# Supporting Information

## A Novel SERS Nanoprobe for Ratiometric Imaging of Hydrogen Peroxide in Living Cells

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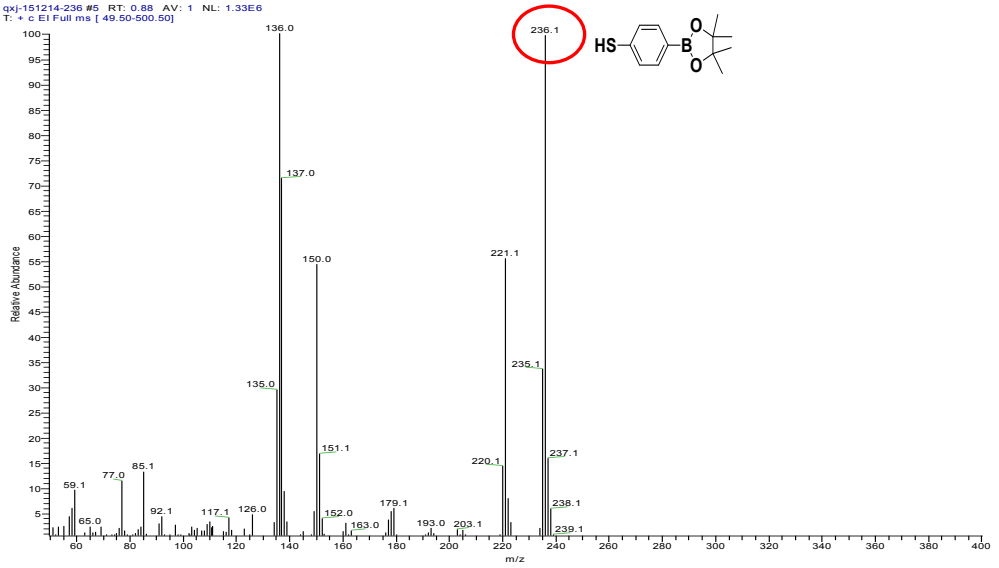
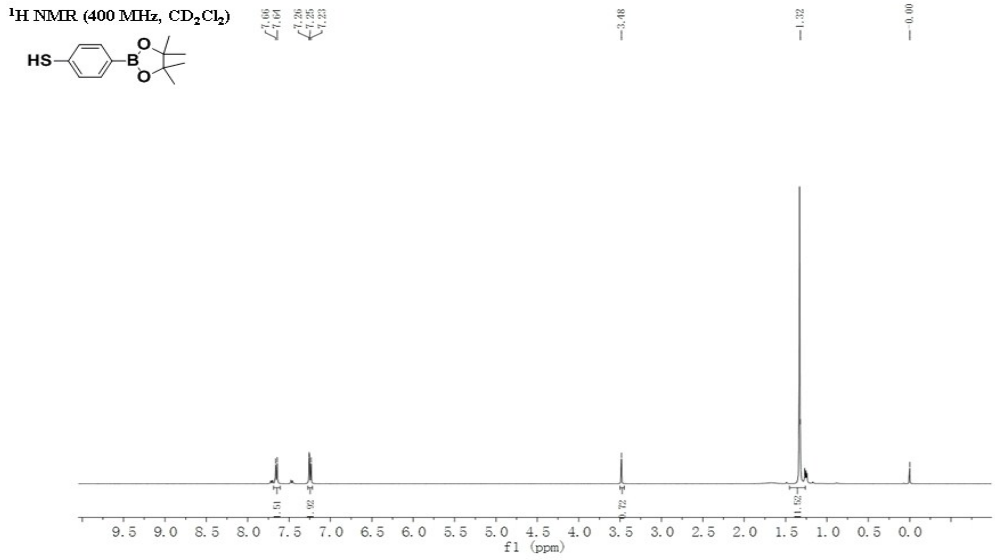
**Reagents and Instrumen**t.

