Microwave-Assisted Synthesis of BSA-Protected Small Gold Nanoclusters and Their Fluorescence-Enhanced Sensing of Silver(I) Ions

(Supporting Information)

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All chemicals are obtained from commercial suppliers and used without further purification. The molecular weights of the BSA and AuNCs@BSA are determined by using an Autoflex III MALDI-TOF mass spectrometer (Sinapinic acid is used as the matrix for MALDI and tetrafluoroacetic acid is added to enhance the ionization). The elemental analysis is performed by using an ELAN 9000/DRC ICP-MS system. Infrared (IR) spectra are recorded in solid with a Bruker Vertex 80V FT-IR spectrophotometer in a resolution of 4 cm^{-1}. The Uv-vis spectra are recorded on a Shimadzu UV-3600 spectrophotometer for solutions with concentration of 10 mg/mL AuNCs@BSA contained in 1 cm × 1 cm quartz cuvettes (4 mL volume).

The synthesis of AuNCs@BSA

The AuNCs@BSA are synthesized by using microwave-assisted method. That is, BSA is reacted with $HAuCl_4$ in the extremely basic condition (pH 12) under microwave irradiation (MWI). The $HAuCl_4$ aqueous (5 mL, 10 mM) is added to BSA aqueous (5 mL, 50 mg/mL) under room temperature. Two minutes later, NaOH solution (0.5 mL, 1 M) is introduced, and the mixture is incubated with the microwave irradiation to keep temperature at 37 °C for 12 h. Growths of AuNCs in BSA as a function of reaction time are followed by fluorescence spectral measurements while 2 h interval is selected to extract sample. And the solutions after synthesis are dialyzed with a 10 kDa cut-off dialysis membrane extensively, against doubly distilled water for more than 24 h with a water change every 4 h, to remove all small molecular impurity. After that, the sample is isolated with the commercially supplied Sephadex G-75 gel (supplied by Pharmacia). Finally, it is subjected to freeze-drying to obtain AuNCs@BSA in the powder form for further characterization and investigations. The re-dissolved AuNCs@BSA in solution emits at 604 nm under 350 nm excitation and the quantum yield of it is found to be 5%, as calculated by using fluorescein in 0.1 M NaOH as a reference (P. L. Xavier, K. Chaudhari, P. K. Verma, S. K. Pal and T. Pradeep, *Nanoscale*, 2010, **2**, 2769).

Fluorescent detection

Fluorescence emission spectra are recorded on a Shimadzu (Japan) RF-5301PC fluorescence spectrophotometer. To reduce the fluctuation in the excitation intensity during measurement, the lamp is kept on for 1 h and the samples are stock-still for 20 min prior to the experiment. Samples for emission measurement are contained in 1 cm × 1 cm quartz cuvettes (4 mL volume). All spectroscopic measurements of AuNCs@BSA are performed in 10.0 mM HEPES buffer solution (pH 7.5) using distilled water, and the concentrations of AuNCs@BSA in the fluorescent experiments is either 1.0 mg/mL or

0.030 mg/mL for different purpose. A fixed excitation wavelength at 350 nm is used. Fluorescence titration is performed by using respective nitrate of metal ion. For the pH titration of AuNCs@BSA, the spectra are monitored in solutions of various pH containing 0.1 M $NaNO_3$ to keep the ionic strength.

