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Hydrophobic Interaction Enables Rapid Enrichment of Volatile Metabolite on Au/TiO_2 based SERS Substrate for Ultrasensitive Bacteria Detection

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Experimental Section

Materials and Instruments. Ti sheets (0.1 mm thickness, 99.6% purity) were supplied by Baosheng Hardware Co., Ltd. (Baoji, China). Ethyl alcohol, chloroauric acid ($HAuCl_4$), sodium hydroxide (NaOH), hydrofluoric acid (HF), sulfuric acid (H_2SO_4), ammonium fluoride (NH_4F), toluene, sodium chloride (NaCl), and glycerol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Phosphoric acid (H_3PO_4), octadecylphosphonic acid (ODPA), and 4-mercapto-phenylboronic acid (4-MPBA) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Phenyl acetonitrile (PA) were purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Dimethyl disulfide (DMDS) was purchased from McLin Biochemical Technology Co., Ltd. (Shanghai, China).

The morphologies of samples were characterized using a field-emission scanning electron micro-scope (FE-SEM, S4800, Hitachi, Japan). X-ray diffraction (XRD) patterns were acquired using an X'Pert spectrometer (PANalytical Empyrean, Holland) using a CuKα X-ray source. UV-visible diffuse reflectance spectra were obtained using a spectrometer (Lambda 750S, Perkin-Elmer, USA). Contact angle was measured using a contact angle measuring instrument (DH-HV1351UM, DAHENG Imaging, China).

SERS measurements. SERS measurements were conducted using a Raman microscopy spectrometer (LabRAM HR, HORIBA Scientific, France). The Raman spectra were measured using a 638-nm laser (12.5 mW) with a 50×objective. The acquisition time was 5 s and the slit aperture size was 200 μm.

Bacteria Culture. In this study, Gram-negative *E. coli* was used as the typical foodborne bacteria. Prior to the experiment, all glassware was sterilized by autoclaving at 121 ℃ for 20 min. The number of *E. coli* was determined using the standard colony counting method. Typically, for enumeration of *E.coli*, bacteria contained solution were first decimal dilutions to suitable concentration. Then, 100 μL of the bacteria contained solution was spread on solid LB medium and cultured at 37 ^{o}C for 24 h. During this 24 h culturing, one bacterial cell can grow into one bacterial colony. After the incubation, plates from three successive dilutions with 30~300 *E.coli* colonies per plate were counted to obtain the final colony forming units per 100 μL bacteria contained solution. As a result, one bacterial colony counted on the solid LB medium represents one bacterial cell in the original bacteria contained solution.

Supporting Figures

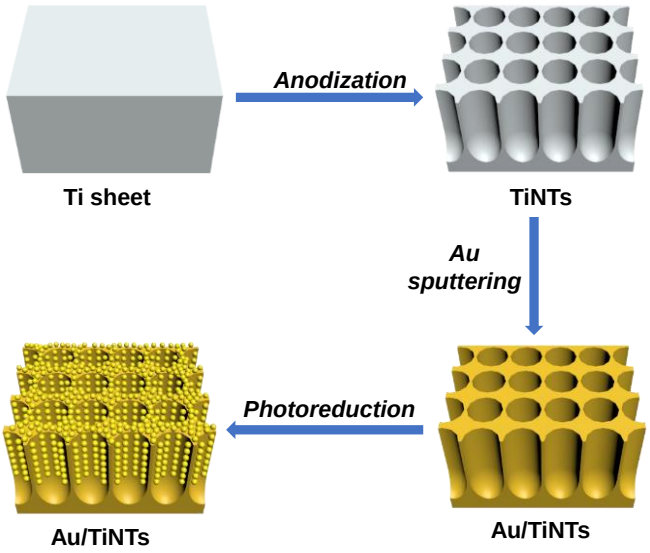

Figure S1. Schematic of preparation procedure for AuNPs/TiNTs.

