Supporting Information

Red Light-Triggered Release of ROS and Carbon Monoxide for Combinational Antibacterial Application

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Contents

1. Experimental Section

1.1.Materials

Pyrrole, zirconium (IV) oxide chloride ($ZrOCl_2 \cdot 8H_2O$), propanoic acid, nitric acid, dichloroacetic acid, and sodium hydroxide were purchased from Sinopharm Chemical Reagent Co., Ltd. 2'-Hydroxyacetophenone, 4-formylbenzoic acid, and palladium chloride were purchased from Energy Chemical. Polyvinyl pyrrolidone (PVP) was purchased from Thermo Fisher Scientific Inc. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma Aldrich and used as received. Singlet oxygen sensor green (SOSG) for $^1O_2$ detection was purchased from Shanghai Maokang Biotechnology Co., Ltd. 2',7'-Dichlorodihydrofluorescein diacetate (DCFH-DA) was purchased from Aladdin Bio-Chem Technology Co., Ltd. 2',7'-dichlorodihydrofluorescein (DCFH) was prepared by deacetylation of the DCFH-DA in an alkaline solution and used immediately after preparation.[1, 2] The enhanced bicinchoninic acid (BCA) protein assay kit was purchased from Beyotime Biotechnogy. CO-specific probe, ally-Flu (AFCO), was prepared following literature procedures.[3] All other reagents and solvents were purchased from Sinopharm Chemical Reagent Co., Ltd. and used without further purification unless otherwise stated. Water was deionized on a Milli-Q® direct water purification system to obtain a specific resistance of 18.2 MΩ cm. *Staphylococcus aureus* (*S. aureus*, ATCC 25923), *Escherichia coli* (*E. coli*, ATCC 25922), and methicillin-resistant *S. aureus* (MRSA, USA300 LAC) cells were used for antimicrobial studies. Sheep red blood cells (purchased from Jiangsu Kejing Biological Technology Co., Ltd.) were used for hemolysis test. Unless otherwise stated, **PCN-222@PVP** and **PCN-CO@PVP** were at the same concentration based on the **PCN-222** in the antibacterial biological experiments, and **HOB-BA** concentration was 1.1-fold to the corresponding **PCN-222** in **PCN-CO@PVP**.

1.2.Characterization and Instruments

^1H and ^{13}C NMR spectra were acquired on a 400 MHz Bruker nuclear magnetic resonance (NMR) spectrometer. Dimethyl sulfoxide-d_6 (DMSO-d_6) was used as solvent. High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) spectra were performed on Waters XEVO® G2-XS-TOF equipped with an electrospray interface. High-performance liquid chromatography (HPLC) analysis was performed with a Shimadzu HPLC

system, equipped with an LC-20AP binary pump, an SPD-20A UV-vis detector, and a Symmetry C18 column. Fluorescence spectra were recorded on an F-4600 (Hitachi) spectrofluorometer. UV-vis absorption spectra were acquired on a UV-3600i Plus UV-vis spectrophotometer (Shimadzu). X-ray diffraction patterns (XRD) were collected on a Japan Rigaku Miniflex 600 rotation. Gas sorption measurements were conducted using an automatic volumetric adsorption equipment (Micrometritics ASAP 2020). Fourier-transform infrared (FT-IR) spectra were recorded on a Bruker Tensor II IR spectrometer. Scanning electron microscopy (SEM) was conducted on a Gemini SEM 500 electron microscopy (Zeiss) at an acceleration voltage of 3 kV. Transmission electron microscopy (TEM) observations were performed on a JEOL-2010 electron microscope at an acceleration voltage of 200 kV. Dynamic light scattering (DLS) and zeta potential measurements were conducted on a Zetasizer Nano ZS (Malvern). Inductively coupled plasma-atomic emission spectrometer (ICP-AES) was performed using ICAP 7400 (America, Thermo Fisher Scientific Inc). Confocal laser scanning microscopy (CLSM) images were acquired using a Leica TCS SP5 microscope.

1.3.Experimental details

***Dynamic light scattering (DLS) and Zeta Potential*.** DLS was used to measure the intensity-average hydrodynamic diameter distributions of **PCN-222**, **PCN-222@PVP,** and **PCN-CO@PVP** at 25 °C. Zeta potential was measured in a folded capillary Zeta cell DTS1070. The Smoluchowski equation was used to calculate the zeta potential. Measurements were performed three times for each sample. Error bars represented the standard deviation of three measurements.

Transmission electronic microscopy (TEM) of PCN-222, PCN-222@PVP, and PCN-CO@PVP. TEM samples were prepared by placing 10 μL of **PCN-222**, **PCN-222@PVP** or **PCN-CO@PVP** aqueous dispersion on copper grids coated with Formvar and carbon films. Finally, TEM photography was directly taken with a JEOL 2010 electron microscope at an acceleration voltage of 200 kV.

X-ray diffraction (XRD) of HOB-BA, PCN-222, PCN-222@PVP, PCN-CO@PVP, and PVP. XRD patterns were recorded on a Japan Rigaku Miniflex 600 rotation anode X-ray diffractometer equipped with graphite monochromatized CuKα radiation (λ = 1.54 Å).

N_2 adsorption-desorption isotherms of PCN-222 and PCN-CO. Before gas adsorption measurement, **PCN-222** or **PCN-CO** samples were thoroughly washed with acetone twice. Then, samples were dried in a pre-heated 85 °C oven for 30 min. Resulting powder (40 mg) was then activated at 150 °C for 12 h for N_2 adsorption measurement. The surface area, pore size, and pore distribution of **PCN-222** or **PCN-CO** were determined from Brunauer-Emmett-Teller (BET) isotherms collected with a N_2 atmosphere at 77 K, 1 atm.

Fourier transform infrared spectroscopy (FT-IR). The spectra were collected over 64 scans with a spectral resolution of 4 cm^{-1} in the range of 4000-400 cm^{-1}.

UV-vis absorption and Fluorescence emission spectra. UV-vis absorption spectra were acquired on a UV-3600i Plus UV-vis spectrophotometer. UV-vis absorption spectra of **HOB-BA**, **PCN-222@PVP**, and **PCN-CO@PVP** (45 μg mL^{-1}) with or without 650 nm light irradiation were recorded at room temperature. Fluorescence spectra were recorded on an F-4600 (Hitachi) spectrofluorometer. The released ROS and $^1O_2$ levels of **PCN-222@PVP** or **PCN-CO@PVP** (15 μg mL^{-1}) were measured in vitro using the fluorescence probes 2′,7′-dichlorodihydrofluorescein (DCFH, 40 μM) or singlet oxygen sensor green (SOSG, 5 μM) with or without 650 nm light irradiation. The fluorescence spectra of DCFH were recorded and collected from 495 to 800 nm using an excitation wavelength of 488 nm. Moreover, the fluorescence spectra of SOSG were recorded and collected from 510 to 800 nm using an excitation wavelength of 504 nm.[4]

Detection of CO release from PCN-CO@PVP. The released CO from **PCN-CO@PVP** (5 μg mL^{-1}) with 650 nm light irradiation was measured with CO-specific fluorescence probes, ally-Flu (AFCO, 10 μM) and palladium chloride ($PdCl_2$, 10 μM). The fluorescence spectra of AFCO were recorded and collected from 500 to 800 nm using an excitation wavelength of 490 nm.

Also, the CO-releasing concentration was monitored by a commercially available CO detector (Drager Pac6500). Briefly, the measurements were carried out in a closed desiccator equipped with a Pac6500 CO detector, and a scintillation vial containing **PCN-CO@PVP** aqueous dispersion (50 μg mL^{-1}, 10 mL). The lid was sealed, and the dispersion was irradiated or not with a 650 nm LED lamp (26 mW cm^{-2}). The CO detector read the CO concentration in the gas phase, and the data was recorded over time. Assuming that the gas and liquid phases

reached an equilibrium, and the pressure in the desiccator remained at 1 atm, the amount of released CO (*Nco*) was calculated using the following eq (1).

$$N_{CO} = \frac{pV_g}{RT} + cV_l = p\left(\frac{V_g}{RT} + \frac{V_l}{k}\right) \quad (1)$$

Where p is the partial pressure of CO; V_g and V_l are the volumes of the gas phase (350 mL) and liquid phase (10 mL); R is the gas constant (0.0821 atm·L·mol^{-1}K^{-1}); T is the temperature (298.15 K); c is the CO concentration in the liquid phase, and k is Henry's law constant of CO in water (1052.63 atm·L·mol^{-1} at 298.15 K).

***In vitro antibacterial assay*.** Antibacterial capacity was studied by the standard colony-forming unit (CFU) counting method.[5] Briefly, *Staphylococcus aureus* (*S. aureus*, ATCC 25923) and methicillin-resistant *S. aureus* (MRSA, USA300 LAC) were used as representative Gram-positive bacterial strains, and *Escherichia coli* (*E. coli*, ATCC 25922) was used as representative Gram-negative bacterial strain.

For each bacterial strain, 1-2 individual colonies were inoculated into fresh Tryptic Soy Broth (TSB) media and incubated at 37 °C for 16-18 h. Then, 40 μL of culture medium was diluted with fresh TSB (100-fold) and regrown at 37 °C to mid-log phase. Bacterial cells were then harvested and washed twice with sterile phosphate buffered saline (PBS) via centrifugation (8500 rpm for 5 min; 4 °C), adjusted with sterile PBS to a suitable bacterial concentration (OD_{600} = 0.5-0.7). Varying concentrations of aqueous dispersions (100 μL) of **HOB-BA**, **PCN-222@PVP**, and **PCN-CO@PVP** in PBS buffer were added into each zero-dilution well in a 96-well microplate. 50 μL of adjusted bacterial suspension was inoculated into each zero-dilution well of a preset microplate, to achieve 5×10^5 CFU mL^{-1} in each well (150 μL). The microplate was then incubated at 37 °C for 10 min, followed by irradiation for 30 min under 650 nm (26 mW cm^{-2}). In addition, the control microplate was kept under dark. After that, the treated bacteria were further incubated at 37 °C for 30 min and diluted with sterile PBS buffer (100×), followed by plating the dilutions (20 μL) onto TSB agar plates for overnight incubation at 37 °C to form visible colonies.

***CLSM observation of live/dead bacteria staining with SYTO 9 and Propidium Iodide (PI) staining assay*.**[5] To further demonstrate the membrane permeability changes, *S. aureus* or *E. coli* cells were further cultured at 37 °C for 3 h after the treatment of **HOB-BA**, **PCN-**

222@PVP or **PCN-CO@PVP** (0.126 mg mL^{-1}) with or without 650 nm light irradiation (26 mW cm^{-2}) for 30 min. Then, SYTO 9/PI probes (2.4 μM and 12 μM) were added to the bacterial dispersion, followed by incubation at 37 °C for 20 min. Afterward, the bacteria dispersions were centrifuged at 8500 rpm for 3 min and washed thrice with 1 mL of PBS. At last, the bacteria were resuspended with PBS and were imaged on a Leica SP-5 confocal laser scanning microscope. The green channel of SYTO 9 was excited at 488 nm and collected from 515 nm to 550 nm, while the red channel of PI was excited at 543 nm and collected from 600 nm to 650 nm. *S. aureus* and *E. coli* cells treated with PBS buffer were used as the negative control.

Observation of the interaction between samples and bacteria by CLSM. *S. aureus* or *E. coli* cells in the mid-logarithmic phase of growth were diluted with PBS to 1×10^6 CFU mL^{-1}. Bacterial cells (80 μL) were incubated with 120 μL of **PCN-222@PVP** or **PCN-CO@PVP** dispersion (0.060 mg mL^{-1}) at 37 °C for 1 h. The bacteria were collected and washed twice with PBS to remove the excess samples in the media. After that, the cells were redispersed in PBS and then were taken on a Leica SP-5 CLSM. The red channel was excited at 405 nm and collected from 650 nm to 695 nm, which was characteristic emission of **PCN-222**. Bacteria treated with PBS buffer was employed as control.

