Electronic Supplementary Information (ESI)

Molecularly imprinted polymer-based SERS sensing of Transferrin in human serum

Xinyi Wang [a], Anran Liu [a,*], Songqin Liu [a]

[*]Corresponding authors:

Dr. Anran Liu, Jiangsu Engineering Laboratory of Smart Carbon-Rich Materials and Device (CMD), Jiangsu Province Hi-Tech Key Laboratory for Bio-medical Research, School of Chemistry and Chemical Engineering, Southeast University, Nanjing, 211189, P. R. China. Email: liuar@seu.edu.cn

a. Jiangsu Engineering Laboratory of Smart Carbon-Rich Materials and Device (CMD), Jiangsu Province Hi-Tech Key Laboratory for Bio-medical Research, School of Chemistry and Chemical Engineering, Southeast University, Nanjing, 211189, P. R. China.

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1. Materials and methods

1.1 Chemicals and materials

Chloroauric acid trihydrate ($HAuCl_4 \cdot 3H_2O$), sodium hydroxide (NaOH), sodium chloride, disodium phosphate dihydrate, sodium phosphate dibasic dodecahydrate, acetonitrile (ACN), anhydrous ethanol, methanol were bought from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Cetyltrimethylammonium bromide (CTAB) was purchased from J&K Scientific Co., Ltd. (Beijing, China). Hydroquinone, 4-formylphenylboronic acid (FPBA), sodium cyanoborohydride ($NaBH_3CN$), PBS buffer (tablets, pH 7.4), ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), 1,6-hexanediamine, anhydrous sodium acetate (NaAc) were acquired from Macklin Biochemical Co., Ltd. (Shanghai, China). Silver nitrate, sodium borohydride ($NaBH_4$), (3-aminopropyl)triethoxysilane (APTES), Isobutyltriethoxysilane (IBTES), tetraethyl orthosilicate (TEOS), 4-Mercaptophenylboronic acid (4-MPBA) were acquired from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Aqueous ammonia (28%), acetic acid (HAc), ethylene glycol were purchased from Ling-Feng (Shanghai, China). Horseradish peroxidase (HRP), bovine serum albumin (BSA), albumin from chicken egg white (OVA), D-glucose, human transferrin (TRF), acid phosphatase (ACP) were all from Sigma-Aldrich (St. Louis, MO, USA). Human serum was from Keygen Biotech. Inc (Nanjing, China). All these reagents were used without further purification. Deionized water was prepared using a Milli-Q purification system with a resistivity of 18.2 MΩ. Silicon wafers (3 mm × 3 mm) were from Crystal Chemical Co., Ltd. (Nanjing, China).

1.2 Apparatus

Transmission electron microscopic (TEM) characterization was carried out on a Talos F200X TEM (Thermo Fisher, USA). UV-Vis extinction spectra were recorded on a Cary 60 UV-Vis spectrophotometer (Agilent. USA). Zeta potentials of nanomaterials were collected by a NanoBrook Omni Zeta potential analyzer (Brookheaxen, USA). Fourier transform infrared spectrum (FT-IR) was carried out on a Nicolet 4700 FT-IR Spectrometer (Thermo, USA) equipped with an attenuated total reflection setup. X-ray diffraction (XRD) patterns were recorded by a Rigaku Ultima IV X-ray diffractometer (Japan). Raman spectra were recorded on the InVia Raman Microscopy (Renishaw, UK) with 20 multiple telephoto and using the 785 nm laser. All Raman spectra were the average of 5 repeated measurements with corrected baseline.

1.3 Preparation of boracic acid-functionalized Fe_3O_4 MNPs

The preparation process based on a previously reported method[1] is shown in **Scheme 1A**, including four steps: 1) preparation of Fe_3O_4 MNPs; 2) preparation of Fe_3O_4@SiO_2 MNPs; 3) preparation of Fe_3O_4@SiO_2@NH_2 MNPs; 3) preparation of Fe_3O_4@SiO_2@FPBA MNPs. The detailed procedures are as

follows.

1.3.1 Preparation of Fe_3O_4 MNPs

The synthesis of Fe_3O_4 MNPs was based on a previously reported method. In short, 2.0 g of $FeCl_3 \cdot 6H_2O$, 13.0 g of 1,6-hexanediamine, and 4.0 g of anhydrous NaAc were mixed with 60 mL of ethylene glycol in a polytetrafluoroethylene-lined autoclave and reacted at 198 ℃ for 6 h. The obtained Fe_3O_4 MNPs were washed with ultra-pure water and anhydrous ethanol for three times each, and finally dried at 50 ℃ in a vacuum overnight.

1.3.2 Preparation of Fe_3O_4@SiO_2 MNPs

200 mL of anhydrous ethanol and 7.5 mL of aqueous ammonia (28%) were added into a 500 mL round-bottomed flask, and then mechanically stirred at a suitable rate. 1.4 mL of TEOS was added immediately, and mechanically stirred for 20 mins at 40 ℃. Ultrasonic dispersion of 200 mg Fe_3O_4 MNPs into 20 mL of anhydrous ethanol, and addition of the resulting suspension to the aforementioned flask were followed by mechanically stirring at 40 °C for 20 minutes. The obtained Fe_3O_4@SiO_2 MNPs were collected by a magnet, washed with ultra-pure water and anhydrous ethanol for three times each, and finally vacuum dried at 50 ℃ overnight. Fe_3O_4@SiO_2 MNPs were sealed at room temperature in a dry centrifuge tube for further use.

1.3.3 Preparation of Fe_3O_4@SiO_2@NH_2 MNPs

The above Fe_3O_4@SiO_2 MNPs were dispersed into 100 mL of absolute alcohol by ultrasonication in a 250 mL round-bottomed flask. Then, 3 mL of APTES was added to the flask and mechanically stirred overnight at 80 ℃ to generate amino-functionalized Fe_3O_4@SiO_2 MNPs. The obtained Fe_3O_4@SiO_2@NH_2 MNPs were collected by a magnet, washed with ultra-pure water and anhydrous ethanol for three times each, and finally vacuum dried at 50 ℃ overnight. The products were sealed at room temperature in a dry centrifuge tube for further use.

1.3.4 Preparation of Fe_3O_4@SiO_2@FPBA MNPs

100 mg of Fe_3O_4@SiO_2@NH_2 MNPs were ultrasonically dispersed into 50 mL of methanol in a 100 mL three-necked round-bottom flask, followed by 400 mg of FPBA and 300 mg of $NaBH_3CN$. Then, the mixture was mechanically stirred at room temperature for 24 h. The obtained through magnet collection Fe_3O_4@SiO_2@FPBA MNPs were washed with ultra-pure water and anhydrous ethanol for three times each, and finally vacuum dried at 50 ℃ overnight. The products were sealed at room temperature in a dry centrifuge tube for further use.

