

Supporting information

**Peptide-Directed Synthesis of Chiral nano-bipyramides for Controllable
antibacterial application**

Panpan Chen^{1#}, Gaoyang Wang^{1#}, Changlong Hao¹, Wei Ma¹, Liguang Xu¹, Hua Kuang¹,
Chuanlai Xu¹, Maozhong Sun^{1*}

*¹International Joint Research Laboratory for Biointerface and Biodetection, State Key Lab of Food Science and
Technology, School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, 214122, PRC;*

*Corresponding Author: smz@jiangnan.edu.cn

#The authors contributed equally to this paper.

Chemicals and instruments

Materials

Hydrogen tetrachloroaurate (III) hydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, 99.5%), sodium borohydride (NaBH_4 , 98%), silver nitrate (AgNO_3 , 99%), *L*-ascorbic acid (99%), cetyltrimethyl ammonium bromide (CTAB, $\text{C}_{19}\text{H}_{42}\text{BrN}$, 95%), cetyltrimethyl ammonium chloride (CTAC solution 25% in water) and trisodium citrate (99%) were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Thiolate PEG (SH-PEG) were purchased from Yanyi Biotechnology Corporation (Shanghai, China). Trypsin-EDTA (0.25%), fetal bovine serum and MTT were obtained from Gibco Life Technologies (Grand Island, USA). All glassware and teflon-coated magnetic stirring bars were thoroughly cleaned with aqua regia and rinsed well with purified water. All chemical materials were dissolved in distilled water (18.2 M Ω). The water was prepared by a Milli-Q water purification system from Millipore (Massachusetts, USA).

Instruments

Transmission electron microscopy (TEM) was performed with a JEOL JEM-2100 (Hitachi, Tokyo, Japan) microscope operating at 200 kV. The CD signals were characterized by a CHIRASCAN CD spectrometer from Applied Photophysics (Surrey, UK) with an optical path length of 1 cm. X-ray photoelectron spectra (XPS) were acquired with a Kratos Analytical Axis Ultra system (Manchester, UK). Powder X-ray diffraction (XRD) patterns were acquired by a Bruker D8 (Germany) equipped. UV-visible (UV-vis) absorption spectra were determined with a Shimadzu UV-vis 3101 spectroscope (Hitachi, Tokyo, Japan). Fluorescence spectra were acquired with a Carl Zeiss F-7000 (Hitachi, Tokyo, Japan).

Preparation of the *D*-/*L*-GBPs

The *D*-/*L*-GBPs were synthesized by a seed-mediated method. Briefly, a citrate-stabilized seed solution was made by adding freshly prepared ice-cold NaBH_4 solution (0.1 M, 0.625 mL) into an aqueous solution composed of HAuCl_4 (0.1 M, 0.025 mL), trisodium citrate (0.05 M, 1 mL) and water (9.625 mL) under vigorous stirring. Then

the solution was placed in an oil bath at 80°C and gently stirred for 90 min to obtain a gold seed solution. The resultant seed solution was kept in a water bath at 25°C for at least 2 h. At the same time, an aqueous growth solution containing 10 mL 100 mM CTAB aqueous solution, 0.2 mL 10 mM silver nitrate, 2 mL 1 M hydrochloric acid and 0.15 mL 100 mM ascorbic acid was prepared, and then added to 100 µL gold seed solution to the growth solution, and allowed to stand at 30°C for 2 h. It was then centrifuged the solution at 8000 ×g for 10 min, and the precipitate was dissolved in 1 mL of a 1 mM CTAB solution to obtain a gold bipyramid solution. Then, 1 mL of a 100 mM CTAB solution and 0.3 mL of a 10 mM HAuCl₄ solution was added to 20 mL aqueous solution, stirred and then 0.1 mL of a 100 mM ascorbic acid solution was added. To obtain the growth solution, 0.08 mL of a 1mM chiral dipeptide (*D*-/*L*-CF) solution was added to the growth solution and finally, 0.4 mL of a gold bipyramids solution was added, and the mixture was left to stand at 30°C for 2 h to obtain the chiral *D*-/*L*-GBPs.

