Supporting Information

An Accurate and Ultrasensitive SERS Sensor with Au-Se Interface for Bioimaging and in situ Quantitation

Xiaoxiao Li[a], Xiaoyan Duan[a], Lu Li*[a], Sujuan Ye[b], Bo Tang*[a]

[a] X. Li, X. Duan, Pro. L. Li, Pro. B. Tang College of Chemistry, Chemical Engineering and Material Science, Key Laboratory of Molecular and Nano Probes, Ministry of Education, Collaborative Innovation Center of Functionalized Probes for Chemical Imaging in Universities of Shandong, Institute of Molecular and Nano Science, Shandong Normal University, Jinan 250014, P. R. China.

[b] Dr. S. Ye College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, People's Republic of China

*E-mail: tangb@sdnu.edu.cn; lilu5252@163.com;

Table of Content

EXPERIMENTAL SECTION

Materials.

Trisodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) , sodium borohydride ($NaBH_4$), ascorbic acid (AA), sodium dodecyl sulfate (SDS) and hydrogen tetrachloroaurate(III) ($HAuCl_4 \cdot 4H_2O$, 99.99%) were purchased from China National Pharmaceutical Group Co.,Ltd. (Shanghai, China); Recombinant human matrix metalloproteinase-2 (MMP-2) was purchased from ProSpec-Tany TechnoGene Ltd. (Ness Ziona, Israel); The p-aminophenyl mercuric acetate (APMA) was purchased from Genmed Scientifics Inc. (Arlington, U.S.A); *Aminomethane hydrochloride (Tris-HCl)* was purchased from Beijing Mairybio Biological＆Technology Co.,Ltd. (Beijing, China); N-Hexadecyltrimethylammonium Chloride (CTAC) and glutathione (GSH) were purchased from Aladdin Aladdin Chemical Company Co.,Ltd. (Shanghai, China); The 1,4-benzenedithiol (1,4-BDT) was purchased from energy chemcical Co.,Ltd. (Shanghai, China); Phosphate buffered saline (PBS) and cell culture products were purchased from *Biological Industries Ltd. (Kibbutz Beit Haemek, Israel);* Enhanced cell counting kit-8 (CCK-8) was purchased from Shanghai Saint-Bio Biotechnology Co.,Ltd. (Shanghai, China); All the chemicals were of analytical grade and used without further purification. Purified water was obtained from Wahaha Group Co., Ltd. (Hangzhou, China) .

The TAMRA-modified peptide (TAMRA-Acp-Gly-Pro-Leu-Gly-Val-Arg-Gly-Cys and TAMRA-Acp-Gly-Pro-Leu-Gly-Val-Arg-Gly-{Se-Cys}) were synthesized and purified by NingBo Karabay Biochem Co., Ltd. (NingBo, China).

Instruments.

The transmission electron microscopy (TEM) was carried out on a Hitachi HT7700 transmission electron microscope (JEOL Ltd, Japan) and the samples were prepared via carbon-coated copper grids. The UV-vis absorption spectra were obtained with a Hitachi U-3010 UV-vis spectrophotometer (JEOL Ltd, Japan). In the MTT assay, a microplate reader (Rayto, U.S.A.) was used to measure the absorbance. The SERS and SERS images were obtained on an in Via Raman microscope (HORIBA, France).

Synthesis of gold nanoparticles (AuNPs).

AuNPs were synthesized by citrate reduction method. All the glassware was first cleaned with a mixture of HCl and HNO_3 (ratio of HCl/HNO_3 = 3:1 in volume) and thoroughly rinsed with ultrapure water. The 100 mL of 0.01% (w/w) aqueous chlorauric acid ($HAuCl_4$) solution was constantly stirred and heated to its boiling point. After its boiling point was reached, 1.5 mL of 1% (w/w) trisodium citrate solution was added to the solution quickly, which resulted in a change in solution changed from a pale yellow to a wine-red color. The solution was then refluxed for 30 min to ensure complete reduction. The colloidal solution was then slowly cooled to room temperature with continuous stirring. Transmission electron microscopy (TEM) images indicated the particle sizes are ~25 nm. The prepared gold colloidal solutions were stored in brown glass at 4 °C untill use.