1.4 Optimization of the incubation time and the incubation concentration of TRF templates

The incubation time and the incubation concentration of TRF templates with Fe_3O_4@SiO_2@FPBA MNPs were optimized according to the adsorption isotherms.

To optimize the incubation concentration of TRF templates, 2 mg of Fe_3O_4@SiO_2@FPBA MNPs were dispersed in different concentration of TRF in PBS buffer (100 mM, pH 8.5) by ultrasonication, and then shaken violently at room temperature for 2 h. The resulting TRF-immobilized Fe_3O_4@SiO_2@FPBA MNPs were magnetically collected, and the amount of TRF captured by Fe_3O_4@SiO_2@FPBA MNPs was determined by UV-Vis detection of the supernatants at 280 nm compared to the original protein solutions.

To optimize the incubation time of TRF templates, 2 mg of Fe_3O_4@SiO_2@FPBA MNPs were dispersed in 0.6 mg/mL of TRF in PBS buffer (100 mM, pH 8.5) by ultrasonication, and then shaken violently at room temperature for a period of time (10, 20, 30, 40, 50, 60, 80 mins). The resulting TRF-immobilized Fe_3O_4@SiO_2@FPBA MNPs were magnetically collected, and the amount of TRF captured by Fe_3O_4@SiO_2@FPBA MNPs was determined by UV-Vis detection of the supernatants at 280 nm compared to the original protein solutions.

The binding ability (Q_e, mg/g) of MNPs to target analytes was calculated as follows.

$$Q_e = \frac{V(C_o - C_e)}{W}$$

Among them, C_o, C_e, V, and W are the initial concentration before adsorption, the equilibrium concentration after adsorption, the volume of standard solution (1 mL), and the mass of MNPs (2 mg), respectively.

1.5 Optimization of monomer composition and imprinting time for the preparation of MIPs

1 mg of MIPs and NIPs prepared with 9 different compositions of the monomers of APTES, IBTES and TEOS for certain imprinting time (See **Fig. S9** for the compositions and imprinting time) were dispersed uniformly into 0.5 mL of TRF standard solutions of 1 mg/mL in PBS buffer (100 mM, pH 7.4), and then shaken violently at room temperature for 1 h. The resulting MNPs were magnetically collected and washed 2 times with PBS buffer (100 mM, pH 7.4), followed by dispersing in 0.5 mL PBS buffer (100 mM, pH 7.4). Subsequently, 50 μL of the resulting suspension was added into a microcentrifuge tube, and then the resulting MNPs were magnetically collected. Then, 300 μL of MPBA-Au NRs dispersed in PBS buffer (10 mM, pH 7.4) was added into each microcentrifuge tube, and the MNPs were dispersed uniformly in this solution, shaken for 30 mins at room temperature. The obtained MNPs were magnetically collected and washed 3 times with PBS buffer (10 mM, pH 7.4). Finally, the MIPs-TRF-MPBA-Au NRs sandwich coupling compound was redispersed and the dispersed solution was dropped onto the silicon wafer, dried and analyzed by a Raman spectrometer.

1.6 Determination of imprinting efficiency

2 mg of Fe_3O_4@SiO_2@FPBA MNPs and MIPs were dispersed in 0.6 mg/mL of TRF in PBS buffer (100 mM, pH 8.5) by ultrasonication, and then shaken violently at room temperature for 1 h. After magnetic separation of MNPs, three times with 2mL PBS buffer solution (100 mM, pH 8.5). The obtained MNPs containing template proteins were dispersed into 0.5 mL of ACN:H_2O:HAc = 50:49:1 (v/v) by ultrasonication and shaken violently for 3 h at room temperature, and the amount of TRF captured by Fe_3O_4@SiO_2@FPBA MNPs and MIPs was determined by UV-Vis detection of the supernatants at 230 nm. The imprinting efficiency was calculated by dividing the binding amount of TRF on MIPs by the binding amount on Fe_3O_4@SiO_2@FPBA MNPs. The experiment was repeated three times. The imprinting efficiency was 64.5% that was excellent for macromolecular imprinting[2].

1.7 Optimization of the SERS probe

To optimize the concentration of MPBA for the preparation of SERS probes, different volumes (10, 20, 30, 40, 50 μL) of 10 mM MPBA (dissolved in 0.2 M NaOH) were added to 1 mL Au NRs solution to get different reaction concentrations (100, 200, 300, 400, 500 μM) of MPBA and the mixed solutions were stirred for 60 mins at room temperature. The obtained MPBA-Au NRs were centrifuged at 13000 rpm for 20 minutes, and redispersed in water. Finally, the dispersed solution was dropped onto the silicon wafer, dried and analyzed by a Raman spectrometer.

To optimize the reaction time between MPBA and Au NRs, 30 μL of 10 mM MPBA (dissolved in 0.2 M NaOH) was added to 1 mL Au NRs solution and the mixed solution was stirred for different reaction time (5, 10, 20, 30, 40, 50 mins) at room temperature. The obtained MPBA-Au NRs were centrifuged at 13000 rpm for 20 minutes, and redispersed in water. Finally, the dispersed solution was dropped onto the silicon wafer, dried and analyzed by a Raman spectrometer.

2. Supporting Figures

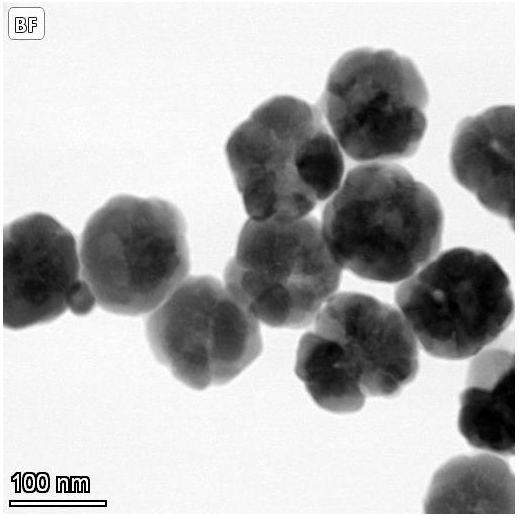

Fig. S1 TEM image of Fe_3O_4@SiO_2@FPBA MNPs.

