were prepared by gradient dilution.

BET NPs and C-BET NPs recognize bacteria by CLSM

First of all, subculture *E. coli* under suitable conditions to reach the logarithmic growth phase. Use an ultraviolet spectrophotometer to adjust the bacterial concentration to 1×10^8 CFU/mL, and serially dilute to 1×10^6 CFU/mL. Then centrifuge at 6000 r for 3 min and discard the culture medium. Prepare 30 μM BET and C-BET NPs with 0.9% normal saline, and add 500 μL to the centrifuge tube and mix thoroughly with a pipette. Next, BET or C-BET NPs and *E. coli* were incubated in 37 °C constant temperature incubator for 2-6 h. PBS as a control group. Subsequently, 20 μL of bacterial suspension was dropped on the center of the confocal petri dish at 2, 4, and 6 h, and a cover glass was placed for CLSM scanning. The interaction between BET/C-BET and *S.aureus* operated in the same way.

Bacterial aggregation test

First of all, subculture *E. coli* under suitable conditions to reach the logarithmic growth phase. Use an ultraviolet spectrophotometer to adjust the bacterial concentration to 1×10^8 CFU/mL, and serially dilute to 1×10^6 CFU/mL. Then centrifuge at 6000 r for 3 min and discard the culture medium. Prepare 30 μM BET and C-BET NPs with 0.9% normal saline, and add 500 μL to the centrifuge tube and mix thoroughly with a pipette. Next, BET or C-BET NPs and *E. coli* were co-incubated in incubator at 37 °C constant temperature. Then, they were taken out and observed under the dark box ultraviolet lamp to observe the bacterial aggregation at 2, 4, and 6 h, respectively. After culturing the *E. coli* to the logarithmic growth phase, use 0.9% normal saline to adjust the bacterial OD_{600} value to 0.3 as the initial bacterial density. BET NPs and C-BET NPs with concentrations of 5, 10, 20, and 40 μM were prepared respectively. Centrifuge and resuspend the bacterial suspension in 1 mL of different concentrations of BET NPs and C-BET NPs, then transfer to sterile cuvettes, measure and record the OD_{600} value every half an hour until 6 h, PBS group as a control.

SEM observation of BET and C-BET co-incubated with bacteria

First, subculture bacteria under suitable conditions to reach the logarithmic growth phase, then resuspend the bacteria in 0.9% saline and adjust the bacterial concentration to 10^8 CFU/mL with an ultraviolet spectrophotometer. The silicon wafer was cut into 6 cm squares and quickly soaked in absolute ethanol for storage after being treated by low-temperature plasma. Next, the silicon wafers were flame-dried with an alcohol lamp and placed in a 24-well plate. 1ml of bacterial suspension was added to each well to completely submerge the silicon wafers. Finally, the 24-well plate was placed in a 37 °C bacteria incubator overnight. After 24 h, the suspension was sucked out, and 1 mL of BET and C-BET were added respectively, and then placed in the bacterial incubator for 4 hours. The PBS group was used as a control. After 4 h, the drug solution was removed, washed three times with PBS, and fixed with 4% paraformaldehyde for 1 hour. Then washed twice with PBS, and dehydrated the cell gradient with 10%, 30%, 50%, 70%, 90%, and 100% ethanol for 10 min each time. Finally, rinse with tert-butanol for 10 min and let it dry naturally. After gold coating, the self-assembly behavior of BET and C-BET on the bacterial surface was detected by scanning electron microscope (SEM).

Antibacterial and cytotoxicity experiments of BET NPs and C-BET NPs *in vitro*

We applied micro broth double dilution method to determine the antibacterial activity *in vitro*. First, add 100μl of TSB to each well of the 96-well plate, then add 100 μL of the drug solution with a concentration of 640 μM to the first well, pipette fully to thoroughly mix the drug and broth, and take 100 μL from the first well and add it to the second well and thoroughly mix it with the broth. Then repeat the above operation until it is diluted to the specified minimum concentration, draw 100 μL and discard. At this time, the drug concentration in each well from left to right is 320, 160, 80, 40, 20, 10, 5, 2.5, 1.25 μM. Finally, add 100 μL of diluted bacterial solution to each well. At this time, the drug concentration of each well from left to right is 160, 80, 40, 20, 10, 5, 2.5, 1.25, 0.625 μM. Finally, a positive control group (only with bacteria liquid and no drugs) and a negative control group (blank broth) were set up, and use neomycin as an antibiotic control group. Each concentration was repeated five times. The 96-well plate was placed in a 37 °C constant temperature incubator, and after 24 h, the absorbance at 600 nm was measured with a multi-mode microplate detection system to observe the growth of bacteria.

BET treatment of a mouse model inoculated with *E. coli*

The animal experiments were carried out in compliance with NIH guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Welfare and Ethics Committee of the National Center for Nanoscience and Technology, Chinese Academy of Sciences, with approval number NCNST2021-2104-0602. Female BALB/c mice between 4 and 6 weeks were divided into five groups, and each group involved six mice. *E. coli* (10^8 CFU/mL) suspended in 100 μL of 0.9% saline was injected through the tail vein in four groups and 100 μL of 0.9% saline in the blank group. Experimental group was intravenously injected with BET (150 μL, 100 and 200 μM) NPs 0.5 h later. Control group was intravenously injected with Neomycin (150 μL, 200 μM) and PBS (150 μL), respectively. Then, health condition of mice was monitored every 3 h for the first 24 h, and then every 8 h for up to 72 h. The mice were euthanized immediately if they showed signs of inability to eat and drink, respiratory distress, or mobility loss. All mice were sacrificed at day 8 to collect major organs for observation. Meanwhile, serum samples were harvested from test animals at day 0, 1, and 8. Serum samples were used to detect TNF-α and IL-6. Detailed steps were referred to the ELISA kit protocol.

Safety assessment of BET

Three healthy BALB/c mice were treated with 200 μM of BET (150 μL) every day for 14 d via intraperitoneal injection. Weight was collected every day. After treatment, all mice were sacrificed to collect blood for blood chemistry index testing according to experiment protocol reported before. All major organs were also collected for H&E staining and stored in 4% PFA by Servicebio Technology.

Statistical analysis.

The data were analyzed using the GraphPad Prism8 software statistics package. Results were given in the form of mean ± standard deviation (SD).