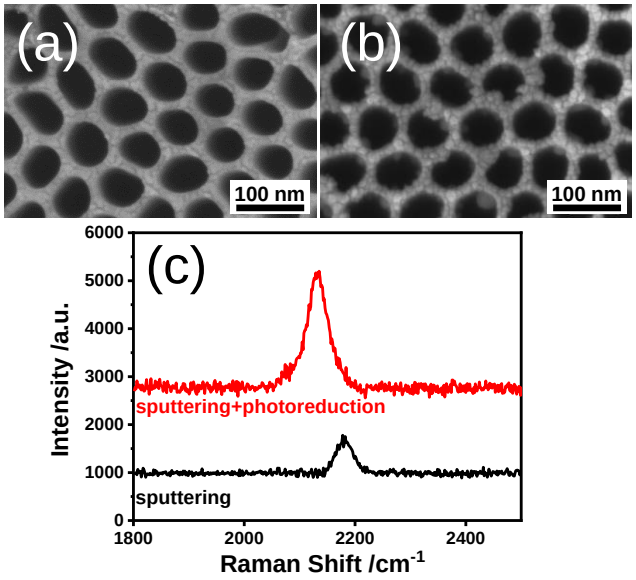

Figure S2. SEM images of AuNPs/TiNTs: (a) sputtering; (b) sputtering and photoreduction, (c) Raman spectra of PA on AuNPs/TiNTs (sputtering) and AuNPs/TiNTs (sputtering and photoreduction).

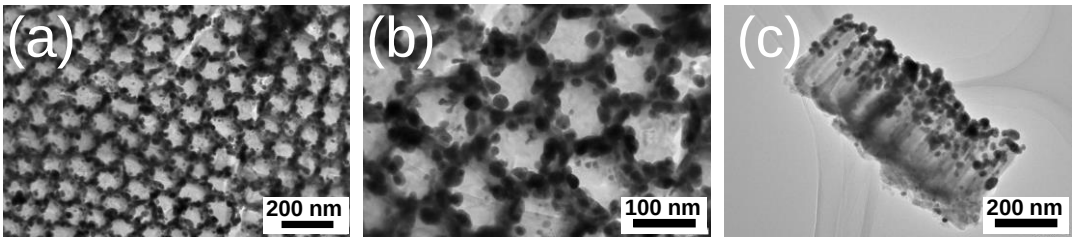

Figure S3. TEM images of AuNPs/TiNTs: (a) top view, (b) magnified top view, and (c) side view.

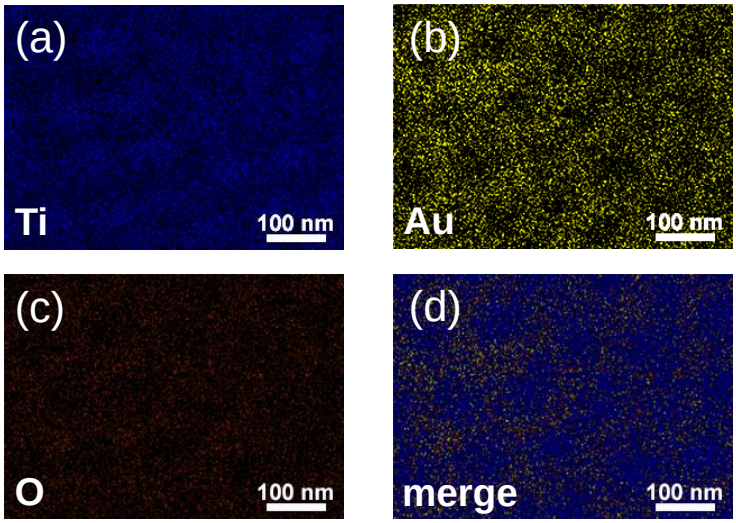

Figure S4. EDS elemental mapping of (a) Ti and (b) Au (c) O and (d) merge imaging for AuNPs/TiNTs.

