Supporting Information

Colorimetric Band-aids for Point-of-Care Sensing and Treating Bacterial Infection

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Materials:

Zirconyl chloride octahydrate ($ZrClO_2 \cdot 8H_2O$), benzoic acid, sodium cyanoborohydride ($NaCNBH_3$), and 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) were purchased from Sigma-Aldrich. Tetrakis (4-carboxyphenyl) porphyrin (TCPP) was obtained from TCI (Shanghai) Industrial Development Co. Sodium periodate ($NaIO_4$), and Lithium chloride (LiCl), bromothymol blue (BTB) were from Aladdin Reagent (Shanghai, China). Chitosan (molecular weight = 5495.1, number-average molecular weight and M_w/M_n = 2.299) was obtained from Golden-shell Biochemical Co., LTD. 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) was obtained from Alfa Aesar. N-hydroxysulfosucnimide sodium salt (sulfo-NHS) was purchased from Pierce Biotechnology. Nitrocefin was obtained from Toronto Research Chemicals Inc. The β-lactamases used here belong to class A (molecular classification system) and group 2 (functional classification system). Ampicillin-resistant *E. coli* (ATCC25922), Ampicillin-resistant *E. coli* (ATCCBAA-2193) were obtained from Chuanxiang Biotechnology, Ltd. (Shanghai, China). All other reagents were of analytical reagent grade and used as received. Ultrapure water (18.2 MΩ; Millpore Co., USA) was used in all experiments and to prepare all buffers.

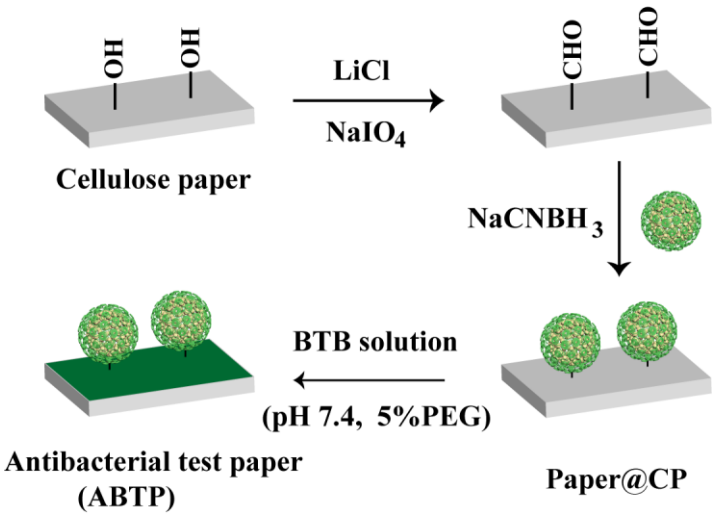
Measurements:

UV absorbance measurements were carried out on a JASCO V-550 UV-vis spectrophotometer equipped with a peltier temperature control accessory. Fourier transforms infrared spectroscopy (FT-IR) was carried out on a BRUKE Vertex 70 FT-IRqW spectrometer. Samples were thoroughly ground with exhaustively dried KBr. Scanning electron microscopic (SEM) images were recorded using a Hitachi S-4800 Instrument (Japan). Transmission electron microscopy (TEM) images were recorded using a FEI TECNAI G220 high-resolution transmission electron microscope operating at 200 kV. Dynamic Light Scatterer (DLS) was made by Malvern Corp, U. K. (ZEN3690). X-ray measurements were performed on a Bruker D8 FOCUS Powder X-ray Diffractometer (XRD) using Cu Kα radiation. X-ray photoelectron Spectroscopy (XPS) measurement was performed on an ESCALAB-MKII spectrometer (VG Co., United Kingdom) with Al KR X-ray radiation as the X-ray source for excitation. Thermo gravimetric analysis (TGA) was performed with a Pyres 1 TGA apparatus (Perkin Elmer, MA).

Experimental Section

Synthesis of PCN-224 Nanoparticles: [1] $ZrOCl_2 \cdot 8H_2O$ solution (10 mL, 15 mg mL^{-1} in N,N-dimethylformamide (DMF)), TCPP solution (20 mL, 2.5 mg mL^{-1} in DMF), and benzoic acid solution (20 mL, 70 mg mL^{-1} in DMF) were added to round-bottom flask and reacted at 90 °C for 5 h under stirring. The product was collected by centrifugation (13000 rpm) and washed with DMF for three times.

Synthesis of CP Nanoparticles: PCN-224 nanoparticles were dissolved in MES buffer (25 mM, pH 6.0) and activated by EDC (2 mg mL^{-1}) and NHS (2 mg mL^{-1}) for 2 h. Then chitosan (2 mg mL^{-1}) was added to the mixture at room temperature with continuous stirring for 20 h. The obtained CP nanoparticles were collected by centrifugation and washed with water for three times.



Scheme S1. The synthetic route of PBA.

Preparation of PBA: The synthetic route of PBA was taken in three steps (Scheme S1):[2]

(i) Filter paper samples were immersed in the oxide solution (0.03 g lithium chloride (LiCl) and 0.084 g sodium periodate ($NaIO_4$) were dissolved in 15 mL water.) and the temperature of the solution was kept at 60 °C for 60 min. The paper was then dried in an oven at 37 °C after rinsed twice with deionized water, then the treated paper samples were sterilized.

(ii) Paper samples were cut into small pieces (5 mm × 5 mm). A low-volume HEPES buffer solution (10 μL, 50 mM, pH = 8.0) containing CP (10 μg/mL, 50 μg/mL, 100 μg/mL, or 200 μg/mL. In sensing experiments, 100 μg/mL was used) and $NaCNBH_3$ (50 mM), was pipetted on the small piece paper. The paper was incubated at 37 °C for 1 h, maintained in humidity, and then washed. The paper@CP was obtained.

