Supporting Information For

AIE-active anthraquinone-derived sonosensitizers with enhanced reactive oxygen species generation for ultrasonic biofilm eradication

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Experimental section

Materials and reagents

Tetrahydrofuran, 2,6-dibromoanthraquinone, 4-borate ester-4,4-dimethoxytriphenylamine, Tetrakis(triphenylphosphine)palladium (Pd $(PPh_3)_4$), malononitrile, titanium tetrachloride ($TiCl_4$), pyridine and Chlorin e6 (Ce6) was purchased from J&K Scientific Co., Ltd. Sodium hydroxide (NaOH) and Potassium carbonate (K_2CO_3) was purchased from Shanghai Wokai Biotechnology Co., Ltd. L-alpha-Dipalmitoyl phosphatidylcholine (DPPC) was purchased from Shanghai Haohong Bio-pharmaceutical Technology Co., Ltd. Paraformaldehyde, dimethyl sulfoxide, dichloromethane (DCM), ethyl acetate, n-hexane, 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) and singlet oxygen sensor green (SOSG), 2,2,6,6-tetramethylpiperidine (TEMP), 5,5-dimethyl-1-pyrroline N-oxide (DMPO) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. Crystal violet was purchased from Ron. The bacterial live/dead staining kit was purchased from Beyotime Biotechnology Co., Ltd. LB agar was purchased from Huankai Microorganisms. Ultrapure water was supplied by a laboratory ultrapure water meter (Pall-cascada III).

Instruments

^1H NMR spectra were conducted with Bruker AVANCE III HD 600 spectrometer. MALDI-TOF was recorded on a MALDI SYNAPT G2-Si mass spectrometer. The absorption spectra were performed on U-3900H spectrophotometer (HITACHI). The fluorescence spectra were carried out on

Fluorolog-3 spectrometer (HORIBA Instruments Incorporated). The fluorescence imaging was performed on inverted fluorescence microscope (NIKON, Ti-S, Japan) and confocal microscope (NIKON, A1/H-TIRFM, Japan). The particle size and zeta potential were measured on a multi-angle particle size and high-sensitivity zeta potential analyzer (DelsaNano C, Beckman Coulter, Inc). The fluorescence intensity and absorbance were measured on a multi-functional microplate detector (Synergy HTX, BioTek). The ultrasound was generated by DJO-2776 sonicator. The morphology and structure of the samples were characterized using a transmission electron microscope (TEM, HT7700, Hitachi, Japan). The morphology of the samples was characterized using a field emission scanning electron microscope (FE-SEM, SU8010, Hitachi, Japan). The absolute photoluminescence quantum yield (PLQY) was measured using an absolute photoluminescence quantum yield spectrometer (C11347-12, Hamamatsu Photonics, Japan).

Synthesis of compound AQ

2,6-Dibromoanthraquinone (220 mg, 0.6 mmol), 4-borate ester-4,4-dimethoxytriphenylamine (646 mg, 1.5 mmol) and Pd $(PPh_3)_4$ (70 mg, 0.06 mmol) were dissolved in a mixed solution of 15 mL THF and 4 mL K_2CO_3 saturated aqueous solution. After removing oxygen from the mixture, fill it with nitrogen three times, then stir overnight at 80°C. After that, the reaction mixture was extracted for three times with ethyl acetate and saturated brine. The crude product was purified using a silica gel column chromatograph (n-Hexane

/DCM=1/1, v/v) and the red solid product was obtained. (320 mg, yield: 66%) ^{1}H NMR (600 MHz, Chloroform-d) δ 8.49 (d, *J* = 1.8 Hz, 2H), 8.33-8.32 (d, *J* = 8.2 Hz, 2H), 7.95-7.94 (dd, *J* = 8.2, 1.9 Hz, 2H), 7.58-7.56 (d, *J* = 8.8 Hz, 4H), 7.13-7.11 (d, *J* = 8.9 Hz, 8H), 7.01-7.00 (d, *J* = 8.7 Hz, 4H), 6.88-6.86 (d, *J* = 8.9 Hz, 8H), 3.82 (s, 12H). ^{13}C NMR (101 MHz, Chloroform-d) δ 183.12, 167.83, 156.37, 149.63, 146.41, 140.26, 134.15, 132.47, 131.38, 130.97, 129.90, 128.84, 128.07, 127.86, 127.14, 124.34, 119.81, 114.88, 68.20, 55.53, 38.75, 30.39, 28.95, 23.77, 23.02, 14.09, 10.99. HRMS-ESI (m/z): $[M+H]^+[C_{54}H_{43}N_2O_6]^+$ calcd. 815.3121, found 815.5089.

Synthesis of compound EQ

Compound AQ (100 mg, 0.12 mmol) and malononitrile (49 mg, 0.74 mmol) were dissolved in 5 mL DCM. After the mixture was stirred at 0 ℃ in for 5 min, titanium tetrachloride (94 μL, 0.86 mmol) was slowly injected into the reaction system using a syringe, and continue stirring at 0 ℃ in for 30 min. Then, pyridine (69 μL, 0.86 mmol) was injected into the above reaction system and stirred for another 30 min. Next, the reaction system was heated to 45 ℃ and refluxed for 24 h. After cooling to room temperature, the reaction mixture was extracted with DCM and saturated brine for three times, then the crude product was further purified by column chromatography (n-Hexane /DCM=1/1, v/v), affording dark green solid. (66 mg, yield: 61%).^{1}H NMR (600 MHz, Chloroform-d) δ 8.39-8.38 (d, *J* = 1.6 Hz, 2H), 8.27-8.25 (d, *J* = 8.3 Hz, 2H), 7.84-7.83 (dd, *J* = 8.4, 1.7 Hz, 2H), 7.49-7.48 (d, *J* = 8.8 Hz, 4H), 7.13-7.11 (d, *J* = 8.9 Hz, 8H), 6.99-6.97 (d, *J*

= 8.8 Hz, 4H), 6.88-6.87 (d, *J* = 8.9 Hz, 8H), 3.82 (s, 12H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.28, 156.87, 150.13, 143.69, 139.66, 131.44, 129.94, 128.98, 128.50, 128.36, 128.23, 127.95, 127.51, 124.73, 81.60, 55.74. HRMS-ESI (m/z): $[M+H]^+[C_{60}H_{43}N_6O_4]^+$ calcd. 911.3346, found 911.6696.

Preparation of nanosonosensitizers AQ@DPPC and EQ@DPPC

AQ (1 mg) and DPPC (10 mg) were dissolved in THF (1 mL), followed by adding slowly to the 10 mL ultrapure water with continuous sonication at a power of 80 W for 3 min. Subsequently, THF was removed using a rotary evaporator, and finally, an aqueous solution of sample AQ@DPPC was obtained. The aqueous solution of nanosonosensitizer EQ@DPPC was prepared following the same procedure. The AQ@DPPC or EQ@DPPC was centrifuged for 10 min with 1000 rpm before use.

For determining the loading capacity and encapsulation efficiency, the above nanosonosensitizer AQ@DPPC or EQ@DPPC dispersion was lyophilized. The freeze-dried AQ@DPPC or EQ@DPPC were dissolved in DMSO and then the absorption spectrum was measured. The loading capacity and encapsulation efficiency of the AQ@DPPC or EQ@DPPC (nanoparticle) were measured by absorption spectroscopy using pre-established standard calibration curves and calculated as follows:

$$\text{Drug loading capacity (DL) \%} = \frac{weight\ of\ AQ\ or\ EQ\ \ in\ the\ nanoparticles}{weight\ of\ the\ nanoparticles} \times 100\%$$

Encapsulation efficiency (EE) %

$$= \frac{weight\ of\ AQ\ or\ EQ\ in\ the\ nanoparticles}{weight\ of\ the\ feeding\ AQ\ or\ EQ} \times 100\%$$

For AQ@DPPC, the loading capacity was determined as 8.7% and the encapsulation efficiency was 92.3%.

For EQ@DPPC, the loading capacity was determined as 8.5% and the encapsulation efficiency was 90.1%.

Theoretical calculation

The ground-state (S_0) and excited-state (S1-S3 and T1-T3) geometries were calculated by the density functional theory (DFT) and time-dependent density functional theory (TD-DFT) method at the basis set of B3LYP/6-31G* using Gaussian 16.

Optical properties

For the absorption spectrum, the stock solution of AQ or EQ (1 mM in DMSO) was diluted to 10 μM in PBS (pH 7.4, 5%DMSO), and its absorption spectrum was measured. AQ@DPPC or EQ@DPPC was diluted to a PBS (pH 7.4) solution with a final concentration of 18 μM (AQ or EQ), and its absorption spectrum was tested.

For the aggregation-induced emission (AIE) effect of AQ or EQ, AQ or EQ was dissolved in THF (good solvent) to a concentration of 1 mM as a stock solution. Then, H_2O (poor solvent) was added into the stock solution of AQ or EQ, and the fluorescence spectra at different THF/H_2O ratios were recorded. The final concentration of AQ or EQ was 10 μM. For AQ, excitation wavelength: 510 nm, slit 10 nm/10 nm. For EQ, excitation wavelength: 530 nm, slit 10 nm/10 nm.

Detection of ROS generation

For detection of total ROS, DCFH-DA was used as a typical probe. DCFH-DA (0.5 mL, 1mM in DMSO) was mixed with NaOH (2 mL, 10 mM in H_2O) for 30 min in dark. After that, the PBS (pH 7.4, 7.5 mL) was added to the above solution to obtain the final concentrations of DCFH to 50 μM. Then, 10 μM samples of AQ/EQ/Ce6 (containing 5%DMSO or 100%DMSO) and 10 μM indicator DCFH were added to PBS solution. The mixture was sonicated in the dark for different time periods (0/5/10/15/20/25/30 min), and the fluorescence intensity at each time point was measured. Ultrasound parameters: 1 MHz, 1.5 W/cm^2, 50% duty cycle.

Serial concentrations (5, 10, 15, 20, and 25 μM) of AQ@DPPC, EQ@DPPC, or Chlorin e6 (Ce6) were prepared in phosphate-buffered saline (PBS). Each sample was supplemented with the ROS indicator DCFH at a final concentration of 10 μM in the dark. Subsequently, samples were subjected to ultrasound irradiation (1 MHz, 1.5 W/cm², 50% duty cycle) for 10 min. The fluorescence intensity of each group was measured using a microplate reader (λ_{ex} = 485 nm, λ_{em} = 528 nm).

The generation of singlet oxygen was assessed using the Singlet Oxygen Sensor Green (SOSG) reagent at a final concentration of 10 μM. Samples containing 10 μM Ce6, AQ@DPPC or EQ@DPPC were incubated with SOSG and subjected to ultrasound irradiation. Fluorescence intensity (λ_{ex} = 485 nm, λ_{em} = 528 nm) was measured at designated time intervals throughout the sonication process. Ultrasound parameters: 1 MHz, 1.5 W/cm^2, 50% duty cycle.

Superoxide anion generation was assessed using dihydroethidium (DHE) as the fluorescent probe at a final concentration of 10 μM. Solutions containing 10 μM of either ce6, AQ@DPPC or EQ@DPPC were incubated with DHE in the dark. The fluorescence intensity (λ_{ex} =535 nm, λ_{em} = 610 nm) was then measured and recorded at various time points under continuous ultrasound irradiation. Ultrasound parameters: 1 MHz, 1.5 W/cm^2, 50% duty cycle.

ESR measurements for ROS generation

ESR was conducted on a Bruker EMX plus-10/12 spectrometer (Germany). Before ESR test, the test samples were first dispersed in the solution, and oxygen in the solution was removed by N_2, them the spin-trapping agents DMPO and TEMP were added for timely testing. The results were obtained by sonication of AQ@DPPC or EQ@DPPC for 10 min at a power of 1.5 W/cm^2, 50% duty circle, 1.0 MHz.

Bacterial cultivation

S. aureus and E. coli were cultured in liquid LB culture medium in a shaking incubator at 37 ℃ and harvested at the logarithmic growth phase by centrifugation at 3000 g for 10 min. After washing with PBS for three times, the bacteria were resuspended in PBS for further use.

