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Electronic Supplementary Information

Modulating the Antibacterial Activity of Gold Nanoparticles by Balancing Their Monodispersity and Aggregation

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Experimental

Materials

L-thioproline (T, Mw = 133.169) and Boc-protected thioproline (B, Mw = 233.28) were from ACMEC biochemical (Shanghai Acmec Biochemical Co., Ltd). Triethylamine and sodium borohydride were from Aladdin (Shanghai Aladdin Biochemical Technology Co., Ltd). Tetrachloroauric acid ($HAuCl_4 \bullet 3H_2O$) and Tween 80 were from Sigma (USA). Polymyxin B, gentamicin, and ampicillin were from Yuanye (Shanghai Yuanye Biotechnology Co., Ltd). All other chemicals and solvents were reagent grade.

Preparation and characterization of TB-Au NPs

We prepared various T and B co-modified Au NPs through one-pot synthesis process. We added T aqueous solution (0.05 mM) with different volumes of B (0.05 mM, T_4B_1 (T:B=0.8 mL:0.2 mL), T_2B_1 (T:B=0.66 mL:0.33 mL), T_1B_1 (T:B=0.5 mL:0.5 mL), T_1B_2 (T:B=0.33 mL:0.66 mL), and T_1B_4 (T:B=0.2 mL:0.8 mL)). We mixed $HAuCl_4$ aqueous solution (120 mM, 0.4 mL), 50 μL triethylamine, 100 μL Tween 80, and sodium borohydride (80 mM, 1 mL) into the solution and kept the final mixture in 15 mL under stirring 1000 rpm for 2 h. We used a dialysis bag (8000-14000Da cut-off, Solarbio) to remove the unreacted chemicals in double-distilled water for 48 h. We prepared bare Au NPs by sodium citrate reduction, and then incubated the ligand (thioproline) with the bare Au NPs to form T-Au NPs by ligand exchange reaction. We obtained powders of the Au NPs by lyophilization, and then redispersed the powders in organic solvents for subsequent experiments. We used trifluoroacetic acid (TFA) or hydrochloric acid (HCl) to remove the Boc group. We dissolved TFA in dichloromethane (DCM) and added B-Au NPs into 25% TFA solution. The Boc protecting group can be removed at room temperature. We used HCl (37%) and ethyl acetate (EA) with a fixed ratio (1:2) and added B-Au NPs in the solution at room temperature for 30 min to remove the Boc group. We characterized the size and the Zeta potential of the Au NPs by a transmission electron microscopy (TEM, F30, FEI company, USA) and a Zetasizer Nano

ZS (Malvern Company, England) respectively. We tested the concentrations of the Au NPs by inductively coupled plasma analysis (ICP, iCAP 6300, Thermo Fisher Scientific, USA). We calculated the number of gold atoms and ligands using the reported methods. The number of gold atoms,

$$N_{Au} = (V_{NP} \times APF)/ V_{Au}, \quad (1)$$

where V_{NP} is the volume of a sphere Au NPs, V_{Au} is the volume of a gold atom, APF is atomic packing factor. The number of thiolate ligands,

$$Ns = N_{Au} \times X, \quad (2)$$

where X is the molar ratio of S to Au in NPs.

Antibacterial effects and cytotoxicity of TB-Au NPs

We evaluated the antibacterial effects of TB-Au NPs on different sensitive and multidrug-resistant (MDR) bacteria to observe minimal inhibitory concentration (MIC). We cultured *Pseudomonas aeruginosa* (*P. a*), MDR *P. a*, *Acinetobacter baumannii* (*A. b*), MDR *A. b*, *Escherichia coli* (*E. coli*), MDR *E. coli*, *Klebsiella Pneumoniae* (*K. p*), and MDR *K. p* in the Luria-Bertani (LB) medium with different concentration of TB-Au NPs at 37 ^{o}C in Corning 96-well plates. After 24 h of incubation, we tested the optical density at 600 nm to directly screen the MIC. We set polymyxin B, gentamicin, and ampicillin as positive controls and the bacterial solution without any addition as a negative control. We found T_1B_1-Au NPs have the lowest MIC among all the Au NPs, so we selected the T_1B_1-Au NPs for the following *in vitro* and *in vivo* experiments. To explore the antibacterial mechanism, we incubated bacteria with T_1B_1-Au NPs at 37 ^{o}C for 4 h and fixed the T_1B_1-Au NPs treated bacteria with 2.5% glutaraldehyde and dehydration by ethanol (30%, 50%, 70%, 80%, 90%, 95%, and 100% (V/V, in water) for scanning electron microscopy (SEM, Regulus8100, Hitachi company, Japan) characterization. We stained the T_1B_1-Au NPs-treated bacteria by 2% phosphotungstic acid for TEM imaging. Furthermore, we stained the cells with propidium iodide (PI) and

SYTO9 (L-13152, Invitrogen) to observe the permeability of cell wall in the presence of T_1B_1-Au NPs by confocal microscopy (Zeiss LSM 710, Germany).