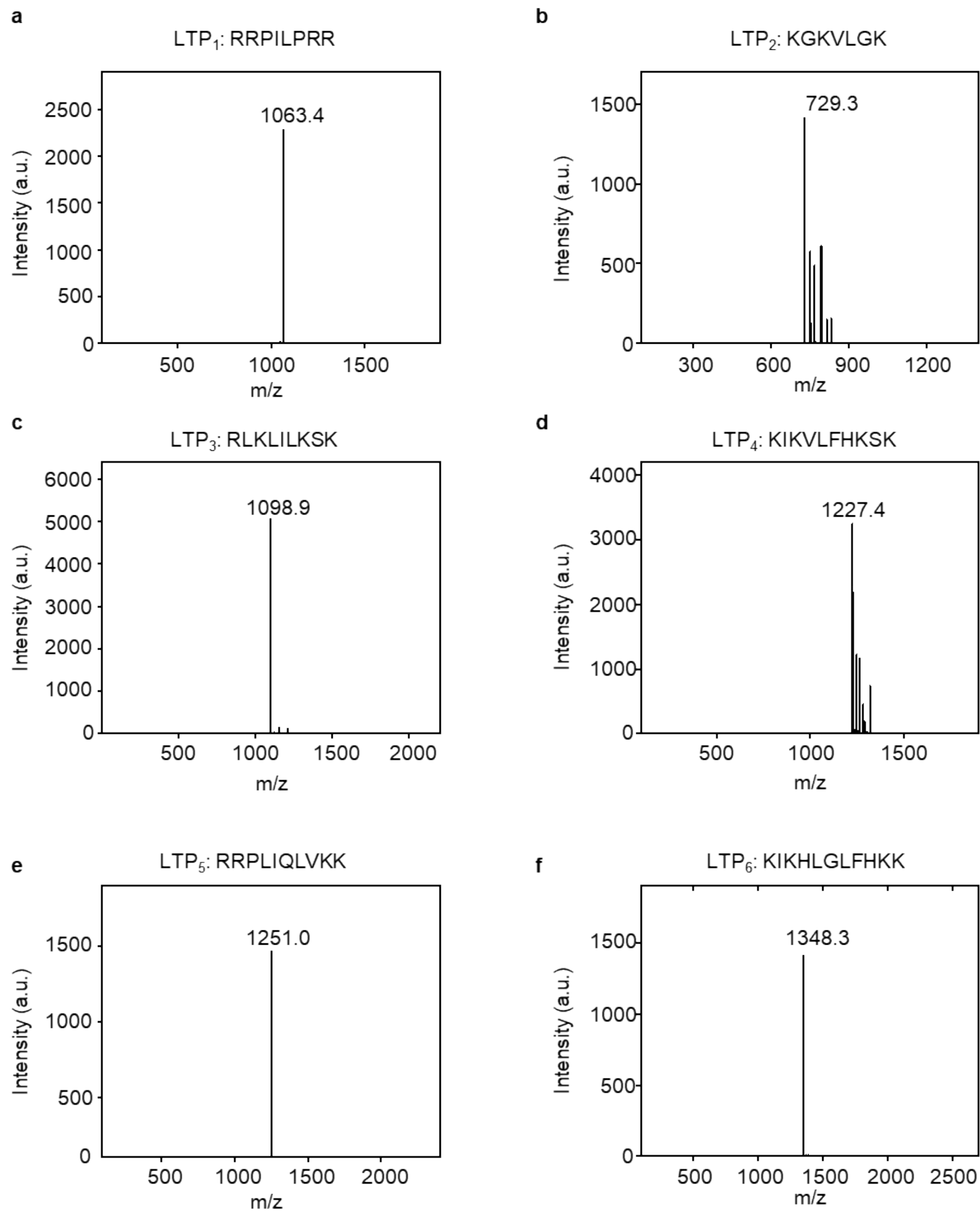


Fig S1. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) of **a)** LTP_1, **b)** LTP_2, **c)** LTP_3, **d)** LTP_4, **e)** LTP_5, **f)** LTP_6.

