Supporting Information

A Carboxylatopillar[5]arene-based pH-triggering Supramolecular Photosensitizer for Enhanced Photodynamic Antibacterial Efficacy

Tao Yue[+], Lei Xia[+], Jia Tian, Baoxuan Huang, Chao Chen, Hongliang Cao*, Weian Zhang*

Shanghai Key Laboratory of Functional Materials Chemistry, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China

Materials

Propionic acid, silver nitrate, methyl iodide, 4-pyridinecarboxaldehyde, pyrrole, glacial acetic acid, ethyl acetate and ethyl acetate were purchased from Shanghai Titan Scientific Co., Ltd. 9,10-Anthracenediylbis(methylene) dimalonic acid (ABDA) was obtained from Sigma-Aldrich Company. Tetrahydrofuran (THF) and toluene were dried by refluxing over sodium shavings and distilled before use. *N*, *N*-Dimethylformamide (DMF), triethylamine (TEA) and dichloromethane (CH_2Cl_2) were dried over CaH_2 and distilled just prior to use. Thiazolyl blue tetrazolium bromide (MTT) was purchased from Aladdin. All other reagents and solvents were of analytical grade and used as received.

Characterization

^1H NMR measurements were recorded at 400 MHz by using a BRUKER AV400 spectrometer in D_2O or DMSO-d_6 with tetramethylsilane (TMS) as an internal reference. Absorption spectra were determined on a SHIMADZU UV-2550 UV spectrophotometer using in quartz Cuvette with 1 cm beam path length. Dynamic light scattering (DLS) measurements were carried out with a BECKMAN COULTER Delasa Nano C particle analyzer. Transmission electron microscopy (TEM) analysis was carried out on a JEOL JEM1400 electron microscope operated at 100 kV. Samples for TEM were prepared by dropping the solution of nanoparticle onto a carbon-coated copper grid which was then dried at room temperature.

Synthesis of tetra(4-pyridyl) porphyrin (H_2TPyP).

Freshly distilled pyrrole (5.04 g, 75 mmol) and 4-pyridinecarboxaldehyde (8.04 g, 75 mmol) were dissolved in propionic acid (150 mL), and the resulting solution was refluxed at 140 °C for 1.5 h in the dark. After the solvent was removed under reduced pressure, the residue was redissolved in DMF (220 mL) and was refluxed for 30 min. After being cooled to room temperature, the mixture was kept at −20 °C overnight to precipitate. The precipitate was filtered off, and the solid residue was carefully washed with ether, and dried under vacuum to give 1.77 g of purple solid (2.9 mmol, yield 15%).

Synthesis of *meso*-tetra(*N*-methyl-4-pyridyl)porphyrin (TPyP). H_2TPyP (200 mg, 0.33 mmol) was dissolved in 30 mL of *N*, *N*-dimethylformamide and 9 mL of methyl iodide was added to the mentioned solution. The obtained solution was stirred for 10 h at room temperature. After evaporating the solvent under vacuum at room temperature, the resulting powder was recrystallized in a mixture of methanol and ethyl acetate to give 240 mg of solid (0.2 mmol, yield 60%). ^{1}H NMR (ppm): -3.11 (s, 2H, NH), 4.73 (s, 12H, N-CH_3), 8.99-9.01 (d, 8H, m), 9.20 (s, 8H, β), 9.48-9.50 (d, 8H, o).

Synthesis of silver tetra(*N*-methyl-4-pyridyl)porphyrin (AgTPyP).

TPyP(1.0 g, 0.8 mmol) was dissolved in 60 mL of distilled water, and a small amount of NaOH solution (0.1 mol/L) was added to the mentioned solution to adjust it to slightly alkaline. Then silver nitrate (0.5 g, 2.9 mmol) was added into the solution. The result solution was refluxed for 2 h. After the reaction was finished, KCl was added into above solution. After stirring for several minutes, the supernatant was collected by centrifugation, and then freeze-dried. The dried power was further purified by dissolving and washing by using hot MeOH. Final product **AgTPyP** was obtained by drying under vacuum. Yield: 0.78 g, 70.5%.

