Facile synthesis of silver-rich Au/Ag bimetallic nanoparticles

with highly active SERS property

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1. Experimental Sections

Materials and Reagents

Rabbit IgG, monoclonal Anti-rabbit IgG (Fc specific) antibody (designated as Ab1), atto-610 labeled polyclonal Anti-rabbit IgG (designated as Ab2), lipoic acid N-hydroxysuccinimide ester (LPA-NHS), sodium thiophenolate (TP), Poly(diallyldimethylammonium chloride) solution (PDDA, 20 wt.% in H_2O), tetrachloroauric(III) acid trihydrate ($HAuCl_4 \cdot 3H_2O$) and silver nitrate were purchased from Sigma-Aldrich. All other reagents were of analytical grade and used without further purification. Ultrapure water was obtained from a Millipore purification system ($\geq$18 MΩ, Milli-Q, Millipore). Phosphate-buffered saline (PBS; 0.01 M, pH 7.4) was used to dissolve Rabbit IgG and anti-Rabbit IgG, the washing buffer was the addition of 0.05% Tween-20 and 0.1% NaN_3 to PBS. The blocking buffer was an ethanolamine-tris buffer, which was prepared by dissolving 1.2 g of ethanolamine and 0.121 g of tris base in 20 mL of water and further adjusted pH to 8.4 using 1 M HCl solution.

Preparation of silver nanoparticles

Briefly, 36 mg of $AgNO_3$ was dissolved in 200 mL water, and the solution was heated to a slight boiling state (~95℃)in a flask equipped with stirring and reflux condensation device. Then, 4 mL 2% trisodium citrate solution (80 mg) was added rapidly, and the whole system was kept for 1 h at 90℃ under vigorous magnetic stirring to obtain a gray-green transparent solution. The concentration of the silver nanoparticles was estimated to be 5.5×10^{-11} M (given that the silver nanoparticles had a spherical-like shape with ~50 nm diameter, estimated from the TEM image)

Preparation of Au/Ag island films

Au/Ag island films were fabricated by electrodepositing silver islands on gold slides and then magnetron sputtering a gold layer with 10 nm thickness. Electrochemical deposition was performed in a thermostated cell at room temperature, using a conventional three-electrode system. The gold slide was the working electrode, a platinum and an Hg/Hg_2SO_4 electrode were used as the auxiliary and reference electrodes, respectively. Silver was deposited from a cyanide free plating bath (0.1 M $AgNO_3$ + 0.1 M EDTA + 0.05 M NH_4NO_3 and several milliliters of concentrated ammonia were added to ensure the pH value was 9–10). The silver islands were prepared using multi-current pulse plating with the first pulse to a current density of 30 mA cm^{-2} for 100 ms followed by a train of pulses of 5 mA cm^{-2} for 10 ms separated by a rest time of 1 s (zero current) [1].

Characterization and SERS measurement

Extinction spectra of the as-synthesized bimetallic nanoparticles were detected at a Shimadzu UV-3600 UV-vis-NIR spectrometer. TEM images were taken with a FEI-Tecnai G20 transmission electron microscope (FEI). HRTEM and high angle annular dark-field scanning TEM (HAADF-STEM) investigations were carried out using a FEI-Tecnai F20 (FEI) with 200 kV accelerating voltage. X-ray diffraction (XRD) patterns were recorded at a Bruker AXSD8 ADVANCE X-ray diffractometer (Cu Kα radiation,λ = 0.15406 nm) at room temperature. SERS spectra were measured on a Jobin Yvon/HORIBA HR500 Raman spectrometer equipped with an integral Olympus BX 41 confocal microscope with a 50× objective lens. The excitation sources included an air-cooled argon ion laser (532 nm, 20 mW) and a Diode Pumped Solid State Laser (785 nm, 100 mW), both were equipped with a holographic notch filter with a grating of 1200 lines/mm. Raman scattering was detected with 180 geometry using a multichannel air-cooled (-70℃) charge-coupled device (CCD) camera. For the detection of protein in this work, the laser power at the sample was ~1.5 mW, and the accumulation time was 30 s. For the SERS detection of TP, the accumulation time was 10 s.
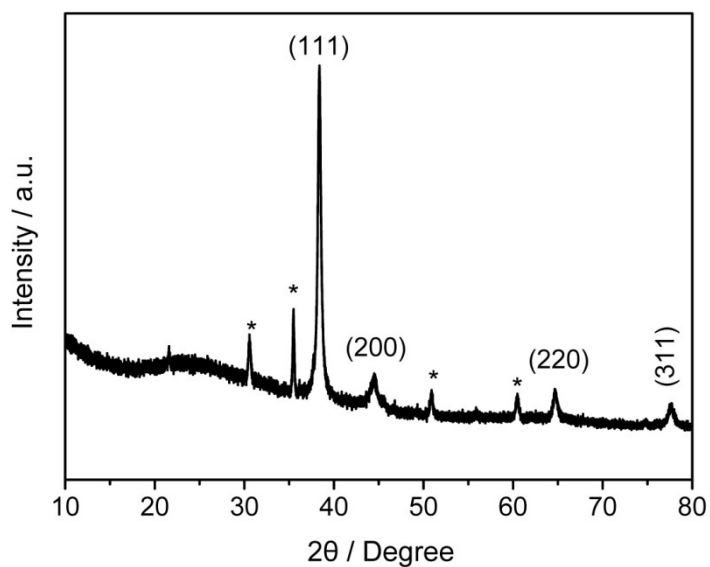
2. Figures and Descriptions