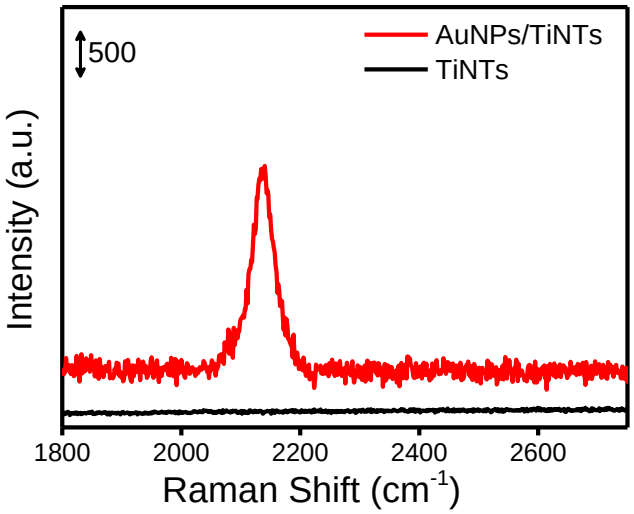

Figure S5. SERS spectra of PA on TiNTs, and AuNPs/TiNTs.

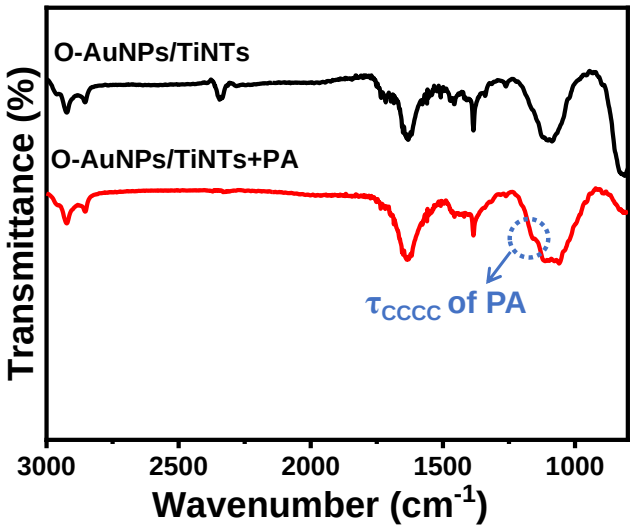

Figure S6. FTIR spectra of O-AuNPs/TiNTs and O-AuNPs/TiNTs+PA.

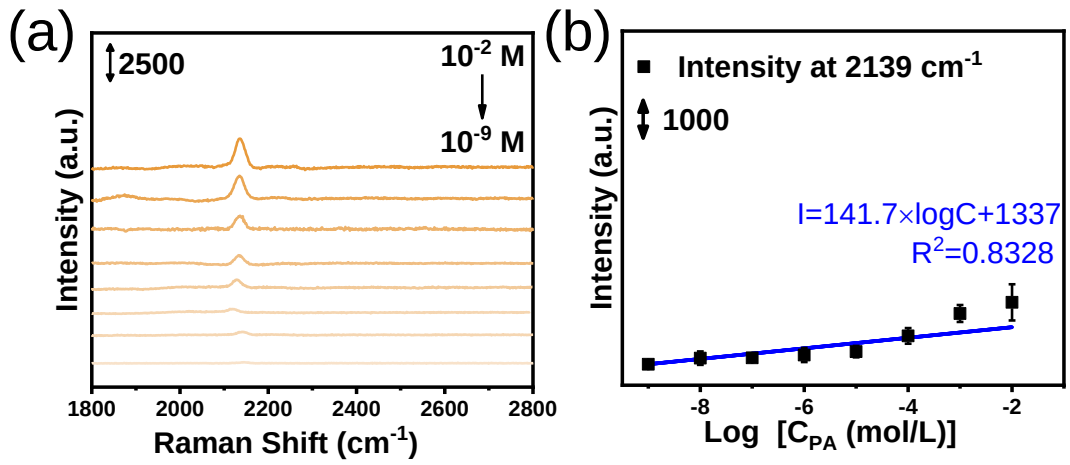

Figure S7. (a) SERS spectra of PA at different concentrations using AuNPs/silicon as the SERS substrate. (b) Linear calibration plot between I_{2139} and logarithm of PA concentration.