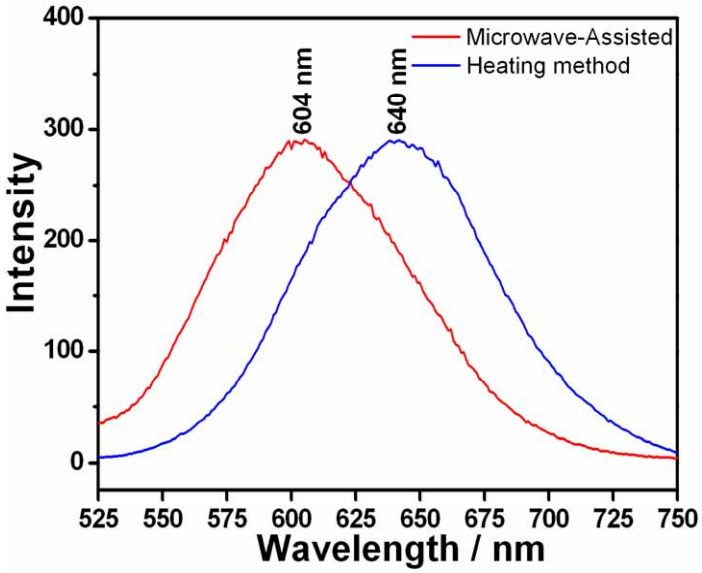

Fig. S1 Fluorescence spectral comparison of AuNCs@BSA produced by the typical heating method and the microwave-assited synthesis, large emission gap ($\Delta\lambda$ = 36 nm) can be observed between them (λ_{ex} = 350 nm).

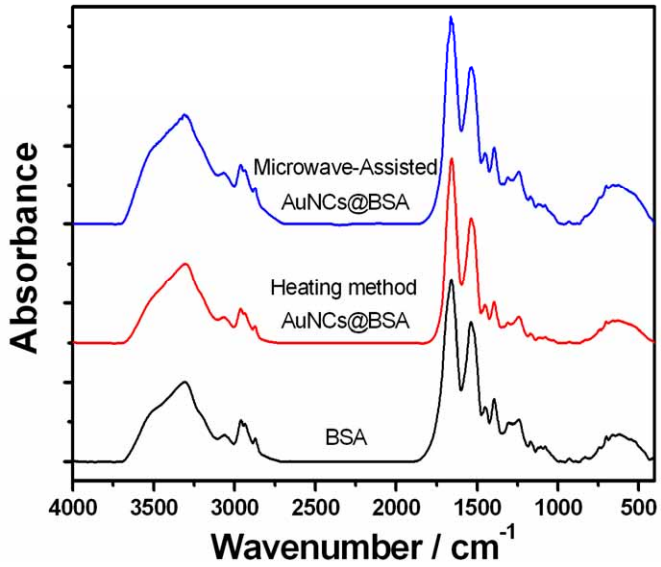

Fig. S2 The FT-IR spectra in monitoring the synthesis of AuNCs@BSA and comparing to products obtained by the different synthesis methods. The black trace depicts the spectrum of pure BSA, the red trace is the spectrum of AuNCs@BSA which is synthesized by heating method and the blue trace is the spectrum of AuNCs@BSA produced by the microwave-assisted method. In the spectra, the bending and stretching vibrations of the peptide backbone which characterize the secondary structure are monitored, but the positions in both AuNCs@BSA do not undergo too much change (red and blue trace),

indicating that the secondary structural of the capping BSA molecules do not change a lot undergo the synthesis.