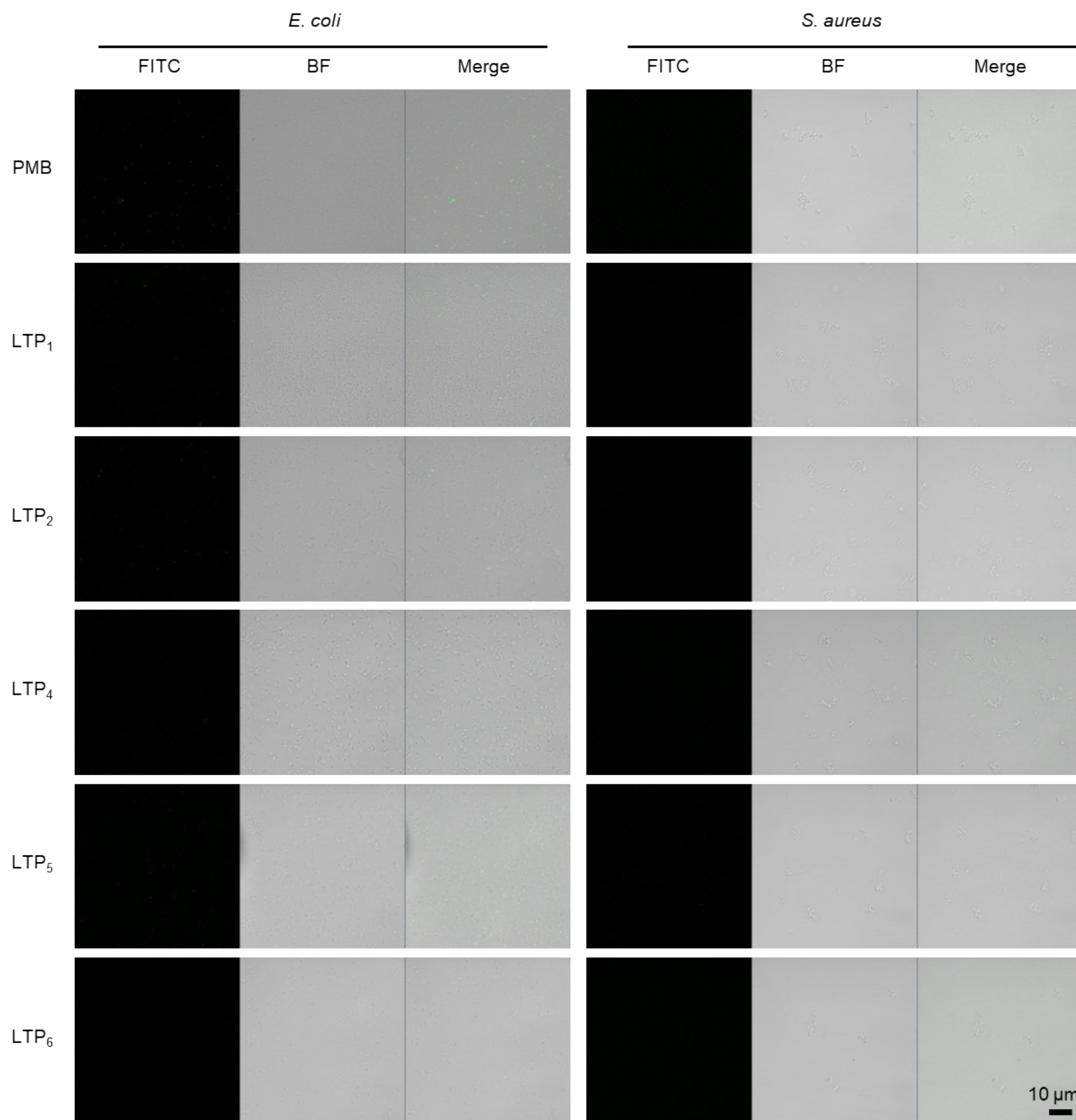


Fig S2. Confocal laser scanning microscopy (CLSM) images of peptides (30 μM) treated 10^6 CFU/mL of Gram-negative *E. coli* and Gram-positive *S. aureus*. Scale bar, 10 μm.

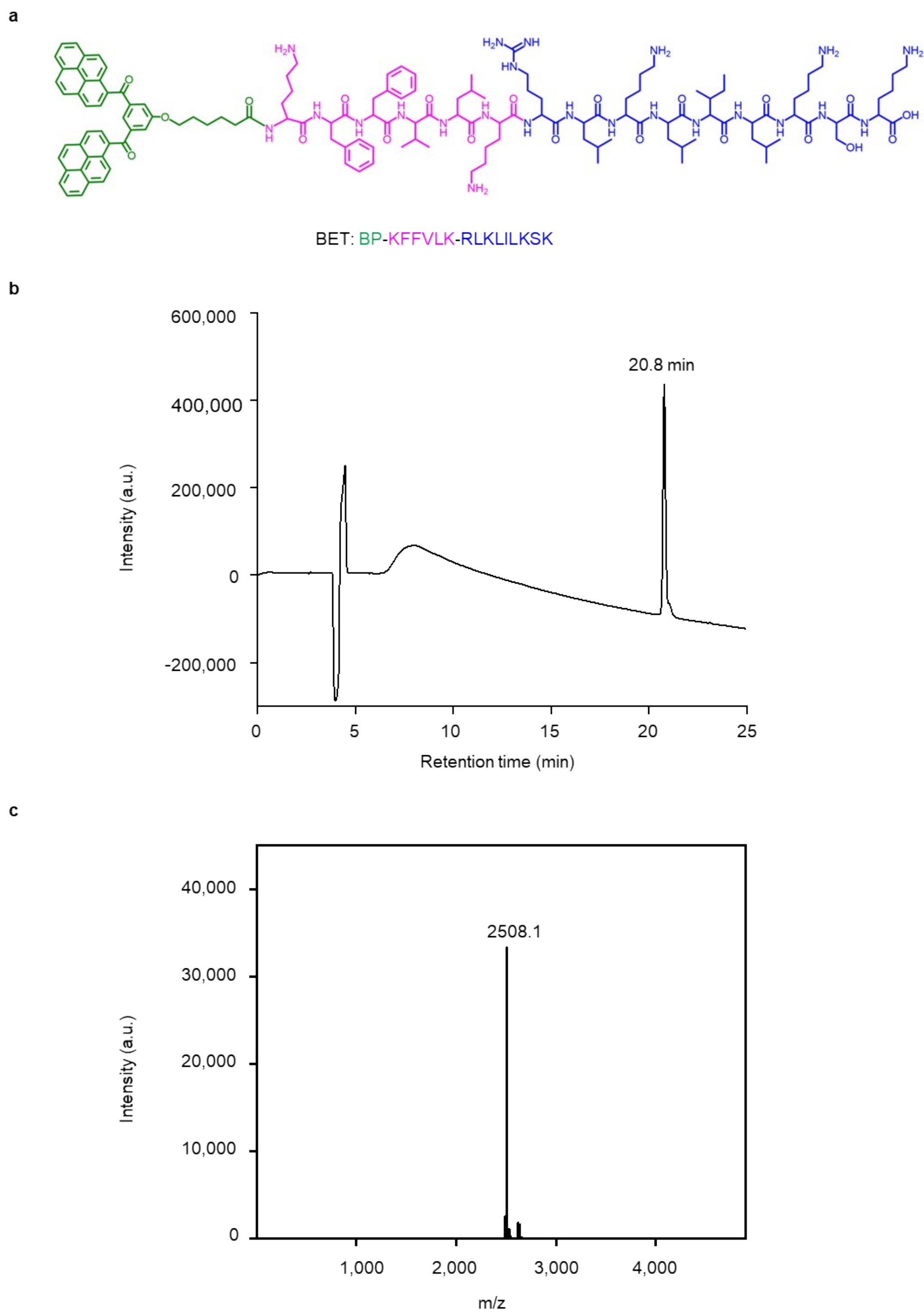


Fig S3. a) Molecular structure of BET peptide, **b)** HPLC spectra and c) MALDI-TOF-MS. The method of HPLC spectra was as follows: solvent A, 0.1% trifluoroacetic acid in 100% water; solvent B, 0.1% formic acid in 100% acetonitrile; 0 min, 5% solvent B, 25 min, 70% solvent B.