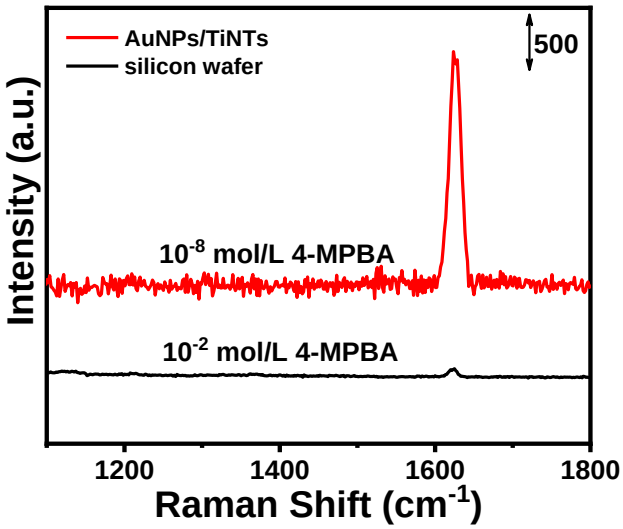

Figure S8. Raman spectra of 4-MPBA on AuNPs/TiNTs and silicon.

The Raman enhancement factor (EF) was calculated using the following eq:[1]

$$EF=\left(\frac{I_{SERS}}{I_{bulk}}\right)\left(\frac{N_{bulk}}{N_{SERS}}\right) \quad (1)$$

where I_{SERS} and I_{bulk} are the intensities of the 1623 cm^{-1} (boronic acid) band of 4-MPBA adsorbed on AuNPs/TiNTs and bare silicon wafer, respectively. N_{bulk} and N_{SERS} are the corresponding number of 4-MPBA molecules. First, 10 μL of 4-MPBA was added to the surface of the Si wafer and AuNPs/TiNTs (2.5×2.5 mm^{-2}), and the corresponding Raman signals are shown in Figure S8.N_{bulk} was calculated using the following equation:

$$N_{bulk}=\left(\frac{C\times V\times S_{Las}}{S_{Sub}}\right)\times N_A=1.926\times10^{11} \quad (2)$$

where C represents the molar concentration of the 4-MPBA solution (10^{-2} mol/L), V is the volume of 4-MPBA solution (10 μL), S_{Las} is the area of laser spot size (2 μm^2), S_{Sub} is the area of the substrate (6.25 mm^2), N_A is Avogadro's number (6.02× 10^{23} mol^{-1}). N_{bulk} was estimated to 1.926×10^{11}. N_{sers} was calculated as the same way to be 1.926×10^{5}. Substituting these values of the above variables into equation (1), EF could be concluded to be around the calculated to be around 4.0×10^{7}.

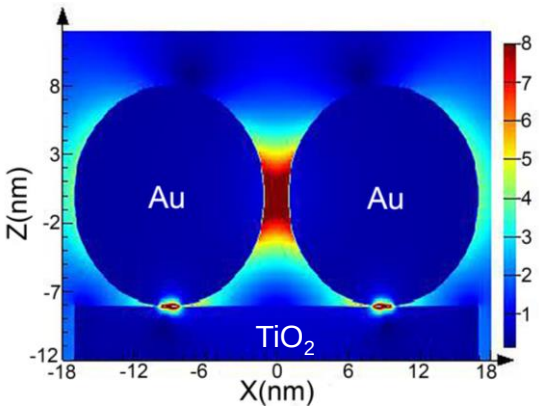

Figure S9. Intensity of electric field distribution around the hotspot on AuNPs/TiNTs.