The human aortic fibroblasts (HAF) cells were from Science Cell (6120) and human umbilical vein endothelial cells (HUVEC) were from American Type Culture Collection (PCS-100-010). We cultured HAF cells with T_1B_1-Au NPs in DMEM (10% fetal bovine serum and 1% penicillin-streptomycin) at 37 ^oC with 5% CO_2. After 24 h of incubation, we assessed the morphologies of the cells by inverted microscope (DMI 6000B, Leica, Germany). We incubated HUVECs (1000 cells per well) with different concentrations of T_1B_1-Au NPs to evaluate the cell viability by a cell counting kit (CCK8, Dojindo, Japan) on a 96-well culture plate. After the incubation, the cells in each well were rinsed thoroughly by culture media to remove the residue nanoparticles to ensure the accuracy of the data. After 24 h of incubation, we measured the absorbance at 450 nm by a microplate reader (Tecan infinite M200) and calculated using the following equation:

$$\text{Cell viability (\%)} = (OD_{T1B1}-OD_{blank})/(OD_{control}-OD_{blank}) \times 100\% , \quad (3)$$

where OD_{T1B1} is the absorbance of the cells treated with T_1B_1 (0 to 64 μg/mL), OD_{blank} is the absorbance of DMEM (negative control), and $OD_{control}$ is the absorbance of cells without any treatment.

We used Student's *t*-test to analyze the data. $P < 0.01$ was considered as significantly different. The data were from the mean of each treatment group (Mean ± SD, n=3).

Table S1 The antibacterial effect of T, B, and Au NPs.

MIC (μg mL^{-1})	*E.coli*	MDR *E.coli*
T	>128	>128
B	>128	>128
Au NPs	>128	>128

Table S2 Average diameter and Zeta potential of the A-Au NPs.

	Diameter (nm)	Zeta Potential (mV)
A-Au NPs	6.4±1.4	-27.4

Table S3 The antibacterial effect of A-Au NPs.

MIC (μg mL^{-1})	*E.coli*	MDR *E.coli*
A-Au NPs	>64	>64

Table S4 Average diameter and Zeta potential of the B-Au NPs and the deprotected Au NPs.

	Diameter (nm)	Zeta Potential (mV)
B-Au NPs	15.61	-7.32
B-Au NPs + HCl/CE	179.90	-29.65
B-Au NPs + TFA/DCM	435.70	-23.63

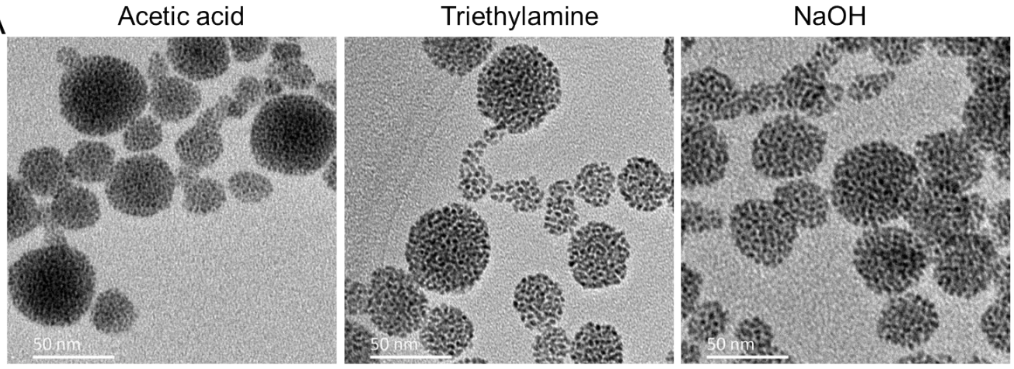
Table S5 The antibacterial effect of B-Au NPs after Boc deprotection by different methods.