Synthesis of Au Core-Shell nanoparticles

Synthesis of Au cores. Au cores were synthesized by a seed-mediated method. Typically, seed solution was firstly prepared by vigorous mixing of 4.5 mL water, 5 mL CTAC solution (0.2 M), 450 μL of $NaBH_4$ solution (0.02 M) and 515 μL of $HAuCl_4$ (4.86 mM). The seed solution was allowed to stay at 30°C for 1 hour and further diluted by 10 times. Then seed growth solution was prepared by mixing 10 mL of CTAC solution (0.1 M) ,515 μL of $HAuCl_4$ (4.86 mM) and 75 μL of ascorbic acid (0.04 M). The 100 μL of diluted seed solution was added into the seed growth solution under sonication and kept darkness for two days to obtain highly uniform spherical nanoparticles. The size of the Au cores obtained at this stage was about 22 nm.

Synthesis of Au Core-Shell. The Au cores (1 nM) were washed once to remove excess CTAC and then redispersed in water. The 1,4-BDT powder was dissolved in ethanol. Then 400 μL of 2 mM 1,4-BDT molecule solution was slowly added to the 10 mL Au core (1 nM) solution under ultrasonication. The 1,4-BDT molecule-modified cores were washed three times to remove excess molecules and then redispersed in CTAC solution(0.1 M). The gold shell was prepared by adding 120 μL of Raman reporter modified core solution into the mixed growth solution of 2 mL CTAC solution (0.1 M), 100 μL of ascorbic acid (0.04 M), and 100 μL of HAuCl4 (4.86 mM) under vigorous sonication.

Synthesis of Au-Se SERS nanoprobes , Au-S SERS nanoprobes and Au-Se-CS SERS nanoprobes

A mass fraction of (10%) sodium dodecyl sulfate (SDS) was added to the AuNPs and Au Core-Shell nanoparticles solution to a final mass fraction of 0.1%. The mixture was shaken for about 30 min. The thiol and selenol -modified peptide chains with TAMRA were added into the mixture and the final concentration of thiol and selenol -modified peptide chains were 1 nM. The mixture under mildly shaking was saved in the dark for 48 h to complete the synthesis of the Au-Se probe and the Au-S probe. Excess reagents were removed by centrifugation at 10,000 rpm for 20 min. Then, the red precipitate was washed and centrifuged three times. The prepared Au-S probe and Au-Se probe solutions were stored in brown glass at 4 °C untill use.

AuNPs' Peptide Loading.

To evaluate peptide loading on the surface of AuNPs, the amount of selenol-modified peptides and thiol-modified peptides loaded on the AuNPs were determined through a reported protocol[1]. Generally, mercaptoethanol was added (final concentration =100 mM) to Au−Se SERS nanoprobes and Au−S SERS nanoprobes, and the mixture was stirred overnight at room temperature in the dark for ligand replacement. Afterward, the released peptides with TAMRA modification were collected via centrifugation, and the fluorescence intensity of the supernatant solution was measured with a fluorescence spectrometer with 560 nm excitation and 582 nm emission, and converted to molar concentrations of the peptides by interpolation from a standard linear calibration curve that was obtained with known concentrations of peptide. By dividing the molar concentrations of peptides by the original concentration of the nanoprobes, the amount of peptides on per nanoprobe was determined. All experiments were performed six times to check reproducibility. This results are listed in table S1, which are similar to those reported in the literature[2].

Raman Response of Au-Se SERS nanoprobes and Au-S SERS nanoprobes to MMP-2

The Au-Se SERS nanoprobes and the Au-S SERS nanoprobes (1 nM) were incubated with 10 μL different concentrition activated MMP-2 at 37 °C, 2 h, respectively.

Cell Culture

A549 cells were cultured at 37℃ in a humidified atmosphere of 5% CO_2 and 95% air using DMEM supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. Cells were passaged every 2-3 days.