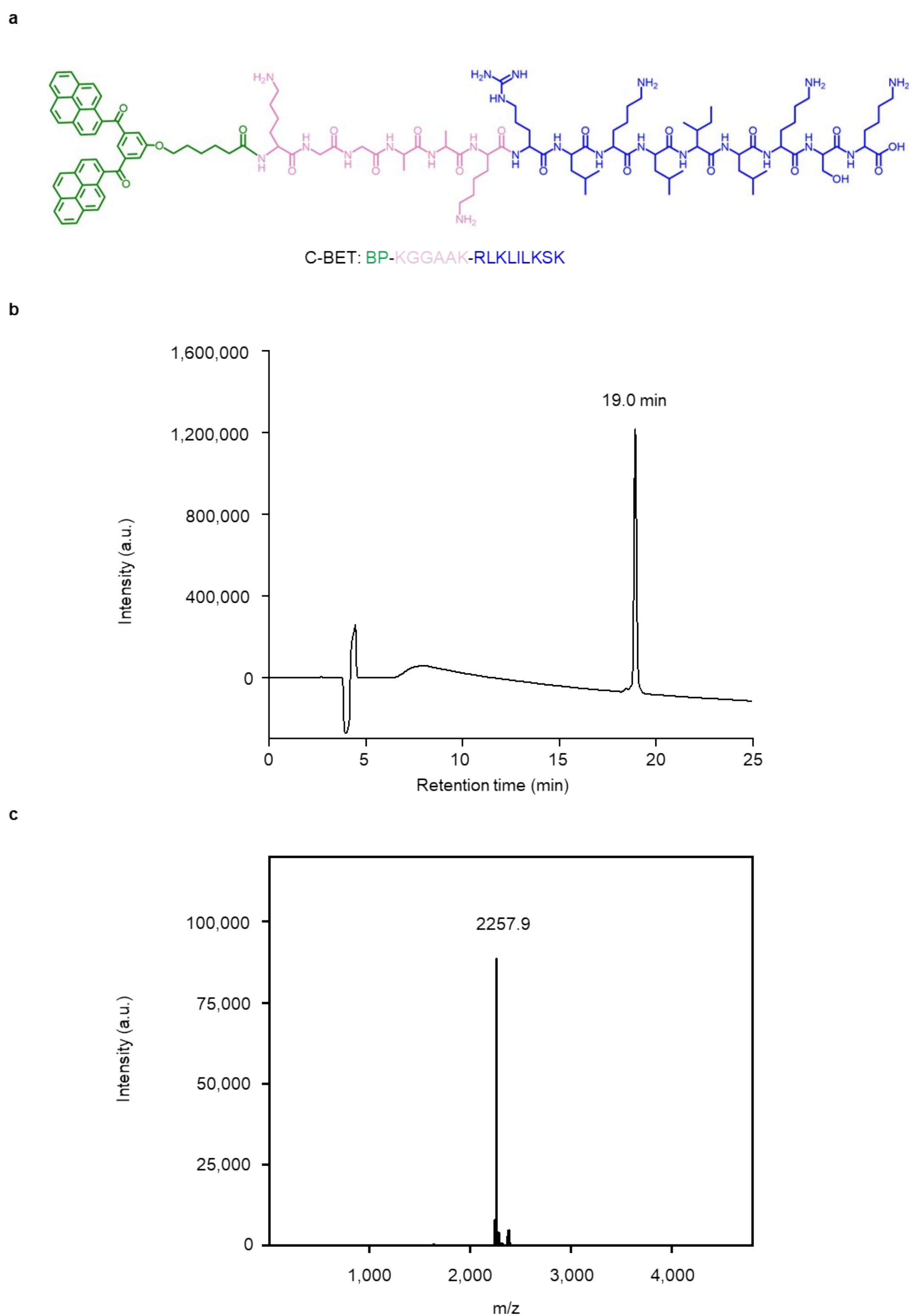


Fig S4. a) Molecular structure of C-BET peptide, **b)** HPLC spectra and c) MALDI-TOF-MS. The method of HPLC spectra was as follows: solvent A, 0.1% trifluoroacetic acid in 100% water; solvent B, 0.1% formic acid in 100% acetonitrile; 0 min, 5% solvent B, 25 min, 70% solvent B.

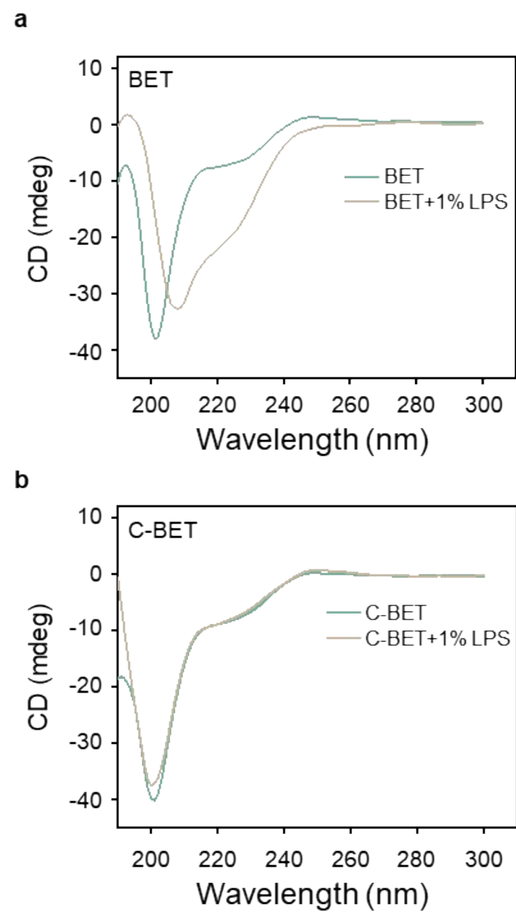


Fig S5. CD spectrum of **a**) BET (30 μM) and BET (30 μM) + LPS (1 μg/mL) and **b)** C-BET (30 μM) and C-BET (30 μM) + LPS (1 μg/mL).

