

## Supporting Information

### Internal Standard Optimization Advances Sensitivity and Robustness of Ratiometric Detection Method

Yuning Jiang, Jiaying Cao, Sen Hu, Tao Cheng, Hanyu Wang, Xiaoyu Guo, Ye Ying, Xinling Liu, Feng Wang, Ying Wen, Yiping Wu\*, Haifeng Yang\*

*The Education Ministry Key Lab of Resource Chemistry, Joint International Research Laboratory of Resource Chemistry, Ministry of Education, and Shanghai Key Laboratory of Rare Earth Functional Materials, College of Environmental and Geographic Sciences, Shanghai Normal University, Shanghai 200234, China.*

\*Email: yipingwu@shnu.edu.cn (Yiping Wu); hfyang@shnu.edu.cn (Haifeng Yang).

### Experimental Section

#### Materials

Chloroauric acid ( $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$ ) was bought from Sinopharm Chemical Reagent Co., Ltd (Shanghai, P. R. China). 3-Aminopropyl triethoxysilane (APTES), and trisodium citrate were purchased from Sigma-Aldrich Company. Para-aminothiophenol (pATP) and sodium nitrite ( $\text{NaNO}_2$ ) were supplied from Macklin Corp (Shanghai, China). 4-Mercaptopyridine (4-Mpy) and hydrochloric acid (HCl) were from Shanghai Macklin Biochemical Technology Co., Ltd (Shanghai, P. R. China). The ITO conductive glass was purchased from Weiguang Corp. (Shenzhen, China). All reagents were analytical grade and used without further purification. All aqueous solutions were prepared with ultrapure water (18.25  $\text{M}\Omega \cdot \text{cm}$ ) from the Shanghai Miaokang Milli-Q system.

## **Preparation of Au/ITO**

Gold nanoparticles were synthesized using Frens' method.<sup>1</sup> Firstly, 250  $\mu\text{L}$  of 0.1 mol/L  $\text{HAuCl}_4$  was added to a conical flask containing 100 mL of ultrapure water and heated to boiling for 10 minutes. Secondly, 1 mL of freshly prepared 1% trisodium citrate solution was quickly injected into the above mixture. This caused the solution to change color from light yellow to wine-red. The resultant Au NPs colloidal was boiled for 30 min under stirring and then cooled to room temperature. It was stored at 4°C for subsequent use.

ITO glasses were successively cleaned with acetone, isopropyl alcohol, and ultrapure water under ultrasound.<sup>2</sup> Then, they were dried in an oven at 60°C for 1 h. The cleaned ITO glass was cut into small pieces (2 cm  $\times$  0.5 cm) and heated to boiling in the activation solution ( $\text{NH}_3 \cdot \text{H}_2\text{O}/\text{H}_2\text{O}_2/\text{H}_2\text{O} = 1:1:5$  in volume ratio) for 30 min. Following, the activated ITO was immersed in 10% (v/v) APTES solution for 16 h to assemble amino groups. After carefully washed with ultrapure water and dried under nitrogen, the ITO glasses with amino groups were incubated in 1 mL Au NPs colloidal solution for 18 h to obtain Au/ITO substrates.<sup>3</sup>

## **In situ SERS monitoring of pATP coupling reaction**

The Au/ITO substrate was placed in a solution of  $10^{-3}$  mol/L of pATP in ethanol for 30 minutes to connect pATP to the substrate surface via the Au-S bond. The resulting pATP/Au/ITO was washed with pure ethanol to remove any physical adsorption molecules and then dried in a vacuum. To observe the dimerization process of pATP to generate DMAB in situ, a Raman imaging microscope (Thermo DXR2xi, U.S.A.) equipped with three laser excitation sources (532, 633, and 785 nm) was used. A temperature-dependent SERS experiment was conducted using the temperature controller instrument (Instec mk2000, UK), with a heating rate set at 10 °C/min.

## **SERS imaging and detection of nitrite**

The pATP/Au/ITO chips were submerged in a  $\text{NaNO}_2$  solution for 5 minutes in the absence of light, with its pH adjusted to 2 using HCl.<sup>4</sup> Afterward, the chips were removed, rinsed with deionized water, dried under nitrogen, and immediately tested.