Figure S1 XRD pattern of the obtained BNPs, the peaks for ITO are marked with *
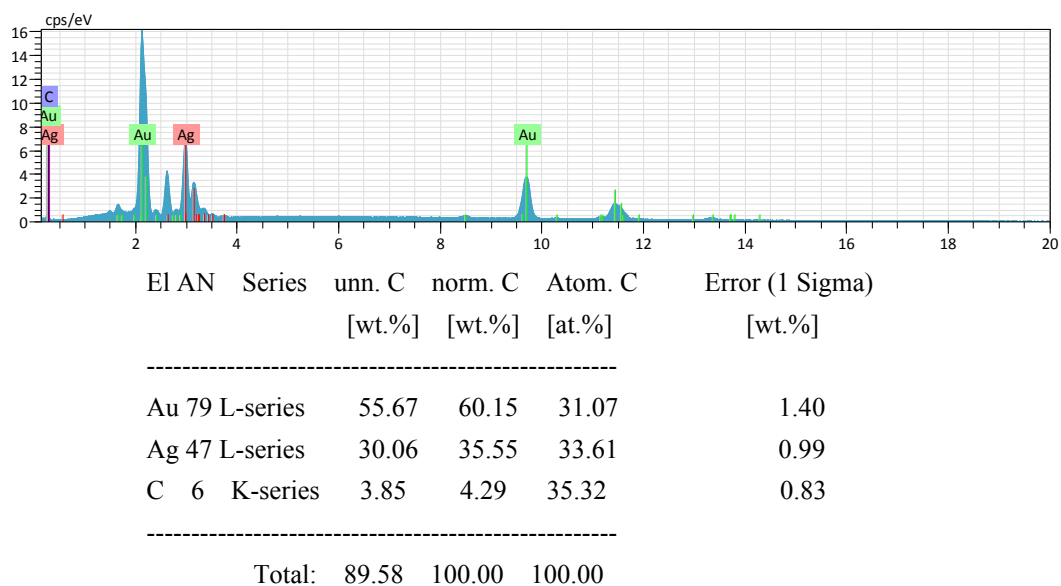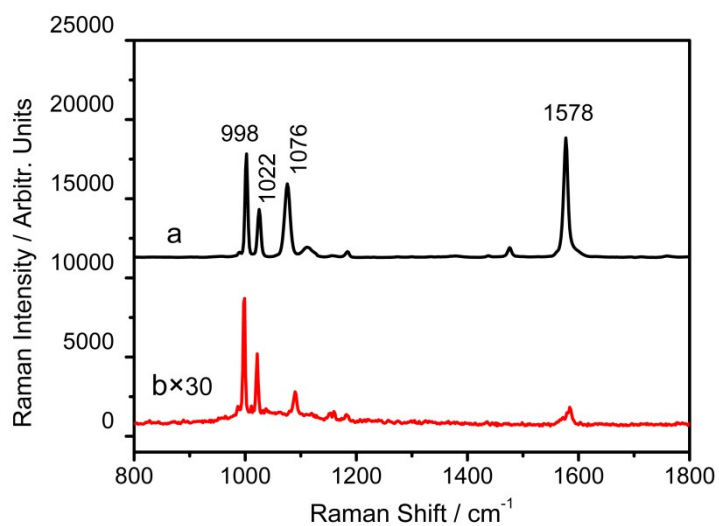

El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (1 Sigma) [wt.%]
Au	79	L-series	55.67	60.15	31.07	1.40
Ag	47	L-series	30.06	35.55	33.61	0.99
C	6	K-series	3.85	4.29	35.32	0.83
		Total:	89.58	100.00	100.00	