(iii) Paper@CP was coated with BTB solution (0.5 mg/mL) containing 5 wt% PEG and dried at 70 °C.

Cargo loading and acid-responsive release: CP was soaked in 5 mL HEPES buffer (pH 6.0) containing 1 mg mL^{-1} rhodamine B. After 24 h incubation, the mixture solution was centrifuged and washed with HEPES buffer (pH 7.4) to remove the excess and adsorbed molecules. All the washing solutions were collected to calculated the loading amount of rhodamine B. Subsequently, to observe the acid-responsive release of payloads, UV-vis spectra measurement was carried out. Rhodamine B loaded CP was dispersed in HEPES buffer (pH 6.0). Aliquots (200 μL) were taken from the suspension at predetermined time intervals to quantify the released molecules with the change of absorbance at 553 nm.

Acid-responsive color changes of PBA: PBA was dripped with 50 μL of buffers with different pH (pH 6.0, 7.0, 8.0). Instantaneous color changes are recorded by photography. Similarly, various concentrations of bacterial solution was dripped onto the synthesized test paper, and the obvious yellow was shown. Accordingly, the change of absorbance at 615 nm was monitored to present the acid responsiveness when BTB and CP was mixed in the solution.

Nitrocefin reaction catalyzed by β-lactamase: The nitrocefin reaction was carried out at room temperature using nitrocefin as substrate in the presence of β-lactamase in 50 mM HEPES buffer. The absorbance of the color reaction was recorded with UV-vis spectra. Typically, 400 μL of reaction systems containing 16 μL of nitrocefin (9.68 mM, DMSO) and 5 μL of β-lactamase (45 U/mL) with different reaction time were used. After nitrocefin was loaded into the CP (named as CP-N), the β-lactamase solution (0.02 - 0.3 U/mL) or *E.coli* solution (0 - 10^8 /mL) was mixed with CP-N. The change of absorbance at 486 nm was recorded to show the chromogenic reaction.

For observing the chromogenic reaction on the test paper, 50 μL of bacterial droplets were placed on test paper and reacted for 2 h or 4 h to observe the color change of test paper.

Bacterial culture and antibacterial experiments: DS *E. coli* and DR *E. coli* on the Luria–Bertani (LB) agar plate was respectively transferred to 20 mL broth and incubated at 37 °C for 24 h with stirring at 180 rpm.

For antibacterial experiments, paper@CP with different concentration of CP was placed on the bottom of wells, and the bacteria were diluted with broth to 10^6 cfu·mL^{-1}. In vitro photodynamic studies, the irradiation by 638 nm laser was maintained for 10 minutes (0.2 W/cm^2). After 12 hours of co-incubation under dark conditions, the survival percent of bacteria was calculated by the plating technique.

β-lactamase-responsive color changes of PBA: PBA was rinsed with 50 μL of *β*-lactamase solution (pH 7.4, 1 U/mL) at 37 ºC. Keep it moist for 4 hours, then color of PBA was recorded by photography. Accordingly, various concentrations of bacterial solution was dripped onto PBA or $PBA_{\text{no nitrocefin}}$. After 4 hours at 37 ºC, color changes of PBA indicated bacterial infections. For quantifying colorimetric products, images were sent to a computer and analyzed using NIH Image J software. The image was split into its color channels and the green color channel was selected and inverted. The green channel was selected because it is the complimentary color of red. The color intensity of each sample was quantified. To eliminate background interference, the color intensity was calibrated by subtracting the mean intensity of DS *E. coli* group on each side of DR *E. coli* group.

Cell Toxicity Assays: Cell viability was measured using the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma-Aldrich) MTT assays. L929 cells were cultured in DMEM (Gibco BRL) medium supplemented with 5% FBS, in a 5% CO_2 humidified environment at 37°C. For the MTT assay, cells

were plated at a density of 10^4 cells per well on 96-well plates, followed by introduction of nanoparticles 12 h later. After 24 h, the cells were treated with 10 μL MTT (5 mg/mL in PBS) for 4 h at 37 °C and then were lysed in DMSO for 10 min at room temperature in the dark. Absorbance values of formazan were determined using an Ultraspec 2000 (Pharmacia Biotech) at 660 nm (reference wavelength) and 490 nm. The following equation was used to calculated cell viability:

$[A]_{test}/[A]_{control} \times 100\%$, where $[A]_{test}$ was the absorbance of the test cells with treatments, and $[A]_{control}$ was the absorbance of cells treated with PBS. Three replicates were done for each treatment group.

Animal models: Healthy female Kunming mice (20-25 g) were purchased from the Laboratory Animal Center of Jilin University (Changchun, China), and handling procedures were according to the guidelines of the Regional Ethics Committee for Animal Experiments. To fabricate the wound infection model, about 5 mm^2 wound was operated in the back of mice. Then 50 μL of DS *E. coli* or DR *E.coli* suspension (1 x 10^8 CFU) were respectively placed on the wound. After four hours, various treated test paper were placed on the wound sites. When red color was observed on the PBA, the irradiation by 638 nm laser was maintained for 10 minutes. Finally, white medical tapes were dressed on the wound to avoid falling off.

To investigate the anti-infective effect, photos of the wounds of the mice were taken each day, and test paper was changed every two days. After 3 days, all mice were sacrificed, and the tissues of wounds were harvested. On one hand, the number of bacteria in the tissues was quantified. For determining the amount of bacteria, wounds

from five groups of mice were separated and homogenized in sterile PBS. Aliquots of diluted homogenized intestinal tissues were placed on agar, on which the grown colonies were counted for analysis. On the other hand, the tissues were dissected to make paraffin sections for further hematoxylin and eosin (H&E) staining.

