Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Functional polypyrrole/silica composites as photothermal agents for targeted killing of bacteria

1. Materials

Pyrrole, Tetraethyl orthosilicate (TEOS) and (3-aminopropyl) triethoxysilane (APTES) were purchased from Sigma-Aldrich. Fluorescein isothiocyanate (FITC) and glutaraldehyde (GTA) were obtained from Alfa Aesar. Ferric chloride hexahydrate ($FeCl_3$•$6H_2O$, Beijing Chemical reagent Corporation, China), Polyvinyl alcohol (PVA-124, Guangzhou Medicine Corporation, China) were used directly without further purification. *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) bacterial strains were purchased from Chuanxiang Biotechnology, Ltd (Shanghai, China). Water throughout all experiments was obtained by using a Milli-Q water system.

2. Synthesis of polypyrrole nanoparticles

1.5 g PVA was dissolved in 20 mL de-ionized water at 60 ^oC, and then cooled to room temperature. $FeCl_3$•$6H_2O$ (0.23M) was added to the stirred PVA solution, the system changed from a clear state to a yellow one. After 1 hour equilibration, pyrrole monomer (0.1M) was introduced and the temperature was controlled at 4^oC.The mixture solution was stirred for 4 hours. After the completion of polymerization, the resulting nanoparticles were separated from the dispersion solution by centrifugation and washed several times with hot water to remove impurities. The obtained PPy nanoparticles were resuspended with water by ultrasonication.

3. Synthesis of PPy/SiO_2 composites and functionalization

In a typical silica-coating procedure, 0.5 mL of as-prepared PPy nanoparticles was added to 45 mL of isopropanol. Under continuous ultrasound treatment, TEOS (30 μL) and ammonia solution (1.5 mL) were consecutively added to the reaction mixture. The reaction was allowed to proceed for 1 h. The resulting PPy/SiO_2composites were purified by repeated centrifugation at 10000 rpm for 5 min and by washing with water in order to remove reaction impurities. Finally, the samples were ultrasonically redispersed.

PPy/SiO_2-GTA complex was prepared as follows. Firstly, 4 μL APTES in 40μL methanol was added to PPy/SiO_2 under stirring for 5 h to get amino modified particles. After removing of excess APTES, GTA (0.25%) was then added to the solution and stirred at room temperature for 24 h to get PPy/SiO_2-GTA complex. The complex was separated by centrifugation and supernatant was measured by UV spectrometer. As for fluorescein-labelled nanoparticles, 1 mg FITC was first reacted with 22 μL of APTES in 1 mL ethanol for 2 h in the dark, and then 100 μL was added to the solution of PPy/SiO_2 with the same condition before.

4. Characterization

SEM images were obtained with a Hitachi S-4800 FE-SEM.FT-IR analyses were carried out on a Bruker Vertex 70 FT-IR spectrometer. Dynamic light scattering (DLS) measurements were performed with a vertically polarized He-Ne laser (DAWN EOS, Wyatt Technology), and the scattering angle was fixed at 90^o. Absorption spectra were measured on a JASCO V-550 UV/vis spectrometer. Optical densities at 600 nm (OD600) were determined in an Amersham Biosciences Ultrospec 500 pro spectrometer. A diode laser (LSR808NL-2000) with wavelength of 808 nm was

used for the laser irradiation experiments.

5. Bacterial culture and photothermal killing of bacteria experiments

Monocolony of *E. coli* and *S. aureus* on the solid Luria-Bertani (LB) agar plate was transferred to 5 mL of liquid LB culture medium in the presence of ampicillin (50 μg mL^{-1}) and grown at 37 ^{o}C for 12 h under 180 rpm rotation. Then the bacteria were diluted with broth to 10^5 cfu mL^{-1}. The as-prepared bacteria solution (500 μL) was mixed with nanoparticles for 10 min. The obtained mixture was irradiated under NIR laser (808nm, 1.5W) for 5 min. Then, the solution was placed on solid medium by spread plate method and cultured for 24 h before observing the number of the bacteria colonies. A control experiment was performed in parallel without NIR irradiation.

6. Fluorescence assay

The 500 μL(~10^5 cfu mL^{-1}) of the bacteria suspension was incubated with 50 μg mL^{-1} of FITC labelled PPy/SiO_2-GTA. The interaction was carried out by vortexing for 10 min. After that, the bacteria were stained with DAPI for 10 min and washed twice with PBS. Fluorescence images of the stained bacteria were taken with a fluorescence microscope. The assay for the photothermal effect on bacteria viability was carried out as follows: after 10 min interaction, the solution was immediately subjected to NIR laser irradiation (808 nm, 1.5W) for 5 min. Then, the bacteria were stained with PI for 10 min and washed with twice with PBS. The dead bacteria cells were also visualized with fluorescence microscope.