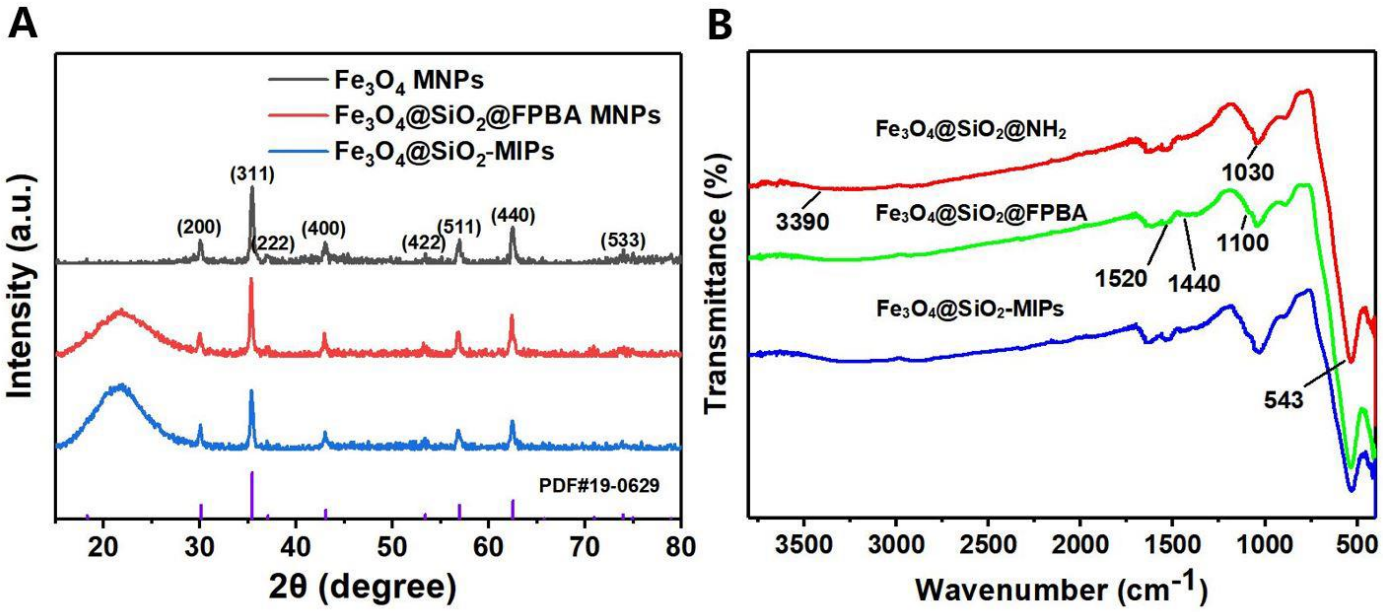

Fig. S2 (A) XRD spectra of Fe_3O_4 MNPs, Fe_3O_4@SiO_2@FPBA MNPs, and Fe_3O_4@SiO_2-MIPs. (B) FT-IR spectra for Fe_3O_4@SiO_2@NH_2 MNPs, Fe_3O_4@SiO_2@FPBA MNPs, and Fe_3O_4@SiO_2-MIPs.

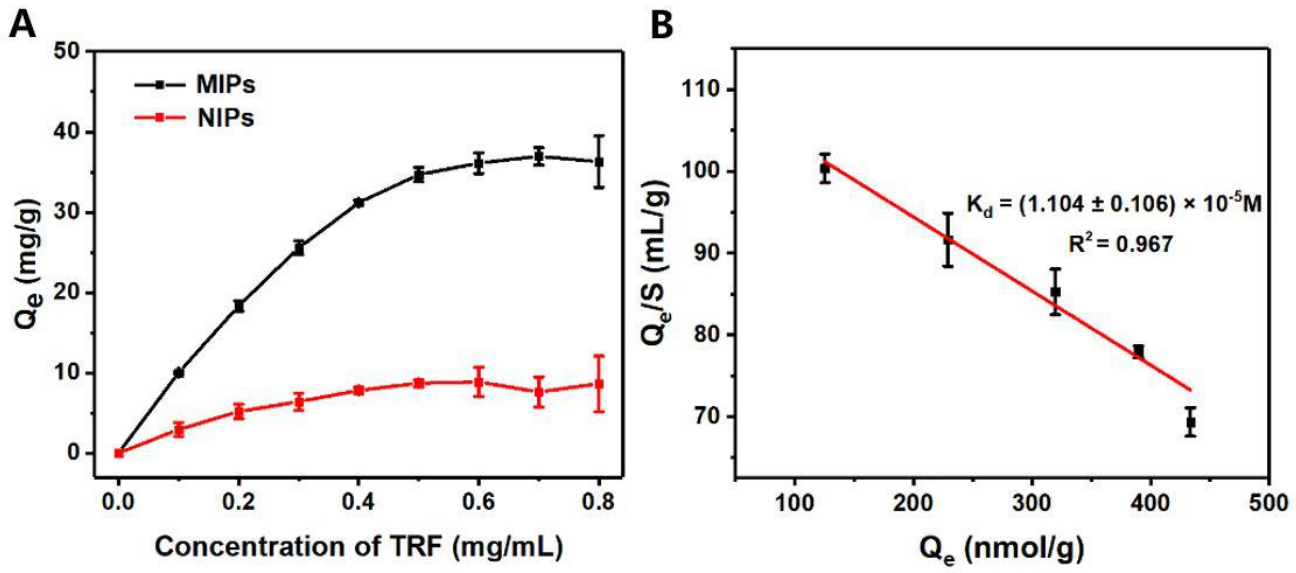

Fig. S3 (A) Binding isotherms for the binding of TRF-imprinted MNPs and non-imprinted MNPs to TRF. (B) Scatchard plot for the binding between Fe_3O_4@SiO_2-MIPs and TRF. The error bars represent the standard deviation of three parallel experiments.