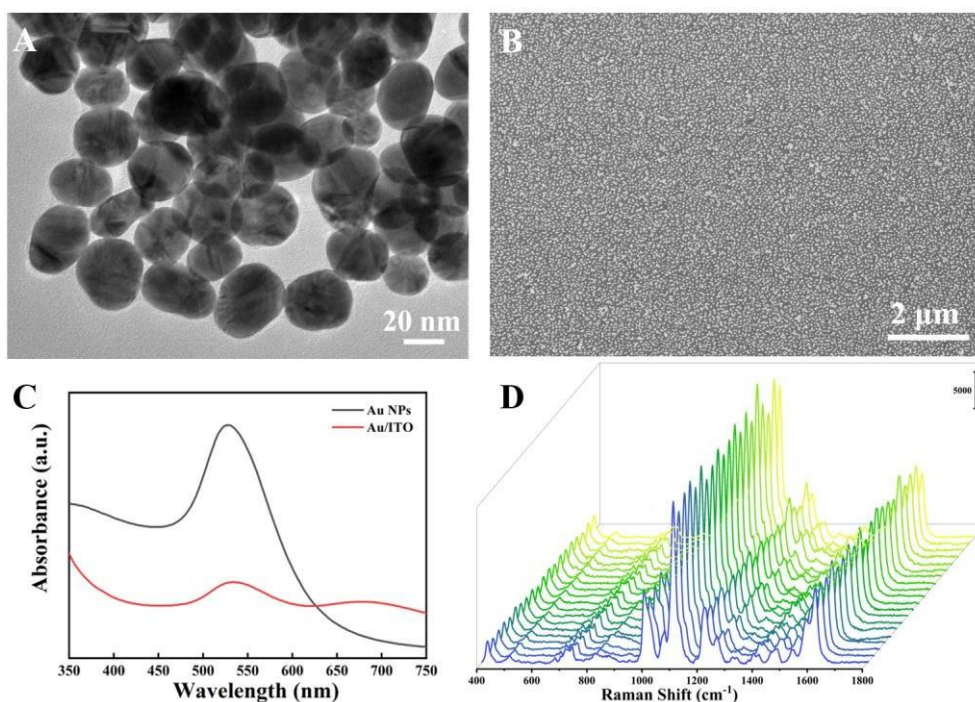
Water samples were gathered from the lake of Xuesi Park at Shanghai Normal University. To create spiked samples, nitrite was added to the water samples in varying concentrations of 10, 25, and 50  $\mu\text{mol/L}$ , respectively. These samples were then purified using centrifugation and filtration to eliminate impurities. SERS imaging and nitrite detection on the pATP/Au/ITO substrate were conducted using a 0.5 mW laser at 785 nm with a 50 $\times$  objective.

### **Characterization of Au/ITO**

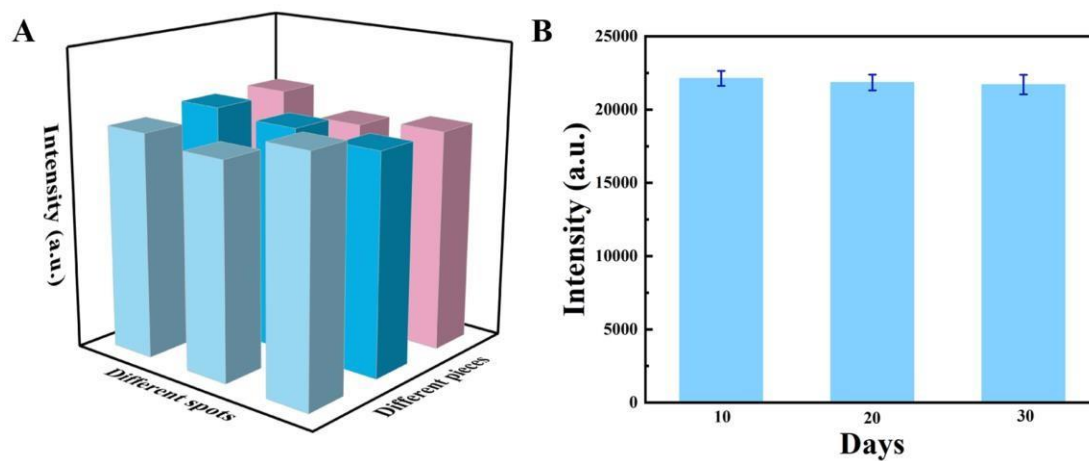
The morphology of Au NPs and Au/ITO were measured on scanning electron microscopy (FESEM, Hitachi S-4800, Japan) and transmission electron microscope (TEM, Model JEM-2100 EXII, Japan). UV-vis spectrometer (UV-1800, Shimadzu, Japan) was used to record the SPR spectrum.