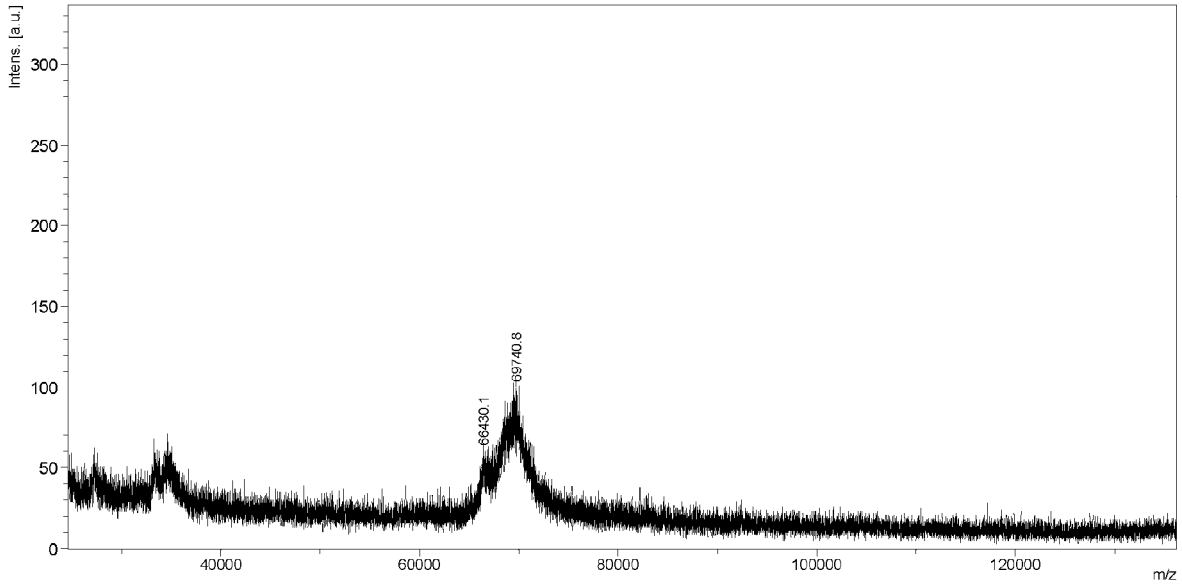

Fig. S3 MALDI/TOF mass spectrum of AuNCs@BSA produced by the microwave-assisted synthesis.

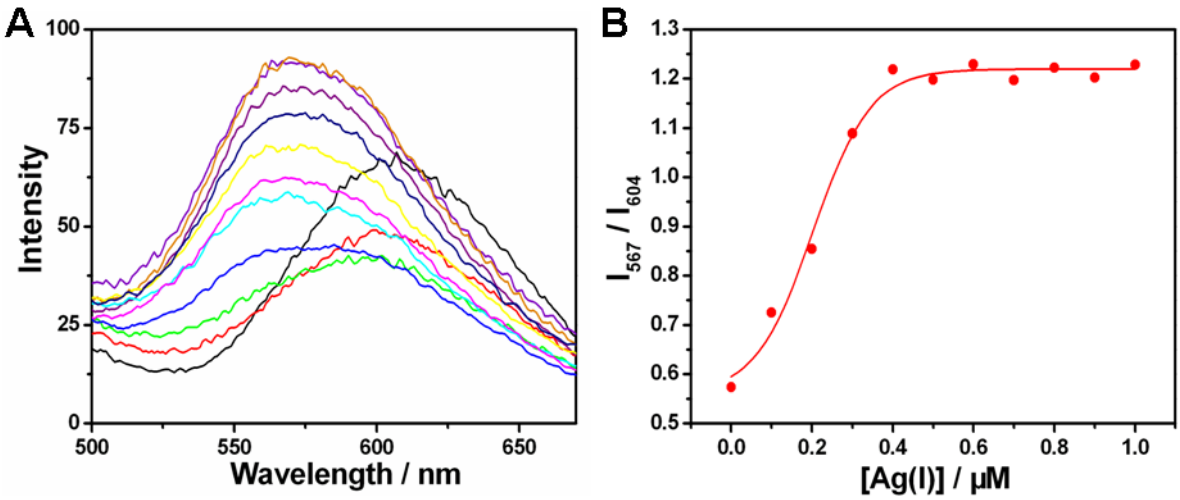

Fig. S4 Fluorescence spectra of Au_{16}NCs@BSA (0.030 mg/mL) in buffer solution (pH 7.5) upon addition of various amounts of $AgNO_3$ (0–1.0 μM, λ_{ex} = 350 nm).

