Supporting Information for

Design of Raman Reporter-embedded Magnetic/Plasmonic Hybrid Nanostirrers for Reliable Microfluidic SERS Biosensor

Bingfang Zou, [a,b] *Shiyun Lou,* [a] *Jie Duan,* [a] *Shaomin Zhou* [a*], *Yongqiang Wang* [a*]

[a]*Key Laboratory for Special Functional Materials of the Ministry of Education, Henan University, Kaifeng 475004, P. R. China.*
[b]*School of Physics and Electronics, Henan University, Kaifeng 475004, P. R. China.*
Email: wangyq@henu.edu.cn, smzhou@henu.edu.cn.

Table of Contents

1. Supplementary Experimental Section.

Carboxyl groups-modified SERS nanotags. The SERS nanotags were synthesized by decorating TFTP on spiky Au nanospheres. Spiky Au nanospheres were firstly synthesized according to previous report. 5 mL of Au nanospheres (~50 nm), 10 mL of PVP (0.05 wt%), 4 mL of $AgNO_3$ (10×10^{-3} M), and 2 mL of KI (20×10^{-3} M) were added in order in 100 mL of H_2O at room temperature. After stirring for 2 min, 900 μL of $HAuCl_4$ (25×10^{-3} M) and 50 mL of L-AA (20×10^{-3} M) were injected into the above solution. The reaction was allowed to proceed for 10 min. The product was collected by centrifugation, and washed with H_2O, and redispersed in 50 mL of H_2O, and 1ml of TFTP (10^{-3} M) and 1ml of HS-PEG-COOH (10^{-4} M) were added and incubated for 2 hours. Subsequently, 1 mL of PEG-SH (10^{-3} M) was added to the mixture and further incubated for 30 minutes. The carboxyl modified-SERS nanotags were centrifuged and washed with H_2O repeatedly and redispersed in 50 mL of H_2O for further use.

Carboxyl groups-modified magnetic hybrid nanostirrers. Carboxyl groups were introduced on magnetic hybrid nanostirrers by treating the functionalized amino groups on magnetic hybrid nanostirrers with SAA. Firstly, the amino groups were attached on magnetic hybrid nanostirrers (10 mg) using ethanol solution (30 mL) containing 1 ml APTES for 24 h, and then they were dried in vacuum oven. And then magnetic hybrid nanostirrers with amino groups were treated in DMF containing SAA (80 mg) for 24 h in mixer, and the resulting carboxylate-modified magnetic hybrid nanostirrers were washed with ethanol and deionized water for 20 h.

Antibodies Conjugating Procedure. Taking carboxyl groups modified magnetic hybrid nanostirrers as example, sulfo-NHS plus EDC crosslinking reaction was then used to functionalize magnetic hybrid nanostirrers with amine-reactive sulfo-NHS eater. Typically, magnetic hybrid nanostirrers (10 mg) were firstly dispersed in 0.8 ml BS buffer (5 mM, pH 7.2) in a 1.5 mL centrifuge tube, and then 50 μL of 4.5 mM EDC and 50 μL of 1.2 mM sulfo-NHS were added in turn, and then the centrifuge tube was sealed and place on rotating incubator at 4 °C for 5 min. After that, the supernatant was discarded to remove unreacted reagents under external magnet, and the magnetic hybrid nanostirrers were redispersed in 400 μL PBS buffer (10 mM, pH 7.4). 0.1 ml of IL-6 capture antibody (0.5 mg/mL in PBS solution) was added into the solution and incubated at 4 °C overnight, and the nonspecific binding sites of magnetic hybrid nanostirrers were blocked with BSA solution (1 %), and the active sites were stopped with 5 μL ethanolamine. Finally, magnetic hybrid nanostirrers were washed with PBS solution several times by vortex, and finally redispersed in 1 mL PBS solution and stored at 4 ℃ for further use. The SERS nanoprobes was synthesized by conjugating detective antibodies on carboxyl groups-modified SERS nanotags in a similar method, and the obtained IL-6 antibody conjugated SERS nanoprobes were redispersed in 1 mL PBS solution and stored at 4 °C for further use.