Antibacterial assays of planktonic bacteria

Gram-negative bacteria *E. coli* and Gram-positive bacteria *S. aureus* were chosen as the model for the antibacterial assays. Each bacterial experiment is grouped as follow: (1) Control group (without US and sonosensitizer). (2) Only US group

(without sonosensitizer). (3) AQ@DPPC (AQ's final concentration is 5, 10, 15, 20 μM). (4) EQ@DPPC (EQ's final concentration is 5, 10, 15, 20 μM). (5) AQ@DPPC+US (AQ's final concentration is 5, 10, 15, 20 μM). (6) EQ@DPPC+US (EQ's final concentration is 5, 10, 15, 20 μM). (7) Ce6 (Ce6's final concentration is 5, 10, 15, 20 μM). (8) Ce6+US (Ce6's final concentration is 5, 10, 15, 20 μM). Different concentrations of Ce6, AQ@DPPC or EQ@DPPC and 10^6 CFU·mL^{-1} bacterial suspension were added to a 96-well plate. The above experimental groups were subjected to sonication or no sonication, followed by incubation at 37 °C for 18 h. The incubated bacterial suspension was diluted 100,000-fold and spread on plates. The plated dishes were cultured in an incubator for 16 h, and colony counts were performed. Ultrasound parameters: 1 MHz, 1.2 W/cm^2, 10 min, 50% duty cycle.

Intra-bacterial ROS measurement and Fluorescence imaging

The generation of reactive oxygen species (ROS) by the nanosonosensitizers inside bacteria was investigated using the fluorescent probe DCFH-DA. The test organisms were Gram-negative *E. coli* and Gram-positive *S. aureus.* AQ@DPPC/EQ@DPPC (AQ's or EQ's final concentration is 10 μM) and bacterial suspension (10^8 CFU·mL^{-1}) were incubated on a 37 °C incubator for 4 h. Subsequently, PBS solution containing 10 μM DCFH-DA was added, and the mixture was incubated on a 37 °C incubator in the dark for 30 min. After centrifugation, the supernatant was discarded, and an equal volume of PBS solution was added for resuspension, followed by US. US parameters: 1 MHz,

1.5 W/cm^2, 10 min, 50% duty cycle. Finally, the fluorescence intensity was measured by the multi-functional microplate detector. Bacterial fluorescence imaging was performed using a laser scanning confocal microscope (NIKON, A1/H-TIRFM, Japan1).

Bacterial live/dead staining assay

The antibacterial performance of the nanosonosensitizers was evaluated using the DMAO/PI bacterial live/dead staining kit. Groups are divided as follow: (1) Control group. (2) Only US group. (3) AQ@DPPC group. (4) EQ@DPPC group. (5) AQ@DPPC+US group. (6) EQ@DPPC+US group. The final concentration of AQ or EQ in the solution are specified as 10 μM. Bacterial suspension (10^8 CFU·mL^{-1}) were cultured with or without AQ@DPPC or EQ@DPPC in a 37 °C incubator for 4 h. Then, the above solution was centrifuged, the supernatant was discarded, and the pellet was resuspended in PBS, followed by US or without US. Ultrasound parameters: 1 MHz, 1.5 W/cm^2, 10 min, 50% duty cycle. The samples were then stained for 30 min, and fluorescence imaging was performed using a confocal microscope. For DMAO, E_x=465-495 nm, while E_m=512-552 nm. For PI, E_x=540-580 nm, while E_m=600-660 nm.

Biofilms ablation effect

Biofilms were characterized using the DMAO/PI bacterial live/dead staining kit. Groups are divided as follow: (1) Control group. (2) Only US group. (3) AQ@DPPC group. (4) EQ@DPPC group. (5) AQ@DPPC+US group. (6) EQ@DPPC+US group. Bacterial suspension (500 μL, 10^8 CFU·mL^{-1}) was

incubated on a 37 °C incubator for 48 h to form bacterial biofilms. The medium was aspirated, and the biofilms were gently washed twice with PBS. PBS solution containing AQ@DPPC/EQ@DPPC (AQ's or EQ's final concentration is 15 μM) was added, and the mixture was incubated on the incubator for 4 h. The above solutions were subjected to sonication or no sonication. US parameters: 1 MHz, 1.5 W/cm^2, 10 min, 50% duty cycle. The solution was carefully aspirated, and 4% paraformaldehyde (PFA) was added to fixation for 20 min. Then, the PFA was aspirated, and the biofilms were gently washed twice with PBS. Live/dead bacterial staining reagent was added for staining for 30 min, and finally, confocal fluorescence imaging was performed. For DMAO, E_x=465-495 nm, while E_m=512-552 nm. For PI, E_x=540-580 nm, while E_m=600-660 nm.

Crystalline violet staining assay

Groups are divided as follow: (1) Control group. (2) Only US group. (3) AQ@DPPC group. (4) EQ@DPPC group. (5) AQ@DPPC+US group. (6) EQ@DPPC+US group. Bacterial suspension (10^8 CFU·mL^{-1}) was placed in a 24-well plate and incubated at 37 °C for 48 h to form biofilms. The medium was aspirated, and the biofilms were gently washed twice with PBS. 1 mL of PBS solution containing AQ@DPPC/EQ@DPPC (AQ's or EQ's final concentration is 15 μM) was added, and the above solutions were subjected to sonication or no sonication. US parameters: 1 MHz, 1.5 W/cm^2, 10 min, 50% duty cycle. After incubation in the incubator for 4 h, the solution was aspirated, and the biofilms were gently washed twice with PBS. Bacterial biofilms from all experimental

groups were fixed with absolute ethanol for 30 minutes. The ethanol solution was subsequently aspirated, and samples were air-dried at ambient temperature prior to downstream staining procedures. Then, 1 mL of 0.1% crystal violet (CV) solution was added, and the plate was placed in a 37 °C incubator for 30 min. The solution was aspirated, and the biofilms were gently washed twice with PBS. The crystal violet stain retained within the biofilms was subsequently dissolved using 95% ethanol to ensure complete dye solubilization. The absorbance of the biofilm solution at 580 nm (OD_{580}) was measured using a microplate reader.

SEM analysis of bacterial biofilms

The detailed procedure for sample preparation and SEM observation is as follows. Bacterial suspension (1 mL, 10^8 CFU/mL) was mixed with 10 μM AQ@DPPC or EQ@DPPC in a 1.5 mL centrifuge tube and incubated at 37 °C for 30 min. For US group, the mixture was subjected to ultrasound irradiation (1 MHz, 1.5 W/cm^2, 10 min, 50% duty cycle). After treatment, the bacteria were collected by centrifugation (5000 rpm, 10 min), washed twice with PBS, and fixed overnight with 4% paraformaldehyde. The fixed samples were then dehydrated using a graded ethanol series (30%, 50%, 70%, 90%, and 100% twice), followed by air-drying at room temperature. Finally, the dried specimens were sputter-coated with a thin gold layer and imaged using a field emission scanning electron microscope (FE-SEM).

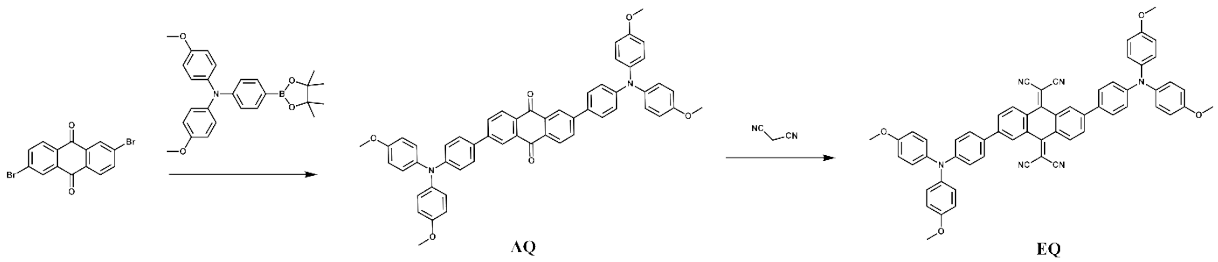
Extraction of orange juice from fruits

Fresh oranges were purchased from local markets in Guangzhou, China. The juice

extraction procedure was performed as follows: Orange pulp was homogenized using a commercial juicer to obtain a pulp suspension. This suspension was then centrifuged at 1,000 rpm for 10 min to remove coarse pulp components. The supernatant underwent secondary centrifugation at 5,000 rpm for 10 min to eliminate residual particulates and impurities, yielding a relatively clear juice for subsequent experiments.

Food bacterial inactivation by sonodynamic

For bacterial inactivation experiments in beverages, freshly extracted orange juice was first sterilized by ultraviolet irradiation for 20 min. The juice was divided into two portions: one portion was artificially contaminated with Escherichia coli at 10^6 CFU/mL, while the other served as an uncontaminated control. The contaminated juice was allocated to six experimental groups: (1) Untreated control. (2) Ultrasound-only (US). (3) AQ@DPPC nanoparticles. (4) AQ@DPPC + US. (5) EQ@DPPC nanoparticles. (6) EQ@DPPC + US. Final concentrations of AQ and EQ were standardized at 15 μM. Ultrasound parameters were uniformly applied at 1.5 W/cm² intensity, 50% duty cycle, 1 MHz frequency, and 10 min duration. Following treatment, all samples were stored at 4°C. At designated timepoints (4, 24, and 48 h), aliquots were serially diluted 30,000-fold in sterile PBS, plated on agar, and incubated at 37°C for 24 h for colony-forming unit (CFU) enumeration.



Scheme S1. Synthetic route of sonosensitizer AQ and EQ

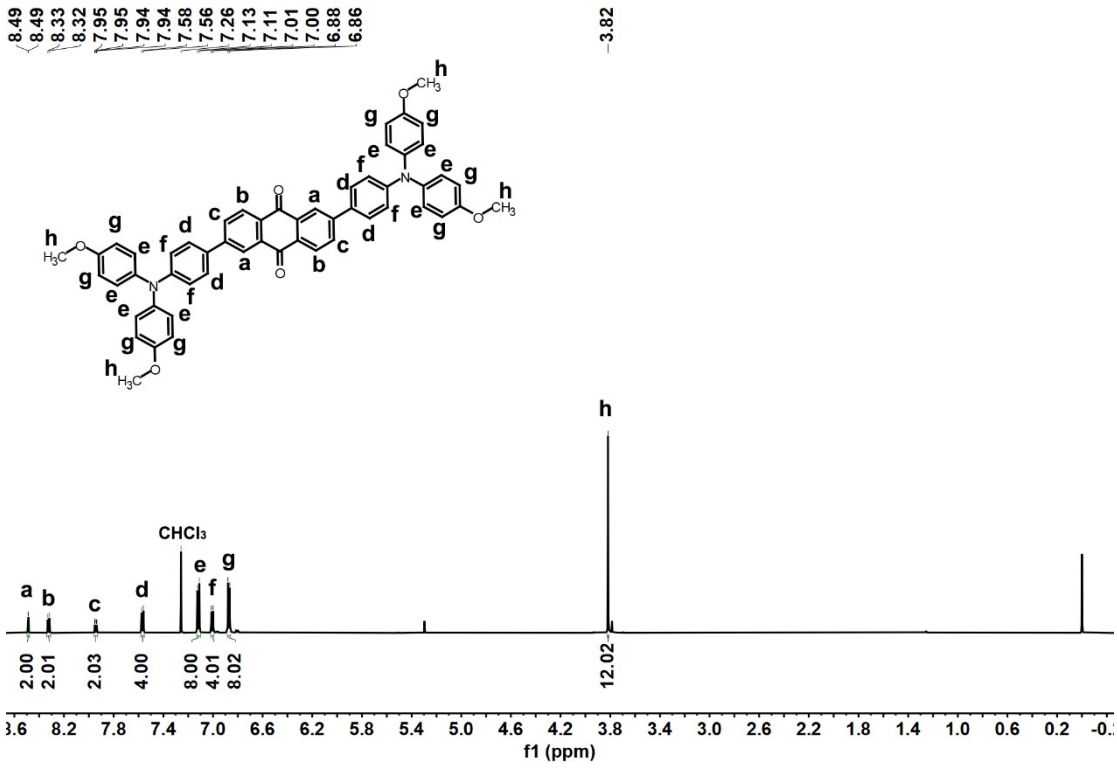


Fig. S1 ^{1}H NMR spectrum of Compound AQ (in $CDCl_3$).