Study of the interaction between samples and bacteria through* zirconium (*Zr) relative content detection by Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES). Firstly, 1 mL of **PCN-222@PVP** or **PCN-CO@PVP** (0.060 mg mL^{-1}) was mixed with 1 mL of *S. aureus* or *E. coli* bacterial solution and incubated for 1 h in an incubator at 37 °C, and then washed three times with PBS. After that, the resulting mixture was digested with 2 mL of concentrated nitric acid/concentrated hydrochloric acid (v/v, 1/3) at 120 °C following dilution with 4% dilute nitric acid to 3 mL, and 0.45 μm water membrane was used to filter insoluble matter after fully sonicating. Finally, the relative content of Zr was detected with ICP-AES.

Intracellular reactive oxygen species (ROS) assay. The intracellular ROS levels of *S. aureus* or *E. coli* cells were measured by a ROS-sensitive fluorescence probe, 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA).[6, 7] *S. aureus* or *E. coli* cells in mid-logarithmic phase were diluted in PBS to 2×10^8 CFU mL^{-1} and mixed with DCFH-DA (final

concentration: 0.5 mM). The bacteria were incubated for 30 min at 37 °C, and then collected and washed twice with PBS to remove the residual DCFH-DA. After that, the bacteria were mixed with aqueous dispersions of **HOB-BA**, **PCN-222@PVP** or **PCN-CO@PVP** (final concentration: 0.060 mg mL^{-1}), and were exposed to 650 nm light irradiation for 30 min. The bacteria were further incubated at 37 ^{o}C for 30 min upon completion. The fluorescence intensities were recorded using an excitation wavelength of 488 nm and collected from 500 nm to 550 nm.

Scanning electron microscopy (SEM) observation of bacteria. To investigate the antibacterial mechanism, SEM was applied to observe the morphological changes of bacteria (*S. aureus* or *E. coli*) with different treatments. Following the same procedures of antibacterial assay as detailed above, the resultant bacteria mixture was collected and washed with PBS for three times. Then, the treated bacteria were fixed with 4% glutaraldehyde at 4 °C overnight. The fixed bacterial cells were washed thrice with sterile PBS and dehydrated in a series of graded ethanol solutions (30, 50, 70, 80, 95, and 100% ethanol, respectively) for 15 min. Finally, the final samples were dropped onto gold-plated silicon wafers and then dried in a vacuum condition overnight.

Transmission electron microscopy (TEM) observation of bacteria. *S. aureus* or *E. coli* cells were inoculated and cultured at 37 °C in TSB or Luria-Bertani (LB) media, and then diluted in sterile PBS to 2×10^{9} CFU mL^{-1}. 5 mL of bacterial suspension and 5 mL of **HOB-BA**, **PCN-222@PVP** or **PCN-CO@PVP** in PBS buffer (0.060 mg mL^{-1}) were mixed and incubated for 10 min at 37 °C, following irradiation for 30 min under 650 nm (26 mW cm^{-2}). The resultant bacteria mixture was incubated for 30 min at 37 °C, then collected and washed with PBS for three times. The treated bacteria were fixed with 5% glutaraldehyde and 4% paraformaldehyde in PBS at 4 °C overnight. The fixed bacterial cells were washed thrice with sterile PBS and stained with a solution of 2% osmium tetroxide (100 μL) overnight. Afterward, the samples were rinsed thrice with sterile PBS (20 min each, 1 mL), and then dehydrated in a series of graded ethanol solutions (30, 50, 70, 80, 95, and 100% ethanol) following acetone rinsing thrice (20 min each, 1 mL). The resulting samples were incubated in epoxy resin solutions for 1 h (epoxy resin/acetone = 1/1, v/v, 500 μL) and then for 3 h (epoxy resin/acetone = 3/1, v/v, 500 μL). After that, the samples were embedded in a fresh

resin solution (100%) for 36 h. Upon completion, a new batch of resin (250 μL) was added, and the samples were cured at 70 °C for two days. Finally, the cured samples were sliced into 70 nm-thick sections using an ultramicrotome equipped with a diamond knife (Leica UC7) and placed on copper grids for TEM observations.

Deoxyribonucleic acid (DNA) and protein leakage assays. After the treatment of **HOB-BA**, **PCN-222@PVP** or **PCN-CO@PVP** (0.060 mg mL^{-1}) with or without 650 nm light irradiation for 30 min, the resultant mixture of *S. aureus* or *E. coli* cells were centrifuged, filtered with a membrane (0.22 μm), and the supernatants were collected. DNA concentrations were quantified by measuring the optical values of the collected supernatants at the wavelength of 260 nm (OD_{260}). The protein contents in the supernatants were measured using an enhanced BCA protein assay kit. The absorbance intensity at 562 nm was recorded and the protein concentrations were calculated against a standard calibration curve using bovine serum albumin (BSA) as a model protein. Bacteria with the addition of 1% Triton X-100 (TX-100) or PBS buffer were employed as the positive and negative control, respectively.

Hemolysis assay. The hemolytic activity of **HOB-BA**, **PCN-222@PVP** or **PCN-CO@PVP** with or without 650 nm light irradiation were tested against sheep red blood cells (2%). First, the erythrocyte dispersion was centrifuged at 2,000 rpm for 10 min, and PBS was used to resuspend the cells three times to remove hemoglobin. The cells with 1% TritonX-100 (TX-100) and PBS buffer were used as the positive and negative control, respectively. After mixing an equal volume of erythrocyte suspension and PBS, micelles, or TX-100, the cells were incubated at 37 °C for 1 h. After centrifugation at 2000 rpm for 10 min, the supernatants (100 μL) were transferred to a 96-well plate. And the absorbance at 576 nm was collected to calculate the percentage of hemolysis using the following equation:[8]

$$\text{Hemolysis\%} = (A_{576,\ \text{treated}} - A_{576,\ \text{blank}}) / (A_{576,\ \text{control}} - A_{576,\ \text{blank}}) \times 100\%$$

where $A_{576,\ \text{treated}}$ is the absorbance in the presence of micelles, $A_{576,\ \text{control}}$ is the absorbance in the presence of 1% TX-100, and $A_{576,\ \text{blank}}$ is the absorbance of the plate with an identical volume of PBS, respectively.

Cytotoxicity assay. L929 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) and antibiotics (100 units/mL penicillin and 0.1 mg/mL streptomycin) at 37 °C in a humidified atmosphere with 5% carbon dioxide (CO_2),

and the cell viability was determined by standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, L929 cells were seeded on a 96-well plate at a density of 10,000 cells/well in 100 μL of DMEM medium. After 24 h incubation, DMEM medium was replaced by 100 μL of fresh medium containing **HOB-BA**, **PCN-222@PVP** or **PCN-CO@PVP** with or without 650 nm light irradiation. After 24 h incubation, MTT reagent in 10 μL of PBS buffer (5 mg mL^{-1}) was added into each well, and the cells were further incubated with 5% CO_2 for 4 h at 37 °C. The culture medium in each well was removed and replaced by 100 μL of DMSO. The absorbance values were recorded at the wavelength of 490 nm by a microplate reader (Thermo Fisher Scientific). The cell viability was calculated using the following equation:

$$\text{Cell viability\%} = (A_{490,\text{ treated}} - A_{490,\text{ blank}})/ (A_{490,\text{ control}} - A_{490,\text{ blank}}) \times 100\%$$

where $A_{490,\text{ treated}}$ and $A_{490,\text{ control}}$ are the absorbance values of cells in the presence and absence of micelles. $A_{490,\text{ blank}}$ is the absorbance value of the plate with an identical volume of MTT solution without cells.

Stability assay. **PCN-CO@PVP** solutions with a concentration of 0.045 mg/mL were individually prepared in pure water, DMEM/FBS (10%), and PBS. Changes over the course of one week under dark were monitored using UV-vis and DLS to characterize the chemical and physical stability of **PCN-CO@PVP** in various media.

Statistical Analysis. Data are presented as mean ± standard deviations and were analyzed using Prism 8.0 software (GraphPad, San Diego, California, USA) and student's t-test. Data were considered statistically significant if p values lower than 0.05 (****p<0.0001, ***p<0.001, **p<0.01, *p<0.05).

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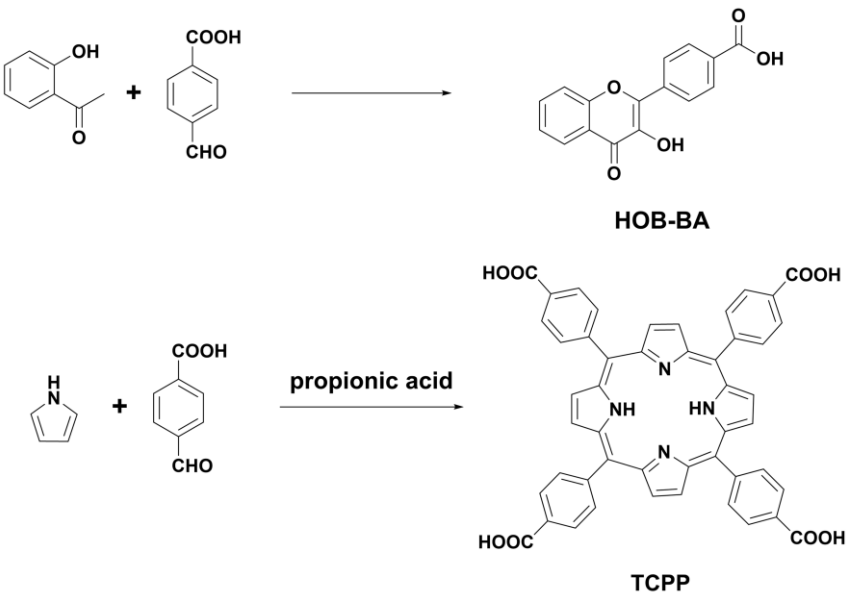
2. Supplementary Results

2.1.Synthetic routes of **HOB-BA** and **TCPP**



Scheme S1. Synthetic routes of **HOB-BA** and **TCPP**.

