Electronic Supplementary Material (ESI) for Chemical Science.

Supplementary information

In situ SERS imaging of protein-specific glycan oxidation on living cells to quantitatively visualize pathogen-cell interaction

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Experimental section

Materials and reagents. Chloroauric acid ($HAuCl_4 \bullet 4H_2O$) was obtained from Shanghai Chemical Reagent Company (China). 5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB) was purchased from Sigma-Aldrich (USA). Ascorbic acid (AA), trisodium citrate and hydrogen peroxide (H_2O_2, 30%) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Chloroplatinic acid (H_2PtCl_6) and galactose oxidase (GO) were purchased from Shanghai Macklin Biochemical Co., Ltd (China). Thiol PEG 2K amine (HS-PEG-NH_2) was purchased from Shanghai To Yong Bio (China). Fetal bovine serum (FBS), Roswell Park Memorial Institute-1640 Medium (RPMI-1640), trypsin, phosphate buffered saline (pH 7.4) containing 136.7 mM NaCl, 2.7 mM KCl, 8.72 mM Na_2HPO_4, 1.41 mM KH_2PO_4, 1 mM $CaCl_2$, and 1 mM $MgCl_2$ (PBS+) and phosphate buffered saline (pH 7.4) containing 136.7 mM NaCl, 2.7 mM KCl, 8.72 mM Na_2HPO_4, 1.41 mM KH_2PO_4 (PBS-) were purchased from KeyGen Biotech (China). Benzyl-2-acetamido-2-deoxy-α-D-galactopyranoside (BAG) was obtained from Santa Cruz Biotechnology Inc. (USA). MCF-7 and MCF-10A cells were obtained from KeyGen Biotech. Co. Ltd. (Nanjing, China). *Fusariun graminearum* (*FG*) was obtained from Mingzhoubio Co. Ltd. (Ningbo, China). Fluorescent soybean agglutinin (FITC-SBA) was purchased from Vectorlabs (USA). Calcein AM/PI cell viabilityassay Kit was purchased from Beyotime Biotechnology (Shanghai, China). Cuprous chloride (CuCl) and carbendazim (CBZ) were obtained from Aladdin Chemical Reagent Co., Ltd (Shanghai, China). Quercetin (QCT) and cefoperazone (CFP) were purchased from Meryer Biochemical Technology Co., Ltd (Shanghai, China). All these reagents were used as received without further purification. All aqueous solutions were prepared using ultrapure water (≥18 MΩ, Milli-Q, Millipore). The DNA sequences in Table S1 (5' to 3') were synthesized and purified by Sangon Biotech Inc. (Shanghai, China), where FAM is 5-carboxyfluorescein.

Apparatus. Transmission electron microscopic (TEM) images were acquired by a FEI Talos F200x electron microscope (USA) operating at 200.0 kV, which was equipped with STEM and EDS detectors for element mapping analysis. Dynamic light scattering (DLS) measurements were performed on a 90 Plus/ BI-MAS equipment (Brook haven, USA). The UV-vis absorption spectra were recorded using a UV-vis spectrophotometer (Nanodrop-2000C, Nanodrop, USA). Fluorescence spectra were recorded on a F-7000 fluorescence spectrophotometer (Hitachi, Japan). Zeta potential analysis was performed on a Zetasizer (Nano-Z, Malvern, UK). Confocal fluorescence imaging of cells was performed on a SP8 STED 3X confocal laser scanning microscope (CLSM) (Leica, Germany). The cell concentration was

determined using a Countess II FL automated cell counter (Life Technologies, USA). The cell viability was measured on a Varioskan Flash fluorescent microplate reader (Thermo Fisher Scientific, USA). Raman images were collected on a Renishaw inVia confocal Raman microscope (Renishaw, UK) using 633 nm excitation.

Preparation of 50-nm AuNPs. 50-nm AuNPs (Au50) were synthesized by adding 2.25 mL of trisodium citrate (1%, w.t.) into 300 mL of boiling $HAuCl_4$ solution (0.01%, w.t.) under vigorous stirring. [S1] After 20 min of boiling, the mixture was cooled down and stored at 4 °C. The concentration of AuNPs was determined using UV-vis absorption spectrometry. [S2]

Preparation of Au@PtNPs. After the as-prepared Au50 solution (60 mL) was vigorously stirred in a round-bottom flask, 2.94 mL of H_2PtCl_6 (1 mM) was added and heated to 68 °C. Under agitation, freshly prepared ascorbic acid (AA) solution (10 mM, 1.48 mL) was added dropwise with a speed of 80 µL/min. The mixture was kept at 80 °C for 30 min, and then cooled to room temperature. After washing by centrifugation (5000 rpm) with deionized water, the Au@PtNPs were obtained. The platinum shell thickness of Au@PtNPs was controlled by changing the amount of H_2PtCl_6 added. [S3]

Preparation of Au@Pt-DTNB/Apt. The Au@PtNPs (1 nM, 1 mL) were dispersed in HS-PEG-NH_2 (MW 2000) solution with a molar ratio of 1:5000 and incubated for 5 min at room temperature. After washing by centrifugation with deionized water, Apt-SH (10 µM, 50 µL) was added into the obtained HS-PEG-NH_2 modified Au@Pt NPs and stirred at room temperature for 30 min. After removal of free Apt-SH by centrifugation with deionized water, the dispersion was incubated with DTNB (10 mM, 50 µL) for 20 min at room temperature. Afterward, the mixture was washed by centrifugation with PBS-, and the obtained Au@Pt-DTNB/Apt was resuspended to 1 mL PBS- for further use. Au@Pt-DTNB/Ran was prepared with the same procedure by replacing Apt-SH with Ran-SH.

***In vitro* response of Au@Pt-DTNB/Apt to ROS.** The ROS solution was prepared by mixing 1 M of $CuCl_2$ with 1 mM of H_2O_2 in the PBS-, which was immediately centrifuged at 14000 rpm for 10 s to collect the supernatant. The concentration of the ROS solution was set equal to the H_2O_2 concentrations in the mixture. 10 µL of different concentrations of ROS solutions were mixed with 10 µL of 5 M Au@Pt-DTNB/Apt at room temperature for 5 min.

