Electronic Supplementary Material (ESI) for ChemComm.

Ratiometric Imaging and Selective Biosensing of Nitric Oxide in Live Cells Based on Trisoctahedral Gold Nanostructures

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Contents:

1. Experimental Section

Chemicals and Reagents. O-nitroaniline, ammonium thiocyanate (NH_4SCN), bromine (Br_2), sodium bromide ($NaBr_2$), potassium hydroxide (KOH), sodium chloride (NaCl), magnesium sulfate ($MgSO_4$), sodium dithionite ($Na_2S_2O_4$), hydrogen peroxide (H_2O_2, 30%), sodium nitrite ($NaNO_2$), sodium hypochlorite solution (NaClO, ~10%), sodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO4) were purchased from Aladdin Chemistry Co. Ltd (China). Gold(III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$,99%), potassium superoxide (KO_2), dimethyl sulfoxide (DMSO), methyl triazolyl tetrazolium (MTT), ascorbic acid (AA), lipopolysaccharides (LPS) were obtained from Sigma-Aldrich (USA). KCl, $MnCl_2 \cdot 4H_2O$, $CdCl_2$, $PbCl_2$, $NiCl_2$ $6H_2O$, $MgCl_2$, $CaCl_2$, $BaCl_2$, $CoCl_2$ $6H_2O$, $FeCl_3$, $NaNO_3$, $CuCl_2 \cdot 2H_2O$, NaOH, chloroform were obtained from Sinopharm Chemical Reagent Co. Ltd (China). Hexadecyltrimethylammonium chloride (CTAC) were obtained from Shanghai Civi Chemical Technology Co. Ltd (China). Ultrapure water (18.2 MΩ cm^{-1}) produced with a Milli-Q gradient system (Millipore, Billerica, MA) was used in all experiments. All of the chemicals were used without further purification.

For the selective test, the ROS and RNS species were obtained according to the article before. [S1] H_2O_2 solution (10 μM) was prepared by diluting 30% H_2O_2. Hydroxyl radical (•OH) was generated by the Fenton reaction (Fe^{2+}/H_2O_2 = 10 μM/60 μM). Hypochlorite anion (ClO^-) was provided by NaClO (10 μM). Superoxide anion (O_2^-) was derived from dissolved KO_2 (10 μM) in the DMSO solution. The first singlet oxygen ($^1O_2$) was prepared by H_2O_2 (10 μM) reacting with NaClO (10 μM). Peroxynitrite ($ONOO^-$) was produced by H_2O_2 (10 μM) and $NaNO_2$ (10 μM). Nitroxyl (HNO) were obtained by adding the Angeli's salt ($Na_2N_2O_3$) to the solution of S-nitroso-N-acetyl-DL-penicillamine. Nitrite (NO_2^-) and nitrate (NO_3^-) were obtained by dissolve sodium nitrite and sodium nitrate in ultrapure water.

Preparation of NO solution. We prepared the NO solution as previous methods. [S2] In specific, 1 mM PBS (10 mL) solution was bubbled with NO gas for 1 h to remove the oxygen. Then it was bubbled with NO gas for 30 min. The saturated solution should

contain 1.8 mM NO at room temperature and the desired concentration were achieved by following dilution with deoxygenated PBS solution. The concentration of the diluted NO solution was further calibrated using Griess Method. [S3]

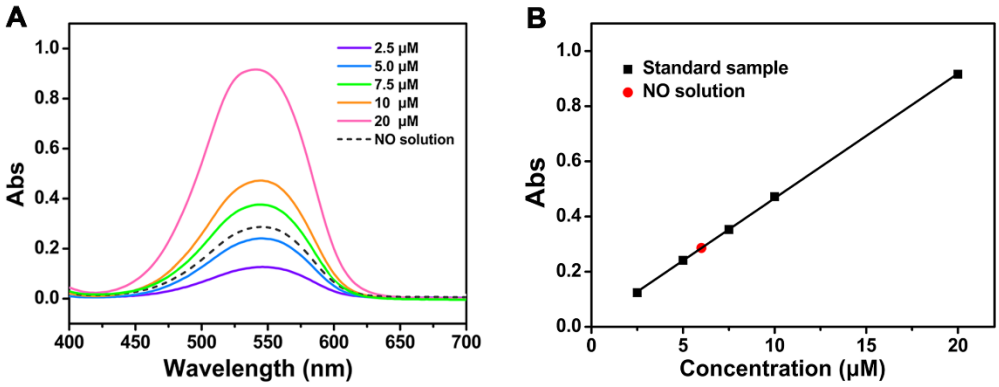

Figure S1. (A) UV-vis spectra of standard NO_2^- samples at different concentrations and NO sample. (B) Plot of absorption peak at 540 nm versus different concentrations of NO and NO_2^-.