Figure S2 EDS analysis of the Au/Ag BNPs

Table S1   EDS analysis of the Au/Ag BNPs with different $HAuCl_4$ added

Volume of $HAuCl_4$ solution (mL)	Normalized atom percentage (at.%) In the presence of citrate		Normalized atom percentage (at.%) In the absence of citrate	
	Ag	Au	Ag	Au
20	89.6	10.4	76.5	23.5
40	74.3	25.7	58.4	42.6
60	65.8	34.2	10.7	89.3
80	54.2	45.8	2.5	97.5
100	49.6	50.4	/	/
120	32.8	67.2	/	/
140	10.2	89.8	/	/
180	5.1	94.9	/	/
200	1.7	98.3	/	/

EF calculation

Fig. S3 (a) SERS spectrum of TP obtained on the BNPs thin film, (b) normal Raman spectrum of solid TP

Calculation of ASEF values for the BNPs thin film

Enhancement factor (EF) of the BNPs thin film can be calculated using the following equation:[2]

$$EF = \frac{N_{bulk} I_{surf}}{N_{surf} I_{bulk}} \qquad (1)$$

where I_{surf} and I_{bulk} denote the intensities for the strongest band of the TP adsorbed on the surface of BNPs and the solid TP, respectively. N_{surf} and N_{bulk} represent the numbers of the corresponding surface and solid molecules effectively excited by the laser beam, respectively.

Calculation of N_{bulk}

The number of TP molecules excited in the bulk solid, N_{bulk}, can be calculated as following equation (2):

$$N_{bulk} = \frac{\pi \left(\frac{d_{spot}}{2}\right)^2 D \rho_{TP} N_A}{M_{r,TP}} \qquad (2)$$

where $d$spot is the diameter of circular laser spot, D is the depth of the incident laser beneath the surface of TP solid, ρ_{TP} and $M_{r,TP}$ are the density and molecular weight of TP, respectively, N_A represents the Avogadro constant. In this study, the laser spot was a circle with diameter of ~4 μm, and the depth the laser could reach was about 19 μm, the density (1.079 g/cm³) and molecular weight (132.16 g/mol) of solid TP. The calculated value of N_{bulk} equals to 1.9×10^{12}.

Calculation of N_{surf}

For the TP molecules adsorbed on the surface of BSSC thin films, assuming that the TP molecules are fully adsorbed and adopt a standing-up orientation on Ag surface, then the area occupied by one TP molecule is considered to be equal to the cross-sectional area of the molecule. The numbers of the TP molecules effectively excited by the laser beam on the surface of the BSSC thin films, N_{surf} could be calculated as following equation (3):

$$N_{surf} = \frac{\pi \left(\frac{d_{spot}}{2}\right)^2}{A_{cs,TP}} \qquad (3)$$

where d_{spot} has the same definition as equation (2), $A_{CS,TP}$ is the cross-sectional area of the TP molecule diameter of circular laser spot, which is ~0.20 nm². The calculated

value of N_{bulk} equals to 1.71×10^8.

EF calculation

A baseline correction was conducted for EF value calculation at each spectrum, and Raman intensity at 1578 cm^{-1} was used to calculate the EF. The I_{surf} and I_{bulk} at 1573 cm^{-1} were 7572 and 42. Thus, the EF was calculated to be 2.3×10^6.
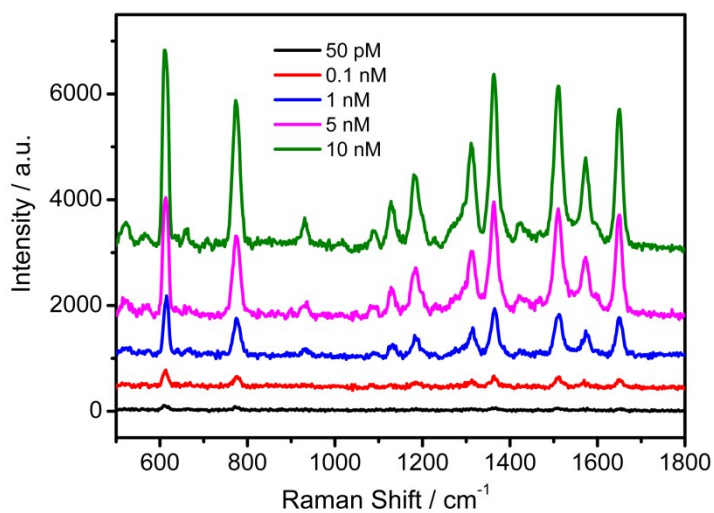

