Supporting Information

Hydrogen-producing Hyperthermophilic Bacteria Synthesized Size-controllable Fine Gold Nanoparticles with Excellence for Eradicating Biofilm and Antibacterial Applications

Experimental section

**Materials.** Hydrogen tetrachloroaurate (III) (HAuCl₄·3H₂O) were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). H₂O₂ was obtained from Beijing Chemicals (Beijing, China). 2,2’-Azinobis (3-ethylbenzthiazoline-6-sulfonate) (ABTS) and crystal violet were obtained from Aladdin Chemistry Co., Ltd. (Shanghai, China). E. coli (ATCC 25922) and S. aureus (ATCC 25923) bacterial strains were obtained from Chuanxiang Biotechnology, Ltd. (Shanghai, China). The type strain is CBS-ZT (=DSM 26941=T=CGMCC 1.5180T) was a kind gift provided by professor Zuoming Zhang, Jilin University. Ultra-pure water (18.2 MΩ; Millipore Co., USA) was used throughout the experiment. All reagents were used as received without any further purification.

**Measurements and characterizations.** Fluorescence measurements were measured using a JASCO FP-6500 spectrofluorometer (JASCO International Co., Japan). The UV/Visible absorption spectra were carried out on a JASCO V550 UV/Visible spectrophotometer (JASCO International Co., LTD., Tokyo, Japan), equipped with a Peltier temperature control accessory. Scanning electron microscope (SEM) images were recorded using a HITACHI S-4500 instrument. The SEM samples were prepared by depositing a dilute aqueous dispersion of the as-prepared samples onto a silicon wafer. The transmission electron microscopy (TEM) images were recorded using a FEI TECNAI G2 20 high resolution transmission electron microscope operating at 200 kV. X-ray diffraction (XRD) spectra were obtained from a D8 Focus diffractometer (Bruker) at a scanning rate of 0.2° min⁻¹ by using Cu-Kα radiation (λ=0.15406 nm). The operation voltage and current were kept at 40 kV and 40 mA. The X-ray photoelectron spectra (XPS) measurements were carried out using an ESCAlab220i-XL electron spectrometer from VG scientific using 300 W AlKα radiations.

![Figure S1. HRTEM images of representative images of individual (a)AuNPs-small, (b) AuNPs-moderate and (c) AuNPs-big.](image-url)
Figure S2. XPS analysis surveys of (a) B-AuNPs-small, (b) B-AuNPs-moderate and (c) B-AuNPs-big.

Figure S3. Wide-angle powder XRD pattern of B-AuNPs nanocomposites. The wide-angle X-ray diffraction pattern exhibits five peaks, which could be indexed as the (111), (200), (220), (311) and (222) reflections of the face centered cubic structure of crystalline Au.

Figure S4. Antibacterial activity studies of the B-AuNPs on both (a) E. coli and (b) S. aureus.
Figure S5. Typical photographs of A: (1) B-AuNPs in 0 M NaCl; (2) B-AuNPs in 0.1 M NaCl; (3) B-AuNPs in 0.5 M NaCl; (4) B-AuNPs in 1 M NaCl. B: TEM of B-AuNPs-small after 1 M NaCl treated. C: Particle size distributions of B-AuNPs-small after 1 M NaCl treated.
Figure S6. Typical photographs of A: (1) B-AuNPs in 0 M HCl; (2) B-AuNPs in 0.1 M HCl; (3) B-AuNPs in 0.5 M HCl; (4) B-AuNPs in 1 M HCl. B: TEM of B-AuNPs-small after 1 M HCl treated. C: Particle size distributions of B-AuNPs-small after 1 M HCl treated.

Figure S7. The peroxidase-like activities of the B-AuNPs after 1 M NaCl or 1 M HCl treated for 12 h.
Figure S8. Steady-state kinetic assay and catalytic mechanism of B-AuNPs. The velocity \( v \) of the reaction was measured using B-AuNPs (5 µg/mL, Au content) in 500 µL of 25 mM PB buffer pH 4.0 at 37 °C. Double-reciprocal plots of activity of (a) B-AuNPs-small, (b) B-AuNPs-moderate, c) B-AuNPs-big at a fixed concentration of one substrate versus different concentration of the second substrate for \( \text{H}_2\text{O}_2 \) or ABTS. Details were described in experimental section.
**Figure S9.** Survival rate of (a) *E. coli* and (b) *S. aureus* treated with H$_2$O$_2$ at different concentration with or without different size of B-AuNPs nanocomposites (5 μg/mL, Au content).

**Figure S10.** The plate samples showing colonies of (a) *E. coli*; (b) *E. coli* treated with B-AuNPs-big; (c) *E. coli* treated with B-AuNPs-moderate; (d) *E. coli* treated with B-AuNPs-small; (e) *E. coli* treated with H$_2$O$_2$; (f) *E. coli* treated with B-AuNPs-big and H$_2$O$_2$; (g) *E. coli* treated with B-AuNPs-moderate and H$_2$O$_2$; (h) *E. coli* treated with B-AuNPs-small and H$_2$O$_2$.

**Figure S11.** The plate samples showing colonies of (a) *S. aureus*; (b) *S. aureus* treated with B-AuNPs-big; (c) *S. aureus* treated with B-AuNPs-moderate; (d) *S. aureus* treated with B-AuNPs-small; (e) *S. aureus* treated with H$_2$O$_2$; (f) *S. aureus* treated with B-AuNPs-big and H$_2$O$_2$; (g) *S. aureus* treated with B-AuNPs-moderate and H$_2$O$_2$; (h) *S. aureus* treated with B-AuNPs-small and H$_2$O$_2$. 


Figure S12. Typical SEM images of (a) E. coli; (b) E. coli treated with B-AuNPs-big; (c) E. coli treated with B-AuNPs-moderate; (d) E. coli treated with B-AuNPs-small; (e) E. coli treated with H$_2$O$_2$; (f) E. coli treated with B-AuNPs-big and H$_2$O$_2$; (g) E. coli treated with B-AuNPs-moderate and H$_2$O$_2$; (h) E. coli treated with B-AuNPs-small and H$_2$O$_2$. The scale bars are 1 µm.

Figure S13. Typical SEM images of (a) S. aureus; (b) S. aureus treated with B-AuNPs-big; (c) S. aureus treated with B-AuNPs-moderate; (d) S. aureus treated with B-AuNPs-small; (e) S. aureus treated with H$_2$O$_2$; (f) S. aureus treated with B-AuNPs-big and H$_2$O$_2$; (g) S. aureus treated with B-AuNPs-moderate and H$_2$O$_2$; (h) S. aureus treated with B-AuNPs-small and H$_2$O$_2$. The scale bars are 500 nm.
Figure S14. Biofilm dispersal study of *S. aureus* after treatment with different size of B-AuNPs (50 μg/mL, Au content) and 1 mM H$_2$O$_2$. Under fluorescence microscopy green and red stains indicate viable and dead cells, respectively, scale bar = 25 μm.

Figure S15. Histologic evaluation of tissues from mice treated with normal saline (Control) and B-AuNPs (Treatment).

Figure S16. Time-dependent biodistribution of B-AuNPs after subcutaneous injection.
Figure S17. Immunogenic response of mice treated with normal saline and B-AuNPs.