Apparatus and Measurements. DABT was determined using ^1H NMR, ^{13}C NMR, and mass spectroscopy. ^1H NMR spectra were recorded on a Bruker spectrometer (500 MHz) and mass spectra were measured on an Agilent 6890. The AuHTs were characterized using a JEM-2100 transmission electron microscope (TEM, JEOL Ltd., Japan) and a JSM- 1788F PRIME scanning electron microscope (SEM, Hitachi, Japan). UV-vis spectra were measured through a UH-5300 spectrophotometer (Hitachi, Japan). For SERS measurement, Thermo Scientific DXR Raman Microscope were used. A 780 nm laser was used for all the measurements. In the vitro experiment, a 10× (NA 0.4) microscope objective with a working distance of 1.3 mm and spot focused laser was used. The laser power and acquisition time were 5 mW and 3 s, respectively. For cells image, a 50× (NA 0.75) microscope with a 0.38 mm working distance and spot focused laser was used. The laser power and acquisition time were 0.1 mW and 1s. The dark-field and bright-field images were also collected from this instrument. For Raman imaging experiment, approximately 30 min was needed for each imaging with a spatial resolution of ~1.35×10^5 nm^2.

Synthesis 3, 4-diamiobenzenethiol (DABT). The organic nitric oxide probe was synthesized as follows. Briefly, o-nitroaniline (13.8 g, 0.1 mol) and ammonium thiocyanate (18.3 g, 0.24 mol) were first dissolved in 110 mL of methanol. The reaction mixture was then cooled to 3-5°C. Next, 17.6 g of bromine was added to a 20 mL methanol that had been saturated with sodium bromide. The mixture was then slowly added to the previous reaction bottle during 45 min while the temperature of the reaction mixture was keeping at 3-5°C. The reaction slurry was stirred for one hour at 0-5°C using ice bath. The ice bath was then removed and the reaction slurry was warm to room temperature. Next, it was poured in ice-water mixture (500 mL) and stirred until the ice disappeared. The solids were filtered, washed three times with cold water and dried. Finally, 4-thiocyano-2-nitroaniline was obtained.

Secondly, 4-amino-3-nitrobenzenethiol was synthesized. 4-thiocyano-2-nitroaniline (3.51 g, 18 mmol) was slowly added to a stirred ethanol (100 mL) contained 6 g potassium hydroxide at 10°C. The mixture was continuously stirred for another 30 min at room temperature. Then, a solution of sulfuric acid (10%) in ethanol was added carefully until the color of the mixture changed from dark violet to orange. pH was adjusted to ~6.0-7.0. The mixture was then poured into water (400 mL) and extracted with ethyl acetate. The organic layer was washed with saturated solution of sodium chloride, dried with magnesium sulfate, and evaporated. A red solid was obtained.
Finally, 4-amino-3-nitrobenzenethiol (3.4 g, 20 mmol) was dissolved in 50% ethanol (300 mL). Then, sodium dithionite (13.93 g, 80 mmol) was added. The solution was refluxed for 1 h and then cooled to room temperature. After the solution was extracted with chloroform, the aqueous layer was evaporated. The resulting solid was extracted with methanol and then evaporated. Then, the crude solid was obtained and purified through column chromatography on silica. Finally, a yellow power was obtained.

Preparation of Au/DABT nanoprobe. Trisoctahedral gold nanostructures were prepared using a previous method. An aqueous solution containing CTAC (30 mL, 0.01 M) was added into an aqueous $HAuCl_4$ (30 mL, 0.6mM) at room temperature. Then, a freshly prepared solution of ascorbic acid (1.2 mL, 0.1M) was quickly added to the

solution. After a slightly shaking, the mixture was placed at 30°C. The color of the solution turned to light pink. The as-prepared product was centrifuged and the precipitant was washed several times using water remove CTAC. The final volume of the aqueous solution of AuHTs was 6 mL.

To prepare the Au/DABT nanoprobe, a freshly prepared solution of DABT in ethanol (0.2 mL, 20 mM) was added into the aqueous solution of AuHTs (4 mL). Then, after stirred for 1 h, excess DABT was removed from the AuHTs through centrifugation and re-suspension in phosphate-buffered saline (PBS, 20 mM, pH=7.4). The Au/DABT nanoprobe were obtained for further experiments.