Incubating SERS nanoprobes in cells

A549 cells of 1×10^6 mL^{-1} were seeded in each confocal dish, the Au-S SERS nanoprobes, Au-S SERS nanoprobes or the Au-Se-CS SERS nanoparticles were then added into each dish. After incubation at 37 ℃ for different times, the cells were sent to a confocal Raman spectrometer for detection.

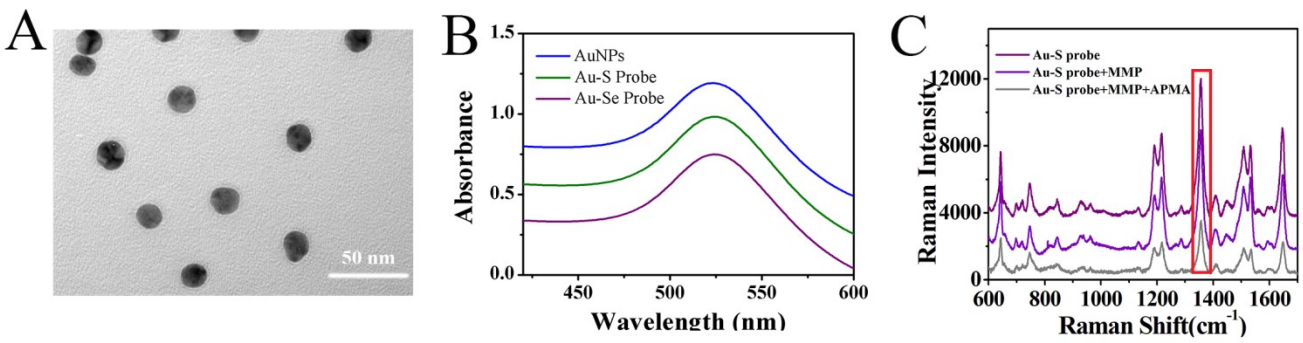
Cell viability experiment

Cell viability experiment was determined by Cell Counting Kit-8 (CCK8). A549 cells were dispersed in replicate 96-well microtiter plates (1×10^6 cells/well), respectively. The plates were kept in 5% CO_2/95% air incubator at 37 °C for 24 h. Then the initial medium was discarded and the cells were incubated with naked-AuNPs of 1 nM, Au-S probe of 1nM and Au-Se probe respectively for 3, 6, 9, 12, 24 and 48 h. After being washed with PBS, the CCK-8 mixed solution (100mL DMEM and 10 μL CCK-8 mixed solution) were added to each well and further incubated for 4 h. The absorbance at 490 nm was detected with a RT 6000 microplate reader.

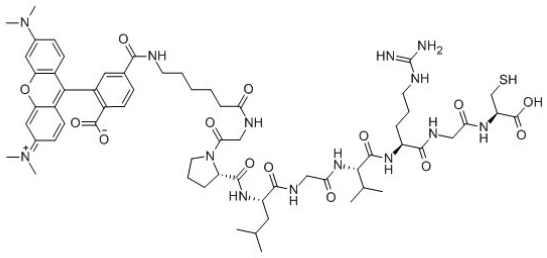
SERS Measurements

SERS spectrum and SERS imaging pictures were obtained using a confocal Raman microscope (LabRAM HR Evolution, HORIBA) equipped with a grating with 600 grooves/cm. SERS spectrum and SERS imaging experiments were acquired at 25 °C using He-Ne laser operating at λ = 633 nm and a 50× long objective lens. For each Raman spectrum and imaging, the laser output power was 10 mW, the collecting time was 5 s.