Then, 2 μL of the mixture was dropped onto a clean silicon wafer, which was subjected to Raman detection. The Raman spectra acquisition mode was the static scan type at a center wavenumber of 980 cm^{-1} with parameters of 1-s exposure time, 1-time accumulation, 17 mW laser power, 633 nm excitation.

The calculation process of the LOD. The LOD was calculated with a traditional and typical approach reported by the previous references.[S4] The Raman measurement for blank samples was executed with three parallel tests, which exhibited an average Raman intensity (I_B) of 2392.3 (a.u). with a standard deviation (S_B) of 198.4. As the signal-to-noise ratio value (k_1) for the calculation of LOD was normally set as 3, the smallest detectable signal (I_R) could be calculated as: $I_R = I_B + k_1S_B$ = 2987.4. The linear regression equation of Raman intensity to ROS concentration in Fig. 2B was: I = 627.0 lg c +8963.3. Thus, when the I equaled to 2987.4, the corresponding c value was the LOD, which was calculated to be 0.29 pM.

Cell Culture. MCF-7 and MCF-10A cells were cultured in RPMI 1640 medium supplemented with 10% FBS, penicillin (100 μg/mL), and streptomycin (100 μg/mL) at 37 °C in a humidified atmosphere containing 5% CO_2, respectively.

Pathogen Culture. *Fusariun graminearum* (*FG*) used in this study is also known by the name of its anamorph *Gibberella zeae (Schweinitz) Petch*. Its teleomorph number is 20273, and the biosafety level is Level 1. It was cultured in a culturing solution containing potato powder (0.5%), dextrose (2%), monopotassium phosphate (0.3%), magnesium sulfate (0.15%) and thiamine•HCl (0.01%) under pH 6.0 ± 0.2 at 25 °C. The concentration of *FG* was measured by bacterial counting plates.

Raman Imaging. MCF-7 or MCF-10A cells seeded on confocal dishes were firstly incubated with 400 μL Au@Pt-DTNB/Apt (0.5 nM) at 37 °C for 40 min. After washing with PBS+ for three times, the Au@Pt-DTNB/Apt bound cells were incubated with different concentrations of GO, *FG* or drug-treated *FG*, and then directly subjected to Raman imaging on a confocal Raman microscope.

The Raman imaging acquisition mode was the static scan type at a center wavenumber of 980 cm^{-1} with parameters of 1-s exposure time, 1-time accumulation, 17 mW laser power, 633 nm excitation and 3 μm × 3 μm step. The Raman images of cells were generated using signal at point review mode at 1337 cm^{-1} by a WiRE 3.4 software with black to red color scale. The average Raman intensity of cells was obtained from statistics mean value by dividing

the total red channel value with the total cell membrane length within the chosen area in the Raman images using Photoshop CS6 software. The Raman spectra were recorded at 100 different randomly chosen locations on the cell membrane in each Raman image.

Cell Viability. After the normal or Au@Pt-DTNB/Apt treated MCF-7 cells were incubated with *FG* or drug-treated *FG* (1×10^6 pcs/mL) for 120 min, 100 μL of the combined Live/Dead cell-staining solution (containing calcein AM and propidium iodide (PI)) was added into the cells to incubate at 37 °C for 30 min. The cells were subjected to CLSM imaging and analyzed with a fluorescent microplate reader (Ex/Em=494/517 nm for Calcein AM, Ex/Em=535/617nm for PI). By calculating the relative calcein AM/PI fluorescence values of the control groups with the experimental groups, the cell viability of each group was obtained.

CLSM Imaging. MCF-7, MCF-10A and BAG treated MCF-7 cells were separately seeded on four-well confocal dishes and cultured overnight. To image MUC1 on cell membrane, the MCF-7 and MCF-10A cells were respectively incubated with FAM-Apt (1.25 μM) or FAM-Ran (1.25 μM) at 37 °C for 40 min. To image Gal/GalNAc on cell membrane, the MCF-7, MCF-10A and BAG treated MCF-7 cells were respectively incubated with FITC-SBA (100 μg/mL) at 37 °C for 30 min. After washing with PBS+ for three times, the cells were subjected to CLSM imaging by collecting the emission signal from 500 to 580 nm under 488 nm excitation.

Statistical Analysis. All data were analyzed using a student's test at a significance level of $p > 0.05$ (NS), $p < 0.05$ (*) and $p < 0.01$ (**)

Supporting figures and tables

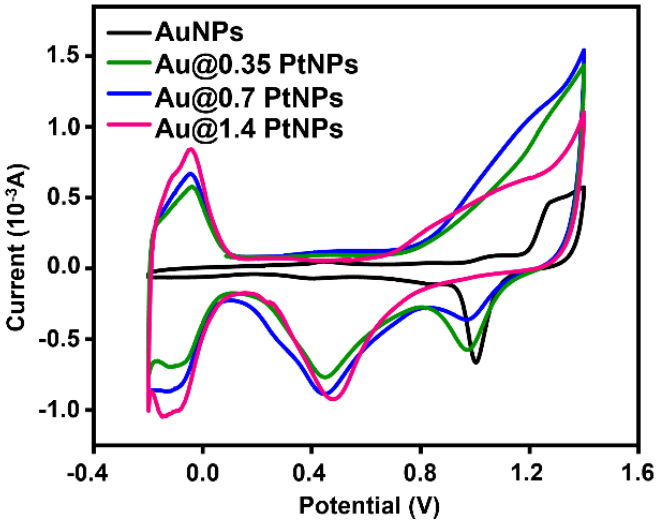


Fig. S1. Cyclic voltammograms of AuNPs, Au@0.35PtNPs, Au@0.7PtNPs and Au@1.4PtNPs modified glassy carbon electrodes in 0.5 M H_2SO_4. Scan rate: 100 mV s^{-1}.