Fabrication of AgTPyP@P[5] complex

P[5] was synthesized according to the previous report. P[5] aqueous solution was added into AgTPyP aqueous solution under ultrasonic stirring. After incubation in a shaking

incubator under room temperature for 30 min, the AgTPyP@P[5] complex with a predetermined molar ratio (quaternary ammonium salt/P[5] = 1:1) was obtained.

$^1O_2$ generation assay

Typically, ABDA solution (50 μL, 1 mg/mL) was added to AgTPyP@P[5] in 3 mL PBS with different pH values. The solution was irradiated with 420 nm light source for different time, and the absorption intensity of ABDA at 380 nm was recorded.

***In Vitro* cell viability assay**

MTT assay was used to assess the cell viability. The mouse fibroblast L929 was seeded in 96-well plate at a determined cell density of about 5×10^3 cells per well. After incubation in a humidified at 37 °C and CO_2 (5%) for 24 h, the cells were washed with PBS (pH = 7.4). And then each well was added to fresh medium (100 μL) and incubated with 10 μL of the prepared samples with varying concentrations. After 48 h of incubation, the medium in each well was removed and washed with PBS, and then the culture medium was replaced with a fresh culture medium containing MTT (5 mg/mL) for a further incubation of 4 h. Finally, the culture medium containing MTT was replaced with 150 μL DMSO to dissolve the precipitates, and measured the absorbance of solution at 560 nm by a THERMO Multiskan MK3 spectrometer, a spectrophotometric microplate reader. Finally, cell viability was calculated by comparing the obtained absorbance intensity. The results were presented as a mean and standard deviation for each sample.

Live/dead assay and scanning electron microscopy (SEM) characterization

The obtained log-phase bacteria were divided into four groups at random: (1) PBS; (2) PBS + L (light); (3) AgTPyP@P[5]; (4) AgTPyP@P[5] + L. After adding into 100 μL of live/dead dye and staining for 15 min, the samples were washed with PBS for three times. Finally, the live/dead assay was performed through confocal laser scanning microscopy (CLSM).

The suspended bacterial solution was fixed with 2.5% glutaraldehyde at 4 ℃ for 12

h, and then dehydrated with different concentrations of ethanol (50, 70, 80, 90, 100%) for 10 min. The sample was dropped on the mica plate and observed by SEM after spraying gold.

In vitro antibacterial activity

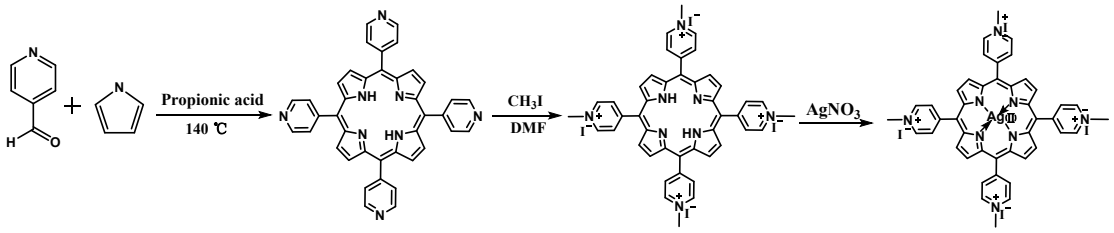
Overnight cultures of bacterial suspensions were subjected to centrifugation (5000 rpm for 3 min) and suspended into sodium acetate buffer (pH = 5.5) and sodium phosphate buffer (pH = 7.4) to a determined concentration of $\approx 10^6$ CFU/mL, respectively. Furthermore, 90 μL of the bacterial suspension was seeded into a 96-well plate, followed by the addition of AgTPyP@P[5] with different concentrations, respectively. The treated bacteria group was irradiated with 420 nm for 10 minutes, and the mixture (10 μL) in each well was transferred to a new 96-well plate, which contained 90 μL of fresh LB broth. After incubation at 37 °C for additional 16 h, the bacterial growth conditions were confirmed according to the OD_{600} value recorded by a microplate reader.

