Magnetic Separation and Immunoassay of Multi-antigen Based on Surface Enhanced Raman Spectroscopy

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1. Synthesis and Preparation

γ-Fe_2O_3 was prepared by oxidizing Fe_3O_4 nanoparticles in the aqueous phase. A total of 2.6 g of $FeCl_3$ and 1.0 g of $FeCl_2$ was successively dissolved in a solution of 0.43 mL of concentrated HCl (12 M) in 12.5mL of H_2O. The solution was added into 125 mL of a 1.5 M NaOH solution with vigorous stirring. The generated black precipitate was collected by a magnet, and the supernatant was removed from the precipitate by decantation. After being washed three times with ultrapure water, 250 mL of a 0.01 M HCl solution was added into the precipitate to neutralize the anionic charges on the nanoparticles. The resulting colloidal was again isolated by the magnet and washed three by ultrapure water. The fresh Fe_3O_4 nanoparticles were dissolved in 0.01M HNO_3 and heated with stirring at 90~100^oC for 1 h to completely oxidize the particles to γ-Fe_2O_3. The solution was cooled to room temperature and washed three with ultrapure water, yielding a magnetite-like, stable suspension of γ-Fe_2O_3.

γ-Fe_2O_3/Au core/shell nanoparticles were synthesized by deposition of Au on the preformed γ-Fe_2O_3 nanoparticles using a modification of Lyon's iterative hydroxylamine seeding procedure. First, the γ-Fe_2O_3 colloidal was diluted to 1.1mM in ultrapure water, and 1mL was stirred with 1mL of 0.1 M sodium citrate for 5 min, the solution was diluted with 20mL H_2O, after stirring 15min, 0.05ml of a 1.5M NaOH solution was added. Next, 0.1mL of $NH_2OH{\cdot}HCl$ solution of 80mM was added. Then, 1% $HAuCl_4$ was incrementally added dropwise upon appropriate stirring each for 2mL. Three additions (each for $NH_2OH{\cdot}HCl$ and $HAuCl_4$) were totally performed during the reaction, and the stirring continued for at least 1h after each addition. The UV and SEM ofγ-Fe_2O_3 nanoparticlesandγ-Fe_2O_3/Au core/shell nanoparticles were presented in Fig.S1.