Fruit preservation models: As an example, the infected tomato model was constructed and PBA was attached on the infectious sites. The OD_{600nm} of bacterial solution was fixed to 0.5, then a sterile cotton bud was dipped into bacterial solution, and a fresh tomato was infected by swabbing with the cotton bud and to keep the infected area moist. The infected area is about 0.25 cm^2. The tomatoes were respectively infected by DS *E. coli*, DR *E. coli*, and their mixture strains (DS *E. coli*: DR *E. coli* = 99:1).

All data were expressed in this article as mean standard deviation (SD). All figures shown in this article were obtained from several independent experiments with similar results.

Safety statement s: no unexpected or unusually high safety hazards were encountered

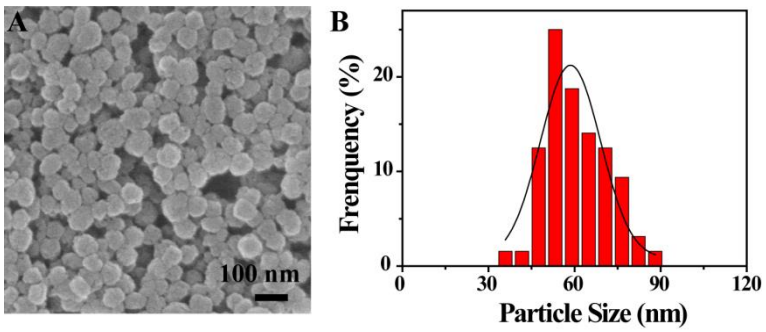


Figure S1 The typical SEM images (A) and corresponding size distribution (B) of PCN-224.

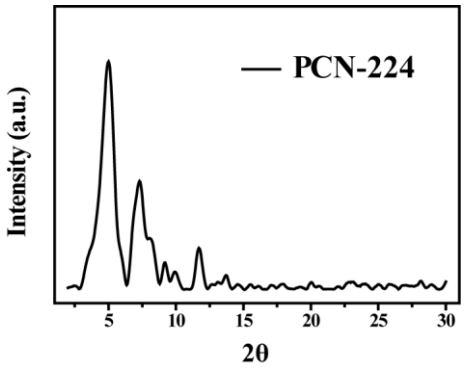


Figure S2 XRD patterns of PCN-224.

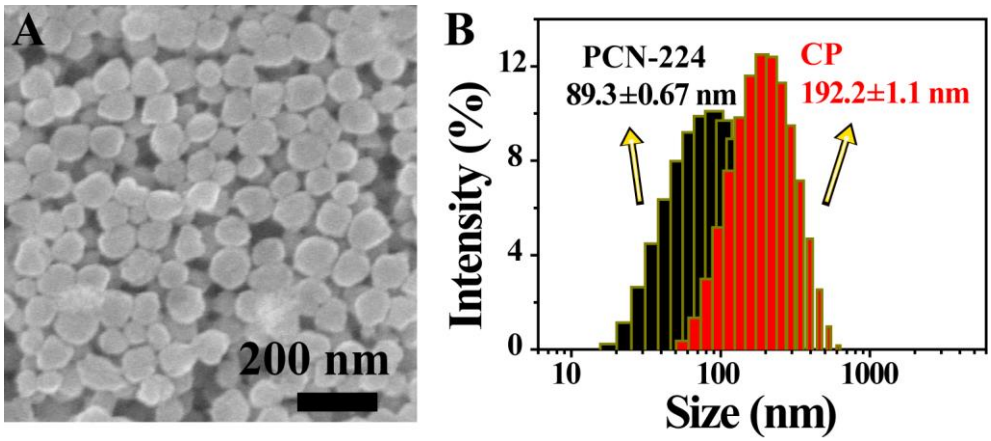


Figure S3 (A)The typical SEM images of CP. (B) The hydrodynamic diameter of PCN-224 and CP measured in water.

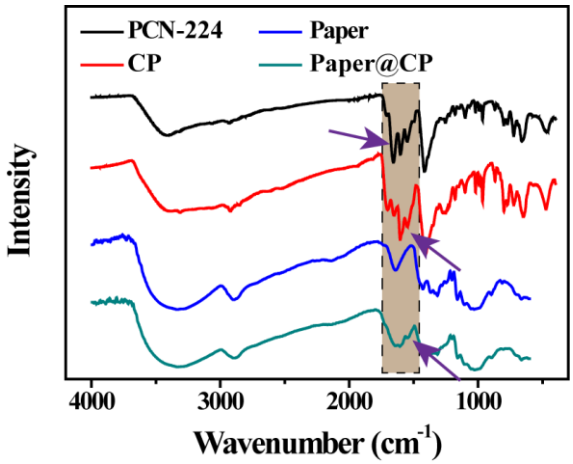


Figure S4 The FT-IR spectra of PCN-224, CP, cellulose paper, and paper@CP.

