Electronic Supplementary Information

Peptide-based aggregation-induced emission bioprobe for selective detection and photodynamic killing of Gram-negative bacteria

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Experimental

Materials and characterization

All chemical and biological supplies were purchased from Sigma-Aldrich. The peptide polymyxinB-SH was purchased from ChinaPeptides Co., Ltd. (Shanghai, China). UV-vis absorption spectra were recorded on a Shimadzu UV-1700 spectrometer. Photoluminescence (PL) spectra were recorded on a Perkin-Elmer LS 55 spectrofluorometer. Bioimaging study was performed by confocal laser scanning microscopy (CLSM, Zeiss LSM 710, Jena, Germany).

Synthesis of Mal-Pra

To the solution of 2-chlorotrityl choloride resin (0.516 g, 0.5 mmol) in solid phase tube contained 15 mL of DCM, removed DCM after 5 min swelling. Then, Fmoc-L-propargylglycine (335 mg, 1.0 mmol) with 10 mL DCM in the tube was added, and pH value was adjusted by adding 0.33 mL of DIEA. After shaking for 2 h

at room temperature, the reaction mixture was washed by DCM (5×5 mL), then 5 mL (DCM: MeOH: DIEA=17:2:1) mixed solution in solid phase tube was added and incubated for 10 min. Removed the mixed solution and washed with 10 mL DCM for five times, then washed with 10 mL DMF for five times. After that, 15 mL piperidine (20% piperidine in DMF) was added. The reaction mixture was shaken for 30 min. Then the mixture was washed by 10 mL of DMF for five times, to which was added 6-maleimidocaproic acid (211 mg, 1.0 mmol), 1 mmol HBTU (379 mg, 1.0 mmol), 0.33 mL DIEA and 15mL DMF into the solid phase tube. The mixture was reacted for another 2 h at room temperature. After that, the mixture was washed by 10 mL of DMF for five times and washed by 10 mL of DCM for five times, then added 10 mL of 1% TFA. The reaction mixture was shaken for 0.5 h, and then concentrated under reduced pressure. The desired residue was purified by reversed-phase high-performance liquid chromatography (HPLC) on a Zorbax 300SB-C18 column (4.6 mm i.d. ×150 mm), with a 10-70% acetonitrile gradient in 0.05% trifluoroacetic acid (TFA)-water, at a flow rate of 8 mL/min (32 min), with detection at 220 nm. A white solid of compound Mal-Pra was obtained in 80% yield.

Synthesis of AIE-DCM-2Mal

The compound AIE-DCM-2Mal was synthesized by the click reaction. To the solution of Mal-Pra (7.35 mg, 24 μmol) and AIE-DCM (5 mg, 6 μmol) in the mixture of tetrahydrofuran (300 μL) and dimethyl sulfoxide (1 mL), and the pH value was adjusted to 7-8 with *N*,*N*-diisopropylethylamine. After that, $CuSO_4$•$5H_2O$ (6.5 mg, 0.026 mmol) and L-ascorbic acid sodium salt (10 mg) were dissolved in 1 mL of

double distilled water respectively, and 100 μL of each solution was added to the reaction mixture. Then the mixture was stirred at room temperature for 8 h under nitrogen. The solution was separated and purified by using HPLC on a Zorbax 300SB-C18 column (4.6 mm i.d. ×150 mm), with a 10-70% acetonitrile gradient in 0.05% trifluoroacetic acid (TFA)-water, at a flow rate of 8 mL/min (32 min), with detection at 220 nm. A orange solid of compound AIE-DCM-2Mal was obtained in 75% yield.

Synthesis of AIE-DCM-2polymyxinB

The compound AIE-DCM-2polymyxinB was synthesized by the Michael addition reaction. To 1 mL of DMF solution, polymyxinB-SH (8 mg, 7.2 μmol) and AIE-DCM-2Mal (2.5 mg, 3 μmol) were added. Then the pH value of the mixture was adjusted to 6.5 by TEA. The mixture was stirred at room temperature for 8 h under nitrogen. Afterward, the final product was purified by HPLC on a Zorbax 300SB-C18 column (4.6 mm i.d. ×150 mm), with a 10-70% acetonitrile gradient in 0.05% trifluoroacetic acid (TFA)-water, at a flow rate of 8 mL/min (32 min), with detection at 220 nm.

