Supporting Information

Antibody-Free Discrimination of Protein Biomarkers in Human Serum Based on Surface-Enhanced Raman Spectroscopy

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Experimental details

1. Materials.

PTCA, bovine serum albumin (BSA), pepsin (Pep), Egg white albumin (EA), Trypsin (Try), Hemoglobin (Hem), Horseradish Peroxidase (HRP), Myoglobin (Myo), Cytochrome C (Cyt c), glucose oxidase (GO), Lysozyme (Lys), $AgNO_3$, and poly(diallyldimethylammonium chloride) (Mw = 200,000−350,000, 20 wt% aqueous solution) were obtained from Sigma-Aldrich. 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), and N-hydroxysuccinimide (NHS) were obtained from J&K Chemical Co. Alpha-fetoprotein (AFP) was obtained from chemiluminescence kit purchased from Beckman Coulter (Product Name: AFP (300 Test Kit); Part Number: 33211; Series Name: ACCESS®). The phosphate buffered saline (PBS; 0.01 M, pH 7.2) used in this study contained 0.8% NaCl, 0.02% KH_2PO_4, 0.02% KCl, and 0.12% $Na_2HPO_4 \cdot 12H_2O$. All chemicals were analytical-grade reagents and used without further purification. Milli-Q water was used in the study. The clinical serum sample was obtained from a patient at the Third Hospital of Jilin University (P.R. China).

2. Preparation of self-assemble chips

Ag hydrosol was synthesized by a conventional synthetic route reported by Lee and Meisel. In brief, 36 mg of $AgNO_3$ was dissolved in 200 mL of water (1.0mM aqueous), and then the solution was heated to 98 °C with rapid stirring under reflux. A 4 mL solution of 1% sodium citrate was added to the solution, and the solution was boiled for 40 min. The Ag nanoparticles with the diameter of 60-70 nm were prepared. Subsequently, glass slides were immersed in a boiling solution prepared by mixing 30% H_2O_2 and 98% H_2SO_4 with a volume ratio of 3:7. After rinsing, the hydroxylated glass slides were obtained. Then they were soaked in a 0.5% PDDA solution for 40 min. After repeated rinsing with water and drying with nitrogen gas, the PDDA-coated slides were soaked in silver hydrosol for 4 h. Eventually, a layer of Ag NPs was assembled on the glass surface by electrostatic interaction.

The glass chips were subsequently immersed into an PTCA aqueous solution (10^{-4} M) for 12 h. The chips were rinsed three times with Milli-Q water and dried with nitrogen gas.

3. Activate carboxyl groups of PTCA

The glass chips were immersed in an aqueous solution of EDC/NHS with concentration of 0.1M. After 3 h, the chips were rinsed three times with Milli-Q water and dried with nitrogen gas. The chips were stored in the plastic centrifuge tube under a nitrogen atmosphere.

4. Bonding with proteins

Subsequently, the proteins were immobilized by pipetting 400 μL of proteins solution at different concentrations onto chips. The reaction was allowed to progress overnight at 4 °C or for 4 h at 37 °C. The chips were rinsed three times with PBS and stored in the plastic centrifuge tube under a nitrogen atmosphere.

5. SERS Characterization and Analysis

All SERS spectra were measured on a Jobin Yvon/HORIBA LabRam ARAMIS Raman spectrometer equipped with an HeNe Laser (632.8 nm). The typical exposure time for each chips was 1 s with one-time accumulation with a 0.3D filter. The grating scale is 1800 l mm^{-1}, the spectra resolution is 1.6 cm^{-1}. And the spectra sampling distance is 0.5 cm^{-1}.

6. Serum test

Briefly, the serum samples were immobilized by pipetting 100 μL of serum with 400 μL PBS buffer onto chips, as well as AFP. The bonding reaction was allowed to progress overnight at 4 °C or for 4 h at 37 °C. Then the chips were rinsed three times with PBS and stored in the plastic centrifuge tube under a nitrogen atmosphere.

