Supplementary Information

Ultrasensitive Detection of Carcino-embryonic Antigen by Using Novel Flower-Like Gold Nanoparticles SERS Tags and SERS-active Magnetic Nanoparticles

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Experimental Section

Materials. Hydrogen tetrachloroaurate(III) trihydrate ($HAuCl_4 \cdot 3H_2O$, 99.99%) and Cetyltrimethylammonium chloride (CTAC, ≥96%) were bought from Alfa Aesar. 4-Mercaptobenzoic acid (4-MBA, 99%), N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), and Tetrakis(hydroxymethyl)phosphonium chloride (THPC, 80% aqueous solution) were purchased from Sigma-Aldrich. 3-Aminopropyltriethoxysilane (APTES, 98%) and Teraethyl orthosilicate (TEOS) were purchased from Aladdin. Ammonium hydroxide (25.0 ~ 28.0%), L-ascorbic acid (AA, ≥ 99.7%) and Ethanol (≥ 99.7%) were obtained from Sinopharm Chemical Reagent Co., Ltd. Carcino-embryonic Antigen (CEA) and anti-CEA antibody were bought from Shanghai Linc-Bio Science Co. LTD. Bovine serum albumin (BSA) was purchased from Biosharp. Borate buffer solution (BBS, 2 mM, pH=9) was served as the buffer solution. Ultrapure Millipore water (18.2 MΩ cm^{-1}) was used as the solvent throughout.

Synthesis of flower-like gold nanoparticles. Gold nanoflowers (AuNFs) were synthesized by using the reduction of $HAuCl_4$ with AA in CTAC surfactant. Briefly, 10 mL of 10 mM $HAuCl_4$ aqueous solution was mixed with 100 mL of 200 mM CTAC aqueous solution under vigorous stirring, followed by adding 5 mL of 300 mM AA aqueous solution into the mixture and stirred vigorously for 2 min. The solution was then left undisturbed for 3 h at 18°C to grow AuNFs. The as-synthesized colloid was centrifuged at

900 rpm for 5 min to remove the excess reactants. The precipitates were collected and washed three times. Finally, these particles were re-dispersed with BBS to 10 mg/mL.

Preparation of AuNFs SERS tags. 750 μL of 1 mM 4-MBA was added into 5 mL colloidal AuNFs under vigorous stirring and the resultant mixture was allowed to react overnight. After purification (900 rpm for 5 min), the AuNFs were linked with anti-CEA (50 μL, 2 mg/mL) by covalent bonding *via* the esterification of NHS (372 μL of 100 mM) with EDC (150 μL of 100 mM). The purified produces were further blocked by BSA (500 μL, 3% in BBS) for 1 h, followed by centrifugal purification for three times. Finally, the AuNFs SERS tags were obtained by re-dispersing the precipitates into 4 mL BBS.

Preparation of GMNPs supporting substrates. Magnetic nanoparticles (MNPs), Fe_3O_4, were synthesized by chemical codeposition of $FeCl_2$ and $FeCl_3$ in aqueous solution, following the synthesis method proposed by Kang et al (K. Kang, J. Choi, J. H. Nam, S. C. Lee, K. J. Kim, S.-W. Lee and J. H. Chang, *Journal of Physical Chemistry B*, 2008, 113, 536-543). The as-synthesized MNPs colloid was finally dried in a vacuum oven at 70°C and then the powder was dispersed to 10 mg/mL by water. The Fe_3O_4@SiO_2@Au particles (GMNPs) were synthesized by three steps. Firstly, the Fe_3O_4 particles were coated with silica shell by hydrolysis of TEOS in an alkaline environment, followed by an aminosilylation of APTES. Secondly, Au seeds (3~5 nm) were absorbed onto the aminated Fe_3O_4@SiO_2 nanoparticles to form Fe_3O_4@SiO_2@Au seeds structure. In the final step, the Au seeds on the surface of Fe_3O_4@SiO_2 grew to be a rough gold shell on the Fe_3O_4@SiO_2. After purification, 3 mL GMNPs in BBS was obtained. The GMNPs supporting substrates were prepared by linking anti-CEA onto the GMNPs. Briefly, 1 mL of 5-fold diluted GMNPs was mixed with 20 μL of 1.1 mg/mL anti-CEA antibodies and incubated for 12 h at 4°C. Then the GMNPs were blocked by 500 μL of 3% BSA. Finally, 3 mL GMNPs supporting substrates was obtained by magnetic separation and re-dispersion with BBS.