Characterization

The morphology and size of the *D*-/*L*-GBPs were determined by TEM. Samples were prepared by drying a drop of the relevant dispersion onto a carbon-coated copper grid at room temperature. The prepared samples were then analyzed using a scanning/transmission electron microscope at an accelerating voltage of 200 kV and UV-Vis-NIR spectra of the *D*-/*L*-GBPs was recorded between 400 and 900 nm. The samples were measured in a quartz cuvette using the pure solvent as control. CD spectra was recorded with a CHIRASCAN CD spectrometer. The remaining product was dried for XRD, XPS, and FTIR characterization studies.

Cell viability assay

Primary uterine fibroblast cells (PCs cells) were cultured in DMEM medium supplemented with 10% fetal bovine serum, 100 units/mL penicillin and 100 mg/mL streptomycin at 37°C and 5% CO₂. We first evaluated the cytotoxicity of chiral GBPs on the PCs cells. The cells in logarithmic growth phase were cultured in 96 well plates

for 24 hours and then, different concentrations of Au NBP, *DL*-GBPs, *L*-GBPs and *D*-GBPs solutions (200 μ L aliquots) were added to 96 well plates and then exposed to NIR light (0.2, 0.4, 0.8 and 1.2 W/cm² for 10 min) and a group were treated with PBS as controls. After incubation for 48 h, 10 μ L of a 5 mg/mL MTT solution was added to each well and mix well. After 4 hours, the absorbance of each well was measured at 570 nm using a multimodal microplate reader. We designated the viability of control cells as 100% to estimate the relative viability of cells treated with the surface modified GBPs.

***In vitro* antibacterial assay**

Staphylococcus aureus was used as a model strain to evaluate the antibacterial properties of chiral *D*-/*L*-GBPs. For antibacterial tests, 100 μ L of bacterial suspensions ($2 \times$ Mixture of *Staphylococcus aureus* in trypsin soybean broth (TSB)), 100 μ L of Au NBPs, *DL*-GBPs, or *D*-/*L*-GBPs (55 μ g/mL) and 100 μ L of PBS were added to each well of the 96 well plate. The plates were vortexed for 3 min, then place it an incubator for another 3 hours, and then we used an 808 nm laser (0.8 w/cm² for 10 min). After irradiation, the mixture was incubated at 37°C for another 12 hours. Finally, the turbidity of the sample was evaluated at OD_{600nm} by measuring the optical density value at 600 nm (OD_{600nm}). Bacterial suspensions cultured with unirradiated samples were used as controls. In the standard plate counting determination, the bacterial suspension was continuously diluted with sterile PBS, and 100 μ L of each diluted sample was coated onto the trypsin soybean agar plate. After incubation at 37°C for 12 hours, the colonies formed on the surface were counted to evaluate the bacterial concentration. For the live/dead staining assay, the treated bacteria were stained with live/dead bacterial viability kit components and inspected under a confocal fluorescence microscopic imaging system.

Bacterial morphology study

SEM images were taken to examine the morphology of bacteria and biofilms treated with PBS, Au NBPs, *DL*-GBPs, *D*-GBPs and *L*-GBPs and NIR irradiation.

Staphylococcus aureus solutions from different treatments were dropped onto silicon wafers, then fixed with 2% glutaraldehyde at room temperature for 3 hours, and then treated with a series of ethanol solutions (50%, 70%, 90%, 95% and 100%, 10 min each step). After drying under a flow of nitrogen, the silicon wafer was coated with ultra-thin gold by sputtering and imaged at 3.0 kV using a HITACHI Su 8010 instrument (Hitachi, Tokyo, Japan).

***In vivo* antibacterial assay**

All animal experiments were performed with ethical compliance and were approved by the Committee on Animal Welfare of Jiangnan University (JN No. 20210515b0901114).

Mouse skin infection and treatment

Female mice aged 6 weeks were used for *in vivo* antibacterial testing. The bacterial inflammation model was established using *Staphylococcus aureus*. The mice were first anesthetized with 2% Pentobarbital Sodium and after shaving, were disinfected with iodophenol. Then, 100 μ L of *Staphylococcus aureus* was injected subcutaneously into the shaved back of each mouse (10^6 CFU/mL). After 24 hours, obvious infection with abscess formed under the skin. Then, the mice were anesthetized, and 100 μ L of *D-/L-*GBPs solution was injected directly into the infected abscess, which was then irradiated with an 808 nm laser for 10 min and the thermal images of the rats were recorded with an infrared thermal imaging camera. After 8 days, the mice were euthanized, and their skin was exposed for photography. Also, the abscess sites were taken for histological H&E staining, and analyzed by a standard plate counting method.