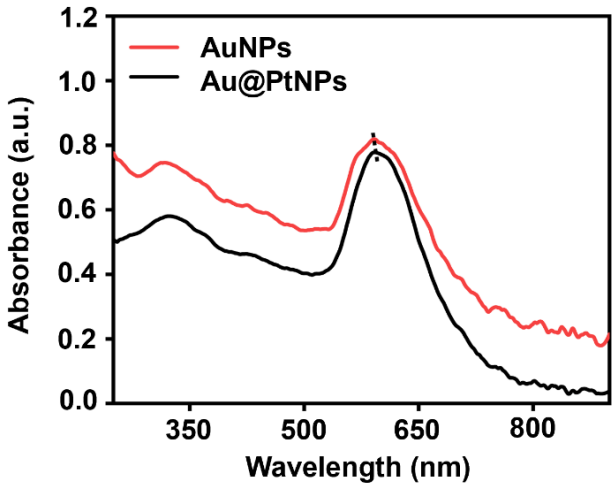


Fig. S2. UV/Vis spectra of synthesized AuNPs and Au@PtNPs dispersions.

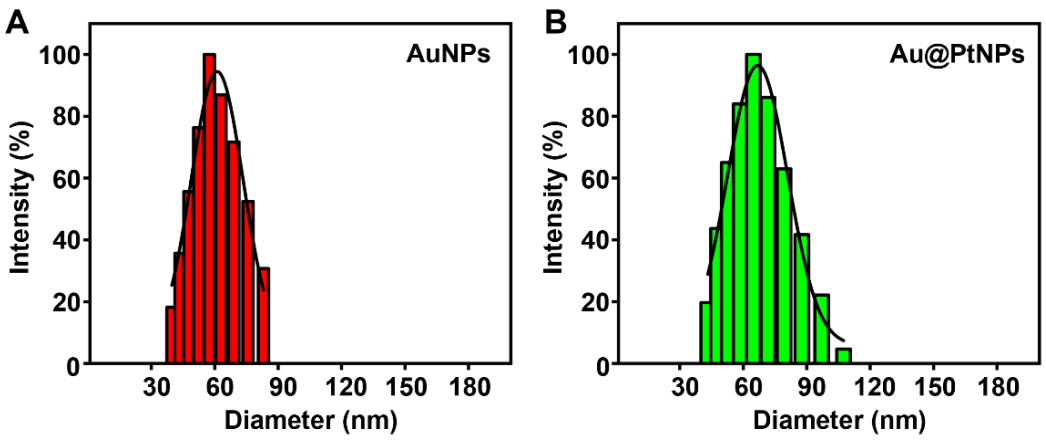
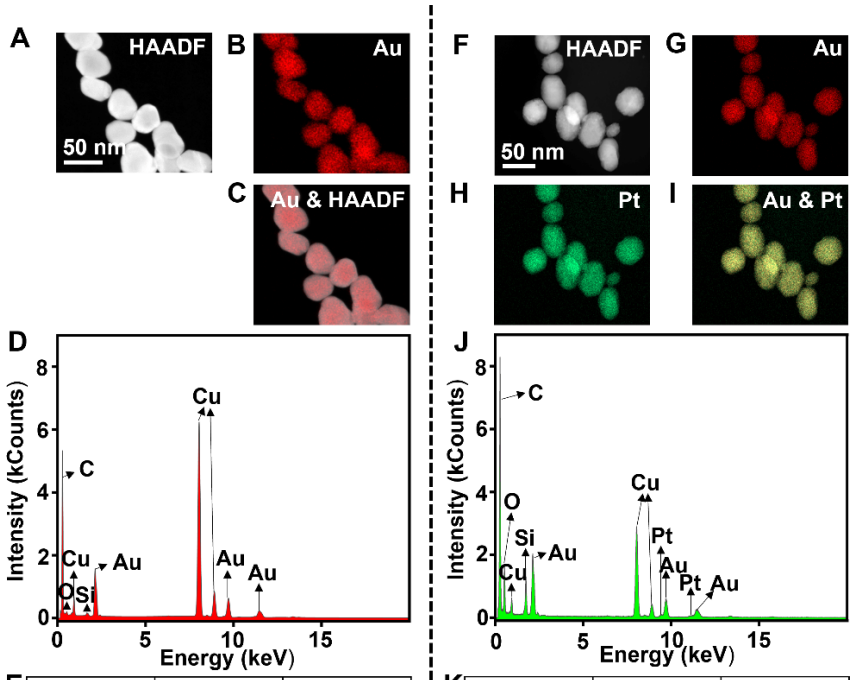


Fig. S3. DLS analysis of (**A**) AuNPs and (**B**) Au@PtNPs.



E

Element	Atomic (%)	Weight (%)
Au	82.1	98.3
Pt	0.0	0.0
O	17.9	1.7

K

Element	Atomic (%)	Weight (%)
Au	35.2	79.0
Pt	4.5	10.0
O	60.3	11.0

Fig. S4. (**A**) HAADF-STEM image of AuNPs. (**B**) EDS elemental mapping of Au. (**C**) Merged image of (**A**) and (**B**). (**D**) Integrated EDS drift corrected spectrum of AuNPs. (**E**) Elemental percentage of AuNPs. (**F**) HAADF-STEM image of Au@PtNPs. (**G**), (**H**) EDS elemental mapping of Au (**G**) and Pt (**H**). (**I**) Merged image of (**G**) and (**H**). (**J**) Integrated EDS drift corrected spectrum of Au@PtNPs. (**K**) Elemental percentage of Au@PtNPs.

