Electronic Supplementary Information for:

Phenylboronic acid functionalized gold nanoparticles for highly sensitive detection of *Staphylococcus aureus*

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1. Experimental Section

1.1. Chemicals

Hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$) was obtained from Sigma-Aldrich Co. (USA). Disulfide phenylboronic acid (dithiodialiphatic acid-3-aminophenylboronic acid (see inset of Figure S1), Bor, MW 448.1) was synthesized according to previously reported method[S1] and characterized by matrix-assisted laser desorption ionization time-of flight mass spectrometry (MALDI-TOF-MS) and Fourier-transform infrared spectroscopy (FT-IR) (see Figure S1 and S2). Peptide CALNN (purity 97%, measured by HPLC) was purchased from Scilight Biotechnology Ltd. Co. (Beijing, China). *Staphyloccocus aureus* Rosenbach (*S. aureus*) and correspondent practical samples were obtained from China-Japan Union Hospital of Jilin University (Jilin, China). *Escherichia coli* DH5R (*E. coli*) bacterial strain was purchased from Dingguo Ltd. (Beijing, China). *Enterobacter cloacae* (*E. cloacae*) and *Bacillus subtilis* (*B. subtilis*) bacterial strains were purchased

from China Center for Virus Culture Collection, the Institute of Microbiology, Chinese Academy of Sciences (Beijing, China). Baird-Parker agar base and egg-yolk tellurite emulsion were purchased from Qingdao Hope Bio-Technology Ltd. Co. (Qingdao, China). Fetal bovine serum (FBS) was obtained from Gibco Co. (USA), and pure cow milk and drinking water were purchased from a local Wal-Mart supermarket. All other used reagents were analytical grade. Milli-Q water (18.2 MΩ.cm) was used in all experiments. All the reactions were carried out at room temperature (RT, 25 °C) except specifically mentioned.

1.2. Instrumentations

The MALDI-TOF-MS analysis was done on a LDI-1700 laser desorption ionization time-of-flight mass spectrometer (American Linear Scientific Inc., USA) using a N_2 laser (337 nm). The FT-IR analysis was obtained on an IFS 66V/S vacuum FT-IR spectrometer (Bruker, Germany). Transmission electron microscopy (TEM) micrographs were obtained on a HITACHI H-600 transmission electron microscope (Hitachi, Japan) operating at an accelerating voltage of 100 kV. Dynamic light scattering (DLS) experiments were carried out on a Malvern Nano-ZS Zatasizer (Malvern, UK).

1.3. Synthesis and functionalization of gold nanoparticles (GNPs)

13 nm GNPs were prepared by reduction of $AuCl_4^-$ with citrate in aqueous solution according to the traditional Frens-Turkevich method.[S2,S3] The Bor-functional GNPs (Bor-GNPs) were prepared by following procedure: (1) 100 μL of Bor (0.25 mg/mL Bor in 0.2 M NaOH solution) was firstly added to 1 mL of GNPs (4.8 nM) solution

drop by drop, and incubated for 24 h; (2) certain amounts of CALNN were introduced to the mixture and given the final molar ratio of Bor to CALNN at 20:1, 15:1, 10:1 and 5:1 respectively; (3) the mixtures were incubated for another 24 h; and (4) the Bor-GNPs were purified by repeated centrifugation (9600 g, 3 times), redispersed in water, and stored at RT. The pure CALNN modified GNPs were synthesized according to our previous procedure.[S4,S5]

1.4. Bacteria culturing

All bacterial strains were cultured following manufacturers' culturing guidelines. Typically, *E. coli* and *S. aureus* strains were cultured in Luria-Bertani (LB) culture medium (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl). *B. subtilis* and *E. cloacae* strains were cultured in nutrient medium (10 g/L tryptone, 3 g/L beef extract, 5 g/L NaCl). After culturing overnight with shaking at 37 °C (*E. coli*, *S. aureus* and *E. cloacae*) or 30 °C (*B. subtilis*), all of these bacterial strains were centrifuged at 5000 g for 8 min, washed with phosphate buffered saline (PBS, pH 7.4), and then resuspended in PBS at a concentration about 10^9 cells/mL, respectively. In all experiments, the concentrations of bacteria were determined by optical density at 600 nm (OD_{600}, 1 OD_{600}=10^8 cells/mL).