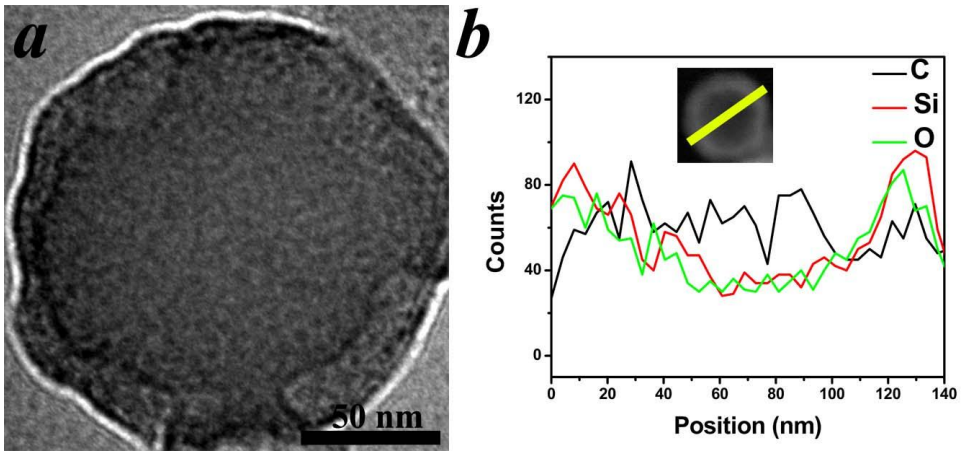

Figure S1. TEM image (a) and EDX spectrum profile-scanning data (b) of PPy/SiO_2 composites.

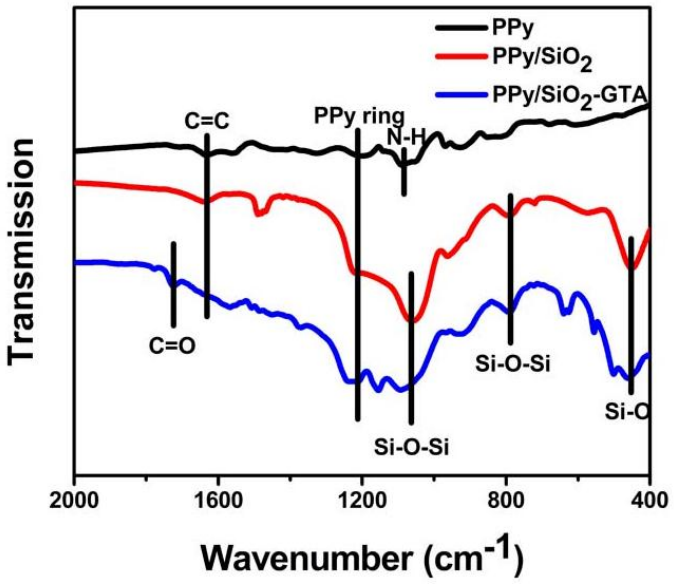

Figure S2. FI-IR spectra of PPy nanoparticles, PPy/SiO_2 and PPy/SiO_2-GTA composites.

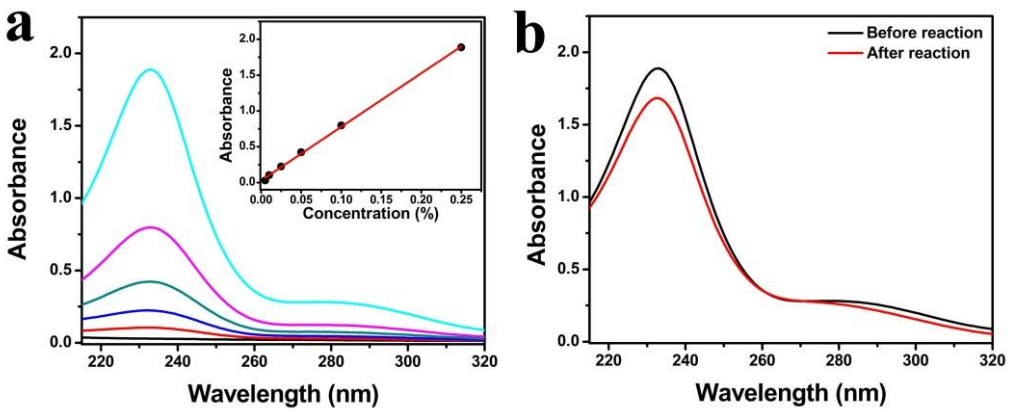

Figure S3. (a) Standard linear calibration curve of glutaraldehyde aqueous solution. (b) UV spectrum of glutaraldehyde aqueous solution before and after reaction.