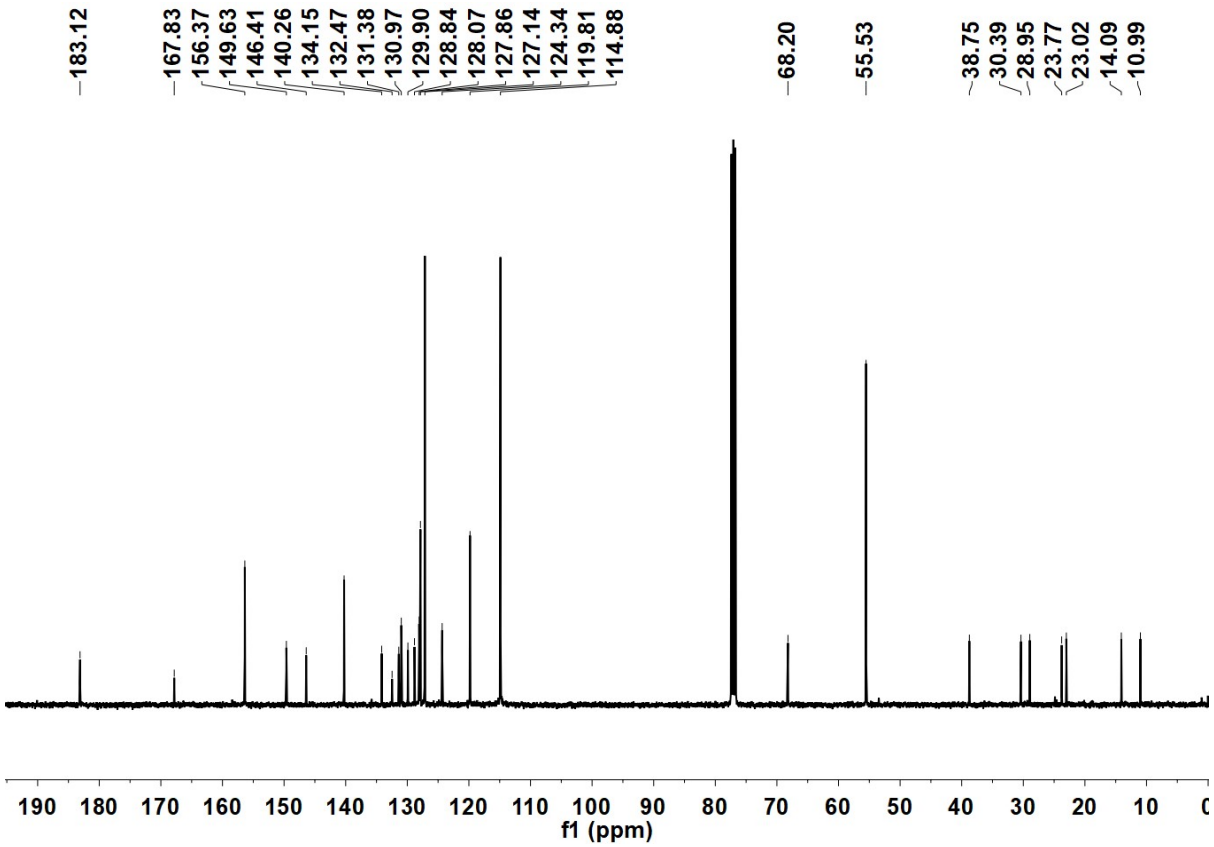


Fig. S2 ^{13}C NMR spectrum of Compound AQ (in $CDCl_3$).

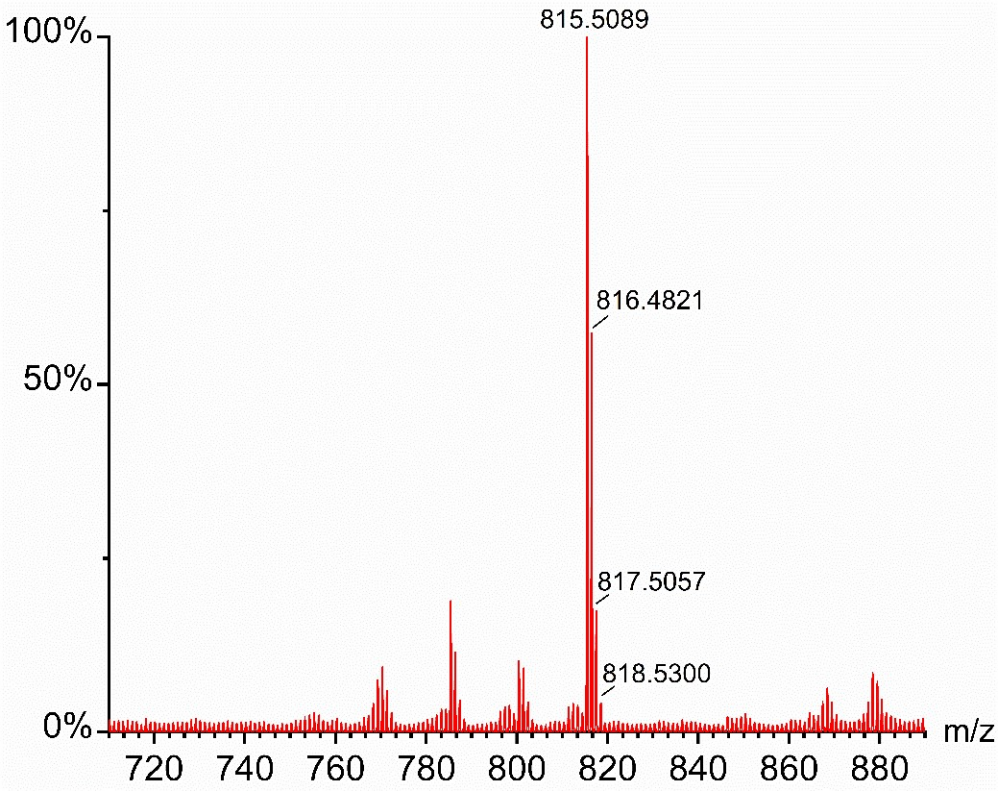


Fig. S3 MALDI TOF mass spectrum of Compound AQ.

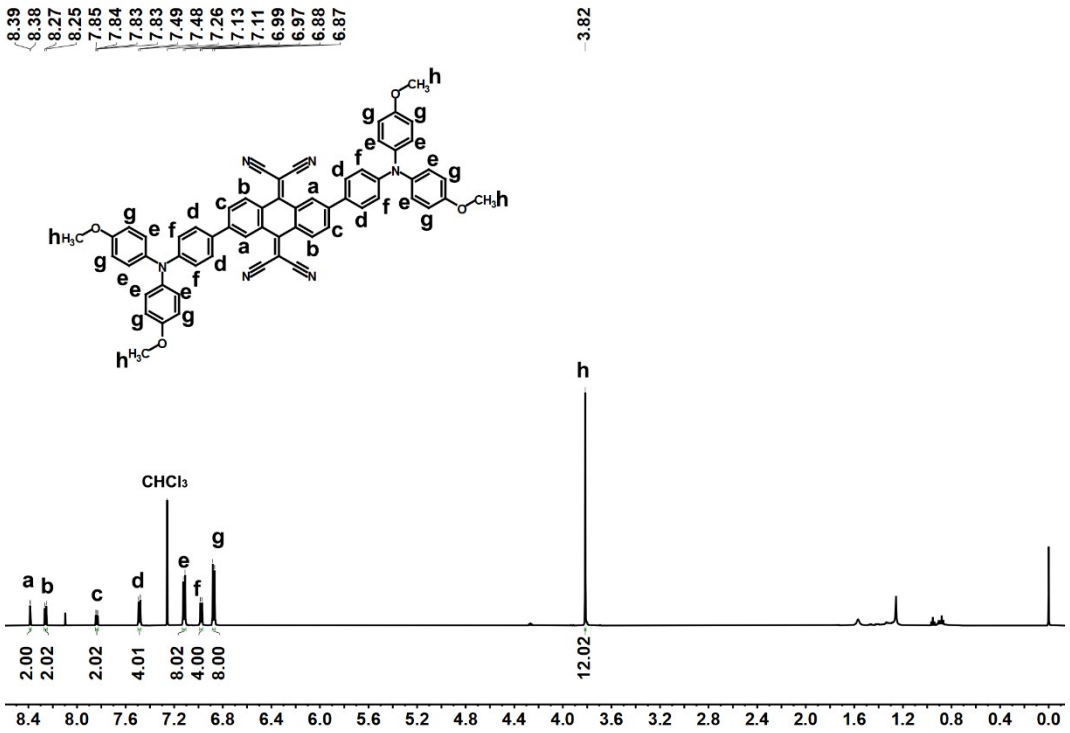


Fig. S4 ^{1}H NMR spectrum of Compound EQ (in $CDCl_3$).

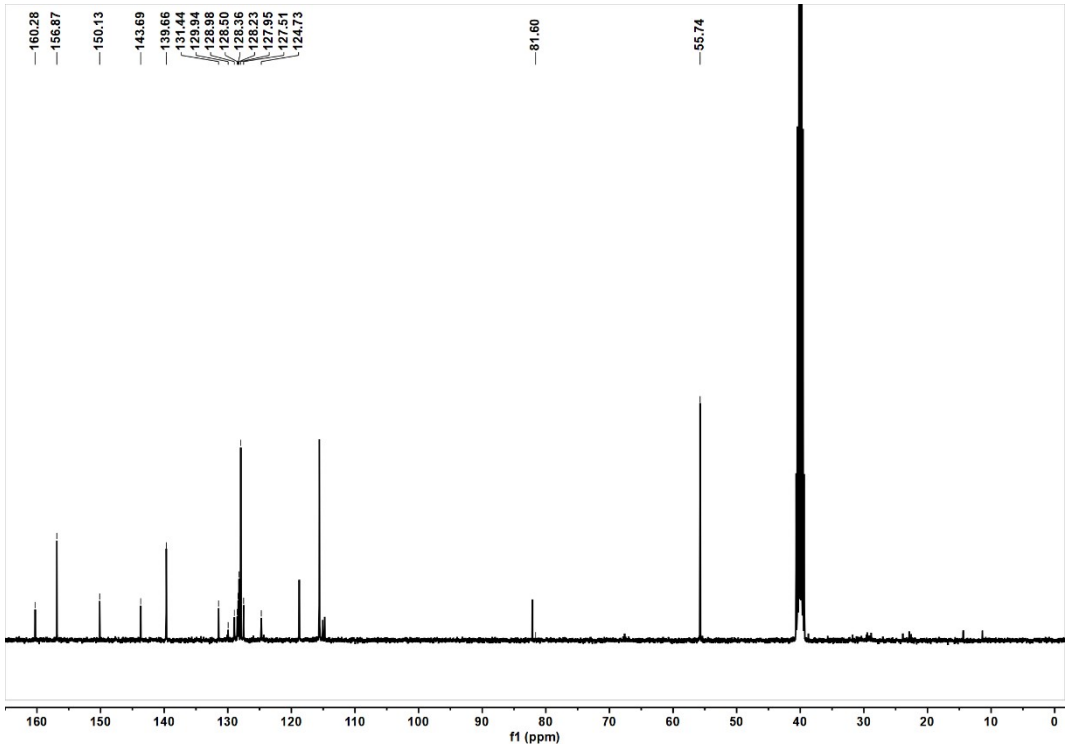


Fig. S5 ^{13}C NMR spectrum of Compound EQ (in d_6-DMSO).

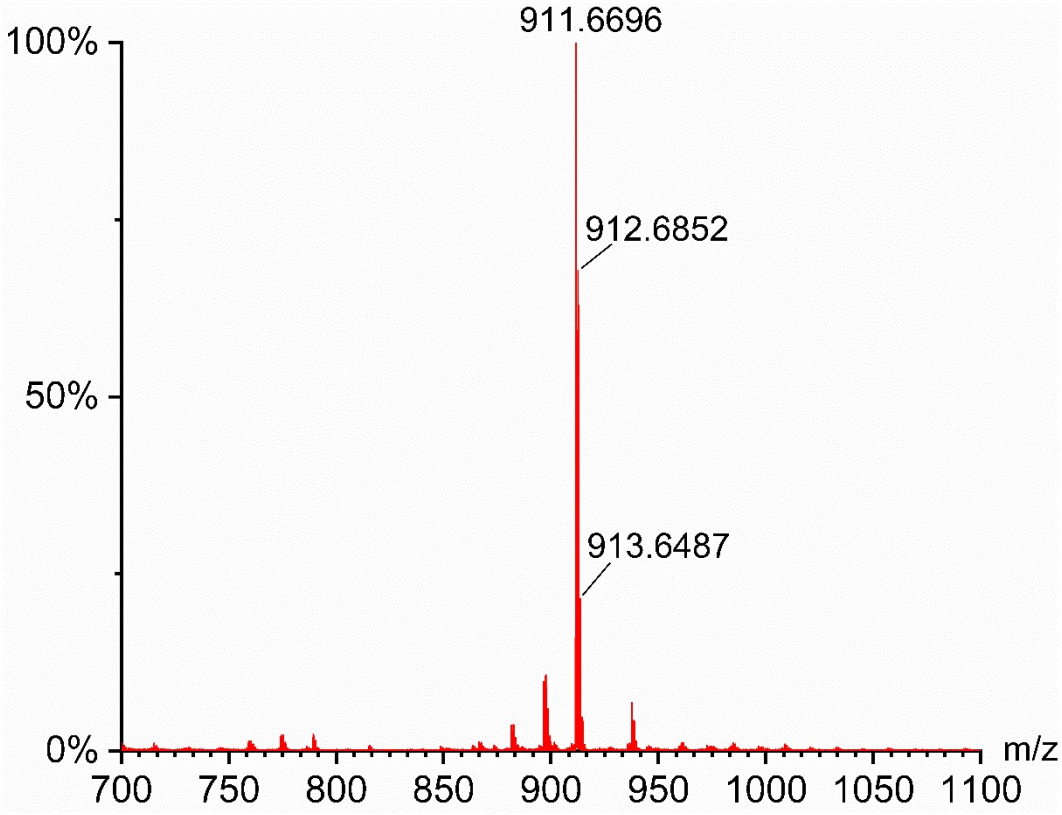


Fig. S6 MALDI TOF mass spectrum of Compound EQ.