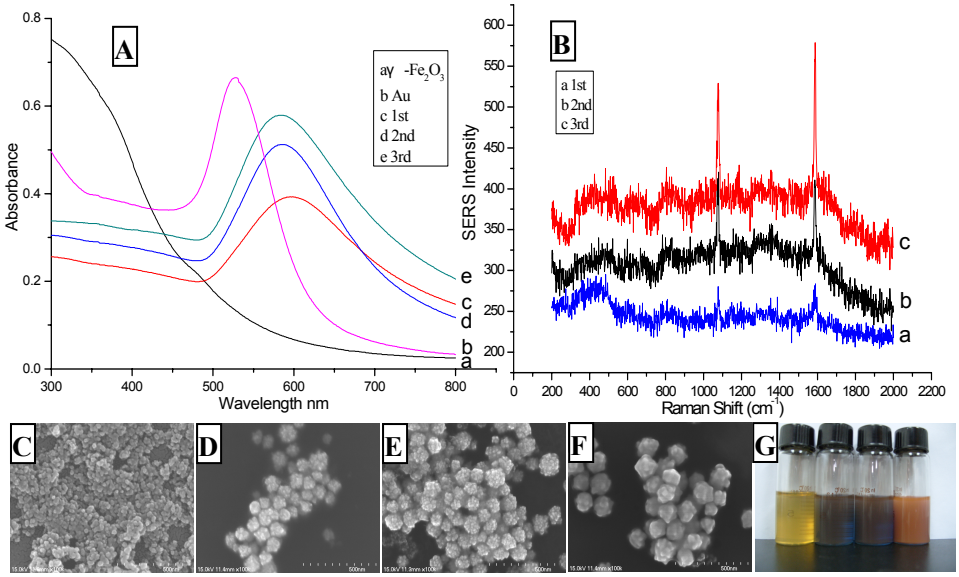

Fig.S1[A]UV-vis spectra of γ-Fe_2O_3 (a), pure Au(b), γ-Fe_2O_3/Au with1~3 Au iterative (c~e); [B] SERS spectra of 4-MBA (C=10^{-5}M) absorbed on γ-Fe_2O_3/Au with 1~3 Au iterative(a~c) in colloid sample. [C~F] SEM images of γ-Fe_2O_3/Au with (C)zero (pure γ-Fe_2O_3) (D)one (E)two (F)three incremental additions of Au^{3+} to the γ-Fe_2O_3/H_2O solution.[G] the color of C,D,E,F nanoparticles, respectively.

2 Preparation of Immunoγ-Fe_2O_3/Au Nanoparticles for Bioseparation and Immunoassay

The γ-Fe_2O_3/Au nanoparticles were separated from the solution by centrifugation and resuspended with 2.0mL of borate buffer. Next, 10μL of 3.34mg/mL Polyclonal antibody (goat anti-mouse or goat anti-rabbit IgG) was added to the above nanoparticles and incubated at room temperature for 2h. After being purified by centrifugation and resuspended with 2.0mL of borate buffer, 20μL of BSA (5%) was added to the immunoγ-Fe_2O_3/Au nanoparticles to block active sites between antibodies. The solution was incubated for 1h at room temperature and then again centrifugated and resuspended in 2.0mL of borate buffer. The resulting immuno-γ-Fe_2O_3/Au nanoparticles were stored at 4°C.

3. Preparation of Raman Reporter-Labeled Immunogold Nanoparticles

The Au nanoparticles were prepared according to Frens'method. In a typical process, 100mL of 0.01% HAuCl4 aqueous solution was heated to boiling with vigorous stirring, to which 1mL of a 1% trisodium citrate solution was added. The mixture was then kept boiling for 30 min.

Afterward, the solution was allowed to cool to room temperature with continuous stirring. The resulting red Au colloidal was about 35 nm in diameter.

The Raman reporter-labeled immunoassay Au nanoparticles were prepared by following a procedure reported by Ni et al. with a slight modification. A total of 2.5μL of 1mM probe molecule (4-MBA) in ethanol was added to 1.0mL of Au nanoparticles, and the resultant mixture was allowed to gently shake for 1h, the reporter-labeled nanoparticles were then separated from the solution by centrifugation at 5000g for 10min and resuspended with 1.0mL of borate buffer. Next, 5μL of 3.34mg/mL Polyclonal antibody (goat anti-mouse or goat anti-rabbit IgG) was added to 1.0mL of MBA-labeled Au nanoparticles with gentle agitation. After incubation at room temperature for 2h, the MBA-labeled immunogold nanoparticles were purified by centrifugation and resuspended with 1.0mL of borate buffer. Then 10μL of BSA (5%) was added to the above MBA-labeled immunogold nanoparticles to make sure that no bare sites on Au nanoparticles were left. The mixture was incubated for 1h at room temperature and then centrifugated and resuspended in 1.0mL of borate buffer.

4. Immunoassay protocol

The SERS-based immunoassay was carried out according to a new method that we called magnetic separation immunoassay, as show in Scheme 1. Typically, 0.15ml antigens solution, 1ml immunoγ-Fe_2O_3/Au nanoparticles and 1ml MBA labeled immunogold nanoparticles were mixed in a weighing bottle (40×25mm), after the weighing bottle had been gently shaken at room temperature for about 3h on a shaker. After collecting 12h by a small magnet (diameter about 5mm) under the weighing bottle, upper liquid was discarded, and the sediment was resuspended in 2ml H_2O under ultrasonic oscillation. Repeat this process with twice and the sediment resuspended in 1ml H_2O, the resultant mixture was pipetted onto a silicon wafer with 3 times for SERS detection.