Calculation of Analytical Enhanced Factor (AEF). AEF of AuHT was estimated by binding raman standard molecule, MBA, on the surface of AuHT, and calculated through equation AEF= (I_{SERS}/N_{SERS}) / (I_{RAMAN}/N_{RAMAN}). I_{SERS} and I_{RAMAN} means the intensity at 1074 cm^{-1} of MBA. N_{SERS} and N_{RAMAN} means the molecule number of MBA in detection volume of solution-phase sample (V_f). V_f was calculated by equation V_f = (depth of focus) × (focus area) = $(1.4\, n\lambda/N_A^2) \times \pi(0.4\, \lambda/2N_A)^2$, in which n is refractive index, λ is excitation wavelength, and N_A is numerical aperture. MBA molecules assembled on the AuHT nanoparticles through Au-S chemical bonding was assumed to form a monolayer, the surface concentration of MBA was estimated to be 3.3 molecule/nm^2. Finally, AEF was calculated to be 7.96×10^6. [S4]

Cellular culture and MTT assays. PC 12 cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum and 1% penicillin/streptomycin (P/S) solution. The cells were incubated at 37°C in a humidified 5% CO_2-containing atmosphere. For the SERS imaging, PC12 cells were dispersed in 6 cm glass bottom dishes and incubated for 24 hours. Then the Au/DABT nanoprobes were added into the culture medium and incubated for another 4 h. Next, the culture medium was removed and the cells were washed three times with PBS (20 Mm, pH=7.4). Finally, the dish was put into a small incubator on the microscope stage for SERS imaging.

The cytotoxicity of the Au/DABT nanoprobe was measured using MTT assay. PC 12

cells were planted in a 96-well plate and incubated with culture medium for 24 h. Then the culture medium was removed. New culture medium containing different concentrations of Au/DABT nanoprobe was added and incubated for 48 h. After removing the culture medium, 20 μL of MTT solution (5 mg/mL) was added into each well, followed by incubating for another 4 h to allow the formation of formazan dye. Then, 180 μL of DMSO was added to terminate the reaction after removing the medium. Absorbance at 490 nm was measured through a Varioskan LUX-multimode microplante reader (Thermo Fisher Scientific, USA) and the cell viability was determined by calculating the absorbance result. Cell viability (%) = the absorbance of experimental group/the absorbance of blank control group × 100%.

2. Synthetic route DABT (Figure S1)

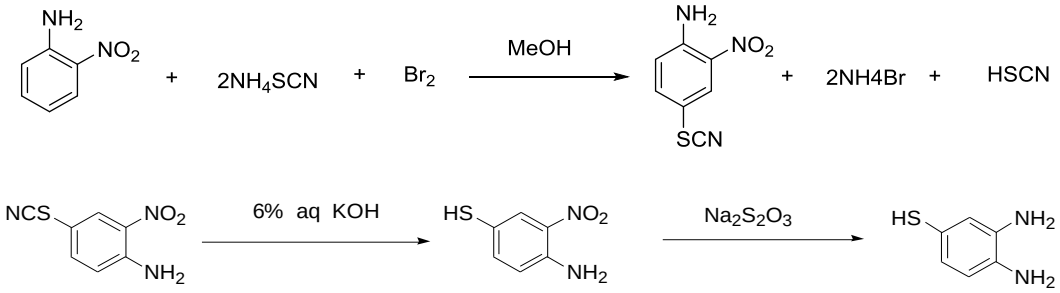

Figure S2. Synthetic route for DABT.

3. ^{1}H NMR, ^{13}C NMR, and MS data of DABT (Figure S2-S4)

