Electronic Supplementary Information

Antimicrobial Peptide with the Aggregation-Induced Emission (AIE) Luminogen for Studying Bacterial Membrane Interactions and Antibacterial Actions

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Experimental Section

Materials

Dimethylphenylphosphine (DMPP, 99%), 1-bromo-1,2,2-triphenylethylene (98%), 4-(hydroxymethyl)phenylboronic acid (98%), methacryloyl chloride (98%) and tetrakis(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$, 97%) were purchased from J&K Scientific (Beijing, China). Thiolated antimicrobial peptide (CysHHC10, Sequence: Cys-Lys-Arg-Trp-Trp-Lys-Trp-Ile-Arg-Trp-NH_2) was purchased from ChinaPeptides Co., Ltd. (Shanghai, China). *Escherichia coli* (*E. coli*, ATCC 25922), *Pseudomonas aeruginosa* (*P. aeruginosa*, ATCC 15692), *Staphylococcus aureus* (*S. aureus*, ATCC 25923) and *Staphylococcus epidermidis* (*S. epidermidis*, ATCC 12228) were obtained from the American Type Culture Collection. All other regents were purchased from J&K Scientific or Aladdin Reagent Co., and were used without further purification.

Synthesis of 4-(1,2,2-triphenylethenyl)benzenemethanol (TPEOH)[1]

1-Bromo-1,2,2-triphenylethylene (2.0 g, 6.0 mmol) and 4-(hydroxymethyl)phenylboronic acid (1.35 g, 9.0 mmol) were dissolved in the mixture of tetrahydrofuran (40 mL) and 1.2 M potassium carbonate aqueous solution (20 mL). The mixture was stirred at room temperature for 30 min under argon gas, followed by the addition of $Pd(PPh_3)_4$ (50 mg, 4.3×10^{-3} mmol). The reaction mixture was heated to reflux for 18 h. After cooling down to room temperature, the mixture was poured into 150 mL of doubly distilled water and extracted three times with 50 mL of ethyl acetate. The organic layer was combined and dried over anhydrous sodium sulfate. After

removing the solvent under reduced pressure, the residue was chromatographed on a silica gel column using dichloromethane/hexane (v/v = 1:2) as the eluent to give a white powder. ^{1}H NMR (600 MHz, $CDCl_3$-*d*, δ, ppm): 4.60 (CH_2-OH, 2H), 6.98 - 7.15 (aromatic protons, 19H).

Synthesis of 4-(1,2,2-triphenylethenyl)benzenemethyl methacrylate (TPEMA)

TPEOH (0.6 g, 1.6 mmol) and triethylamine (0.3 g, 3.0 mmol) were dissolved in 20 mL of dichloromethane. Under cooling, methacryloyl chloride (0.3 g, 3.0 mmol) was added and the whole mixture was stirred at room temperature for 12 h. After filtration, the filtrate was washed twice with 30 mL of deionized water and dried over Na_2SO_4. The solvent was removed, and the residue was chromatographed on a silica gel column using dichloromethane/hexane (v/v = 1:3) as the eluent to give a white powder. ^{1}H NMR (600 MHz, $CDCl_3$-*d*, δ, ppm): 1.99 (CH_3-C, 3H), 5.14 (CH_2-O, 2H), 5.60 (CH_2=C, 1H), 6.16 (CH_2=C, 1H) and 7.00 - 7.17 (aromatic protons, 19H).

"Click" synthesis of tetraphenylethene (TPE)-functionalized CysHHC10 (TPE-AMP) via thiol-ene addition

TPEMA (55.6 mg, 0.13 mmol) and CysHHC10 (100.0 mg, 0.065 mmol) were dissolved in 20 mL of *N*,*N*-dimethylformamide (DMF) in a 50-mL round bottom flask. One hundred μL of DMPP (1.8 mg, 0.013 mmol) stock solution (18.0 mg/mL in DMF) was added into the reaction mixture. After sealing, the reaction mixture was stirred at room temperature for 48 h, followed by dialysis (MWCO = 500 Da) against methanol for 3 days. The solution was filtrated and concentrated using a rotary evaporator. The product

was then dissolved in 30 mL of deionized water, passed through a syringe filter (0.2 μm), and lyophilized. HRMS (MALDI-TOF): m/z 1976.4620 ([M + H^+], calcd 1977.0258). HPLC analyzed purity: 90.1%.