Mouse peritonitis infection and treatment

Eight groups of female mice (five in each group) were infected by intraperitoneal injection of 0.5 mL bacterial suspension (7.5×10^8 CFU per mouse). One hour after infection, mice from each group received a single intraperitoneal injection of 100 μ L *D-/L-*GBPs solution. The survival rate was observed 48 hours after infection and analyzed

by nonparametric rank tests. Once the infected mice died, the heart, liver, spleen, lung, and kidneys were removed and homogenized in sterile PBS to obtain a single-cell suspension. continuous dilution of each suspension was plated onto *Staphylococcus aureus* chromogenic agar plates for colony counting. In addition, the body weight of each mouse was recorded, and the number of white blood cells (WBC), neutrophils (NEU) and lymphocytes (LYM) from the different treatment groups were counted.

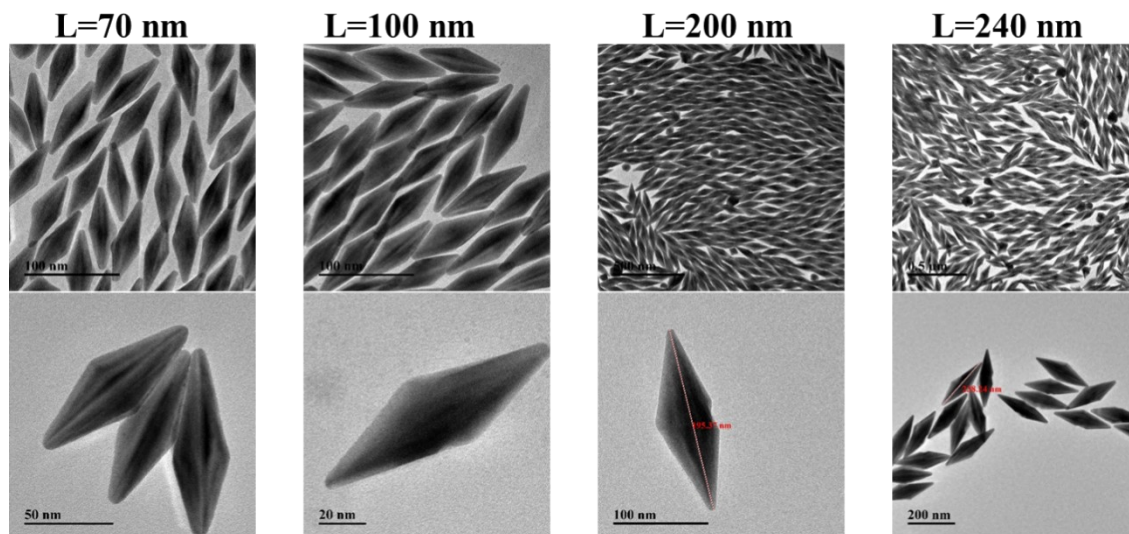
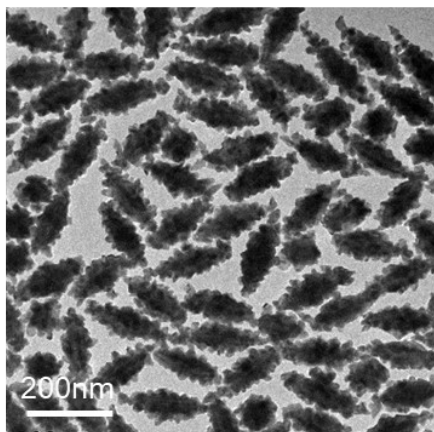


Figure S1. TEM images of biconical seeds (Au NBPs) with different lengths.

A



B

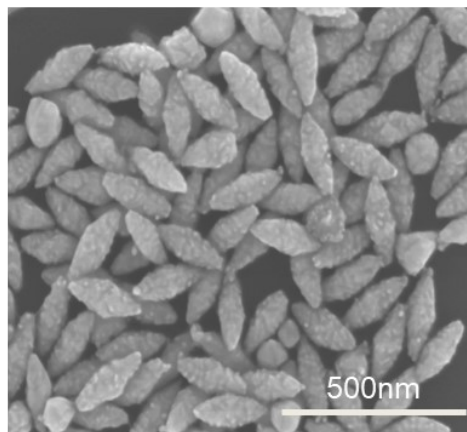


Figure S2. The (A) TEM and (B) SEM images of *L*-GBPs.

