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Electronic Supporting Information:

An intelligent alkyne-tag for Raman imaging of living cells: Graphdiyne-encapsulated Au nanosphere

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[1] Y. Tao and W. Jia contributed equally this work.

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Experimental Methods

Chemicals. $HAuCl_4 \cdot 3H_2O$, $NaBH_4$, hexadecyltrimethylammonium chloride (CTAC), L-ascorbic acid, NaClO, tetrahydrofuran, tetrabutylammonium fluoride (TBAF) were purchased from Alfa Aesar and used without further purification. Hexakis-[(trimethylsilyl)ethynyl]-benzene (HEB-TMS, >98%) was purchased from Xianfeng Nano Company (Nanjing, China).

Characterizations. The surface characteristics of the GQDs were examined by X-ray photoelectron spectroscopy (XPS), which was measured with an ESCALAB 250 XPS spectrometer (VG Scientifics) using a monochromatic Al Kα line at 1486.6 eV. Binding energies were calibrated with respect to the C 1s peak at 284.6 eV. Peak fit analysis was performed using the XPS PEAK program. TEM images were obtained on a JEOL JEM-2100F TEM operating at an accelerating voltage of 200 kV.

Synthesis of AuNSs. 50 μL of 0.05 M $HAuCl_4$ was mixed with 5 mL of 0.1 M CTAC solution. Then, 200 μL of freshly prepared 0.02 M $NaBH_4$ was quickly added under vigorous stirring. The mixture was diluted 10 times using 100 mM CTAC solution after 2 h. 900 μL of the diluent and 40 μL of 0.1 M ascorbic acid were mixed in 10 mL of 25 mM CTAC solution. Then, 50 μL of 0.05 M $HAuCl_4$ was injected under vigorous stirring. The mixed solution was left undisturbed for 30 min. Afterwards, 2.5 μL of the mixed solution, 40 μL of 0.1 M ascorbic acid, and 10 mL of 25 mM CTAC solution were added together. Subsequently, 50 μL of 0.05 M $HAuCl_4$ was introduced. After 1 h, 10 μL of 10% NaClO was added. Finally, 6.25 μL of 0.05 M $HAuCl_4$ was injected under stirring. After 24 h, the formed Au NSs were collected by centrifugation (8000 rpm) and dispersed into 10 mL of water.

Synthesis of GDY sheet. We obtained HEB monomer by treating HEB-TMS with TBAF using the reported procedures.[1] Clear copper plates were placed into a mixed solution of 100 mL acetone, 5 mL pyridine and 1 mL tetramethylethylenediamine (TMEDA) in a round-bottom flask. Then, 10mg HEB was dissolved with 50ml acetone and added slowly in 4 hours into the flask mentioned above. The mixture was kept at 50 °C under Ar protection for at least 24 h. Finally, the copper plates were washed with heated acetone and DMF in turn to remove the unreacted monomer and oligomer. GDY was removed from the copper plates by sonification. Then the samples were washed by acetone, dimethyl formamide (DMF), ethanol, and water. At last, the obtained GDY was dried in N_2 environment.

Fabrication of Au@GDY. First, we cut the as-prepared GDY sheets into small-sized fragments. 10 mg of GDY powder was mixed with 1 mL of concentrated HNO_3, 1.2 mL of H_2SO_4 in ice water bath. 10 mg of $KMnO_4$ powder

was then added into the mixture under vigorous stirring. The mixture was kept at 80 °C for 24 h. After being cooled to room temperature, NaOH solution was added into the mixture to adjust pH to 8.0. The suspension was centrifuged (10000 rpm) for 10 min, washed using water, and dispersed into water. Then, the GDY suspension was dialyzed using 3500 Da dialysis bag. The GDY fragments were obtained. Next, 1 mL of Au NSs suspension was mixed with 7 mL GDY fragments suspension (100 μg mL^{-1}) and allowed stirring for 1 h to wrap GDY on Au NSs. Then, the mixture was centrifuged (8000 rpm) for 10 min. Lastly, the precipitate was dispersed into 12 mL of water.

Surface functionalization of aptamer-Au@GDY. Aptamer S2.2 (5'-GCA GTT GAT CCT TTG GAT ACC CTG G-$(CH_2)_6$-NH_2-3') was covalently conjugated to GDY via the formation of the amide between the –COOH group of GDY and the –NH_2 moiety at S.2 by EDC/NHS chemistry. We firrst activated GDY in the EDC/NHS mixture (5 mM EDC, 10 mM NHS in PBS, pH 7.4) by dispersing 1 mL Au@GDY suspension and stirring for 30 min. Then, 10 mL of S2.2 (1 mM) was added to Au@GDY suspension and stirred for 4 h at ambient temperature. S2.2-Au@GDY was collected by centrifugation, washed with copious PBS, and dried in vacuum at ambient temperature.