All of the reagents used in this study were of analytical grade and were used without further purification. Ultrapure water obtained from a Millipore water purification system (18 M Ω) was used in all assays. gold (III) chloride hydrate ($HAuCl_4 \cdot 3H_2O$), cetyltrimethylammonium bromide (CTAB), silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$), ascorbic acid, and dehydrated alcohol were purchased from J&K Scientific. 4-Mercaptophenylboronic acid, 4-Mercaptophenol (4-MP), pinacol, cyclohexane, hydrogen peroxide ($H_2O_2$, 30 wt.%), were purchased from the Aladdin Chemical Company (Shanghai, China). Human cervical carcinoma cancer cell (HeLa cell) was obtained from the American Type Culture Collection (Manassas, VA). Transmission electron microscopy (TEM) were captured by a Tecnai G2 X-Twin (FEI Co.) instrument operating at 200 kV. Zeta potential experiments were performed at room temperature using a Malvern Zeta Sizer Nanoseries (Nano ZS90). The absorption spectra were measured by a Hitachi U-4100 spectrophotometer (Kyoto, Japan). MTT assay uses a Synergy™ 2 Multi-Mode Microplate Reader (Bio-Tek, Winooski, TV). SERS spectra were detected using a Raman microscope (InVia, Renishaw, Gloucestershire, UK) was used. This microscope uses 532nm, 633nm,785 nm point source laser, piezo-controlled stage for micrometer-resolved spatial mapping and a 1 in. CCD detector for spectral resolution of 1.07 cm-1 . An infinity-corrected 50 objective was used. Each spectrum was analyzed by least-squares analysis with Wire 3.4 Software (Renishaw).

**Synthesis of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzenethiol (MPBE)**[1]

4-Mercaptophenylboronic acid (2.0 mmol, 465 mg) and pinacol (2.0 mmol, 237mg) were dissolved in cyclohexane (10 mL) at room temperature. Magnesium sulfate (about 2 g) was added and the reaction mixture was stirred over night at room temperature. The reaction mixture was filtered and the filtrate was evaporated to give MPBE in 98 % yield (540 mg) which was pure enough for the subsequent experiments. $^1$H NMR(400MHz, $CD_2Cl_2$) δ 7.65(d,J=7.5Hz, 2H), δ 7.24(d,J=7.4Hz, 2H), δ 3.48(s, 1H), δ 1.33(s, 12H). MS(EI+) m/z calcd for $C_{12}H_{17}BO_2S$ : 236.10; found: 236.1.




**Preparation of GNR/MPBE or GNR/4-MP Nanoconjugates**

GNRs with the longitudinal LSPR peak at 630 nm were synthesized using a seed-mediated method in aqueous solution.[2] Seeds were synthesized first. 0.24 mL of 0.01 M ice-cold sodium borohydride was quickly added into a 4 mL stirring water solution containing 0.25 mM HAuCl4•3H2O and 0.1 M CTAB. The seeds formed well after 5 min stirring. Next, 0.32 mL of 1 mM $HAuCl_4$•3H2O was added into an 16 mL of 0.1 M CTAB aqueous solution, and 6.4 μL of 100 mM $AgNO_3$, and then 0.112 mL of 100 mM ascorbic acid was added into the solution to reduce the $Au^{3+}$ to $Au^{+}$. We utilized an improved single-step method based on Ma et al. When the solution turned colorless, it was mixed with 19.2 μL of the previously prepared seed solution. After 2 hours' growth at room temperature, the GNR solution was centrifuged twice at 7000 rpm to

remove the excess CTAB and then redispersed into the DI water, the final concentration of GNRs is estimated to be ca 0.86 nM. To obtain GNRs/MPBE nanoconjugate, 1 mM ethanol solution of 4-PBME (or 4-MP) was added to the as prepared GNRs and the mixture was incubated for 12 h. The contents were then centrifuged at 8000 rpm at room temperature for 8 min twice to remove free MPBE (or 4-MP) and finally dispersed in 10 mL water solution. The GNRs/MPBE (or GNRs/4-MP) nanoconjugate were then obtained for the subsequent analytical experiments.