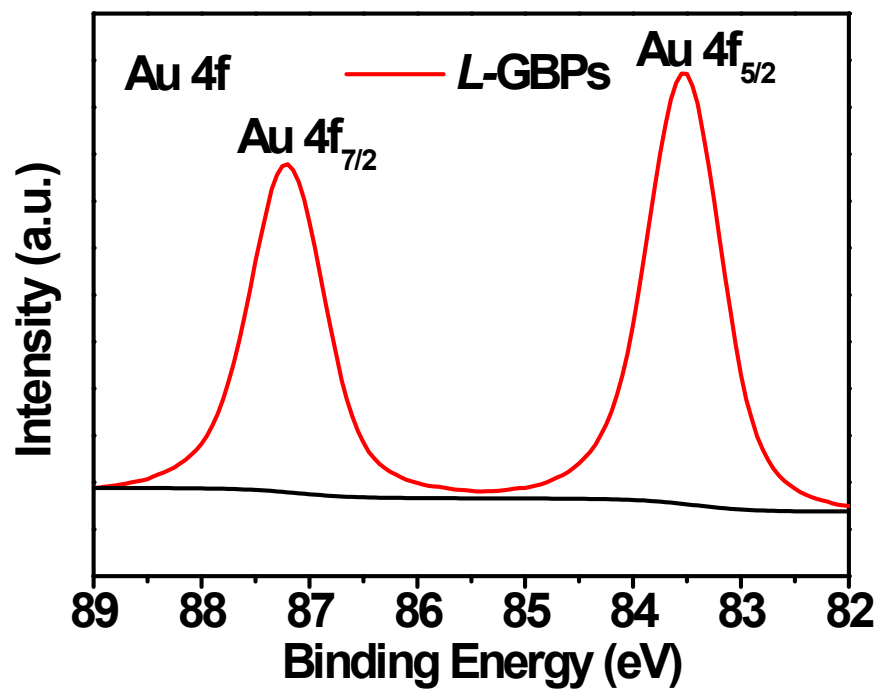


Figure S3. The XPS spectrum of *L*-GBPs.

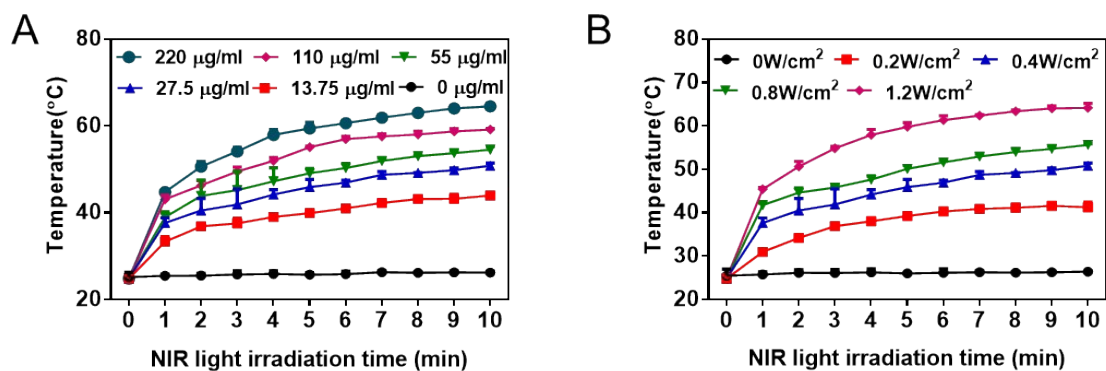


Figure S4. (A) The temperature of *D*-GBPs solutions with different concentrations increased under NIR irradiation (808 nm laser, 0.8 W/cm²). (B) Temperature evolution curve of *D*-GBPs solution under different intensity NIR irradiation. The concentration of *D*-GBPs solution is 55 µg/mL.