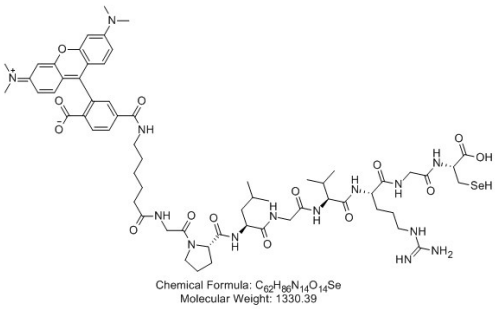
SUPPLEMENTAL FIGURES



D TAMRA—Peptide—SH:



TAMRA—Peptide—SeH:



Chemical reaction:

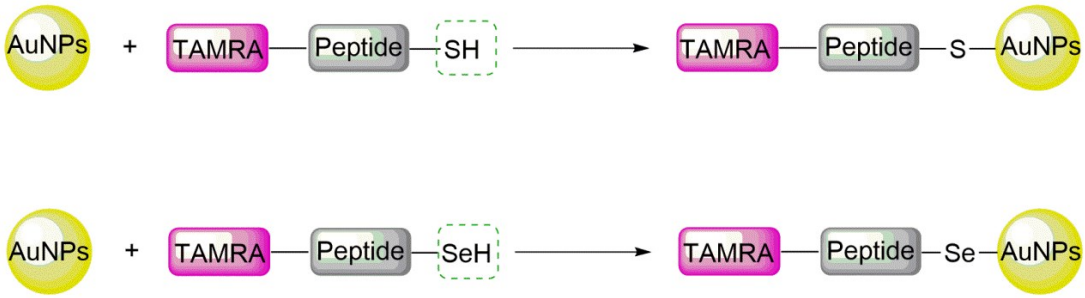


Figure S1. (A) TEM images of AuNPs. (B) UV-Vis spectra of the Au NPs, Au-Se SERS nanoprobes and Au-S SERS nanoprobes. The final concentration of nanoprobe for UV-Vis analysis is 10^{-7} mol/L. (C) Raman spectra of Au-S SERS nanoprobes , Au-S SERS nanoprobes with inactivated MMP-2 and activated MMP-2 (10 ng/mL). (D) The structural formulas of the two peptides and the chemical reaction between AuNPs and peptides.