2.2 ^{1}H NMR, ^{13}C NMR spectra, HPLC, and HR-ESI-MS analysis for **HOB-BA**

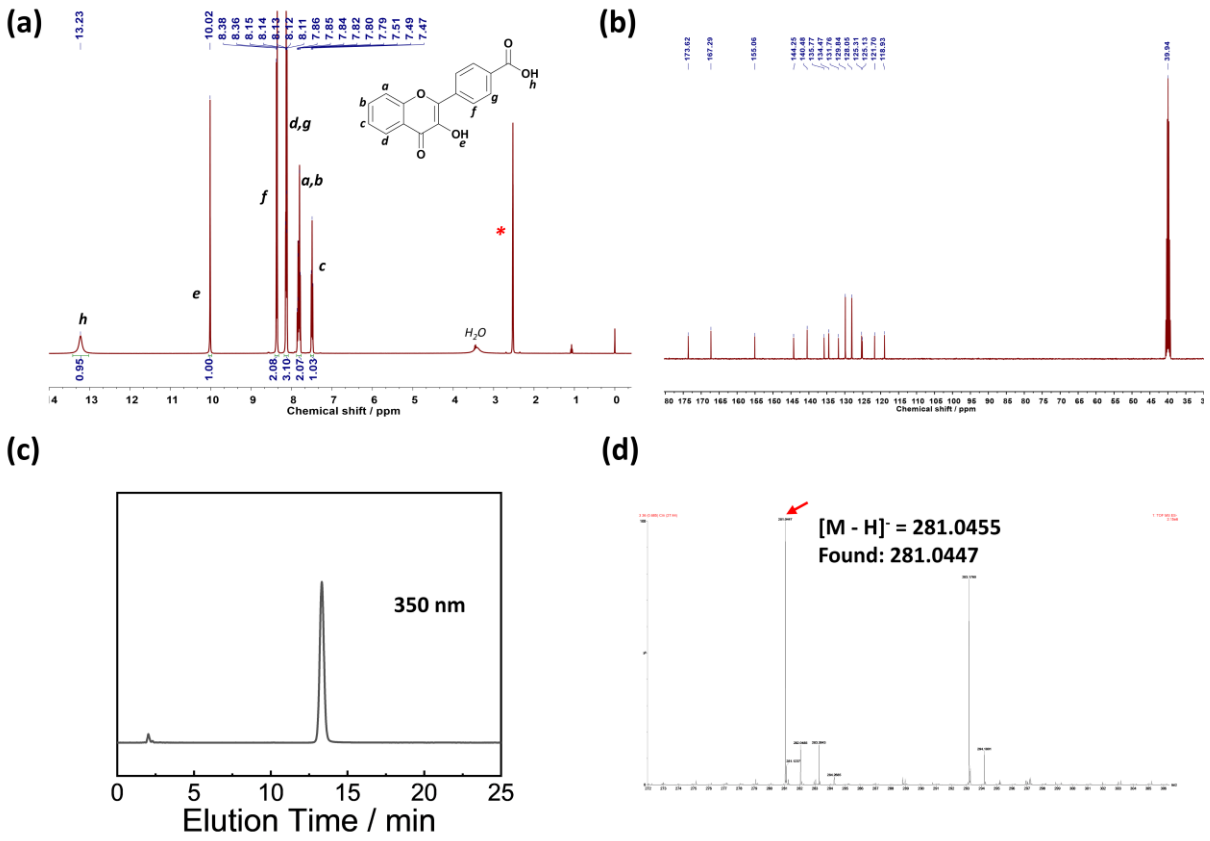


Figure S1. (a) ^{1}H and (b) ^{13}C NMR spectra recorded in DMSO-d_6 for **HOB-BA**. (c) HPLC elution profile detected at 350 nm and (d) HR-ESI-MS analysis for **HOB-BA.**

2.3 UV-vis absorption spectra

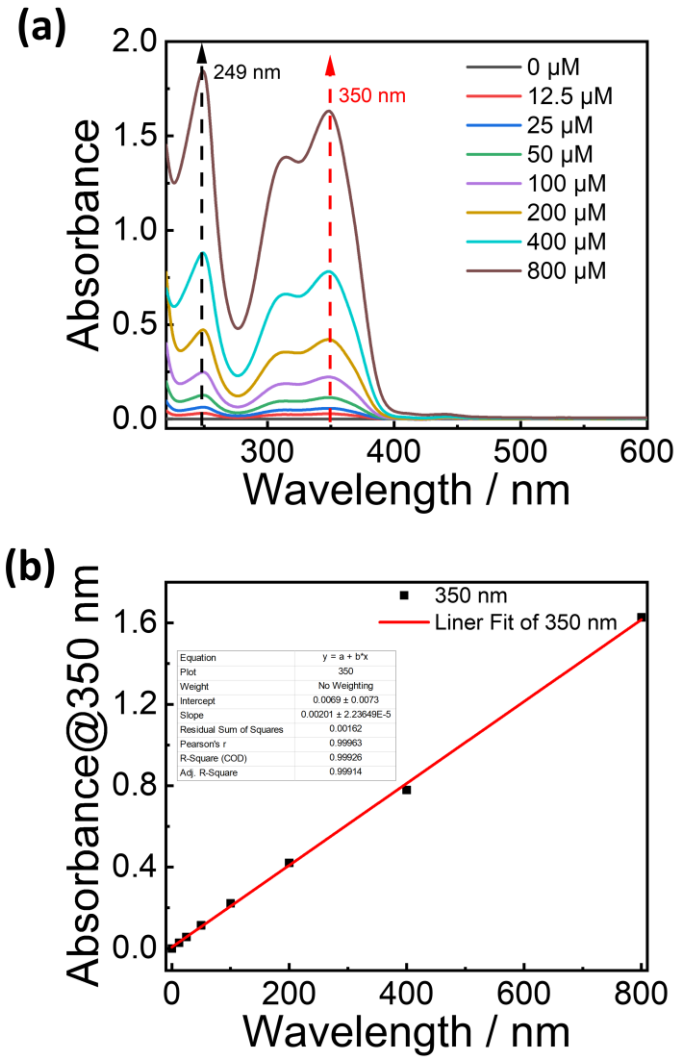


Figure S2. (a) UV-vis absorption intensity changes of **HOB-BA** with concentration (0-800 μM) and (b) the fitted absorption intensity-concentration standard curve at 350 nm.

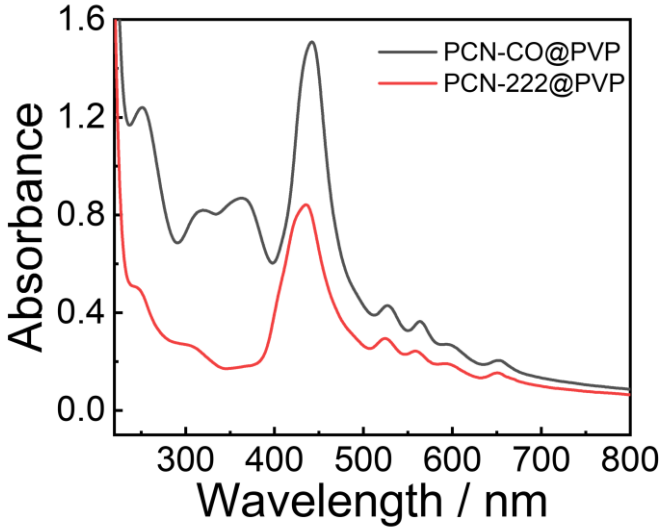


Figure S3. UV-vis absorption spectrum of **PCN-222@PVP** and **PCN-CO@PVP** aqueous dispersions, which is used to quantify **HOB-BA** content in **PCN-CO@PVP** system.

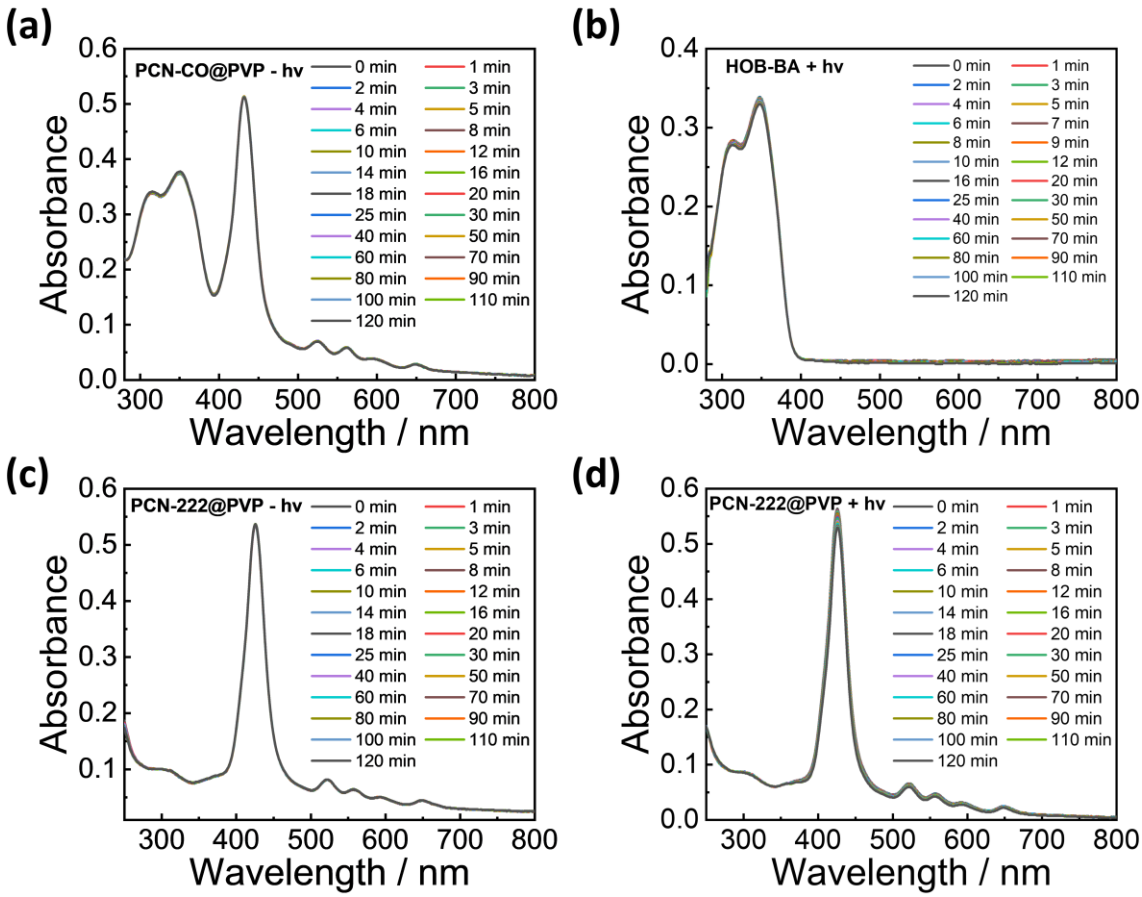


Figure S4. UV-vis spectra of (a) **PCN-CO@PVP**, (b) **HOB-BA**, (c) **PCN-222@PVP**, and (d) **PCN-222@PVP** aqueous dispersions with or without 650 nm light irradiation. **PCN-222@PVP** and **PCN-CO@PVP** are at the same concentration based on **PCN-222**, 0.045 mg mL^{-1}, **HOB-BA**: 0.050 mg mL^{-1}.