1.5. Bacteria detection

To obtain the optimized detection conditions, the effects of pH value of buffer, incubation time, the concentration of Bor-GNPs were considered. For instance, (1) 100 μL of *S. aureus* (10^7 cells/mL) in various 20 mM HEPES buffer with different pH value (7.0, 7.5, 8.0, or 8.5) was incubated with 100 μL of 13 nm Bor-GNPs (7.2 nM)

for 1 h, respectively; (2) 100 μL of *S. aureus* (10^7 cells/mL) in HEPES buffer (pH 7.5) was incubated with 100 μL of 13 nm Bor-GNPs (7.2 nM) for 0.5, 1, 1.5, and 2 h, respectively; and (3) 100 μL of *S. aureus* (10^7 cells/mL) in 20 mM HEPES buffer (pH 7.5) was incubated with 100 μL of Bor-GNPs with different concentration (2.4, 4.8, 7.2 and 9.6 nM) for 1 h, respectively. After reaction with *S. aureus*, unbounded nanoparticles were removed by repeated centrifugation (5000 g, 8 min, 3 times), washed with 300 μL water (3 times), resuspended in 50 μL water and transferred to a 384-well microtiter plate for absorption spectra measurement, respectively. Absorption spectra were measured on a Power WaveTM XS2 microplate spectrophotometer (BioTek Instruments, Inc., USA). In this spectroscopic assay, corresponding reaction of *S. aureus* with CALNN modified GNPs were used as control experiment.

For *S. aureus* detection, a serial 10-fold dilutions range from 10^1 to 10^8 cells/mL were prepared by diluting *S. aureus* in 20 mM HEPES buffer (pH 7.5). Then 100 μL of 13 nm Bor-GNPs (7.2 nM) was mixed with 100 μL of *S.aureus* with different concentration (10^1, 10^2, 10^3, 10^4, 10^5, 10^6, 10^7 and 10^8 cells/mL), respectively. Following 1 h incubation with gentle shaking (130 rpm), the *S. aureus* were washed and detected as descried above. Because of nutritional deficiencies, the proliferation of bacteria in the HEPES buffer is very slow. Therefore, the changes of bacteria concentrations are in the error range of ±5%.

The limit of detection (LOD) is calculated by following equation[S6]:

$LOD=K_D S_b/q_1$

Here, $K_D=3$, S_b was the standard deviation of blank sample, q_1 was the slope of the

calibration line, respectively. In our experiment: calibration line is Y=-3.5+0.36X (R^2=0.996, inset of figure 1), the standard deviation of logarithm of maximum optical intensity of blank sample is 0.2 (n=20), that is meaning log (LOD) = 3 × 0.2/0.36 = 1.67. Therefore, the LOD of the assay is *ca.* 50.

1.6. Selective detection of S. aureus

100 μL of *S. aureus*, *E. coli*, *B. subtilis* and *E. cloacae* (in 20 mM HEPES buffer, pH 7.5) with a concentration of 10^7 cells/mL were mixed with 100 μL of 13 nm Bor-GNPs (7.2 nM), respectively. Following 1 h incubation at RT with gentle shaking (130 rpm), the bacteria were washed and detected as descried above.

For transmission electron microscopy (TEM) analysis, 7 μL of the nanoparticle stained bacteria solutions were dropped onto a 200 mesh copper grid (coated with carbon film), and dried at RT.

1.7. Real samples detection

For preparing real samples, aliquots of 1 mL of drinking water, 50% (wt/wt) FBS, 25% cow milk (wt/wt) and 25% human urine (wt/wt) were incubated with desired concentrations of *S. aureus* (10^2, 10^3 and 10^4 cells/mL), respectively. Following a 1 h incubation at RT with gentle shaking (130 rpm), bacteria were collected by centrifugation (5000 g, 9 min) and the supernatant were discarded. The samples were resuspended in 100 μL 20 mM HEPES buffer (pH 7.5) and detected by Bor-GNP-based assay as described above on bacteria detection.

For the lung fluid of methicillin-resistant *S. aureus* pulmonary infection patient: 3 mL fluid was collected from a male patient, which was divided to 12 equal portions and diluted (*Caution: highly infectious samples*). 6 of them (half of the samples were

spiked with 1×10^5 *S. aureus*) were measured by plate count method or Bor-GNP-based assay, respectively.

1.8. Baird-Parker agar plate count method

Baird-Parker agar plates were prepared following the manufacturers' description. Typically, 95 mL Baird-Parker agar solution were cooled down to 50 °C, then mixed with 5 mL egg-yolk tellurite emulsion to pour plate. The samples were prepared as described above on real samples detection section. Following 1 h incubation at RT with gentle shaking (130 rpm), the samples were centrifuged at 5000 g for 9 min, and resuspended in 1 mL PBS for Baird-Parker agar plate counting. Generally, 1 mL of bacterial sample was inoculated to 3 plates and spread evenly with 300 μL, 300 μL and 400 μL, respectively. After incubated for 24 h at 37 °C, the colonies on the plates were counted, and the total number of each sample was calculated. The processes were repeated three times in each case.