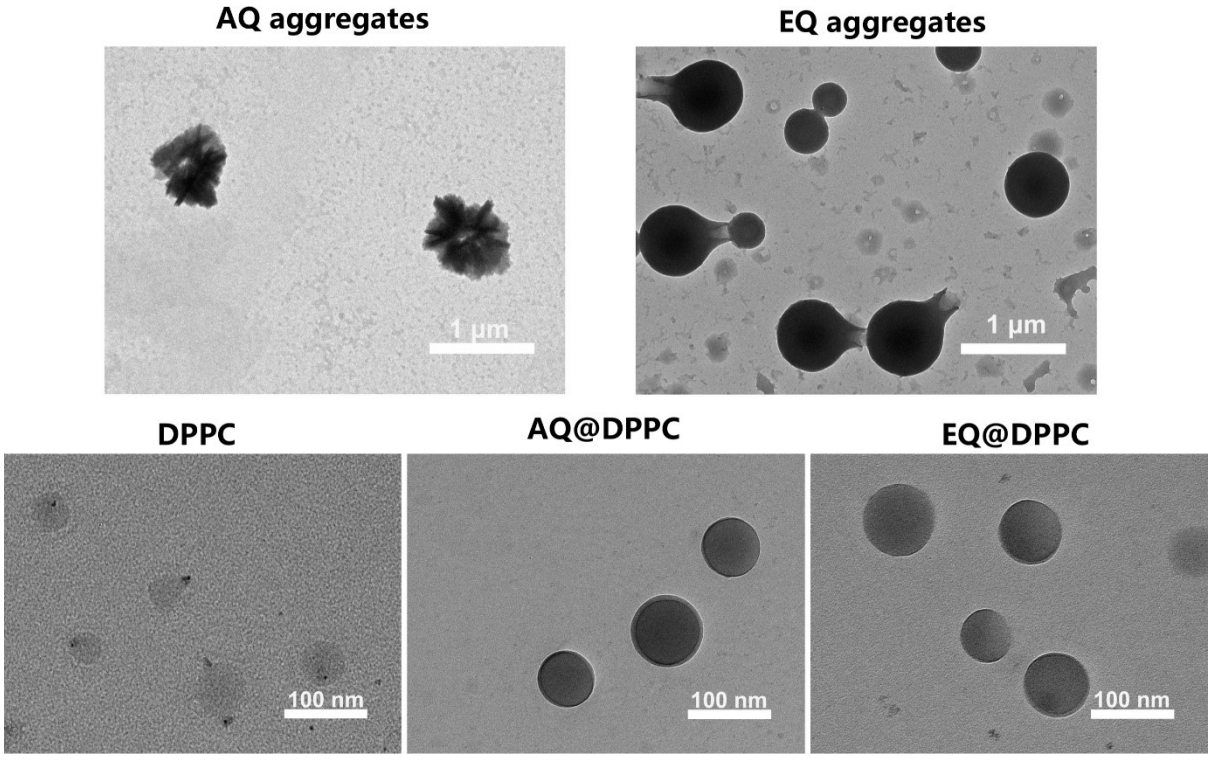


Fig. S7 TEM images of AQ (5%DMSO in water), EQ (5%DMSO in water), DPPC, AQ@DPPC and EQ@DPPC.

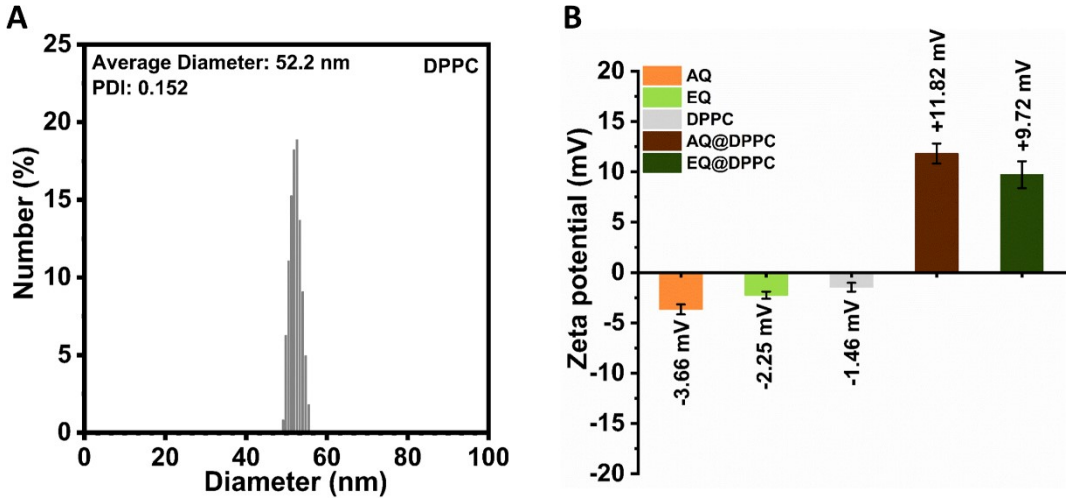


Fig. S8 (A) Average hydrodynamic diameter distribution of DPPC. (B) Zeta potential of AQ, EQ, DPPC, AQ@DPPC and EQ@DPPC.

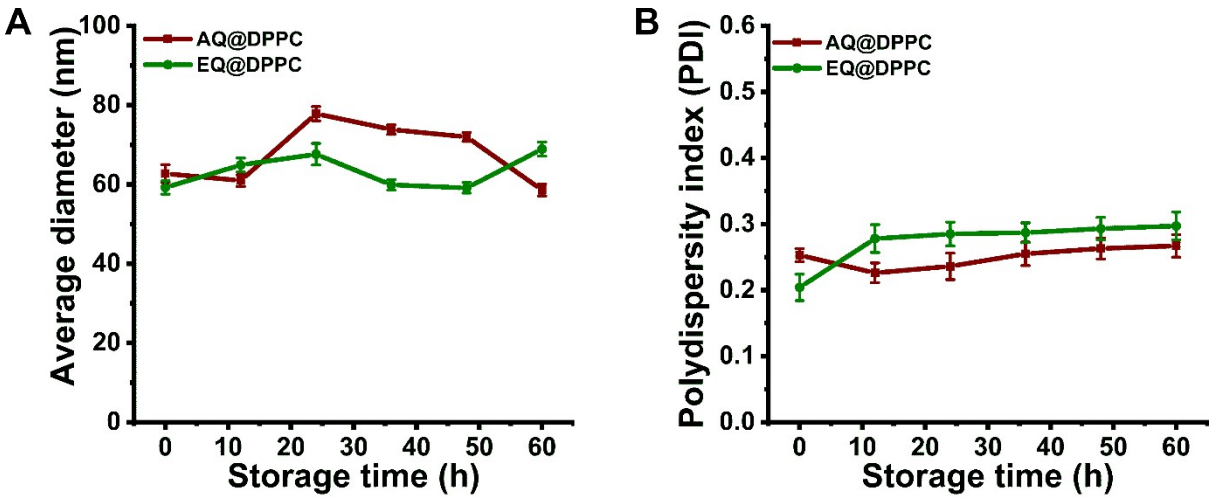


Fig. S9 Stability of nano-sonosensitizers AQ@DPPC and EQ@DPPC. (A) Hydrodynamic average diameter of AQ@DPPC and EQ@DPPC over storage time. (B) Polydispersity index (PDI) of AQ@DPPC and EQ@DPPC over storage time.

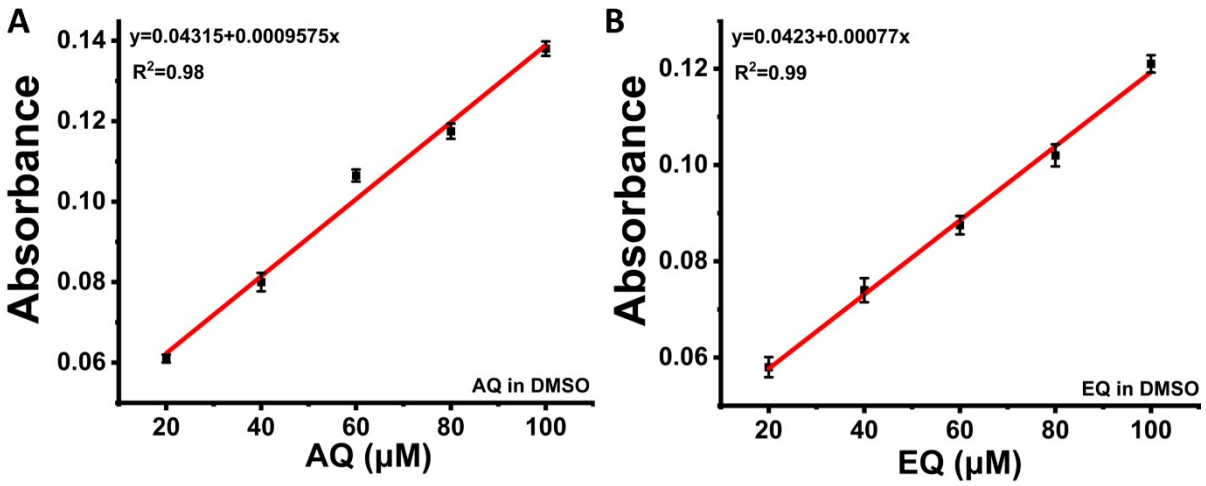


Fig. S10 Linear relationship between absorbance and concentration. (A) Absorbance of AQ at 485 nm as a function of its concentration. (B) Absorbance of EQ at 555 nm as a function of its concentration.