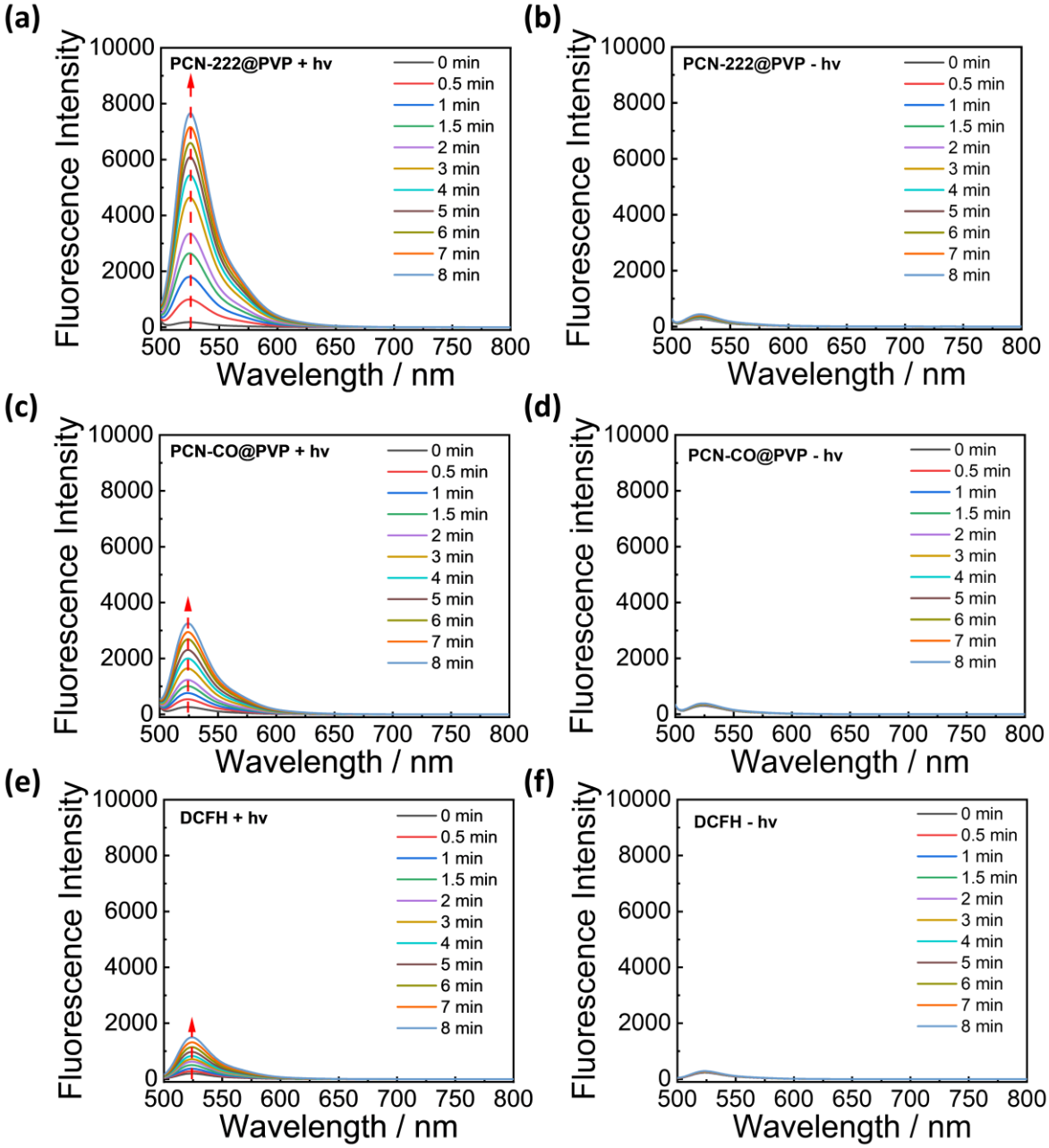


Figure S5. Evolution of fluorescence spectra of DCFH (40 μM, λ_{ex} = 488 nm; slit width: Ex. = 5 nm, Em. = 5 nm) with aqueous dispersions of **PCN-222@PVP**, **PCN-CO@PVP** and DCFH (a, c, e) with or (b, d, f) without 650 nm irradiation (26 mW cm^{-2}). **PCN-222@PVP** and **PCN-CO@PVP** are at the same concentration based on **PCN-222**, 0.015 mg mL^{-1}.

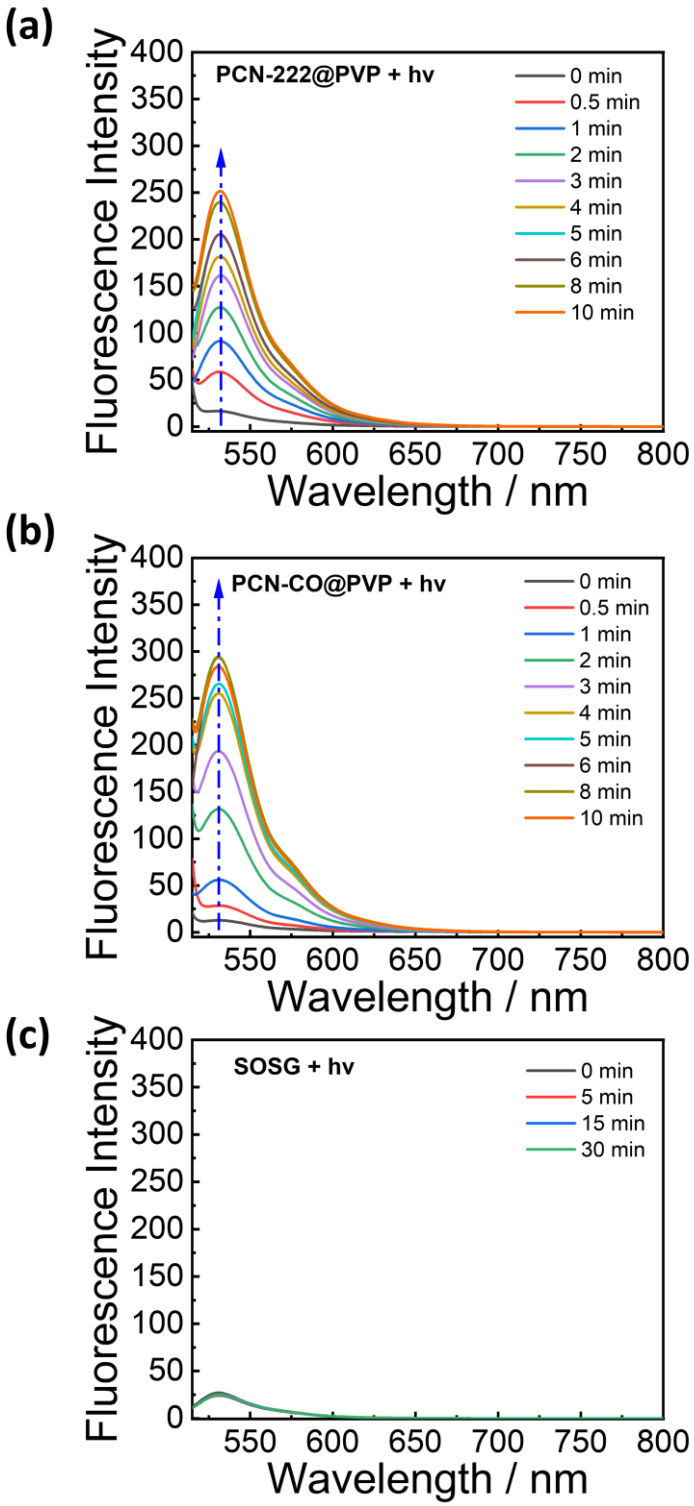


Figure S6. Evolution of fluorescence spectra of SOSG (5 μM, λ_{ex} = 504 nm; slit width: Ex. = 5 nm, Em. = 5 nm) with aqueous dispersions of (a) **PCN-222@PVP**, (b) **PCN-CO@PVP**, and (c) SOSG under 650 nm irradiation (26 mW cm^{-2}). SOSG with 650 nm irradiation is as a control. **PCN-222@PVP** and **PCN-CO@PVP** are at the same concentration based on **PCN-222**, 0.050 mg mL^{-1}.

2.4 HPLC for tracking the photodegradation process of **HOB-BA**

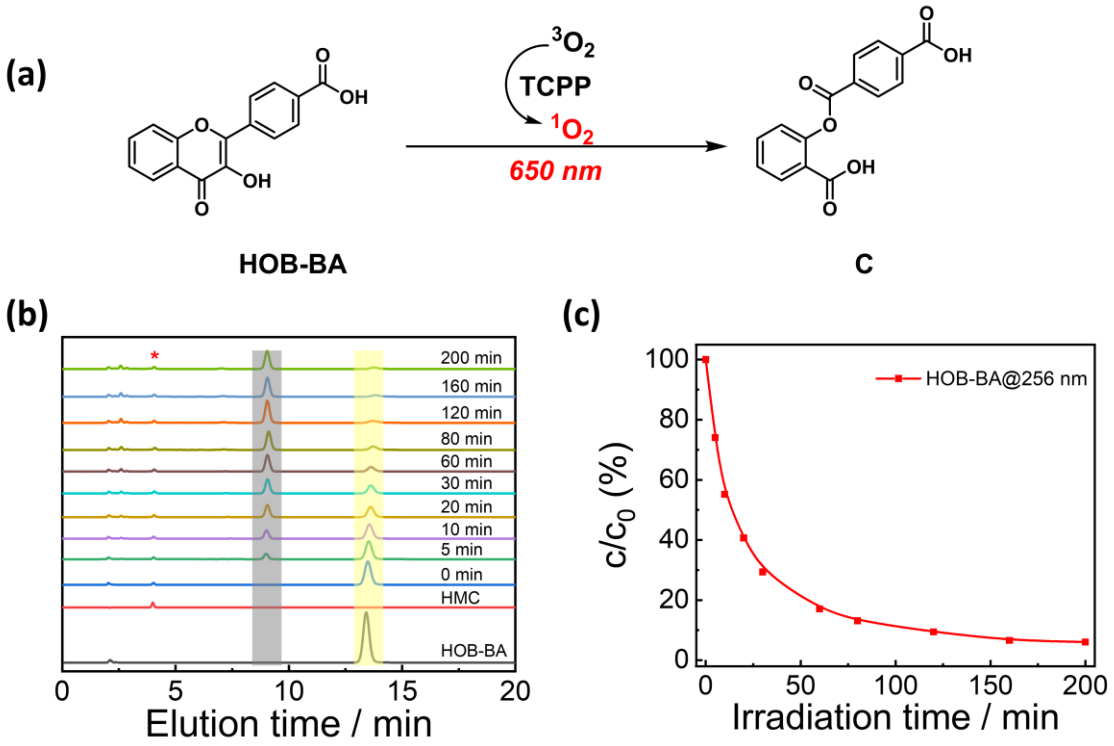


Figure S7. (a) Proposed CO release mechanism through 650 nm light-mediated tandem reactions of **HOB-BA**. (b) HPLC elution profiles of **HOB-BA** (100 μM) under 650 nm light irradiation with time in the presence of **TCPP** (25 μM, CH_3CN: H_2O = 35:65 (containing 0.1% TFA, v/v)). Authentic **HOB-BA** was used for comparison. The asterisk represents the signal of 7-hydroxy-4-methylcoumarion (HMC), which was used as the internal standard. (c) Normalized peak areas against HMC internal standard of **HOB-BA** under 650 nm light irradiation quantified from HPLC results in (b).

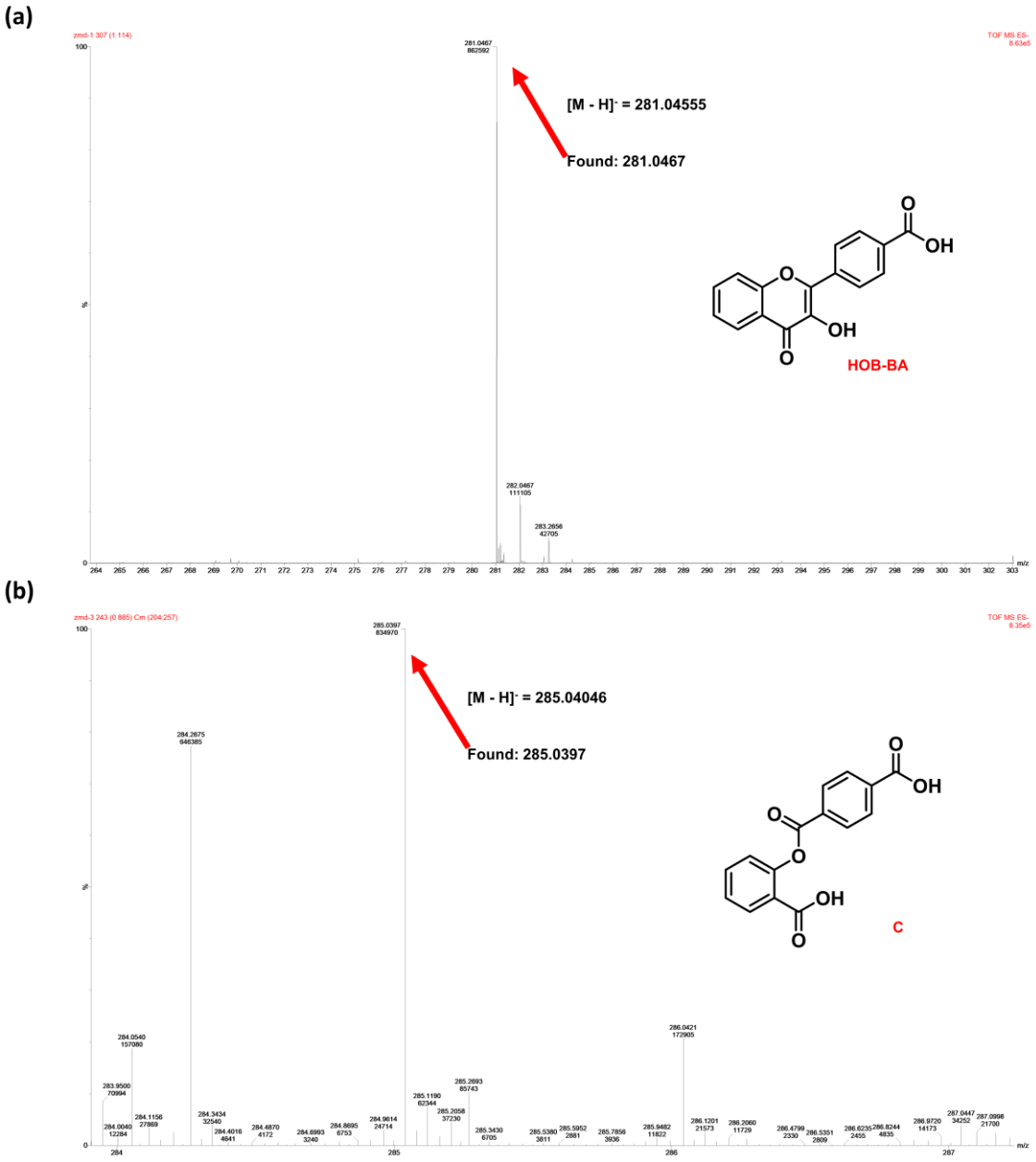


Figure S8. ESI-MS spectrum recorded for (a) **HOB-BA** and (b) compound **C** after **HOB-BA** without or with 650 nm light irradiation for 30 min in the presence of **TCPP** (CH_3CN: H_2O = 35:65).