2. Additional Figures

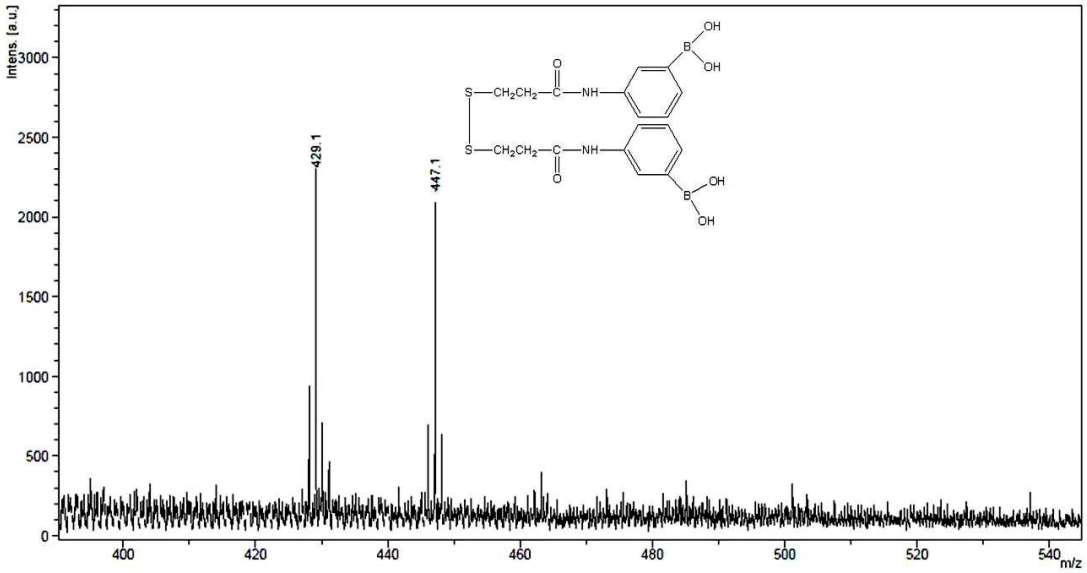

Fig. S1 MALDI-TOF mass spectrum of disulfide phenylboronic acid (Bor). The theoretically molecular weight of Bor is 448.1.

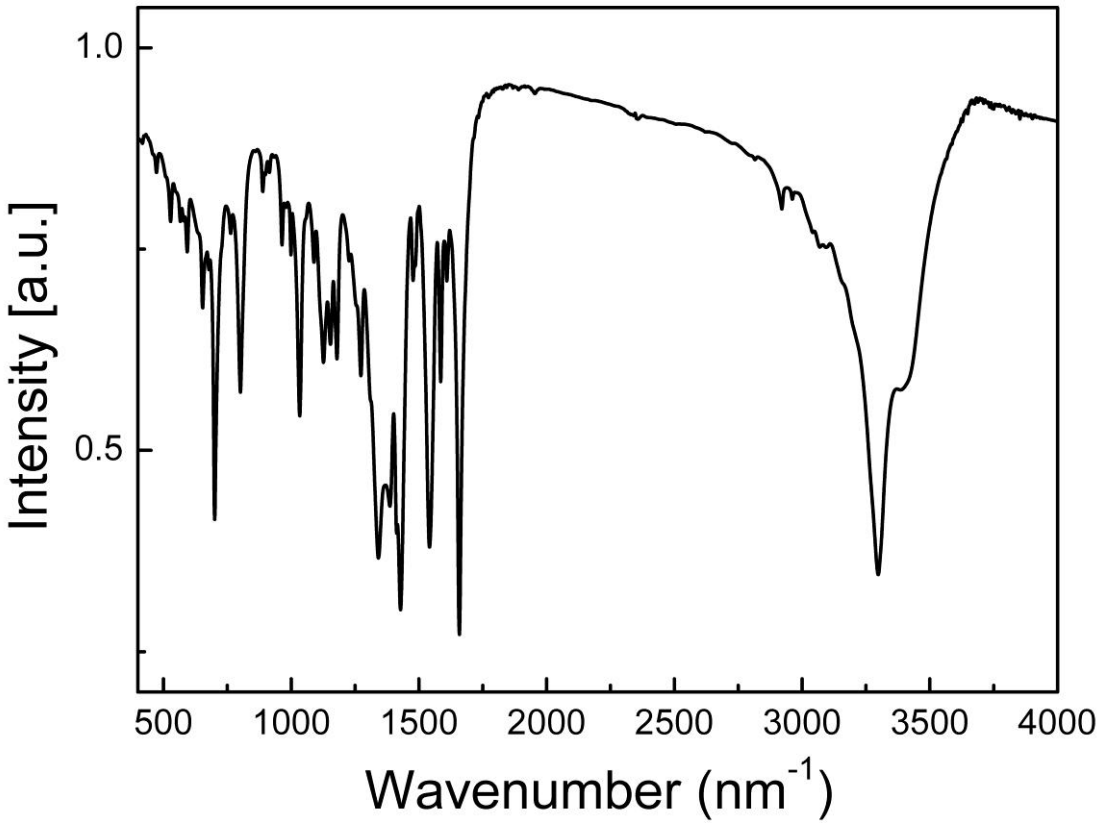

Fig. S2 FT-IR spectrum of disulfide phenylboronic acid (Bor).