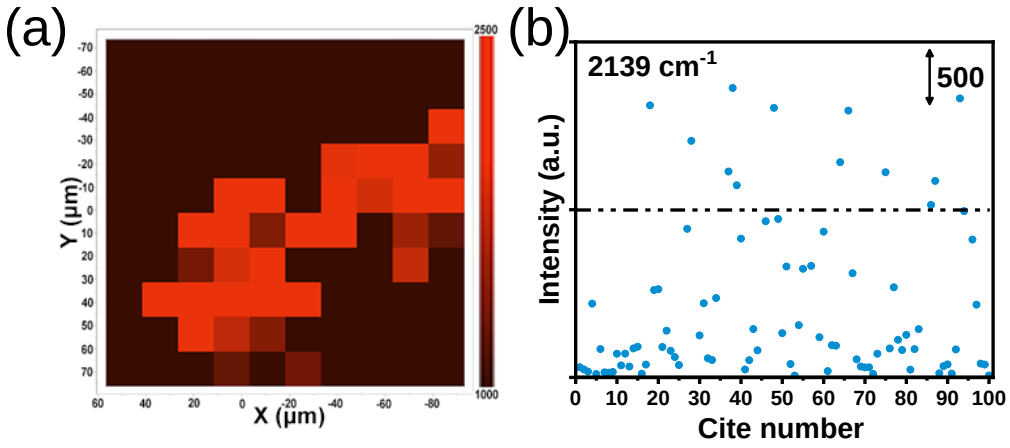

Figure S10. (a) SERS mapping results (at 2139 cm^{-1}) of PA on AuNPs/silicon. (b) Intensity variations of the 2139 cm^{-1} peak from randomly selected 100 points of PA on AuNPs/silicon.

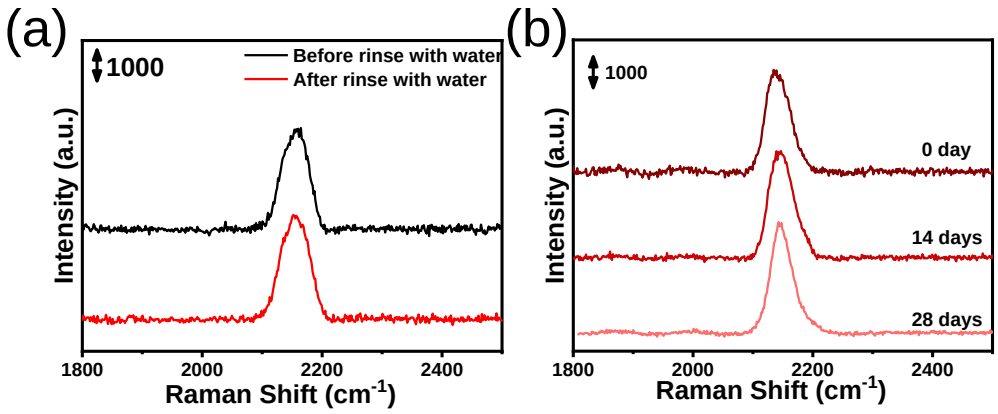

Figure S11. SERS spectra of PA on O-AuNPs/TiNTs (a) after rinsing with water for 30 s and (b) storage at room temperature for 14 and 28 days.

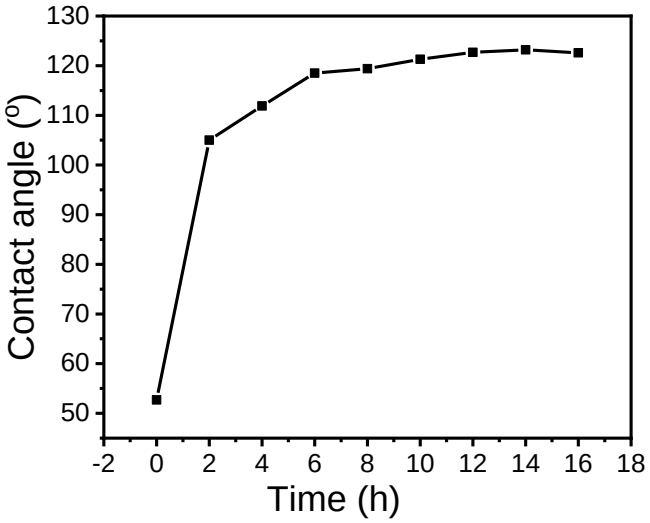

Figure S12. Relationship between the contact angle of O-AuNPs/TiNTs and the reaction time with ODPA.

