A Chiral Signal Amplified Sensor for Enantioselective Discrimination of Amino Acid Based on Charge Transfer-Induced SERS

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Experimental Section

Materials and Reagents. All of the chemicals were of analytical reagent grade unless stated otherwise. *p*-Aminothiophenol (PATP, 97%) and $AgNO_3$ (99%) were purchased from Sigma-Aldrich Co., Ltd. and used without further purification. Enantiomers of amino acid, including phenylalanine (Phe), tryptophan (Trp), and tyrosine (Try), and poly dimethyl diallyl ammonium chloride (PDDA, 20 wt.%, aqueous solution) were obtained from Aladdin Chenmical Co., Ltd. (Shanghai, China). All the other chemicals needed were acquired from Sinopharm Group Co., Ltd. Mono(6-mercapto-6-deoxy)-beta-cyclodextrin (SH-*β*-CD) and Heptakis-(6-Mercapto-6-deoxy)-β-Cyclodextrin (7-SH-*β*-CD) were purchased from Shandong Binzhou Zhiyuan Biotechnology Co., Ltd. Distilled and deionized water from a Milli-Q-plus system with a resistivity greater than 18 MΩ•cm was used throughout the study.

Preparation of Ag-TiO₂-Ag Substrate. The Ag NPs were synthesized by using the classical process of Lee and Meisel to prepare Ag NPs substrates.[1] The Ag NPs substrates were prepared via a self-assembled method on the surface of a hydroxylated quartz glass slide by electrostatic interaction.

Porous TiO_2 NPs were synthesized by using the recipe of Cheng et al.[2] Typically, 2 mL of Ti(OBu)₄ was added to 45mL ethylene glycol with vigorous stirring at room temperature for 8 h. The reaction solution was then quickly transferred into a 200 mL acetone solution containing 2.5 mL water and 0.8 mL acetic acid with vigorous stirring at room temperature for another 3h, and the white precipitate was acquired by centrifugation. After washing with ethanol several times, the titanium glycolate precursor was added to 50 mL water with stirring for 8 h at 70 °C. Finally, the TiO_2 colloids were obtained via being washed with water several times, centrifugated, and redispersed in water.

The TiO_2 NPs were spin-coated on the surface of the prepared Ag substrate. The formed Ag-TiO_2 complex substrates were followed with calcination in a muffle furnace at different temperature under the protection of nitrogen. After hydroxylation of the calcinated TiO_2 substrate, the substrate was immersed in the PDDA solution to contain positive electricity. Then, another layer of Ag NPs was assembled on the surface of TiO_2 substrate by electrostatic interaction, and the Ag-TiO_2-Ag substrate was fabricated.

Preparation of Enantioselective Sensor. The SH-*β*-CD molecules with a concentration of 10^{-3} M

were adsorbed on the prepared Ag-TiO$_2$-Ag substrates for 6 h through the formation of Ag−S bonds. After exhaustive being rinsed by ethanol and drying by nitrogen, the substrates with SH-β-CD were immersed in 10^{-4} M PATP solution for 4 h to replace some of the SH-β-CD, and the SH-β-CD and PATP molecules were co-adsorbed on the substrate. After the substrates were rinsed and dried, the enantioselective sensors were prepared, and then they were immersed in various aqueous solutions of amino acid enantiomers with different concentrations for 6 h to fully interact with the enantiomers. The sensors with different amino acid enantiomers were rinsed by water and then dried before SERS measurements.

Instrumentations and Sample Characterization. UV−vis absorption spectra were recorded on a Cary 5000 UV-vis-NIR Spectrophotometer (Agilent, America) at room temperature. Transmission electron microscopy (TEM) measurements were performed by Tecnai-G220 ST (FEI, America). Surface morphology was observed with an SU8010 field emission scanning electron microscope (FE-SEM, Hitachi, Japan). The crystalline phase was acquired by a Rigaku/Dmax-rB X-ray powder diffraction (XRD, PANalytical, Holland). Dynamic light scattering measurements were carried out using Malvern Zetasizer Nano-ZS 90 instrument at 25 °C under corresponding solution conditions.

