Supporting Information

Dual-functional peptide conjugated gold nanorods for the detection and photothermal ablation of pathogenic bacteria

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Materials

Sodium borohydride ($NaBH_4$), Chloroauric acid ($HAuCl_4 \cdot 3H_2O$), L-ascorbic acid (AA) was obtained from Sinopharm Chemical Reagent Co., Ltd; Cetyltrimethylammonium bromide (CTAB) was obtained from Shanghai Richjoint Chemical Reagent Co., Ltd; Siver nitrate ($AgNO_3$) were purchased from Tianjin Damao Chemical Reagent Factory. Milli-Q water (18 MU cm) was used for all solution preparations.

Au NRs Synthesis

The preparation of Au NRs was using the seed-mediated growth method. Briefly, the seed solution was first synthesized by mixing 0.25 mL of 0.01 M $HAuCl_4 \cdot 3H_2O$ with 7.5 mL of 0.1 M CTAB through the way of inversion in a test tube. Then 0.6 mL of 0.01 M ice-cold $NaBH_4$ was added, along with the rapidly inversion of test tube for two minutes. The color of the seed solution was changed from yellow to pale brow. The final solution was incubated for at least 3 h at 30 ^oC.

After 3 hours, an Au growth solution was prepared by the following method: 4.75 mL of 0.1 M CTAB was mixed with some solutions, including 0.2 mL of 0.01 M HAuCl4, 0.03 mL of 0.01 M $AgNO_3$, 0.032 mL of 0.1 M AA. The color of the solution was changed from yellow to clear. At last, 0.01 mL seed solution was added. In order to removing the excess CTAB, the solution of Au NRs were centrifuging at 12000 rpm for 10 min twice before used. The precipitate was collected and redispersed in the water for further experiments.

Modification of P937 on AuNRs

The peptide-937 was dissolved in the solution of 1,1,1,3,3,3-Hexafluoro-2-propanol; HFIP. Typically, 1 mg of the peptide-937 (WGLHTSATNLYLHGGGC) was added into 1 mL of a solution of 1,1,1,3,3,3-Hexafluoro-2-propanol; HFIP, Shaking 12 h at room temperature to completely dissolved. The obtained solution of peptide-937 was preserved in the fridge (–18 ^{o}C).

Before used, the peptide solution (16.5 μL) was dried at room temperature naturally, and was redispersed in the deionized water (165 μL). A peptide-modified Au NRs (Au@P937 NRs) was prepared by mixing peptide-937 with purified Au NRs at the molar ratio of peptide/Au = 1/50. The mixture was kept more than 12 h to enhance the connection between peptide and gold nanoparticles at 30 ^{o}C. The Au@P937 NRs was then purified by centrifuging at 12000 rpm for 10 min to remove the unreacted peptide and the precipitate was redissolved in deionized water.

Characterizations of P937 on Au@P937 NRs

Ultraviolet-visible (UV-vis) absorption spectra of the Au nanoparticle suspensions were recorded with a UV-1800PC spectrophotometer.

Transmission electron microscopy (TEM) images were acquired using a Tecnai 12 microscope operating at an accelerating voltage of 120 KV. As for bacteria samples, 10 μL of samples was added onto the copper grid placed on the tweezers for 10 min and then dyed by 10 μL 1% bis (acetato) dioxouranium for 2 min, and rinsed by water for 2 min.

The black field TEM images were obtained on a microscopy (Tecnai G2 F30) operated at an acceleration voltage of 200 KV.

X-ray photoelectron spectra (XPS) of the representative Au nanoparticles were recorded on an ESCALAB 250 spectrometer (PHI5000 Versa Probe) using Al Kα radiation at 1486.6 eV. The resolution of the XPS is ± 0.1 eV. The binding energies of Au were calculated with respect to C1s peak of contaminated carbon at 284.6 eV.

The FTIR Spectrometer (Termo Scientifc, Marietta, GA, USA) spectra were recorded using a smart multi-Bounce ARK accessory (Termo Nicolet) equipped with a calcium fuoride crystal.

Zeta potential of samples was measured using a Zetasizer (Nnao ZS90) at a temperature of 25 ºC.