Figure S13. Optical images of water drops on the hydrophobic substrates before and after storage for one year in darkness.

As shown in Figure S13, the contract angle showed a quite slight decrease from 118.4^{o} to 115.5^{o} after one year storage, indicating a stable hydrophobicity of ODPA modified AuNPs/TiNTs.

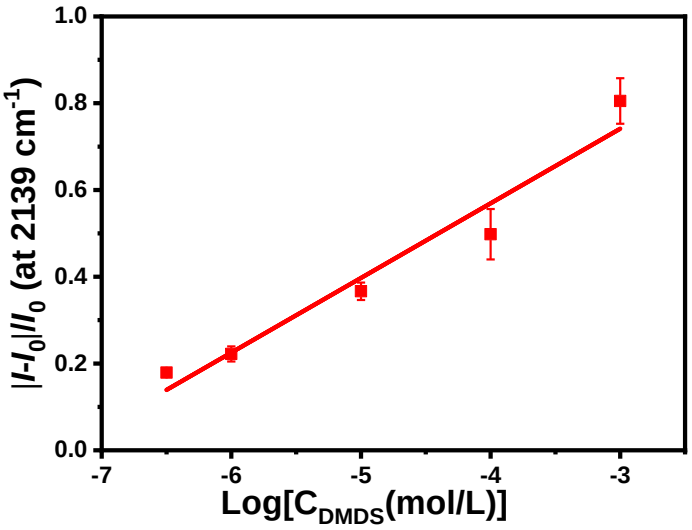

Figure S14. Linear calibration plot between $|I–I_0|/I_0$ of PA at 2139 cm^{-1} and logarithm of DMDS concentration using O-AuNPs/TiNTs as the SERS substrate.

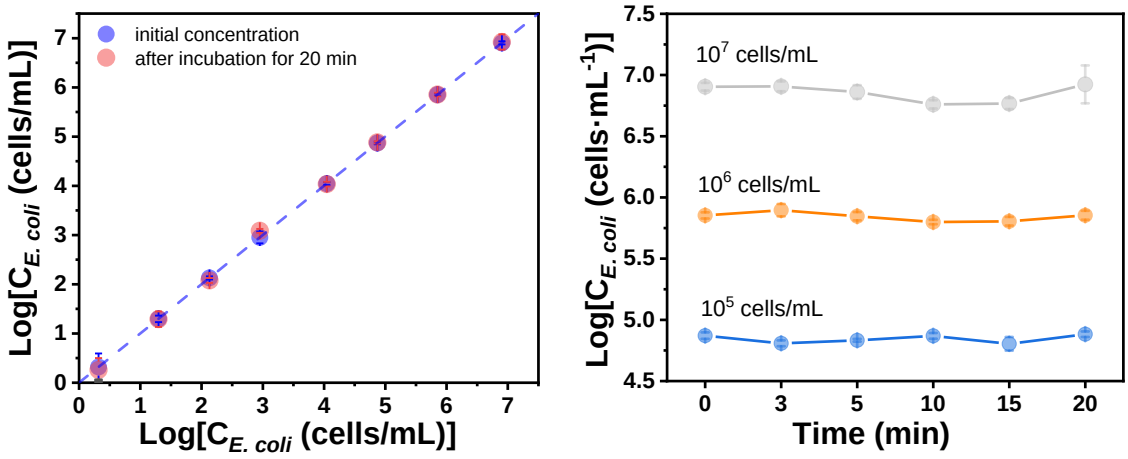

Figure S15. (a) Bacteria concentration before and after incubation in a LB medium at 37 ^{o}C for 20 min. (b) Bacteria growth after incubation in LB medium at 37 ^{o}C for different periods.