In the plate experiment, the diluted bacterial suspension (10^6 CFU/mL) was cultured with AgTPyP@P[5] nanoparticles with 60 μg/mL at different pH values. After culturing 30 min, the mixed solution (100 μL) was plated onto solid agar plates, and was irradiated with 420 nm light.

In vivo antibacterial activity

All animal procedures were performed in accordance with Chinese legislation on the Use and Care of Research Animals (Document No. 55, 2001), and institutional guidelines for the Care and Use of laboratory animals established by the East China University of Science and Technology Animal Studies Committee, and this committee approved the experiments.

To investigated the *in vivo* antibacterial performance of AgTPyP@P[5], four groups of mice were subjected to wound modeling with a wound diameter of about 3 cm. Then the wound was treated with PBS containing MDR *S. aureus* (10^8 CFU) to induce bacterial infection. At a fixed time of each day, different groups of mice were treated with AgTPyP@P[5] nanoparticles (100 μL, 40 μg/mL) or PBS, and the light group was irradiated with a 420 nm light source for 15 min.



Scheme S1. Synthesis of silver tetra(*N*-methyl-4-pyridyl)porphyrin (**AgTPyP**).

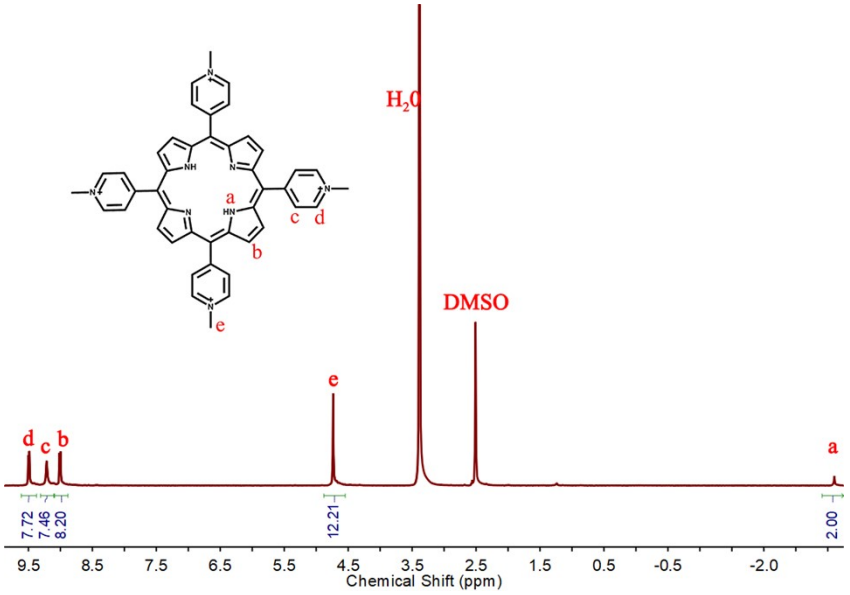


Fig. S1 ^{1}H NMR spectrum of TPyP.