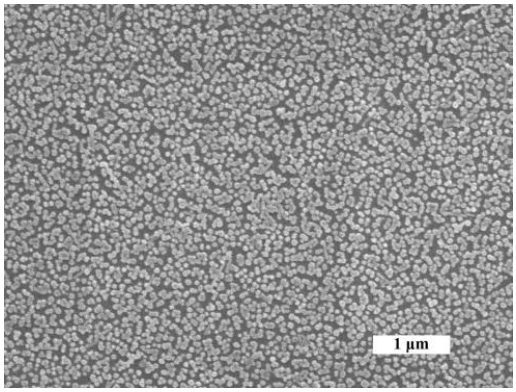

Figure S1. SEM image of the self-assembled silver chip.

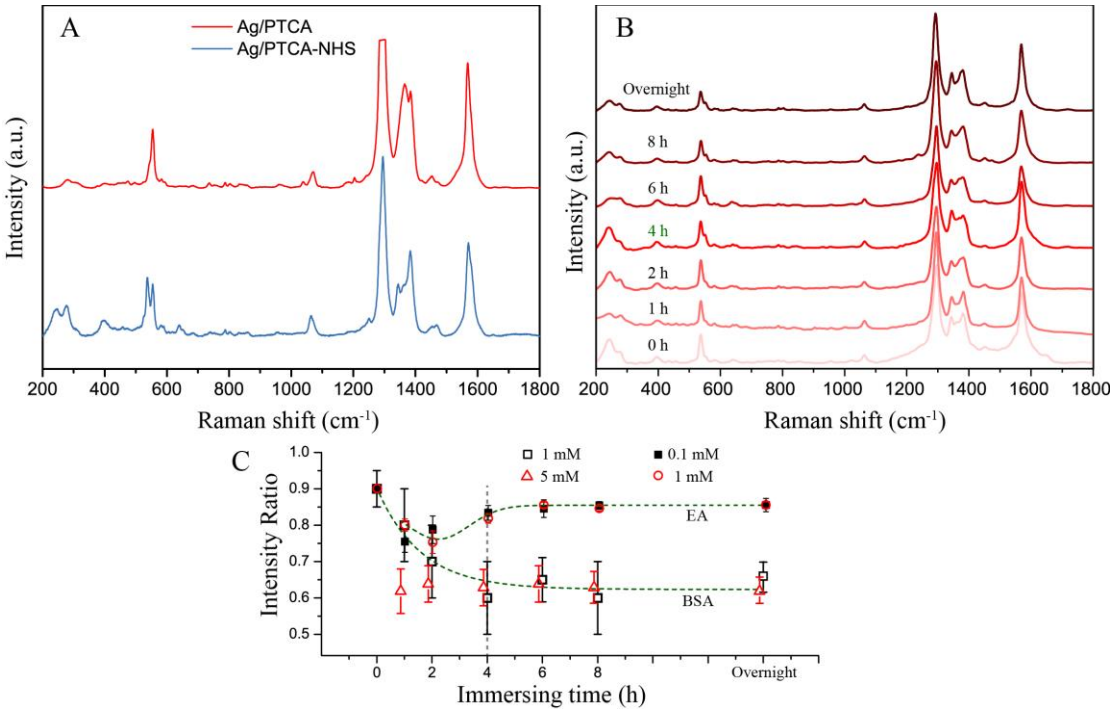

Figure S2. (A) Raman spectrum of Ag/PTCA (red line) and Ag/PTCA-NHS (blue line); (B) SERS spectra of PTCA modified chips with BSA (5 μM) in PBS at different immersion times; (C) Immersing time course of $I_{550}/(I_{550}+I_{537})$ changes of BSA and EA at different concentrations (EA, 0.1■ and 1 mM○; BSA: 1□ and 5 mM△).