**In vitro tests**

A solution of $H_2O_2$ was freshly prepared, and 50 μL of the $H_2O_2$ solution at different concentrations was immediately added to 100 μL of the prepared GNRs/MPBE solution. Following this step, in vitro SERS detection was conducted. All of the experiments were conducted under ambient conditions. The selectivity of the GNRs/MPBE nanosensor was investigated by measuring the SERS responses of possible interferents. SNAP (1 mM) in phosphate buffer (100 mM, pH=7.4) at room temperature was used to generate nitric oxide (NO), •OH was prepared through the reaction of ferrous ammonium sulfate (0.1mM) with $H_2O_2$ (1 mM), $^1O_2$ was prepared based on the reaction of $H_2O_2$ with NaClO. Samples were diluted in water and placed inside capillary and analyzed with Raman microscopy (50x objective, 2s exposure time, laser power: 20mW).

**Cell viability assay**

The biocompatibility of GNRs/MPBE nanoconjugates was examined by the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay. The HeLa cells were seeded onto 96-well microtiter plates and incubated for 24 h at 37 °C. Then, 10 μL of GNR/MPBE nanoconjugates at different concentrations were added respectively, and incubated for 24 h. After that, 5 μL of MTT solution (5 mg/mL) was added into each well and was incubated at 37 °C for another 4 h in the $CO_2$ incubator. The reaction was terminated by adding 150 μL of DMSO after removing the supernatant medium. Then, the formed purple formazan crystals were dissolved by DMSO, and the absorbance of the wells was measured on a microplate reader (Bio-Rad model 680) with a test wavelength of 570 nm. Cells incubated in the absence of nanoparticles were used as a control.

**Cellular studies**

The HeLa cells were propagated in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin and were maintained in an incubator containing 95% air and 5% $CO_2$ at 37 °C. To prepare live cells for imaging and spectroscopic detection, the cells were seeded in 6 cm dishes and allowed to adhere overnight. The growth medium was replaced by a culture medium containing 2.5 nM GNR/MPBE nanoconjugates. After incubating for 1 h, the culture medium was removed, and the cell culture dishes were washed with PBS three times to remove free unbound samples. The culture dish was then mounted to a small incubator on the microscope stage to perform imaging detection, cells were imaged under the Raman confocal microscope with a 633 nm laser (20x objective, 0.5s for each acquisitions, laser power: 10mW).

**Tissue studies**

The cervical tumor tissue slices were prepared from rat frozen slices. All slices samples were obtained from the Laboratory Animal Center of Xiangya Medical School of Central South University. All experimental procedures and study design were reviewed and approved by the Institutional Animal Care Committee of Xiangya Medical School of Central South University, China.

A side of the tissue was cut flat using a vibratingblade microtome. The slice was cultured with GNR/MPBE nanoconjugates (1 mg/mL) at room temperature for 60 minutes, or was first cultured with GNR/MPBE nanoconjugates (1 mg/mL) at room temperature for 60 minutes and then treated with $H_2O_2$ for another 60 minutes at room temperature, or was first treated with NAC at room temperature for 60 minutes and then incubated with GNR/MPBE nanoconjugates (1 mg/mL) for another 60 minutes at room temperature, and finally washed with PBS three times for imaging (484(22x22) acquisitions for each sample, 0.5s for each point, 633nm laser with power : 10 mW).