### **Computational details**

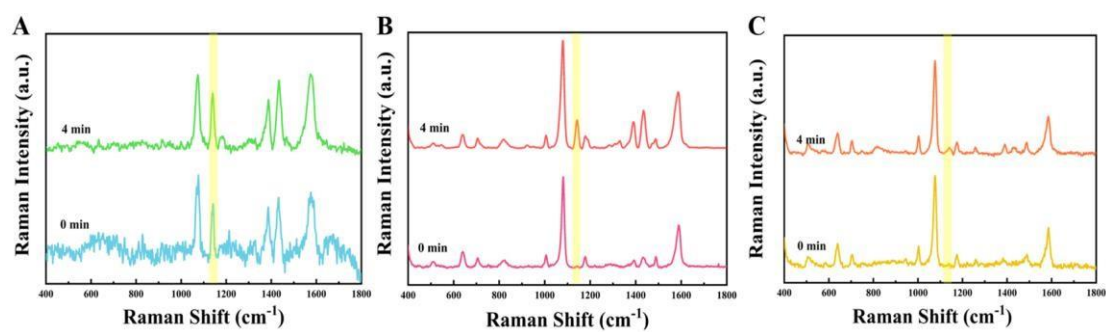
The Gaussian 09 software performed molecular geometry optimizations, molecular electrostatic potential, and vibrational spectra calculations. These calculations were carried out using density functional theory (DFT) methods in combination with 6-311 as a basis set. The optimized structures were used to calculate the vibrational frequencies to ensure no virtual frequencies and to confirm that the structure matched the local minimum on the potential energy surface.



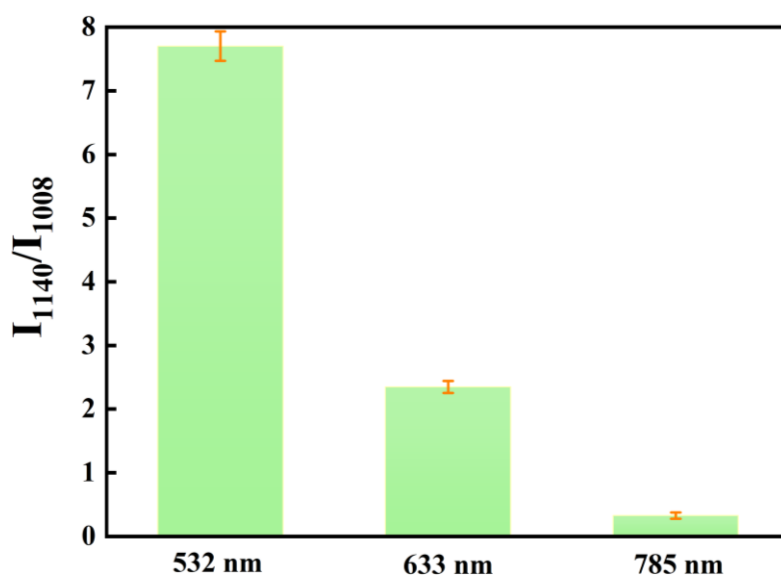
**Figure S1.** Characterization of Au/ITO. (A) TEM image of Au NPs; (B) SEM image of the Au/ITO substrate; (C) the UV-vis absorption spectra of Au NPs and Au/ITO; (D) SERS spectra of 4-Mpy ( $1 \times 10^{-6}$  mol/L) recorded from 20 randomly selected points on Au/ITO.