Determination of antimicrobial properties of TPE-AMP

Minimum inhibitory concentration (MIC) of TPE-AMP was determined by the broth microdilution method. *E. coli*, *P. aeruginosa*, *S. aureus* and *S. epidermidis* were cultured according to the ATCC protocols/specifications. The bacterial suspensions were then dispersed in Mueller Hinton Broth (MHB) to give rise to a final concentration of 5×10^5 bacterial cells per mL. Stock solution of TPE-AMP were prepared in PBS (pH = 7.4), and then serially diluted by 2-fold each time using MHB. Each well of a 96-well plate was filled with 100 μL of medium containing TPE-AMP, to which 100 μL of bacterial suspension (5×10^5 bacterial cells per mL) was added. The plate was incubated at 37 °C for 18 h, and MIC was recorded as the lowest concentration of TPE-AMP that inhibited bacteria growth detected by naked eye.

Fluorescence response of TPE-AMP upon the addition of bacteria

Stock solution of TPE-AMP were prepared in PBS (pH = 7.4) at a concentration of 0.2 mg/mL. *E. coli*, *P. aeruginosa*, *S. aureus* and *S. epidermidis* were cultured according to the ATCC protocols/specifications. The bacterial suspensions were centrifuged at 2700 rpm for 10 min. After removal of the supernatant, *E. coli*, *P. aeruginosa*, *S. aureus* and *S. epidermidis* were dispersed in separate PBS (pH = 7.4) solutions to give rise to a final concentration of 10^7 cell per mL. The bacterial suspensions was then mixed with

stock solution of TPE-AMP with an equal volume. After vortexing, the mixtures were immediately recorded their fluorescence spectra on a Shimadzu RF-5301PC spectrofluorometer with an excitation wavelength of 350 nm. The fluorescence spectra of bacteria were recorded using the bacterial suspensions (10^7 cell per mL) mixed with equal volume of PBS solution. Bacteria treated with TPE-AMP were filtrated through 0.22 μm syringe filter, and were recorded their fluorescence spectra with an excitation wavelength of 350 nm.

Studying the interactions between TPE-AMP and bacteria by flow cytometry

E. coli, *P. aeruginosa*, *S. aureus* and *S. epidermidis* were dispersed in separate PBS (pH = 7.4) solutions to give rise to a final concentration of 10^7 cell per mL. The bacterial suspensions was then mixed with stock solution of TPE-AMP with an equal volume. After vortexing, the fluorescence intensities of bacteria were analyzed on a BD LSR Fortessa Flow Cytometry Analyser equipped with four laser lines (405, 488, 561, and 640 nm). Data were acquired using the 405 nm laser and processed by Summit Software v4.3.

Studying the interactions between TPE-AMP and bacteria by confocal microscopy

E. coli, *P. aeruginosa*, *S. aureus* and *S. epidermidis* were dispersed in separate PBS (pH = 7.4) solutions to give rise to a final concentration of 10^7 cell per mL. The bacterial suspensions was then mixed with stock solution of TPE-AMP with an equal volume. After vortexing, the bacterial suspensions were immediately transferred to glass cover-