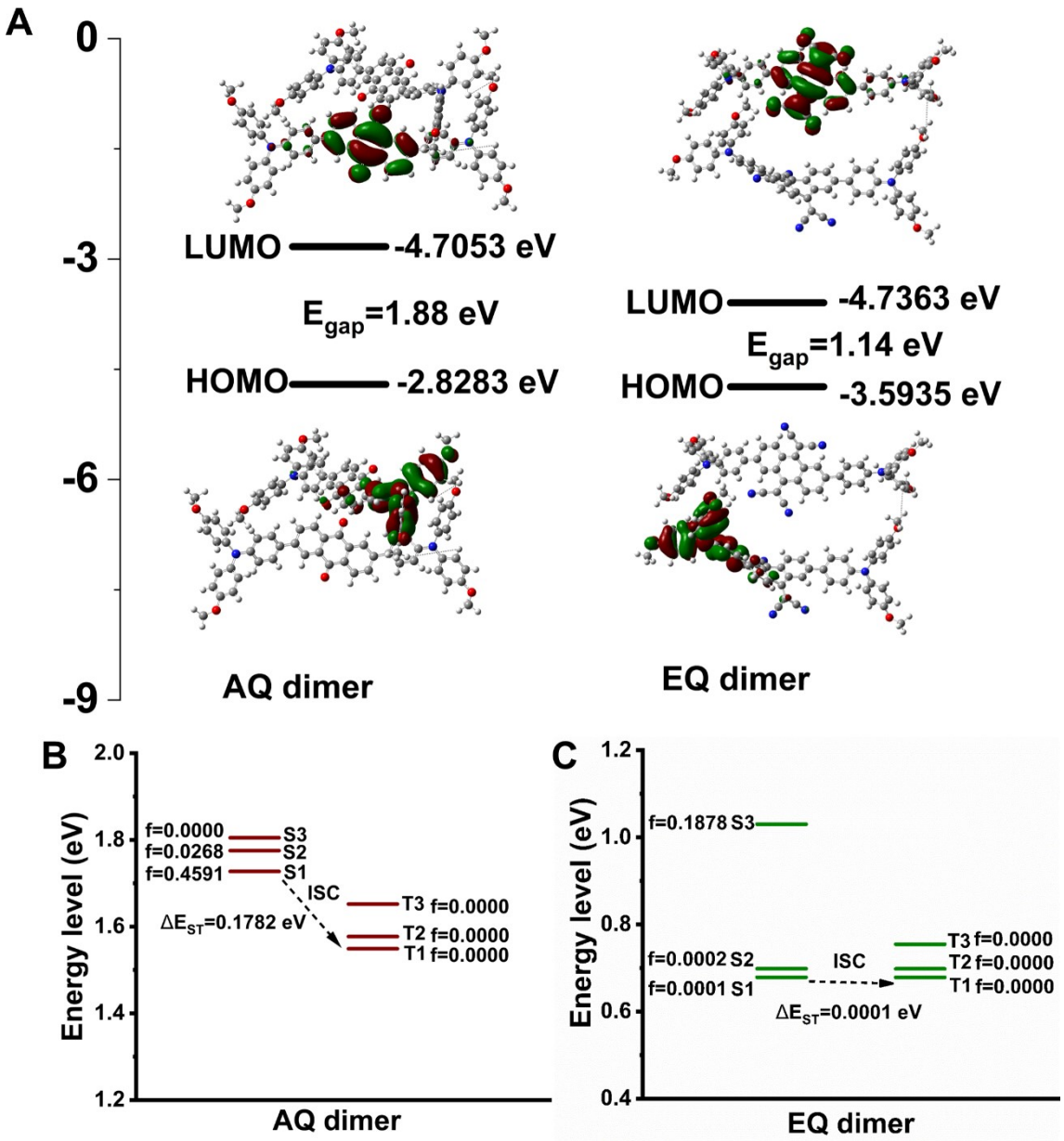


Fig. S11 Theory Calculation of AQ dimer and EQ dimer. (A) Geometrically optimized structures at B3LYP/6-31G* level using Gaussian 16. (B) Energy levels of S1-S3 and T1-T3 of AQ dimer calculated by TD-DFT. (C) Energy levels of S1-S3 and T1-T3 of EQ dimer calculated by TD-DFT.

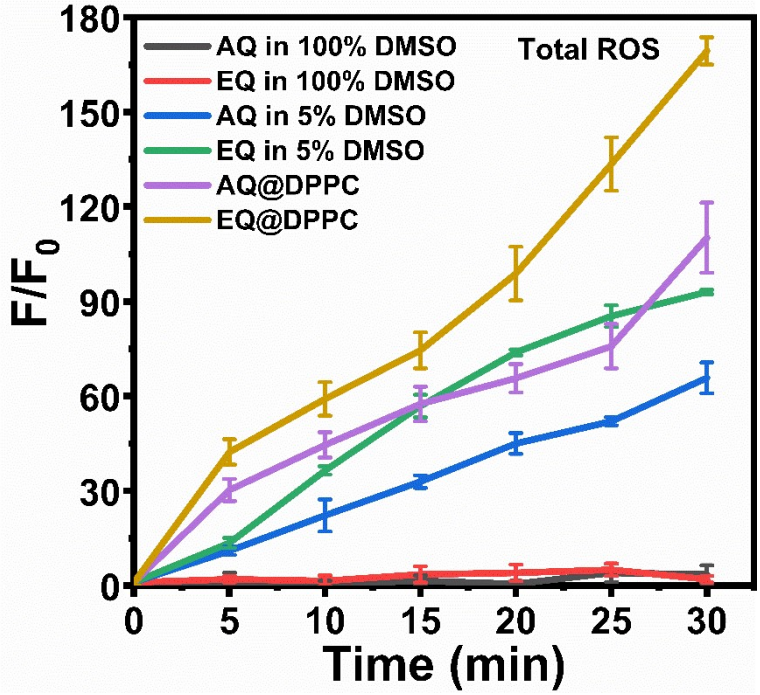


Fig. S12 Plots of F/F_0 for ROS generation in the presence of AQ and EQ in 100%DMSO or 5%DMSO (95% PBS), AQ@DPPC and EQ@DPPC in PBS with different US time; F_0 was the fluorescence intensity of DCFH-DA before stimulation. Ex= 485 nm, Em= 528 nm.

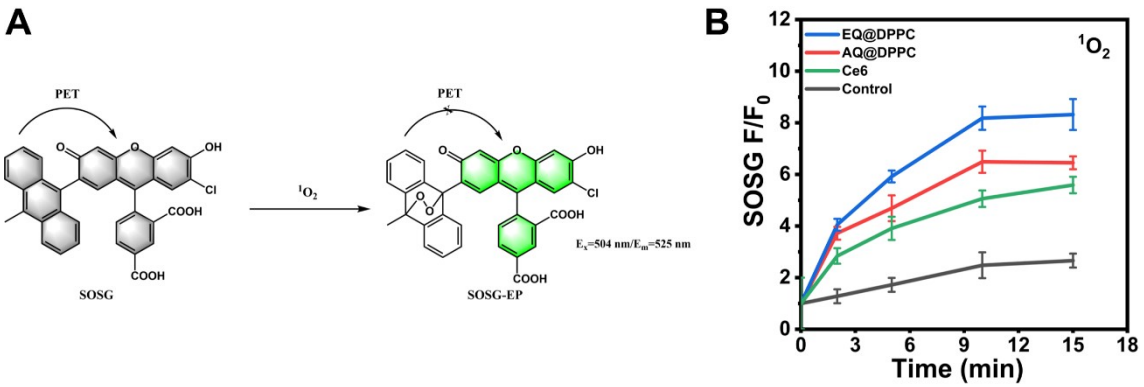


Fig. S13 Singlet oxygen generation test. (A) Response mechanism of singlet oxygen probe SOSG. (B) Plots of F/F_0 for $^1O_2$ generation in the presence of Ce6, AQ@DPPC or EQ@DPPC with different US time. F_0 was the fluorescence intensity of SOSG before stimulation.

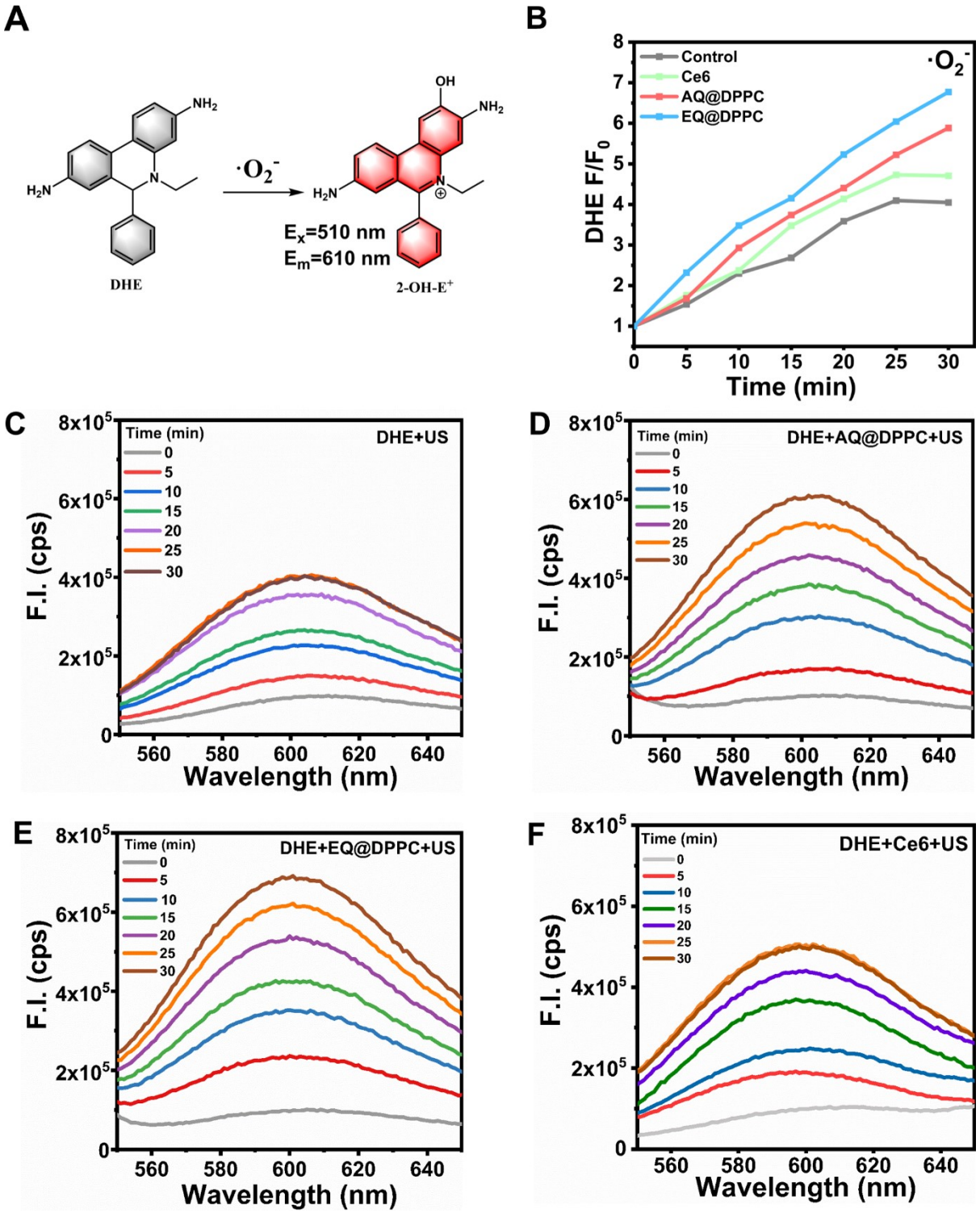


Fig. S14 Superoxide anion generation test. (A) Response mechanism of superoxide anion fluorescent probes DHE. (B) Plots of F/F_0 for $\cdot O_2^-$ generation in the presence of Ce6, AQ@DPPC or EQ@DPPC with different US time. F_0 was the fluorescence intensity of DHE before stimulation. E_x= 535 nm, E_m= 610 nm.

(C) Fluorescence spectra of DHE with different US time. (D) Fluorescence spectra of DHE in the presence of AQ@DPPC with different US time. (E) Fluorescence spectra of DHE in the presence of EQ@DPPC with different US time. (F) Fluorescence spectra of DHE in the presence of Ce6 with different US time.

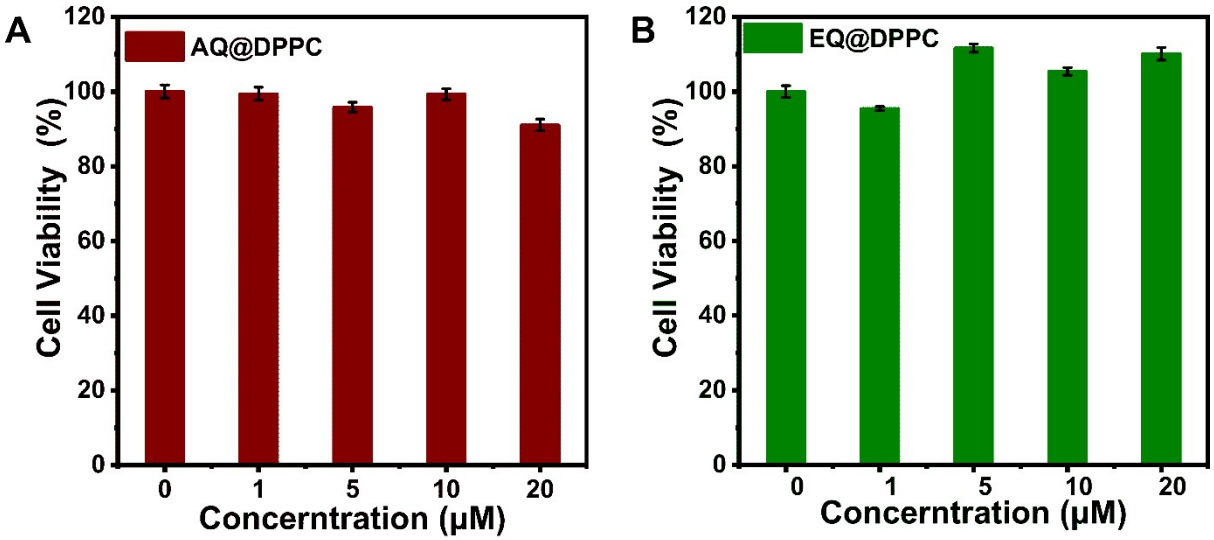


Fig. S15. Cell viability for BMSCs in the presence of the AQ@DPPC and EQ@DPPC at varied concentrations (AQ's or EQ's concentrations). Three independent experiments were conducted, and for each independent experiment, the assays were conducted in eight replicates. Data represent mean ± SD. Error bars represent the standard deviation (SD).