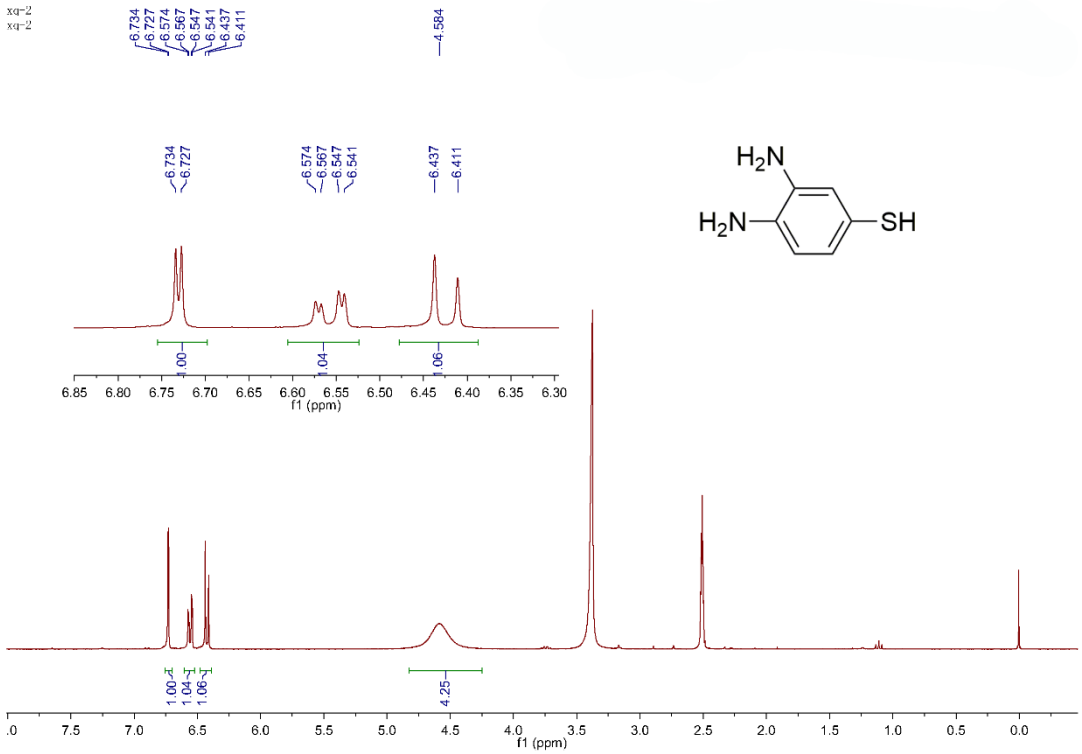

Figure S3. ^{1}H NMR spectrum (500 MHz) of DABT in DMSO-d6.

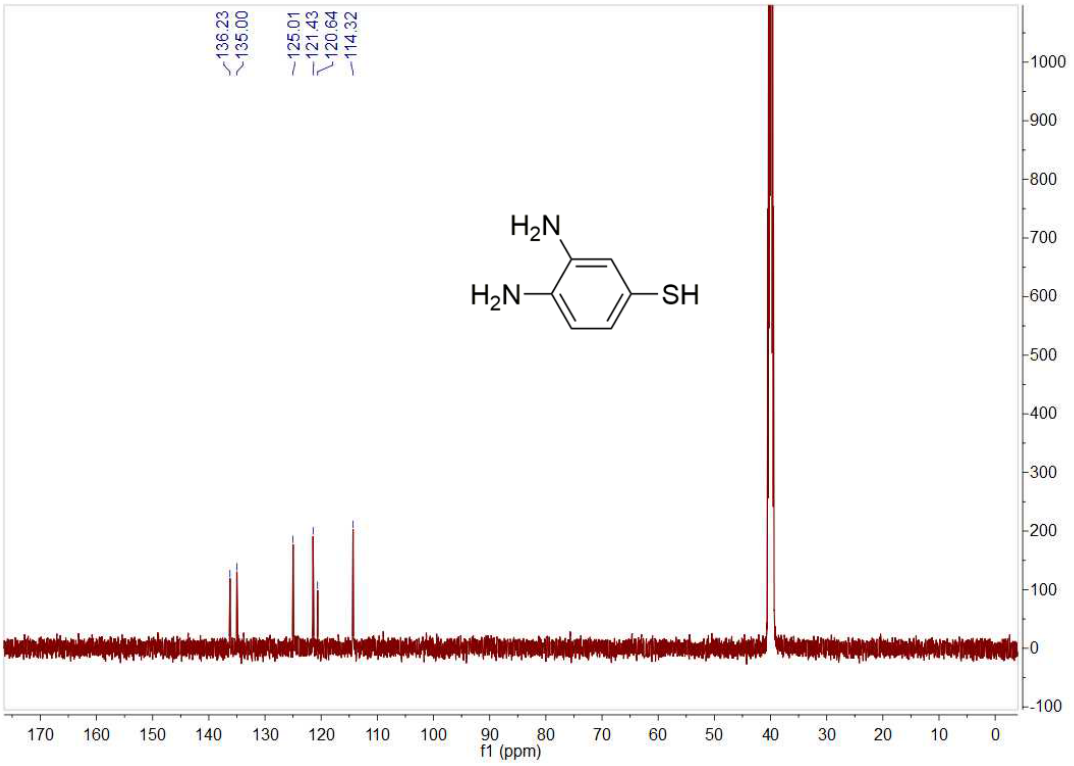

Figure S4. ^{13}C NMR spectrum (500 MHz) of DABT in DMSO-d6.