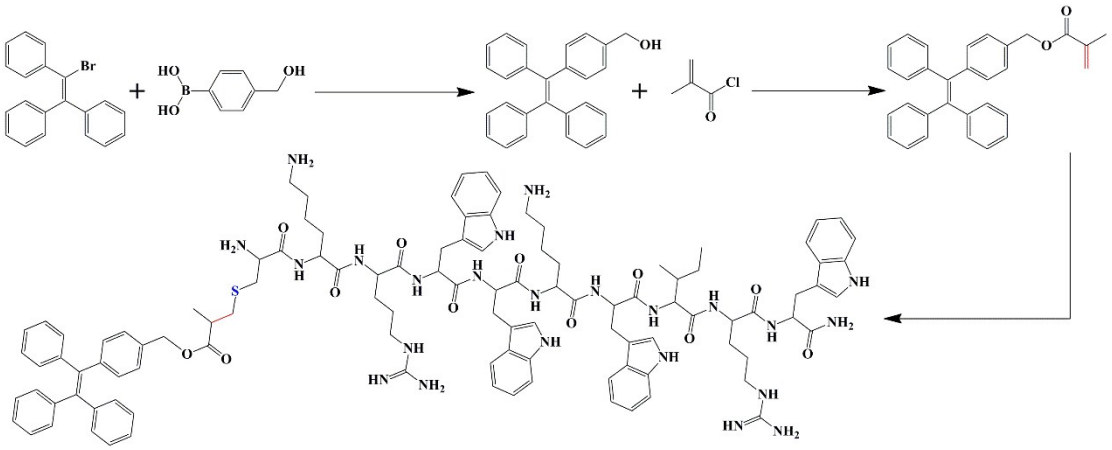
slips without washing. The fluorescence images of bacteria were viewed on a confocal laser scanning microscope (CLSM, Olympus FV1000). Images were acquired using the 405 nm laser and processed by FV10-ASW 4.2 Viewer.

In vivo toxicity of TPE-AMP

For the evaluation of *in vivo* toxicity, TPE-AMP or CysHHC10 was injected into five-week-old ICR mice intravenously through the tail vein at a dose of 5 mg/kg. After 5 days post-administration, liver, heart, lung, kidney and femoral muscle were collected. The tissue were embedded in paraffin and stained with hematoxylin and eosin (H&E) and ED-1 for histological examination.

Characterization

The chemical structures of synthetic compounds were characterized by ^{1}H NMR spectroscopy on a Bruker DRX 400 MHz spectrometer. The optical absorbance for bacterial work was measured on a microplate reader (BioTek Instruments ELX800, USA). The UV-visible absorbance of TPE-AMP was conducted with a Shimadzu UV-2550 spectrophotometer. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) of TPE-AMP was carried out with an AB SCIEX TOF/TOF MS 5800 System (Sciex, Framingham, MA).



Scheme S1. Synthesis route of TPEOH, TPEMA and TPE-AMP.

Table S1. MIC values for CysHHC10 and TPE-AMP

Samples	MIC (μM)			
	E. coli	*P. aeruginosa*	*S. aureus*	*S. epidermidis*
CysHHC10[2]	10.1	20.2	2.5	1.3
TPE-AMP	15.8	31.6	15.8	7.9

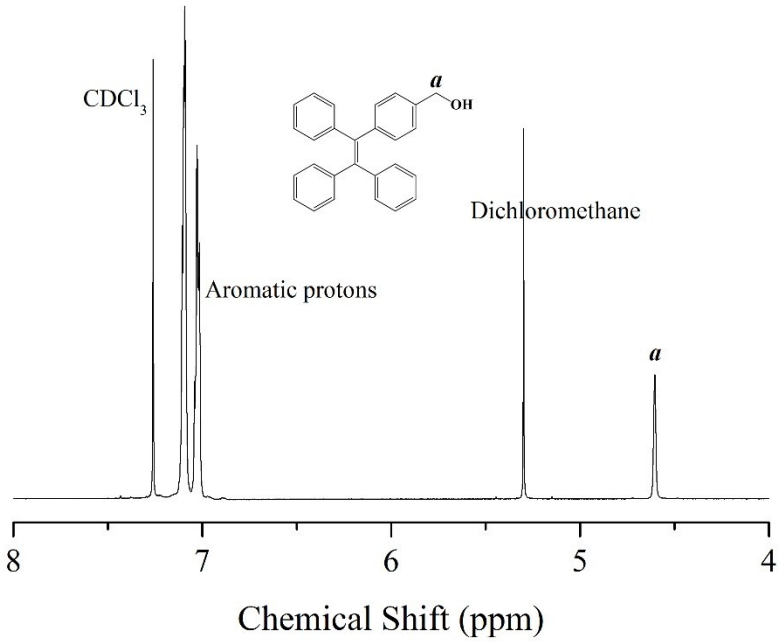


Figure S1. ^{1}H NMR spectrum of TPEOH.