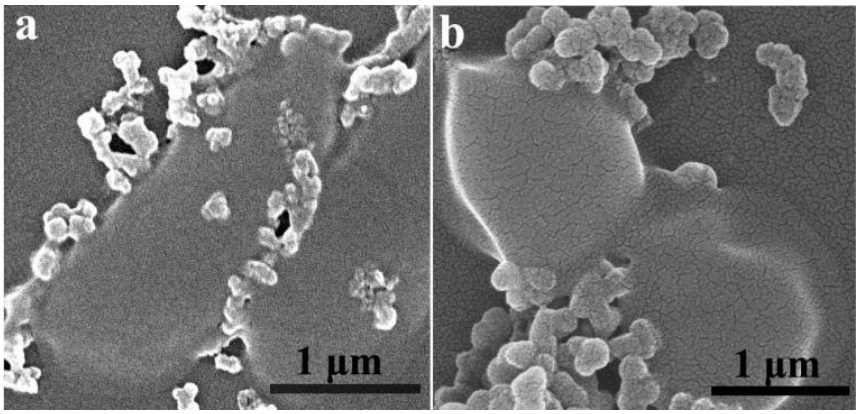

Figure S4. SEM images of *E. coli* (a) and *S. aureus* (b) bacteria incubated with PPy/SiO_2-GTA.