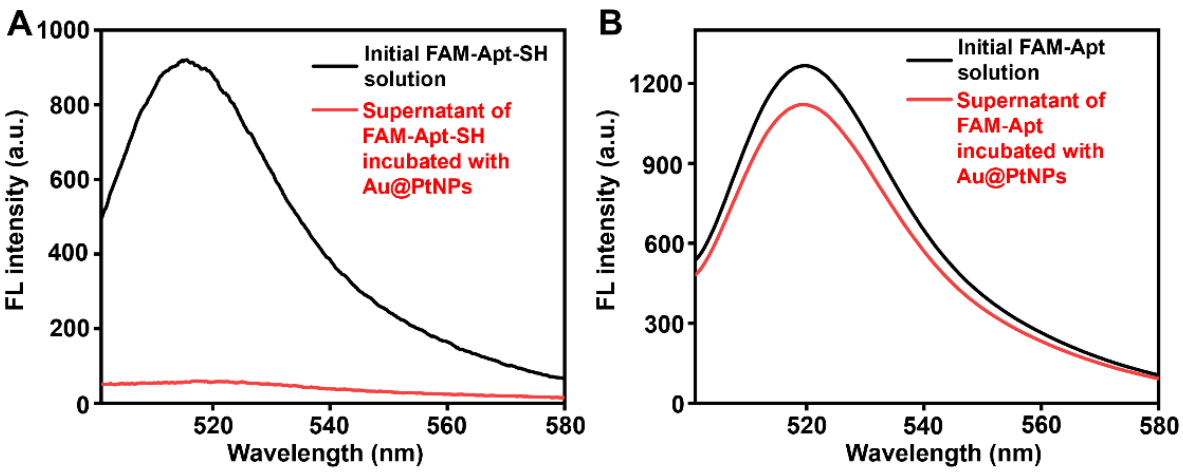


Fig. S5. Fluorescence spectra of (**A**) the initial FAM-Apt-SH solution and supernatant of FAM-Apt-SH after incubated with Au@Pt NPs, and (**B**) the initial FAM-Apt solution and supernatant of FAM-Apt after incubated with Au@Pt NPs.

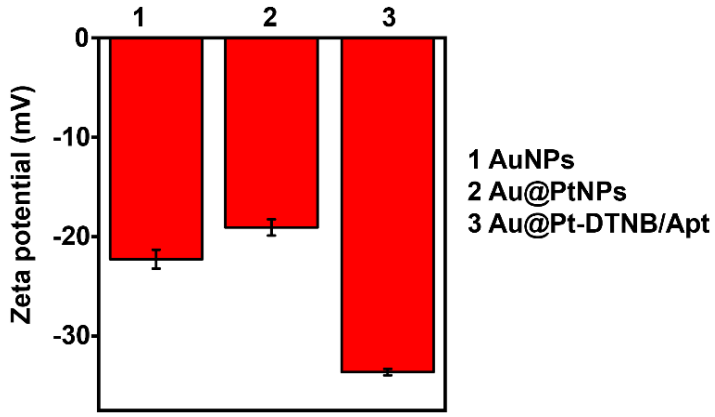


Fig. S6. Zeta potentials of AuNPs, Au@PtNPs and Au@Pt-DTNB/Apt. Error bars represent ± S.D. (*n*=3).

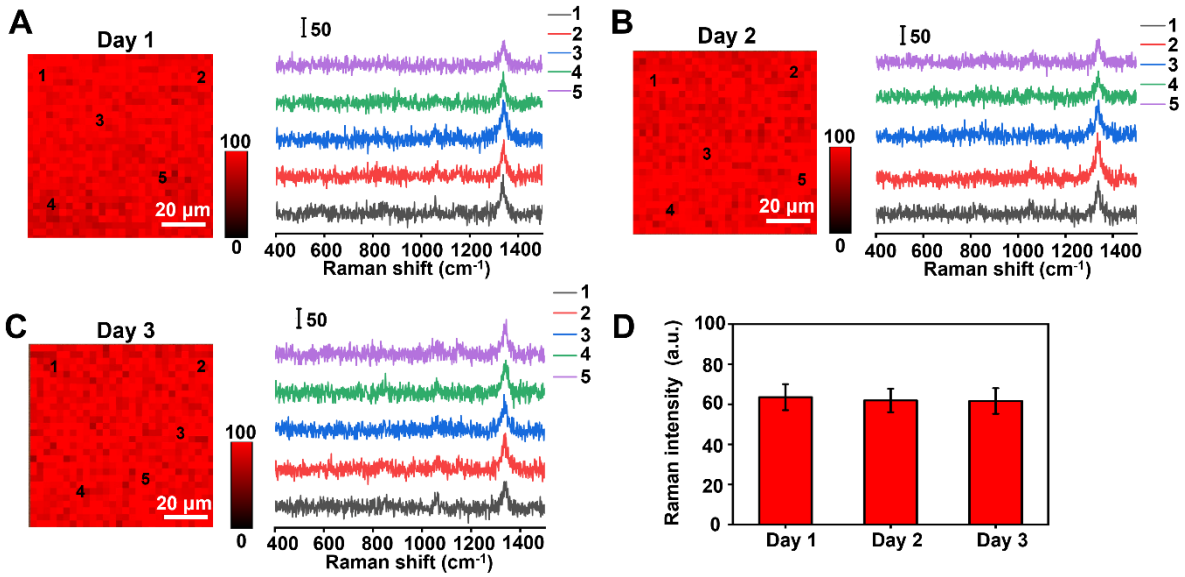


Fig. S7. (**A-C**) Raman images and corresponding Raman spectra at five different locations of Au@Pt-DTNB/Apt solution kept for 1, 2 and 3 days. (**D**) Average Raman intensities at 1337 cm^{-1} of Au@Pt-DTNB/Apt solution kept for 1, 2 and 3 days from all the 729 pixels in (**A-C**). The error bars indicate means ± SD.

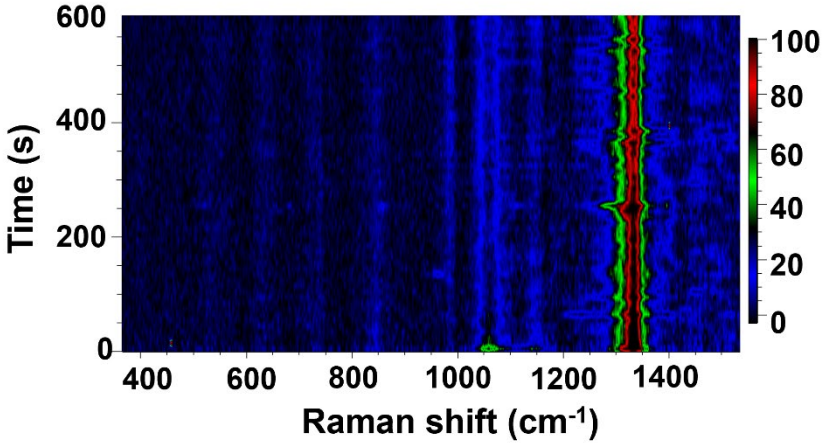


Fig. S8. Time series Raman signals of Au@Pt-DTNB/Apt solution under continuous laser irradiation for 600 s.