Cytotoxicity Assay

MTT assay was carried out to investigate the cytotoxicity of Au@P937 NRs on Human umbilical vein endothelial cell lines (HUVEC) because they can be considered as a relatively reliable and simple in vitro model to predict and evaluate the cytotoxicity of nanoparticles, which are obtained from School of Food and Biological Engineering of Jiangsu University. Briefly, HUVEC cells was grown in a 96-well plate at a concentration of 5×10^3 cells per well 100 μL of culture medium for 24 h at 37 ^oC, 5% CO_2. After that, removed the supernatant and incubated with different concentrations of Au NRs and Au@P937 NRs (0.4 mM-78.8 μg mL^{-1}, 0.2 mM-39.4 μg mL^{-1}, 0.1 mM-19.7 μg mL^{-1}, 0.05 mM-9.85 μg mL^{-1}) for 48 h, respectively. For the cytotoxicity analysis, 10 μL MTT (5 mg mL^{-1}) was added and incubated for 1.5 h. After this, removed all the solution and washed each well with culture solution. Finally, each wall was added 100 μL of dimethylsulfoxide (DMSO) and measured the absorbance at 550 nm determined by a microplate reader (SYNERGY H4).

Table S1 Characteristics of peptides

Peptide	pI	Net charge	Hydrophobicity
GLHTSATNLYLH (GLH)	8.1	0.2	17%
HGTSNLHALTYL (HGT)	8.1	0.2	17%
LLADTTHHRPWT (LLA)	8.1	0.2	17%

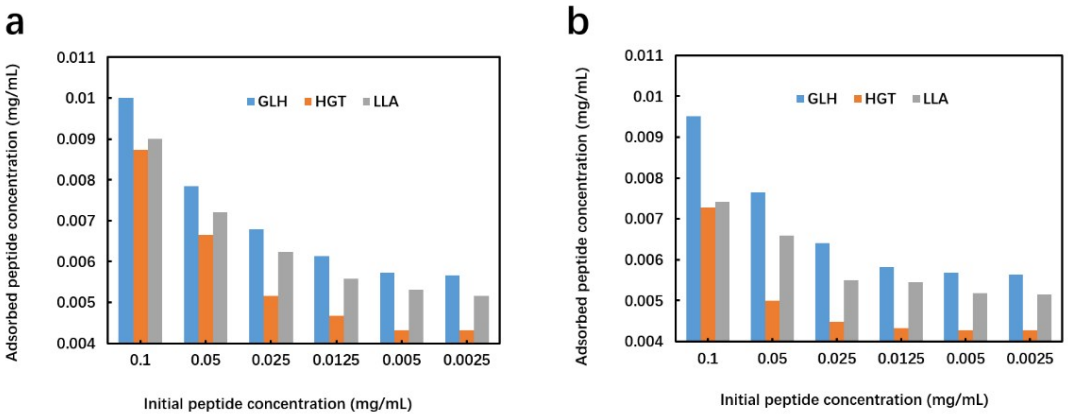

Figure S1. Adsorbed peptide concentration at different initial peptide concentration on the same amount of bacterial. (a) E. coli (b) S. aureus.