Immunoassay protocol. 200 μL SERS tags were added into a tube and mixed together homogeneously with 160 μL supporting substrates, followed by adding the target antigen, CEA. After incubating for 3 h at 4°C under shaking, the products were separated by magnet and washed three times by BBS. The final products were dispersed in 100 μL water for SERS measurements.

Instrumentation. The UV-Vis-NIR spectrophotometer (UV-3600, Shimadzu, Japan) was used to characterize monitor the absorption spectra of the colloids. Transmission electron microscopy (TEM) (HT7700, Hitachi, Japan) and field emission scanning electron microscopy (FESEM) (S-4800, Hitachi, Japan) were utilized to characterize the morphology and distribution of particles. The X-ray powder diffraction (XRD) investigation was carried out by using a Bruker D8 Advance. All the SERS spectra, except single-particle SERS measurements, were recorded by a HRC-10HT Raman Analyzer (Enwave Optronics Inc.), with a 785 nm excitation wavelength, 30 mW excitation power and 5 s acquisition time. Specially, single-particle SERS spectra were recorded by using a custom designed optical system, which consist of an inverted microscopy (eclipse Ti-U, Nikon, Japan) equipped with a dark-field condenser ($0.8 < NA < 0.95$), a 100 W halogen lamp, a true-color digital camera (Nikon DS-fi), and a monochromator (Acton SP2300i) equipped with a spectrograph CCD (CASCADE 512B, Roper Scientific) and a grating (1200 grooves/mm), and a 785 nm excitation laser (3.6 mW of power at sample). The laser was focused onto the sample by using a 60X objective lens (NA=0.70).

Single-particle SERS measurements. For single-particle SERS characterization, the SERS samples were prepared as follows: the as-synthesized pure gold colloids were concentrated to 0.3 mL first, and then 30 μL of 1mM 4-MBA was mixed with 0.1 mL concentrated colloids and stirred for 2 h to ensure the Raman molecules to absorb on the particles sufficiently. After centrifugal purification, the colloid was then diluted 100 times, from which 20 μL liquid was pipetted and coated onto a clean ITO glass for 1 h, followed by water washing and N_2 drying. The sparsely individual nanoparticle was then used for single-particle SERS detection. Fig. S1A shows an dark-field image (×60) of particles randomly distributed on ITO slide. By moving the object stage of microscopy and tuning narrowly the slit of the spectrograph as shown in Fig. S1B, the scattering signal from an individual particle can be collected. Each SERS spectrum was accumulated for 5 s.

SERS-based immunoassay. For SERS immunoassay, 5 aliquots of 10 μL final products were dropped onto silica wafers (5 mm × 5 mm) respectively, and a cylindrical magnet (diameter ~ 1.5 mm, length ~ 10 mm) was placed under each sample point to magnetically concentrate the immune products into a small spot with diameter less than 1 mm. All the SERS detections were collected from the spot, with 30 mW exciting power and 5 s acquisition time.