The signal filtering experiments. According to our proposal, reliable SERS nanoprobe signals could be collected by picking out similar sampling microenvironments. Here, similar sampling microenvironments were determined by exploring the relationship between intensity range of beacon and signal reproducibility of SERS nanoprobes. The detail experiment procedure was described as follows: IL-6 with concentration of 100 pg mL^{-1} was used as example, 1 μL of IL-6 solution and 1 μL of solution containing magnetic hybrid nanostirrers and SERS nanoprobes were injected into microfluidic platform. The two solutions were then mixed within big chamber, and were incubated for 10 minutes at 37 °C under rotating magnetic field. After they were accumulated in small chamber under external electromagnet, extra 5 minutes were proceeded in order to form a stable aggregate. The microfluidic platform was transferred on the objective table of Renishaw confocal Raman microscope. The laser beam with power of 0.5 mW was focused by a 20× objective, and a depth scan were firstly conducted to determine the optimal height. And then random single-point measurements (100 points) were performed with an integration time of 20 s for each spectrum acquisition. The detailed data processing was demonstrated in main text. Extra five independent tests were employed for validating the repeatability as shown in Fig. S9, and their RSD values with $\sigma = 4.3\%$ are 5.8%, 5.9%, 5.7%, 6.1% and 6.1%, respectively.

2. Supplementary Figures

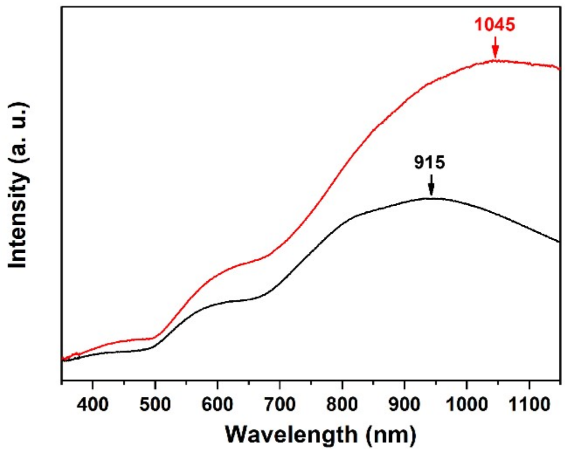

Fig. S1 Extinction spectra of magnetic hybrid nanospheres and nanostirrers.

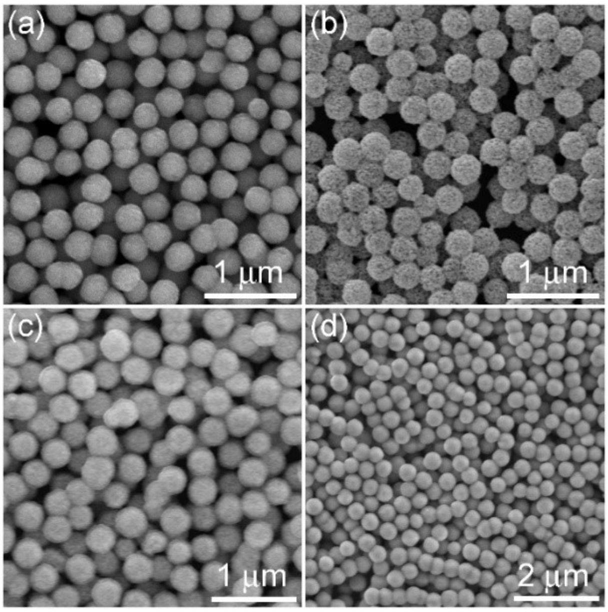

Fig. S2 Characterization of magnetic nanostirrers synthesized by using Fe_3O_4 nanospheres (280 nm) as magnetic cores. SEM images of (a) Fe_3O_4 nanospheres, (b) MBA-modified Fe_3O_4/Au hybrid nanospheres and (c) MBA-modified Fe_3O_4/Au hybrid nanospheres with thin silica coating. (d) Fe_3O_4/Au hybrid nanostirrers by using product (c) as building blocks.

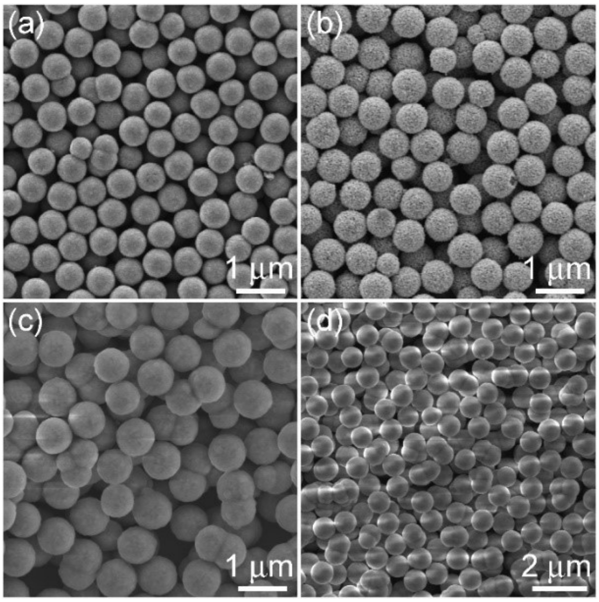

Fig. S3 Characterization of magnetic nanostirrers synthesized by using Fe_3O_4 nanospheres (600 nm) as magnetic cores. SEM images of (a) Fe_3O_4 nanospheres, (b) MBA-modified Fe_3O_4/Au hybrid nanospheres and (c) MBA-modified Fe_3O_4/Au hybrid nanospheres with thin silica coating. (d) Fe_3O_4/Au hybrid nanostirrers by using product (c) as building blocks.