Photophysical property of AIE-DCM-2polymyxinB

Firstly, AIE-DCM-2polymyxinB was dissolved in DMSO and prepared into a stock solution with a concentration of 4 mM, then it was dissolved in 1×PBS buffer (pH=7.4) and prepared into a working solution with a concentration of 20 μM (DMSO/PBS = 1/199). After that, the *E.coli* or *S. enteritidis* suspension (OD600 = 0.5) was centrifuged (8000 rpm, 5 min), and then washed with 1×PBS buffer solution for 3

times. After that, *E. coli* or *S. enteritidis* was mixed with AIE-DCM-2polymyxinB (20 μM), which was incubated in dark for 30 s, followed by recording on a Perkin-Elmer LS 55 spectrofluorometer

Then, 9,10-anthracenediyl-bis(methylene) dimalonic acid (ABDA) was used as the ROS indicator since it could quickly react with ROS to bring about reduction of absorbance. ABDA was dissolved in DMSO and prepared into the stock solution with a concentration of 50 mM, which was added into the AIE-DCM-2polymyxinB solution (DMSO/PBS = 1/199) and the mixed solution (AIE-DCM-2polymyxinB + *E. coli* or *S. enteritidis*) with a probe concentration of 20 μM, respectively. The volume ratio of ABDA stock solution to the probe/probe+bacteria solution was 1:1000. Then, under the white light irradiation of 0.86 W/cm^2, the change of absorption spectrum of ABDA was monitored. Three replicates were performed in each group, and distilled deionized water was used as the control group.

Bacterial culture

The strains used in the experiment were *Escherichia coli* (*E. coli*, ATCC25922), *Salmonella enteritidis* (*S. enteritidis* ATCC13076), *Enterococcus faecalis* (*E. faecalis*, ATCC29212) and *Streptococcus mutans* (*S. mutans*, ATCC25175), respectively, which were donated by Tianjin Medical University. Gram-negative bacteria were cultured in LB medium, while Gram-positive *E. faecalis* and *S. mutans* were cultured in BHI medium. The bacteria were cultured in the corresponding medium at 37 ^{o}C.

Bacterial imaging

After each bacterial suspension (OD600 = 0.5) was centrifuged (8000 rpm, 5 min)

and washed with 1×PBS for 3 times, AIE-DCM-2polymyxinB was added with a final concentration of 20 μM. After 30 s incubation with the bioprobe at room temperature, the bacterial suspension was dropped onto the slide with the coverslip, which was then imaged by CLSM. The fluorescent signal was collected above 560 nm with excitation at 458 nm.

Antibacterial study

Colony-forming unit (CFU) counting method was used to study the antibacterial effect of AIE-DCM-2polymyxinB with and without white light irradiation. In the "Probe" group, the mixed solution of 1 mL bacterial suspension (OD600 = 0.5) and AIE-DCM-2polymyxinB at a final concentration of 20 μM was incubated in the dark for 30 s. Then the mixture was centrifuged (8000 rpm, 5min) and washed with 1×PBS for 3 times. After washing, the bacteria were re-suspended in the liquid LB/BHI medium in the 24-well plate. The total volume of the bacteria suspension was kept at 500 μL. In the "Probe + Light" group, after incubation with AIE-DCM-2polymyxinB (20 μM) for 30 s, the bacterial suspensions in 24-well plate were exposed to white light irradiation (0.86 W/cm^2, 30 s), which was followed by the same experimental procedure as that for "Probe" group.

The aforementionedd bacterial suspensions were serially diluted with 1×PBS buffer. After that, 100 μL portion of the diluted bacterial suspension was spread on the solid BHI agar plate and the solid LB agar plate, followed by incubation at 37 ^{o}C for 18 h. The colony-forming units were subsequently counted.

Statistical analysis

Quantitative data were expressed as mean ± standard deviation (SD). Statistical comparisons were made by ANOVA analysis and two-sample Student's *t*-test. *P* value < 0.05 was considered statistically significant.

Figures

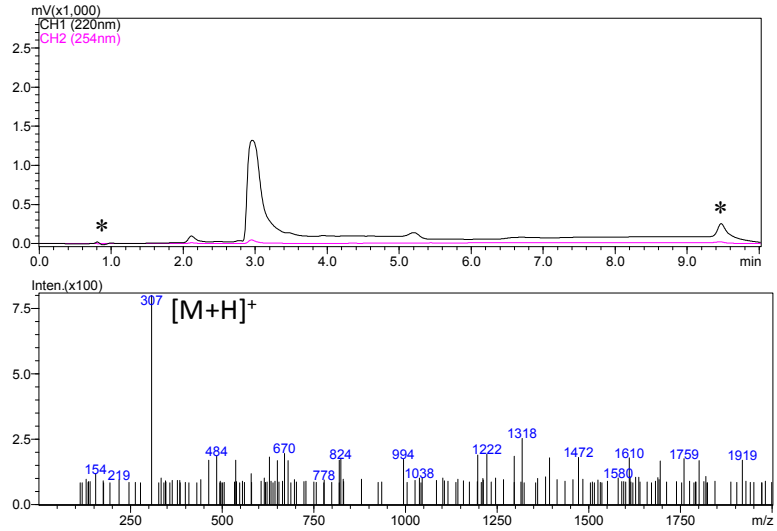

Fig. S1 LC-MS spectra of Mal-Pra (the stars represent systemic peaks).