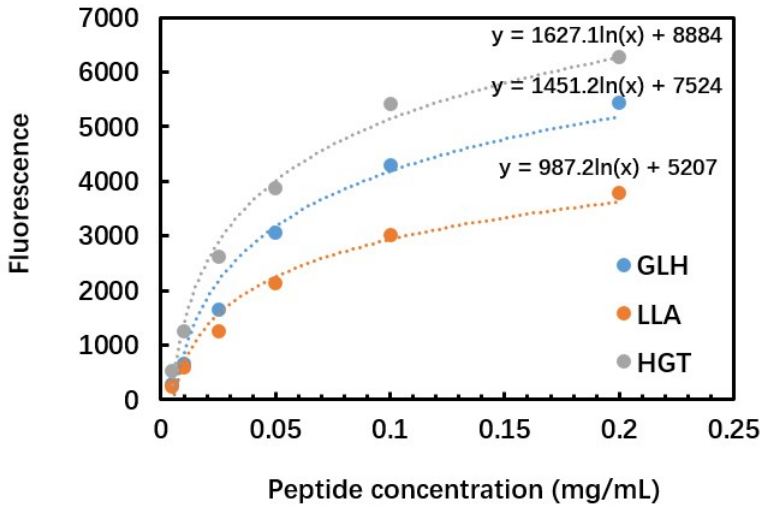

Figure S2. Standard curve determination of peptide concentration

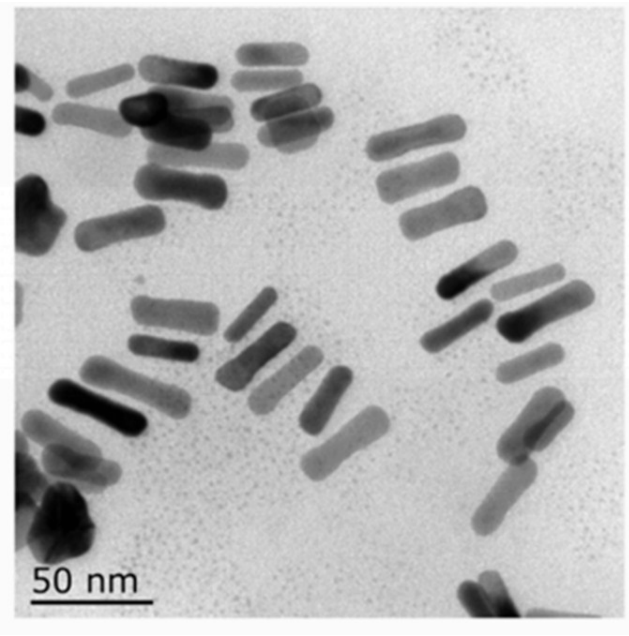

Figure S3. TEM image of as-prepared Au NRs.

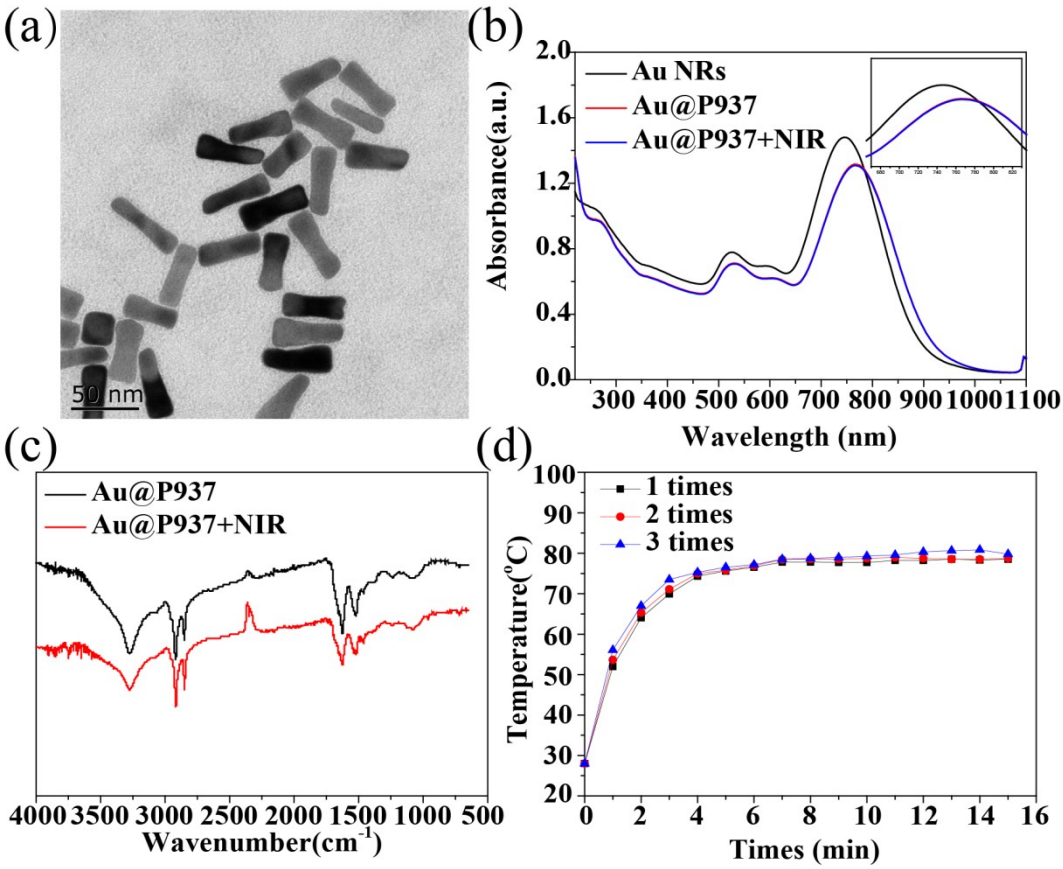

Figure S4. The morphology (a), UV-vis-NIR spectra (b), and FTIR spectra (c) of Au@P937 NRs before and after 808 nm laser irradiation (3 W cm^{-2}) for 15 min; and the heating profiles of Au@P937 NRs for 3 times at the same conditions.