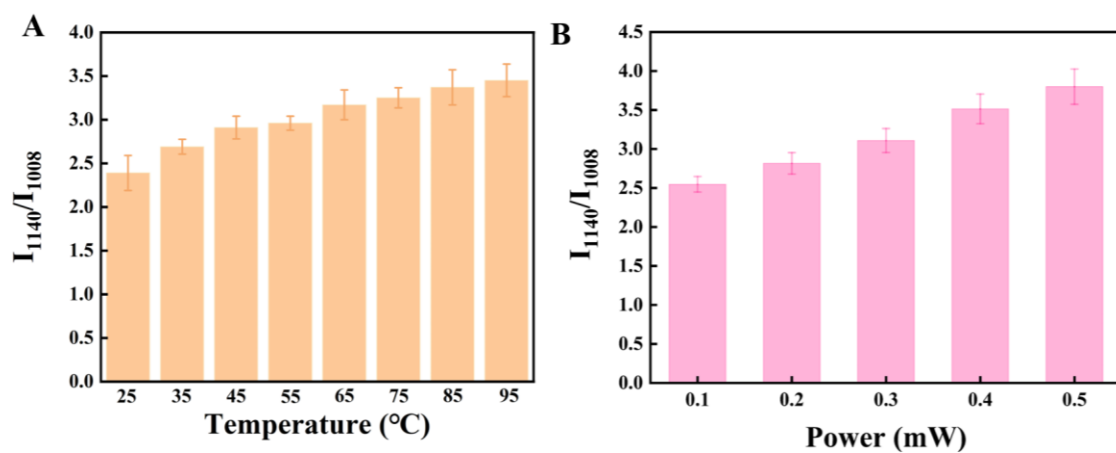
**Figure S2.** SERS intensity of 4-Mpy on Au/ITO substrates with different preparation batches (A) and storage times (B).



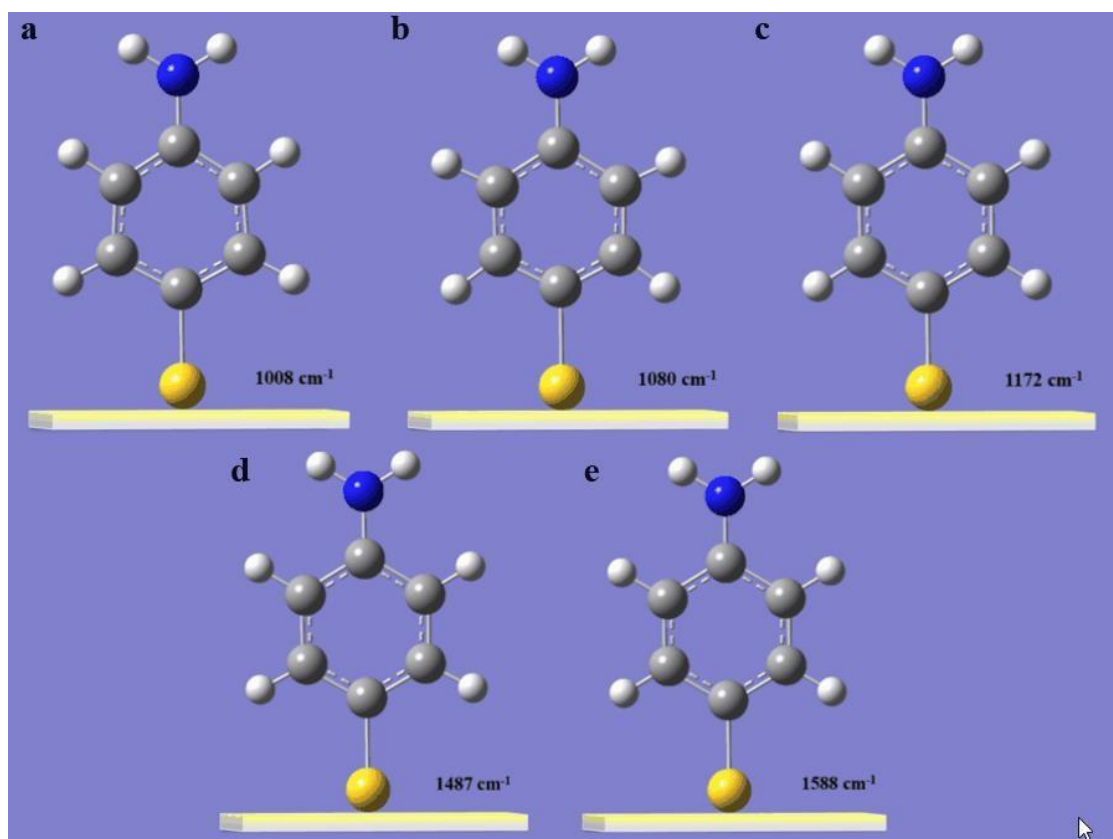
**Figure S3.** The SERS spectra of pATP acquired by using 532 (A), 633 (B), and 785 (C) nm lasers after 4 minutes of exposure. (Laser power set at 0.1 mW, and exposure time of 1 s)



