Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Etchable SERS nanosensor for accurate intracellular pH and hydrogen peroxide sensing in living cells

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EXPERIMENT SECTION

Chemicals. Silver nitrate ($AgNO_3$) and bovine serum albumin (BSA) were purchased from Aldrich. Tetracholoroauric acid ($HAuCl_4 \bullet 4H_2O$) was purchased from Macklin. 4-MPy was purchased from TCI. 4-Mercaptophenylboronic acid was purchased from Amethyst Chemicals. Trisodium citrate dihydrate ($C_6H_5Na_3O_7 \bullet 2H_2O$) was purchased from Energy Chemical. Pinacol was purchased from Heowns. Potassium ferricya-nide [$K_3Fe(CN)_6$] was purchased from Meryer. Hydrogen peroxide (35% w/w) and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Alfa Aesar. 4-hydroxythiophenol (4-HTP) was purchased from Adamas. Ultrapure water (Milli-Q, 18.2 MΩ) was obtained from a Millipore water system. All of the reagents used in this study were of analytical grade and used without further purification.

Synthesis of 4-MPBE. The synthesis of 4-MPBE referred to the modified Tan's method.[1] In brief, 4-mercaptophenylboronic acid (118 mg) and pinacol (150 mg) were dissolved in 5 mL of cyclohexane under vigorous magnetic stirring at room temperature. Then, 1g of magnesium sulfate was added and the reaction mixture was stirred. After overnight reaction, the mixture was filtered and the filtrate was subsequently evaporated at 90 ℃ resulting in 4-MPBE (108 mg). The product was dissolved in deuterated chloroform ($CDCl_3$) for ^1H NMR characterization (Figure S4). The synthesized 4-MPBE was dissolved in ethanol for further using.

Preparation of AMBA pH Nanosensors and AMPE H_2O_2 Nanosensors. The preparation of AMBA pH nanosensors and AMPE H_2O_2 nanosensors is shown in Scheme 1A. The 40 nm gold NPs were synthesized according to the Puntes' method.[2] Briefly, 150 mL of 2.2 mM sodium citrate was refluxed to boiling under vigorous stirring. Then, 1 mL of $HAuCl_4$ (25 mM) was injected and the color of the solution changed from yellow to soft pink in 10 minutes. Then, the temperature was maintained at 90 ℃ and 1 mL of $HAuCl_4$ (25 mM) was added. After 30 minutes, repeat this step (sequential addition of 2 mL of 60 mM sodium citrate and 1 mL of 25 mM $HAuCl_4$) for 2 generations to obtain 40 nm Au NPs.

Next, the silver shell was coated around the Au core according to our previous method.[3] 25 mL of the as-prepared Au colloids were heated to boiling, then 3 mL of an aqueous silver nitrate solution (4.71 mM) and 1 mL of an aqueous sodium citrate solution (1% w/w) were added to the stirred solution dropwise. After 1 h, repeat this step and react for another hour, 50 nm Au@Ag core-shell NPs were achieved. The Au@Ag NPs were subsequently functionalized with 4-MPy (0.05 mM) / BSA (2% w/w) and 4-MPBE (0.05 mM) to get the AMBA pH nanosensors and AMPE H_2O_2 nanosensors, respectively.

Characterization. The morphology and energy-dispersive X-ray (EDX) spectra measurements of the nanosensors were performed with a high-resolution transmission electron microscope (TEM) with the voltage of 200 kV (HRTEM, JEOL JEM-2800, Japan). The extinction spectra were measured with a UV-Vis spectrophotometer (Specord-210 plus, Analytik Jena, Germany).

Cell Culture. MCF-10A cells were cultured in DMEM/F12 supplemented with 5% horse serum, cholera virus, 1% penicillin/streptomycin, human epidermal growth factor (hEGF), rh-insulin and hydrocortisone. SKBR-3 cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin. The cells were cultured in 95% air and 5% CO_2 atmosphere at 37 ℃. For the sake of further SERS imaging, the cells were seeded in 20 mm dishes (Wuxi NEST Biotechnology Co., Ltd) and incubated overnight to adhere. Then, 1 mL of culture medium containing AMBA pH nanosensors/AMPE H_2O_2 nanosensors (150 pM) was added to incubate for 4 h. The cells were washed three times with phosphate-buffered saline (PBS) to remove free NPs outside the cells. Subsequently 1 mL of imaging medium (140 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 20 mM HEPES, pH=7.4, mOsm=300) was added in the dish for SERS imaging.