5. The measurements on the feasibility of the current assay

The MBA (Raman reporter) modified Au nanoparticles was mixed with the immuno γ-Fe_2O_3/Au (with goat anti-rabbit IgG) particles, and then was separated by a small magnet for

about 12 hrs. The SERS and TEM measurements on the sediment were present as the following figures. No observation of SERS signal and Au nanoparticles in TEM image indicated that the Raman probe functionalized Au nanoparticles (with out the antibody) would not adsorb on to the magnetic-plasmonic particles.

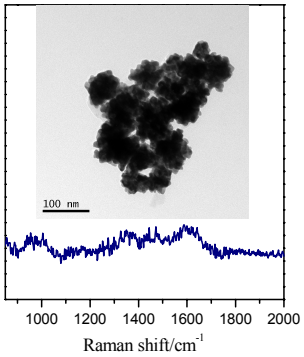

Fig.S2 SERS spectrum and TEM image from the sediment after a magnetic separation was applied on a mixture solution of Raman probe functionalized Au nanoparticles (with out the antibody) and the magnetic-plasmonic particles (with antibody).

6. TEM images on the antigen induced particle aggregation

Small red-shift on absorption peaks was observed after the rabbit IgG was introduced into the mixture solution of MBA and antibody (goat anti-rabbit IgG) modified Au nanoparticles and goat anti-rabbit IgG modified magnetic-plasmonic particles. It indicated that the antigen induced particle aggregation. The TEM images gave the other evidence on the antigen induced particle aggregation (as shown in Fig.S3). The magnetic-plasmonic particles were separated by a magnet in the absence of corresponding antigen (as shown in Fig. S3a), while the MBA and antibody (goat anti-rabbit IgG) modified Au nanoparticles was attached on to the goat anti-rabbit IgG modified magnetic-plasmonic particles in the presence of rabbit IgG (Fig. S3 b).

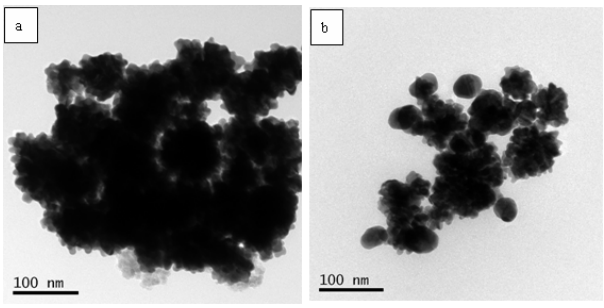

Fig.S3 The TEM images of the sediment collected by a magnet on the mixture solution of MBA and antibody (goat anti-rabbit IgG) modified Au nanoparticles and goat anti-rabbit IgG modified magnetic-plasmonic particles in the absence (a) and presence (b) of rabbit IgG.

7. Specificity of the current assay

1ml MBA labeled goat anti rabbit IgG immuno Au nanoparticles and 1 ml goat anti rabbit IgG immuno Fe_2O_3/Au particles were employed to detect the antigen solution of rabbit IgG (0.1ug/ml) and mouse IgG (1ug/ml) respectively, see the Fig. S4. Although the concentration of mouse IgG was about 10 times of that of rabbit IgG, no SERS signal was detected from the mouse IgG solution because of the absence of corresponding antibody. Therefore, although high concentration of control antigen was mixed into the low concentration of target antigen, no influence of the control antigen was involved in the detection. It suggested the high specificity of current assay.

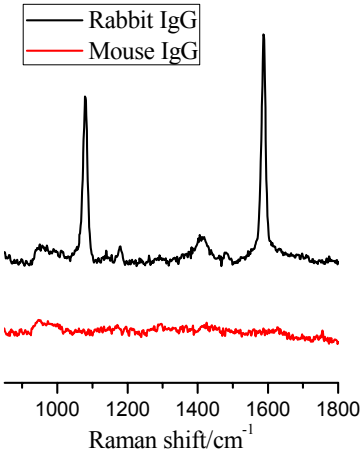

Fig. S4 SERS spectra of immunoassay for different antigen by γ-Fe_2O_3/Au coated with goat anti-rabbit IgG. mouse IgG(1ug/ml), rabbit IgG (0.1ug/ml).