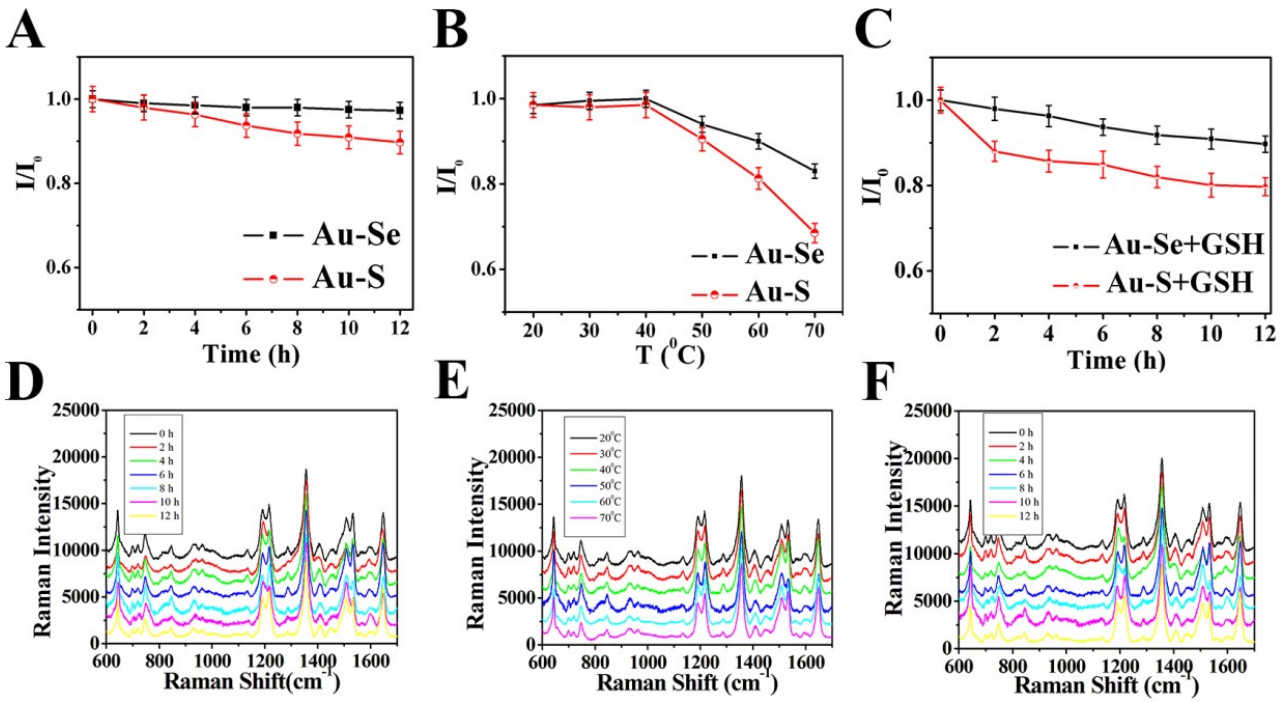


Figure S2. The SERS signal changes of Au-Se SERS nanoprobes and Au-S SERS nanoprobes at different time (A), different temperature (B) and in the prescence of 5 mM GSH at different time (C) Raman spectra of Au-Se SERS nanoprobes at different time (D) Raman spectra of Au-Se SERS nanoprobes at different temperature (E) Raman spectra of Au-Se SERS nanoprobes in the prescence of 5 mM GSH at different time (I, instant Raman intensity; I_0, initial Raman intensity).

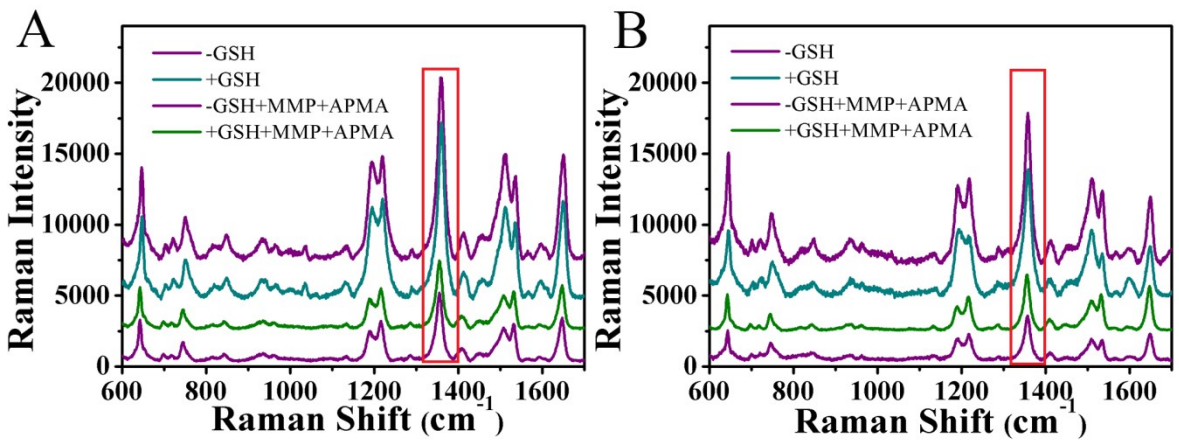


Figure S3. Raman spectra of the Au-Se SERS nanoprobes(A) and Au-S SERS nanoprobes(B) under 5 mM GSH in the presence and absence of activated MMP-2(10 ng/mL).