Etching Procedure. The silver shells of the nanosensors can be etched by a hexacyanoferrate-thiosulphate redox-based solution consisting of potassium ferricyanide [$K_3Fe(CN)_6$, 0.2 M] and sodium thiosulfate ($Na_2S_2O_3$, 0.2 M). 100 μL of $K_3Fe(CN)_6$ and $Na_2S_2O_3$ were mixed with 800 μL of PBS solution.[30] The concentration of the etchant was optimized and 1 mM was chosen as proper etching

concentration (Figure S2). For *in-situ* etching of the cells, 100 µL of the etchant was mixed with 900 µL of culture medium and then added into the dish to react for 5 minutes. After that, the etchant was removed and the cells were washed with PBS for three times, and then 1 mL of imaging medium was added for SERS imaging.

SERS Measurements. SERS spectra were recorded on a Raman spectrophotometer (Horiba HR evolution, 600 grooves/mm grating) with a laser excitation wavelength of 633 nm (HeNe laser). A 10× (NA 0.5) microscope objective, 6.7 mW laser beam and 10 s acquisition time were applied to measure the solution samples. A 50× (NA 0.5) microscope objective, 5.4 mW laser beam and 0.5 s acquisition time were applied to measure the cell samples with scanning step of 1 µm.

Cell Viability Assay. MTT assay was applied to investigate the biocompatibility of the pH/H_2O_2 nanosensors with different SERS substrate (Au@Ag core-shell versus pure Ag NPs) before and after etching. The MCF-10A/SKBR-3 cells were seeded in a 96-well plate with a concentration of 10,000 cells per well and incubated for 24 h. The culture medium containing different concentrations (75-225 pM) of the nanosensors was separately added into the 96-well plate, and the cells without adding NPs were set as control. After further incubation for 4/24 h, the medium was removed and the cells were washed with PBS. In partial wells, 1 mL of etchant was added and incubated for 5 minutes, then the etchant was removed and the cells were washed with PBS. Afterwards, 10 µL of MTT (5 mg/mL in PBS) solution was added to each well. After incubation for another 4 h, the medium was removed, and 100 µL of DMSO was added. Finally, the 96-well plate was placed on a Multiskan FC microplate reader (Thermo Fisher SCIENTIFIC) and measured at a wavelength of 560 nm.