2.5 Antibacterial assay in vitro

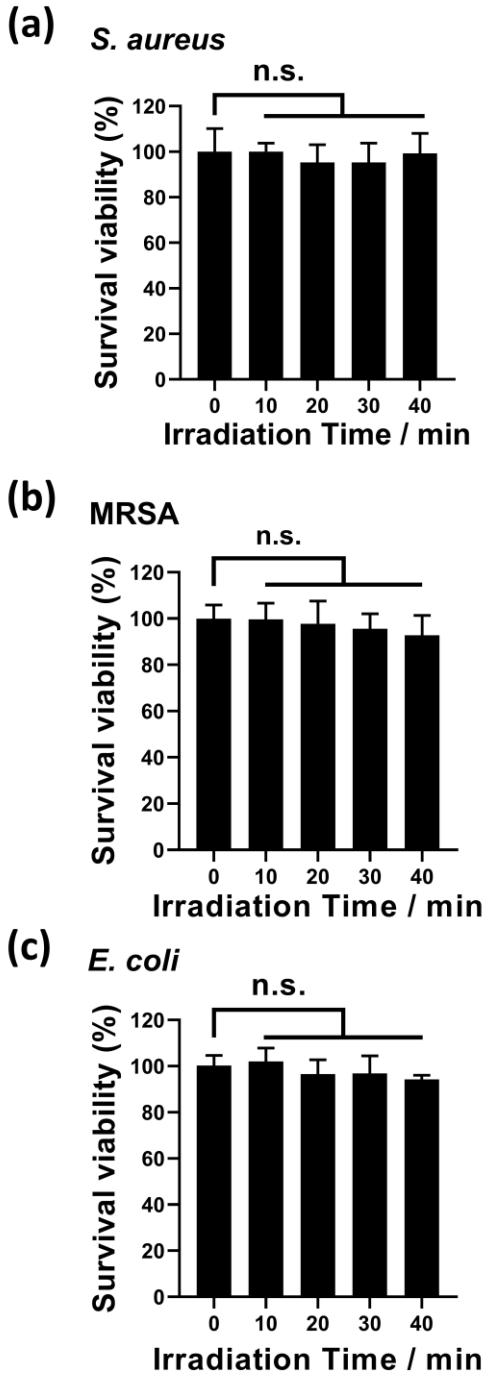


Figure S9. Cell viability of (a) *S. aureus,* (b)MRSA, and (c) *E. coli* bacteria with different irradiation time (650 nm, 26 mW cm^{-2}). Data are shown as mean ± s.d. (n = 3). n.s., not significant.

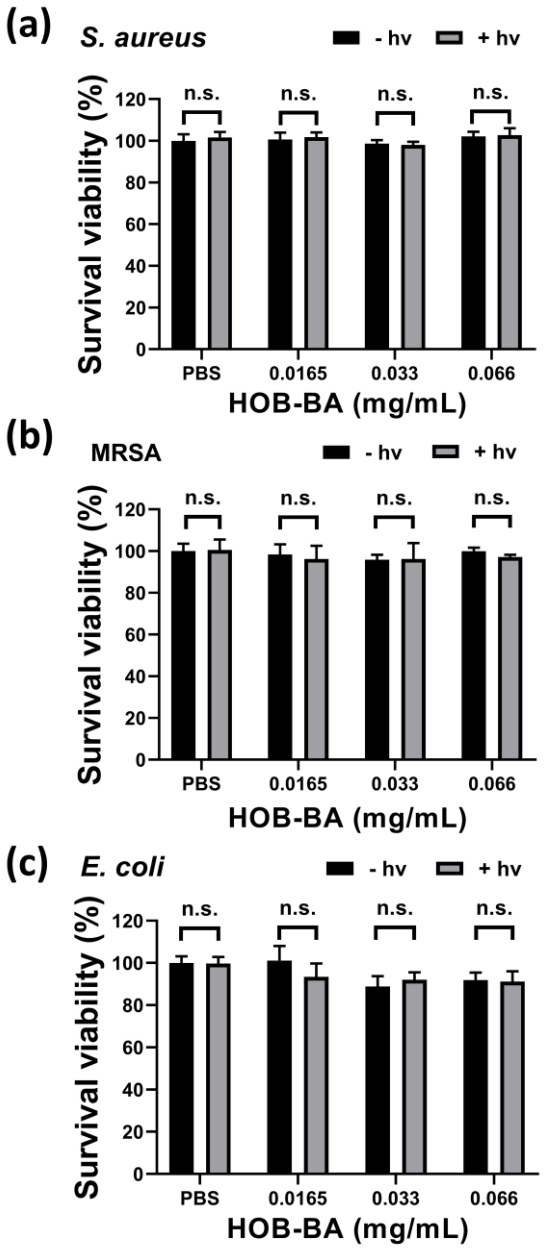


Figure S10. Antibacterial activity of **HOB-BA** with or without 650 nm light irradiation at gradient concentrations against (a) *S. aureus*, (b) MRSA, and (c) *E. coli* counted by colony-forming unit assay. Data are shown as mean ± s.d. (n = 3). n.s., not significant.

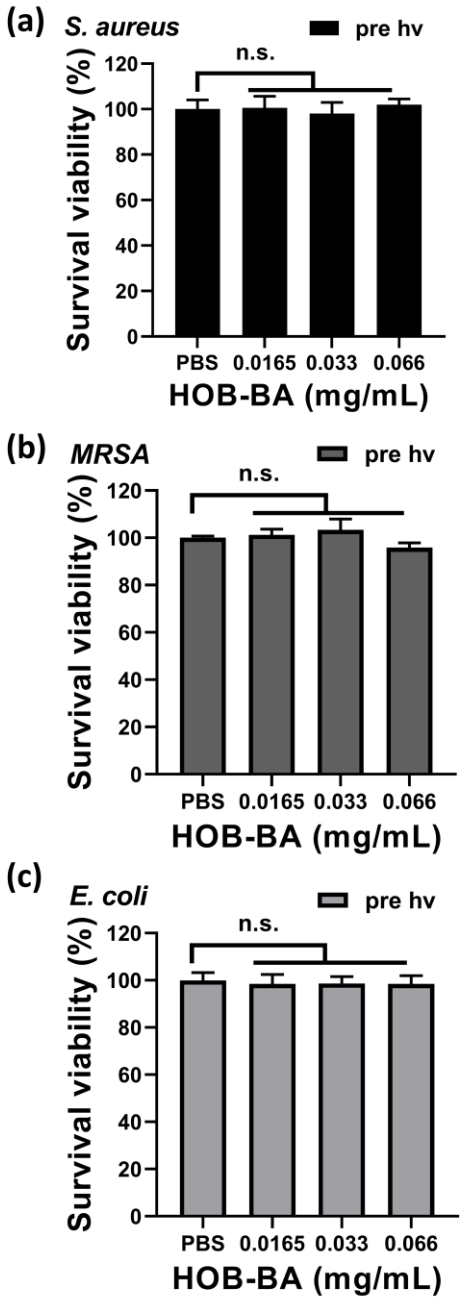


Figure S11. Antibacterial activity of pre-lighted **HOB-BA** at gradient concentrations against (a) *S. aureus*, (b) MRSA, and (c) *E. coli* counted by colony-forming unit assay. Data are shown as mean ± s.d. (n = 3). n.s., not significant.

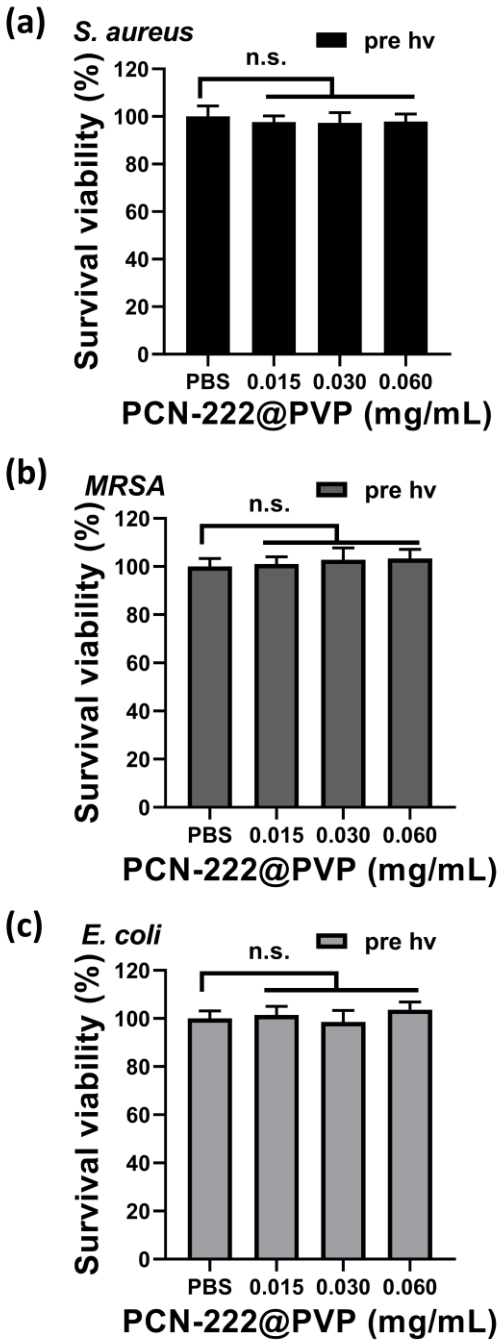


Figure S12. Antibacterial activity of pre-lighted **PCN-222@PVP** at gradient concentrations against (a) *S. aureus*, (b) MRSA, and (c) *E. coli* counted by colony-forming unit assay. The concentration of **PCN-222@PVP** is based on **PCN-222**. Data are shown as mean ± s.d. (n = 3). n.s., not significant.