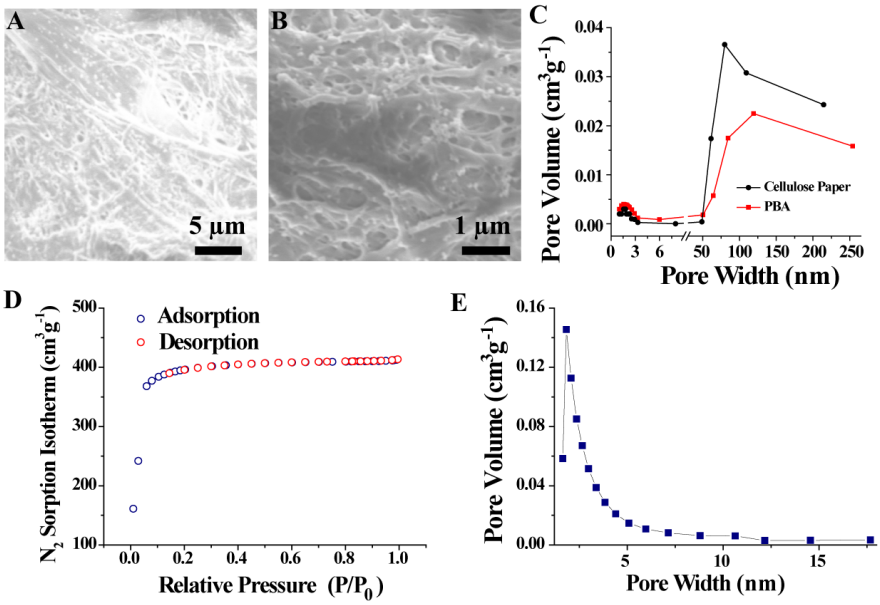


Figure S5 (A) - (B) The typical SEM pictures of PBA. (C) Pore size distribution of cellulose paper and PBA based on BJH analysis. (D) N_2 adsorption isotherms of PCN-224. (E) Pore size distribution of PCN-224.

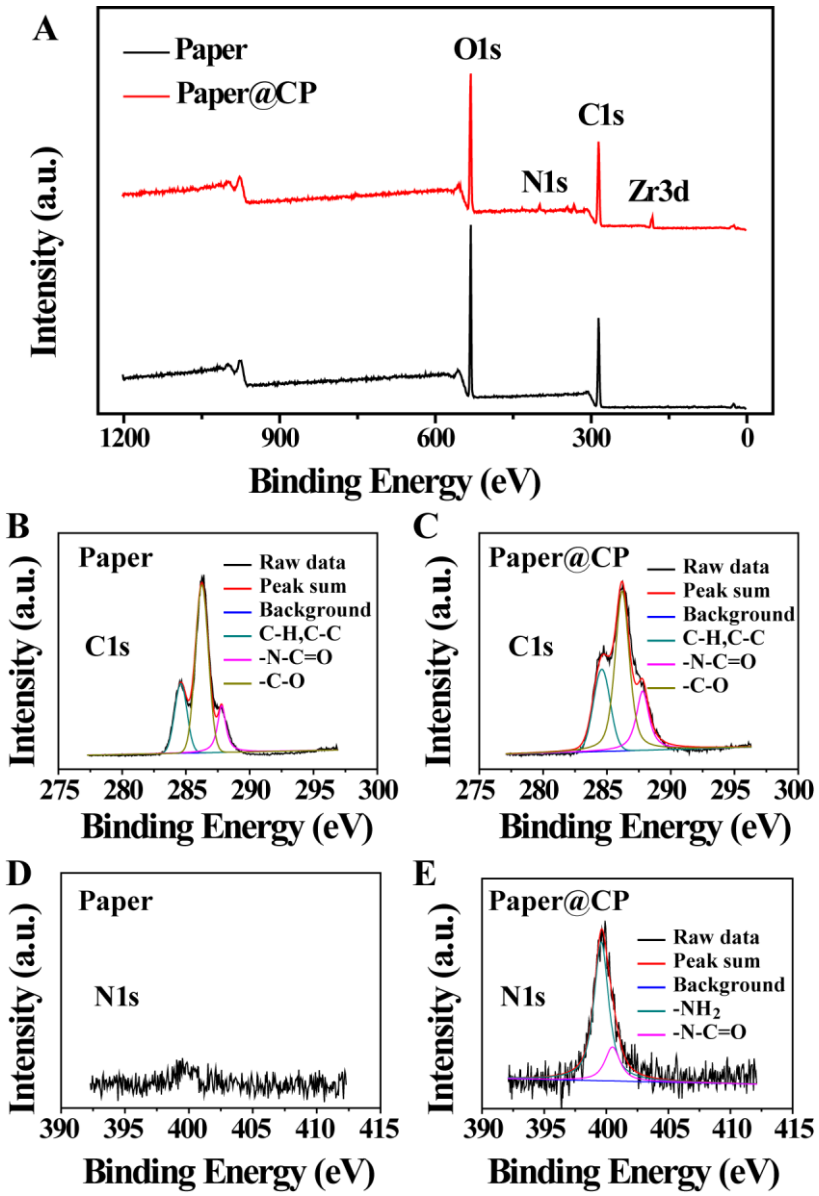


Figure S6 (A) The X-ray photoelectron spectroscopy (XPS) spectra of cellulose paper and paper@CP. (B-E) XPS characterization of the functional groups on paper and paper@CP. The sharp N1s peak at 400.5 eV represented the amides (–N–O=C), and the peaks at binding energies of 287.8 denoted carbonyl (C=O). After the modification of CP, dominant –N–O=C peaks were observed on the surface of paper.