References

1 R. Peng, Y. Si, T. Deng, J. Zheng, J. Li, R. Yang and W. Tan, *Chem. Commun*, 2016, **52**, 8553.

2 N. G. Bastús, J. Comenge and V. Puntes, *Langmuir,* 2011, **27**, 11098.

3 W. Xie, C. Herrmann, K. Kömpe, M. Haase and S. Schlücker, *J. Am. Chem. Soc*, 2011, **133**, 19302.

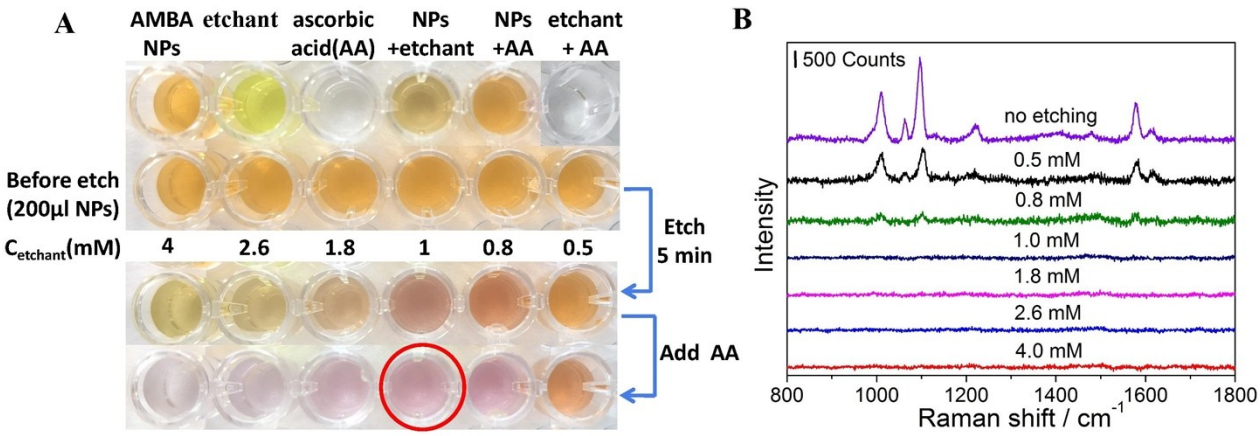

Figure S1: **Plate Optimization of Etching Conditions**. Concentration of the etching solution was optimized in a 96-well plate. The etchant (yellow) consists of 100 µL of $K_3Fe(CN)_6$ (0.2 M) and $Na_2S_2O_3$ (0.2 M) mixed with 800 µL of PBS solution. Ascorbic acid (AA, 20 mM, colorless) can reduce $K_3Fe(CN)_6$ thus was used to quench the etching activity. 200 µL of the AMBA NPs (orange, 150 pM) were added into each well, and then different concentrations of etchant were added. After reacting for 5 min, 10 µL of AA were injected to quench the etching reaction. Complete etching of the silver shells on the Au@Ag NPs was judged by visual inspection of the color (A) and vanished SERS signal (B). 1 mM was chosen as the optimal etching concentration, because the color of the remnant NPs after etching changes to soft pink (just like the original 40 nm gold colloid) and the SERS signal disappeared, indicating that the silver shells are completely etched.

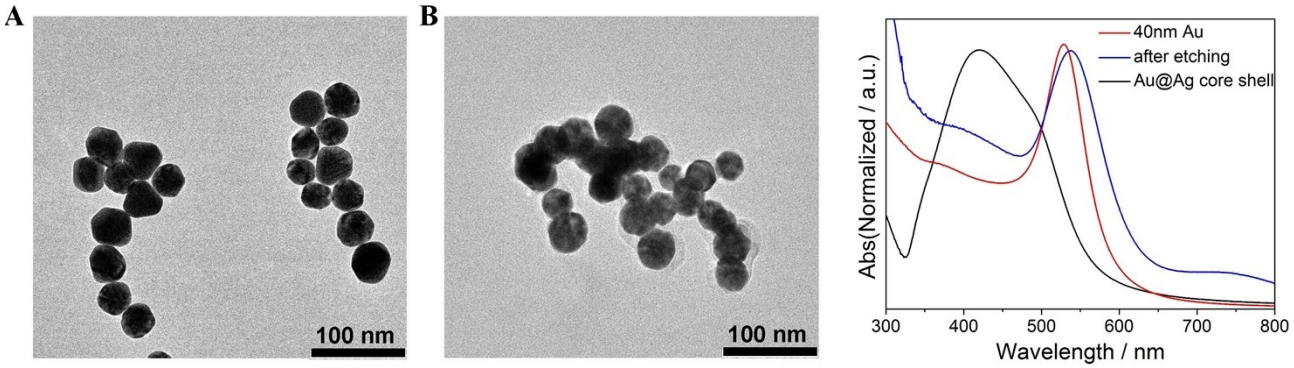

Figure S2: TEM images of (A) 40 nm gold seeds and (B) residual NPs after etching, and UV-Vis extinction spectra (C) of 40 nm Au seeds, Au@Ag core shell NPs and residual NPs after etching, respectively.