SERS Measurement A Horiba XploRA Raman spectrometer equipped with the 532, 638, and 785 nm exciting lasers were used for measurement of all Raman and SERS spectra. Efficient laser powers reaching the samples for these three lasers were: 0.01 mW (532 nm), 3 mW (638 nm), and 15 mW (785 nm). A Raman band of a silicon wafer at 520.7 cm^{-1} was used to calibrate the spectrometer.
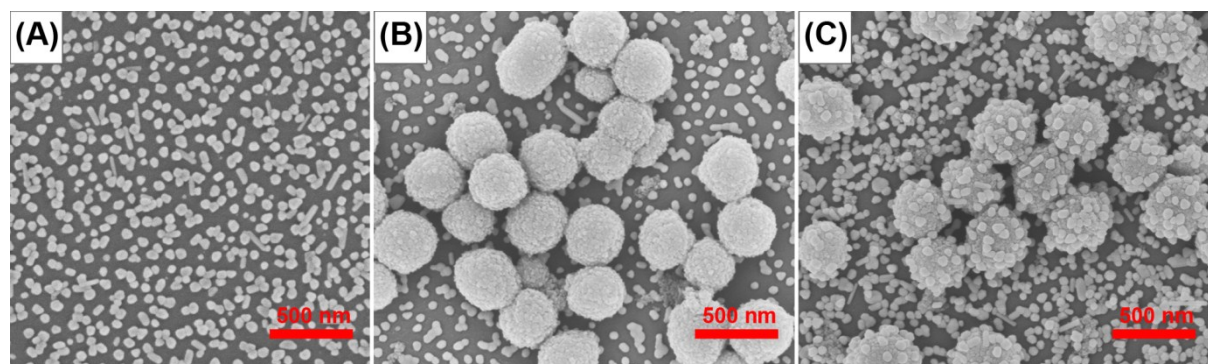
Additional Supporting Graphics

Figure S1. SEM images of the step-by-step fabrication process of Ag-TiO$_2$-Ag substrate: **(A)** Ag, **(B)** Ag-TiO$_2$, and **(C)** Ag-TiO$_2$-Ag
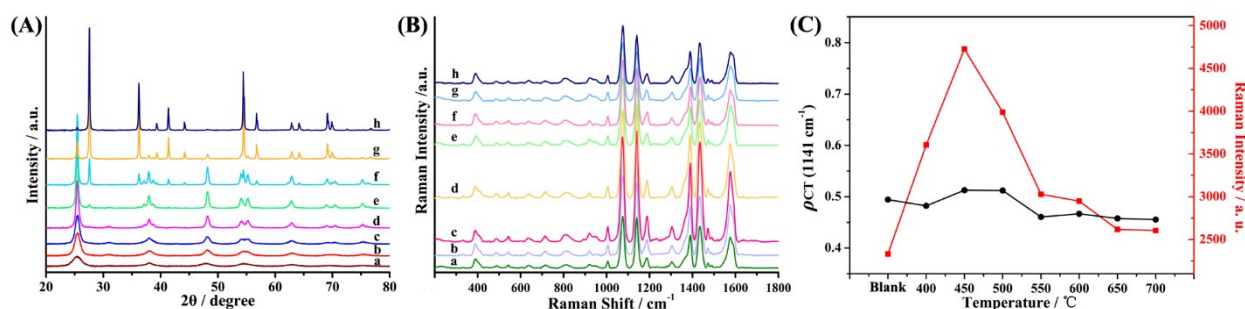

Figure S2. (A) XRD patterns of the prepared TiO$_2$ NPs with different calcination temperatures. **(B)** SERS spectra of PATP molecules adsorbed on the prepared Ag-TiO$_2$-Ag substrates with the TiO$_2$ NPs calcined at different temperatures with the excitation of 638 nm. Calcination temperature: (a) without calcination, (b) 400, (c) 450, (d) 500, (e) 550, (f) 600, (g) 650, (h) 700 ℃. **(C)** Degree of CT (ρ_{CT}) of the b$_2$ mode at 1141 cm^{-1} and the SERS intensity of the a$_1$ mode at 1075 cm^{-1} for PATP as a function of the different calcination temperatures.

For a benzene-like molecule with low-lying unfilled π orbitals with C$_{2v}$ symmetry, a propensity rule can empirically state that the strong and selective enhancement of the non-totally symmetric (b$_2$) modes arises from CT processes from the metal substrates to the adsorbed molecules.[4] The whole intensity of the SERS signal can be derived from both the EM and the CT enhancement, while the b$_2$ modes of the molecules can only be enhanced through CT enhancement. In our enantioselective discrimination system, besides the changes in the whole intensity of the adsorbed PATP molecules, the relative several bands of adsorbed PATP molecules changed obviously, such as the bands at 1139, 1398, 1442 and 1573 cm^{-1}, which are belong to the b$_2$ modes. Therefore, the higher intensities of SERS spectrum of PATP in both b$_2$ and other modes (mainly a$_1$ modes) are, the larger SERS