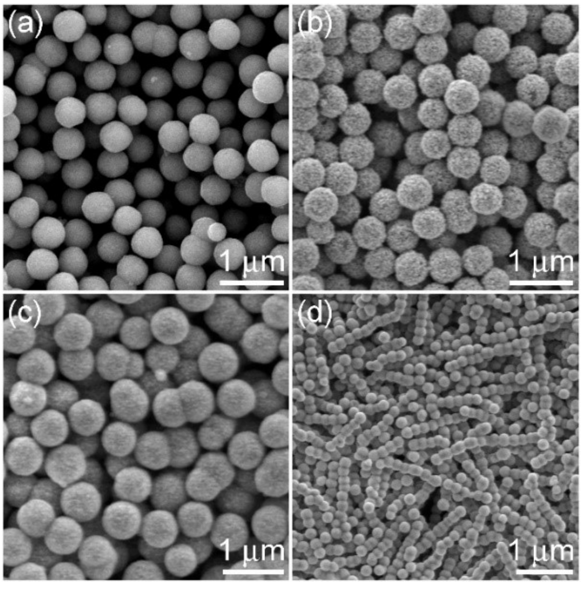

Fig. S4 Characterization of magnetic nanostirrers synthesized by using Fe_3O_4 nanospheres (500 nm) as magnetic cores. SEM images of (a) Fe_3O_4 nanospheres, (b) MBA-modified Fe_3O_4/Au hybrid nanospheres and (c) MBA-modified Fe_3O_4/Au hybrid nanospheres with thin silica coating. (d) Fe_3O_4/Au hybrid nanostirrers by using product (c) as building blocks.

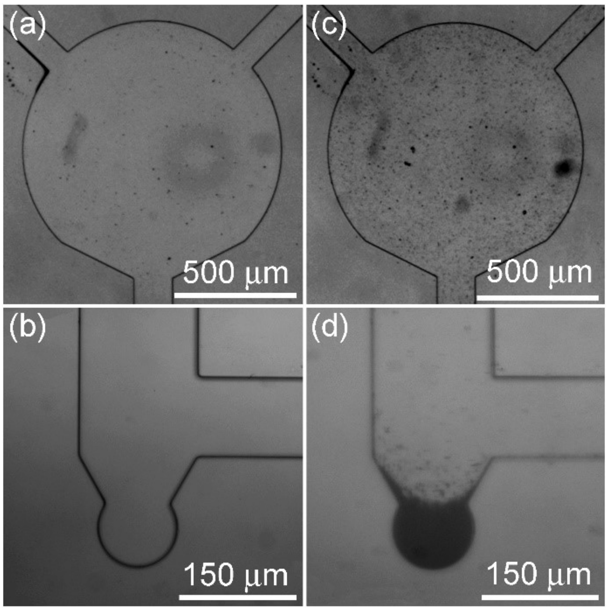

Fig. S5 Optical images of blank (a) big chamber and (b) small chamber, and (c) big chamber filled with sample containing magnetic hybrid nanostirrers and SERS nanoprobes and (d) small chamber filled with SERS nanoprobes conjugated magnetic nanostirrers.

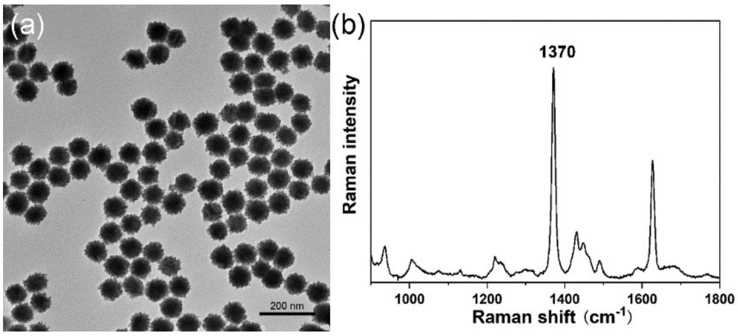

Fig. S6 TEM image of spiky Au nanoparticles and SERS spectrum of TFTP-modified spiky Au nanoparticles.