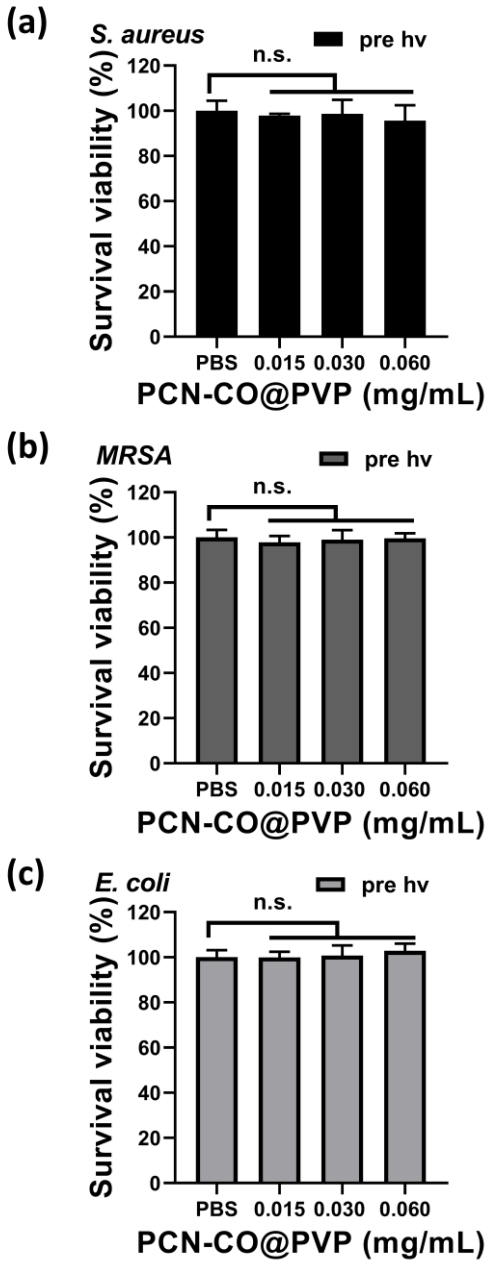


Figure S13. Antibacterial activity of pre-lighted **PCN-CO@PVP** at gradient concentrations against (a) *S. aureus*, (b) MRSA, and (c) *E. coli* counted by colony-forming unit assay. The concentration of **PCN-CO@PVP** is based on **PCN-222**. Data are shown as mean ± s.d. (n = 3). n.s., not significant.

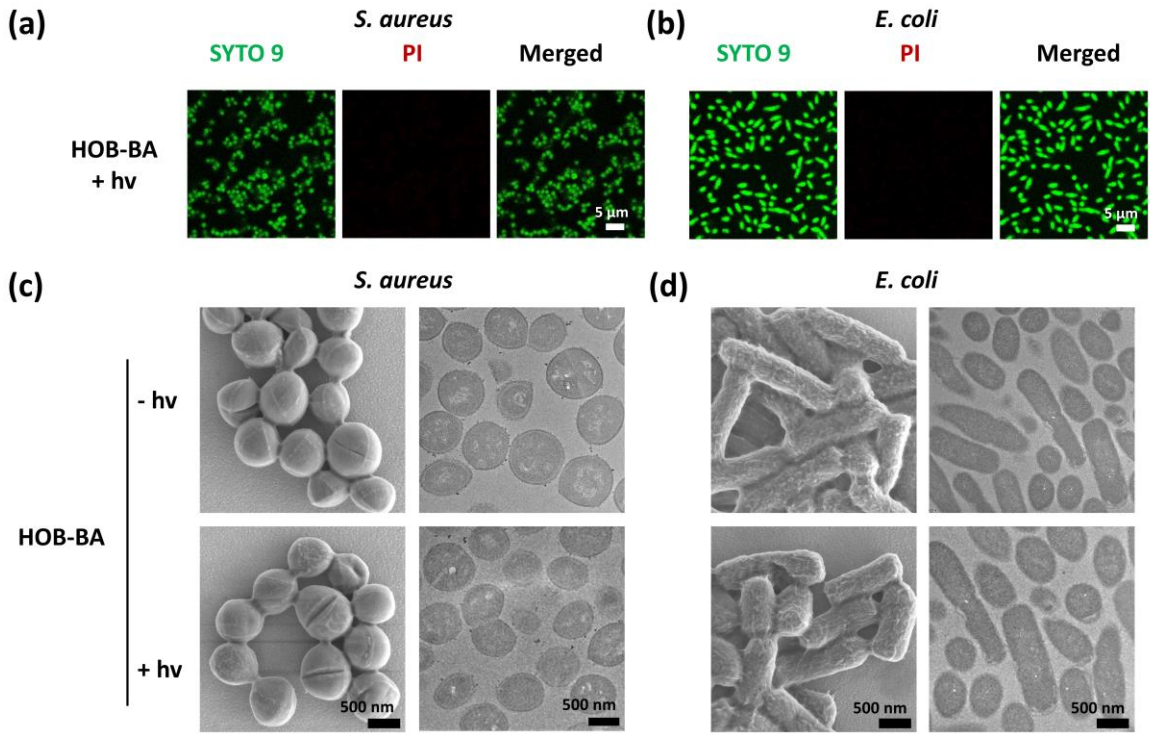


Figure S14. CLSM images of **HOB-BA**-treated (a) *S. aureus and* (b) *E. coli* under 650 nm light irradiation (26 mW cm^{-2}) in the presence of SYTO 9/PI mixed fluorescent dyes. SYTO 9 (green fluorescence, λ_{ex} = 488 nm) stained cells are intact and live, while PI (red fluorescence, λ_{ex} = 543 nm) stained cells are dead due to disruption of the cell membrane. TEM and SEM images of (c) *S. aureus* and (d) *E. coli* incubated with **HOB-BA** with or without 650 nm light irradiation for 30 min. (**HOB-BA**: 0.066 mg mL^{-1}).

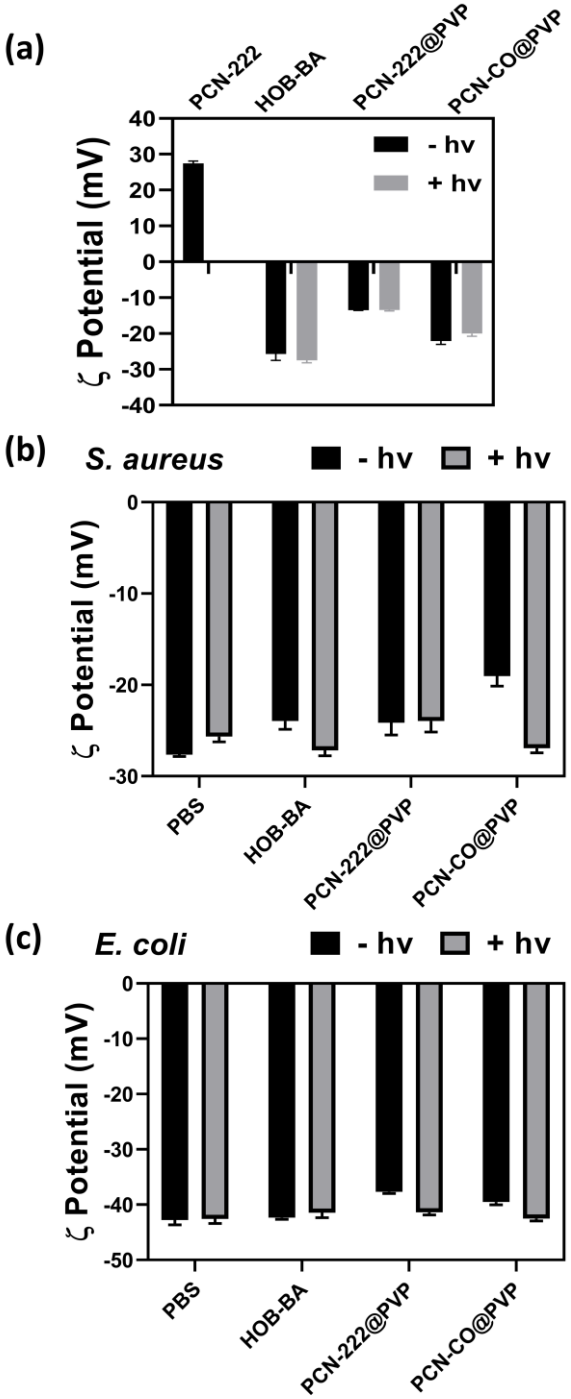


Figure S15. Zeta (ζ) potential of (a) **HOB-BA**, **PCN-222@PVP** and **PCN-CO@PVP** aqueous dispersions, (b) *S. aureus*, and (c) *E. coli* bacteria dispersed in aqueous solution pretreated with **HOB-BA**, **PCN-222@PVP** or **PCN-CO@PVP** with or without 650 nm light irradiation (26 mW cm^{-2}) for 30 min. **PCN-222@PVP** and **PCN-CO@PVP** are at the same concentration based on **PCN-222**, 0.060 mg mL^{-1}, HOB-BA: 0.066 mg mL^{-1}.

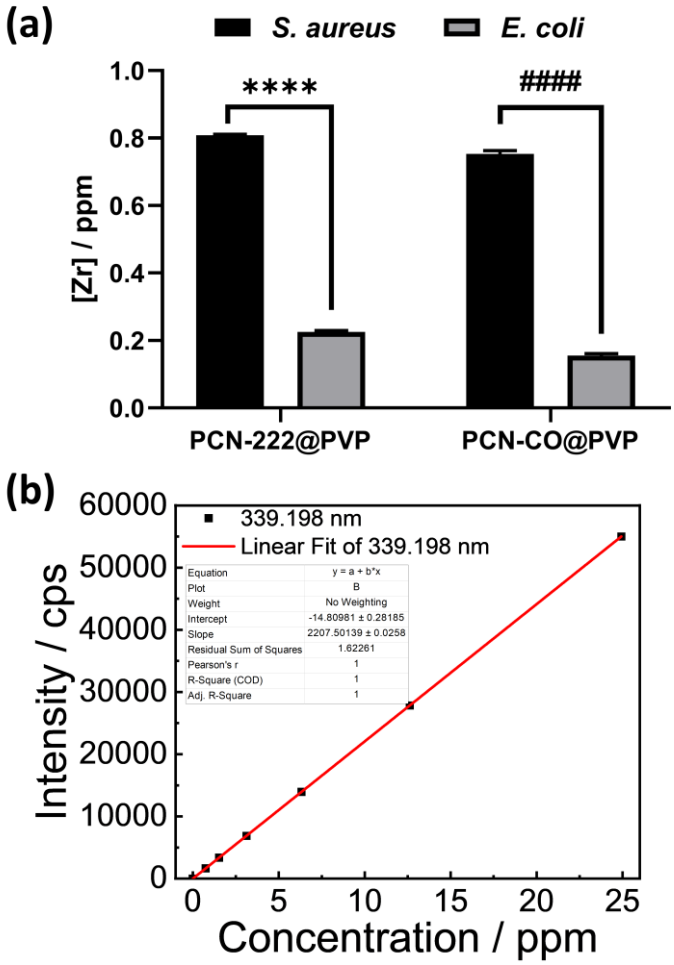


Figure S16. (a) The contents of Zr quantified by ICP-AES of *S. aureus* and *E. coli* incubated with **PCN-222@PVP** or **PCN-CO@PVP** under dark and (b) the calibration curve fitting the emission intensity and concentration of Zr at 339.198 nm. The calibration curve shows a good linear relationship within the test concentration range. Data are shown as mean ± s.d. (n = 3). n.s., not significant; ****$p<0.0001$; $^{\#\#\#\#}p<0.0001$; $^{*,\ \#}$, compared to the **PCN-222@PVP** group or **PCN-CO@PVP** group with 650 nm light irradiation, respectively.