enhancement is obtained which is originated by EM and CT enhancement mechanism. Therefore, the TiO$_2$ NPs prepared at 450 ℃ were selected as optimal conditions, because the whole intensity as well as the b$_2$ modes of PATP is the highest.
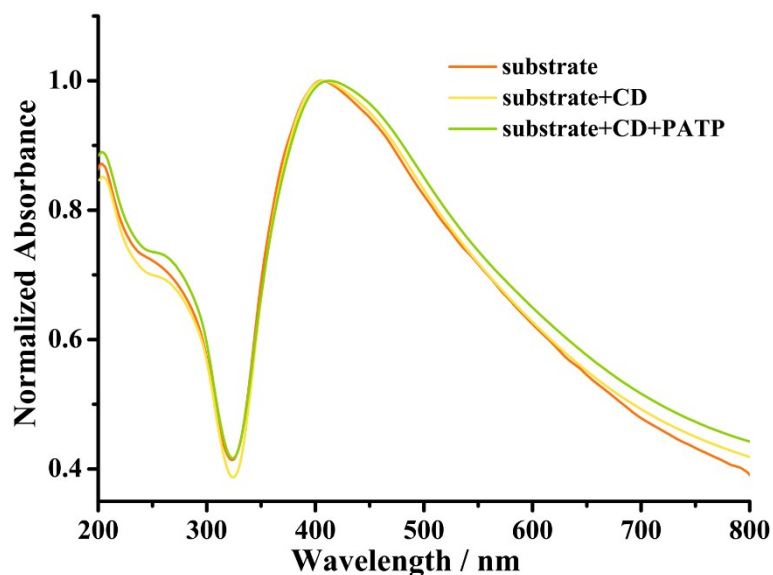

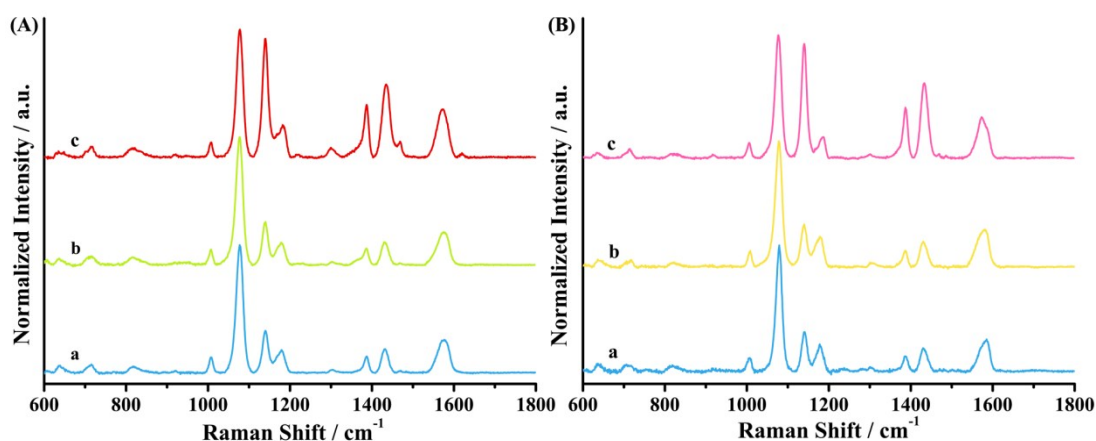**Figure S3.** Normalized UV-vis absorption spectra of the enantiodiscrimination sensing systems