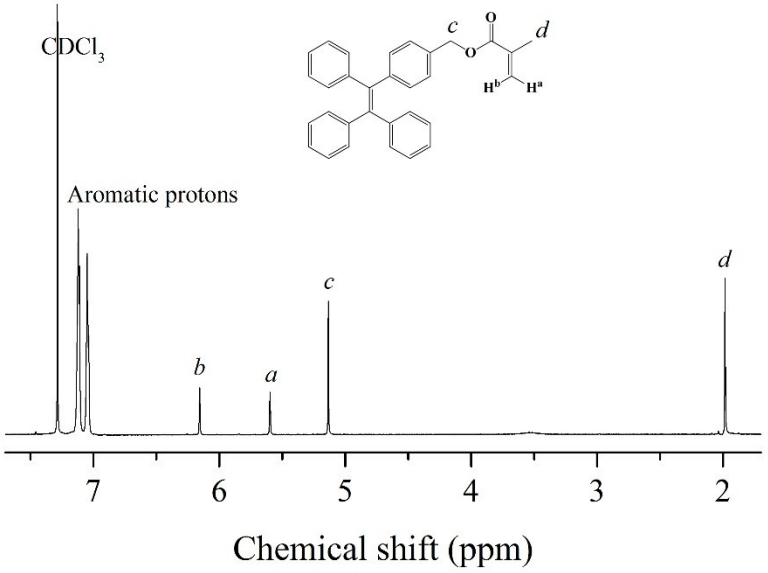


Figure S2. ^{1}H NMR spectrum of TPEMA.

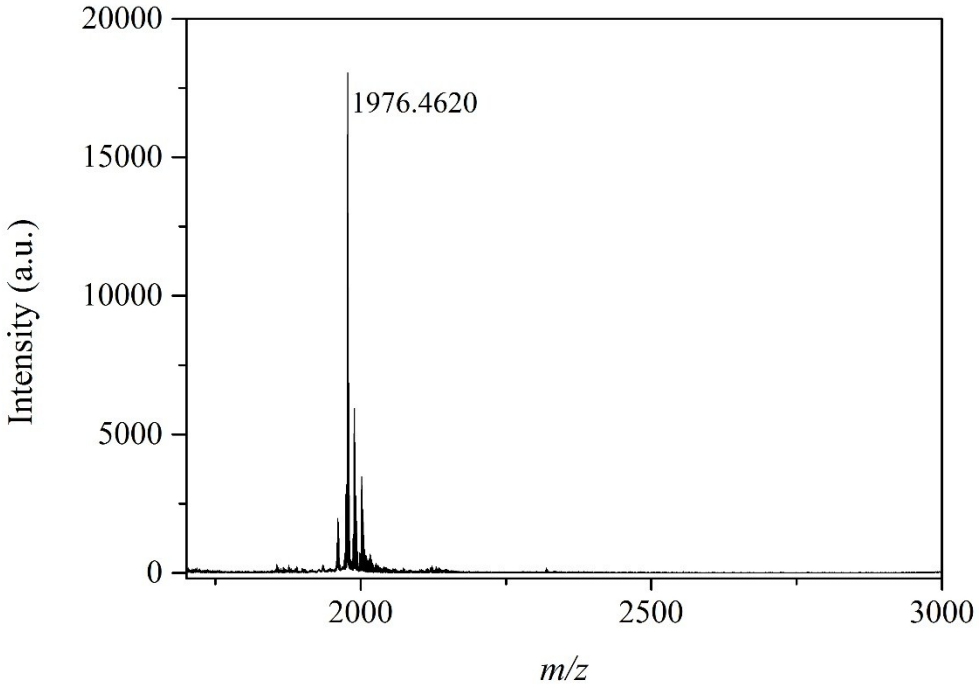


Figure S3. MALDI-TOF mass spectrum of TPE-AMP.