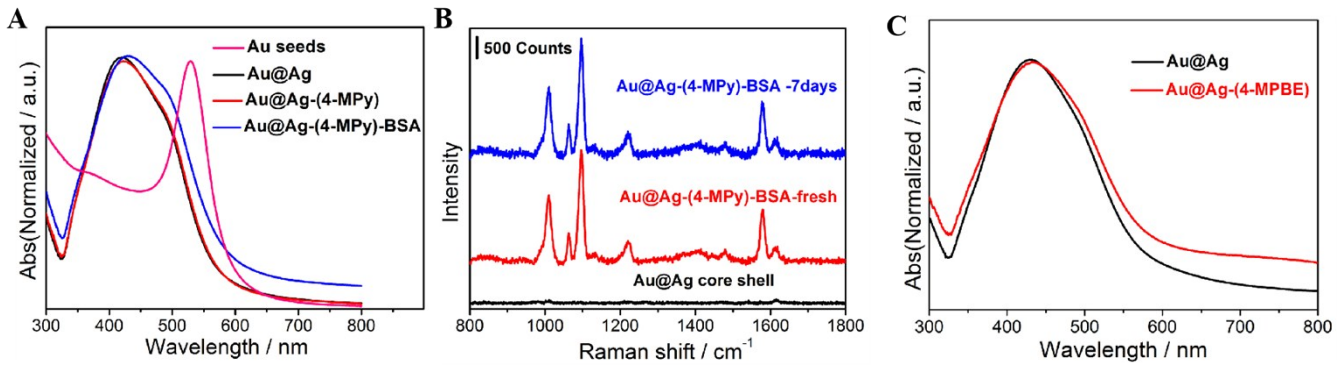

Figure S3: (A) UV-vis extinction spectra of Au@Ag core shell, Au@Ag-(4-MPy), AMB pH nanosensor. SERS spectra of AMB pH nanosensor in PBS solutions of various pH values ranging from pH 4.6 to 8.4 . (B) SERS spectra of Au@Ag core shell NPs, freshly synthetized AMB pH nanosensor and that storaged for 7 days. (C) UV-vis absorption spectra of Au and Ag core shell, Au@Ag-(4-MPBE).

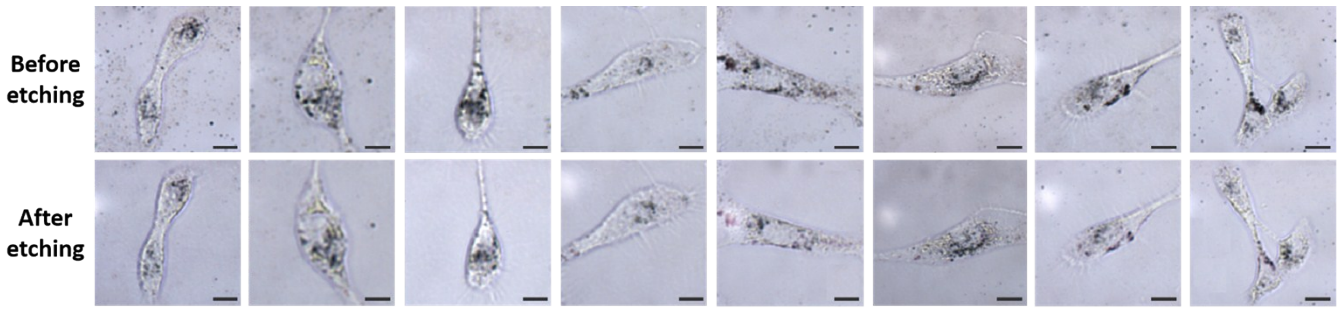

Figure S4: Bright field microscopic images of MCF-10A cells before and after etching. Scale bar: 10 µm.