Figure S4 Raman Spectra of increasing concentrations of R6G on the BNPs (100 mL HAuCl$_4$ added) at 785 nm laser excitation
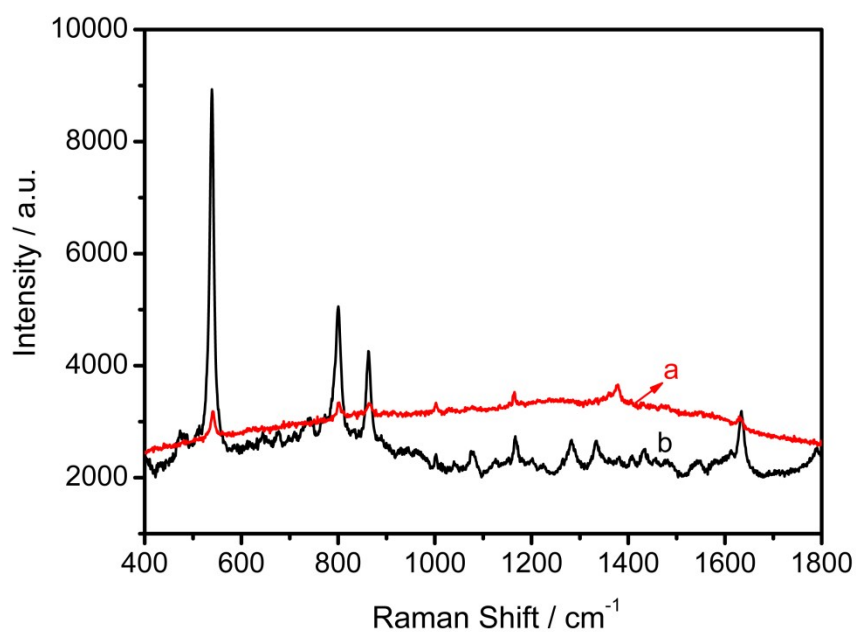
Figure S5 SERS spectra of Atto-610 labeled immunocomplex on Au thin film substrate (a) with and (b) without gold enhancement. The concentration of rabbit IgG is 10 ng·L^{-1}
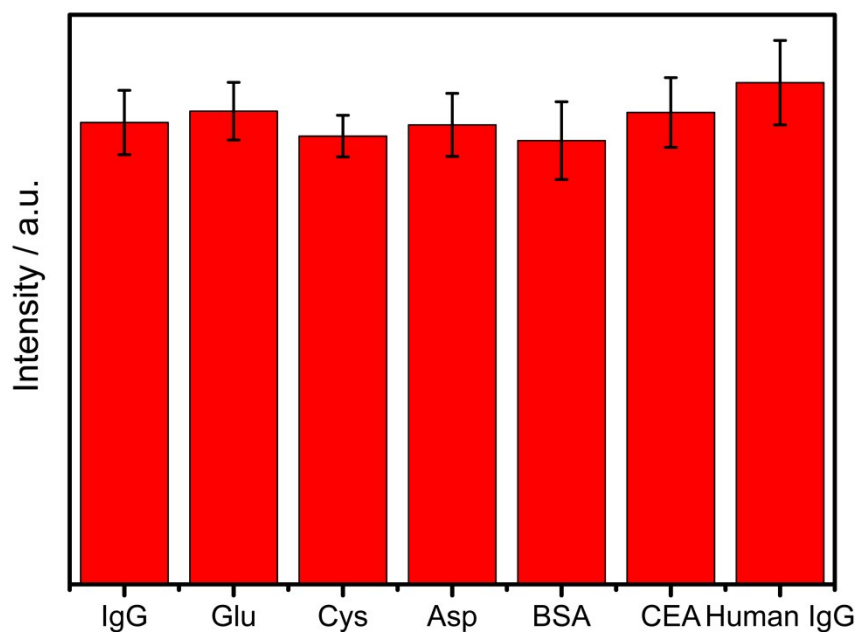

Figure S6 Specificity of the SERS-based immunosensor examined by addition of several interferents to 10 ng·L^{-1} rabbit IgG. Each error bar represents the standard

deviation with n=5.

References

[1] X. Gu, Y. Yan, G. Jiang, J. Adkins, J. Shi, G. Jiang, S. Tian, Using a silver-enhanced microarray sandwich structure to improve SERS sensitivity for protein detection, Anal. Bioanal. Chem., 406 (2014) 1885-1894.

[2] X.-M. Lin, Y. Cui, Y.-H. Xu, B. Ren, Z.-Q. Tian, Surface-enhanced Raman spectroscopy: substrate-related issues, Anal. Bioanal. Chem., 394 (2009) 1729-1745.