Figure S4. SERS spectra of the PATP modified on the enantioselective sensor in Phe (A) and Tyr (B) sensing systems, respectively. In each of the spectra, a to c are the sensors in the deionized water (the blank), in the D enantiomeric solution, and in the L enantiomeric solution with the concentration of 10^{-3} mol/L, respectively. All the SERS spectra are normalized to the band at 1075 cm^{-1} with the 785 nm excitation.
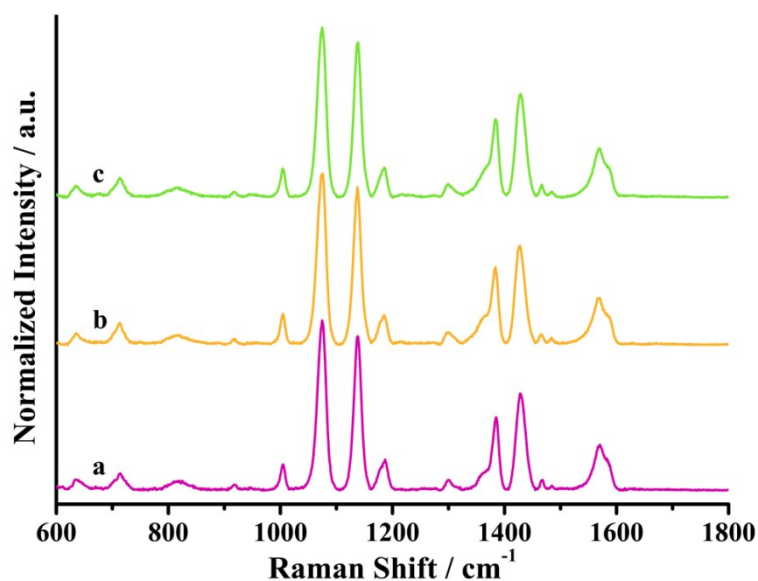
Figure S5. SERS spectra of the PATP modified on the Ag-TiO$_2$-Ag substrate without SH-*β*-CD in the blank system immersed in the deionized water (a), in the aqueous solution of D-Trp (b) and L-Trp (c) with the concentration of 10^{-3} mol/L. All the SERS spectra are normalized to the band with 1075 cm^{-1} with the 785 nm excitation.
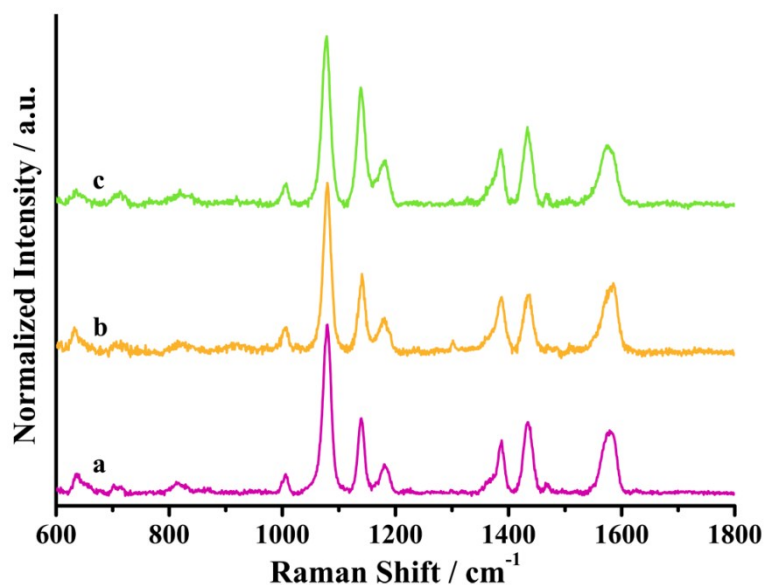