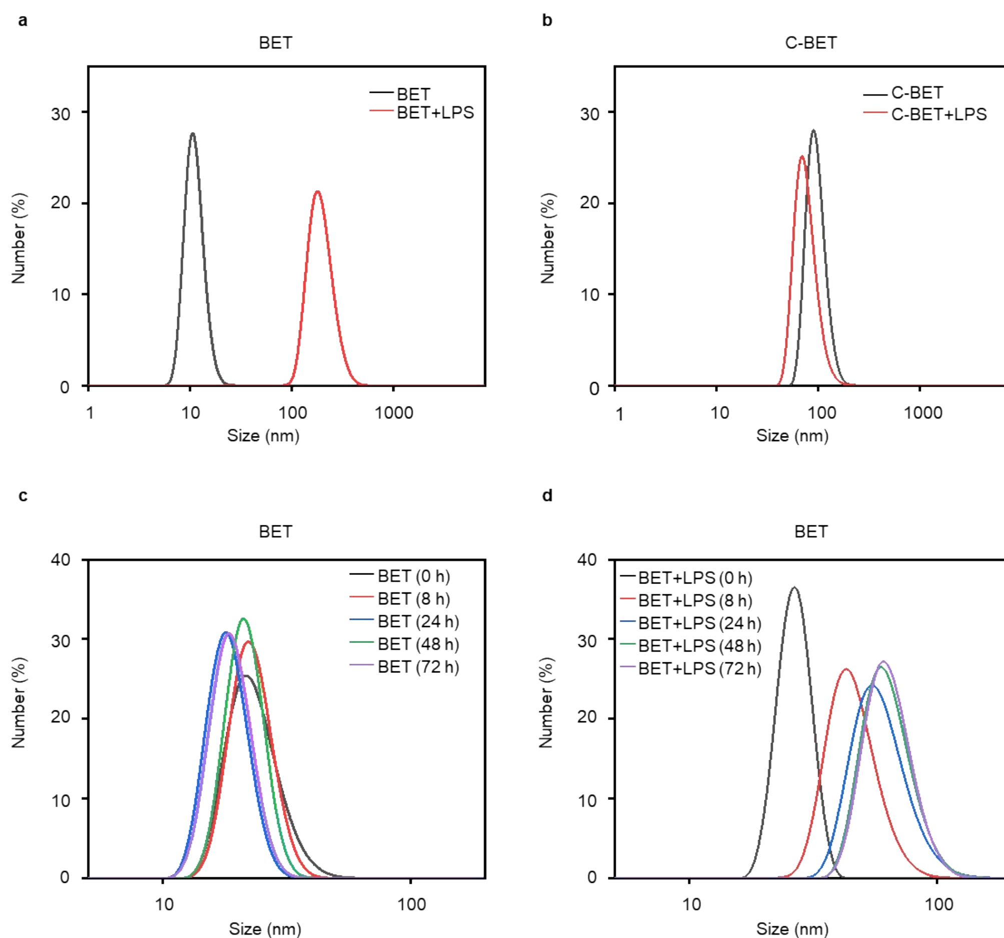


Fig S6. Dynamic light scattering (DLS) of **a**) BET (30 μM) and BET (30 μM) + LPS (1 μg/mL) at day 3. **b)** C-BET (30 μM) and C-BET (30 μM) + LPS (1 μg/mL) at day 3. **c)** BET (30 μM) at different time spans of 0, 8, 24, 48, 72 h. **d)** BET (30 μM) + LPS (1 μg/mL) at different time spans of 0, 8, 24, 48, 72 h.

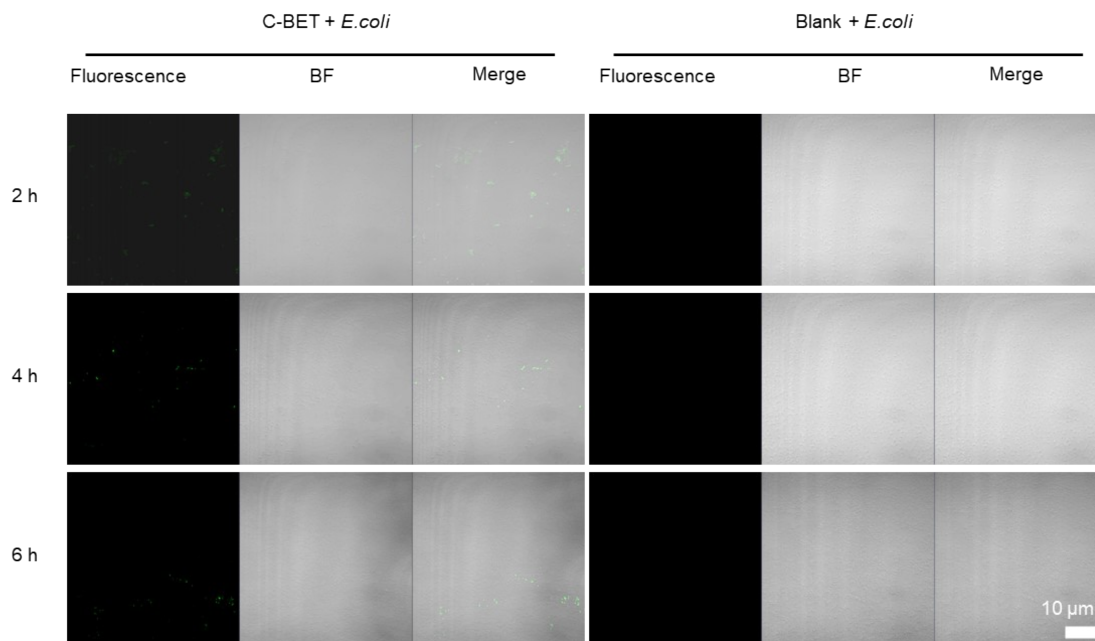


Fig S7. Confocal laser scanning microscope observation of C-BET (30 μM) co-incubated with 10^6 CFU/mL of *E. coli* for 2, 4, and 6 h. Scale bar, 10 μm.

