Supporting Information

Highly Sensitive Detection of *Staphylococcus aureus* by a THz Metamaterial Biosensor based on Gold Nanoparticles and Rolling Circle Amplification

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Experimental section

Reagents and materials

The HPLC-purified oligonucleotides presented in Table 1 were synthesized by Sangon Biotech (Shanghai, China). DNA ligase, DNA ligase buffer, QuickCut™ BamH I, QuickCut™ EcoR I, QuickCut™ Hind III, bovine serum albumin, exonuclease I, exonuclease III, exonuclease buffer, and TaKaRa MiniBEST Bacteria Genomic DNA Extraction Kit Ver. 3.0 were purchased from Takara (Dalian, China). Phi 29 DNA polymerase, Phi29 DNA polymerase buffer, and deoxyribonucleosides were obtained from Thermo Fisher Scientific (USA). AuNPs with an average diameter of 60 nm were obtained from Mengbio (Chongqing, China). Streptavidin-coated magnetic beads (MBs) with a diameter of 1 μm and the MBs de-hybridization solution (95% formamide, 10 mM EDTA, pH 8.2) were obtained from Dynal Biotech, Invitrogen (USA). The four clinical bacterial strains—E. coli, S. aureus, A. baumannii, and P. aeruginosa—were obtained from Southwest Hospital (Chongqing, China). The blood agar plates for the bacterial cultures were obtained from Pang Tong (Chongqing, China), and the positive photoresist (Microposit S1805) was from the Rohm and Haas Company (MA, USA). All chemical reagents were of analytical reagent grade. The high-purity deionized water and PBS buffer used in all experiments were obtained from Boster (Wuhan, China).

Preparation of capture probe-modified MBs

The streptavidin-coated MBs were washed three times with 1×binding and washing (B&W) buffer containing 10 mM of Tris-HCl (pH 7.5), 1 mM EDTA, and 2 M NaCl. The MBs were then re-suspended in 2×B&W buffer, adjusting the suspended MB concentration to 5 μg/μL. 50 μL of the biotin-coated capture probe (1 μM) was added to 50 μL of the MB suspension and incubated at room temperature for 15 min. Capture probes were immobilized on MBs by streptavidin-biotin interactions, and these complexes were then magnetically separated and washed three times with 1×B&W buffer. Finally, the capture probe-modified MBs were re-suspended in 50 μL of deionized water for downstream experiments.

Preparation of nano probe coated with AuNPs

A dry powder of the AuNP probe presented in Table 1 was dissolved in deionized water to form the initial solution (100 μM), and 5 μL of this solution was added to 80 μL of AuNP solution and mixed at 37 °C for 12 h. Then, 12 μL of PBS buffer and 23 μL of deionized water were added to the mixture and incubated overnight at room temperature to combine the AuNPs with the probe. The mixture was then centrifuged for 30 min at 12,000 rpm to remove the supernatant. After washing three times with PBS buffer, the nano probe was dissolved in PBS buffer and stored at 4 °C. The initial concentration of the nano probe was determined to be 10 nM by UV-vis absorption spectrometry.
**Fabrication of THz metamaterial**

The metamaterial manufacturing process mainly consisted of the following steps. (a) The surface of the silicon substrate (thickness of 470 μm, resistivity of 500 Ω·cm) was pretreated by anhydrous ethanol and distilled water, respectively, followed by drying with nitrogen gas flow. (b) A layer of Au film (thickness of 200 nm) was deposited onto the surface of the silicon substrate by magnetron sputtering. (c) A positive photoresist was spin-coated uniformly on the metal film followed by drying on a hotplate at 100 °C for 20 min. (d) Periodic structures were patterned on the silicon substrate with a conventional optical mask. (f) The Au film was wet-etched with a prepared iodine-potassium iodide solution (47 mM I₂, 0.24 M KI, pH 7.2) followed by washing with distilled water. (g) The residual photoresist was removed with acetone and distilled water, respectively.

![Image S1](image.png)

**Figure S1.** Image of the THz metamaterials. The detailed structural parameters were as follows: L=48 μm, W=2 μm, S=2 μm and G=3 μm.