**References**

1 Infinity Discovery, Inc. Patent: WO2008/63300 A2, 2008.

2 J. Pérez-Juste, L. M. Liz-Marzán, S. Carnie, D. Y. C. Chan, P. Mulvaney, Adv. Funct. Mater. **2004**, 14, 571.

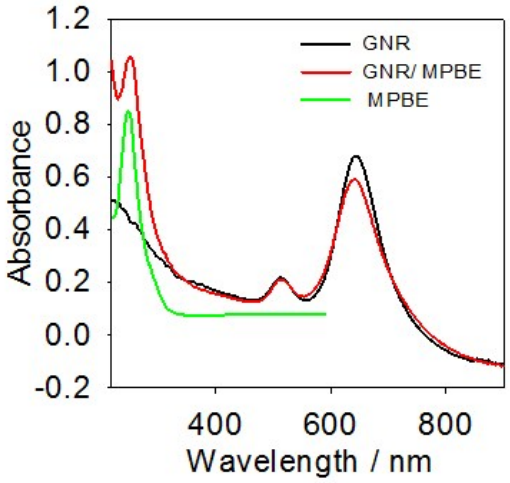



**Figure S1.** UV−vis absorption spectrum of the GNRs (black), GNRs/MPBE nanoconjugate (red) and MPBE (green).

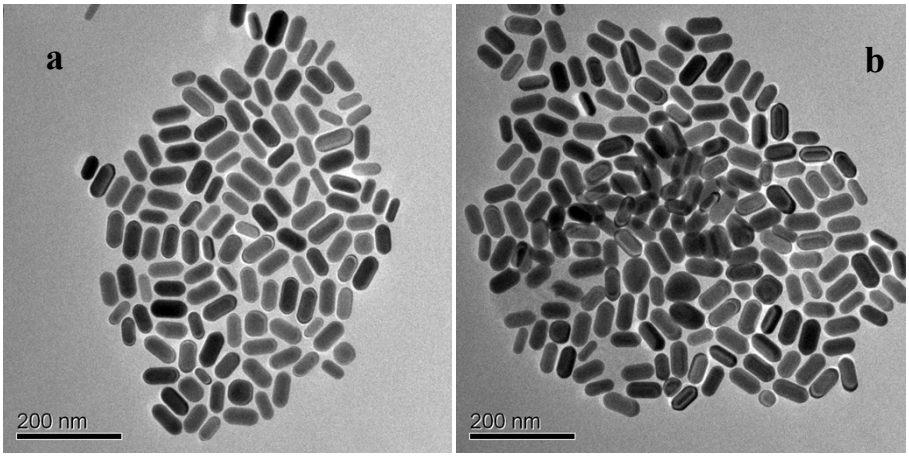



**Figure S2.** Typical TEM images of the GNRs (a) and GNRs/MPBE nanoconjugate (b).

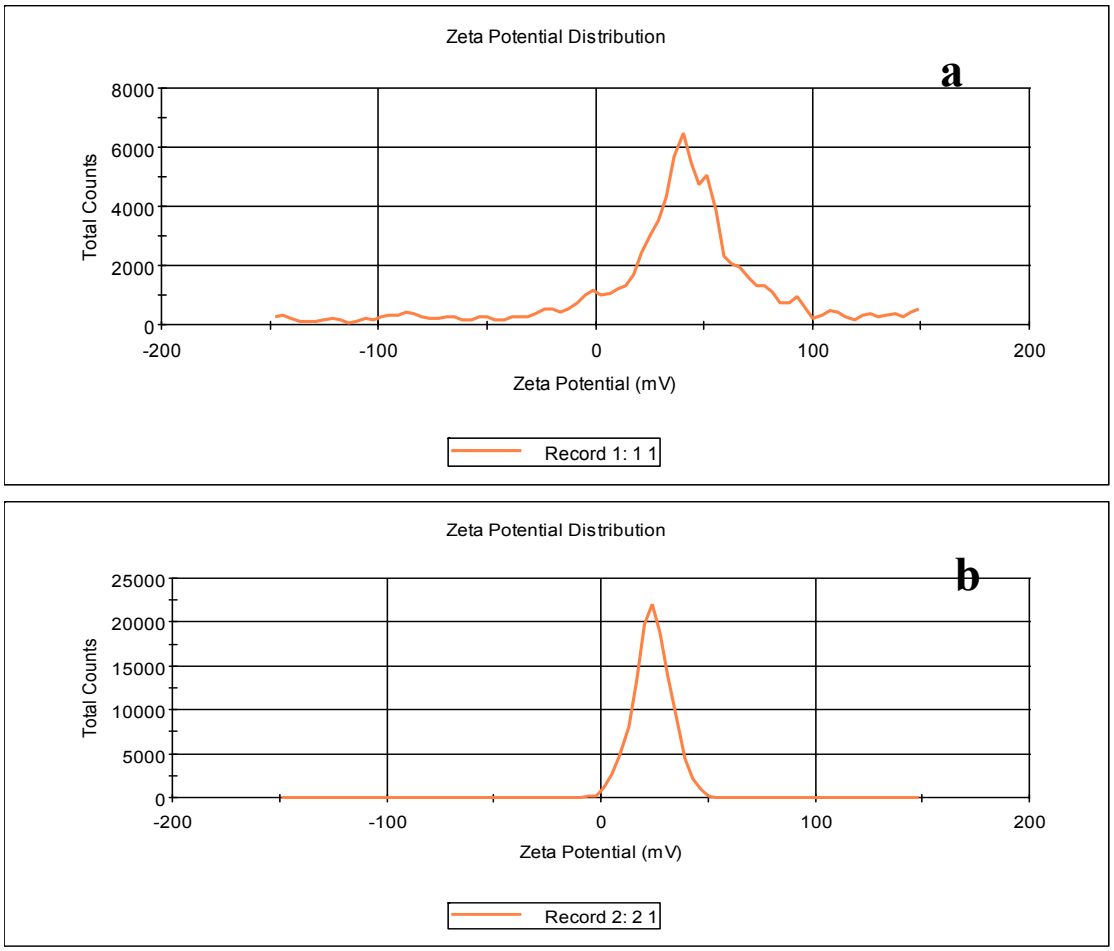