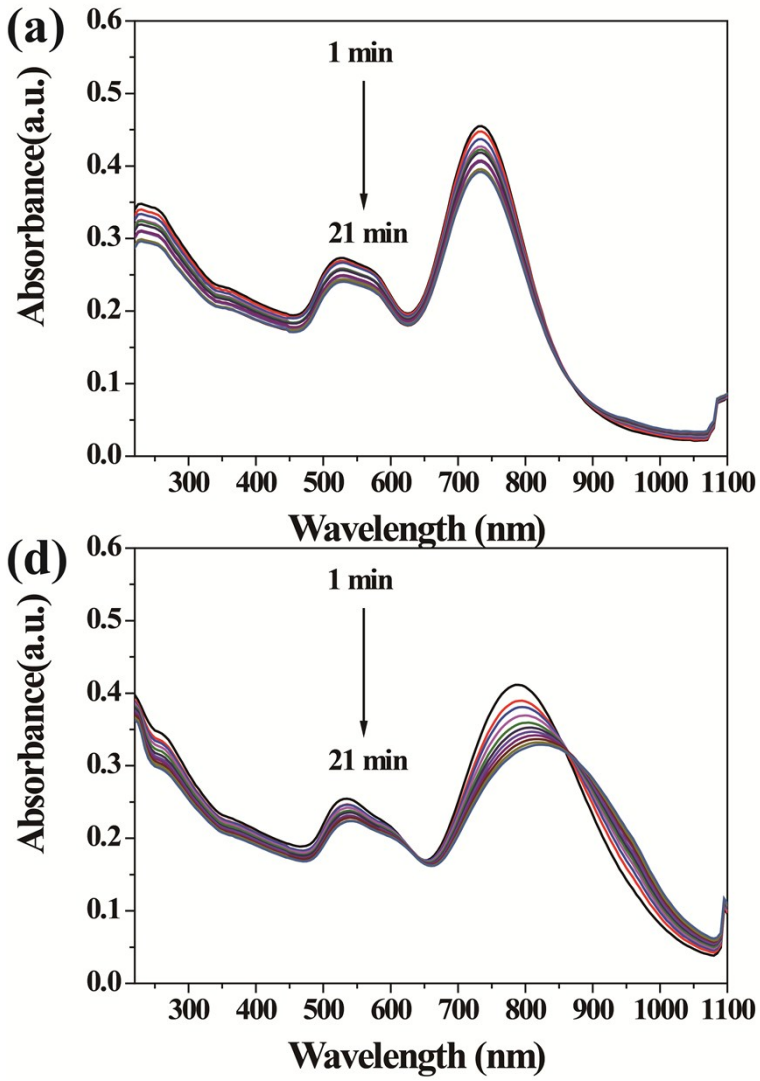

Figure S5. Vis-NIR spectra of (a) Au NRs and (b) Au@P937 NRs in the presence of ~10^5 cfu mL^{-1} *E.coli* at varying times (curves 1−21 correspond to 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21 min, respectively).