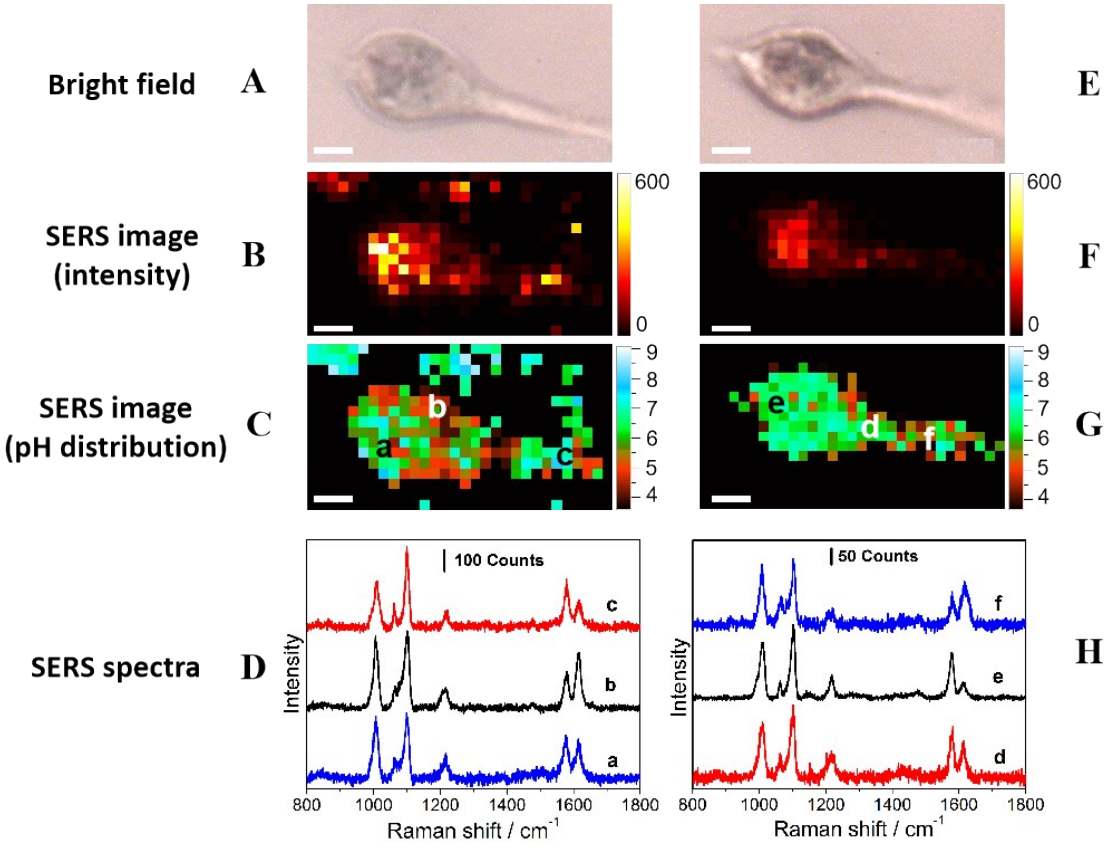

Figure S5: Bright field microscopic images of a MCF-10A cell after incubation with pH nanosensor for 4 h before (A) and after (E) (E) etching; SERS intensity images of the cell before (B) and after (F) etching using the peak at 1577 cm^{-1}; pH distribution images of the cell before (C) and after (G) etching; (D) and (H), typical SERS spectra obtained from different regions of the cell as indicated in (C) and (G), respectively. Scale bar: 10 µm.