Figure S6. SERS spectra of the PATP modified on the Ag substrate with SH-*β*-CD (Ag-CD-PATP) in the blank system immersed in the deionized water (a), in the aqueous solution of D-Trp (b) and L-Trp (c) with the concentration of 10^{-3} mol/L. All the SERS spectra are normalized to the band at 1075 cm^{-1} with the 785 nm excitation.
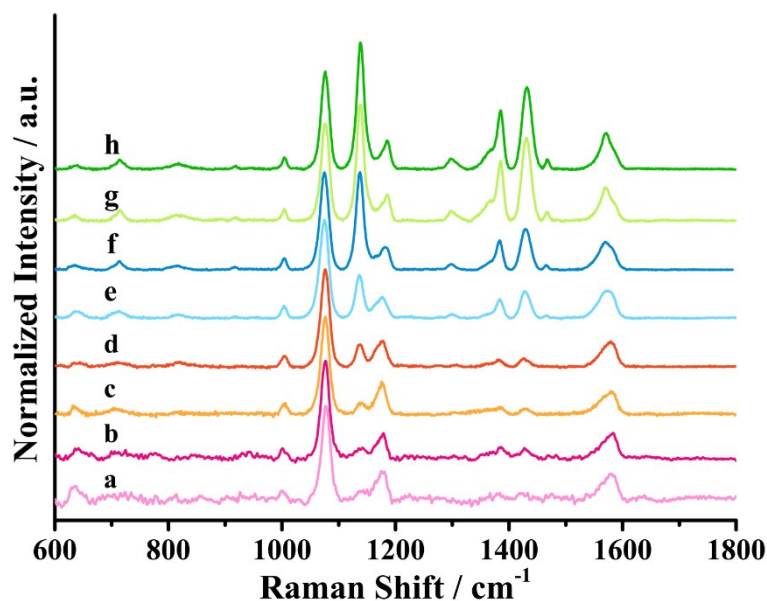
Figure S7. SERS spectra of different concentrations of PATP modified on the enantioselective sensor immersed in the Trp enantiomeric solutions of 10^{-3} mol/L, and a to h represent the sensor with 10^{-6} mol/L PATP in the D-Trp, with 10^{-6} mol/L PATP in the L-Trp, with 10^{-5} mol/L PATP in the D-Trp, with 10^{-5} mol/L PATP in the L-Trp, with 10^{-4} mol/L PATP in the D-Trp, with 10^{-4} mol/L PATP in the L-Trp, with 10^{-3} mol/L PATP in the D-Trp, and with 10^{-3} mol/L PATP in the L-Trp systems, respectively. All the SERS spectra are normalized to the band at 1075 cm^{-1} with the 785 nm excitation.
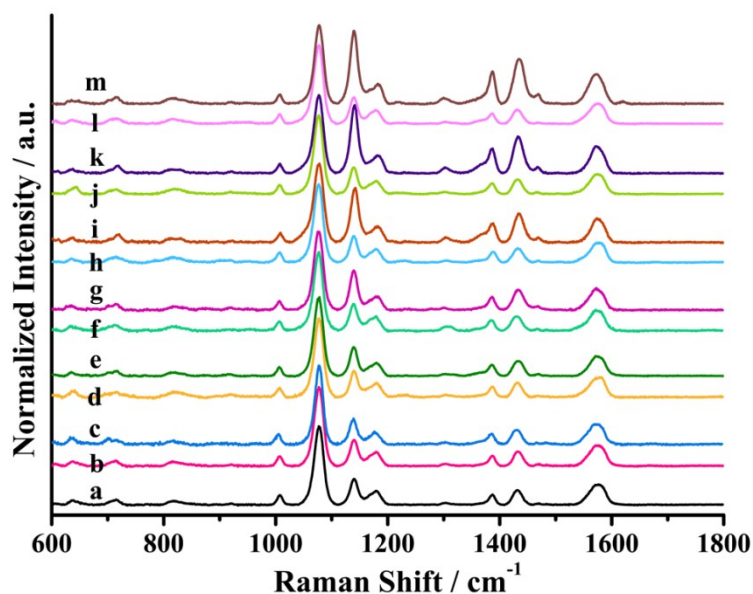

Figure S8. SERS spectra of PATP in the enantiodiscrimination sensing system for discriminating D- and L-Phe enantiomers of different concentrations with the 785 nm excitation; a is the blank one without Phe, and b to m represent the D- and L-Phe enantiomers with the concentrations of 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} mol/L, respectively. All the SERS spectra are normalized to the band at 1075 cm^{-1}.

Details about the degree of CT

The "degree of CT (p_{CT})" was proposed by Professor Lombardi, which was calculated to estimate the contribution of CT to the Raman enhancement in the entire SERS intensity.[3-4] The $\rho_{CT(k)}$ of an individual molecular band in the Raman spectrum (use an index k to identify) can be determined using the following equation:

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

We choose the intensities of two bands in a spectral region for better understanding. One band is totally symmetric with the SERS signal only contributions from SPR, whose intensity is denoted $I^0(SPR)$, and for this line $I^k(SPR) = I^0(SPR)$. While the other band is non-totally symmetric (intensity denoted $I^k(CT)$). It is the measured intensity in the region of the spectrum where CT resonance makes an additional contribution to the SERS intensity excluding the contribution of SPR. In this case, $I^k(SPR)$ is normally be small or zero. We selected two bands, the totally symmetric band (a_1), which is equivalent to the $I^0(SPR)$ in the above formula, and the nontotally symmetric band (b_2),[4-5] which corresponds to the $I^k(CT)$ in the formula, for the evaluation of the contribution of CT mechanism. Considering that the CT contributes to the SERS intensity, the $I^k(SPR)$ is approximately equal to zero under our experimental conditions.

In the SERS spectra of PATP in the two enantiomeric systems, the a_1 mode at 1075 cm^{-1} and the b_2 mode at 1141, 1398, 1435, and 1573 cm^{-1} were selected to be put into the formula of p_{CT} for the investigation of the ρ_{CT} values on the basis of the rule that non-totally symmetric band is fairly intense and relatively isolated from interference by nearby bands. The relative ρ_{CT} values for PATP in the two enantiomeric discrimination system were thus plotted in Figure 4.

References

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2   Y. Cheng, J. Guo, X. Liu, A. Sun, G. Xu and P. Cui, *J. Mater. Chem. A* 2011, **21**, 5051-5056.
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