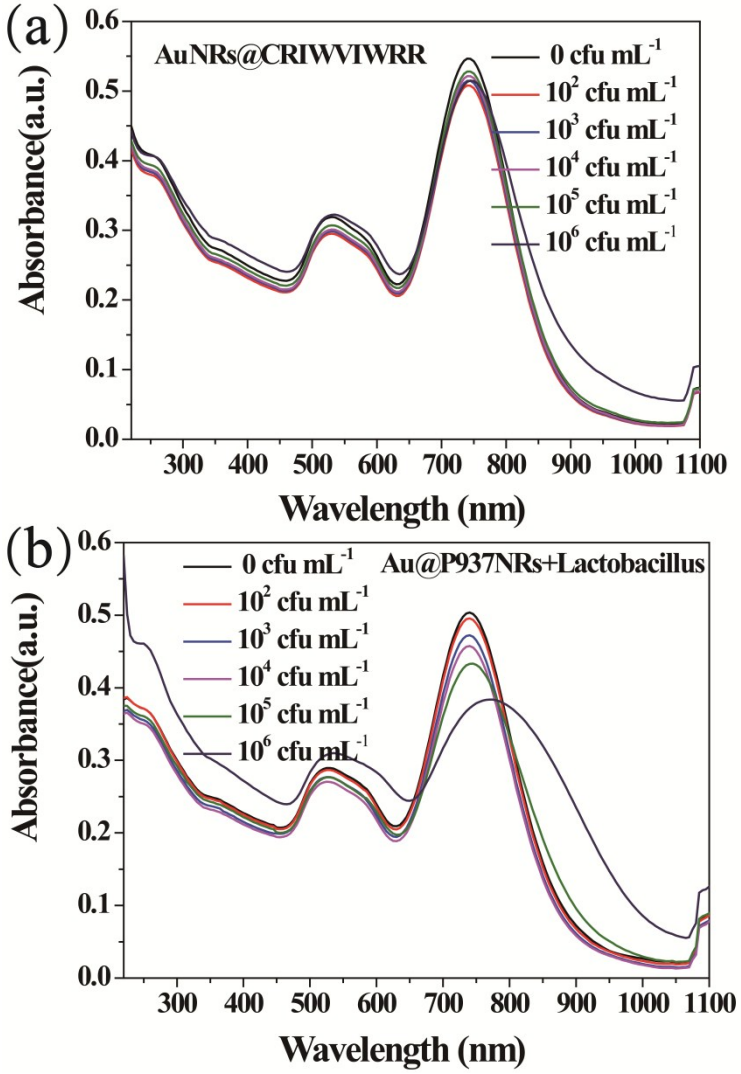

Figure S6. The UV-vis to NIR spectra of (a) Au@CRIWVIWRR NRs incubating with *E. coli* at different concentration (1×10^2, 1×10^3, 1×10^4, 1×10^5, 1×10^6 cfu mL^{-1}) and (b) Au@P937 NRs incubating with *Lactobacillus* at different concentrations (1×10^2, 1×10^3, 1×10^4, 1×10^5, 1×10^6 cfu mL^{-1}).

Figure S6 shows the bad variation of LSPR peak of Au@CRIWVIWRR NRs with decreasing the concentrations of *E. coli* due to the peptide CRIWVIWRR without specific bacterial binding affinity.

Thanks to the good bacterial binding affinity of peptide P937, it is found that the Au@P937 NRs also showed good binding affinity to *Lactobacillus* (BNCC194390) with linear variation of LSPR peak VS *Lactobacillus* concentration. The detection of *Lactobacillus* with Au@P937 NRs at different *Lactobacillus* concentration is shown

in Figure S6b. The calculated detection limit was 98 cfu mL $^{-1}$ with the R^2 of 0.912, revealing that Au@P937 NRs can be used as a sensitive probe for SPR-based detection of bacteria.

Cytotoxicity of Au@P937 NRs

The cytotoxicity of Au@P937 NRs was evaluated via the cell viability of Human Umbilical Vein Endothelial Cells (HUVEC), as shown in Figure S7. It is clear to see that the cell viability incubated with Au NRs was much lower than 70% even at the concentration of 0.05 mM, indicating the strong cytotoxic effect of Au NRs, probably due to the surfactant of CTAB capped on the Au surfaces.[48] However, the cytotoxicity of Au@P937 NRs was significantly inhibited even at the concentration up to 0.4 mM (cell viability higher than 70%), which can be ascribed to the substitution of the CTAB by P937 on the surface of Au NRs, and it therefore significantly reduced the cytotoxicity of Au NRs. The Au@P937 NRs was demonstrated to have a good biocompatibility.