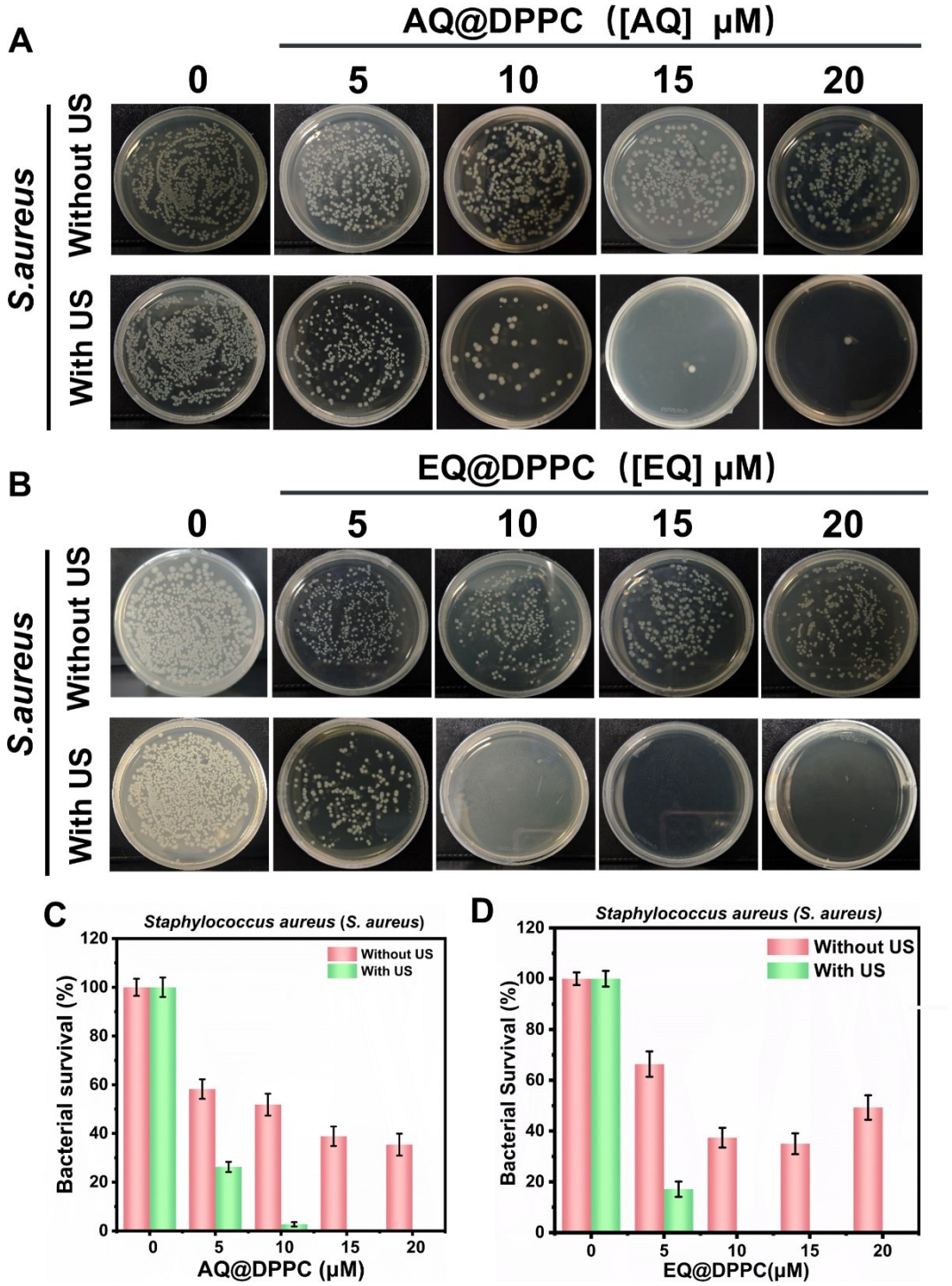


Fig. S16 The photographs of *S. aureus* colonies coated on LB-agar plates under different concentrations of AQ@DPPC (A) and EQ@DPPC (B) with US or without US. Inhibition of *S. aureus* under different concentrations of AQ@DPPC (C) and EQ@DPPC (D) with US or without US.

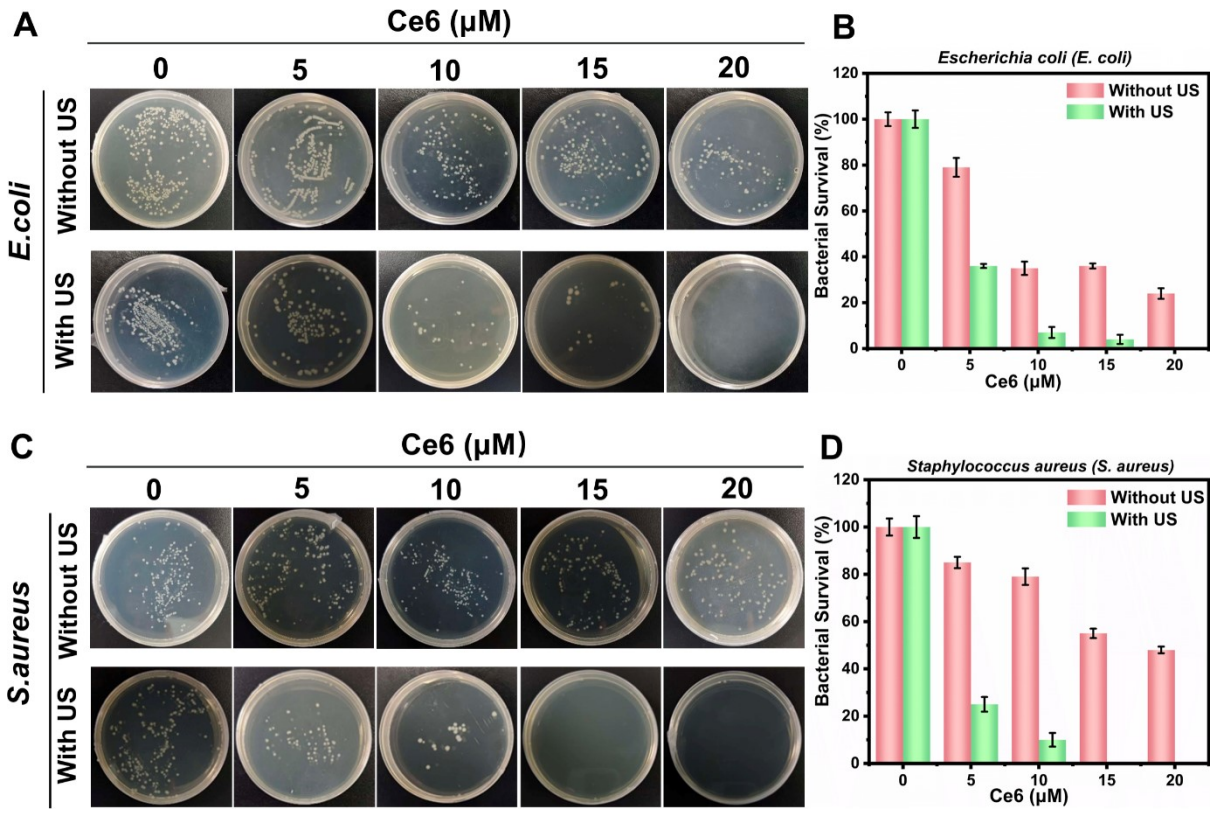


Fig. S17 The photographs of *Escherichia coli* (A) or *S. aureus* colonies (C) coated on LB-agar plates under different concentrations of Ce6 with US or without US. (B) Inhibition of *Escherichia coli* under different concentrations of Ce6 with US or without US. (D) Inhibition of *S. aureus* under different concentrations of Ce6 with US or without US.

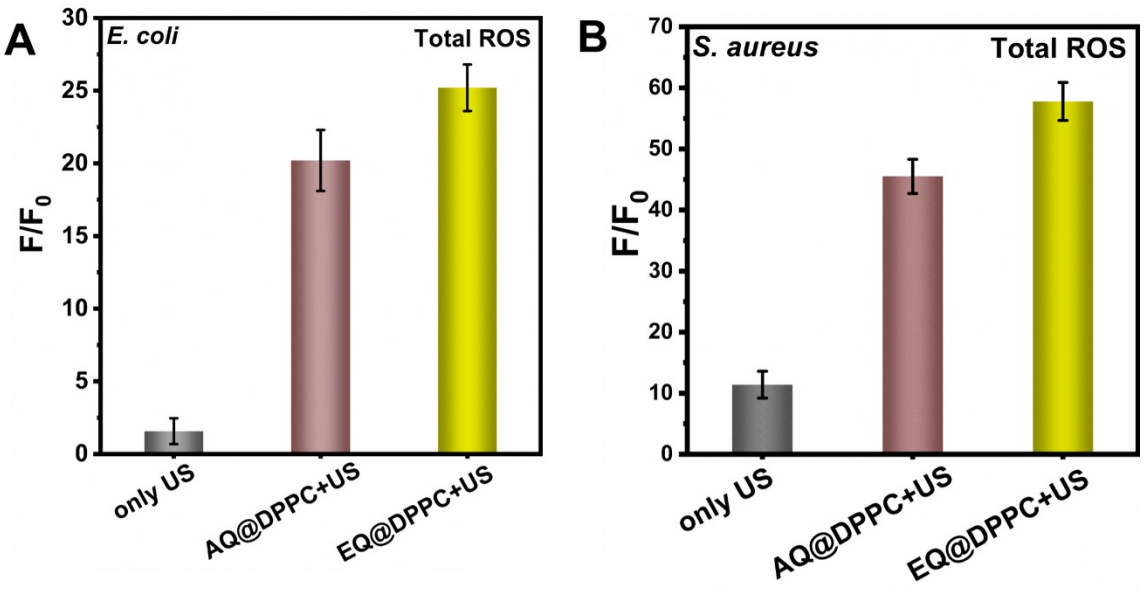


Fig. S18 The ROS generation levels in *E. coli* (A) and *S. aureus* (B) after AQ@DPPC or EQ@DPPC treatments under US stimulation. F_0 represents for the fluorescent intensity of DCFH-DA before US stimulation, while F represents for the fluorescent intensity of DCFH-DA after US stimulation for 10 min.

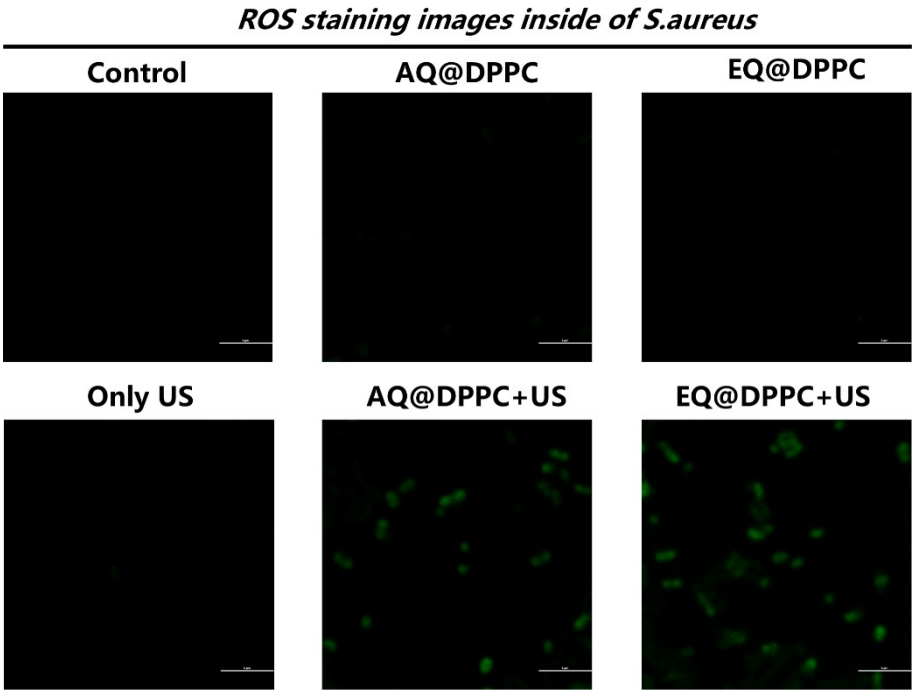


Fig. S19 Fluorescence imaging of intracellular ROS in *Staphylococcus aureus* (stained with DCFH-DA) after various treatments. Scale bar: 5 μm.