MIC (μg mL^{-1})	*E.coli*	MDR *E.coli*
B-Au NPs	32	32
+HCl/CE	32	32
+TFA/DCM	64	64

Table S6 Components of the T-Au NPs, B-Au NPs and TB-Au NPs.

Materials	Molar ratio of sulfur to Au in NPs	Number of molecules modified on the Au NPs
T-Au NPs	0.79:1	12590
T_4B_1	0.81:1	9226
T_2B_1	0.46:1	3410
T_1B_1	0.20:1	603
T_1B_2	0.22:1	506
T_1B_4	0.26:1	284
B-Au NPs	0.24:1	362

A



B

	Triethylamine	Acetic acid	NaOH
Diameter (nm)	71.36±4.05	68.62±6.05	67.85±5.42
Zeta Potential (mV)	-26.2 ±1.87	-30.0±2.83	-27.2 ±1.93

Figure S1. Characterization of T-Au NPs synthesized in different solutions. (A) TEM images and (B) Zeta potential of T-Au NPs synthesized in different solutions.

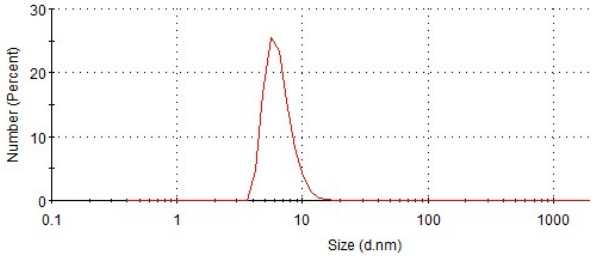


Figure S2. Characterization of A-Au NPs by DLS analysis.

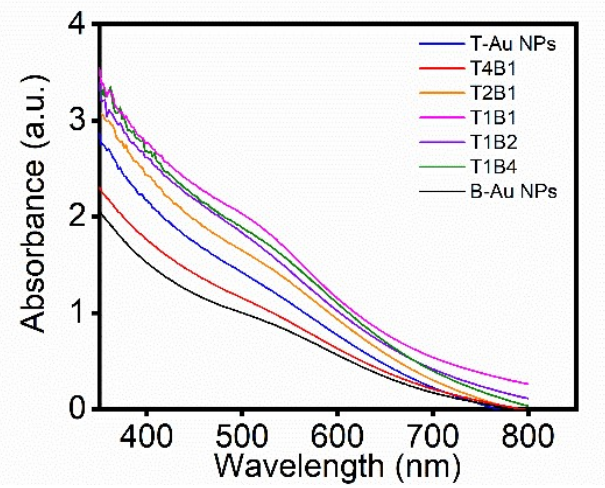


Figure S3. UV-visible spectra of T-Au NPs, B-Au NPs and TB-Au NPs of different T/B ratios.