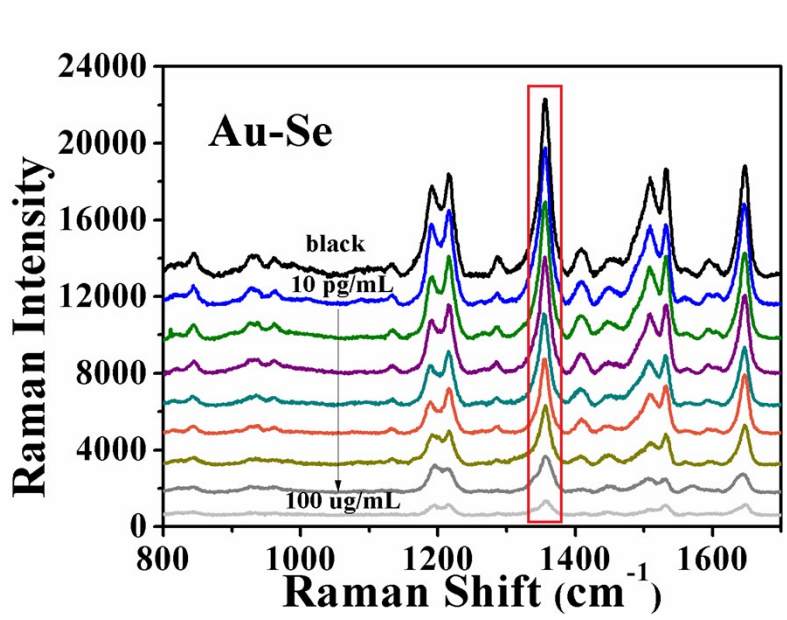


Figure S4. Raman spectra of the Au-Se SERS nanoprobes to different concentrations of MMP-2 (From top to bottom: black, 10 pg/mL, 100 pg/mL, 1 ng/ mL, 10 ng/mL, 100 ng/mL, 1 μg /mL, 10 μg /mL, 100 μg /mL, respectively).

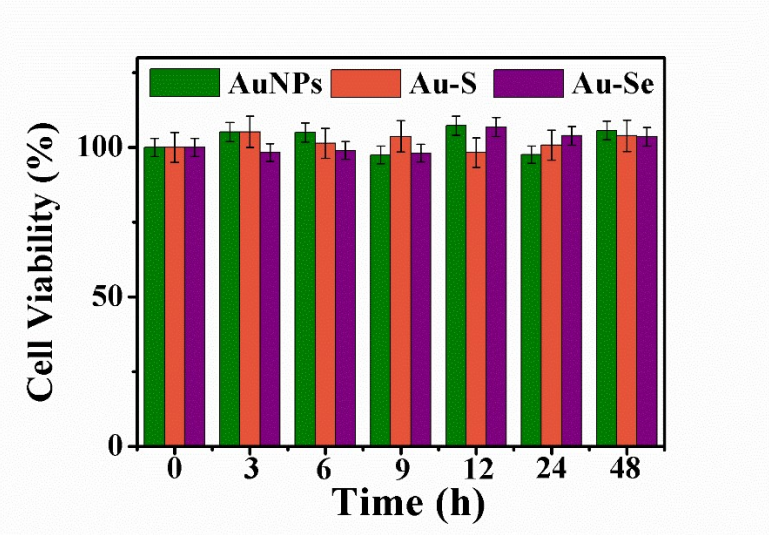


Figure S5. The MTT assay of A549 cells incubated with AuNPs (1 nM), Au-S SERS nanoprobes (1 nM) and Au-Se SERS nanoprobes (1 nM) for different time

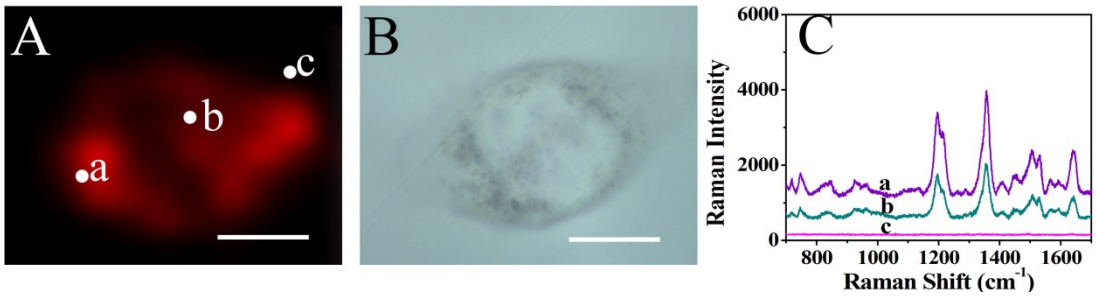


Figure S6. (A) Raman image and (B) bright image of A549 cells using Au-S SERS nanoparticles. (C) SERS spectra acquired from representative locations (a and b) inside and (c) outside of the A549 cells in image A. scale bar =5 μm.