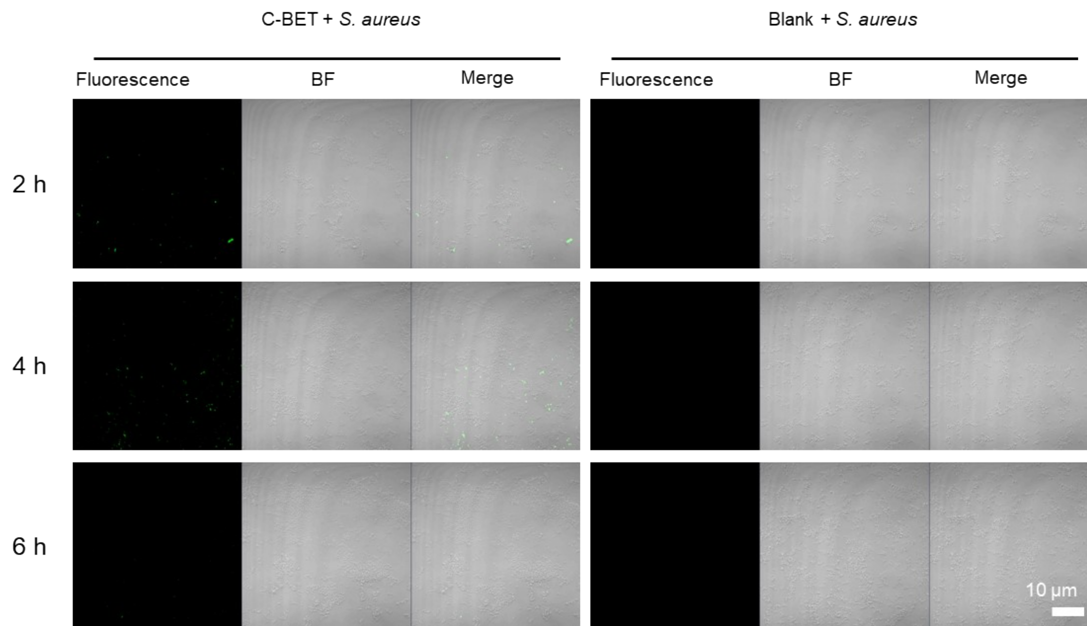


Fig S8. Confocal laser scanning microscope observation of C-BET (30 μM) co-incubated with 10^6 CFU/mL of *S. aureus* for 2, 4, and 6 h. Scale bar, 10 μm.

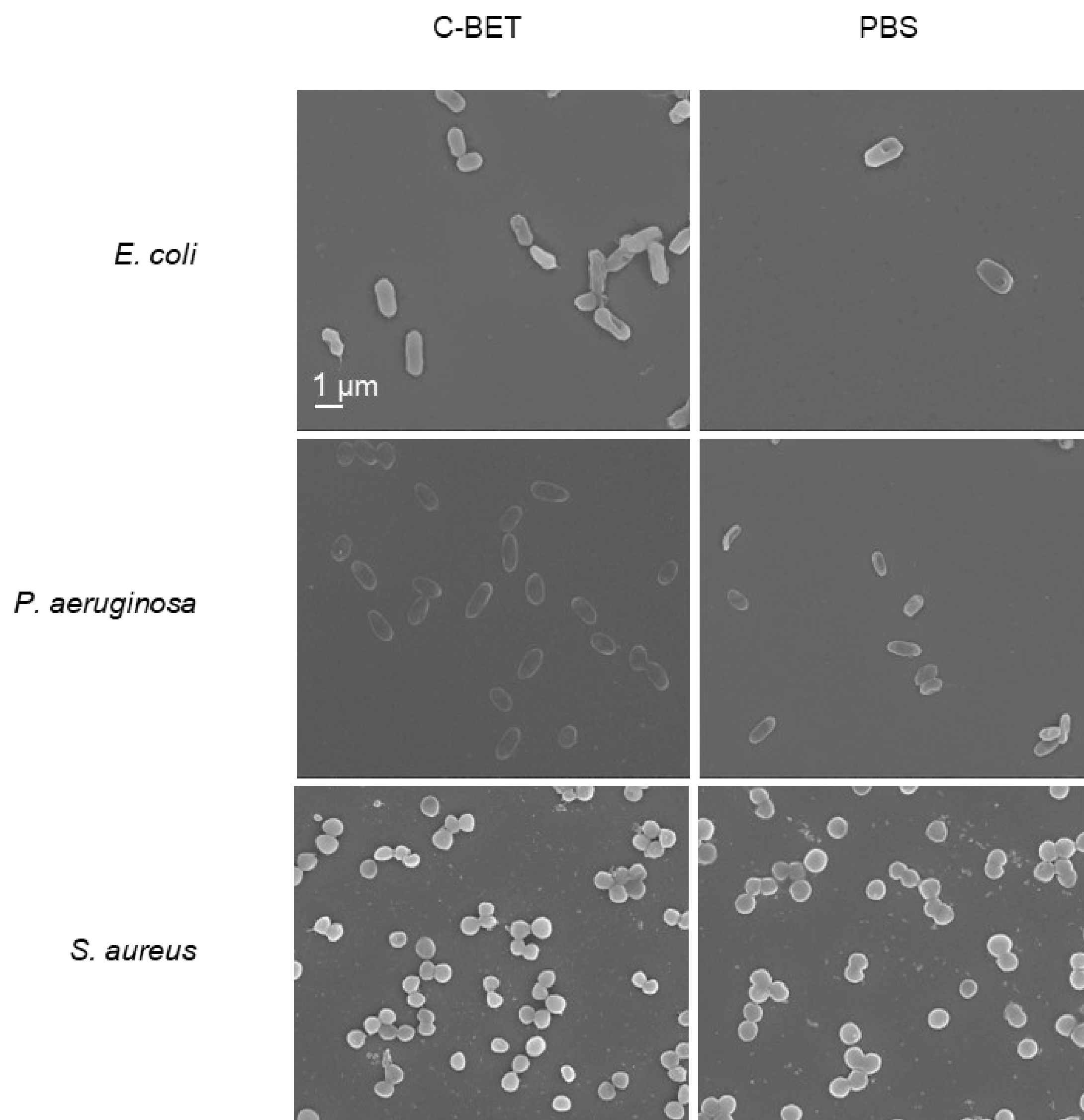


Fig S9. SEM characterization of interaction between C-BET (30 μM) or PBS and three bacteria strains (10^6 CFU/mL). Scale bar, 1 μm.