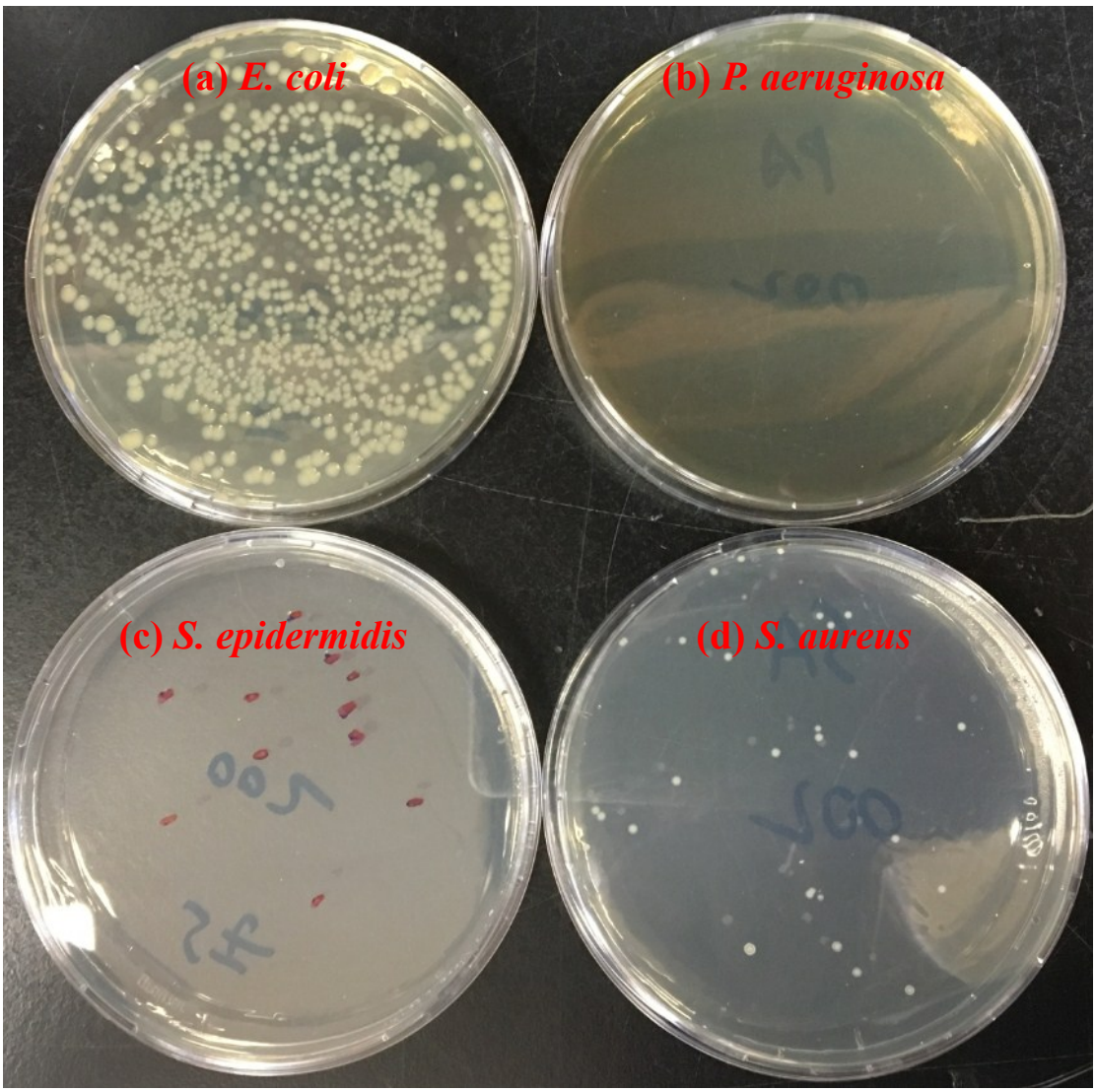


Figure S4. Photograph of agar plates spread with TPE-AMP-treated *E. coli*, *P. aeruginosa*, *S. aureus* and *S. epidermidis*. The colonies of *P. aeruginosa* appear yellow green in color, and cover all of the agar plate. *S. epidermidis* forms a small colony on the agar plate, and the colonies are highlighted in red color.