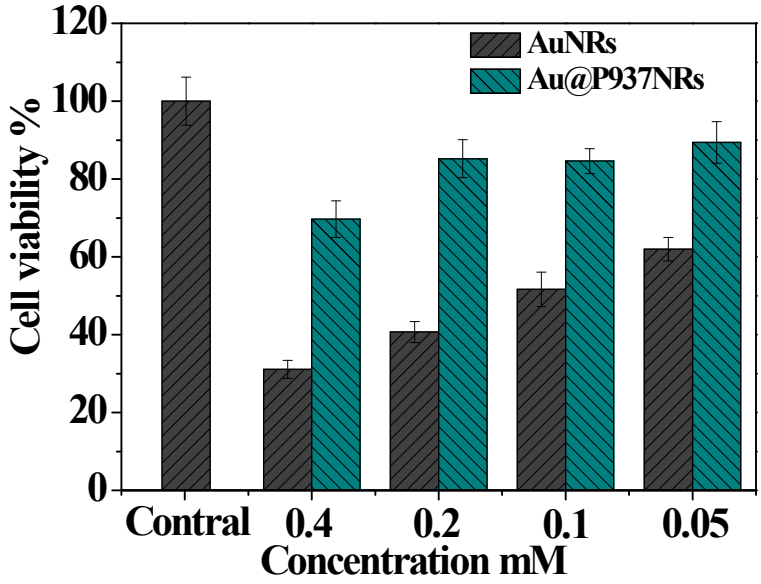

Figure S7. Cell viability of Human Umbilical Vein Endothelial Cells (HUVEC) incubated with Au NRs and Au@P937 NRs at different concentrations.

Table S2 Zeta potential of *E. coli* and *S. aureus*

Samples	Zeta potential (mV)
E. coli	–24.3
S. aureus	–19.7

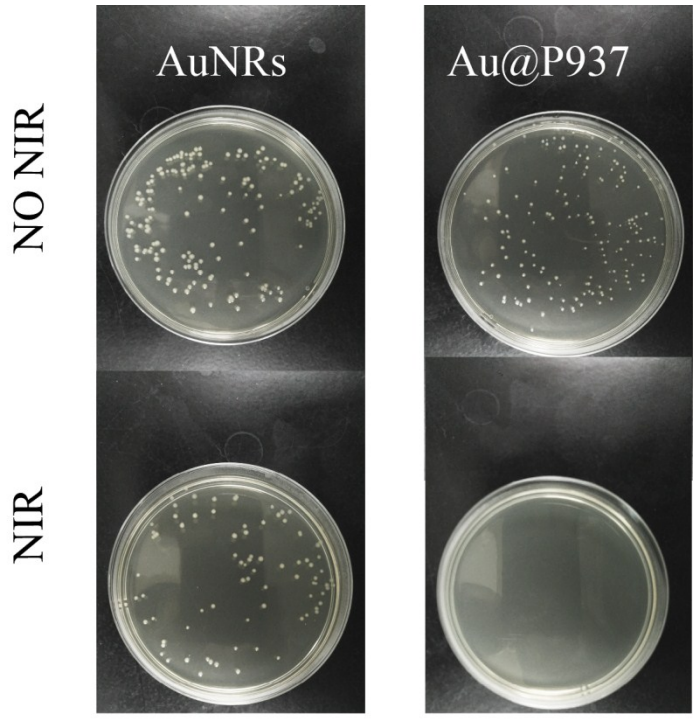

Figure S8. Images of bacteria *(E.coli*) colony incubated with Au NRs (a1 and a2) and Au@P937 NRs (b1 and b2) before and after NIR irradiation (808 nm, 3 W cm^{-2}).

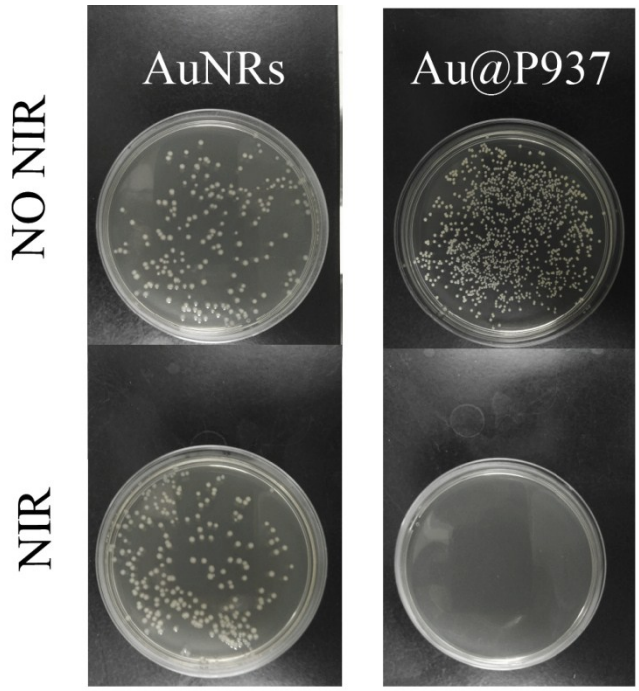

Figure S9. Images of bacteria (*S. aureus*) colony incubated with Au NRs (a1, and a2) and Au@P937 (b1 and b2) before and after NIR irradiation (808 nm, 3 W cm^{-2}).