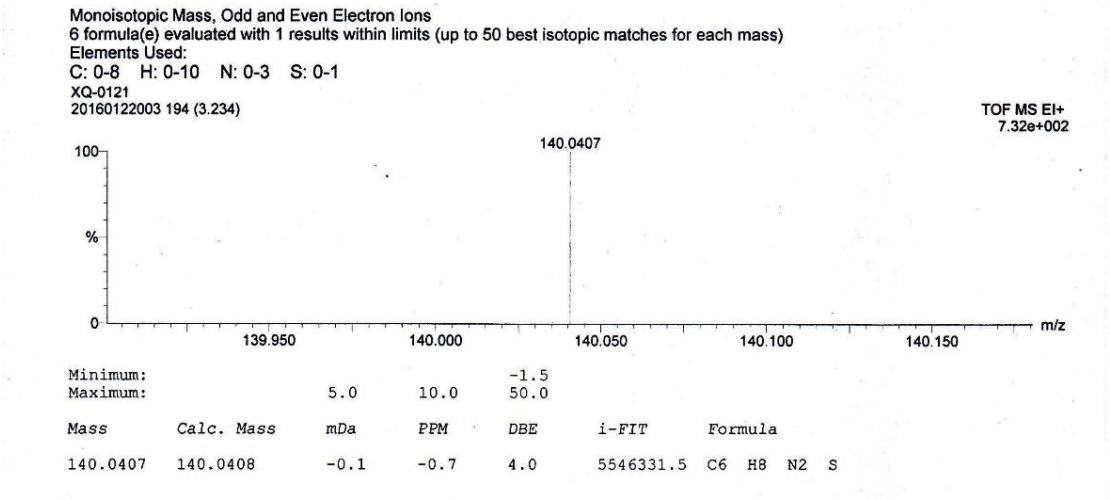

Figure S5. Elemental composition search report by HR-MS for DABT.

4. SERS bands assignment (Table S1)

DABT		BTAH	
1573cm^{-1}	Benzene ring stretching	1573cm^{-1}	Benzene ring stretching
1398cm^{-1}	C-H in-plane bending, C-C stretching	1398cm^{-1}	C-H in-plane-bending, C-C stretching
1365cm^{-1}	Benzene ring stretching	1365cm^{-1}	Benzene ring stretching, Triazole ring stretching
1266cm^{-1}	C-H in-plane bending	1266cm^{-1}	C-H in-plane bending
1250cm^{-1}	C-N stretching	974cm^{-1}	C-S bending
974cm^{-1}	C-S bending	698cm^{-1}	Triazole ring scissoring
500cm^{-1}	C-C-C out-of-plane bending	500cm^{-1}	C-C-C out-of-plane bending
443cm^{-1}	C-H out-of-plane bending	443cm^{-1}	C-H out-of-plane bending
420cm^{-1}	C-S in-plane bending, C-N in-plane bending	420cm^{-1}	C-S in-plane bending, C-N in-plane bending
385cm^{-1}	NH_2 in-plane bending	385cm^{-1}	NH_2 in-plane bending

Table 1. The SERS bands assignment of DABT and BTAH.

5. ^{1}H NMR and MS data of 6-chloro-1H-benzo[d] [1, 2, 3] triazole (Figure S5-S6)