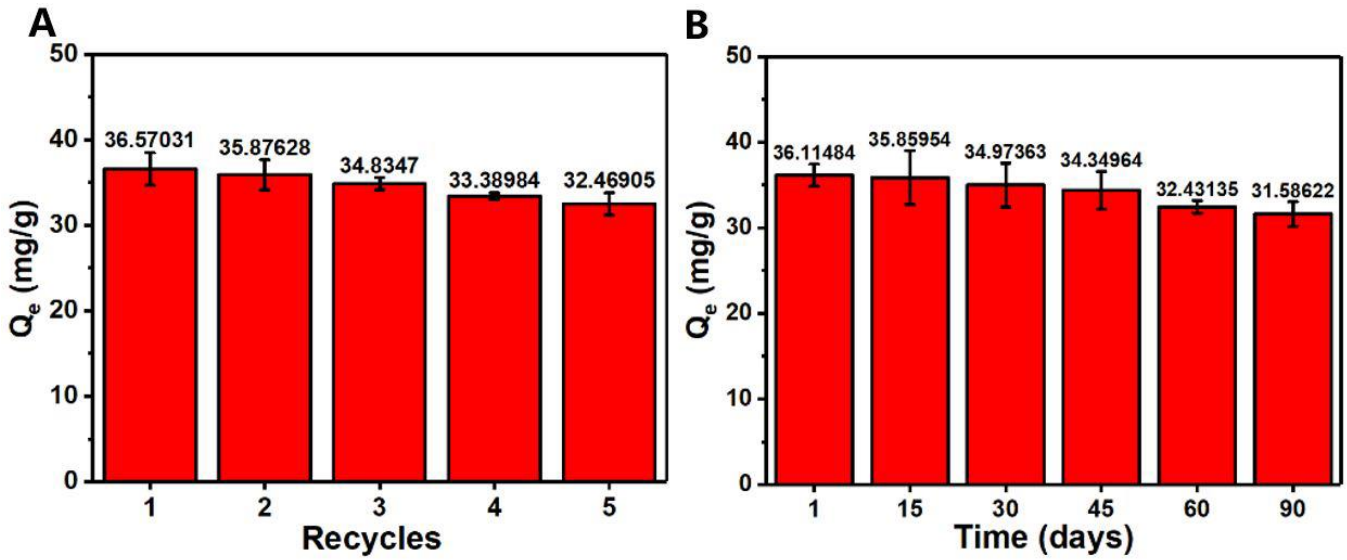

Fig. S4 The amount of TRF adsorbed by Fe_3O_4@SiO_2-MIPs in 0.6 mg/mL TRF (dissolved in 100 mM PBS buffer, pH 7.4) solution (A) with different regeneration times, and (B) at different storage time. The error bars represent the standard deviation of three parallel experiments.

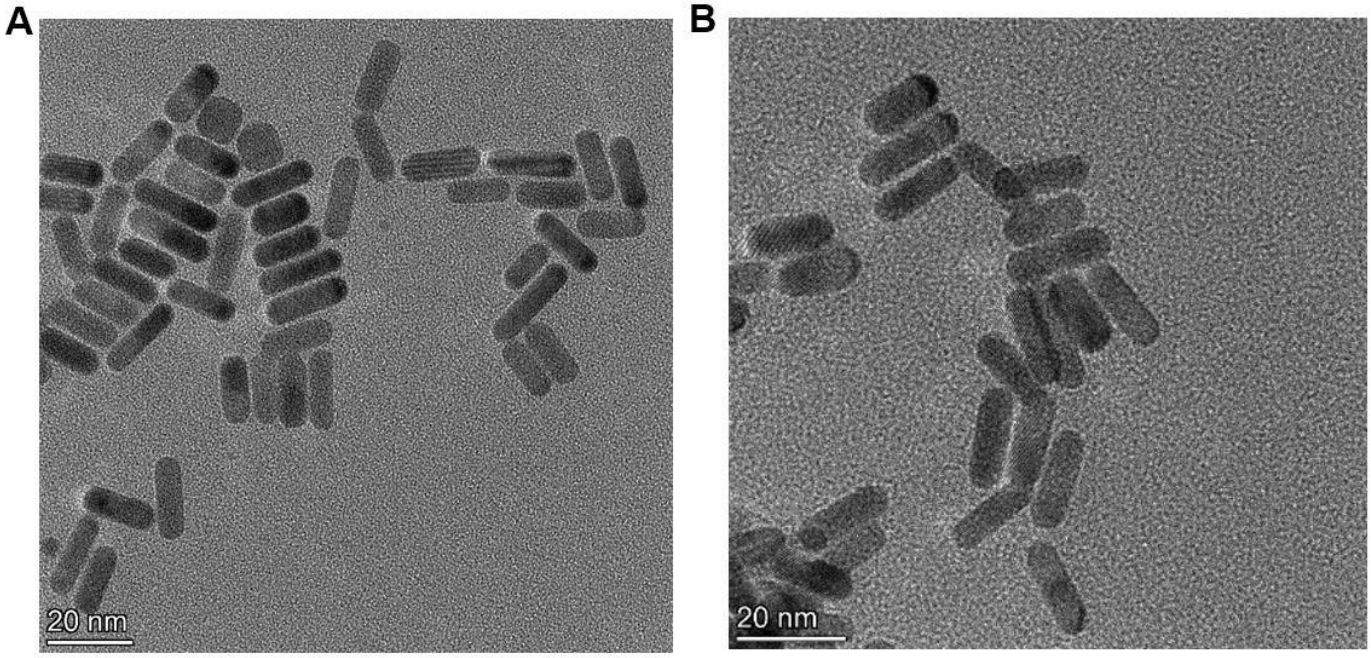

Fig. S5 (A)(B) TEM images of Au NRs.