The FT-IR spectrum is consistent with literature report.[S1]

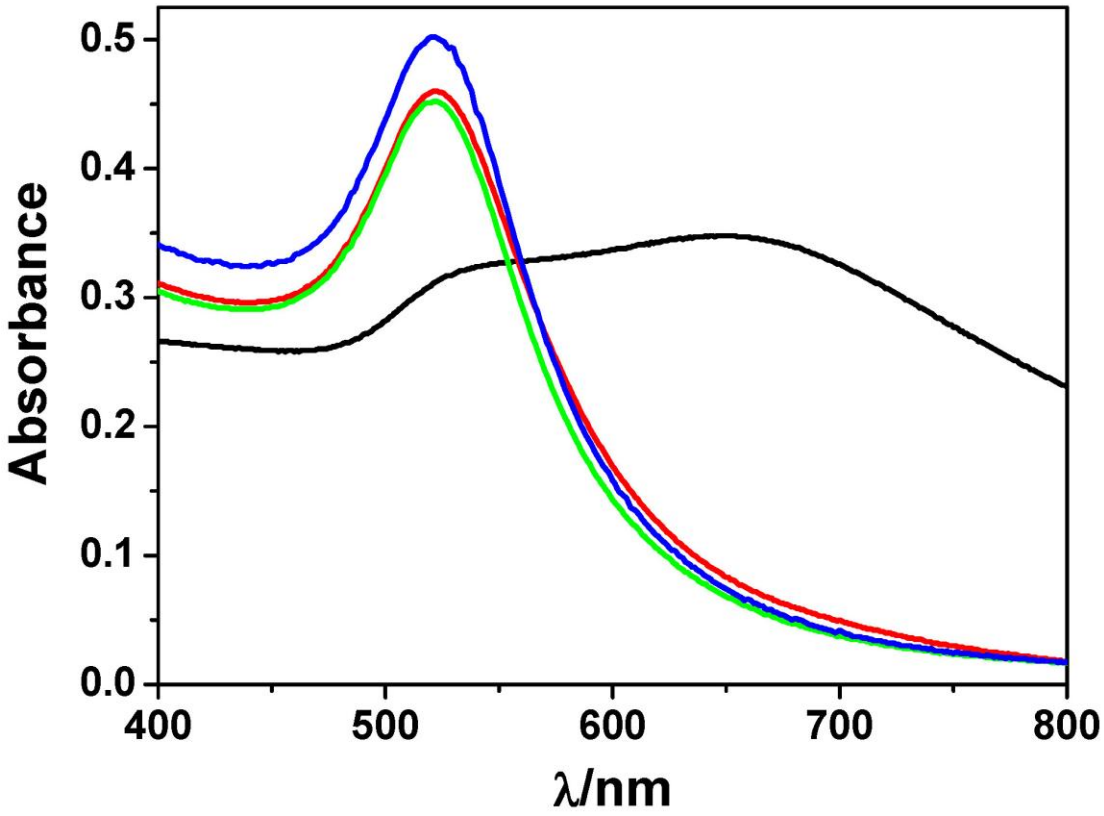

Fig. S3 UV-visible spectra of Bor-GNPs prepared at different molar ratio of Bor to CALNN (20:1 (black line), 15:1 (red line), 10:1 (green line), and 5:1 (blue line)) in 10 mM HEPES buffer (pH 7.5) with 0.15 M NaCl, respectively.