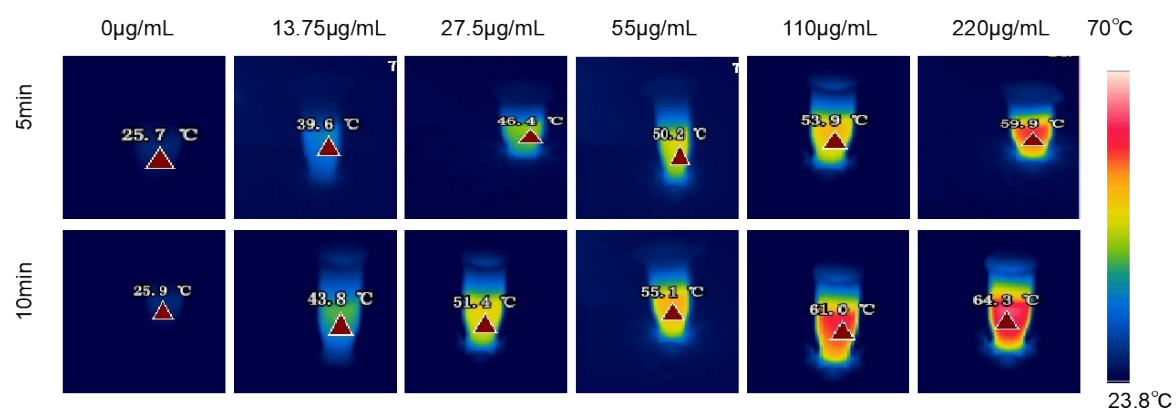


Figure S5. Thermal imaging images of *D*-GBPs solutions with different concentrations under NIR irradiation (808 nm laser, 0.8 W/cm²).

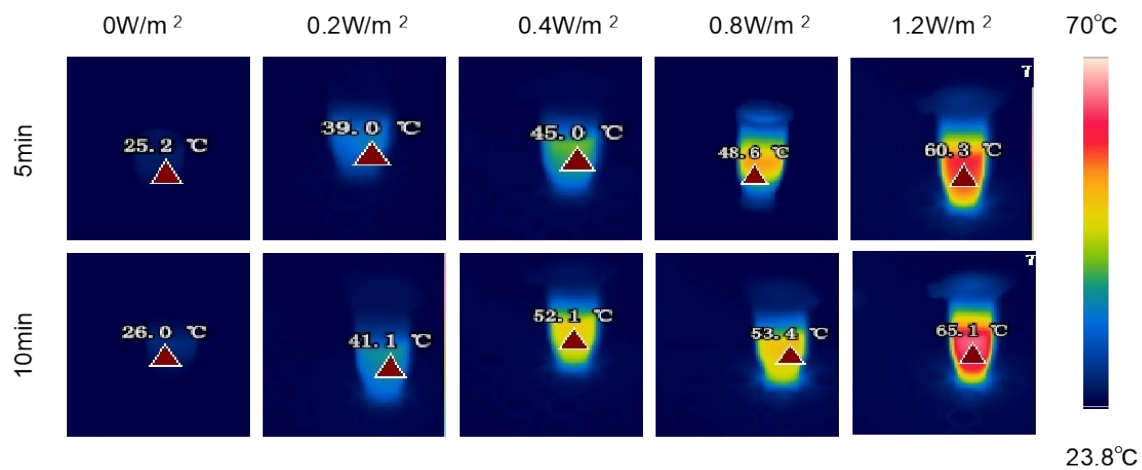


Figure S6. Thermal imaging images of *D*-GBPs solution under different intensity NIR irradiation. The concentration of *D*-GBPs solution is 55 $\mu\text{g/mL}$.

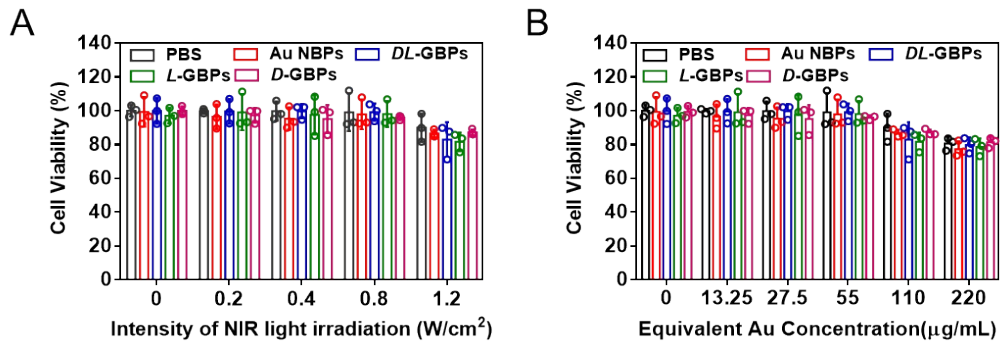


Figure S7. (A) Cell viability of primary uterine fibroblast cells treated with PBS and Au NBPs, *DL*-GBPs, *L*-GBPs and *D*-GBPs were irradiated with different intensities of NIR light. The concentration of Au NBPs, *DL*-GBPs, *L*-GBPs and *D*-GBPs solution was 55 μg/mL, and the irradiation interval was 10 minutes. (B) Cell viability of primary uterine fibroblast cells when treated with different concentrations of Au NBPs, *DL*-GBPs, *L*-GBPs and *D*-GBPs. The intensity and irradiation time interval of NIR light were 0.8 W/cm² and 10 minutes.