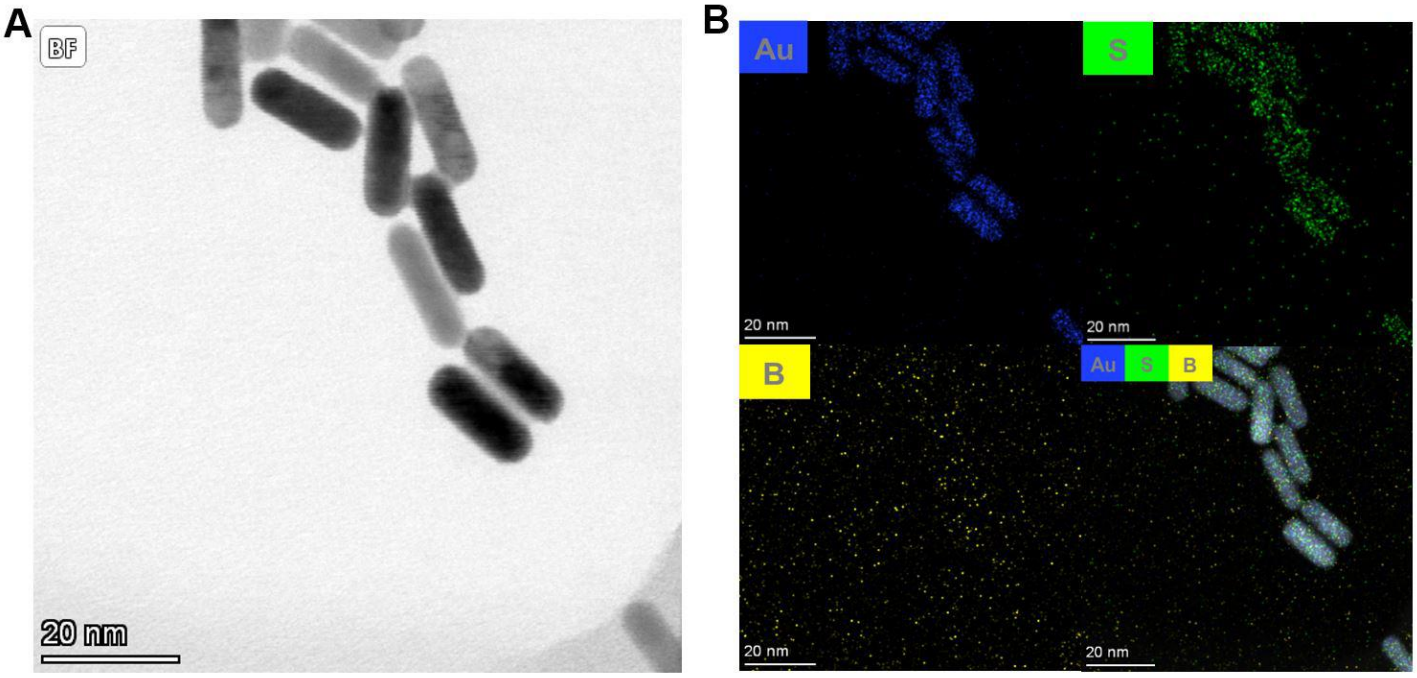

Fig. S6 (A) TEM image of MPBA-Au NRs. (B) EDS elemental mapping of MPBA-Au NRs including Au, B, and S.

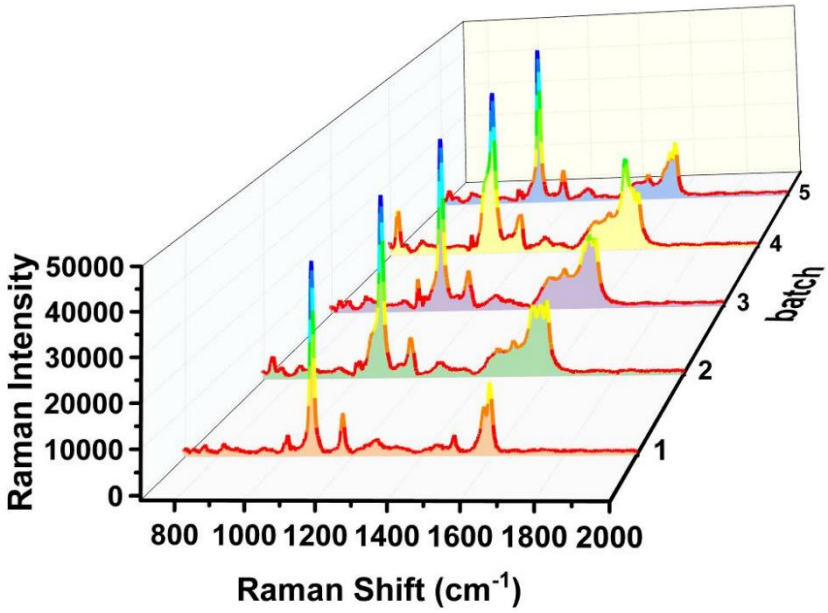

Fig. S7 Raman intensity of five parallel MPBA-Au NRs.

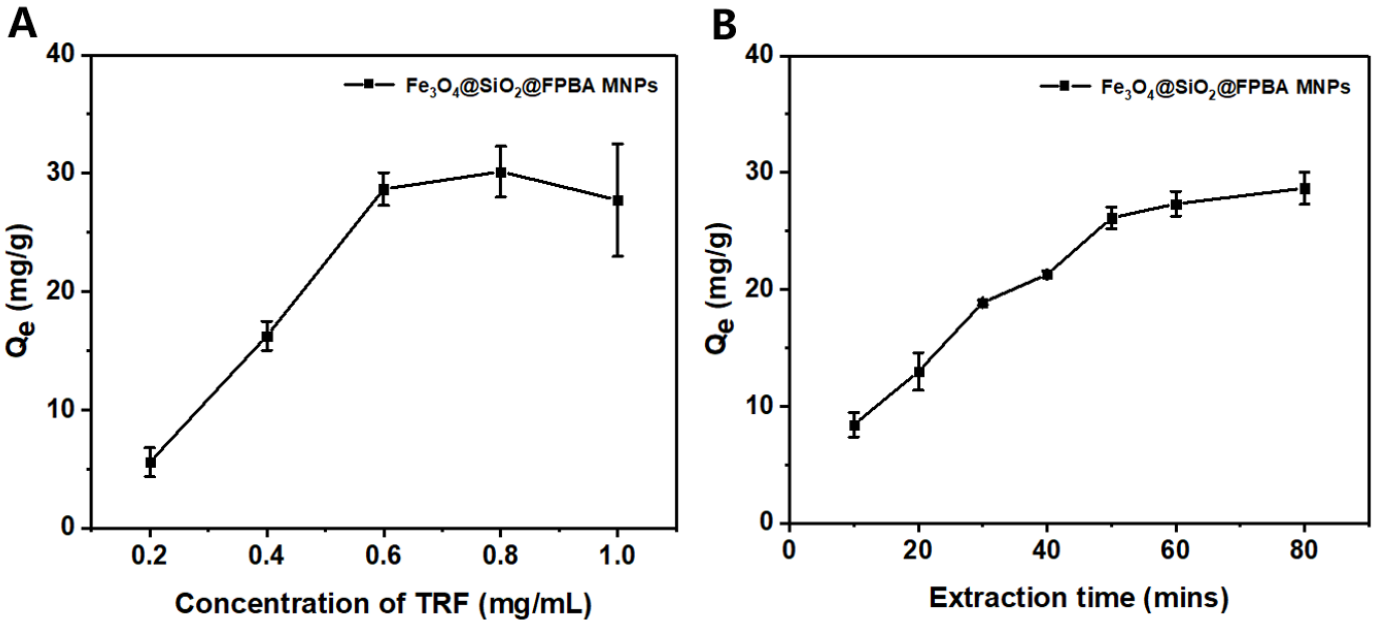

Fig. S8 Optimization of (A) concentration of the template glycoprotein and (B) incubation time between TRF (dissolved in 100 mM PBS buffer, pH 8.5) and Fe_3O_4@SiO_2@FPBA MNPs for the MIP preparation. The error bars represent the standard deviation of three parallel experiments.