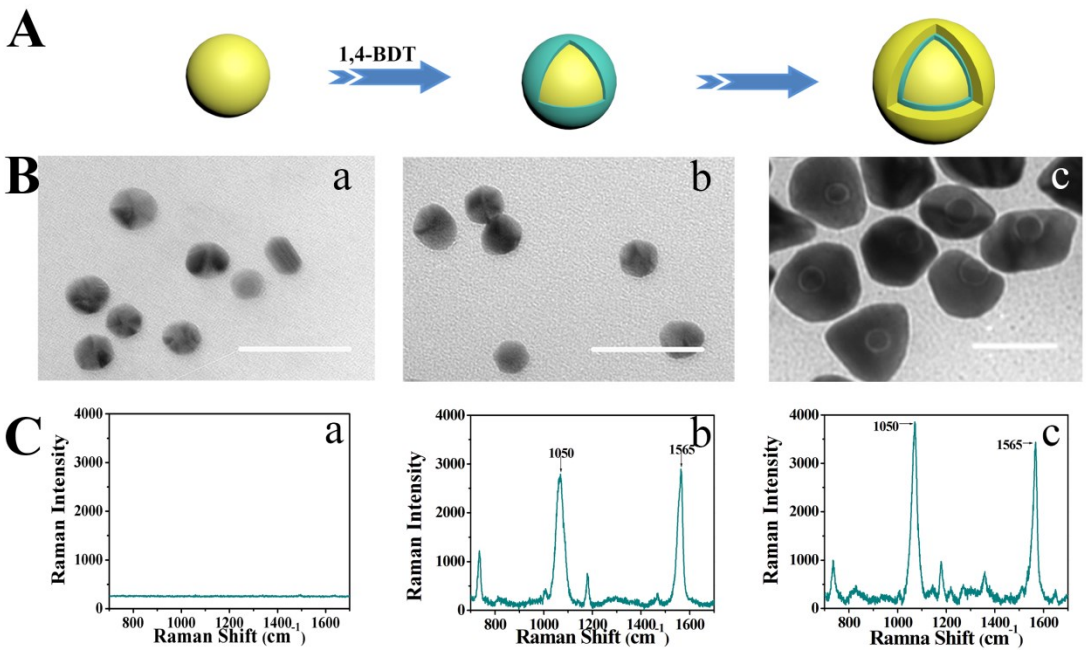


Figure S7. (A) Schematic synthesis procedures of core-shell nanoprobes. (B) TEM images of AuNPs (a), AuNPs modified of 1,4-BDT (b), core-shell SERS nanoprobes (c) (scale bar = 50 nm) (C) Raman spectra of AuNPs (a), AuNPs modified of 1,4-BDT (b), core-shell SERS nanoprobes (c). The characteristic peaks of 1,4-BDT are at 1050 cm^{-1} and 1565 cm^{-1}.