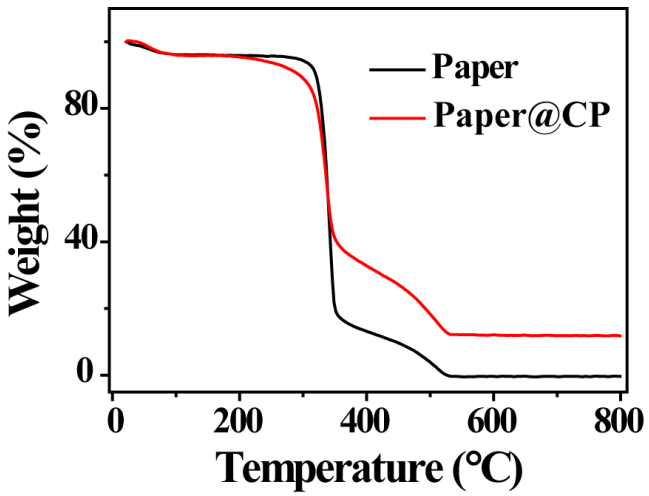


Figure S7 Thermogravimetric Analysis (TGA) of cellulose paper and Paper@CP at a heating rate of 10 °C/min from 50 °C to 800 °C under air atmosphere.

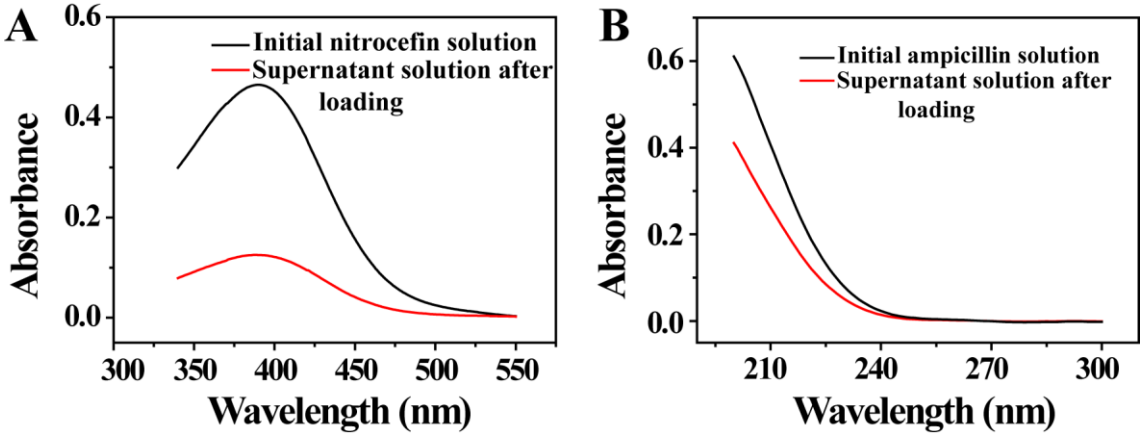


Figure S8 UV-Vis absorption spectra of nitrocefin solution (A), ampicillin solution (B) and their supernatant solution after encapsulation with CP.

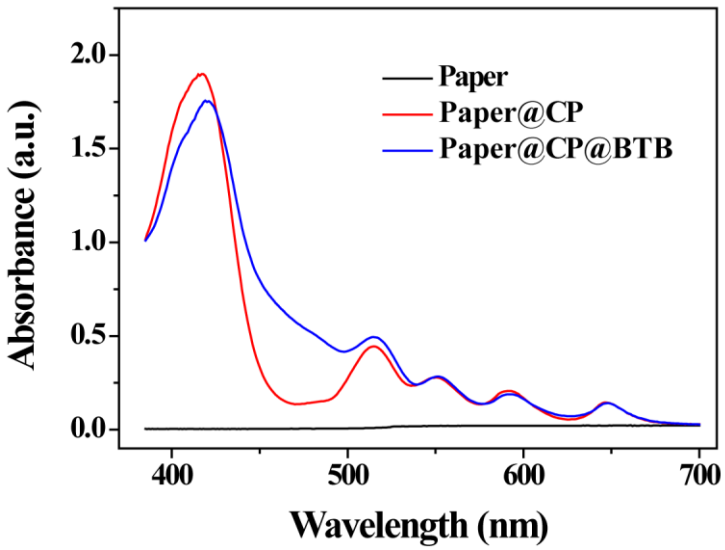


Figure S9 UV-Vis absorption spectra of Paper@CP and Paper@CP@BTB.

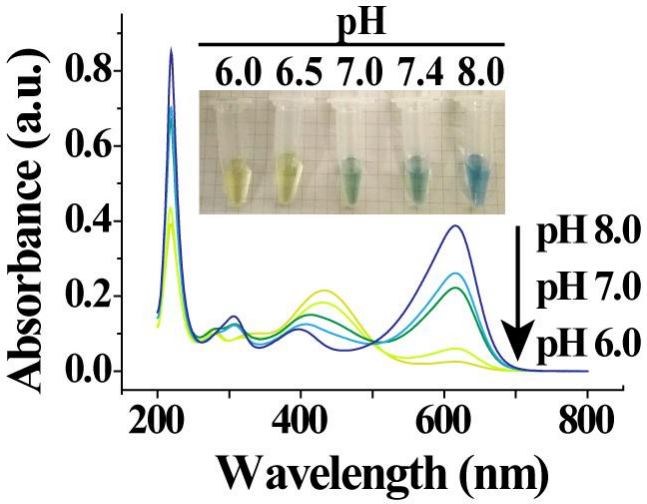


Figure S10 UV−vis absorption spectra of BTB at different pH. The inset pictures show the corresponding color change.

