Human Serum Albumin Stabilized Gold Nanoclusters as Selective Luminescent Probes for *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus*

Po-Han Chan and Yu-Chie Chen*
Department of Applied Chemistry, National Chiao Tung University, Hsinchu 300, Taiwan

Supporting Information

Materials and Methods

All chemicals, including trypsin from bovine pancreas (TPCK treated), BSA, HSA, and α-cyano-4-hydroxycinnamic acid, were purchased from Sigma–Aldrich (St. Louis, MO). Hydrogen tetrachloroaurate(III) tetrahydrate was obtained from Showa (Tokyo, Japan). Trifluoroacetic acid was acquired from Alfa Aesar (Ward Hill, MA). Luria–Bertani broth and tryptic soy broth were obtained from Becton Dickinson (Fanklin Lakes, NJ). Yeast extract was purchased from Alpha Bioscience (Baltimore, MD). Amicon Ultra-4 centrifugal filters were purchased from Millipore (Billerica, MA). Sodium hydroxide was obtained from Riedel-de Haën (Seelze, Germany). Acetonitrile was acquired from Merck (Darmstadt, Germany). *Staphylococcus aureus*, *Streptococcus pyogenes* (GAS117), vancomycin-resistant *Enterococcus faecalis* (VRE), *Pseudomonas aeruginosa*, and pandrug-resistant *Acinetobacter baumannii* (PDRAB) were originally collected from Tzu-Chi Hospital (Hualien, Taiwan), and then provided by Prof. Pei-Jane Tsai (National Cheng-Kung University). *Methicillin-resistant S. aureus* (MRSA) (NCTC10442) was provided by Prof. Pei-Jane Tsai, *Escherichia coli J96* was provided by Prof. Hwei-Ling Peng (National Chiao Tung University), and *Enterobacter cloacae* (NCTC 10005) was obtained from the Institute of Food Science (Hsinchu, Taiwan). All bacteria were cultured based on a previously described standard method (*Nanomedicine*, **2010**, *5*, 755), and the preparation details of the medium are described elsewhere (*Nanomedicine*, **2010**, *5*, 755). Freshly harvested bacteria were used during the sensing

experiments. Bacterial culture should be performed in the hood with laminar flow. Care should be taken when conducting the experiments.

For the production of HSA-AuNCs, tetrachloroaurate(III) tetrahydrate (100 mM, 10 μL) and HSA (0.15 mM, 0.5 mL) were added to deionized water (0.48 mL). The resultant solution was vigorously stirred for 5 min, and the solution pH was adjusted to ~12 by adding aqueous sodium hydroxide (2 N, 10 μL). The mixture was then stirred for another 5 min at room temperature, and placed in a domestic microwave oven (power= 90 W) for 7 heating cycles (5 min/cycle). The mixture was cooled to room temperature between each heating cycle. During heating, the sample vial cap was kept loose. The resultant HSA-AuNCs were filtered from the solution by centrifugation for 8 min using Amicon ultra-4 centrifugal filters at 4500 rpm (rotor radius= 85 mm) to remove excess proteins. The isolated AuNCs were rinsed with deionized water (1 mL × 3), and these centrifugation and rinsing steps were repeated several times. The AuNCs were subsequently lyophilized for storage. Prior to experiments, HSA-AuNCs were dissolved in a given volume of solvent to prepare a sensing solution (1.2 mg/mL).

For bacterial sensing experiments, the generated HSA-AuNCs (1.2 mg/ mL, 100 μL) were added to a bacterial solution (OD_{600} = 1, 900 μL) and then vortex mixed for 1–2 h. After centrifugation at 3500 rpm for 5 min, the resulting solution was investigated under UV light (λ_{max} = 365 nm). The supernatant was analyzed by fluorescence spectroscopy. Trypsin digestion of the HSA-AuNCs was carried out by mixing trypsin (18.75 μg/mL, 500 μL) and HSA-AuNCs (1.2 mg/mL, 500 μL) in ammonium hydrogen carbonate buffer (pH 8), followed by microwave heating (power= 900 W) for 1 min. The tryptic digestion product of HSA-AuNCs without any further treatment was used as the probe solution for bacteria in tube II in Figure 3. The cleaved peptides from the tryptic digest of HSA-AuNCs were collected by centrifugation using an Amicon Ultra-4 centrifugal filter (cutoff mass= 3 kDa). The collected

peptide solution was used as the probes in the bacterial sensing experiments in tube III in Figure 3.