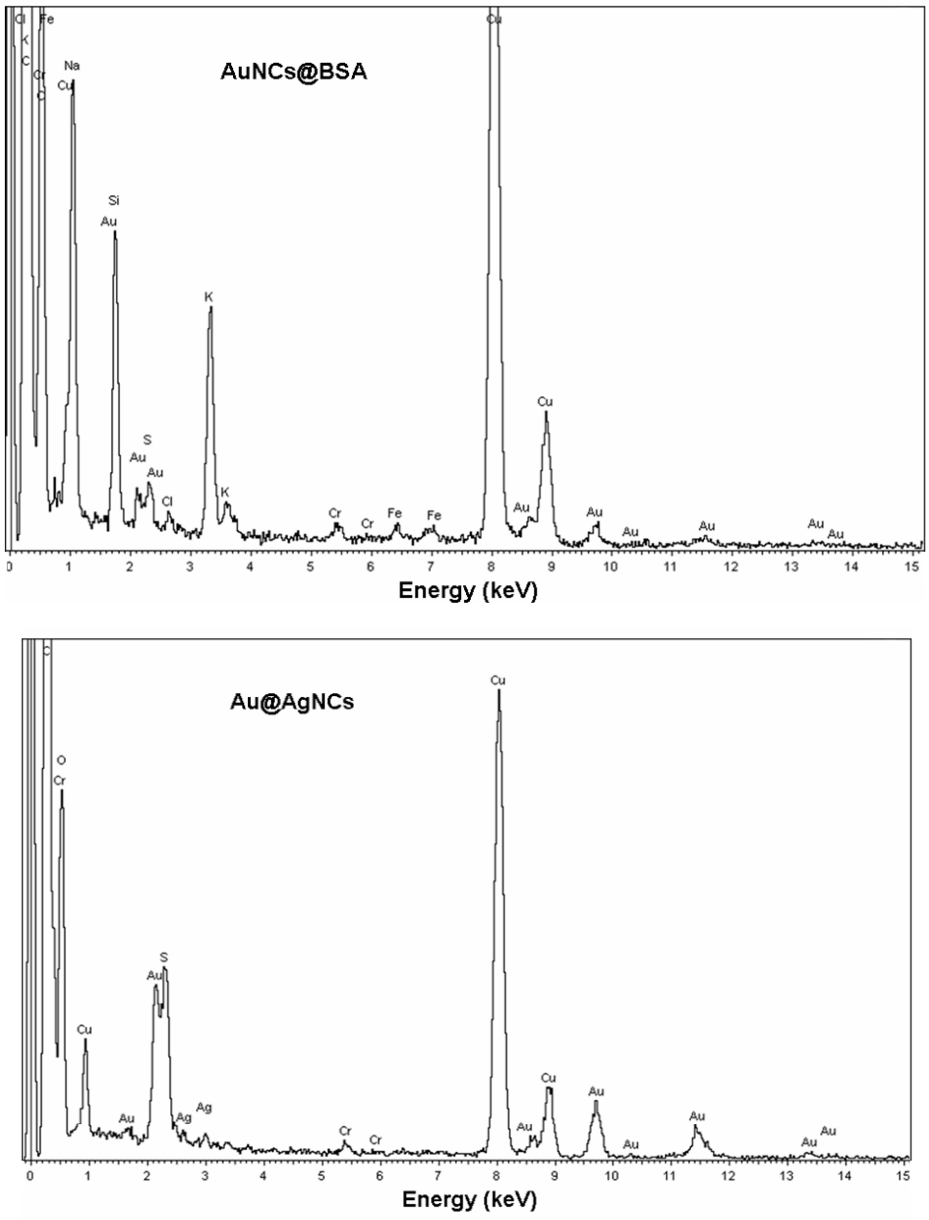

Fig. S5 EDS spectrum of the AuNCs@BSA (up) and Au@AgNCs (bottom), indicating the presence of Ag and Au atoms in the Au@AgNCs with contents of 0.74% AgL and 8.62% AuL. So the ratio of Ag/Au is calculated to be 0.086 as 0.74%/8.62%. The Au@AgNCs is prepared by adding 0.25 mL Ag(I) ions (10 mM) to 10 mL AuNCs@BSA (10.0 mg/mL) solution under stirring for more than 30 min, and then is dialyzed with a 10 kDa cut-off dialysis membrane extensively against doubly distilled water for more than 24 h (with a water change every 4 h). Finally, it is subjected to freeze-drying to obtain Au@AgNCs in the powder form for further characterization and investigations.