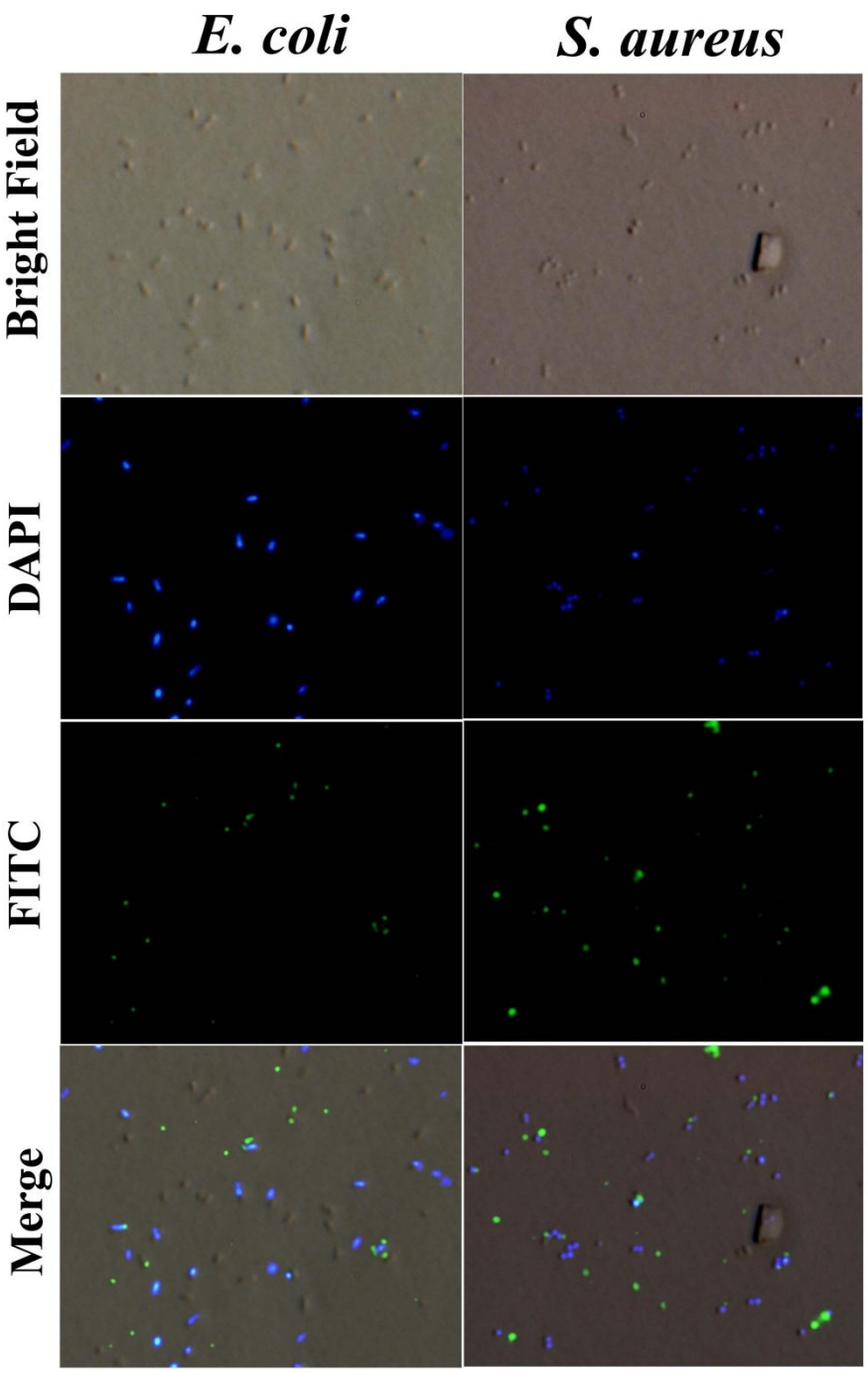

Figure S5. Fluorescence microscopy images of *E. coli* and *S. aureus* incubated with PPy/SiO_2. The green fluorescence of PPy/SiO_2 was not co-localized with blue fluorescence of bacteria, which indicated that PPy/SiO_2 can hardly target bacteria without the modification of GTA.

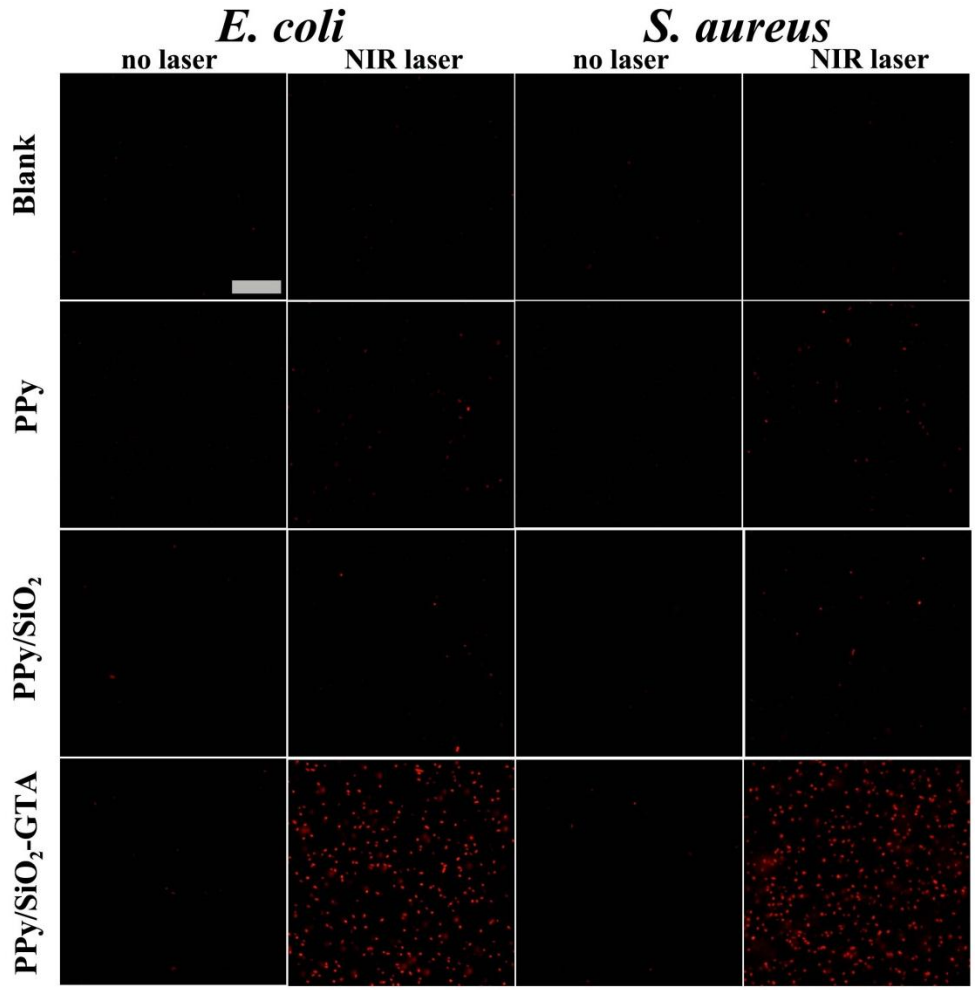

Figure S6. Fluorescence images of *E. coli* and *S. aureus* incubated with nanoparticles before and after photothermal treatment. Bacteria were stained with PI. Red fluorescence cells are representative of dead cells. Scale bar equals 10 μm.