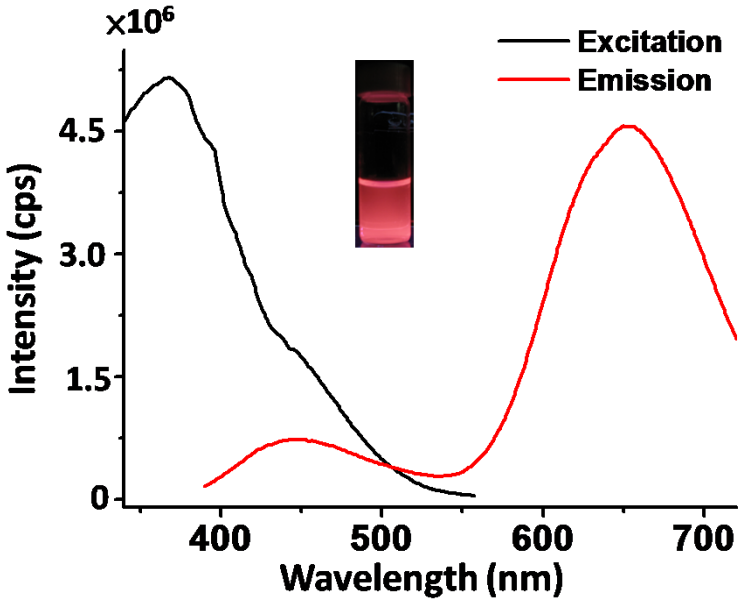

Figure S1. Emission (λ_{ex}= 368 nm) and excitation (λ_{em}= 655 nm) spectra of the HSA-AuNCs. The inset shows the photograph of the HSA-AuNCs under ultraviolet light (λ_{max} = 365 nm).

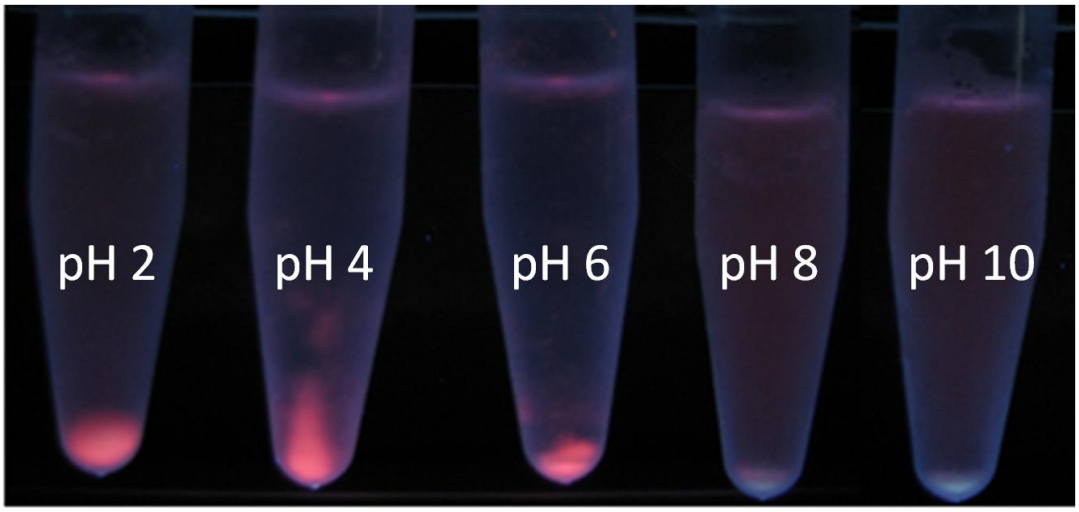

Figure S2. Photographs obtained after using HSA-AuNCs (0.12 mg/mL) as the sensing probes for the samples containing *S. aureus* (OD_{600}= 0.9, 1 mL) at pH 6. The precipitates were resuspended at pH 2, 4, 6, 8, and 10 (from left to right) followed by low-speed centrifugation.