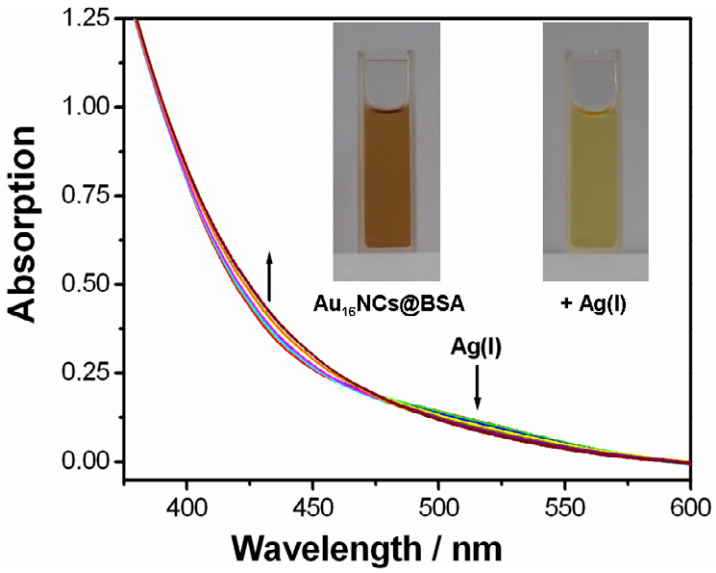

Fig. S6 Uv-vis spectra of Au_{16}NCs@BSA (10.0 mg/mL) in buffer solution (pH 7.5) upon addition of different amounts of $AgNO_3$ (0–100 μM), which are measured 20 min after the corresponding Ag(I) ions are added.

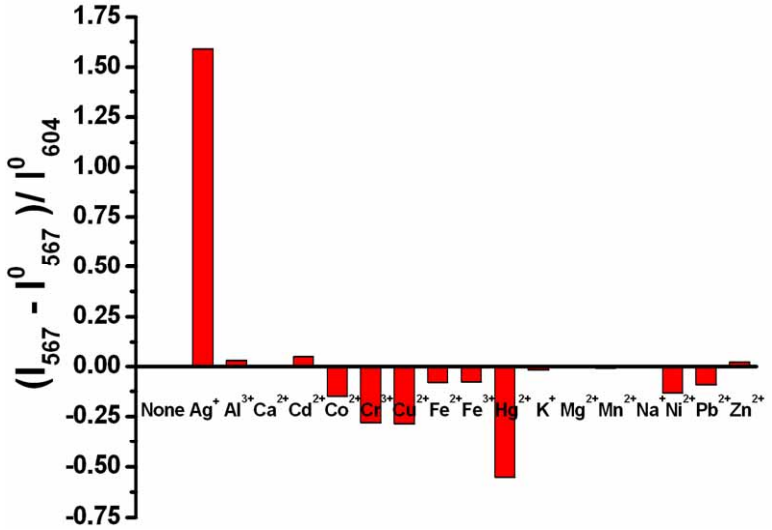

Fig. S7 The fluorescence intensity ratios of Au_{16}NCs@BSA (1.0 mg/mL) in buffer solution (pH 7.5) in the presence of various metal ions (20.0 μM).