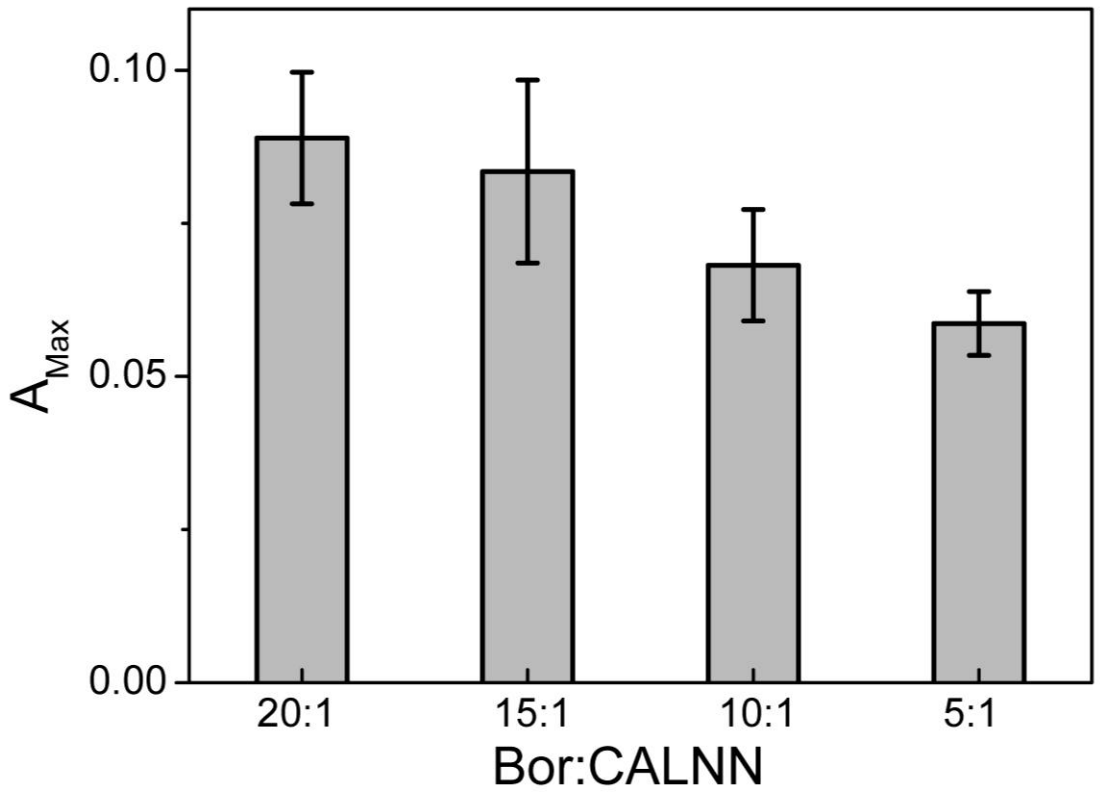

Fig. S4 Effect of molar ratio of Bor to CALNN on the reaction of Bor-GNPs with *S. aureus*. The concentration of *S. aureus* was 5×10^6 cells/mL in 10 mM HEPES buffer (pH 7.5). The concentration of Bor-GNPs was 3.6 nM and the reaction time was 1 h. The error bars mean standard deviations (n=3).

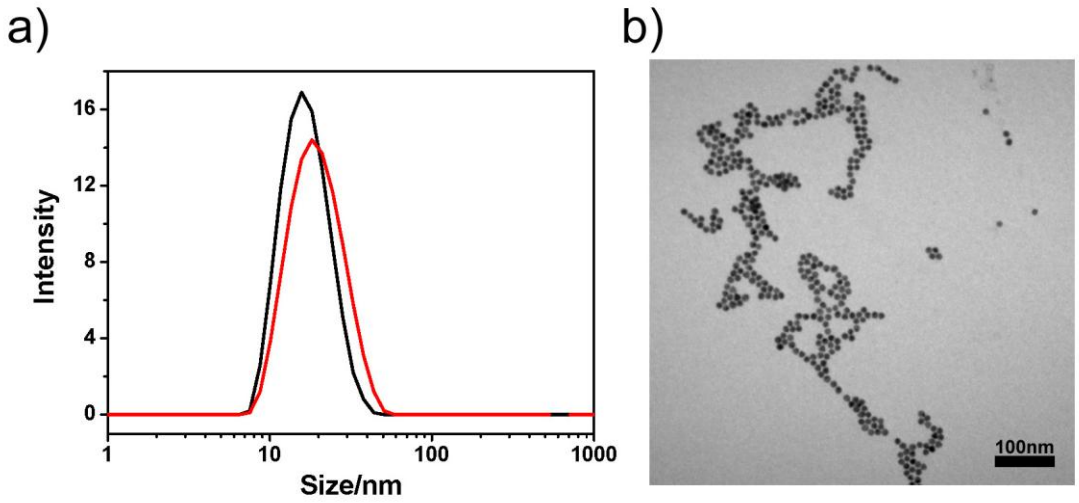

Fig. S5 a) DLS spectra of GNPs and Bor-GNPs, and b) TEM micrograph of Bor-GNPs.