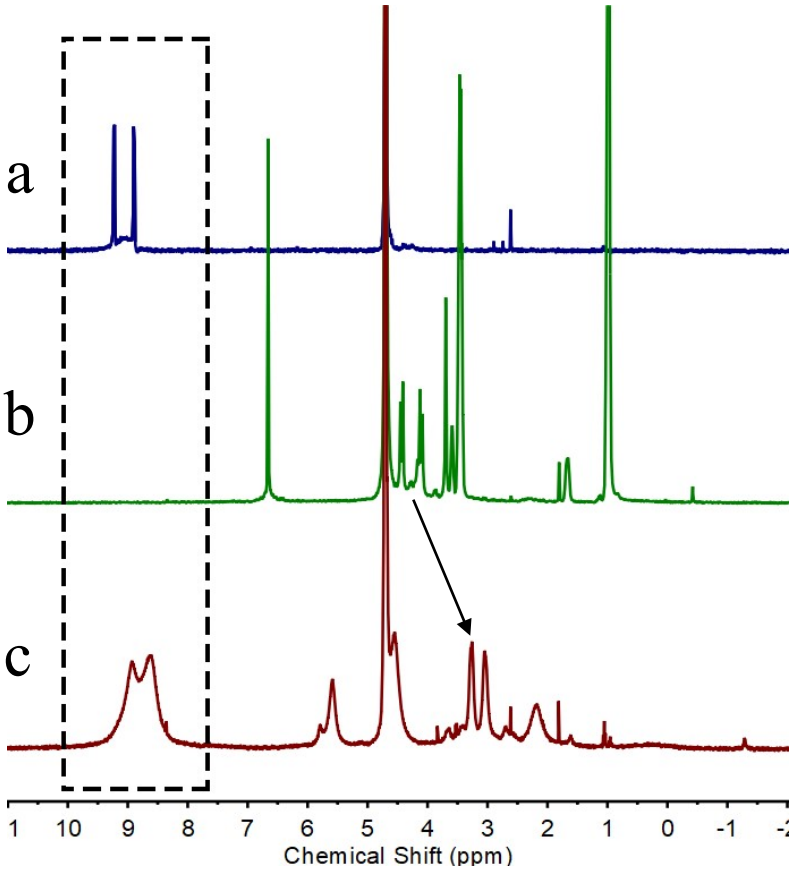


Fig. S2 ^{1}H NMR spectra of a) AgTPyP, b) P[5], and c) AgTPyP@P[5].

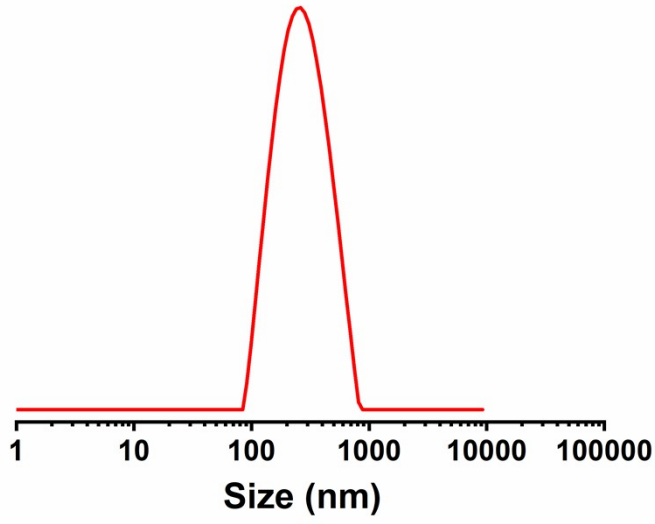


Fig. S3 Size distribution of AgTPyP@P[5] nanoparticles at pH = 7.4.