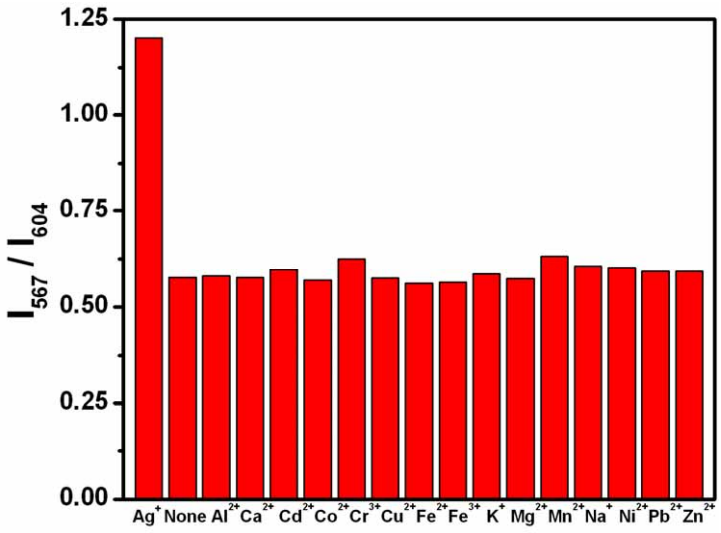

Fig. S8 The fluorescence intensity ratios of Au_{16}NCs@BSA (1.0 mg/mL) in buffer solution (pH 7.5) in the presence of various metal ions (20.0 μM). The high selectivity of Au_{16}NCs@BSA to silver ions can be illustrated more clearly once the intensity ratio of I_{567} / I_{604} is used.

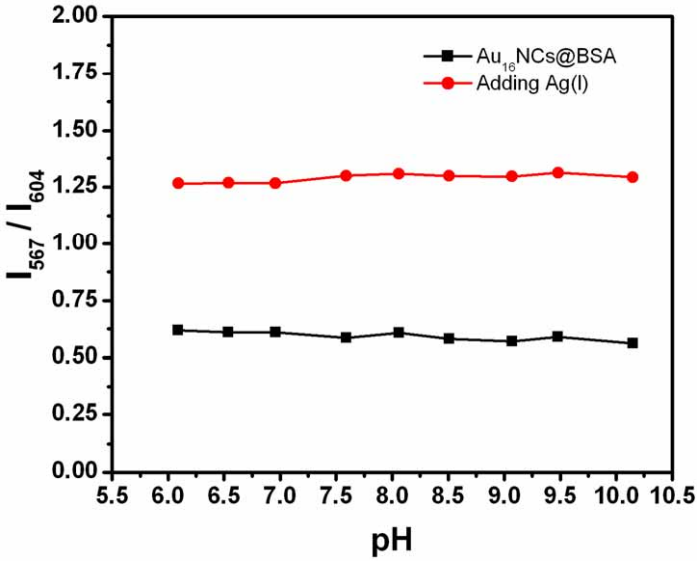

Fig. S9 The fluorescence intensity ratios of Au_{16}NCs@BSA (1.0 mg/mL) in changing with the pH before and after addition of 20.0 μM $AgNO_3$ in buffer solution. It is very stable under the pH changes before and after addition of Ag(I).

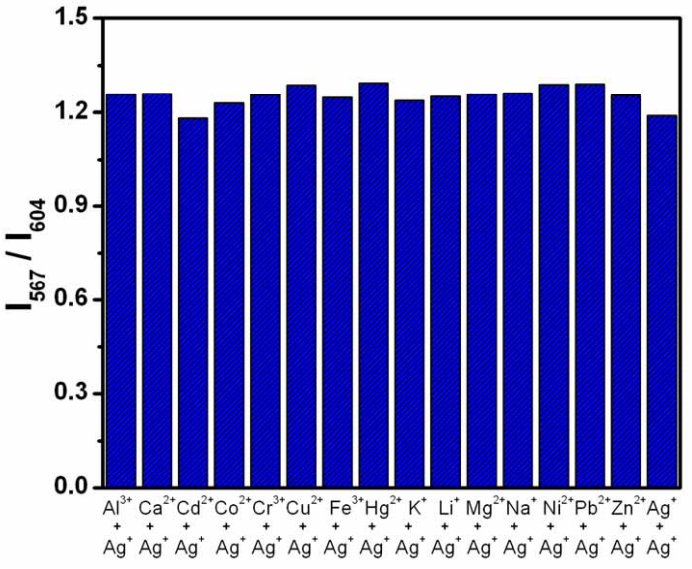

Fig. S10 Fluorescence intensity ratios of AuNCs@BSA (1.0 mg/mL) in buffer solution (pH 7.5) in the presence of Ag(I) (20.0 μM) and the indicated ions (20.0 μM), respectively.