Supporting Information

Understanding of chiral site-dependent enantioselective identification on plasmon-free semiconductor based SERS substrate

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EXPERIMENTAL SECTION

Materials and Reagents. Ti sheets (~0.1 mm thickness, 99.6% purity) were purchased from Baosheng Hardware (Baoji, China). Phenylphosphonic acid (C₆H₇O₃P), o-phosphorylethanolamine (C₂H₃NO₃P), tryptophan (C₁₁H₁₂N₂O₂), phenylalanine (C₉H₁₅NO₂), 3,4-Dihydroxy-L/D-phenylalanine (C₉H₁₄NO₄), histidine (C₉H₁₀N₃O₂), potassium ferrocyanide trihydrate (K₄FeC₆N₆·3H₂O), and iron chloride hexahydrate (FeCl₃·6H₂O) were purchased from Aladdin (Shanghai, China). N-Hydroxysuccinimide (NHS) and N-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) were obtained from Sigma-Aldrich (St. Louis, USA). Other reagents including ammonium fluoride (NH₄F), isopropyl alcohol, ethylene glycol, and lactic acid were purchased from Sinopharm Chemical Reagent Co. Ltd. and used as received without further purification.

Apparatus and Characterization. Morphological and microstructures characterization were carried out using a field-emission scanning electron microscope (SEM, Hitachi SU8000, Japan). SERS measurements were conducted using a Raman microscopy spectrometer (LabRAM HR, HORIBA Scientific, France). XRD patterns were acquired using an X’Pert XRD spectrometer (Philips, USA) using a CuKα X-ray source. X-ray photoelectron spectra (XPS) were recorded on a Perkin-Elmer Physical Electronics 5600 spectrometer using AlKα radiation at 13 kV as excitation source. UV-visible diffuse reflectance spectra were obtained using a Perkin-Elmer spectrometer (Lambda 750S, USA). Ultraviolet visible (UV/Vis) diffuse reflectance spectra were recorded using a Lambda Bio/XLS+ UV-vis spectrophotometer (PerkinElmer, USA) with a 1.0 cm optical path.
Fig. S1. SEM images of top view and side view of (A) TiO$_2$ nanoparticles based membrane, and TiO$_2$ NTs prepared with different anodization periods: (B) 1 min, (C) 2 min, (D) 3 min, (E) 4 min, and (F) 10 min.
Fig. S2. SERS spectra of MB adsorbed on TiO$_2$ NTs with different lengths.
**Fig. S3.** Raman spectra of MB adsorbed on different substrates: silicon wafer and TiO$_2$ NTs.

The enhancement factor (EF) of TiO$_2$ NTs was evaluated using Eq 1.$^{S1}$ $I_{\text{SERS}}$ and $I_{\text{bulk}}$ are the intensities of the 1628 cm$^{-1}$ (C-C stretching vibration) band of MB adsorbed on a SERS-active substrate and bare silicon wafer, respectively. $N_{\text{bulk}}$ and $N_{\text{SERS}}$ are the average number of molecules in the scattering area for non-SERS and SERS measurements. In this study, the number of probe molecules was estimated using Eq. 2 based on the assumption that the probed molecules were distributed uniformly on the substrates. $N_A$ is the Avogadro constant; $C$ is the molar concentration of the probe solution; $V$ is the volume of a droplet; and $S_{\text{sub}}$ is the area of the substrate.

$$EF = \left(\frac{I_{\text{SERS}}}{I_{\text{bulk}}} \right) \left(\frac{N_{\text{bulk}}}{N_{\text{SERS}}} \right)$$

$$N = CVN_A S_{\text{sub}}$$
**Fig. S4.** SERS spectra of $2 \times 10^{-5}$ M MB acquired on ten pieces of TiO$_2$ NTs.
Fig. S5. (A) XPS survey spectra of PA/TiO$_2$ NTs before and after L-Trp modification. (B) Ti 2p signal, (C) O 1s signal, (D) P 2p signal, and (E) N 1s signal before and after L-Trp modified onto PA/TiO$_2$ NTs.
**Fig. S6.** The energy-dispersive X-ray spectroscopy (EDS) mapping images of L-Trp/PA/TiO$_2$ NTs.
Fig. S7. Raman spectra of TiO$_2$ NTs, PA/TiO$_2$ NTs, and L-Trp/PA/TiO$_2$ NTs.
Fig. S8. CD spectra of L/D-Trp.
**Fig. S9.** Raman spectra of TiO$_2$ NTs, L-DOPA/L-Trp/PA/TiO$_2$ NTs, and D-DOPA/L-Trp/PA/TiO$_2$ NTs.
Fig. S10. SERS spectra for sensing (A) L-DOPA and (B) D-DOPA under different recognition time periods. The corresponding Raman intensity for sensing (C) L-DOPA and (D) D-DOPA.

As shown in Fig. S10, the Raman peak intensity at 2158 cm\(^{-1}\) increases with the chiral recognition time, and this trend slows down at 60 min.
Fig. S11. SERS spectra for sensing (A) L-DOPA and (B) D-DOPA under different Fe³⁺ concentrations. The corresponding Raman intensity for sensing (C) L-DOPA and (D) D-DOPA.