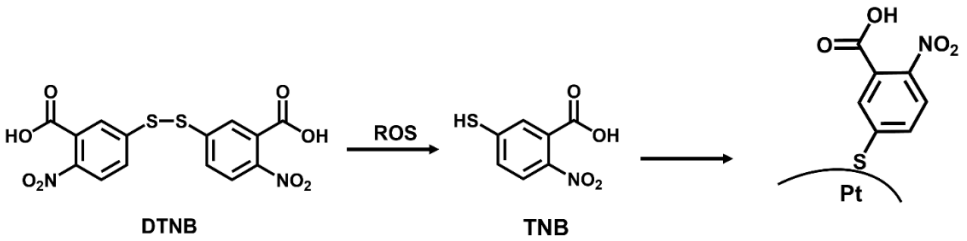


Fig. S9. Illustration of the oxidation of DTNB on Au@Pt-DTNB/Apt nanoprobe by ROS to produce TNB.

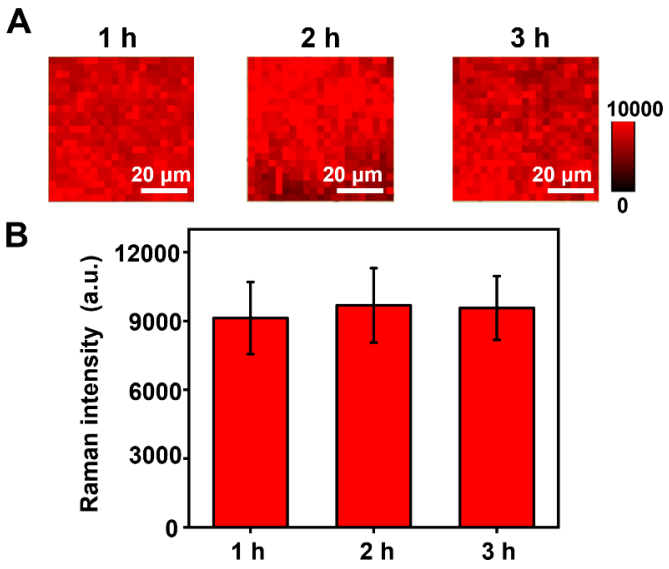


Fig. S10. (**A**) Raman images of Au@Pt-DTNB/Apt in the 1 mM ROS solution for 1 h, 2 h and 3 h. (**B**) Average Raman intensities at 1337 cm^{-1} of Au@Pt-DTNB/Apt in the ROS containing solution kept for 1 h, 2 h and 3 h from all the 441 pixels in (**A**). The error bars indicate means ± SD.

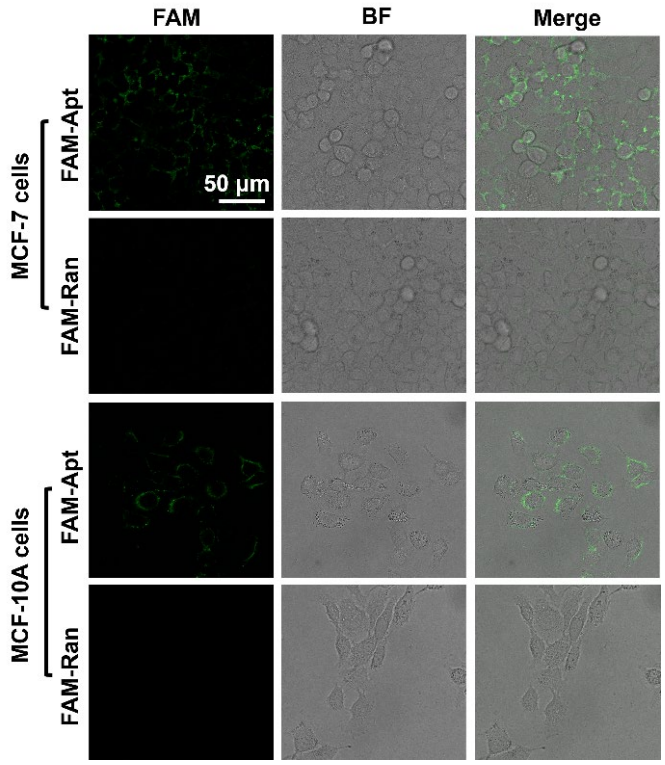


Fig. S11. CLSM images of MCF-7 and MCF-10A cells after incubated with 1.25 μM of FAM-Apt or FAM-Ran at 37 °C for 40 min.

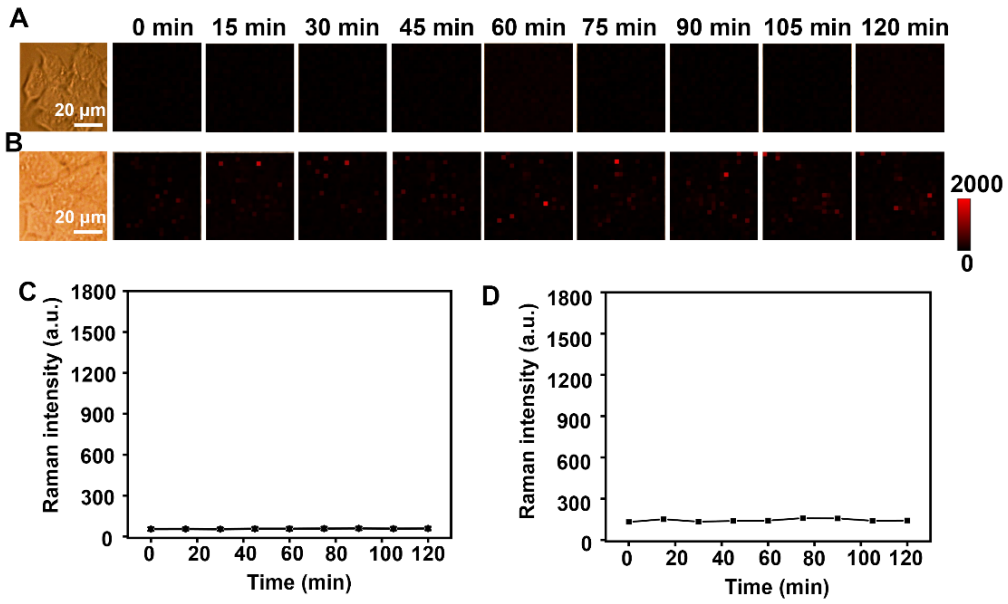


Fig. S12. (**A**) Raman images of MCF-7 cells treated with 0.5 nM Au@Pt-DTNB/Ran for 40 min and then incubated with 30 U/mL GO for different times. (**B**) Raman images of MCF-7 cells treated with 0.5 nM of Au@Pt-DTNB/Apt for 40 min and then PBS+ for different times. (**C, D**) Plots of Raman intensity *vs* incubation time from (**A**) and (**B**), respectively. Error bars represent ± S.D. (*n*=3).