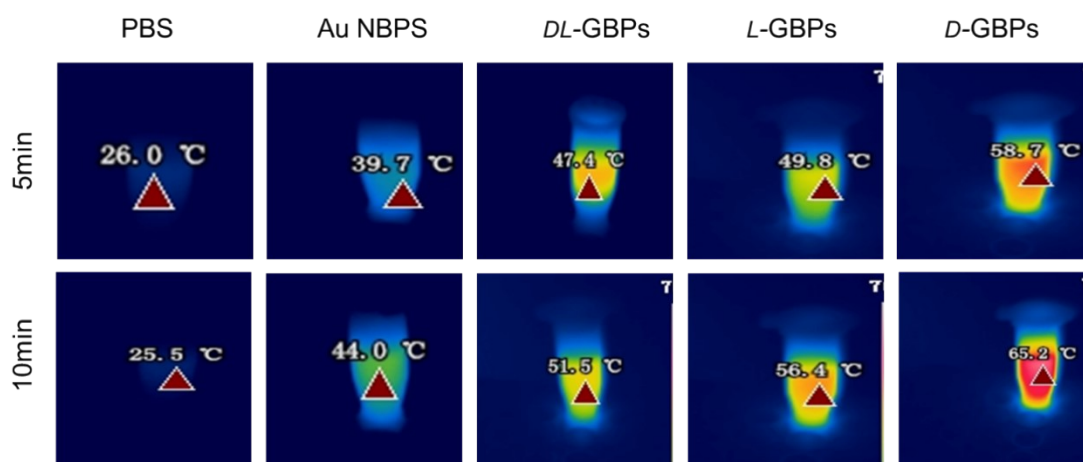


Figure S8. Thermal images of PBS, Au NBPs, *DL*-GBPs, *D*-GBPs and *L*-GBPs under 808nm laser irradiation (0.8 W/cm²).

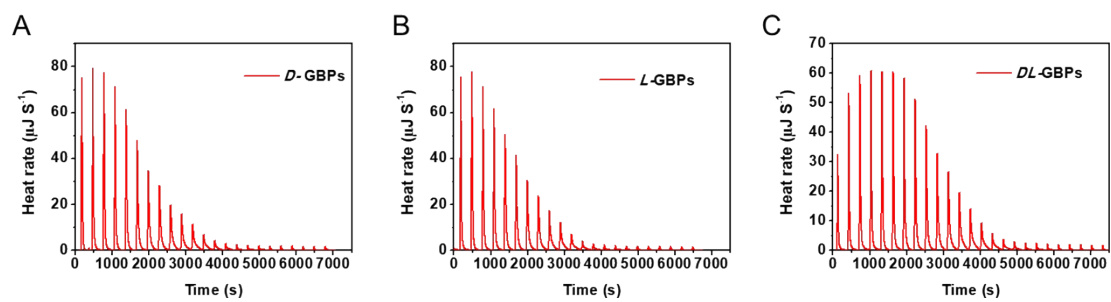


Figure S9. ITC data with respect to time for the titration of (A) D-GBPs, (B) L-GBPs and (C) DL-GBPs into protein A in the cell wall of *Staphylococcus aureus*.