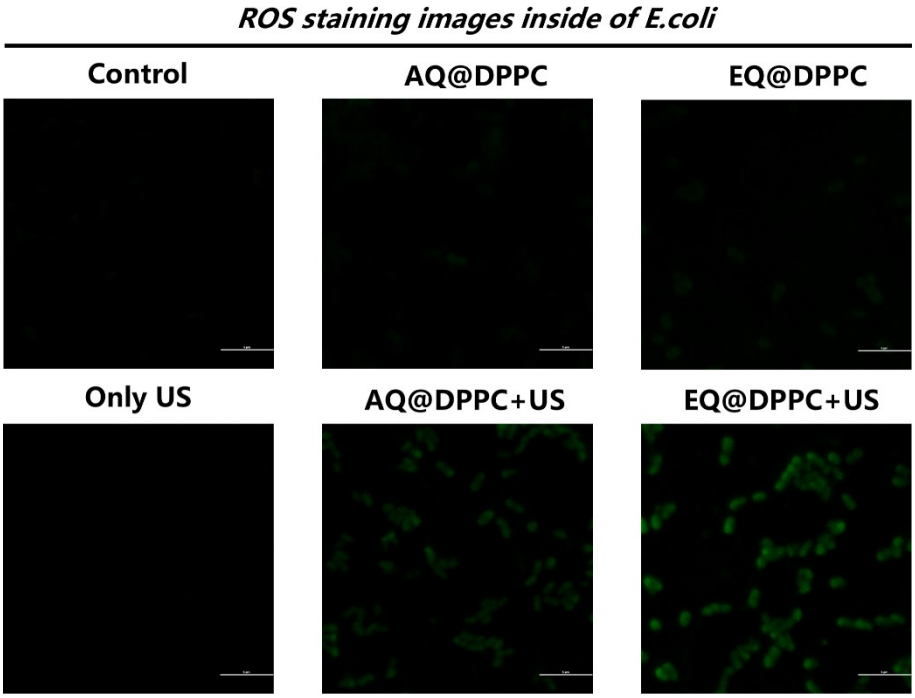


Fig. S20 Fluorescence imaging of intracellular ROS in *Escherichia coli* (stained with DCFH-DA) after various treatments. Scale bar: 5 μm.

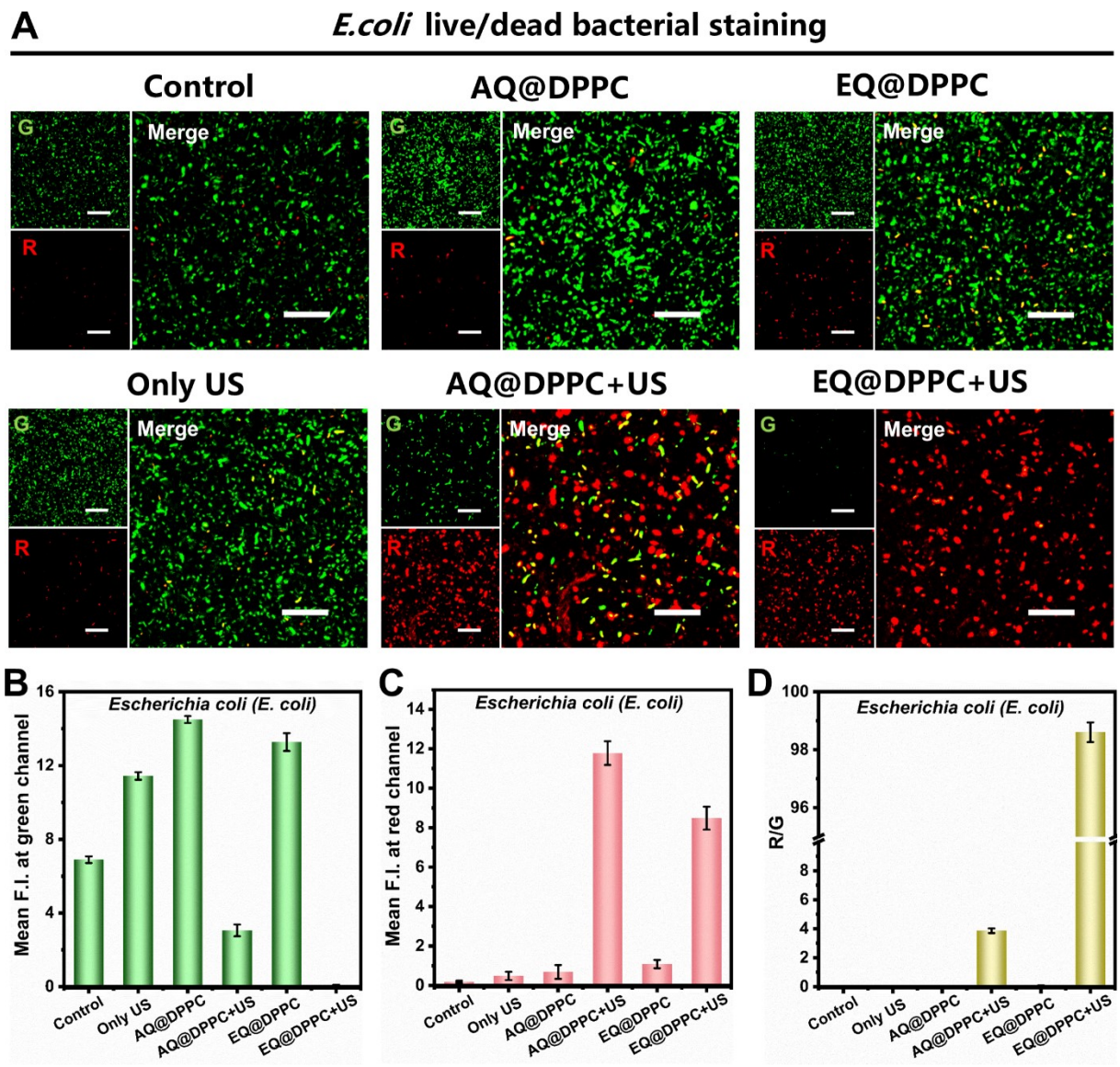


Fig. S21 Live/dead staining of *Escherichia coli.* (A) CLSM images of *E. coli* under different treatments after live/dead staining. Scale bar: 25 μm. (B) Mean fluorescent intensities of green channel under different treatments corresponding to (A). (C) Mean fluorescent intensities of red channel under different treatments corresponding to (A). (D) The mean fluorescent ratio of R/G under different treatments corresponding to (A).

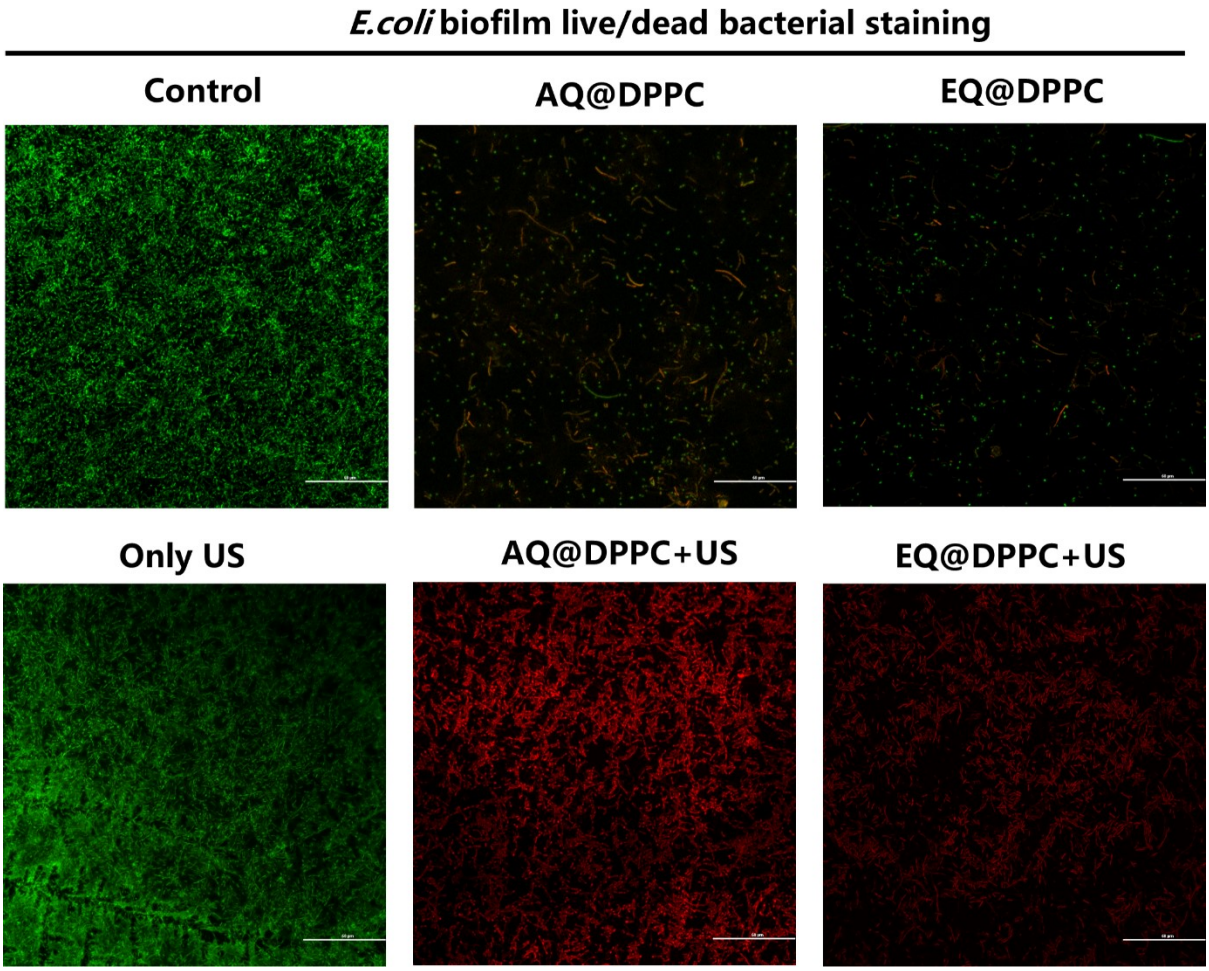


Fig. S22 Z-stacked of maximum fluorescent intensity in live/dead staining of *E. coli* biofilms after different treatments.

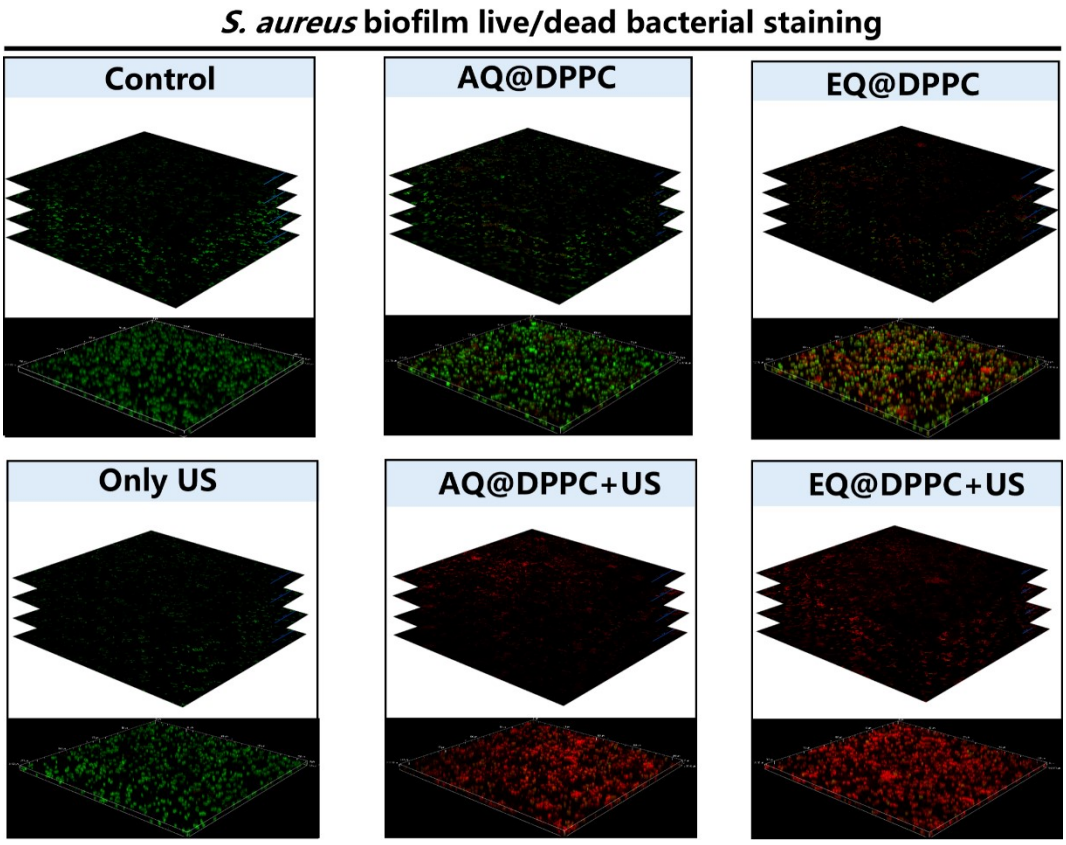


Fig. S23 Live/dead staining of *S. aureus* biofilms. DMAO/PI staining assay of *S. aureus* biofilms after different treatments.