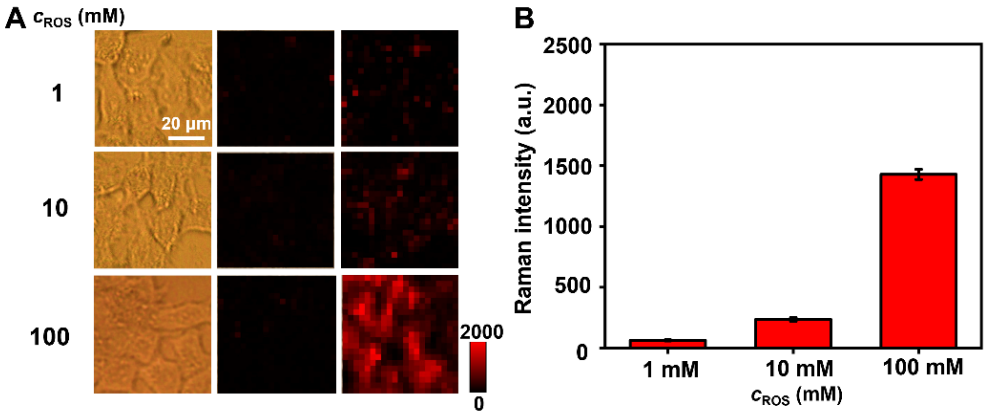


Fig. S13. (**A**) Raman images of MCF-7 cells treated with 0.5 nM of Au@Pt-DTNB/Apt for 40 min and then incubated with different concentrations of ROS. (**B**) Histogram of Raman intensity to different ROS concentrations from (**A**).

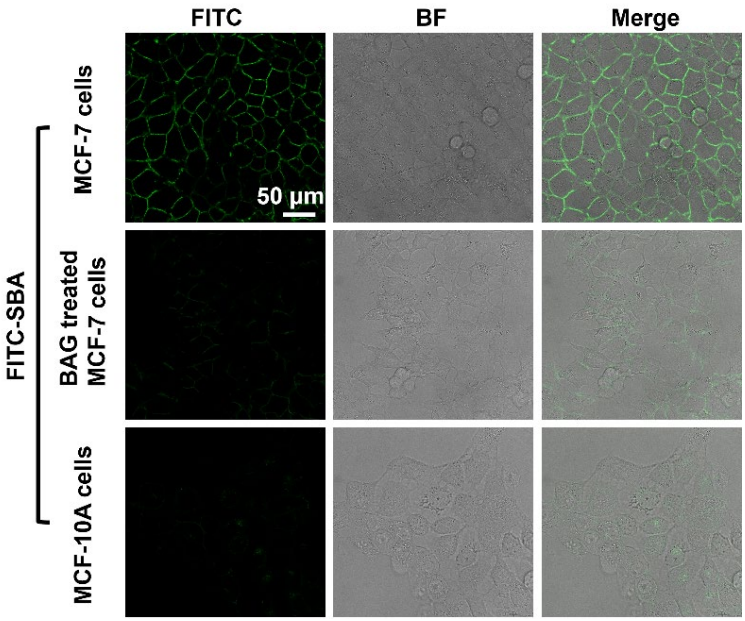


Fig. S14. CLSM images of MCF-7 cells, MCF-7 cells treated with 2.5 mM BAG at 37 °C for 22 h, and MCF-10A cells after incubated with 100 μg/mL of FITC-SBA at 37 °C for 40 min.

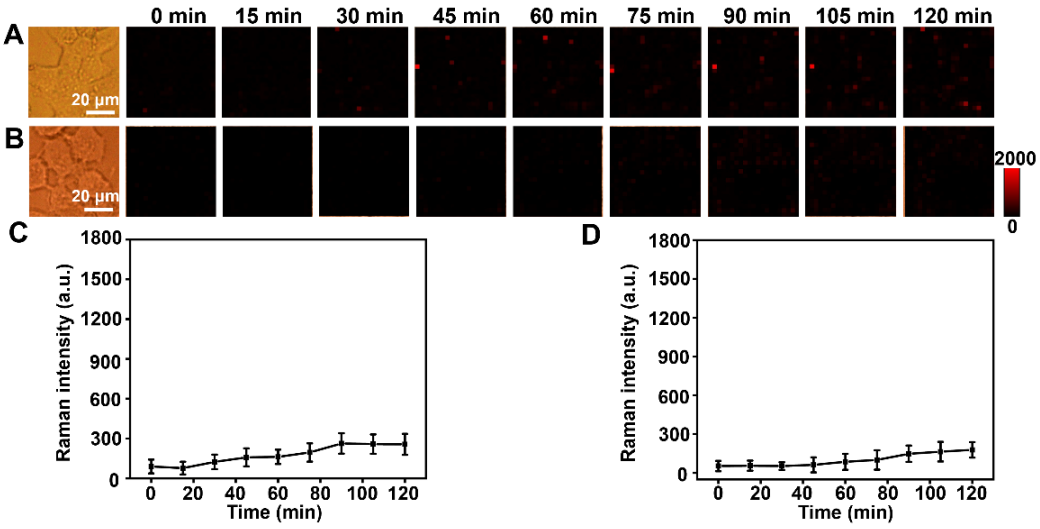


Fig. S15. (**A, B**) Raman images of BAG treated MCF-7 (2.5 mM at 37 °C for 22 h (**A**) and MCF-10A cells (**B**) after incubated with 0.5 nM Au@Pt-DTNB/Apt for 40 min and then 30 U/mL GO for different times. (**C, D**) Plots of Raman intensity *vs* incubation time with GO from (**A**) and (**B**), respectively. Error bars represent ± S.D. (*n*=3).