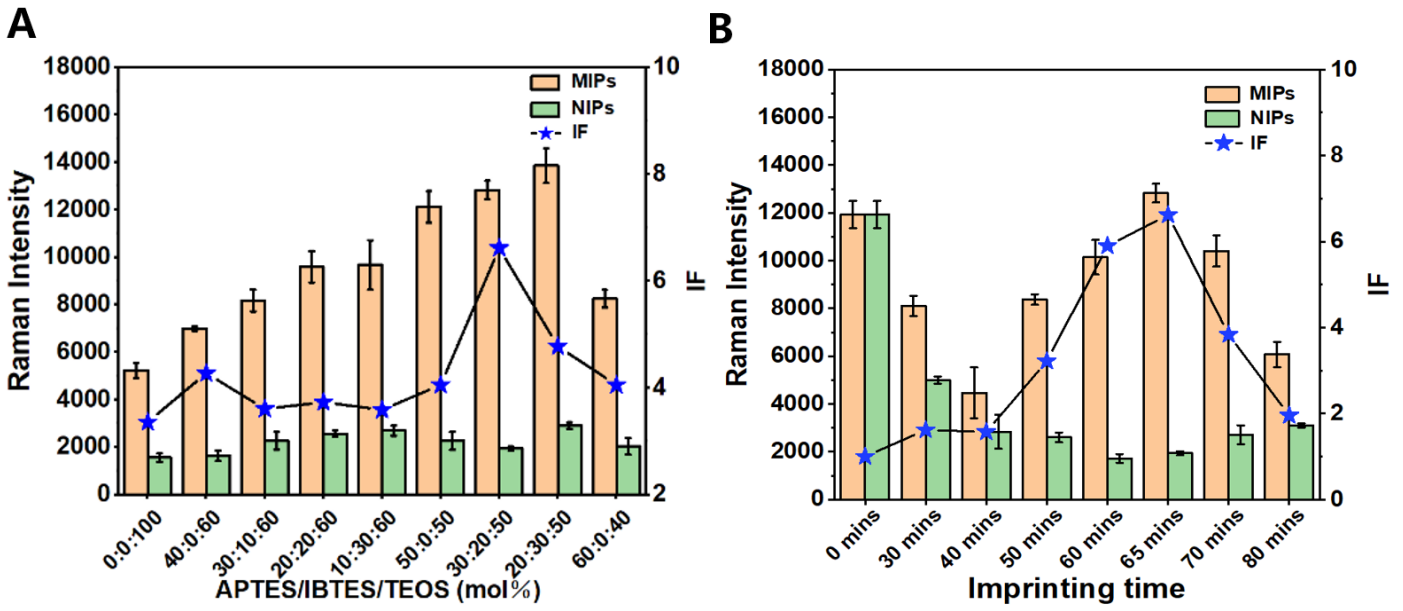

Fig. S9 Optimization of (A) proportions of monomers and (B) imprinting time for the preparation of Fe_3O_4@SiO_2-MIPs and Fe_3O_4@SiO_2-NIPs.

Note: In this work, in order to obtain the best recognition performance, different ratios of functional molecules to crosslinking agents were set under the optimal polymerization time (65 mins). And, different imprinting time was set under the optimal ratio of functional molecules to crosslinking agents (APTES/IBTES/TEOS = 30: 20: 50).

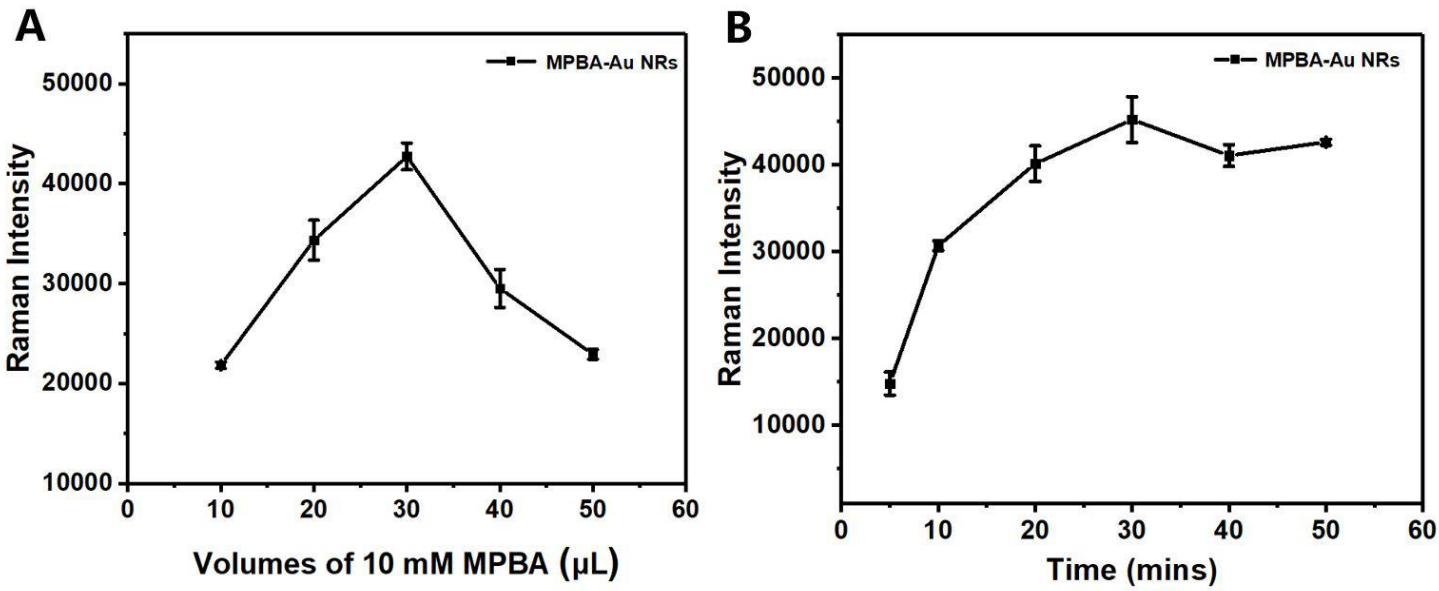

Fig. S10 Optimization of (A) concentration of MPBA and (B) reaction time for the preparation of SERS probes. The error bars represent the standard deviation of three parallel experiments.