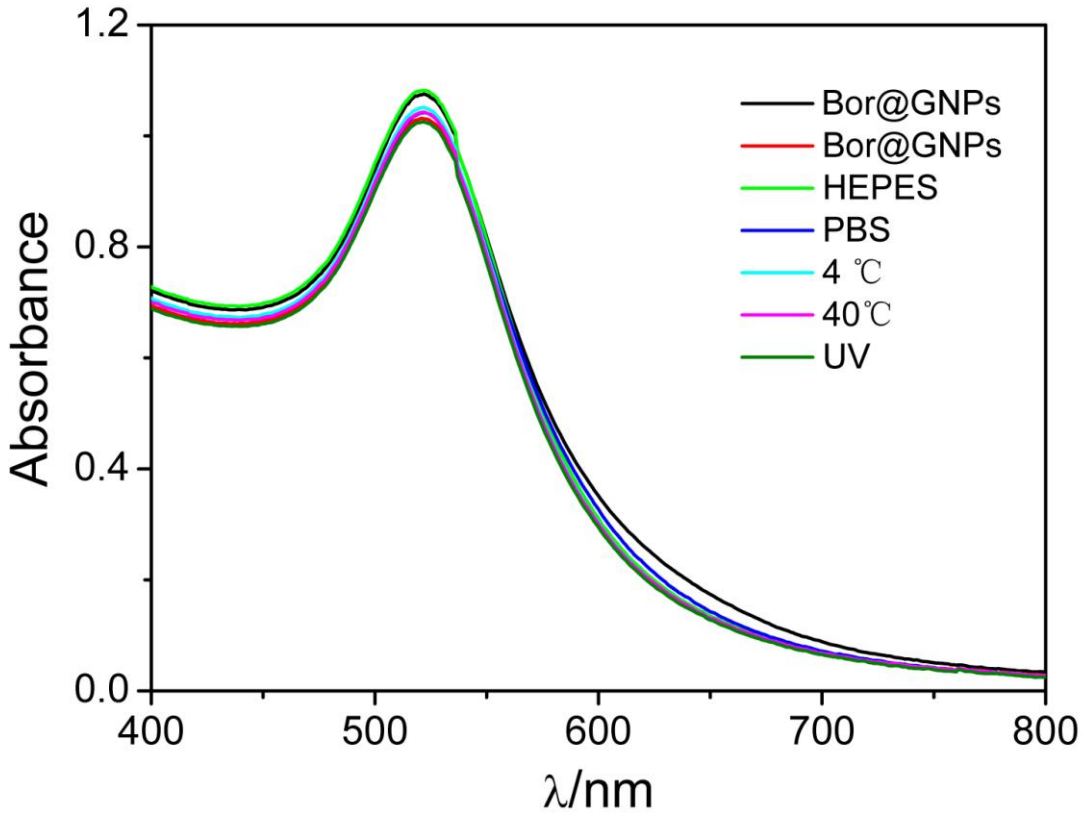

Fig. S6 UV-visible spectra of Bor-GNPs under different conditions: (1) fresh made in H_2O (black line), (2) stored at RT for a month in H_2O (red line), (3) in 10 mM HEPES buffer (pH 7.5) with 0.15 M NaCl (green line), (4) in PBS (10 mM PB plus 0.137 M NaCl, pH 7.5) (blue line), (5) stored at 4 °C for a month in 10 mM HEPES buffer (pH 7.5) (cyan line), (6) incubated at 40 °C for 1 h (magenta line) in 10 mM HEPES buffer (pH 7.5), and (7) irradiated by UV light (5 mW/cm^2) for 1 h (olive line) in 10 mM HEPES buffer (pH 7.5), respectively.

No significant UV-visible spectral changes of Bor-GNPs were observed. This experimental result suggests that the Bor-GNPs has good stability.