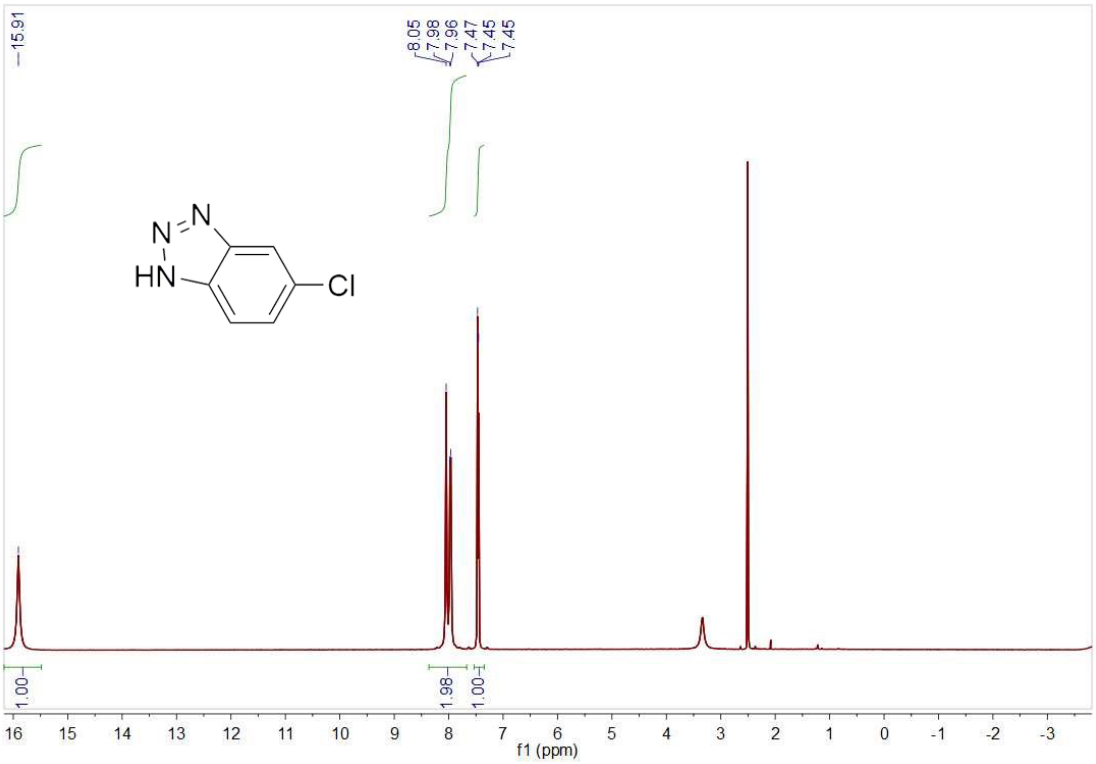

Figure S6. ^{1}H NMR spectrum (500 MHz) of 6-chloro-1H-benzo[d] [1, 2, 3] triazole in DMSO-d6.

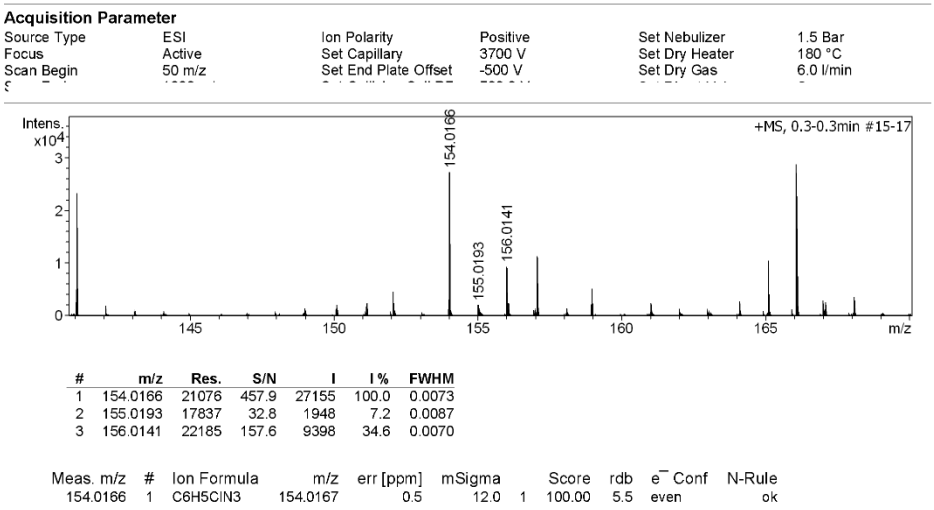

Figure S7. Elemental composition search report by HR-MS for 6-chloro-1H-benzo[d] [1, 2, 3] triazole.