**Figure S3.** Typical Zeta-potential of the GNRs (a) and GNRs/MPBE nanoconjugate (b).

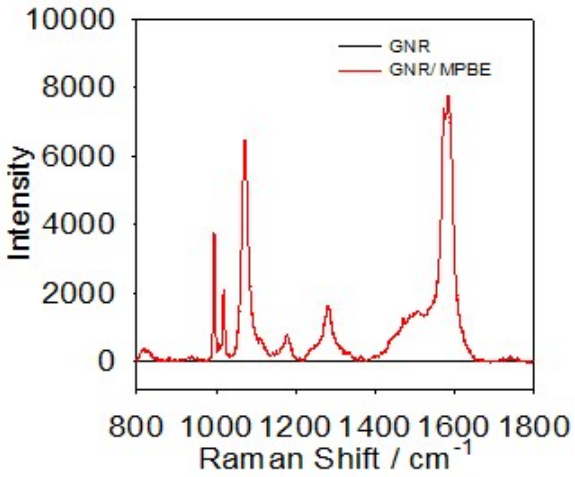



**Figure S4.** SERS response of the GNRs (black) and GNRs/MPBE nanoconjugate (red) with laser 633nm illumination.

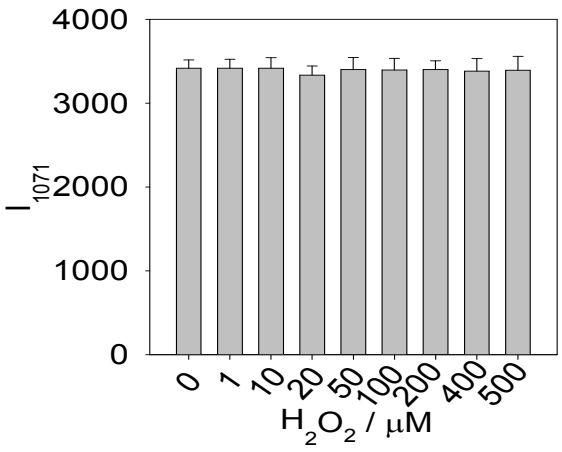



**Figure S5.** Plots of 1071cm$^{-1}$ peak intensities recorded at different $H_2O_2$ concentrations (0, 1, 10, 20, 50, 100, 200, 400, and 500 μM).