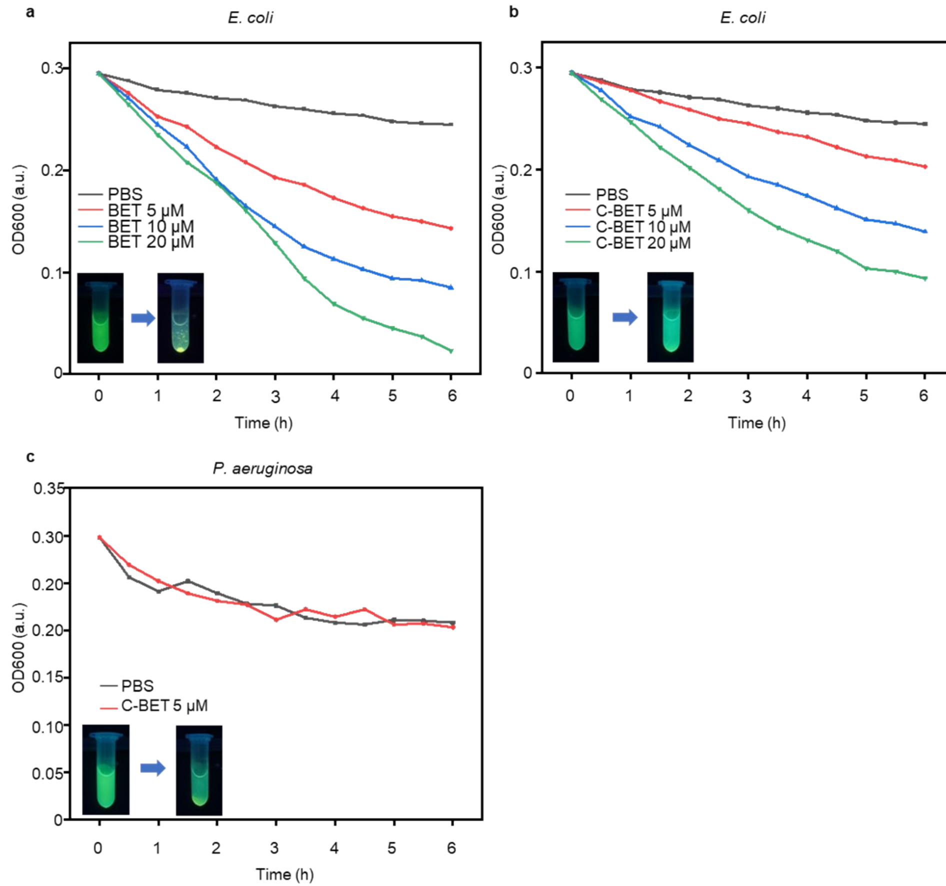


Fig S10. OD_{600} characterization of concentration gradient of **a)** BET, **b)** C-BET treated with *E coli* (10^6 CFU/mL) and **c)** C-BET treated with *P. aeruginosa* (10^6 CFU/mL). Inserted images showed bacteria aggregation under a dark box ultraviolet lamp.

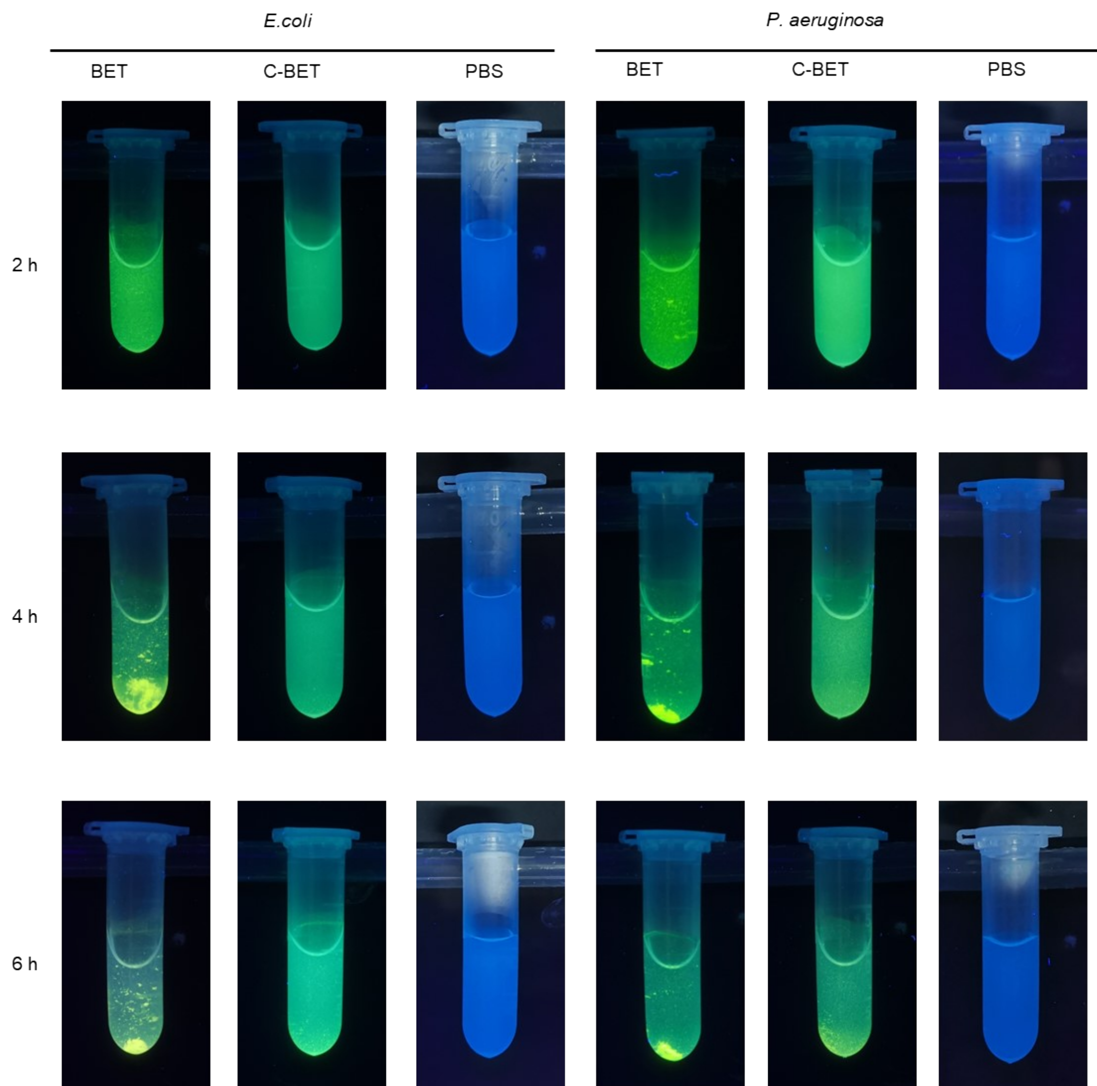
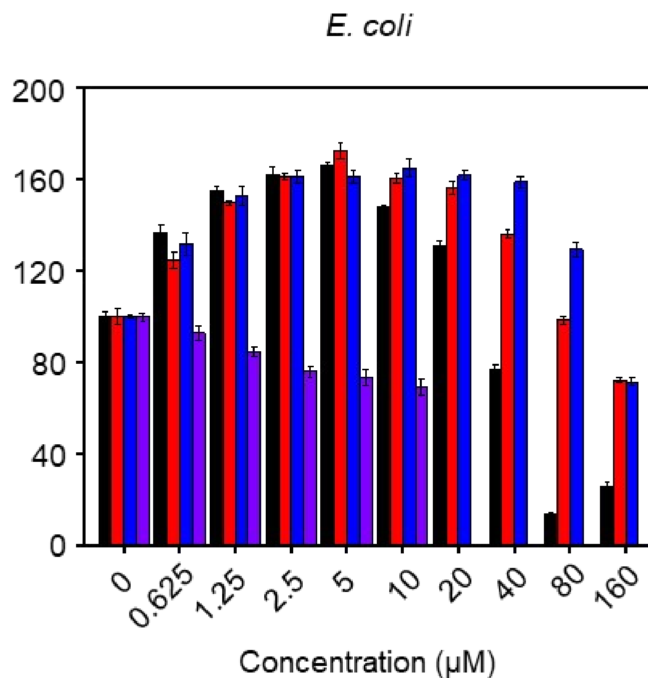