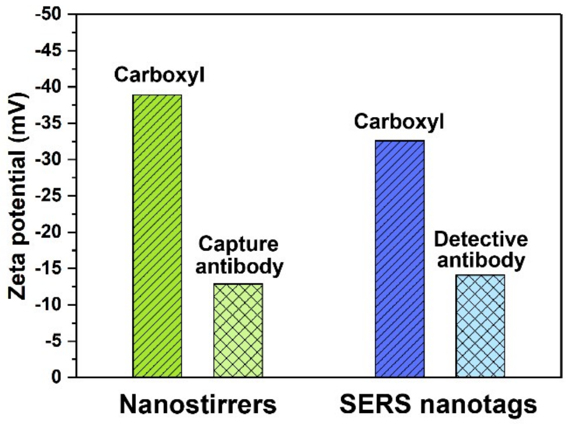

Fig. S7 Zeta potential of magnetic hybrid nanostirrers and SERS nanotags modified with carboxyl and antibodies.

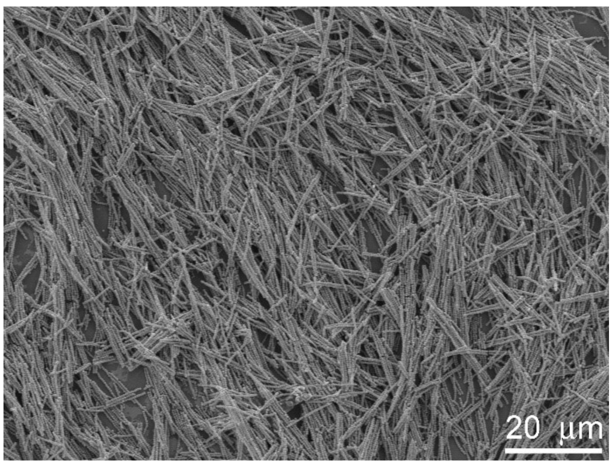

Fig. S8 SEM image of magnetic nanostirrers with average length about 20 µm.

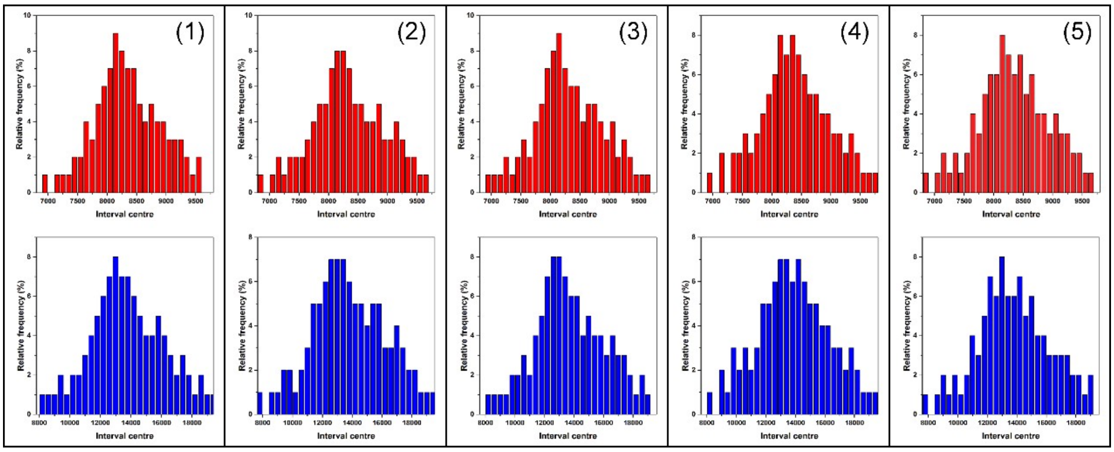

Fig. S9 Column distributions of beacon and SERS nanoprobe signals collected from five tests.

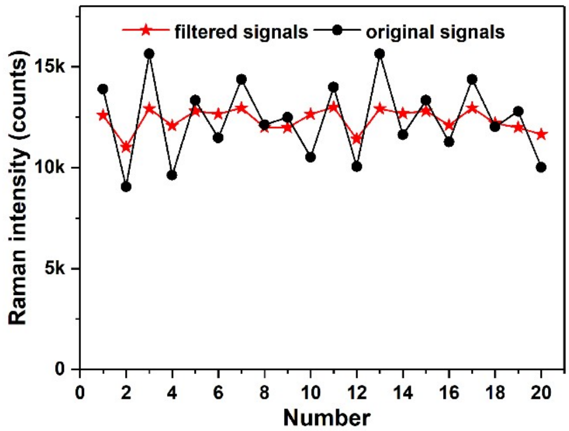

Fig. S10 Average intensity of original (black dots) and filtered (red stars) SERS nanoprobe signals in twenty independent tests (twenty repeats in each test).