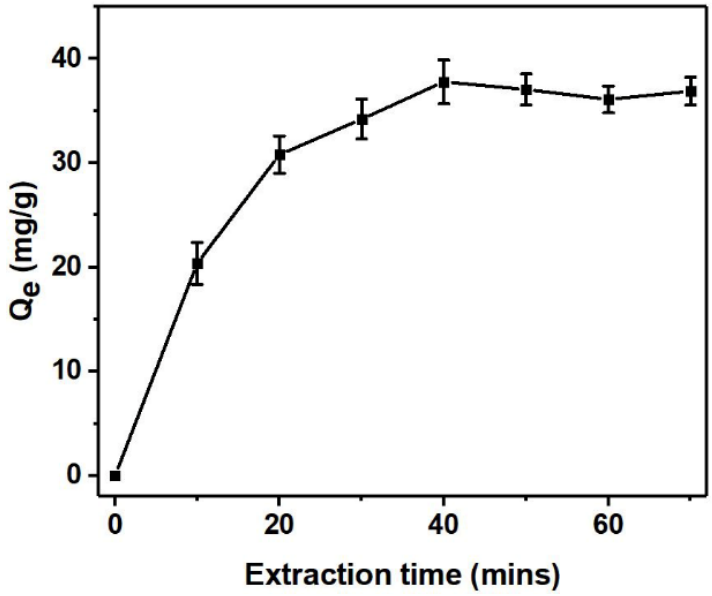

Fig. S11 The amount of TRF adsorbed by Fe_3O_4@SiO_2-MIPs in 0.6 mg/mL TRF (dissolved in 100 mM PBS buffer, pH 7.4) solution with different extraction time. The error bars represent the standard deviation of three parallel experiments.

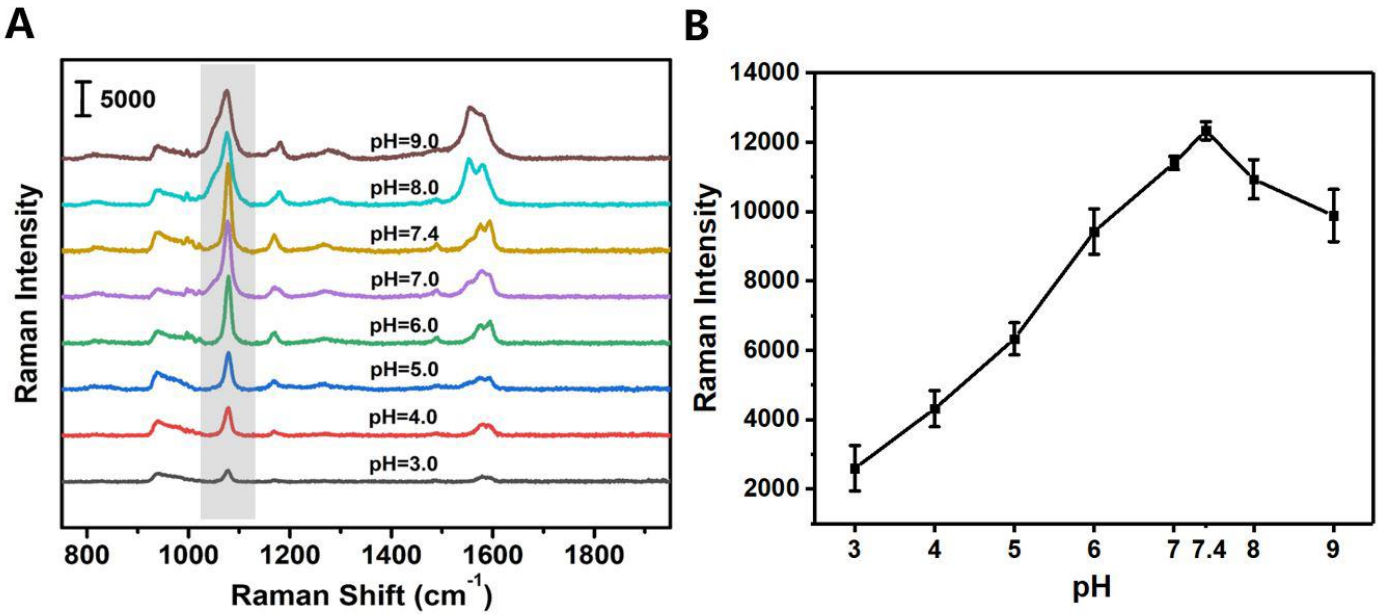

Fig. S12 (A) SERS spectra of the sandwich system detecting 1 mg/mL TRF (dissolved in 100 mM PBS buffer) solution at different pH. (B) Optimization of pH for the detection. The error bars represent the standard deviation of three parallel experiments.

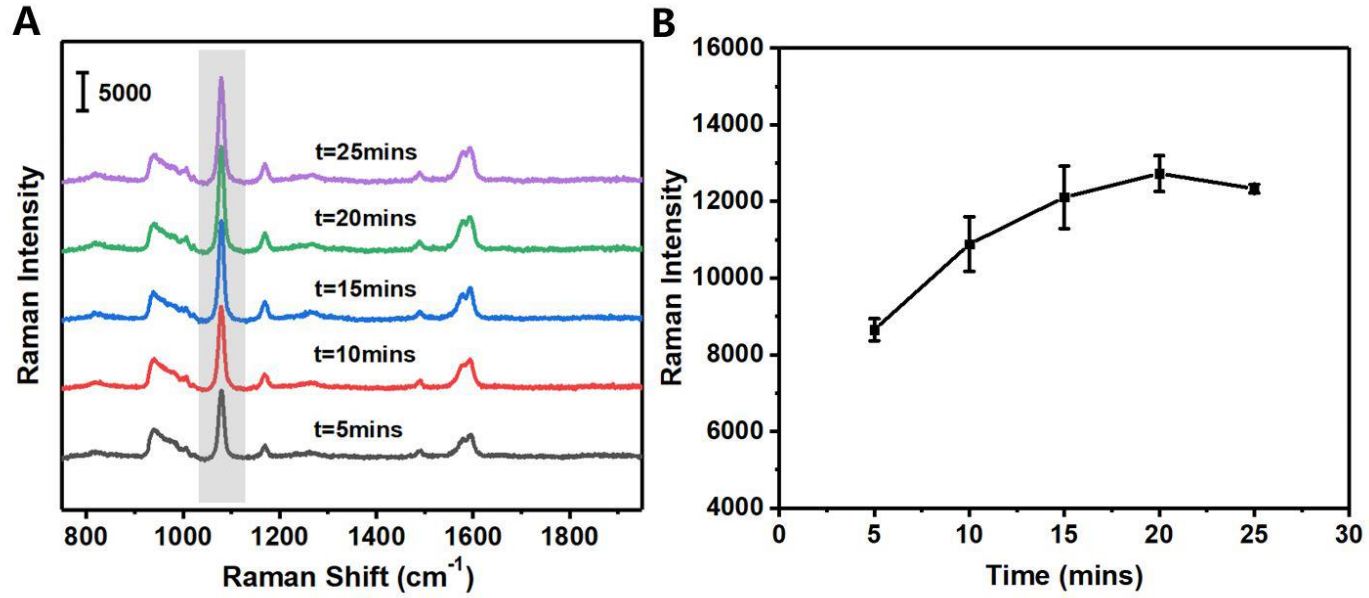

Fig. S13 (A) SERS spectra of the sandwich system detecting 1 mg/mL TRF (dissolved in 100 mM PBS buffer, pH 7.4) solution at different labeling time. (B) Optimization of labeling time for the detection. The error bars represent the standard deviation of three parallel experiments.