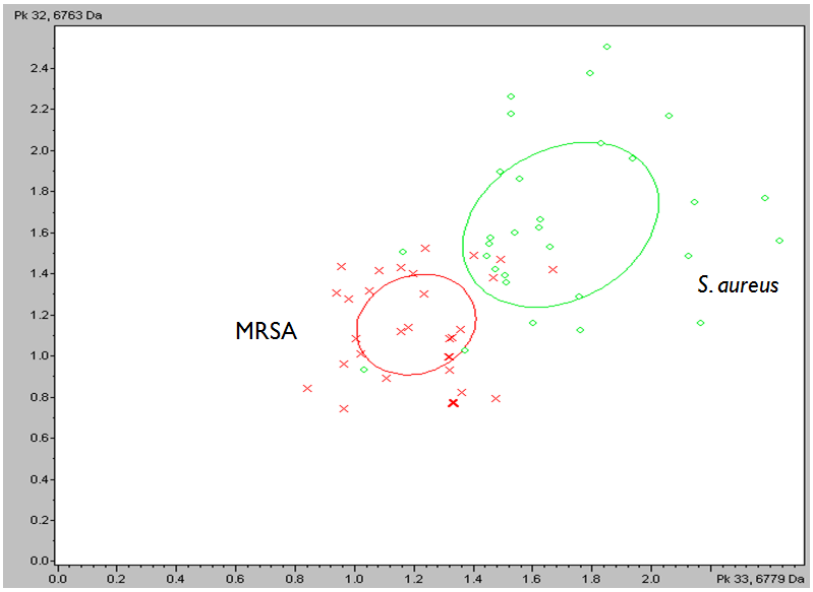

Figure S3. Principal component analysis plot obtained from the data treatment of MALDI spectra of *S. aureus* and MRSA.