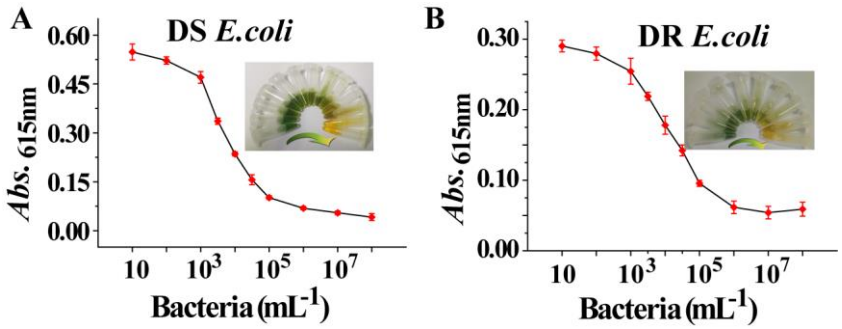


Figure S11 Absorbance change of the mixture of CP and BTB at 615 nm with the number of DS *E. coli* (A) and DR *E. coli* (B). The inset pictures show the corresponding color change of solutions.

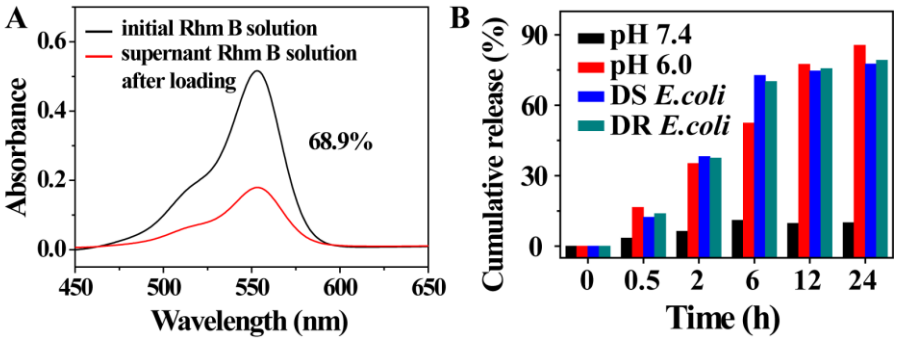


Figure S12 (A) UV-Vis absorption spectra of rhodamine B solution and supernatant rhodamine B solution after encapsulation with CP. (B) Cumulative percentage release of rhodamine B from CP-R in the HEPES buffer at pH 7.4, at pH 6.0, or with DS *E. coli*, or with DR *E. coli*. [*E. coli*] = 0.5 OD.

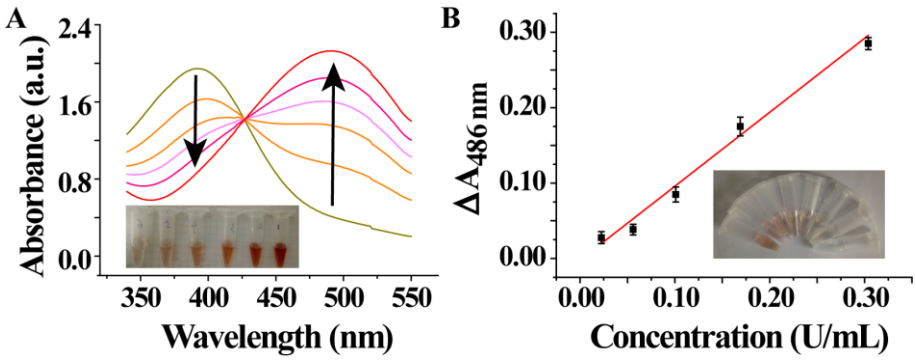


Figure S13 (A) UV−vis absorption spectra after reaction of nitrocefin with β-lactamase for different time. [β-lactamase] = 1 U/mL. The corresponding solution was shown in the inset pictures. (B) Absorbance change of nitrocefin at 486 nm with various concentrations of β-lactamase.

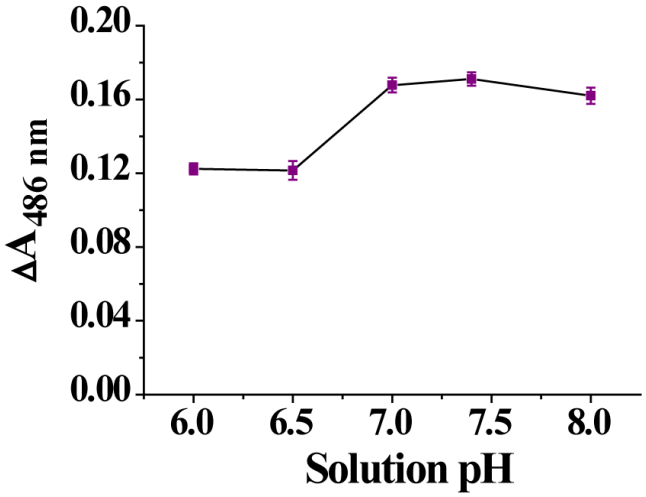


Figure S14 Absorbance changes of the mixture of nitrocefin and CP at 486 nm with the change of pH.

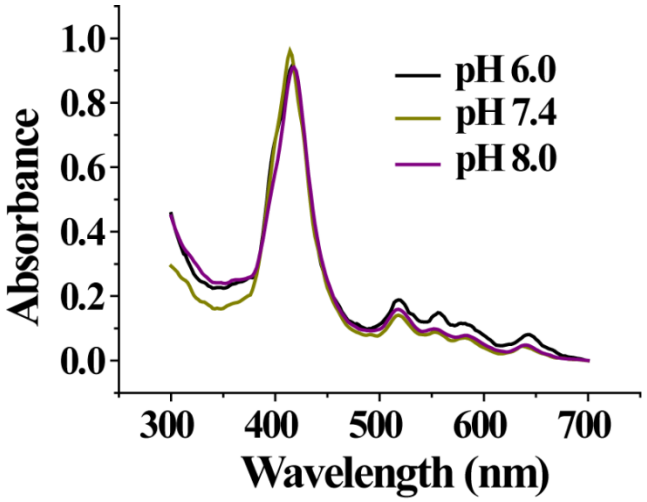


Figure S15 UV-Vis absorption spectra of PCN-224 solution (HEPES buffer) at different pH.