2.6 CLSM observation of bacteria

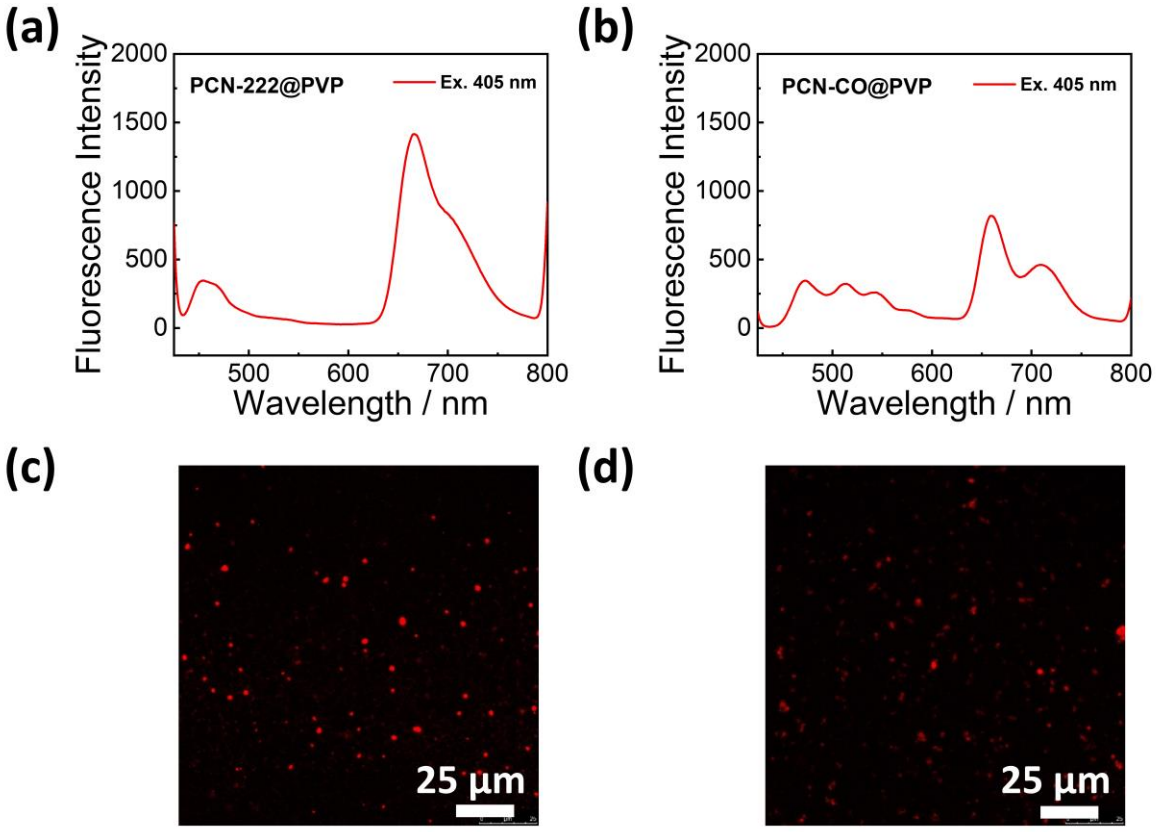


Figure S17. Fluorescence spectra (a, b) and CLSM images (c, d) of **PCN-222@PVP** and **PCN-CO@PVP** aqueous dispersions, respectively (λ_{ex} = 405 nm).

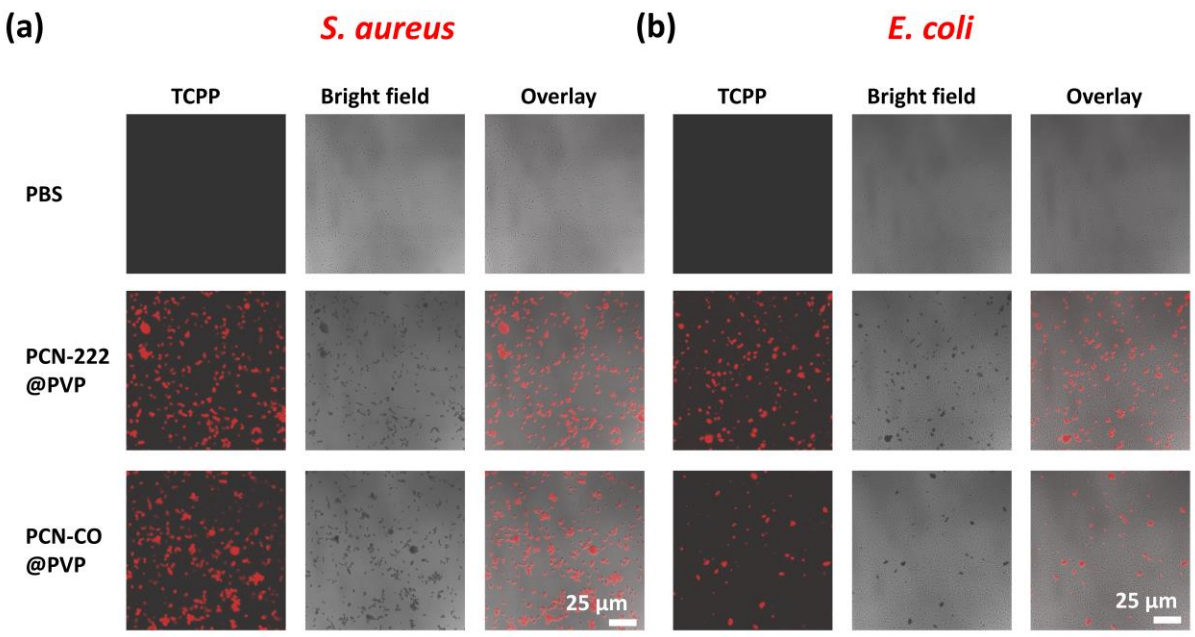


Figure S18. CLSM images of (a) *S. aureus* and (b) *E. coli* after incubated with PBS, **PCN-222@PVP** or **PCN-CO@PVP** aqueous dispersions (λ_{ex} = 405 nm), which were washed three times before imaging. **PCN-222@PVP** and **PCN-CO@PVP** are at the same concentration based on **PCN-222**.

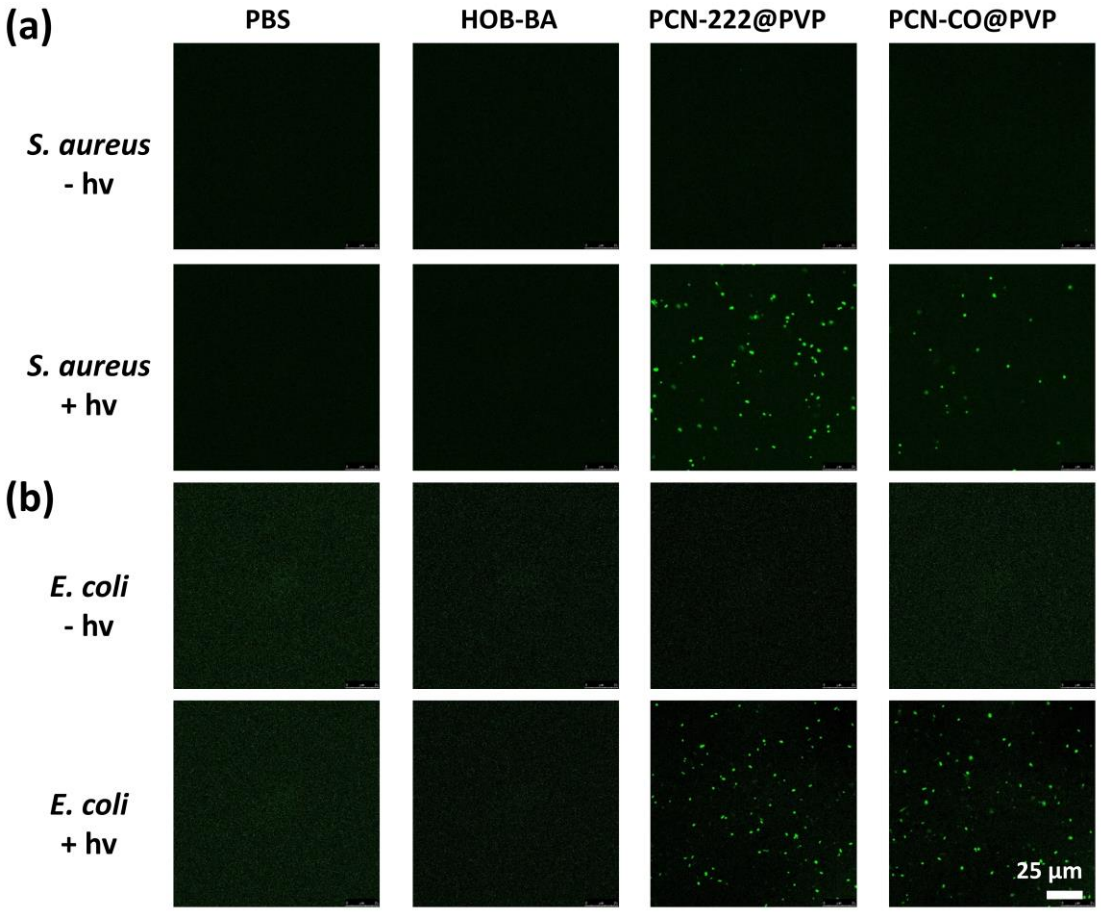


Figure S19. CLSM image of (a) *S. aureus* and (b) *E. coli* bacteria processed with DCFH-DA (λ_{ex} = 488 nm), incubated with PBS, **HOB-BA**, **PCN-222@PVP** or **PCN-CO@PVP** aqueous dispersion irradiated with or without 650 nm light (26 mW cm^{-2}). **PCN-222@PVP** and **PCN-CO@PVP** are at the same concentration based on **PCN-222**, 0.060 mg mL^{-1}, **HOB-BA**: 0.066 mg mL^{-1}.

2.7 Hemolysis assay

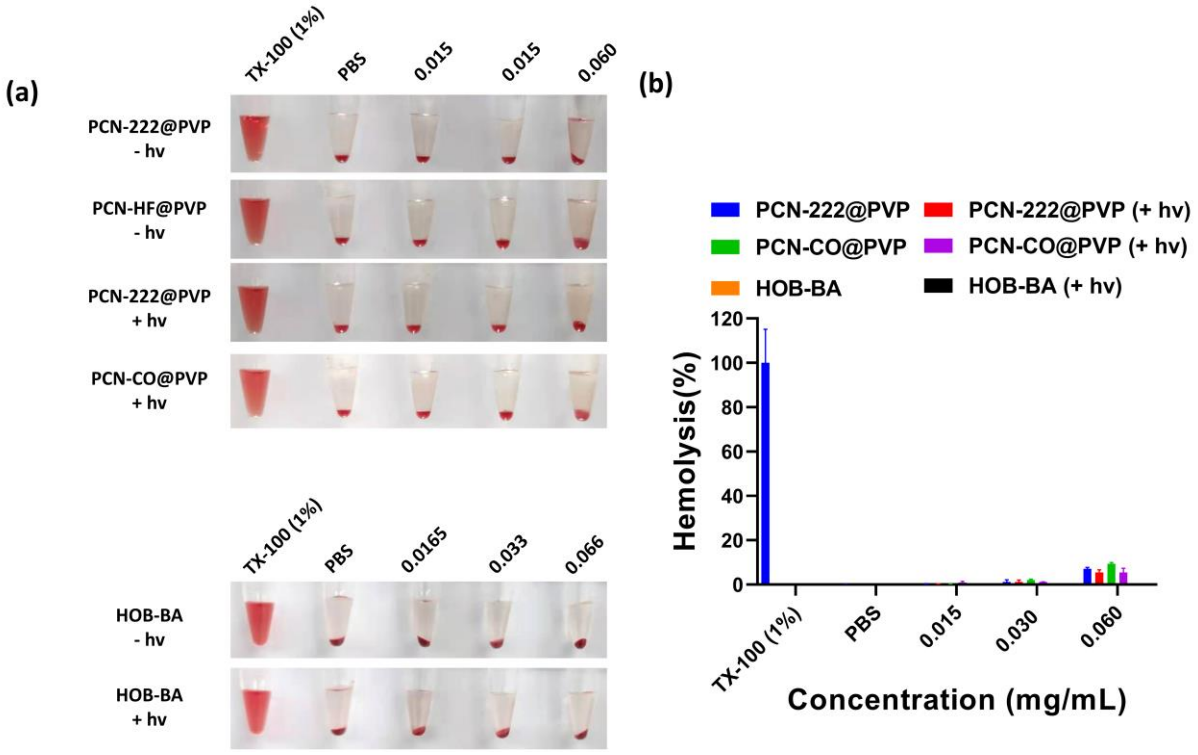


Figure S20. (a) Representative images of sheep red blood cells in the presence of TX-100 (1%), PBS, **HOB-BA**, **PCN-222@PVP** or **PCN-CO@PVP** aqueous dispersion. (b) Hemolytic assay of aqueous dispersions of TX-100 (1%), PBS, **HOB-BA**, **PCN-222@PVP** or **PCN-CO@PVP** calculated from (a). TX-100 (1%) and PBS were used as the positive and negative control, respectively. **PCN-222@PVP** and **PCN-CO@PVP** are at the same concentration based on **PCN-222.** Data are shown as mean ± s.d. (n = 3).