Fig. S1

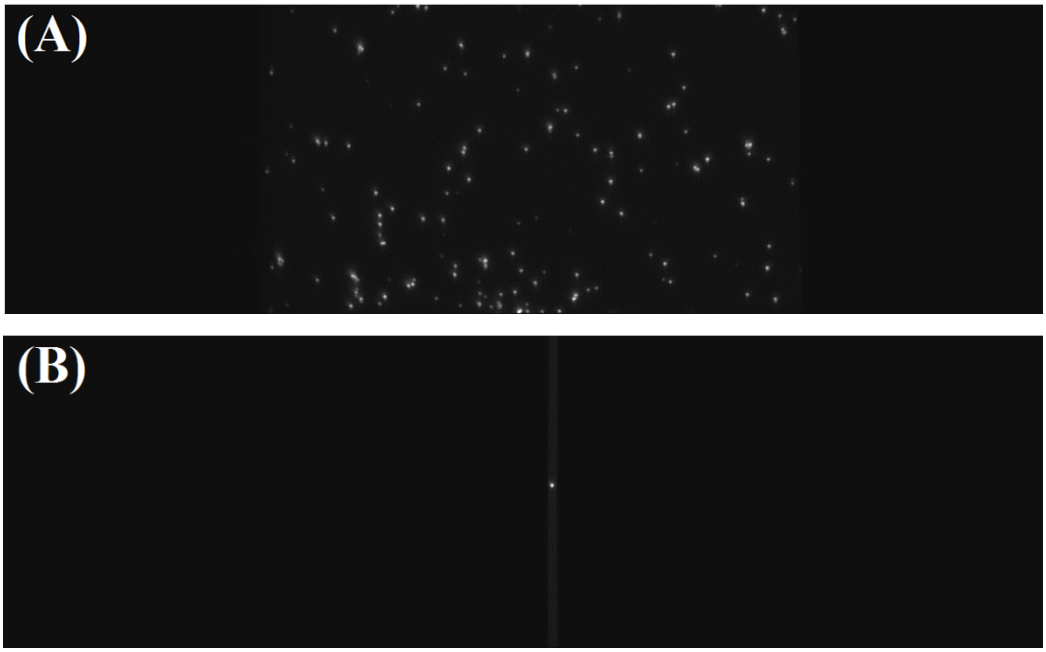

Fig. S1 Dark-field images of AuNFs for single particle SERS detection.

Fig. S2

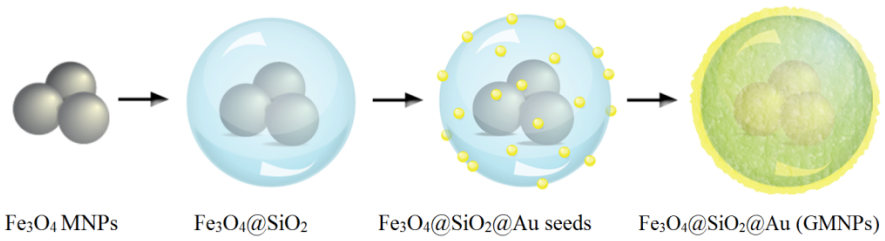

Fig. S2 Sketch maps of the preparation of GMNPs.

Fig. S3

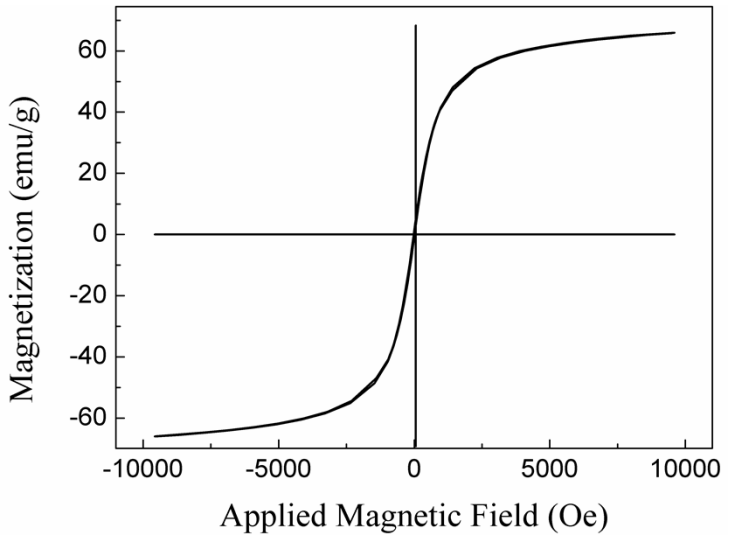

Fig. S3 Magnetic hysteresis curve of Fe_3O_4 nanoparticles.