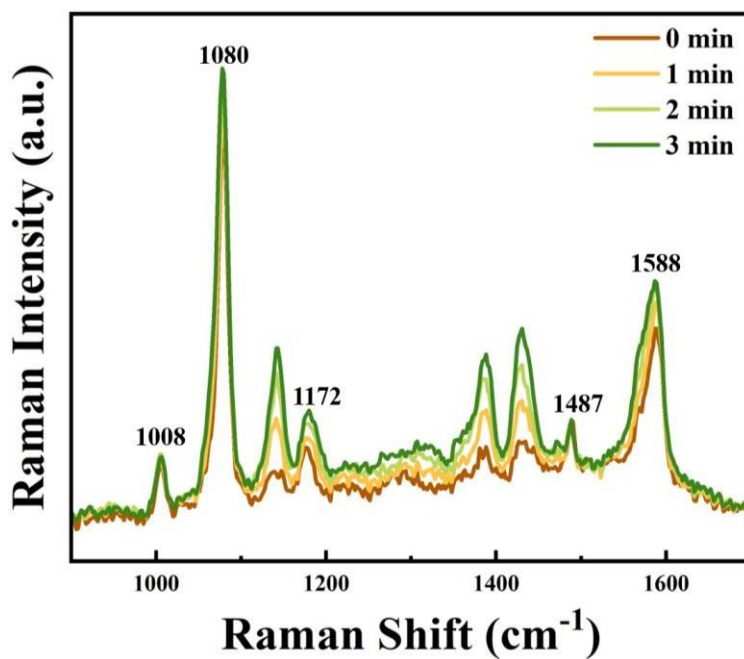
**Figure S4.** The corresponding histogram relative SERS intensity under different laser illumination for duration of 4 minutes. (Laser power set at 0.1 mW, and exposure time of 1 s)



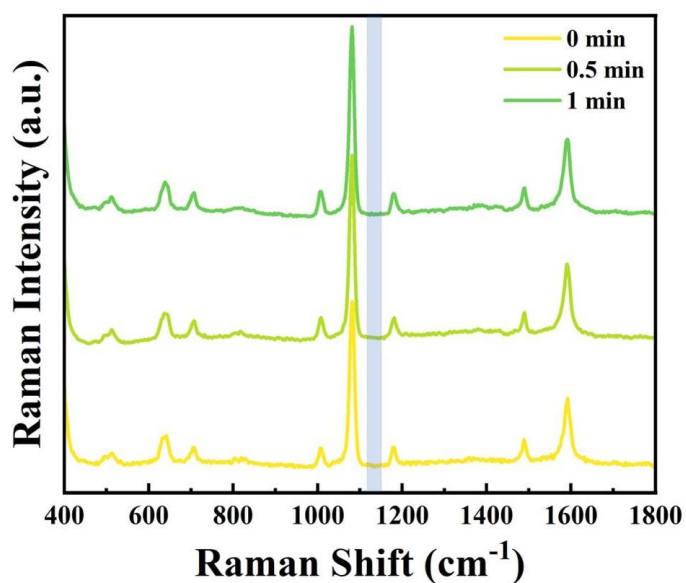
**Figure S5.** The relative intensity of  $I_{1140}/I_{1008}$  under a 633 nm laser with different temperatures (A) and laser powers (B).