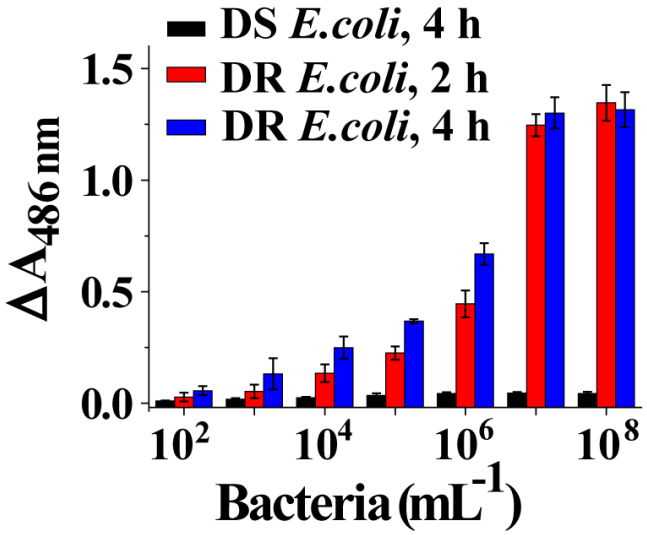


Figure S16 Absorbance change of the mixture of CP-N and BTB at 486 nm with the number of *E. coli*. The absorbance change of the mixture solution at 486 nm with reaction time (ΔA_{486nm}), which was calculated as following equation:

$$\Delta A_{486nm} = A_{486nm,\,t} - A_{486nm,\,t_0}$$

$A_{486nm,\,t0}$ and $A_{486nm,\,t}$ represent the absorbance of mixture solution (CP-N, BTB, and bacteria) at t_0 and t, respectively. t_0 is the time when bacteria was just mixed with CP-N and BTB.

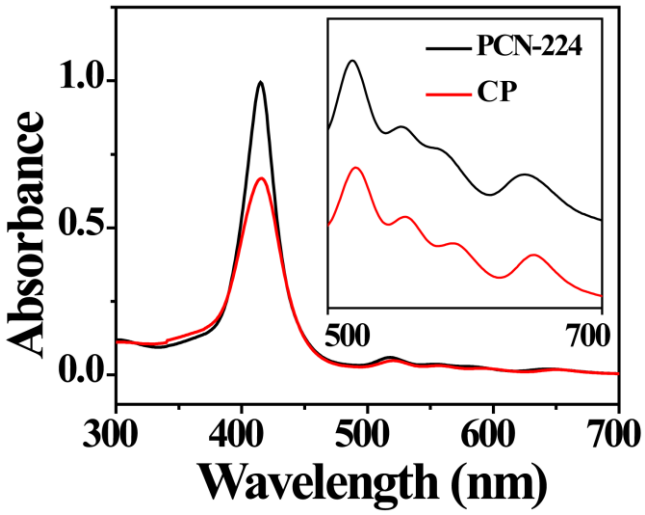


Figure S17 UV−vis absorption spectra of PCN-224 and CP.

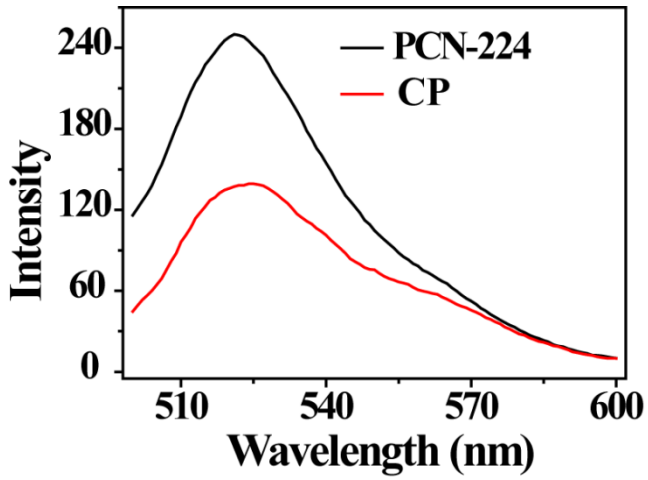


Figure S18 Fluorescence spectra of DCFH-DA incubated with PCN-224 and CP under visible light irradiation of 638 nm.

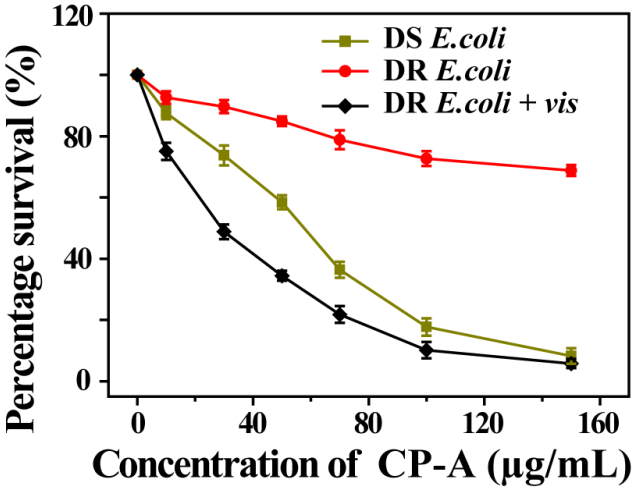


Figure S19 The viability of DR *E. coli* and DS *E. coli* incubated with CP-A with or without light irradiation.