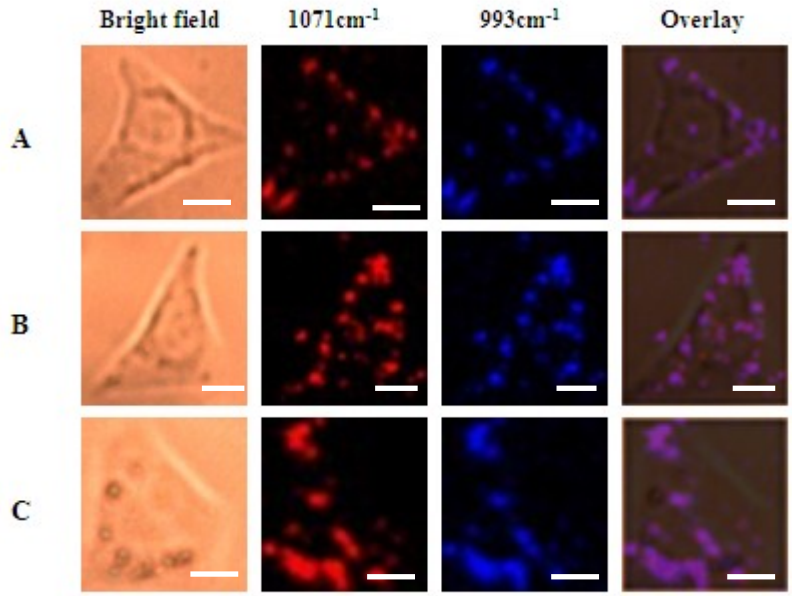



**Figure S6.** Confocal SERS imaging of HeLa cells treated with GNR/MPBE nanoconjugate for different times (A: 30 min, B: 60min, C: 90 min). Images displayed in pseudocolor represent the ratio of SERS intensities collected in 1071cm$^{-1}$ (red) and 993cm$^{-1}$ (blue), respectively, upon laser illumination at 633 nm. The scale bar is 10 μm.

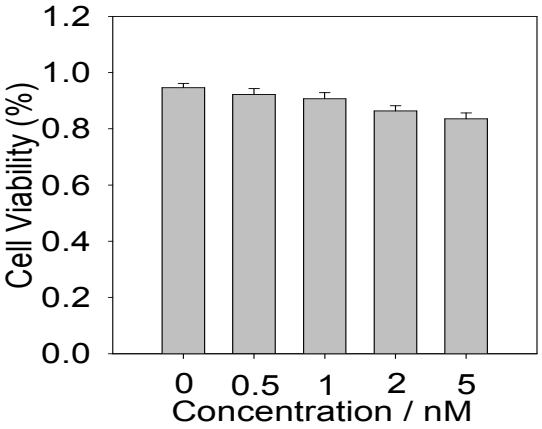



**Figure S7.** Cell viability of HeLa cells incubated with different concentrations (0, 0.5, 1.0, 2.0, and 5.0 nM) of GNR/MPBE nanoconjugates for 24 h. The first bar stands for the control.

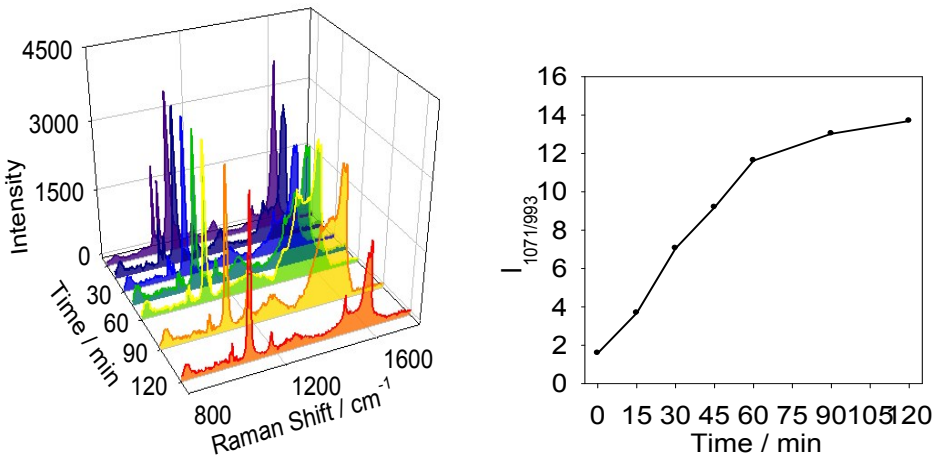



**Figure S8.** SERS response of the GNR/MPBE nanosensor to 1 mM $H_2O_2$. Left: Spectra were acquired before and 15, 30, 45, 60, 90, and 120 min after $H_2O_2$ was added. Right: Kinetic plot of SERS intensity ratios $I_{1071/993}$ of the GNR/MPBE to 1 mM $H_2O_2$ using laser wavelength at 633 nm.