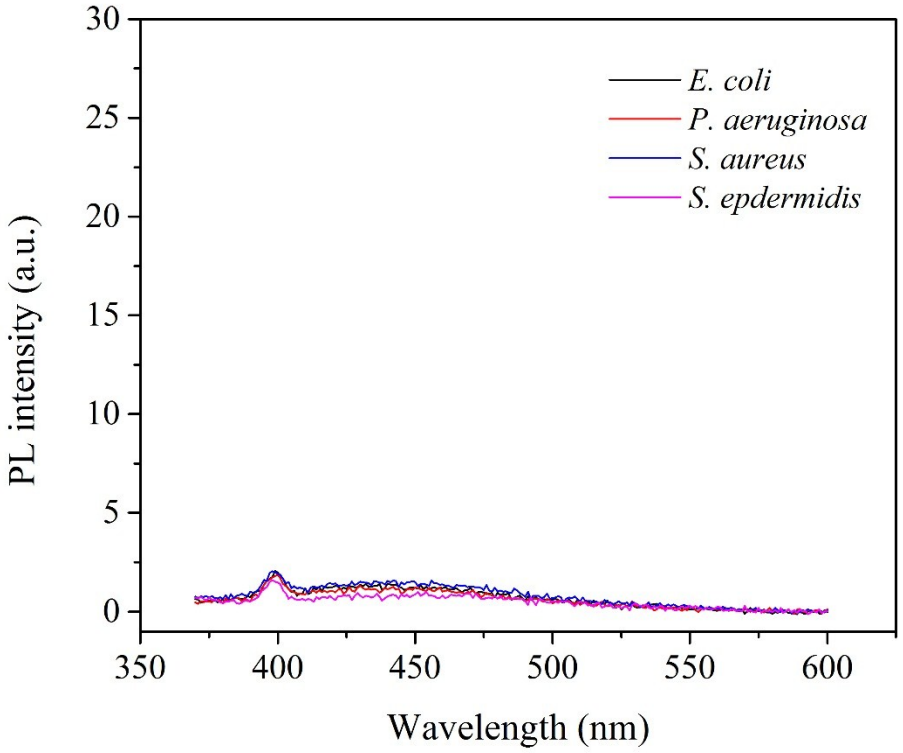


Figure S5. PL spectra of *E. coli*, *P. aeruginosa*, *S. aureus* and *S. epidermidis* in PBS solution (5×10^6 cells/mL, pH = 7.4) with an excitation wavelength of 350 nm.