Fig. S4

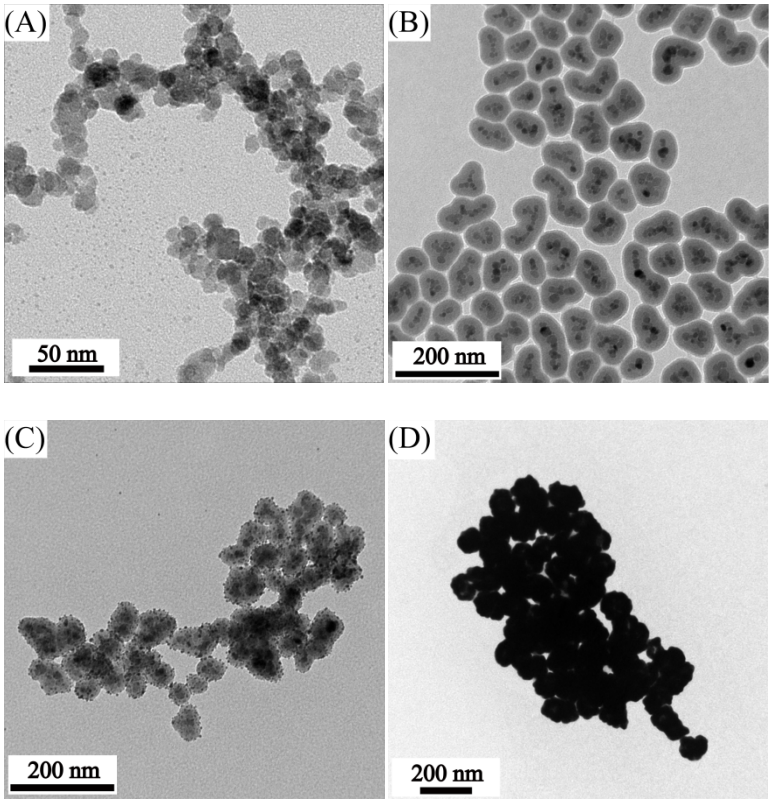

Fig. S4 TEM images of (A) Fe_3O_4 nanoparticles, (B) Fe_3O_4@SiO_2 core-shell nanoparticles, (C) Fe_3O_4@SiO_2@Au seeds, (D) Fe_3O_4@SiO_2@Au shell nanoparticles (GMNPs).

Fig. S5

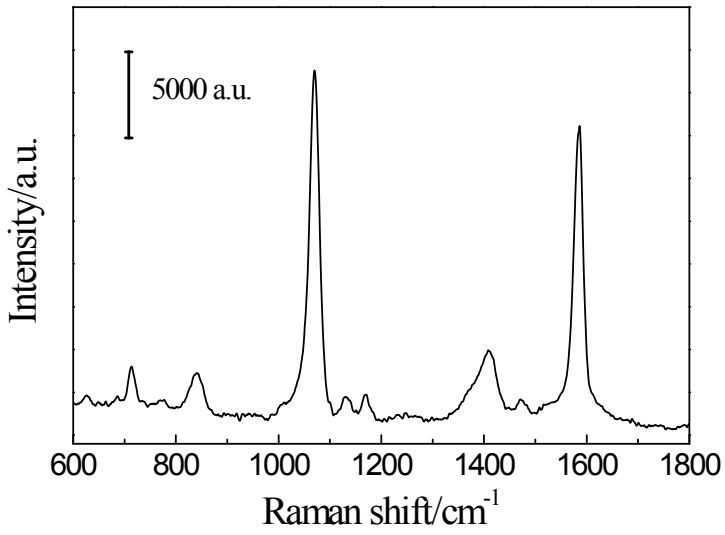

Fig. S5 SERS characterization of GMNPs using 4-MBA as Raman reporter.

Fig. S6

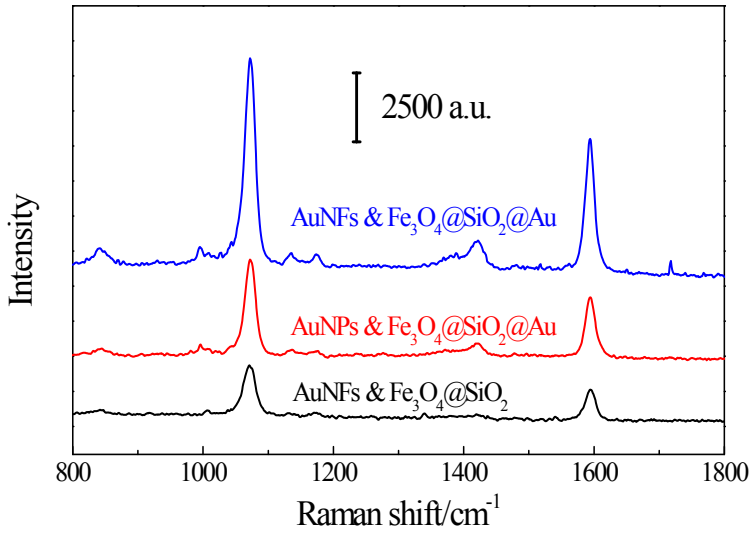

Fig. S6 Immunoassays for 1 ng/mL CEA using different combination of SERS tags and supporting substrates, AuNFs with GMNPs, AuNPs with GMNPs, and AuNFs with silica coated MNPs, respectively.