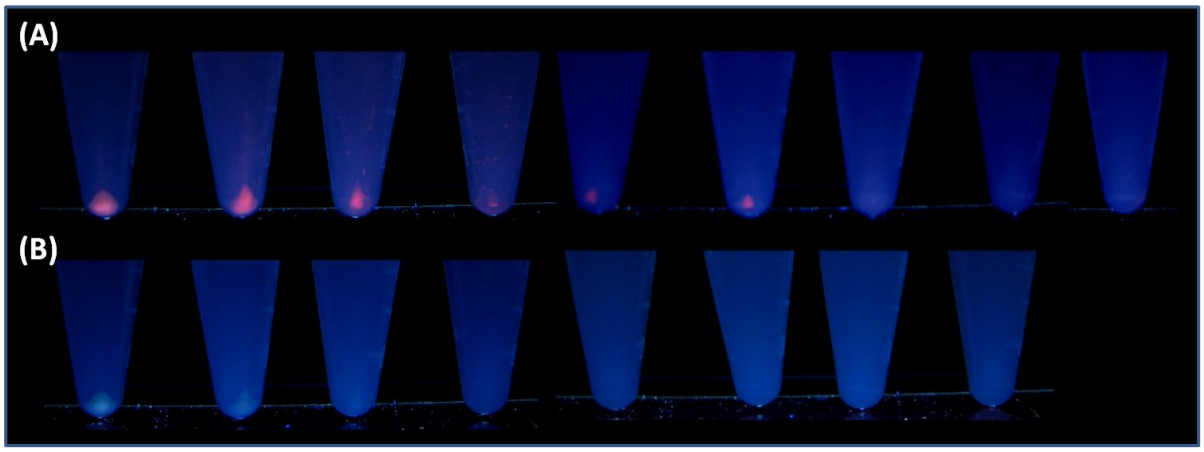

Figure S4. HSA-AuNCs were used as the sensing probes for *S. aureus* spiked in 10-fold diluted urine samples by PBS solution (pH 6). The precipitate in urine was removed before preparing the diluted urine sample. (A) Photograph obtained after vortex mixing the HSA-AuNCs (0.12 mg/mL) with the samples containing different concentrations of *S. aureus* for 1 h, followed by centrifugation at 3500 rpm for 5 min. The concentrations of *S. aureus* in the tubes (1 mL) from left to right were as follows (in cells/mL): 4.2×10^9, 2.1×10^9, 4.2×10^8, 8.4×10^7, 4.2×10^7, 8.4×10^6, 4.2×10^6, 8.4×10^5, and 0. (B) Photograph obtained from control samples by vortex mixing the same bacterial samples as used for panel A without HSA-AuNCs added for 1 h, followed by centrifugation at 3500 rpm for 5 min.

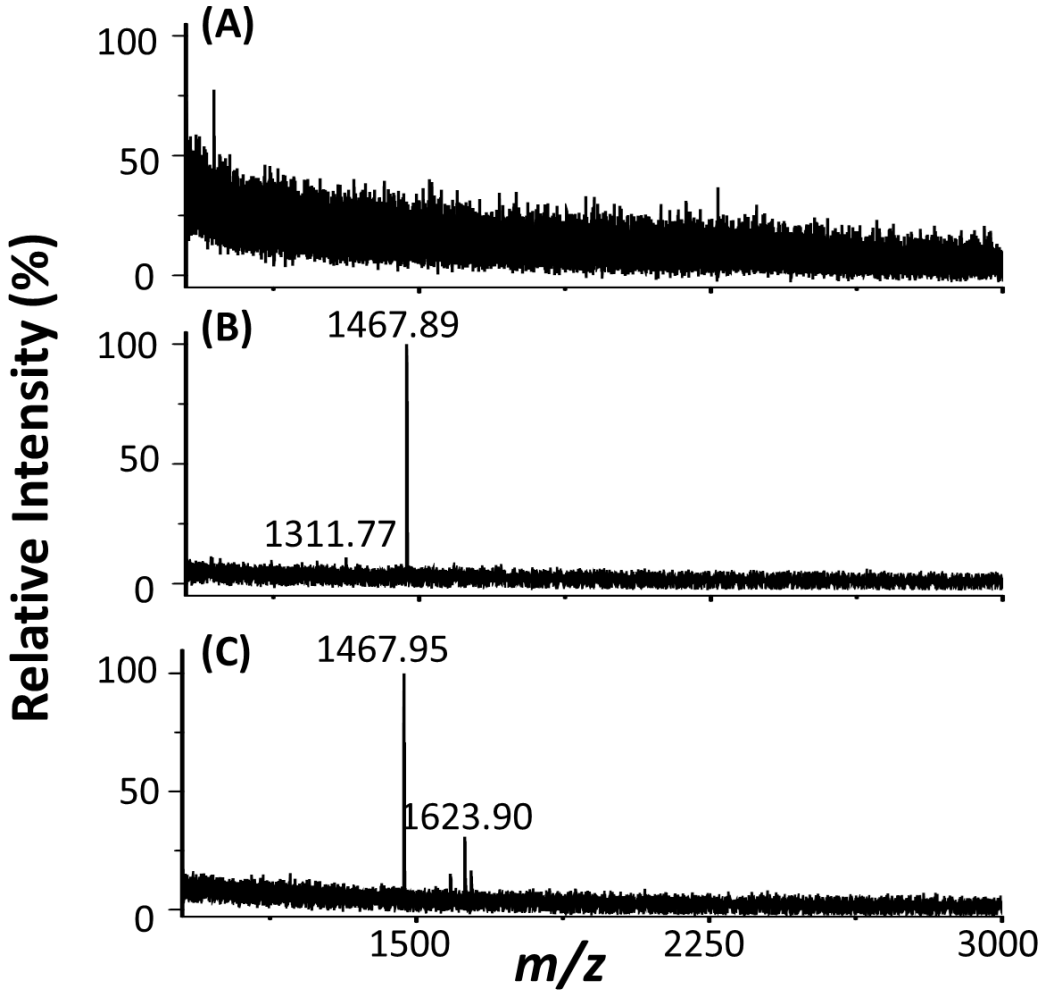

Figure S5. MALDI mass spectra obtained from the supernatants of (A) tube I, (B) tube II, and (C) tube III in Figure 3B. α-Cyano-4-hydroxycinnamic acid was used as the MALDI matrix.