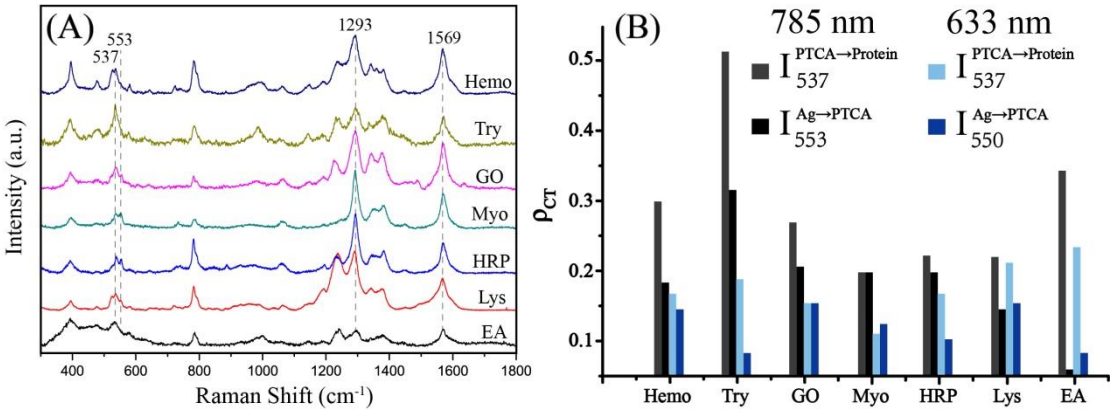

Figure S3. SERS spectra of the chips binding with versatile proteins at excitation of 785 nm (A) and degrees of CT (ρ_{CT}) with different proteins at different excitations of 633, 785 nm (B).

On the basis of our previous work, ρ_{CT}(k) of a k-band can be determined as followed equation:[1,2]

$$\rho_{CT}(k) = \frac{I^k(CT) - I^k(SPR)}{I^k(CT) + I^0(SPR)}$$

Note that one line of I^k(CT) is the intensity of a band which is attributed to SERS intensity by CT resonance. Also, it is important to choose reference bands where there is no CT contribution to SERS intensity, the line of I^k(CT), which originates from SPR. Usually, I^k(SPR) is very small or zero when the CT vibrations are nontotally symmetric. In this system, we selected the ring breathing A_g (A_1) mode at 1293 cm^{-1} as one line of I^0(SPR), which is enhanced in whole spectrum. As for I^k(CT), we selected the band at 537 cm^{-1} and 550 cm^{-1} (b_1 mode) for the investigation of CT of PTCA-to-LUMO of residue (PTCA → Protein) and silver-to-PTCA (Ag → PTCA) individually. Thus, the above equation can be expressed as follows:

$$\rho_{CT}(k) = \frac{I_{Ag\rightarrow PTCA\ \ or\ \ PTCA\rightarrow protein}/I_{1293}}{1 + I_{Ag\rightarrow PTCA\ \ or\ \ PTCA\rightarrow protein}/I_{1293}}$$

As shown in Figure S3B, proteins display different degree of CT under different excitations. It is important to note that the degree of CT decreased with the decrease of the excitation energy which indicates an off-resonance at 633 nm. Another important fact is that the intensity of the band at 537 cm^{-1} is borrowed not only from SPR, but also from the intensity of silver-to-PTCA process.[3] Taken together, we deduced that the gap between HUMO of PTCA and LUMO of the amino acid residue should be lower than 1.96 eV (633 nm), and protein-dependent.

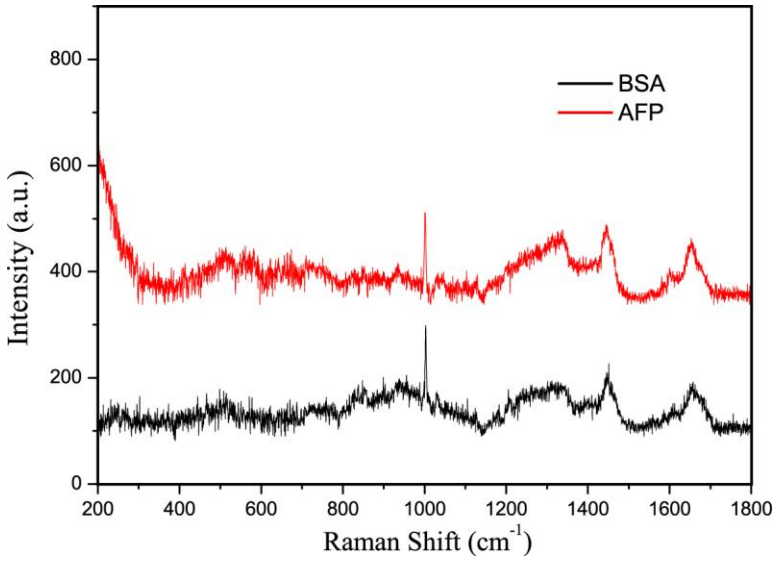

Figure S4. Normal Raman spectra of BSA and AFP.