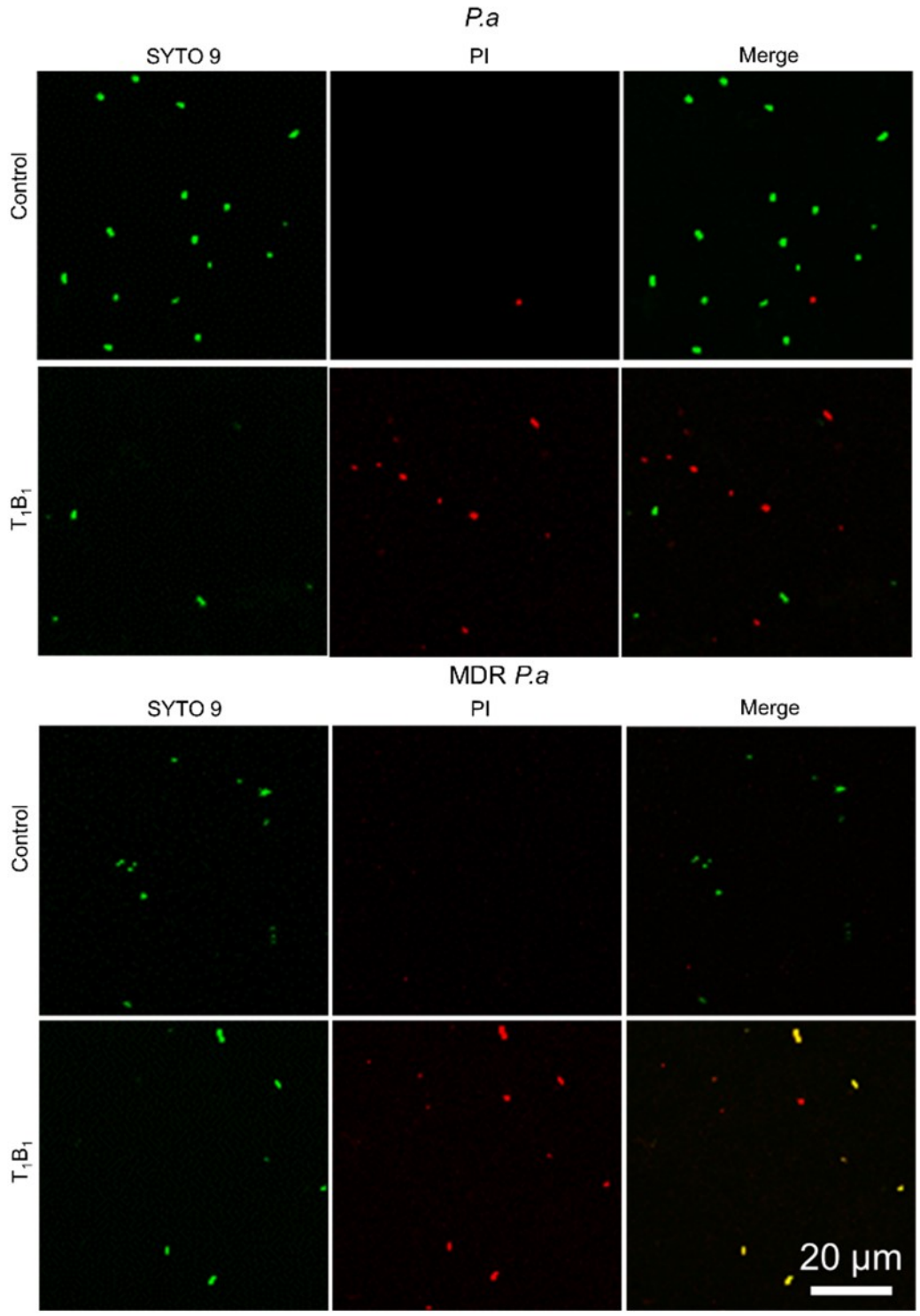


Figure S4. Confocal images of *P. a*- and MDR *P. a*-treated with T_1B_1-Au NPs by live/dead staining.

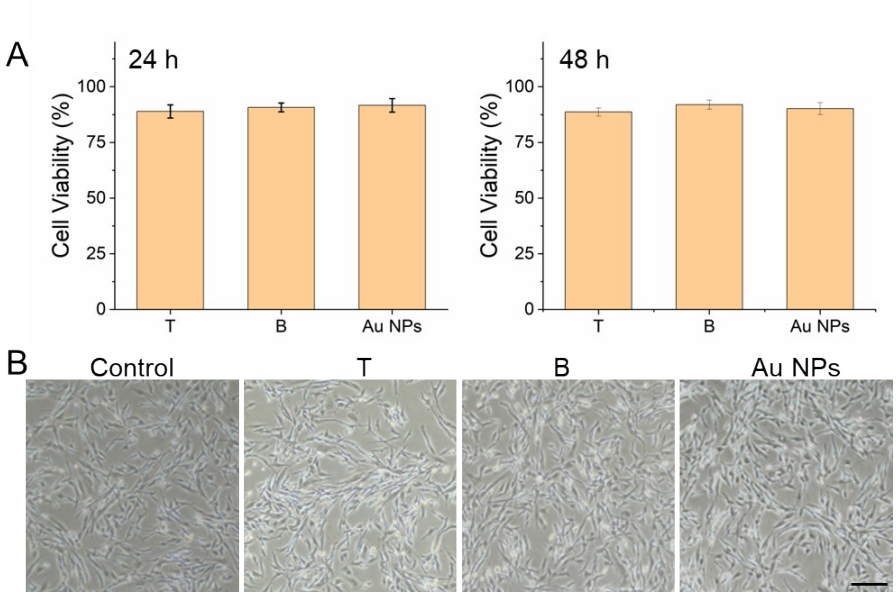


Figure S5. Biocompatibility evaluation of starting materials (thioproline, T; Boc-protected thioproline, B; and Au NPs) *in vitro*. (A) Cell viability of HUVECs under the treatment of different concentrations of T, B, and Au NPs. (B) Morphologies of HAF cells incubated with T, B and Au NPs for 48 h. Scale bar: 100 μm.