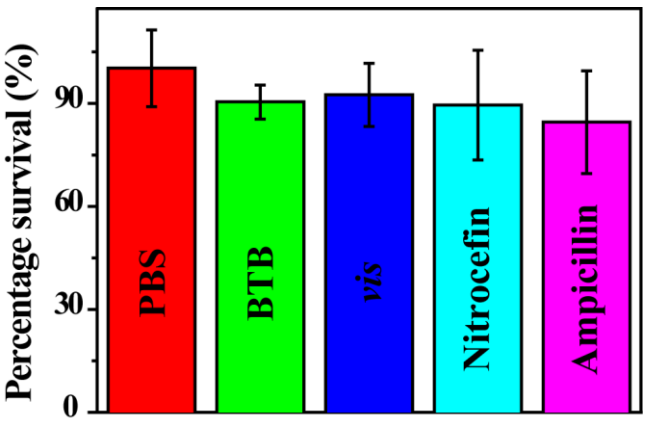


Figure S20 Estimation of L929 cells viability by MTT assays.

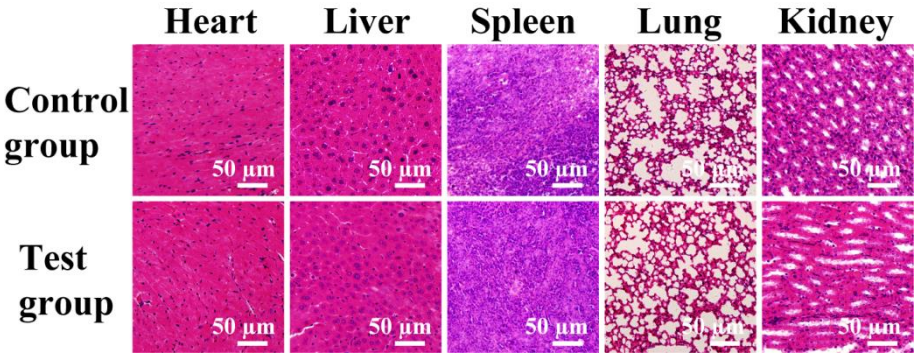


Figure S21 Histological analysis of tissues from mice treated with Paper and PBA.

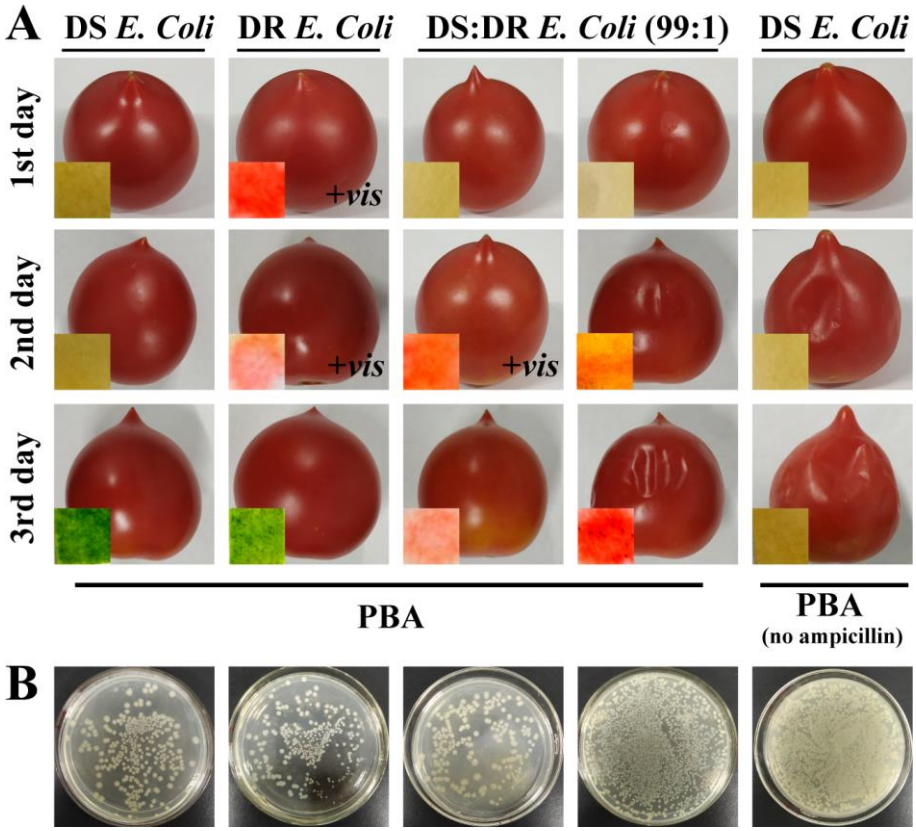


Figure S22 (A) Photographs of infected tomatoes during 3-day treatments. Inset images revealed the color of PBA. When red color was observed on the PBA, the irradiation by 638 nm laser was maintained for 10 minutes. And PBA was changed every day. (B) Bacteria was separated from infected tomatoes after 3-day treatment and then cultured on agar plates.

Reference

1. Park, J.; Jiang, Q.; Feng, D.; Mao, L.; Zhou, H.-C. Size-controlled synthesis of porphyrinic metal–organic framework and functionalization for targeted photodynamic therapy. *J. Am. Chem. Soc.* **2016,** *138*, 3518-3525.
2. Wang, F.; Li, W.; Wang, J.; Ren, J.; Qu, X. Detection of telomerase on upconversion nanoparticle modified cellulose paper. *Chem. Commun.* **2015,** *51*, 11630-11633.
3. Sun, H.; Gao, N.; Ren, J.; Qu, X. Polyoxometalate-based rewritable paper. *Chem. Mater.* **2015,** *27*, 7573-7576.