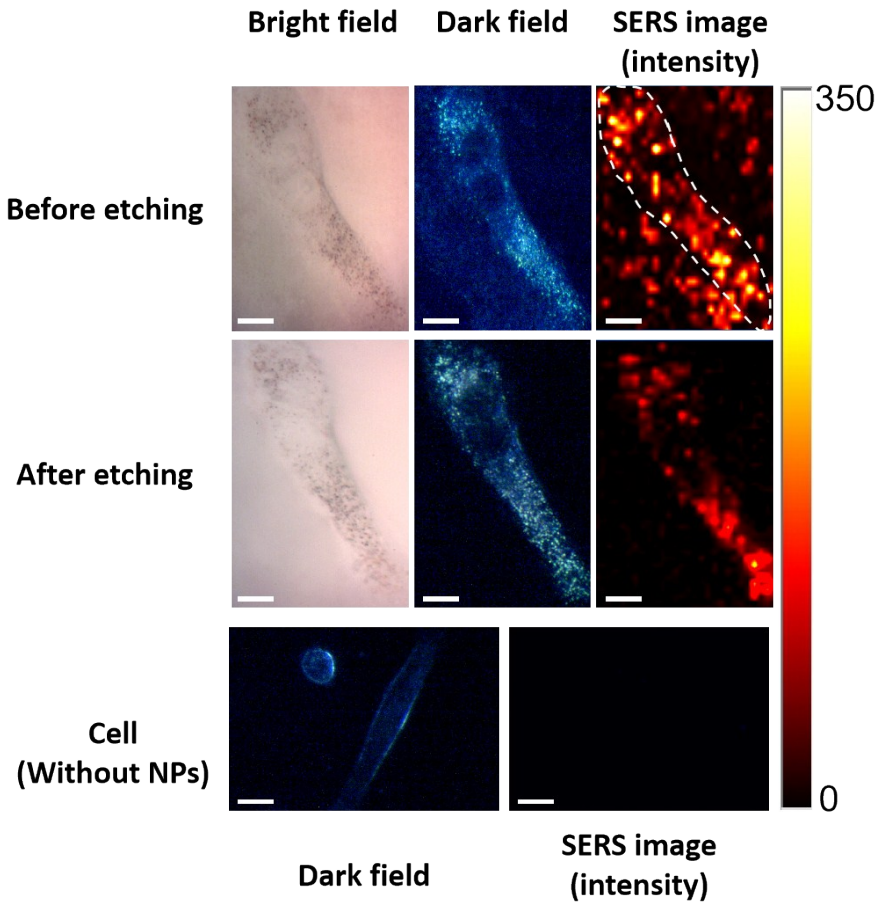

Figure S6: Bright field and dark field photograph of a MCF-10A cell after incubation with the AMBA pH nanosensors for 4 h, and corresponding SERS intensity image using the peak of 4-MPy at 1577 cm^{-1} (upper row), and that after etching (middle row). Dark field photograph of blank MCF-10A cells without NPs and corresponding SERS intensity image using the peak of 4-MPy at 1577 cm-1 (nether row). Scale bar: 10 µm.