2.8 Cytotoxicity assay

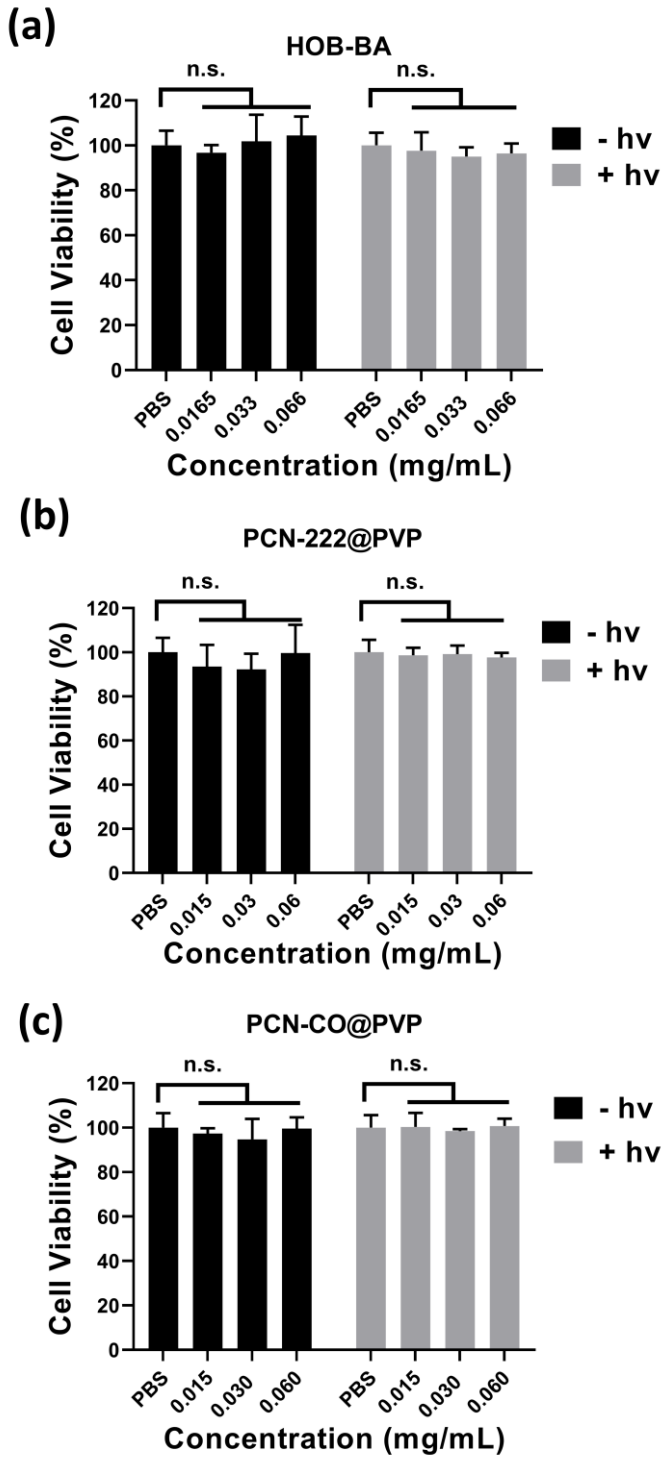


Figure S21. Cell viability of L929 cells as determined by MTT assay after 24 h incubation with (a) **HOB-BA**, (b) **PCN-222@PVP**, or (c) **PCN-CO@PVP** with or without 650 nm light irradiation (26 mW cm^{-2}). **PCN-222@PVP** and **PCN-CO@PVP** are at the same concentration based on **PCN-222**. Data are shown as mean ± s.d. (n = 3). n.s., not significant.

2.9 Stability

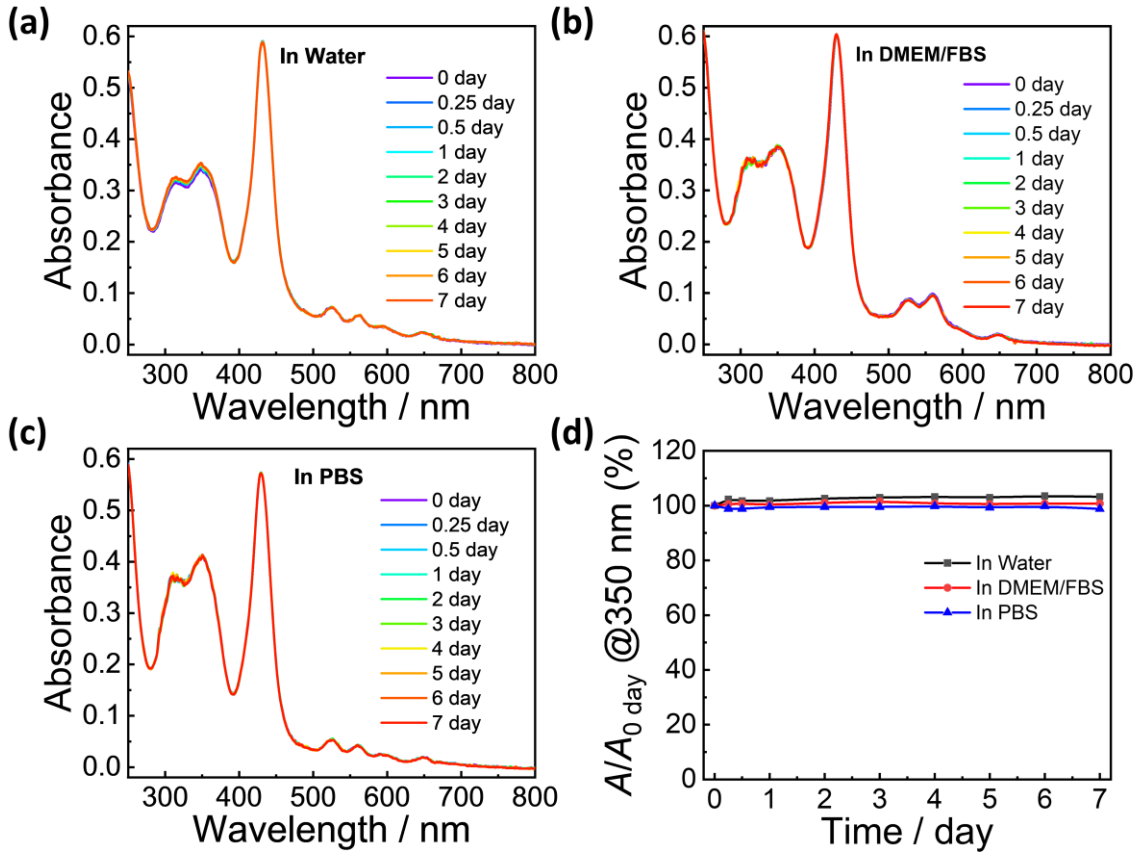


Figure S22. The UV-vis absorption spectra of **PCN-CO@PVP** (0.045 mg mL^{-1}) monitored over the course of one week in (a) water, (b) DMEM/FBS (10%), and (c) PBS under dark. (d) Absorbance intensity changes at 350 nm of **PCN-CO@PVP** in water, DMEM/FBS, and PBS.

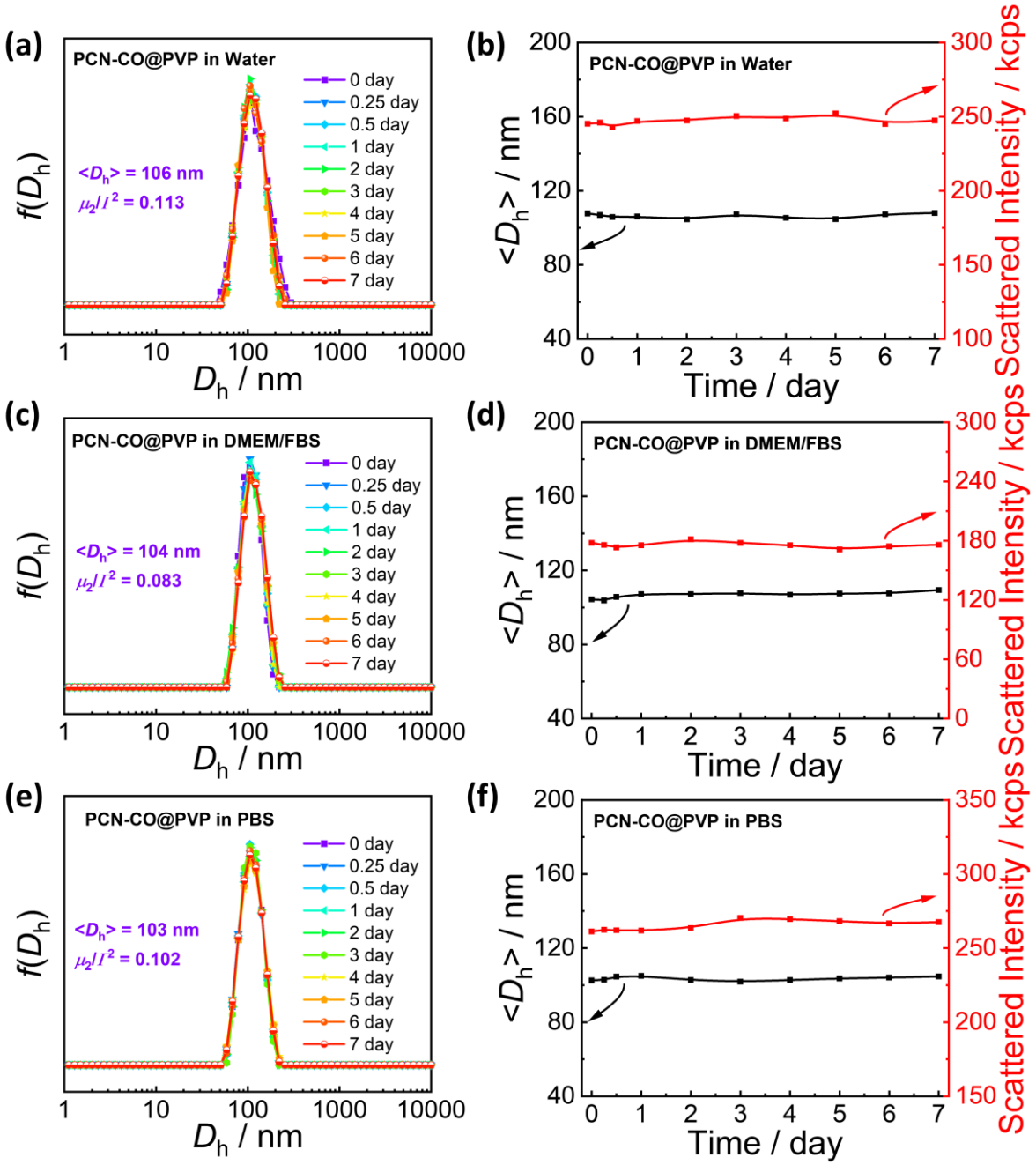


Figure S23. Evolution of intensity-average hydrodynamic diameters, <D_h>, and corresponding scattering intensities of **PCN-CO@PVP** monitored over the course of one week in (a, b) water, (c, d) DMEM/FBS (10%), and (e, f) PBS under dark.