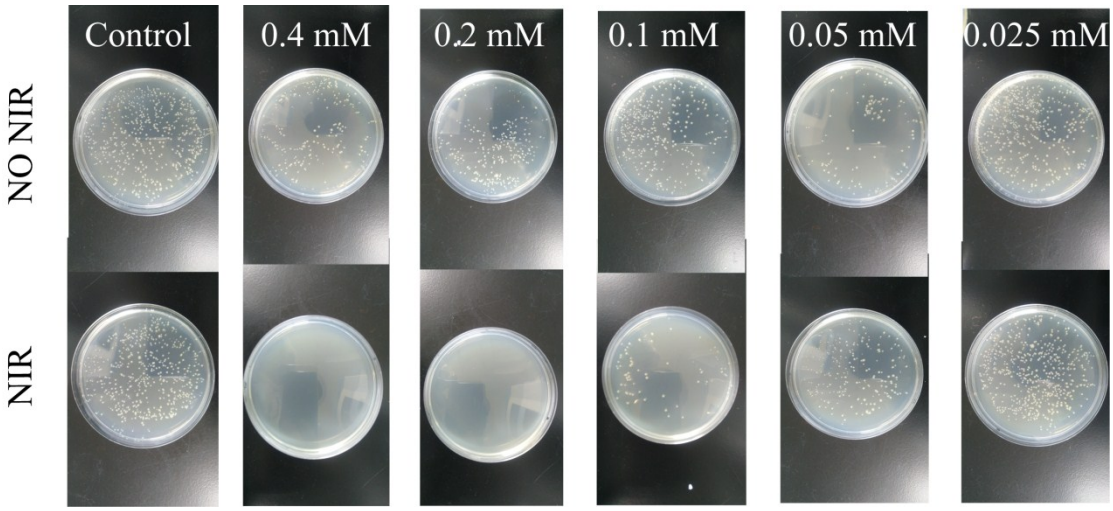

Figure S10. Images of bacteria (*E.coli*) colony incubated with Au@P937 NRs before and after NIR irradiation (808 nm, 3 W cm^{-2}) at the different concentrations.

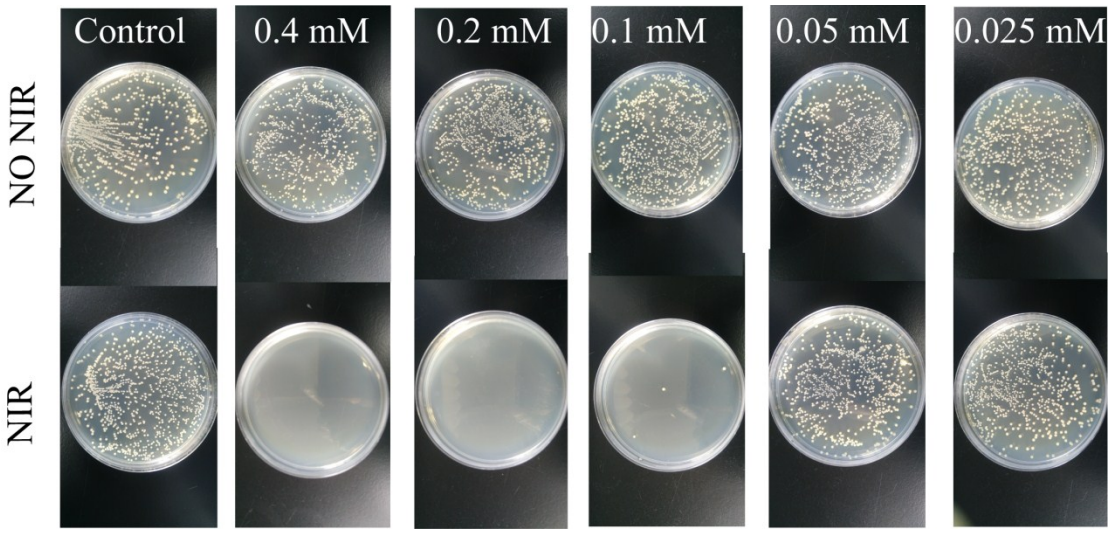

Figure S11. Images of bacteria (*S. aureus*) colony incubated with Au@P937 NRs before and after NIR irradiation (808 nm, 3 W cm^{-2}) at the different concentrations.