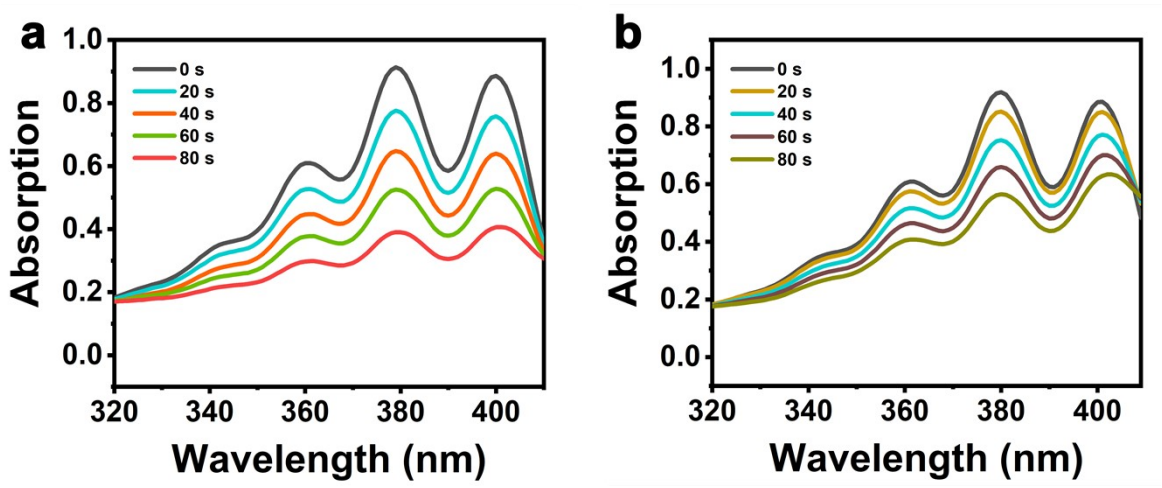


Fig. S4 UV-*vis* absorbance spectra of ABDA with AgTPyP@P[5] at pH = 5.5 (a), and pH = 7.4 (b) upon 420 nm light irradiation.

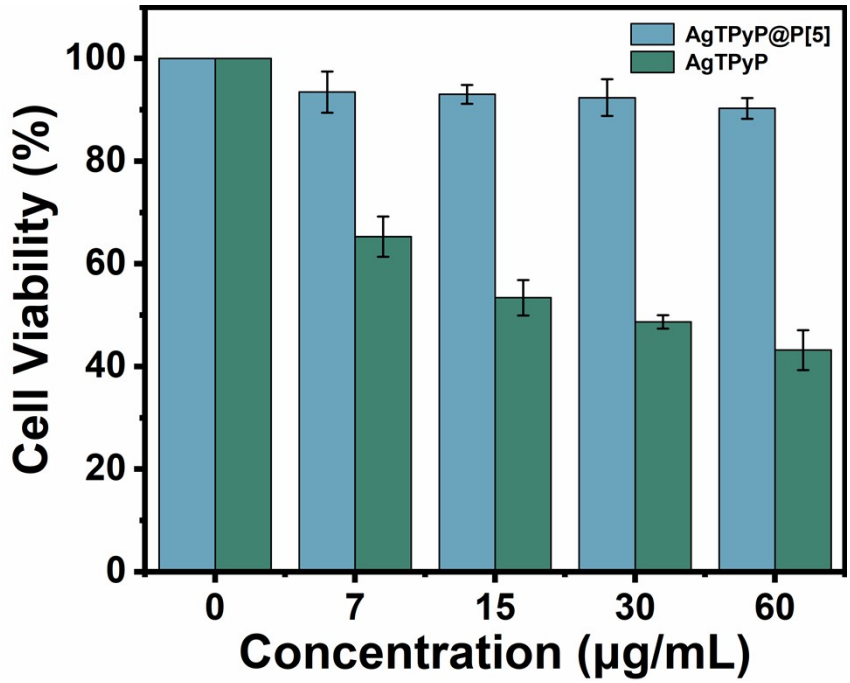


Fig. S5 Cell viability assay of L929 treated with AgTPyP and AgTPyP@P[5].