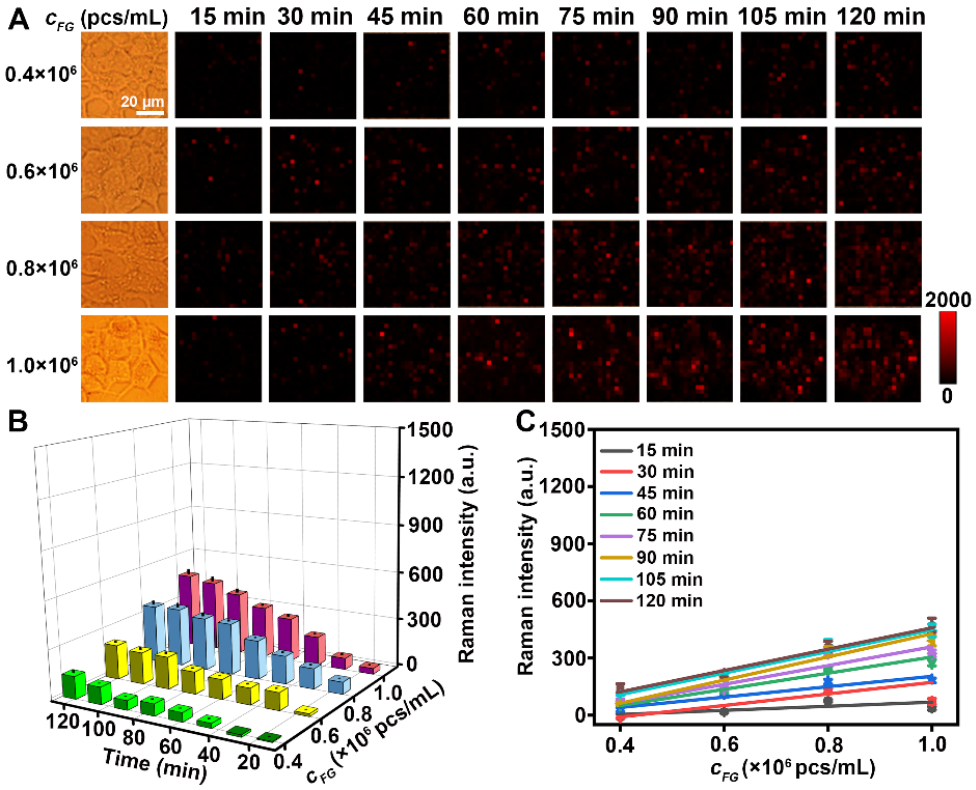


Fig. S16. (**A**) Raman images of MCF-7 cells treated with 0.5 nM Au@Pt-DTNB/Apt for 40 min and then incubated with different concentrations of *FG* for different times. (**B**) 3D histogram of Raman intensity to concentration of *FG* at different interacting times from (**A**). (**C**) Plots of Raman intensity to *FG* concentration at different interacting times from (**B**). The error bars indicate means ± SD (*n* = 3).

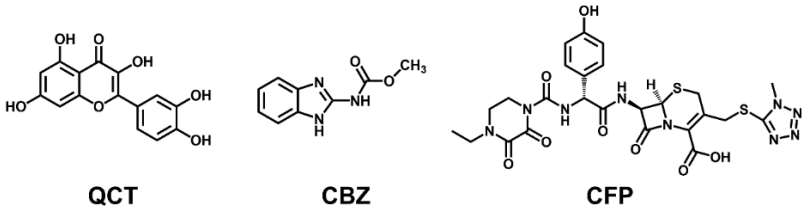


Fig. S17. Illustration of the structures of QCT, CBZ and CFP.