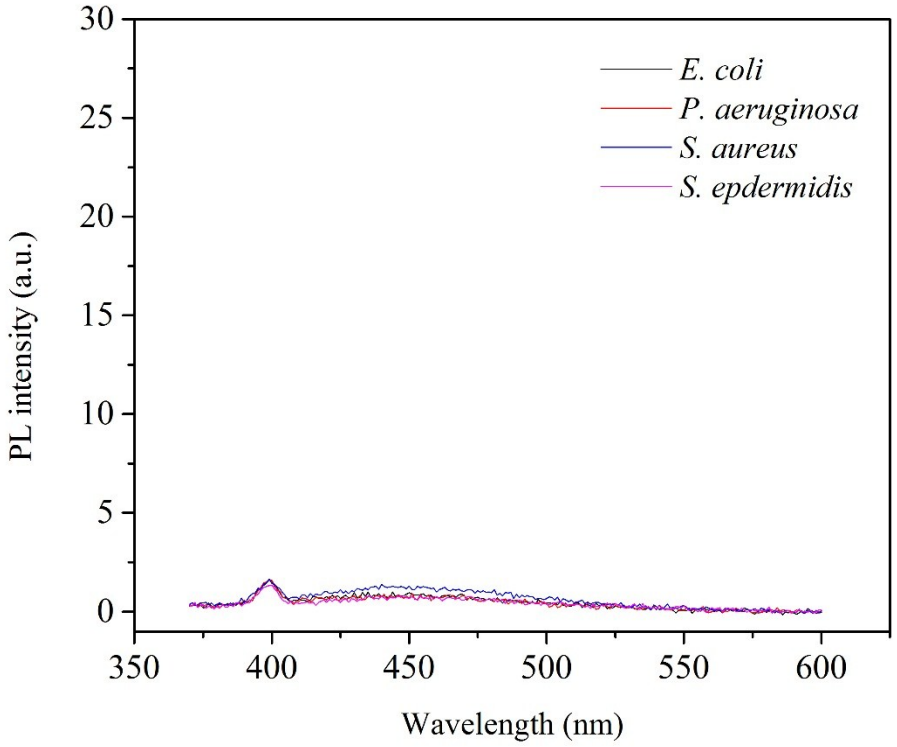


Figure S6. PL spectra of bacteria (*E. coli*, *P. aeruginosa*, *S. aureus* and *S. epidermidis*) and TPE-AMP mixtures in PBS (pH = 7.4) after passing through the 0.22 μm syringe filters with an excitation wavelength of 350 nm.

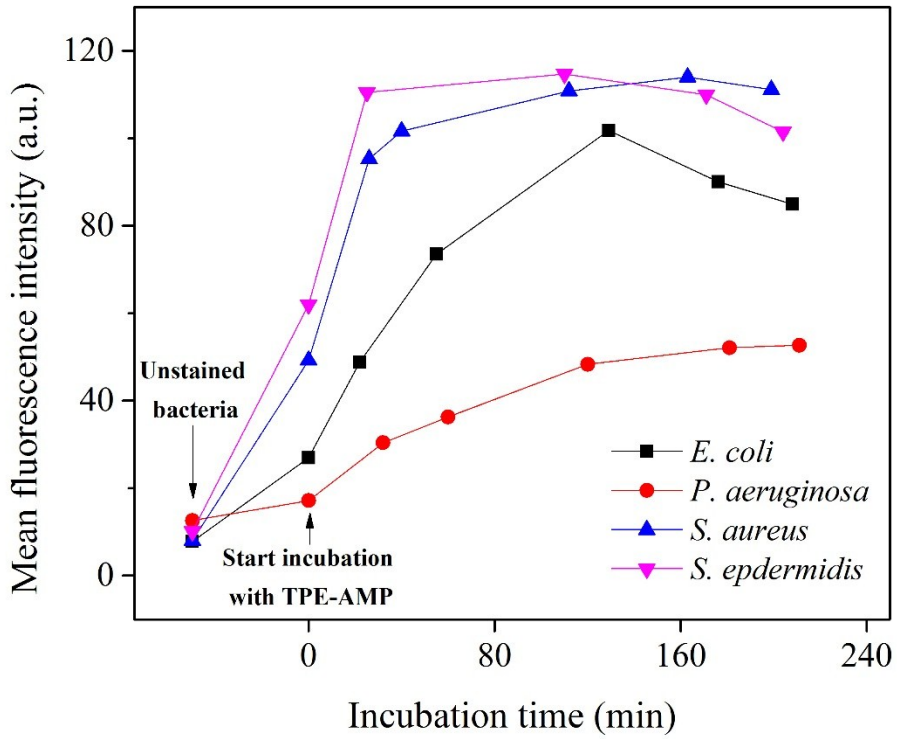


Figure S7. The evolution of the mean fluorescence intensity (MFI) in the flow cytometry histograms of *E. coli*, *P. aeruginosa*, *S. aureus* and *S. epidermidis* after incubation with TPE-AMP.

References

[1] H. Li, X. Zhang, X. Zhang, B. Yang, Y. Yang, Y. Wei, *Polym. Chem.* **2014**, *5*, 3758-3762.

[2] X. Y. Cai, J. Z. Li, N. N. Li, J. C. Chen, E.-T. Kang, L. Q. Xu, *Biomater. Sci.* **2016**, *4*, 1663-1672.