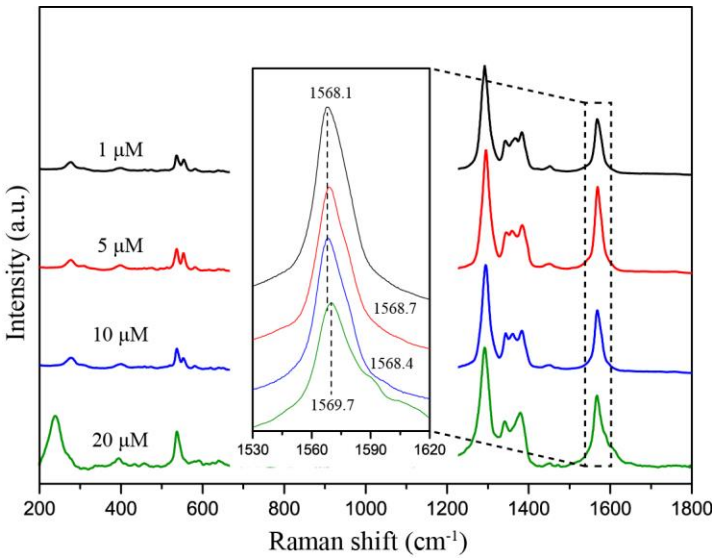

Figure S5. BSA concentration-dependent SERS spectra on the PTCA-modified chips.

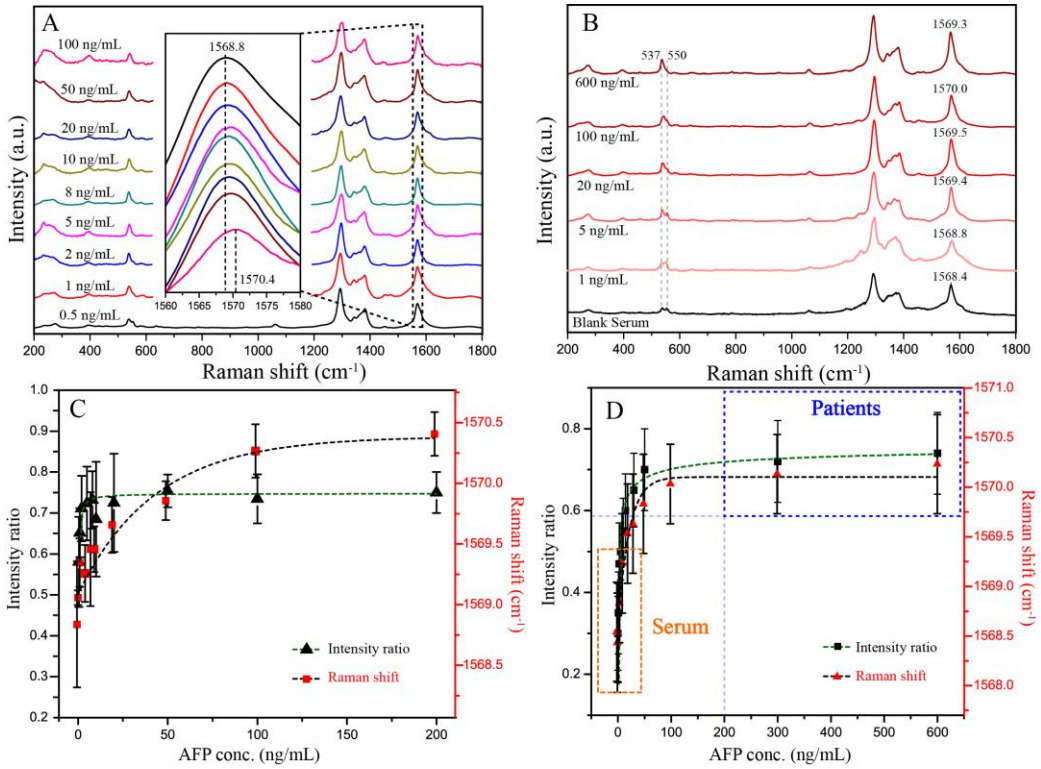

Figure S6. SERS spectra of the PTCA-modified chips with AFP at different concentrations in PBS (A), and in human serum(B); detection curves for AFP in PBS (C) and (D) in serum.