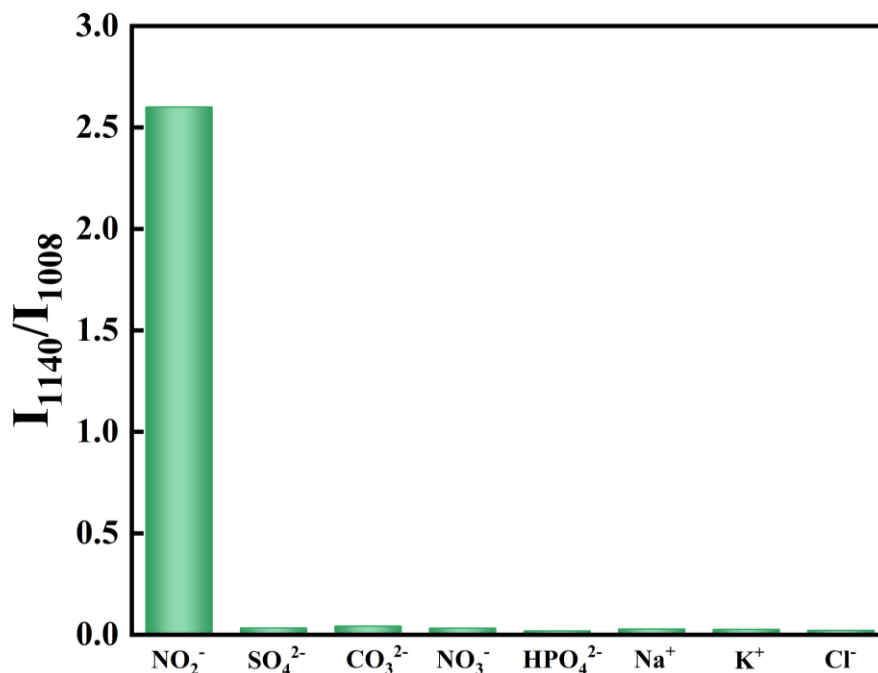
**Figure S6.** The vibration modes at 1008 (a), 1080 (b), 1172 (c), 1487 (d), and 1588 (e)  $\text{cm}^{-1}$  for pATP (The corresponding video is shown in **Supplementary Video 1**).



**Figure S7.** The SERS spectra of pATP were continuously acquired under a 633 nm laser for an exposure duration of 3 minutes (laser power set at 0.2 mW and acquisition time of 0.05 s for each spectrum).



**Figure S8.** The SERS spectra of pATP successively acquired under a 785 nm laser for an exposure duration of 1 minute (laser power set at 0.5 mW, and acquisition time of 1 s for each spectrum).



**Figure S9.** The normalized ratio of I<sub>1140</sub>/I<sub>1008</sub> in the presence of various ions (the concentration of nitrite is 10<sup>-4</sup> mol/L, and the other ions are 10<sup>-3</sup> mol/L).

**Table S1.** Detection of nitrite in lake water by using pATP/Au/ITO-based SERS method.

| Added (μmol/L) | Found (μmol/L) | Recovery (%) | RSD (%) |
|----------------|----------------|--------------|---------|
| 0              | -              | -            | -       |
| 10             | 9.62           | 96 %         | 4.9%    |
| 25             | 25.73          | 103%         | 8.2%    |
| 50             | 53.48          | 107%         | 7.4%    |

## References

1. G. Frens, *Nat. Phys. Sci.*, 1973, **241**, 20-22.
2. J. Luo, Y. Jiang, X. Guo, Y. Ying, Y. Wen, P. Lin, Y. Sun, H. Yang and Y. Wu, *Sen. Actuat. B-Chem.*, 2020, **309**.
3. X. Teng, F. Chen, Y. Gao, R. Meng, Y. Wu, F. Wang, Y. Ying, X. Liu, X. Guo, Y. Sun, P. Lin, Y. Wen and H. Yang, *Anal. Chem.*, 2020, **92**, 3332-3339.
4. C. Zhao, R. Shi, J. Wu, X. Luo and X. Liu, *Biosensors*, 2021, **11**.