6. Response time (Figure S7)

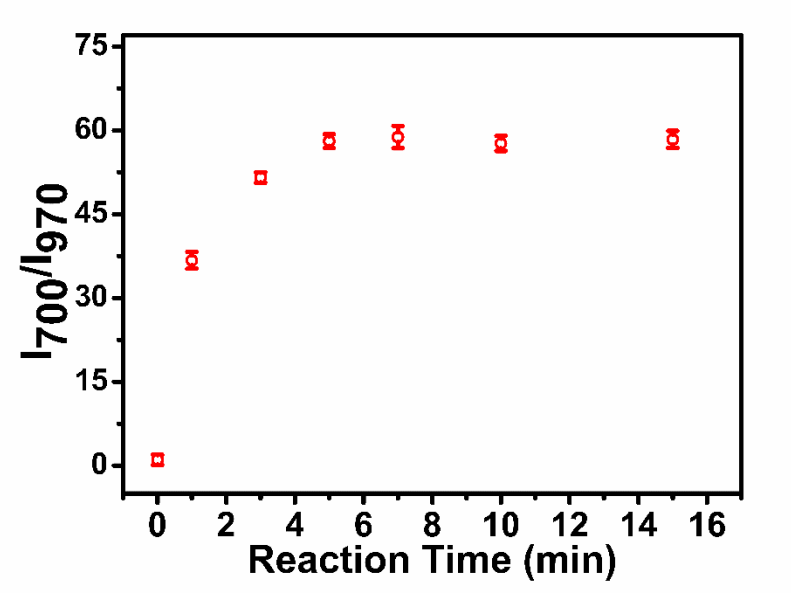

Figure S8. Plots of ratiometric peak intensities I_{698}/I_{974} versus time (1, 3, 7, 10, and 15 min) with NO in PBS buffer (20 mM, pH 7.4). Each point represents the average value from three SERS spectra. Error bars show the standard deviations.

7. Stability of Au/DABT probe (Figure S8)

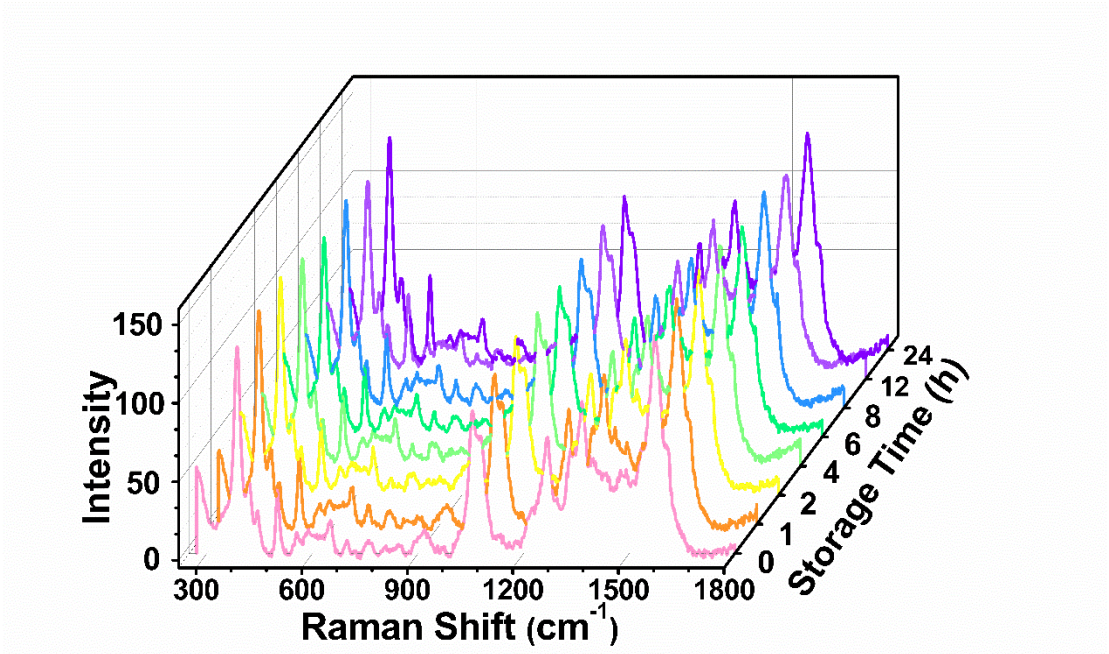

Figure S9. (A) SERS spectra of Au/DABT probe after storage for different time (0, 1, 2, 4, 6, 8, 12, and 24 h) under ambient condition.