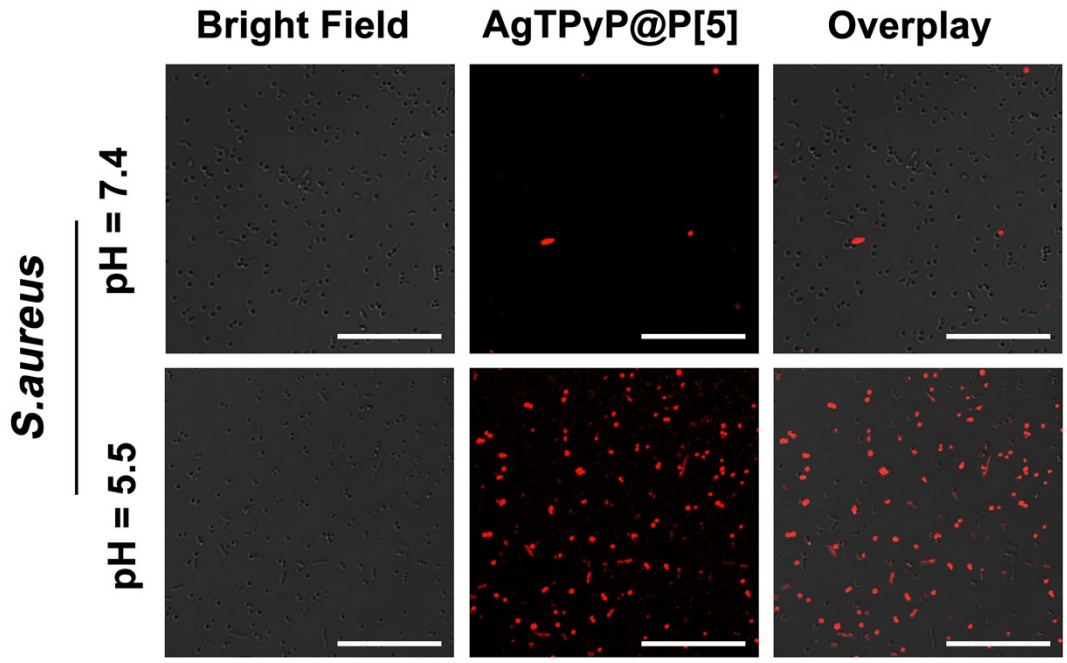


Fig. S6 CLSM images of MDR *S. aureus* after incubation with the AgTPyP@P[5] under different pH conditions for 1 h. Scale bar: 20 μm

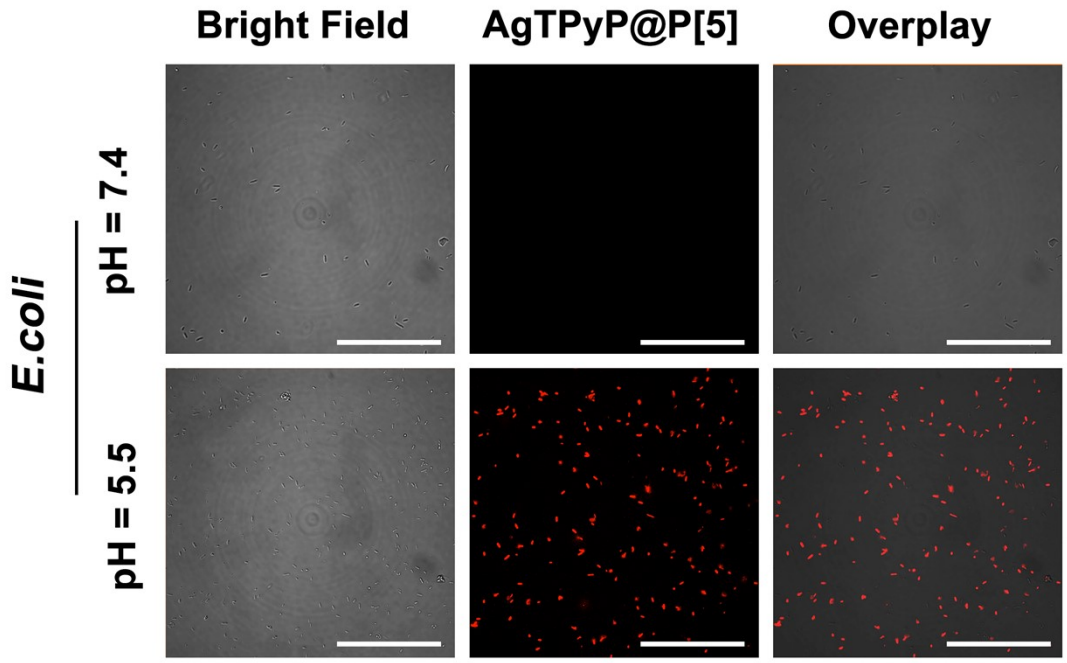


Fig. S7 CLSM images of MDR *E. coli* after incubation with the AgTPyP@P[5] under different pH conditions for 1 h. Scale bar: 20 μm