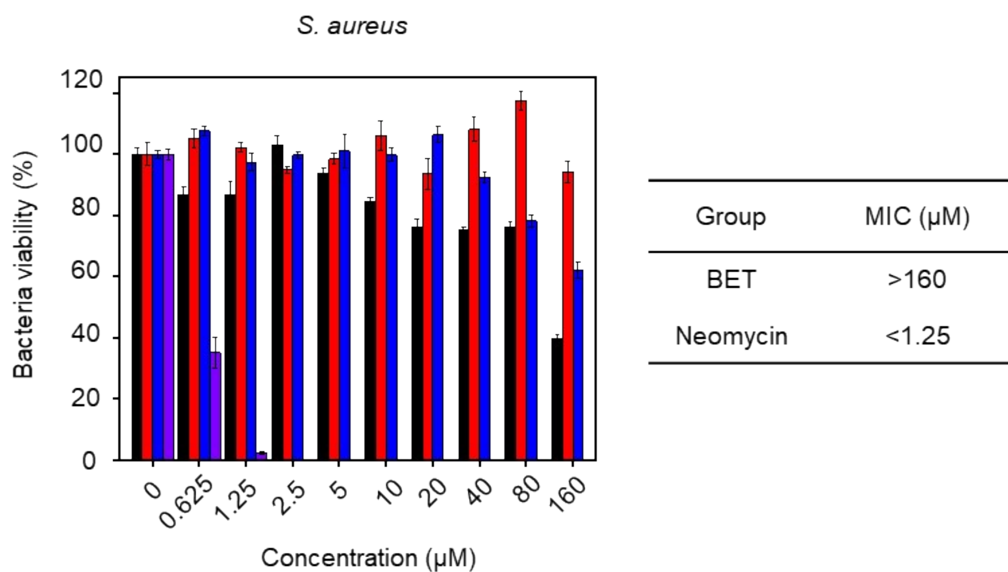
Fig S11. Observation of BET (20 μM), C-BET (20 μM) and PBS treated with *E. coli* (10^6 CFU/mL) and BET (5 μM), C-BET (5 μM) and PBS treated *P. aeruginosa* (10^6 CFU/mL) at 2, 4, and 6 h.

BET C-BET LTP_3 Neomycin



Group	MIC (μM)
BET	>160
Neomycin	<20

Fig S12. Antimicrobial effect of BET, C-BET, LTP_3 and Neomycin treated *E. coli* (10^8 CFU/mL) for 24 h.



Group	MIC (μM)
BET	>160
Neomycin	<1.25

Fig S13. Antimicrobial effect of BET, C-BET, LTP_3 and Neomycin treated *S. aureus* (10^8 CFU/mL) for 24 h.

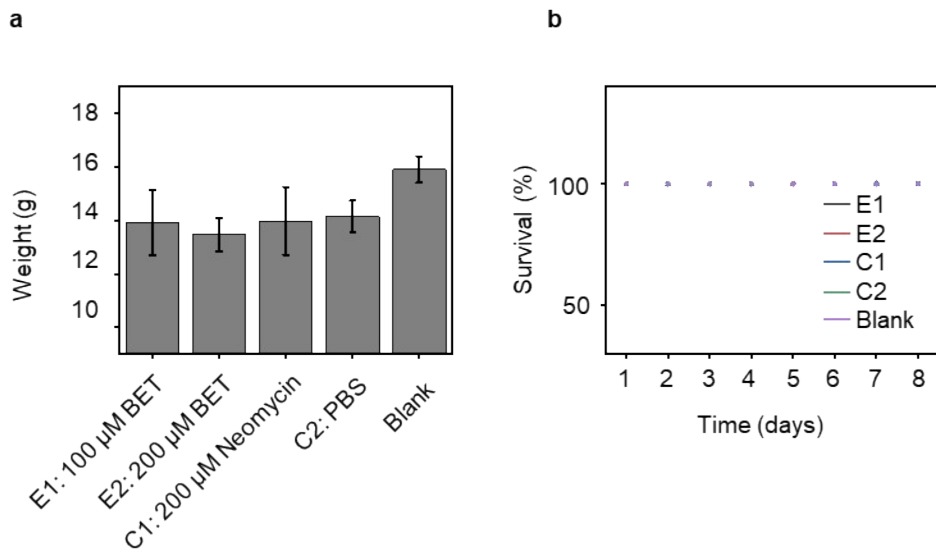


Fig S14. a) Weight of treated mice at end of experiment (day 8). **b)** Survival of mice.