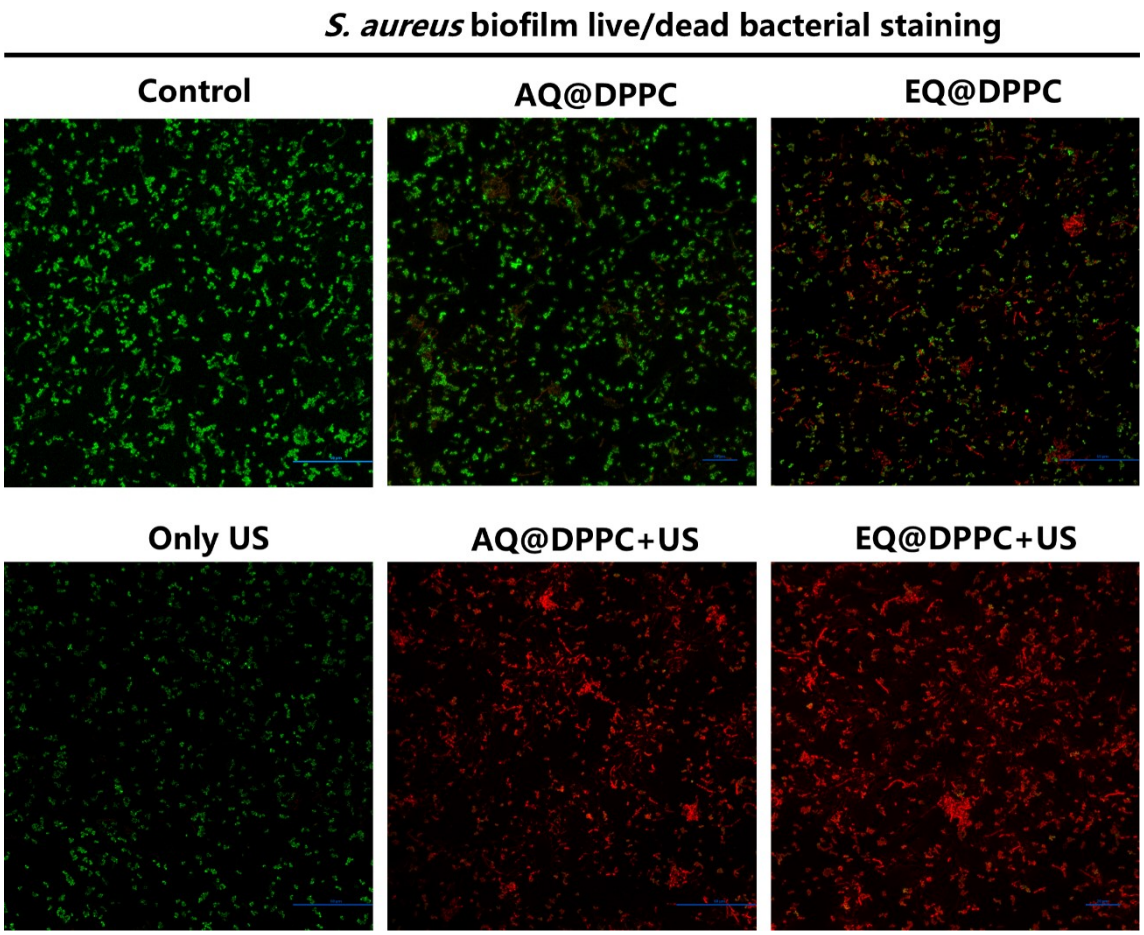


Fig. S24 Z-stacked of maximum fluorescent intensity in live/dead staining of *S. aureus* biofilms after different treatments.

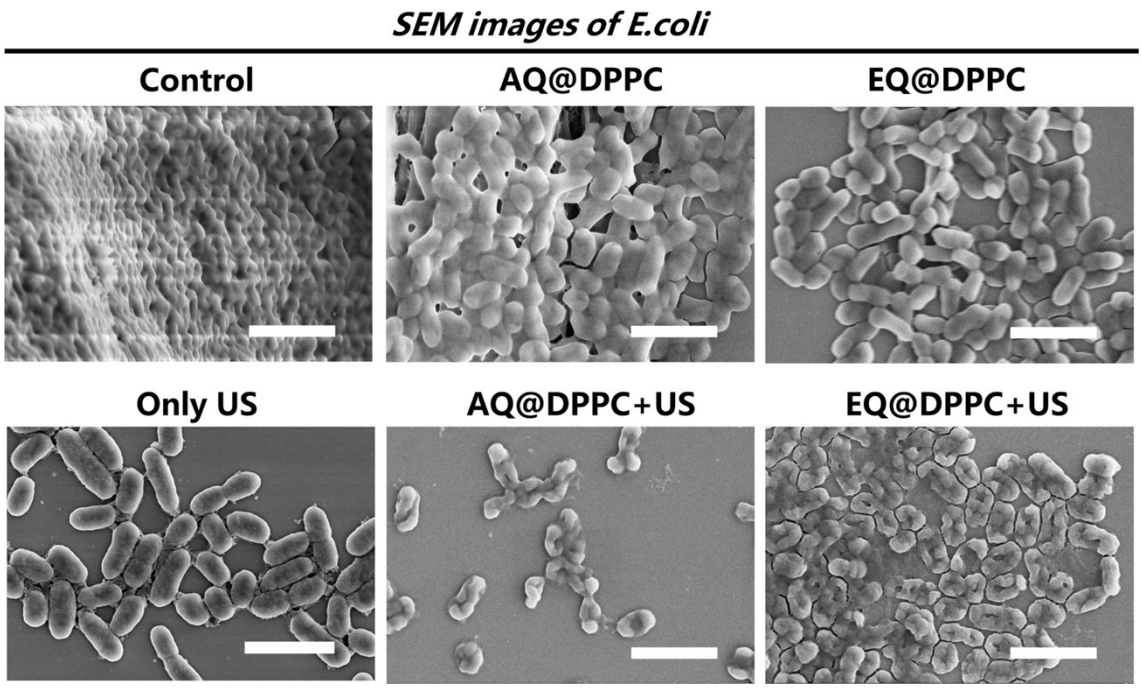


Fig. S25 Scanning electron microscopy (SEM) Images of *Escherichia coli* biofilm after various treatments. Scale bar: 3 μm.

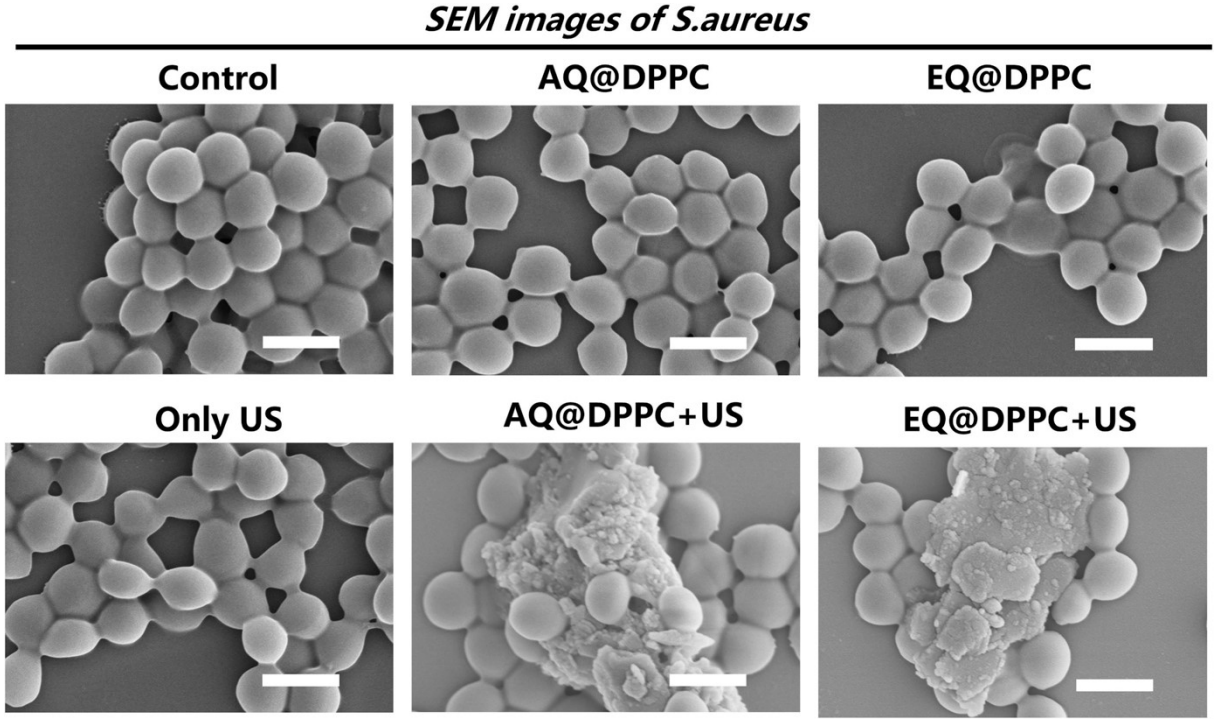


Fig. S26 Scanning electron microscopy (SEM) Images of *Staphylococcus aureus* biofilm after various treatments. Scale bar: 1 μm.

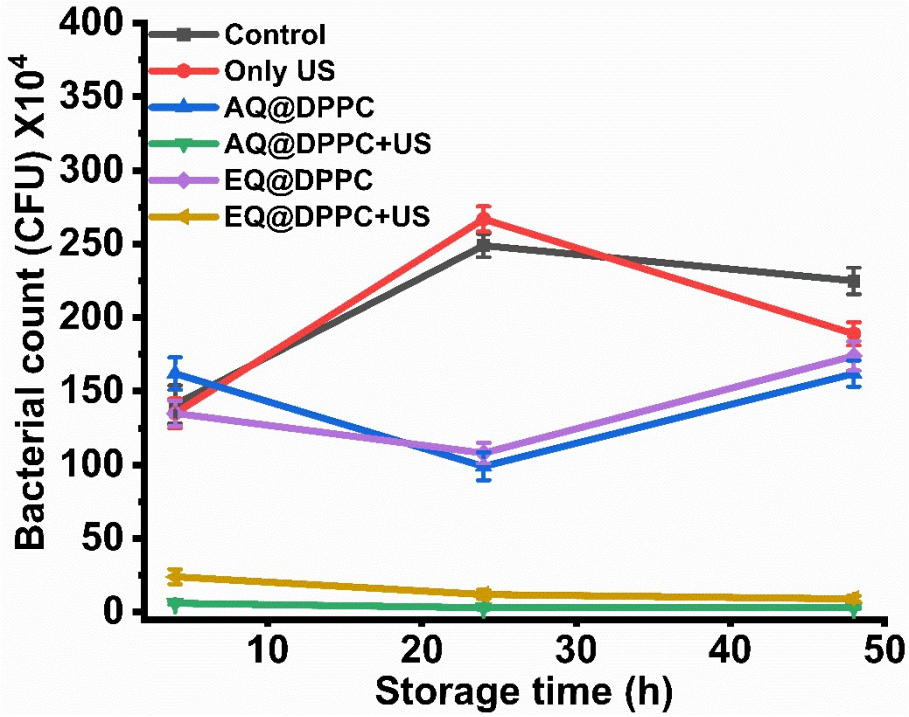


Fig. S27 Bacteria colony counts of *E. coli* in different treatments versus storage time.