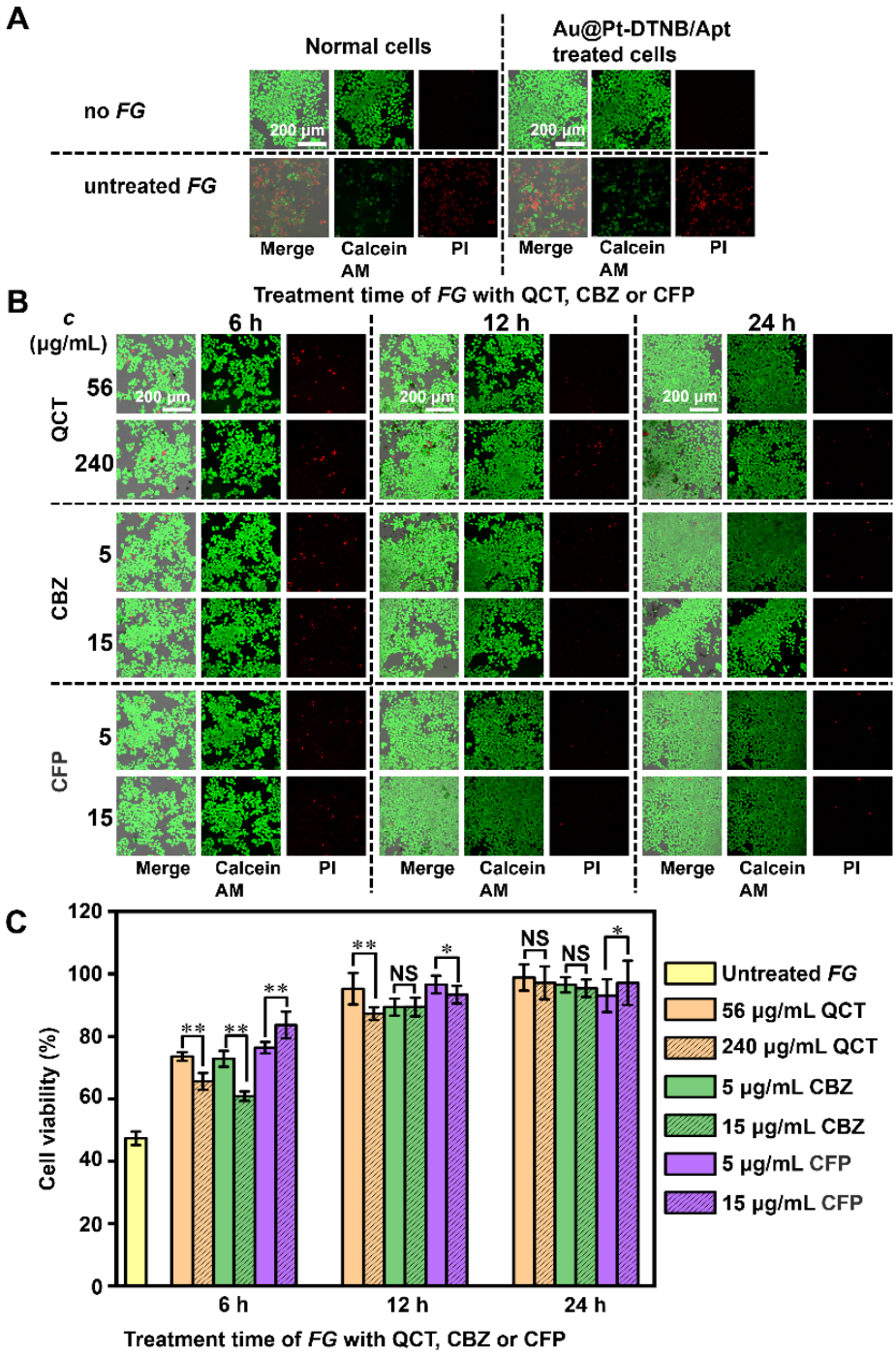


Fig. S18. (**A**) CLSM images of stained normal and Au@Pt-DTNB/Apt treated MCF-7 cells after incubated with PBS+ and untreated *FG* for 120 min. (**B**) CLSM images of stained normal MCF-7 cells incubated with drug-treated *FG* for 120 min. (**C**) Histogram of cell viability to drug treatment time of *FG* from (B) The data were analyzed using a student's test at a significance level of $p > 0.05$ (NS), $p < 0.05$ (*) and $p < 0.01$ (**). The error bars indicate means ± SD ($n = 5$).

Table S1. Nucleic acid sequences information.

Strand	Sequences (5'-3')
Thiol conjugated MUC1 aptamer (Apt-SH)	HS-TTT TTT TTT TTT TTT TTT TTT GCA GTT GAT CCT TTG GAT ACC CTG G
Thiol group conjugated random-sequenced DNA (Ran-SH)	HS-TTT TTT TTT TTT TTT TTT TTT GAG AAC CTG AGT CAG TAT TGC GGA GT
FAM conjugated MUC1 aptamer (FAM-Apt)	FAM-TTT TTT TTT TTT TTT TTT TTT GCA GTT GAT CCT TTG GAT ACC CTG G
FAM conjugated random-sequenced DNA (FAM-Ran)	TTT TTT TTT TTT TTT TTT TTT GAG AAC CTG AGT CAG TAT TGC GGA G
Thiol and 3'-FAM conjugated MUC1 aptamer (FAM-Apt-SH)	HS-TTT TTT TTT TTT TTT TTT TTT GCA GTT GAT CCT TTG GAT ACC CTG G TTT-FAM

Table S2. Linear equations of Raman intensity to GO[1] or *FG*[2] concentrations used to incubate Au@Pt-DTNB/Apt bound MCF-7 cells, and MUC1-specific Gal/GalNAc oxidation (MSGO) activities at different interacting times.

Interacting time	Linear equation of Raman intensity (*RI*) to GO concentration (c_{GO}) [1]		Linear equation of Raman intensity (*RI*) to *FG* concentration (c_{FG}) [2]		MSGO activity of *FG* (U/pcs)
15 min	$RI = -169.69 + 38.37\ c_{GO}$	$R^2 = 0.95$	$RI = -34.19 + 92.60\ (c_{FG}\times10^{-6})$	$R^2 = 0.48$	2.41×10^{-6}
30 min	$RI = -248.29 + 56.56\ c_{GO}$	$R^2 = 0.95$	$RI = -23.10 + 138.58\ (c_{FG}\times10^{-6})$	$R^2 = 0.32$	2.45×10^{-6}
45 min	$RI = -465.23 + 67.80\ c_{GO}$	$R^2 = 0.96$	$RI = -62.68 + 264.71\ (c_{FG}\times10^{-6})$	$R^2 = 0.91$	3.90×10^{-6}
60 min	$RI = -494.22 + 61.29\ c_{GO}$	$R^2 = 0.97$	$RI = -97.14 + 388.72\ (c_{FG}\times10^{-6})$	$R^2 = 0.96$	6.34×10^{-6}
75 min	$RI = -458.50 + 54.21\ c_{GO}$	$R^2 = 0.98$	$RI = -111.52 + 466.90\ (c_{FG}\times10^{-6})$	$R^2 = 0.88$	8.61×10^{-6}
90 min	$RI = -465.47 + 52.27\ c_{GO}$	$R^2 = 0.99$	$RI = -147.54 + 562.32\ (c_{FG}\times10^{-6})$	$R^2 = 0.97$	1.08×10^{-5}
105 min	$RI = -300.64 + 34.90\ c_{GO}$	$R^2 = 0.91$	$RI = -131.67 + 587.95\ (c_{FG}\times10^{-6})$	$R^2 = 0.98$	1.68×10^{-5}
120 min	$RI = -231.30 + 21.77\ c_{GO}$	$R^2 = 0.69$	$RI = -85.53 + 552.57\ (c_{FG}\times10^{-6})$	$R^2 = 0.99$	2.54×10^{-5}

[1, 2] Corresponding linear plots were shown in Fig. 3C and Supplementary Fig. 16C.

Supplementary References

S1 G. Frens, *Nat. Phys. Sci.,* 1973, **241**, 20–22.
S2 W. Haiss, N. T. K. Thanh, J. Aveyard and D. G. Fernig, *Anal. Chem.,* 2007, **79**, 4215–4221.
S3 S. Lin, H. Ze, X. G. Zhang, Y. J. Zhang, J. Song, H. Zhang, H. Zhang, Z. Yang, C. Yang, J. Li and Z. Zhu, *Angew. Chem. Int. Ed.* 2022, **61**, e202203511.
S4 G. Long, and J. Winefordner, *Anal Chem.* 1983, **55**, 712A-724A.