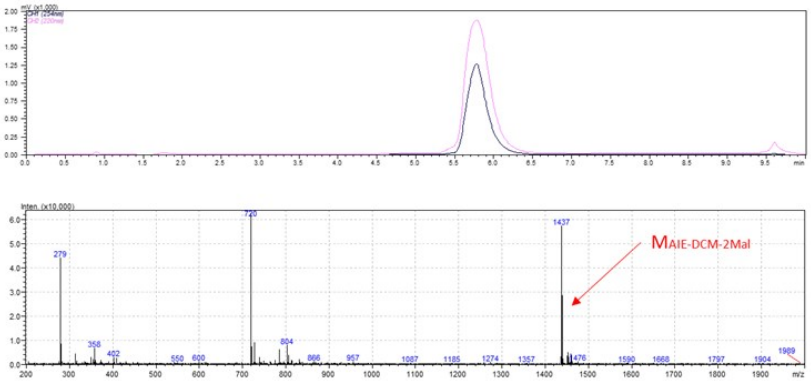

Fig. S2 LC-MS spectra of AIE-DCM-2Mal.

Fig. S3 LC-MS spectra of AIE-DCM-2polymyxinB.

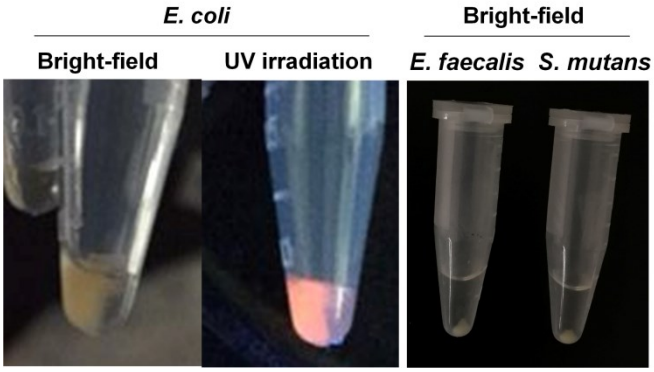

Fig. S4 Photographs of various bacteria after incubation with AIE-DCM-2polymyxinB (20 μM) for 30 s. The bioprobe-incubated *E. coli* was irradiated by a hand-held UV lamp.

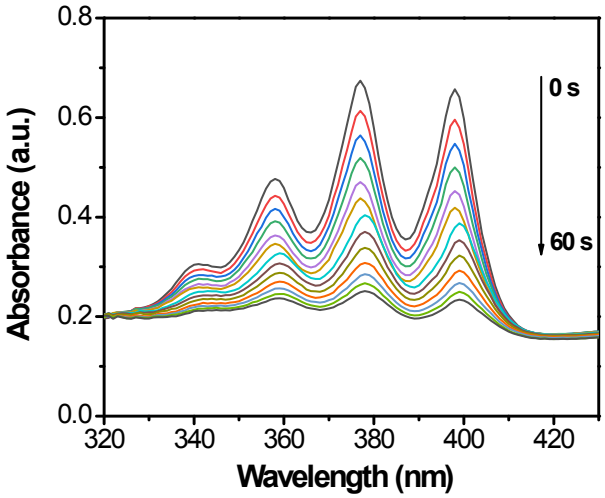

Fig. S5 Absorption spectra of ABDA in the presence of AIE-DCM-2polymyxinB (20 μM) upon white light (0.86 W/cm^2) irradiation for different times.

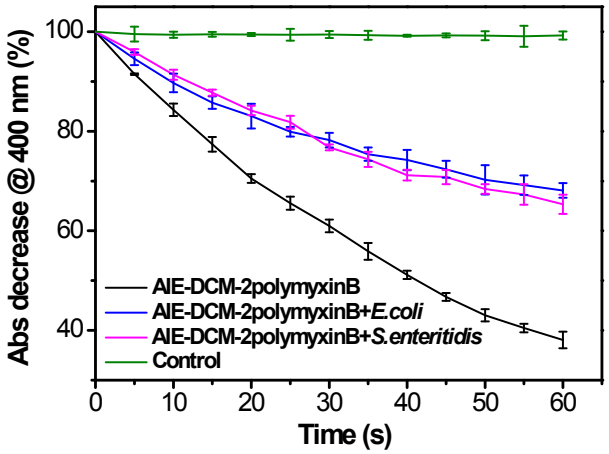

Fig. S6 The absorbance (abs) change of ABDA at 400 nm upon white light (0.86 W/cm^2) irradiation of different solutions as indicated.