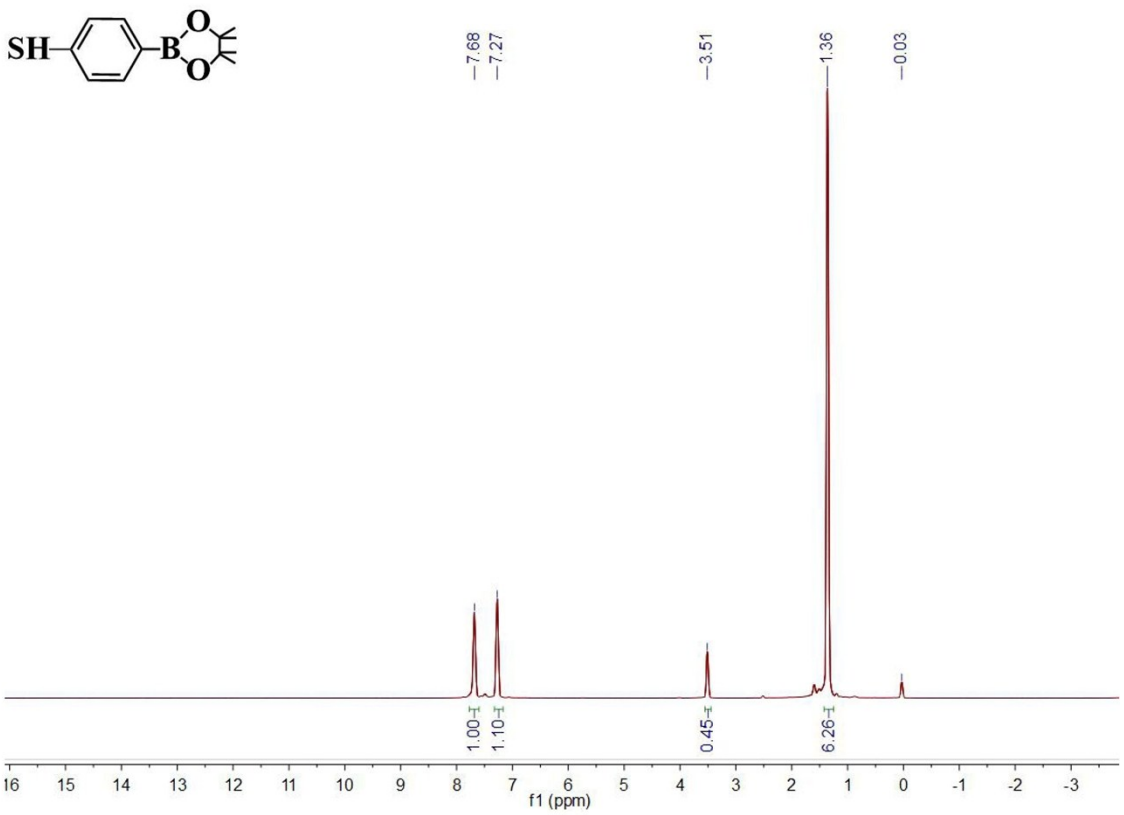

Figure S7: ^{1}H NMR characterization of 4-MPBE. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (s, 2H), 7.27 (s, 2H), 3.51 (s, 1H), 1.36 (s, 12H).

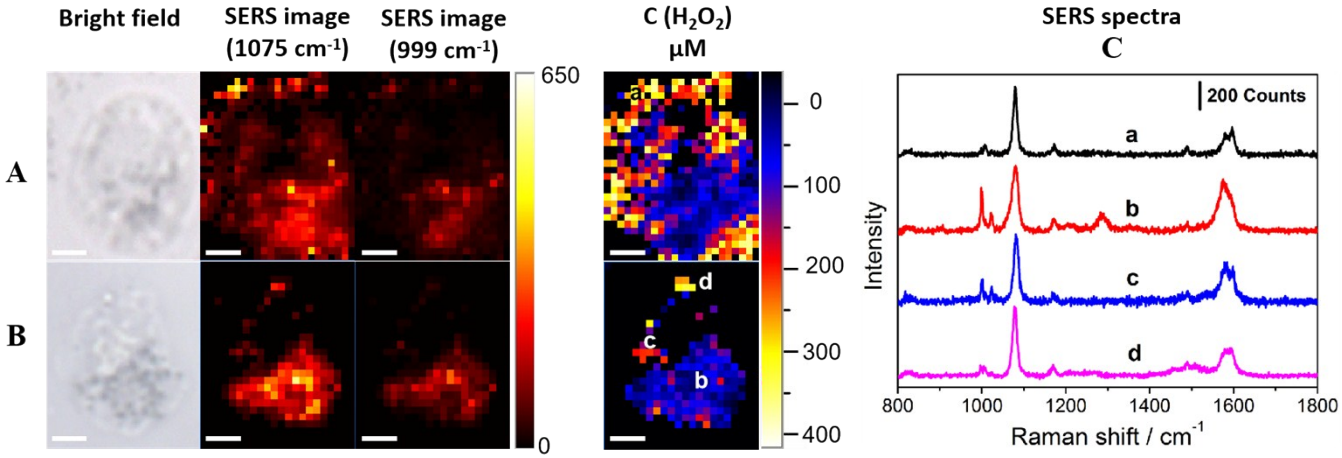

Figure S8: SERS images of a SKBR-3 cell incubated with AMPE H_2O_2 nanosensors for 4 h and treated with 300 μM of H_2O_2 for 2h before (A) and after (B) etching, respectively. (C) Typical SERS spectra obtained from different regions outside and inside the cell as indicated in (A) and (B), respectively. Scale bar: 10 μm.