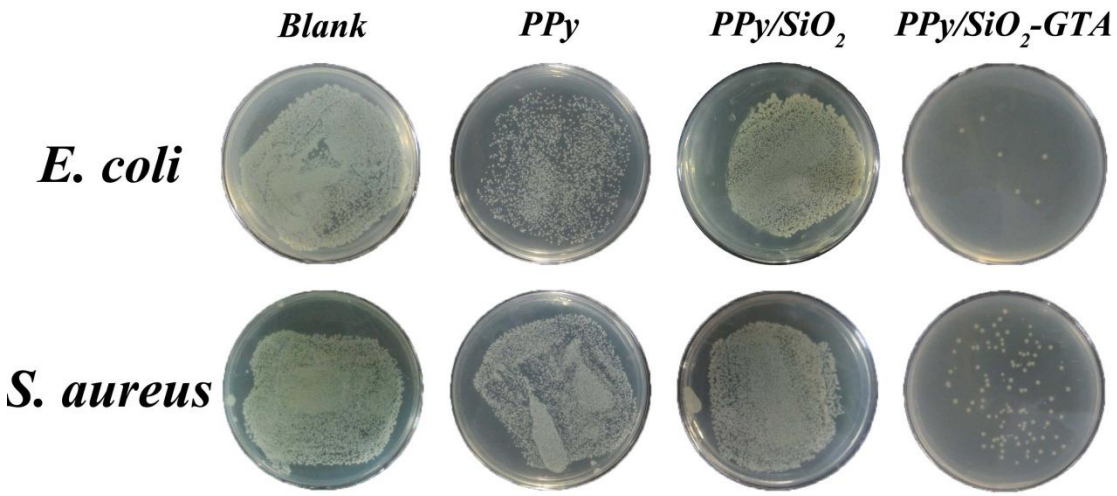

Figure S7. The plate samples showed colonies of *E. coli* and *S. aureus* incubated with various materials under NIR irradiation (1.5 W, 5 min).

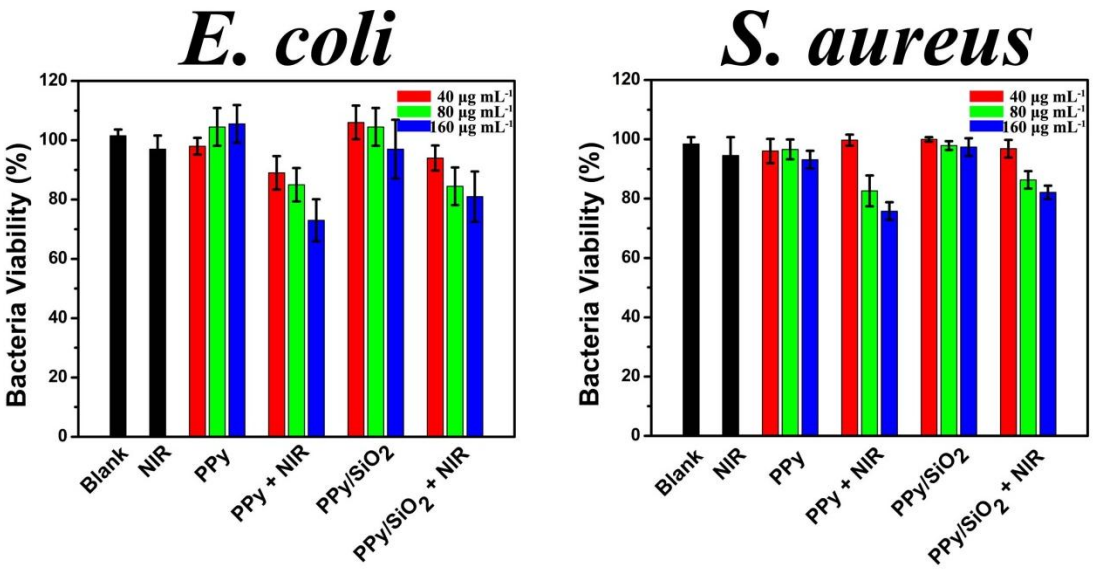

Figure S8. Histogram presented the viability of *E. coli* and *S. aureus* after treatment of various materials with or without NIR irradiation (1.5 W, 5 min).