8. pH dependence of Au/DABT probe (Figure S9)

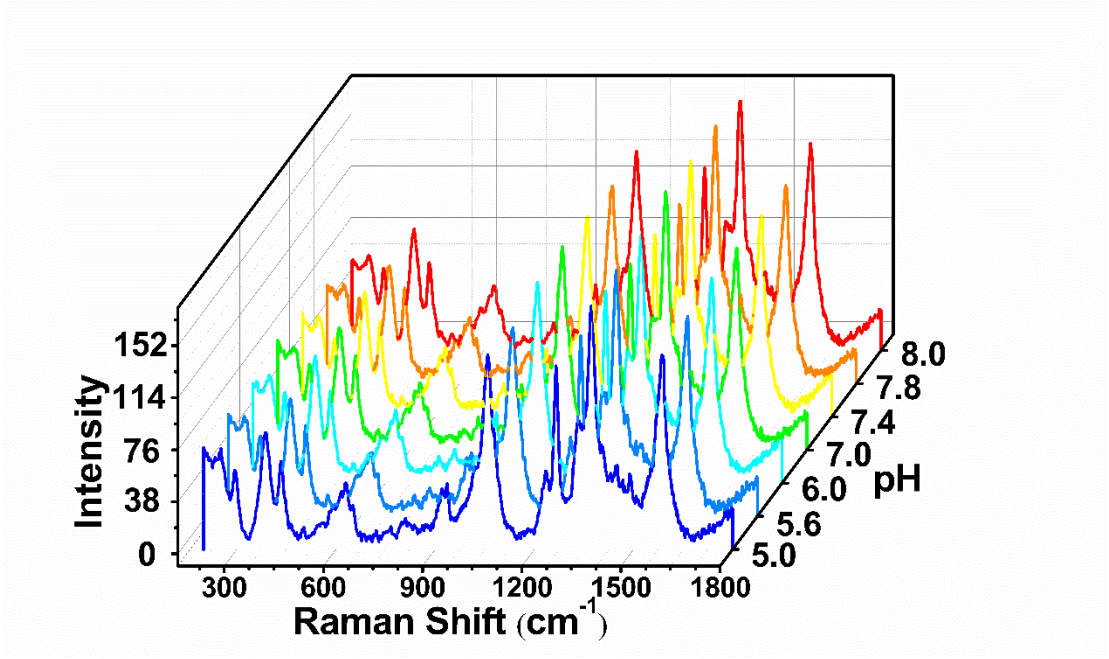

Figure S10. SERS spectra of Au/DABT probe recorded from PBS buffer with different pH (5.0, 5, 6, 6.0, 7.0, 7.4, 7.8, and 8.0).

9. MTT assay (Figure S10)

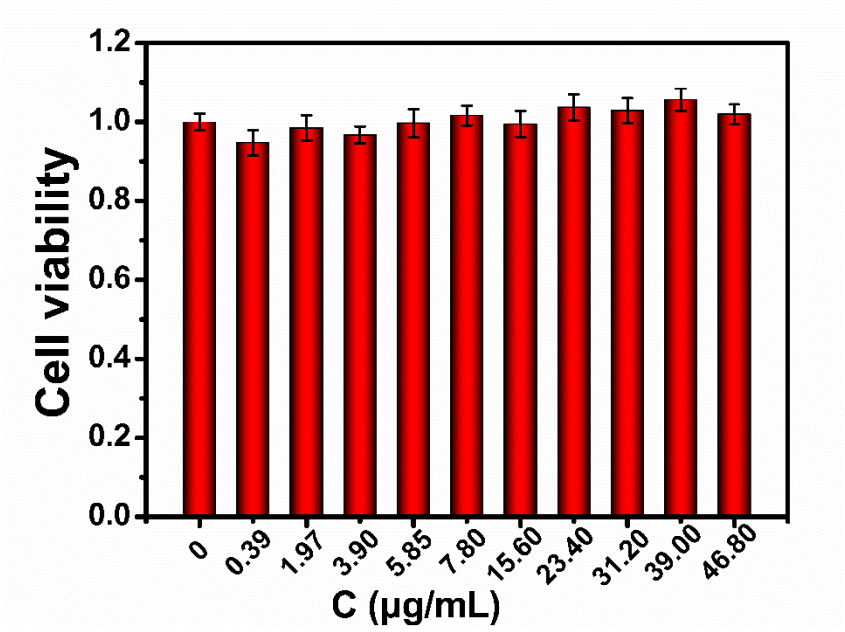

Figure S11. Cell viability values (%) estimated by MTT proliferation tests versus incubation with different concentrations of Au/DABT probe. The error bars equal to the standard deviations (n=3).

Reference

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S2. (a) H. B. Yu, X. F. Zhu, Y. Xiao, W. Zou, L. P. Wang and L. J. Jin, *Anal. Chem.*, 2013, **85**, 7076; (b) M. Sato, T. Nakajima, M. Goto and Y. Umezawa, *Anal. Chem.*, 2006, 78, 8175.

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