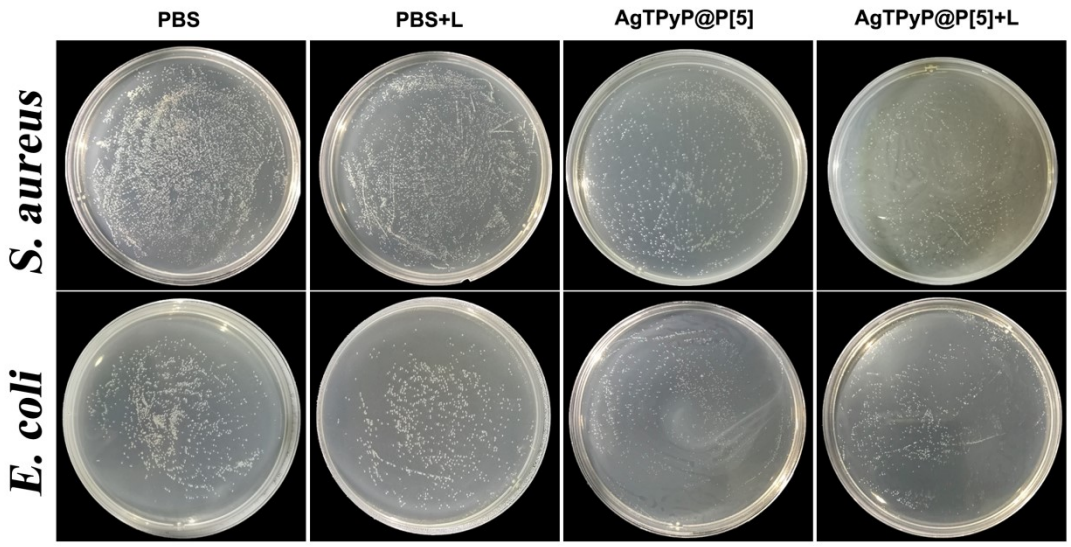


Fig. S8 Bacterial colony units growing on the plates after being treated with AgTPyP@P[5] nanoparticles with or without light irradiation at pH = 7.4.