It can be seen that the SERS signals of PB increased with Fe³⁺ concentration for L-DOPA and D-DOPA. The SERS signal intensity reached a plateau at a Fe³⁺ concentration of 0.25 mM for both cases. At this high concentration of Fe³⁺, the reaction has nearly completed. Further increasing the concentration of Fe³⁺ does not shift the equilibrium too much (The intensity of the signal does not increase with higher concentration of Fe³⁺). As shown in Fig. S11, the SERS substrate with D-DOPA consistently shows a larger PB signal than that of L-DOPA with any concentration of Fe³⁺, suggesting more D-DOPA molecules were captured on the SERS substrate. This result was in agreement with the MD simulation.
Fig. S12. SERS spectra for sensing (A) L-DOPA and (B) D-DOPA under different Fe³⁺ chelation time periods. The corresponding Raman intensity for sensing (C) L-DOPA and (D) D-DOPA.

As shown in Fig. S12, the changes in Raman peak intensity are also influenced by Fe³⁺ chelation time, and a stable value was achieved in 30 min.
Fig. S13. Zeta potentials of TiO$_2$ NTs, PA/TiO$_2$ NTs, and L-Trp/PA/TiO$_2$ NTs.
Fig. S14. SERS spectra of TiO$_2$ NTs, PB/TiO$_2$ NTs, PB/PA/TiO$_2$ NTs, PB/L-Trp/PA/TiO$_2$ NTs, PB/L-DOPA/L-Trp/PA/TiO$_2$ NTs, and PB/D-DOPA/L-Trp/PA/TiO$_2$ NTs.
**Fig. S15.** EDS elemental mapping images of generated PB *via* DOPA recognition on L-Trp/PA/TiO$_2$ NTs.
Fig. S16. (A) X-ray diffraction (XRD) patterns and (B) UV/Visible diffuse reflectance spectra of homochiral SERS substrate before and after PB generation.

The peaks appeared at 24.1° (220), 29.5° (311), 33.5° (400), 43.8° (422), 50.6° (440), 59.9° (622), and 66.1° (640), which can be indexed as the PB cubic space group \textit{Fm\textoverline{3}m} (JCPDS card no. 73–0687).\textsuperscript{S2}

The UV-visible absorbance spectra verify the formation of PB via the broad absorbance band appearing from 480 nm to 800 nm, which can be attributed to the intermetal charge-transfer band from Fe(II) to Fe(III) in PB.\textsuperscript{S3}
**Table S1.** Comparison of various methods for detection of L/D-DOPA.

<table>
<thead>
<tr>
<th>Method</th>
<th>Mechanisms</th>
<th>Sensitivity</th>
<th>Reference</th>
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<tbody>
<tr>
<td>L/D-cysteine modified gold nanoparticle based colorimetric assay</td>
<td>L/D-Cys as chiral selectors and Au NPs as artificial enzyme for enantioselective recognition of L/D-DOPA</td>
<td>1 μM</td>
<td>S4</td>
</tr>
<tr>
<td>L/D-tartaric acid grafted optical fiber probe based plasmon absorption bands shifts</td>
<td>Hydrogen bonding induced wavelength shift of plasmonic metal.</td>
<td>10 nM</td>
<td>S5</td>
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<tr>
<td>Gold nanodendrite modified glassy carbon electrode based differential pulse voltammetry</td>
<td>DOPA enantiomers were distinguished by a noticeable potential difference</td>
<td>0.25 μM</td>
<td>S6</td>
</tr>
<tr>
<td>Homochiral metal-organic frameworks based photoelectrochemical assay</td>
<td>the charge-carrier separation-induced photocurrent variation</td>
<td>0.24 μM</td>
<td>S7</td>
</tr>
<tr>
<td>Chiral CuO/CoO nanofibers based electrochemiluminescence method</td>
<td>CuO/CoO NFs as the catalytic activity center and chiral cysteine as the inducer of chiral recognition for enantioselective catalysis and recognition of L/D-DOPA</td>
<td>0.29 nM for L-DOPA; 0.31 nM for D-DOPA</td>
<td>S8</td>
</tr>
<tr>
<td>Signal amplification based SERS assay</td>
<td>Hydrogen-bonding interactions were amplified through the onsite growth of Prussian blue</td>
<td>0.13 pM for L-DOPA; 0.17 pM for D-DOPA</td>
<td>This work</td>
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</table>
Fig. S17. SEM images of (A, B) bare substrate, (C, D) PB coated by L-DOPA recognition, and (E, F) PB coated by D-DOPA recognition.
Fig. S18. CD spectra of TiO$_2$ NTs, PA/TiO$_2$ NTs, L-Phe/PA/TiO$_2$ NTs, and L/D-Phe.
Fig. S19. SERS spectra of PB for sensing different concentrations of (A) L-DOPA and (B) D-DOPA on L-Phe/PA/TiO₂ NTs. (C) The corresponding Raman intensities at 2158 cm⁻¹. (D) Mechanism for anchoring L-Phe onto PA/TiO₂ NTs.
Fig. S20. SERS spectra of PB for sensing different enantiomers (A) L/D-DOPA, (B) L/D-Phe, (C) L/D-Ala, (D) L/D-Pro, (E) L/D-Glu, and (F) L/D-His.
Fig. S21. Raman intensity at 2158 cm$^{-1}$ towards different enantiomers. Error bars indicate the standard deviation of triplicate tests. The concentration of each enantiomer is 1 mM.
REFERENCES


(S6) H. Lian, S. Huang, X. Wei, J. Guo, X. Sun and B. Liu, Talanta, 2020, 210, 120654.