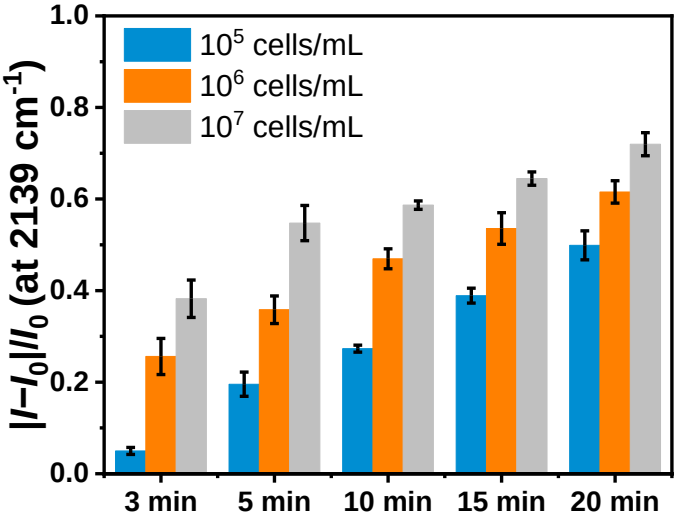

Figure S16. $|I-I_0|/I_0$ of PA on O-AuNPs/TiNTs when exposed to *E. coli* with concentrations of 10^5, 10^6, and 10^7 cells/mL for different time periods.

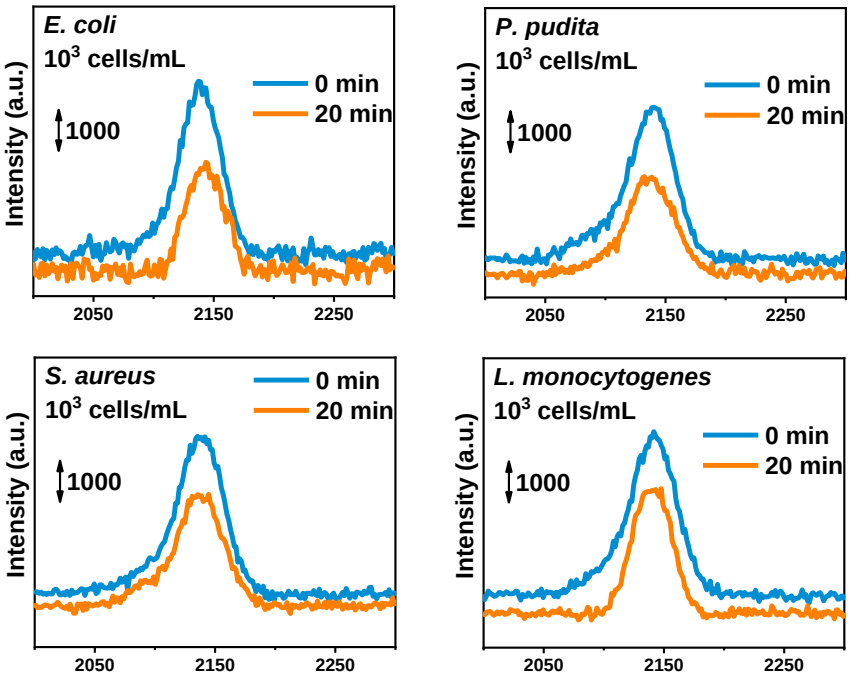

Figure S17. Raman spectra of PA on O-AuNPs/TiNTs before and after exposed to *E. coli* (10^3 cells/mL), *P. putida* (10^3 cells/mL), *S. aureus* (10^3 cells/mL), and *L. monocytogenes* (10^3 cells/mL) for 20 min.

Table S1. Comparison of the proposed method with other SERS methods

Detection time	LOD (cells/mL)	Raman shift cm^{-1}	Assignment	Refs
60 min	10	1188	amide III	S2
30 min	10^2	1264	δ (CH_2) amide III	S3
24 h	10^5	680	ν (C−S)	S4
15 min	-	674	ν (C−S)	S5
20 min	3	2139	ν (C≡N)	This work

References

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