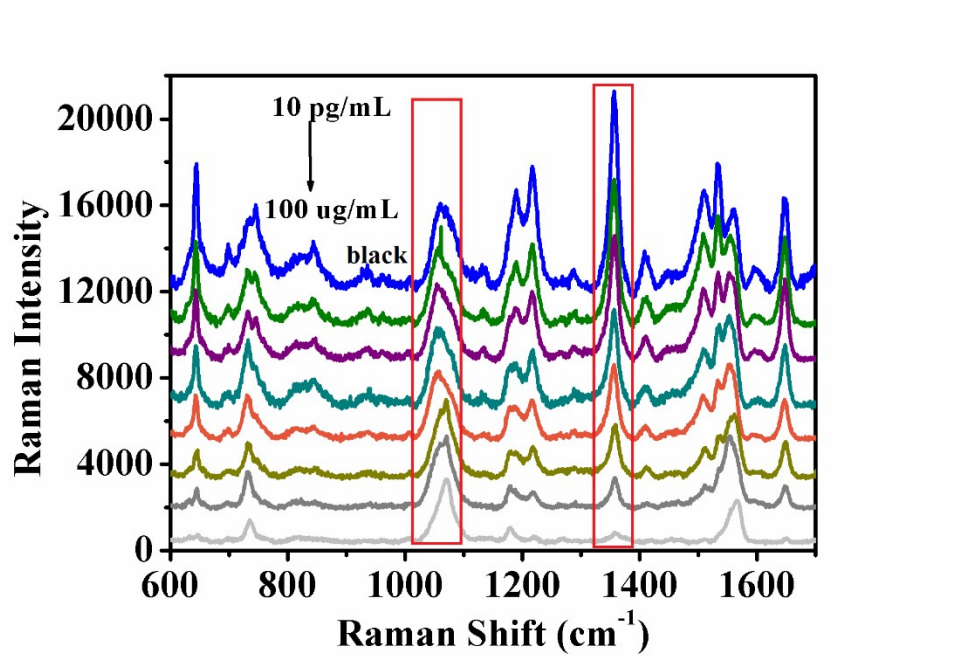


Figure S8. Raman spectra of the Au-Se-CS SERS nanoprobes to different concentrations of MMP-2 (From top to bottom: black, 10 pg/mL, 100 pg/mL, 1 ng/ mL, 10 ng/mL, 100 ng/mL, 1 μg /mL, 10 μg /mL, 100 μg /mL, respectively).

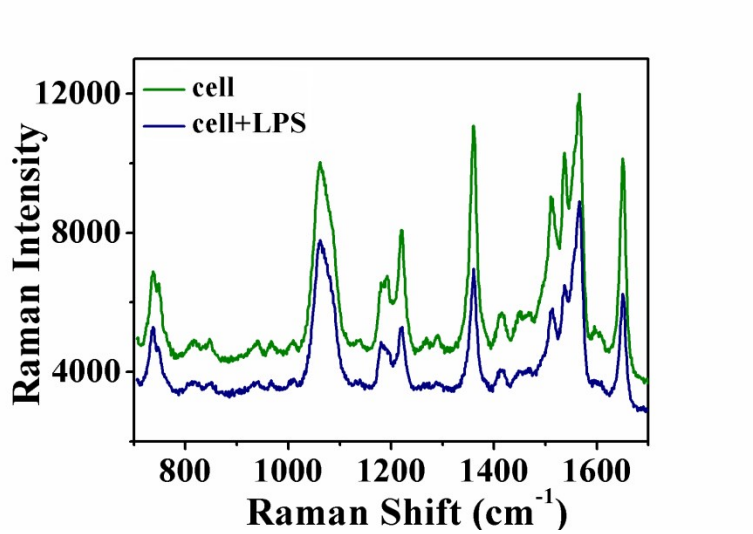


Figure S9. The Raman spectra of Au-Se-CS SERS nanoparticles to MMP-2 secreted by A549 cells before and after LPS stimulation.

Peptide concentration	100 nM	500 nM	1 μM	10 μM
Au-Se SERS nanoprobe (counts)	18 ± 1	98 ± 1	165 ± 1	178 ± 2
Au-S SERS nanoprobe (counts)	10 ± 1	53 ± 1	104 ± 2	170 ± 3

Table S1. The amount of peptide chains loaded on of Au-S SERS nanoprobes and Au-Se SERS nanoprobes.

Table S1 shows that the amount of two types of peptide chains on AuNPs when peptide chains with different concentrations were combined with AuNPs. For comparison, we prepared two types of SERS nanoprobes with 100 peptide chains on each AuNP. Finally, 500 nM of peptides was chosen for Au-Se SERS nanoprobes and 1 μM for the Au−S SERS nanoprobes.

Reference:

[1] Luan, M.; Yu, L.; Li, Y.; Pan, W.; Gao, X.; Wan, X.; Li, N.; Tang, B. Anal. Chem. 2017, 89, 10601-10607.

[2] X. N. Gao, L. L. Jiang, B. Hu, F. P. Kong, X. J. Liu, K. H. Xu, B. Tang. Anal. Chem. 2018, 90, 4719-4724.