The detection curves for AFP in PBS and serum are shown in Figure 6 C and D, respectively. Since both of the frequency shifts and intensity ratios in the present study are independent of protein concentration of the initial sample (Figure S2C), there is no conventional concentration-dependent calibration curve. The lowest detectable AFP concentrations in serum and PBS buffer are 5 ng/mL and 0.5 ng/mL, respectively.

In the clinical diagnosis, the patients whose AFP concentration is higher than 200 ng/mL would be diagnosed with HCC, and thus we used the same value in our analysis. As shown in Figure S6D (the blue region), when the intensity ratio is higher than 0.6 or the Raman shift is higher than 1569.5, the patient would be determined as a HCC patient. Accordingly, we plotted all the results in Figure 4C.

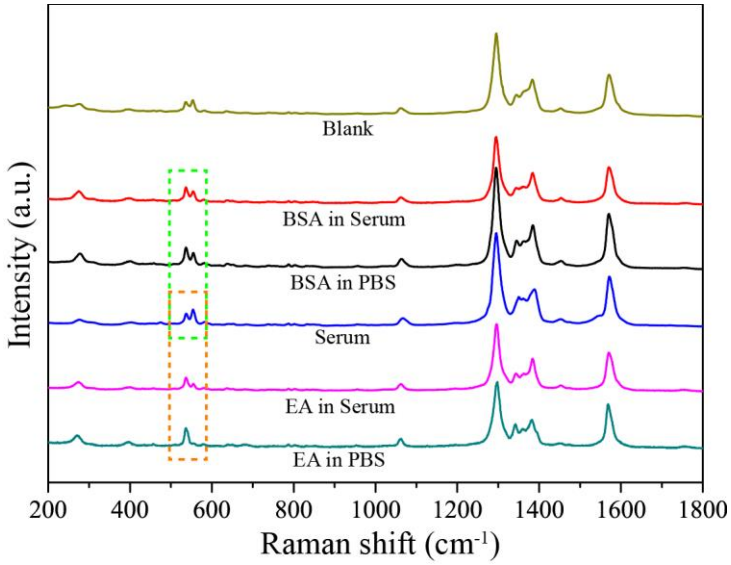

Figure S7. SERS spectra of PTCA modified chips with EA and BSA in the serum and PBS.

The species containing amino groups may indeed interfere with the analytical results. Actually in real practical application, we found the interference from other species in human serum is negligible for AFP detection as shown in Figure S6. Moreover, we choose EA and BSA as two model proteins and serum as a disturber to evaluate the accuracy of the proposed method. As shown in Figure S7, in the green dotted area, the SERS spectra of the BSA in serum and in PBS buffer are almost the same. Although there is a minor difference between the SERS spectra of EA (in serum *vs* in PBS buffer), the fluctuation does not affect the discrimination ability of the present method for EA.

References:

(1) Richter, A. P.; Lombardi, J. R.; Zhao, B. Size and wavelength dependence of the charge-transfer contributions to surface-enhanced Raman spectroscopy in Ag/PATP/ZnO junctions, *J. Phys. Chem. C* **2010**, *114*, 1610-1614.

(2) Wang, X.; Wang, Y.; Sui, H.; Zhang, X.; Su, H.; Cheng, W.; Han, X. X.; Zhao, B. Investigation of charge transfer in Ag/N719/TiO_2 interface by surface-enhanced Raman spectroscopy, *J. Phys. Chem. C* **2016**, *120*, 13078-13086.

(3) Ma, H.; Wang, H.; Li, P.; Wang, X.; Han, X.; He, C.; Zhao, B. Interfacial charge-transfer in TiO_2/PTCA/Ag revealed by surface-enhanced Raman spectroscopy, *J. Phys. Chem. C* **2018**, *122*, 15208-15213.