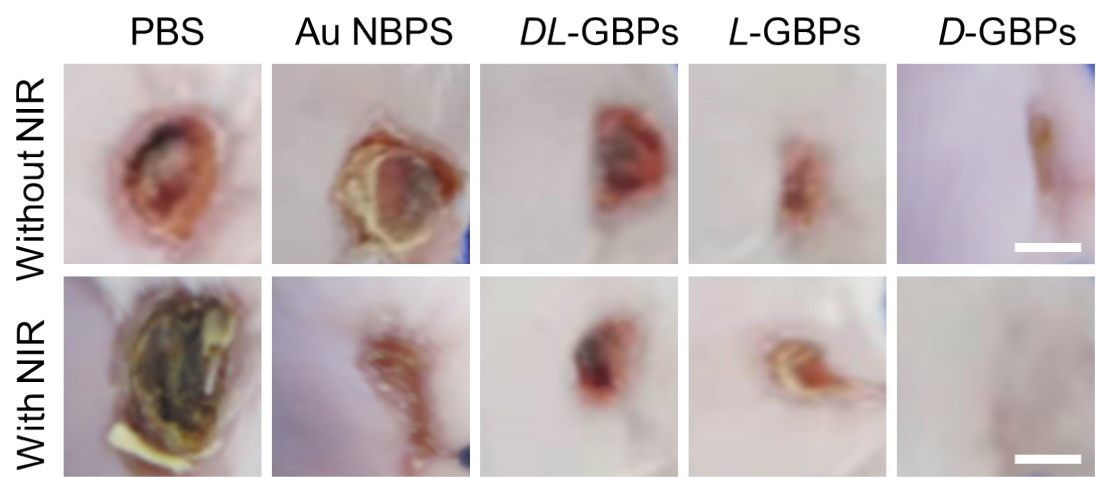


Figure S10. The images of *Staphylococcus aureus* infection sites on scarves after different treatments (scale: 20 μm).

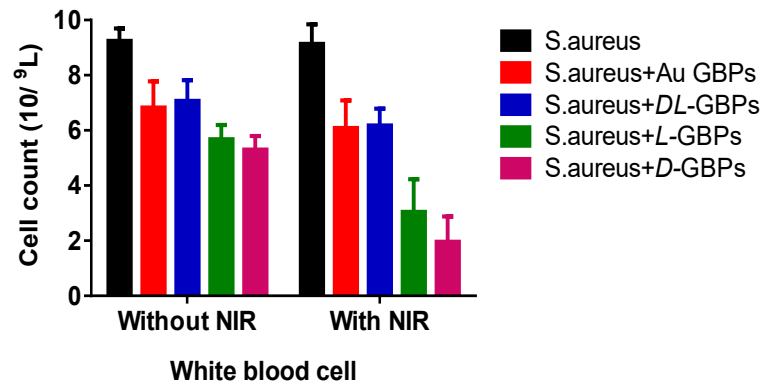


Figure S11. The number of white blood cells (WBC) in the mice sepsis infection model before and after different treatment.

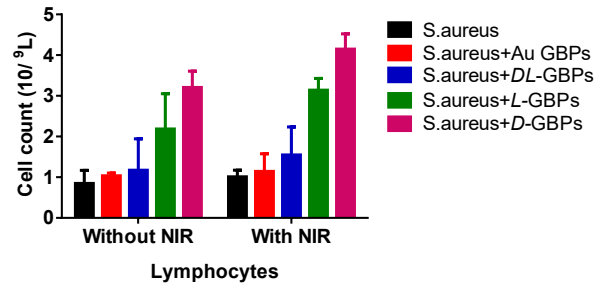


Figure S12. The number of lymphocytes (LYM) in the mice sepsis infection model before and after different treatment.

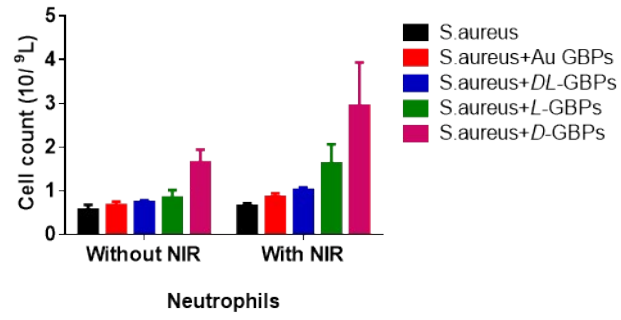


Figure S13. The number of neutrophils (NEU) in the mice sepsis infection model before and after different treatment.

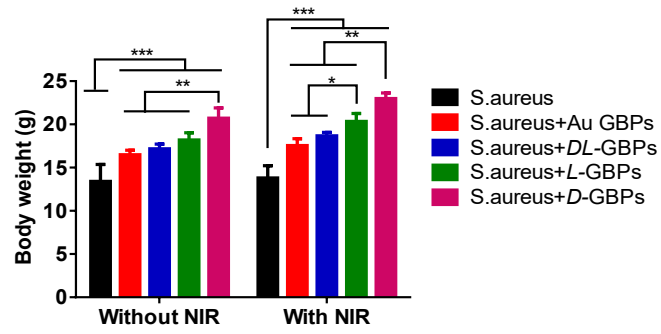


Figure S14. Changes of body weight in the mice sepsis infection model caused by PBS, Au NBPs, *DL*-GBPs, *D*-GBPs and *L*-GBPs. *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$, the statistical significance was determined by nonparametric one-way ANOVA.