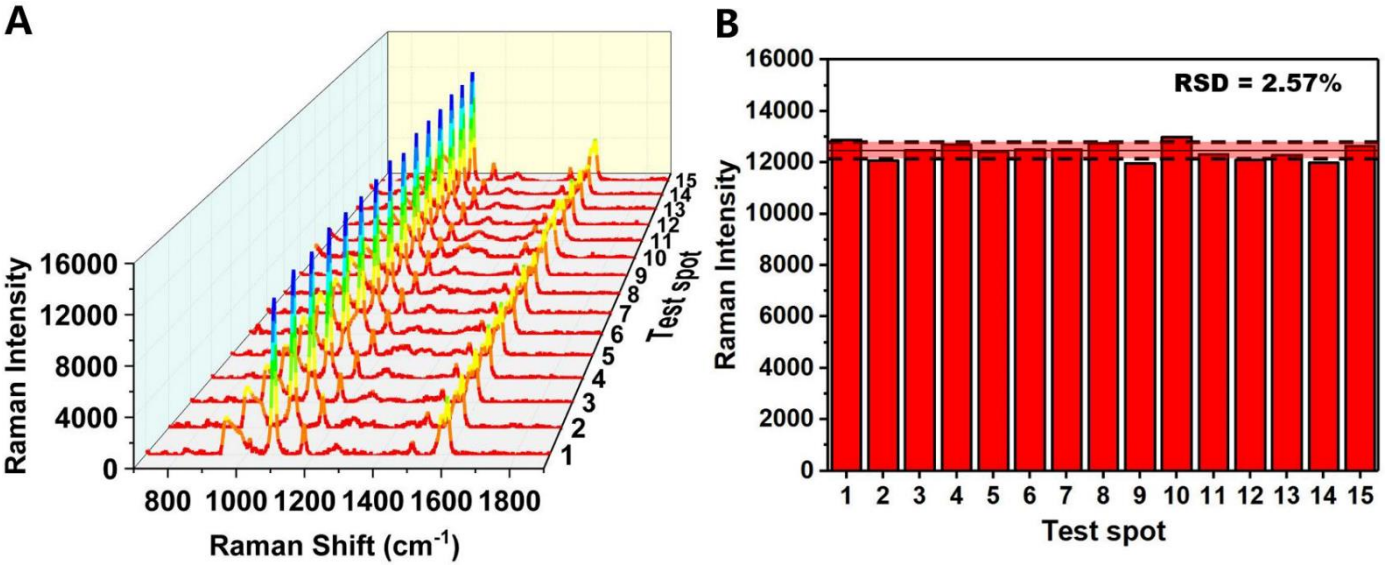

Fig. S14 (A) SERS spectra of the sandwich system detecting 1 mg/mL TRF by fifteen parallel measurements. (B) Corresponding Raman peak intensity at 1080 cm^{-1}.

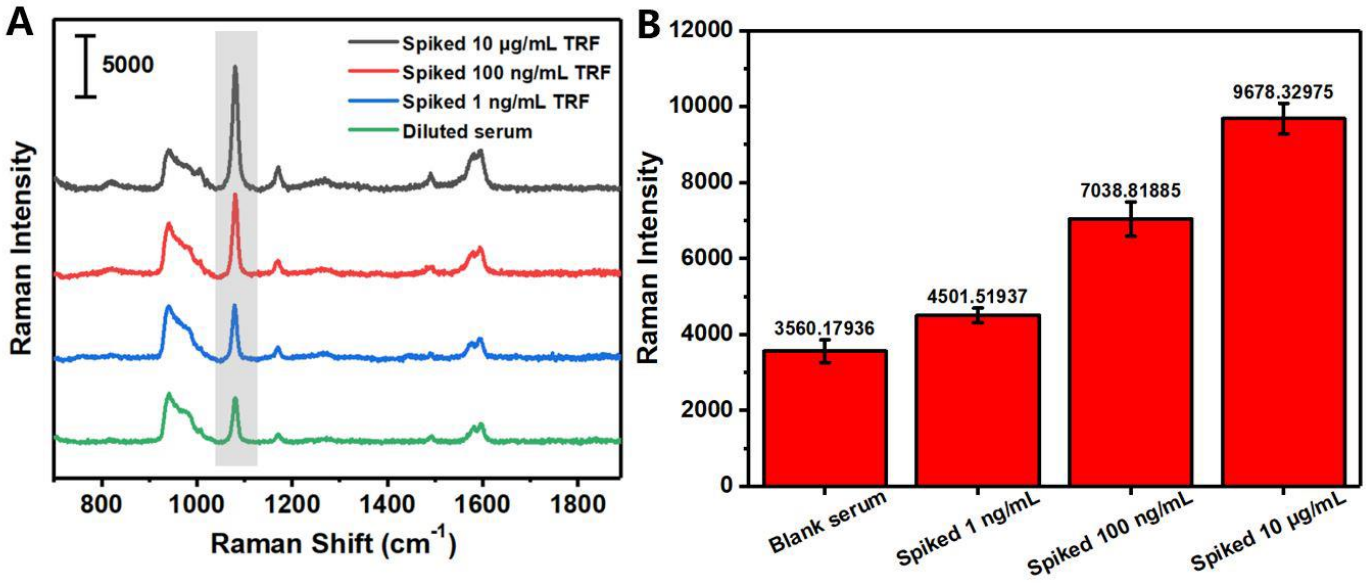

Fig. S15 (A) SERS spectra for detecting serum samples（diluted by 10^7 times with 10 mM PBS buffer, pH 7.4）spiked with different TRF concentrations. (B) SERS intensity for serum samples spiked with different TRF concentrations. The error bars represent the standard deviation of three parallel experiments.

References

1 Y. Ma, W. Li, R. Xing, P. Li and Z. Liu, *ACS Sensors*, 2020, **5**, 2537–2544.

2 H. Zhang, *Adv. Mater.*, 2020, **32**, 116043.