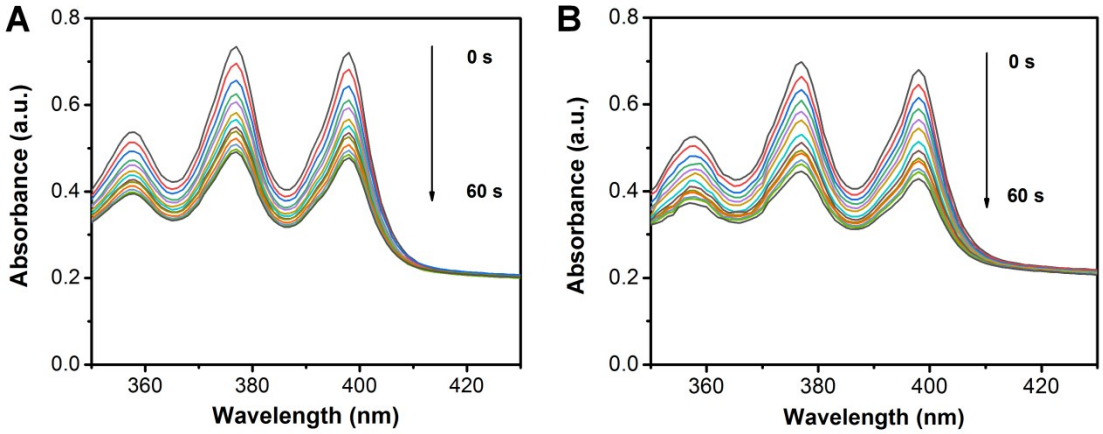

Fig. S7 Absorption spectra of ABDA in the presence of (A) "AIE-DCM-2polymyxinB (20 μM) + *E. coli*" and (B) "AIE-DCM-2polymyxinB (20 μM) + *S. enteritidis*" upon white light (0.86 W/cm^2) irradiation for different times.

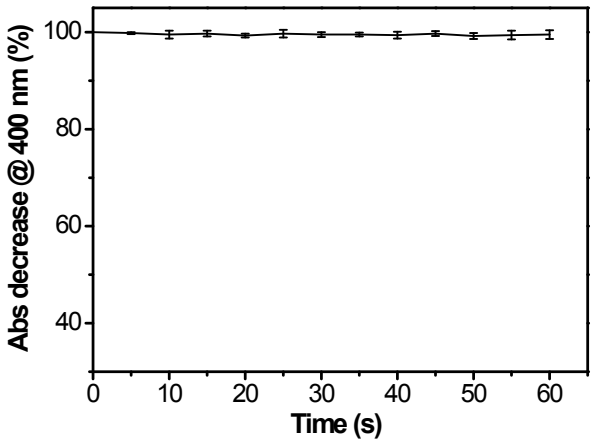

Fig. S8 The absorbance (abs) change of ABDA at 400 nm upon white light (0.86 W/cm^2) irradiation in the presence of *E. coli* only.

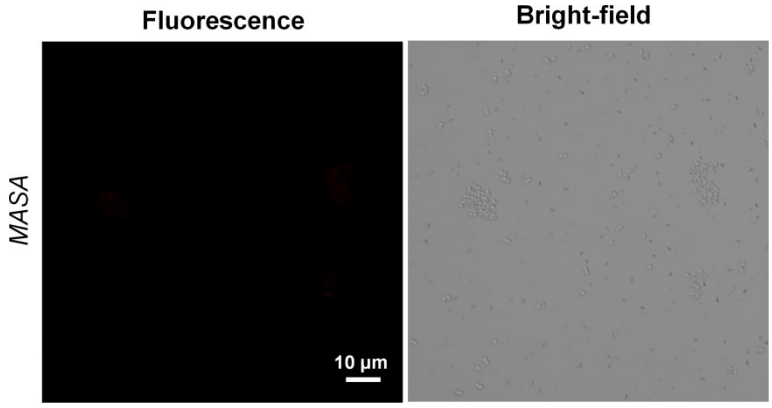

Fig. S9 CLSM images of *MASA* incubated with AIE-DCM-2polymyxinB for 30 s at room temperature, respectively.