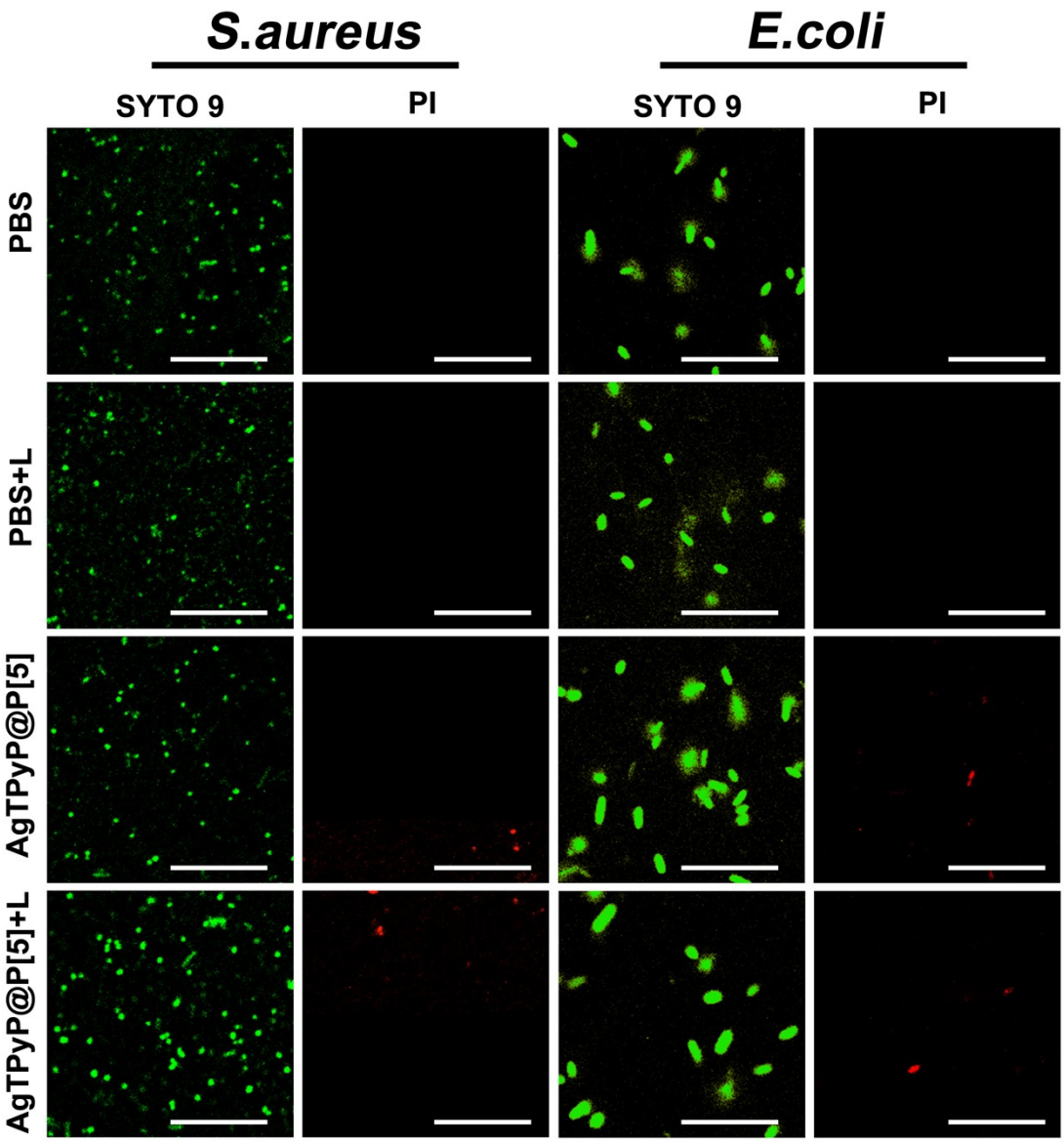


Fig. S9 CLSM images of *S.aureus* and *E. coli* before (control) and after treated with AgTPyP@P[5] with or without light irradiation at pH = 7.4. Scale bar: 20 μm

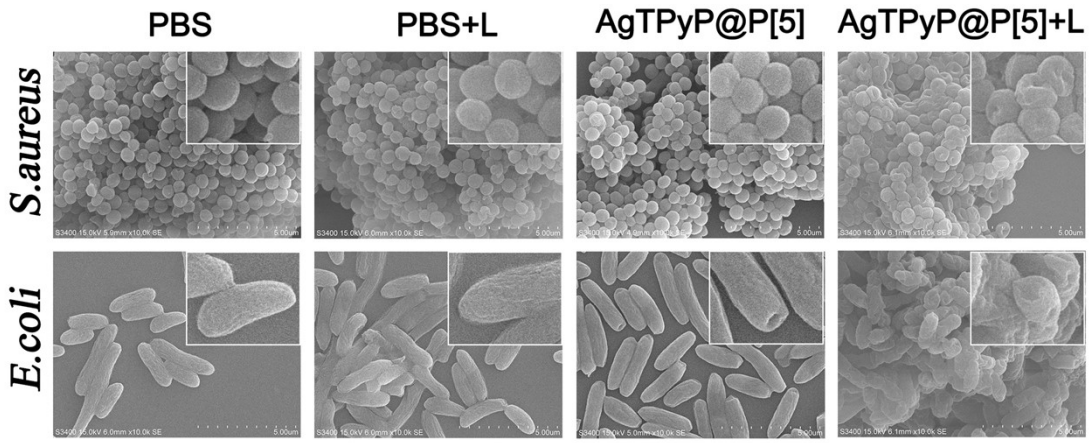


Fig. S10 SEM images of MDR E. coli and MDR S. aureus cells before (control) and after incubated with AgTPyP@P[5] nanoparticles with or without irradiation