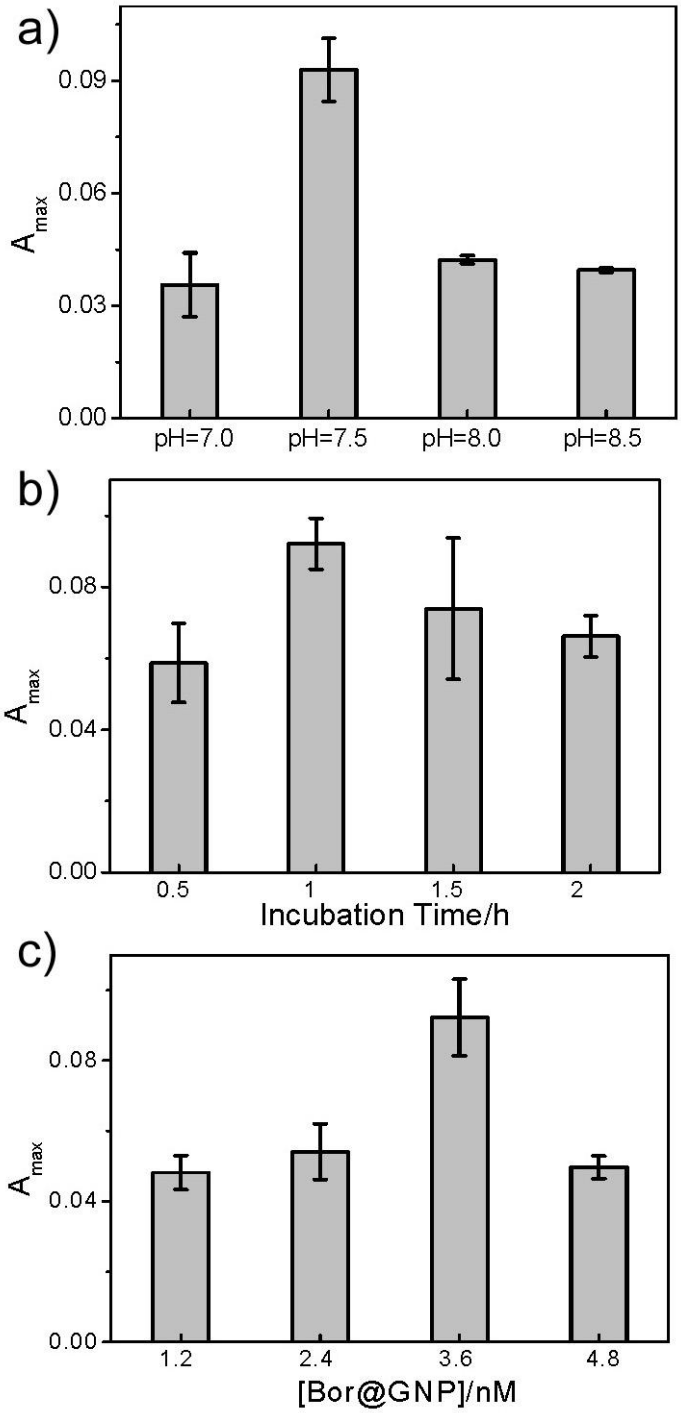

Fig. S7 The optimization results of detection conditions: the effects of a) pH value of buffer, b) incubation time, and c) the concentration of Bor-GNPs on the reaction of *S. aureus* with Bor-GNPs. The concentration of *S. aureus* was 5×10^6 cells/mL in 10 mM HEPES buffer. The error bars mean standard deviations (n=3).