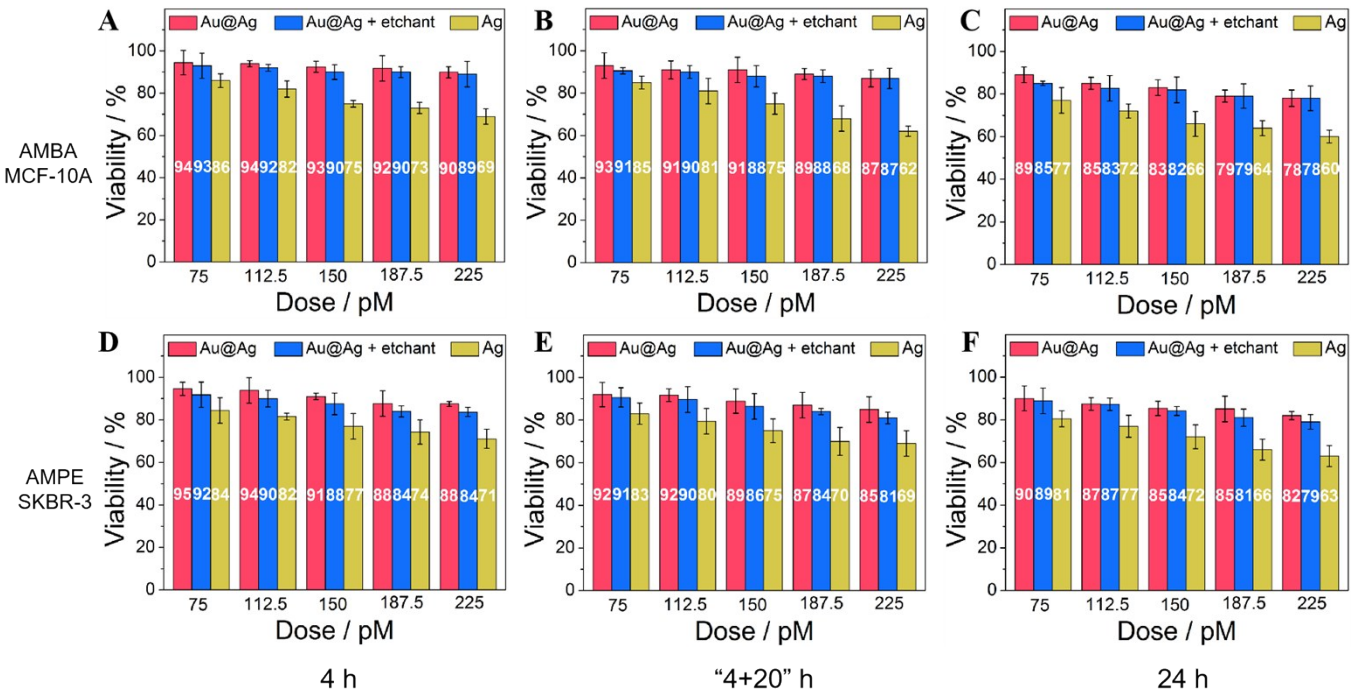

Figure S9: (A, B, C) Cell viability of MCF-10A cells after incubation with different doses of AMBA pH nanosensors for 4 h (4 h co-incubation with NPs) , "4+20" h (4 h co-incubation with NPs, removing free NPs and measuring after another 20 h incubation) and 24 h (24 h co-incubation with NPs), respectively; (D, E, F) Cell viability of SKBR-3 cells after incubation with different doses of AMPE H_2O_2 nanosensors for 4 h, "4+20" h and 24 h, respectively. Red: without etching; Blue: after etching; Yellow: cells incubated with nanosensors using pure Ag NPs (50 nm) as substrates. Similar cell viability (> 85% at a dose of 225 pM of Au@Ag NPs) is observed after 4 h incubation and "4+20" h incubation, indicating that the internalized NPs and the residual extracellular NPs have very low toxicity for the cells. Compared with 4 h co-incubation, the cell viability decreases slightly after 24 h co-incubation, because more silver atoms are oxidized to silver ions; However, the viability of the cells co-incubated with Au@Ag NPs is still close to 80% while that with Ag NPs decreases to ~60%.

Table S1. Raman Mode Frequencies (cm^{-1}) and Assignments of 4-MPy of AMBA pH Nanosensor in PBS Solution.

Vibrational mode	Raman shift (cm^{-1})
Ring breathing	1003
C-H in-plane bending	1094, 1216
N-H deformation	1205
C-C in-plane bending coupled with C-S stretching	1062
Ring deformation with C=C antisymmetric stretch	1577
Ring deformation with C=C symmetric stretch	1614

Table S2. Raman Mode Frequencies (cm^{-1}) and Assignments of 4-MPBE of AMPE H_2O_2 Nanosensor in PBS Solution.

Vibrational mode	Raman shift(cm^{-1})
B–O symmetric stretching	999
B–O–H bending	1021
C-H in-plane bending	1075
Ring deformation with C=C antisymmetric stretch	1573
Ring deformation with C=C symmetric stretch	1586