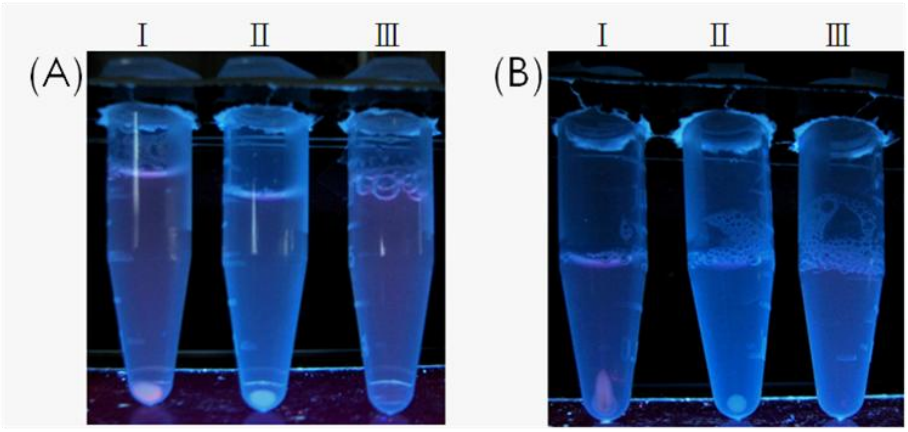

Figure S6. (A) Photograph obtained after using Pep10-AuNCs (1 mg/mL, 0.1 mL) as the sensing probes to trap target bacteria from a PBS solution (pH 6) containing *S. aureus* (OD_{600} = 1, 0.9 mL). Tube I contained the Pep10-AuNCs and *S. aureus*, which were vortex mixed for 2 h and centrifuged at 3500 rpm for 5 min. (B) Photograph obtained after using Pep16-AuNCs (1 mg/mL, 0.05 mL) as the sensing probes to trap target bacteria from a PBS solution (pH 6) containing *S. aureus* (OD_{600} = 1, 0.45 mL). Tube I contained the Pep16-AuNCs and *S. aureus*, which were vortex mixed for 2 h and centrifuged at 3500 rpm for 5 min. Tubes II and III contained *S. aureus* and Pep16-AuNCs, respectively, as control samples.

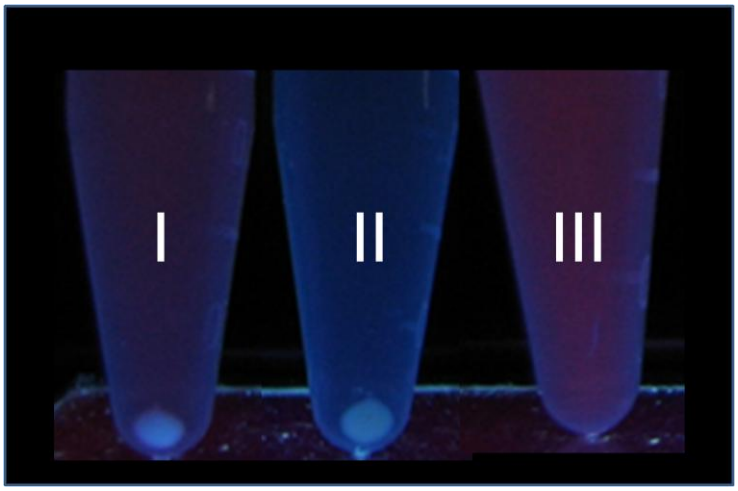

Figure S7. Photograph obtained after using BSA-AuNCs as the sensing probes for target bacteria from the sample containing *S. aureus* (tube I). Tubes II and III were the control samples containing *S. aureus* and BSA-AuNCs, respectively. All samples were vortex mixed for 2 h and centrifuged at 3500 rpm for 5 min.