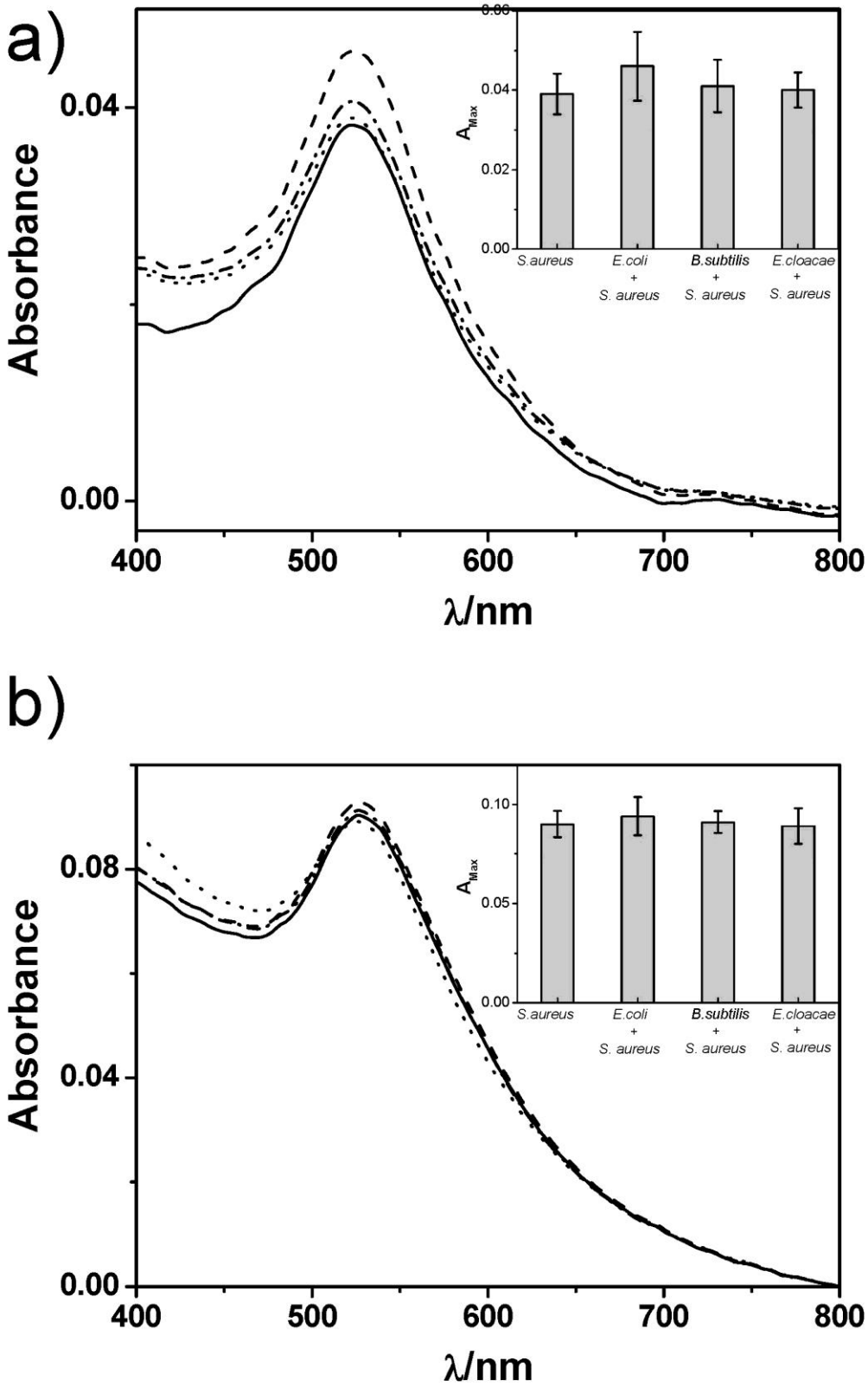

Fig. S8 UV-visible spectra of Bor-GNPs stained *S. aureus* (solid line), *E. coli* with *S. aureus* (dash line), *B. subtilis* with *S. aureus* (dash dot line) and *E. cloacae* with *S.*

aureus (dot line). The concentration of *S. aureus* was (a) 5×10^5 or (b) 5×10^6 cells/mL in 10 mM HEPES buffer (pH 7.5), and the concentration of the competitive bacterium was 5×10^6 cells/mL in 10 mM HEPES buffer (pH 7.5), respectively. The concentration of Bor-GNPs was 3.6 nM and the reaction time was 1 h. Inset shows the maximum optical intensity of Bor-GNPs stained bacteria ($\lambda\approx523$ nm). The error bars mean standard deviations (n=3).

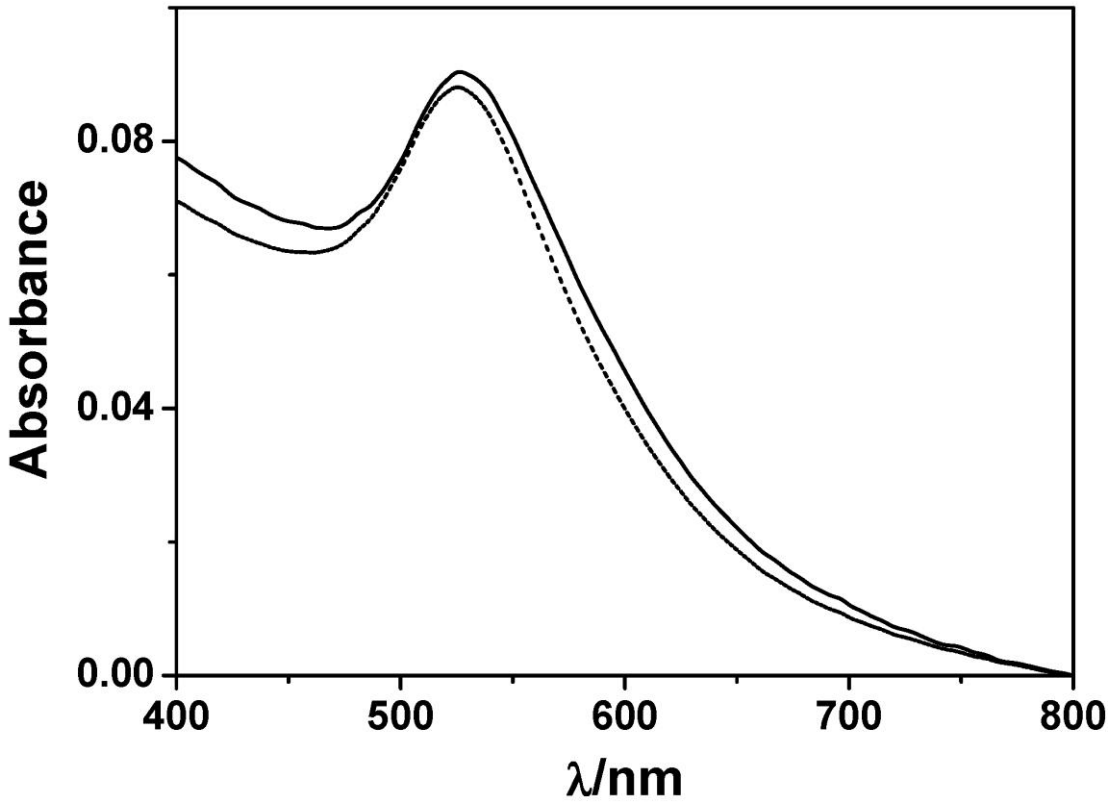

Fig. S9 UV-visible spectra of Bor-GNPs stained *S. aureus* (solid line) and vancomycin treated S. *aureus* (dash line). The concentration of *S. aureus* was 5×10^6 cells/mL in 10 mM HEPES buffer (pH 7.5). The concentration of Bor-GNPs was 3.6 nM and the reaction time was 1 h. The binding efficiency of Bor-GNPs with *S. aureus* can not be significant changed by the vancomycin treatment. The experimental result suggests that the antibiotic-resistant mutation of *S. aureus* might not affect on the assay result since vancomycin is one of main antibiotics for MRSA treatment.

3. Additional References

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