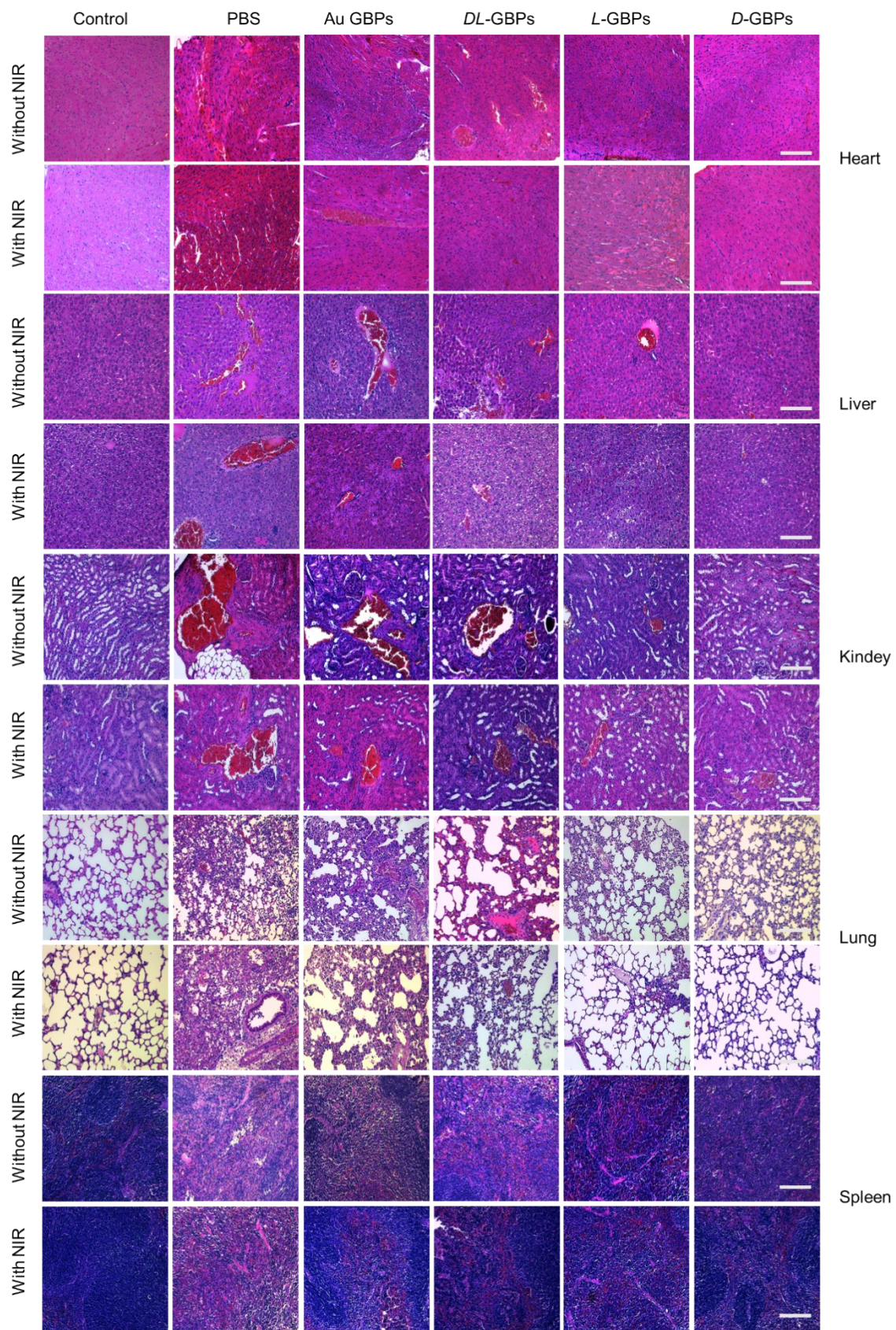


Figure S15. Histopathological changes of different organs before and after different treatment in sepsis model. Amplifier, by 400.