SERS measurement. SERS spectra were recorded on Raman confocal microscope (LabRam HR Evolution) equipped with 50× working objective lens. A 633 nm laser excitation at power of 2.5 mW was used. The SERS spectra were collected with a spectral resolution of 3 cm^{-1} and grating of 600 grooves/mm in the range of 500–3000 cm^{-1}. An integration time of 10 s, and accumulation of 3 was used. All spectra were baseline corrected and smoothed (Savitzky-Golay smoothing, standard values: degree 2) using LabSpec 5 software.

Cell Culture. MCF-7 cells were obtained from Cell Center of Chinese Academy of Sciences (Shanghai, China) and cultured at 37 °C in DMEM medium supplemented with 10% fetal bovine serum (FBS), 4 mM L–glutamine, 100 μg mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin in a 5% CO_2 environment.

MTT assay. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) measurements were performed to evaluate the biocompatibility of the Au@GDY. MCF-7/HepG2 cells (~1×10^4) were first incubated with Au@GDY at a concentration of 0–200 μg mL^{-1} for 24 h, and were repeatedly rinsed with PBS. 10 μL of MTT solution (5 mg mL^{-1} in PBS, pH 7.4) was then added into each well and incubated for 4 h at 37 °C. Afterwards, 100 μL of dimethylsulfoxide (DMSO) was added into each well. Absorbance was recorded at 550 nm on a Synergy 2 microplate reader (Biotek, USA).

Raman imaging of living cells. The as-prepared Au@GDY was diluted using DMEM. The final concentration of this suspension was controlled to be 100 μg mL^{-1}. After growing to 50% confluence, MCF-7 cells were washed with PBS (0.145 M NaCl, 1.9 mM NaH_2PO_4, 8.1 mM K_2HPO_4, pH 7.4). Then, MCF-7 cells were cultured at 37 °C in 6 mL DMEM medium suspended with 1 mL of the above Au@GDY suspension in a 5% CO_2 environment. After 12 h, the cells were washed with PBS. Afterwards, MCF-7 cells were transferred to a sterilized silicon and treated with Karnovsky fixation solution for Raman measurement. For targeted Raman imaging with aptamer functionalized Au@GDY, MCF-7 and HepG2 cells were cultured at 4 °C in 6 mL DMEM medium suspended with 1 mL of S2.2-Au@GDY suspension (100 μg mL^{-1}) in a 5% CO_2 environment for 0.5 h. For Raman imaging of cells, Raman confocal microscope (LabRam HR Evolution) equipped with 50× working objective lens was used. A 633 nm laser was selected for excitation at power of 2.5 mW. The laser line was scanned with a spacing of 2 μm over an area of 75 × 50 μm^2. Exposure time of 4 s was used.

FDTD Simulations. FDTD simulation (Lumerical, Canada) was performed to investigate the optical properties of the proposed structures. The Au core was modeled as an Au nanosphere with 100-nm diameter. A total-field scattered-field (TFSF) light source with the wavelength range 400–1100 nm was selected. The perfectly matched layer (PML) boundary condition was applied for the x, y, and z directions and the mesh size was set to 1 nm. The Au dielectric constant was taken from the CRC database provided in the software.

DFT Calculations. The charge distribution of Au@GDY, band gap of GDY, and Fermi level of Au were calculated using the plane-wave spin-polarized density functional theory (DFT) as implemented in $DmoL^3$ code. The generalized gradient approximation (GGA) in Perdew–Burke–Ernzerhof (PBE) with Grimme methods for DFT-D correction was used as the exchange–correlation energy function. The primitive cell of GDY containing 18 carbon atoms was employed as a model of GDY. For Au@GDY, we modelled the 21×21 Å^2 unit cell of Au (111) plane coated with GDY. The modulus unit cell vector in the z direction was set to be 20 Å. The convergence tolerance of energy, maximum force, maximum displacement, and Gaussian electron smearing width for geometry optimization were set to be 1.0×10^{-5} hartree, 0.002 hartree per Å, 0.005 Å, and 0.005 eV, respectively. We used 1 × 1 × 1 Monkhorst–Pack k-point mesh to sample the Brillouin zone.

REFERENCES

[1] Zhou, J.; Gao, X.; Liu, R.; Xie, Z.; Yang, J.; Zhang, S.; Zhang, G.; Liu, H.; Li, Y.; Zhang, J.; Liu, Z. Synthesis of Graphdiyne Nanowalls Using Acetylenic Coupling Reaction. *J. Am. Chem. Soc.* **2015**, *137*, 7596−7599.

Figures

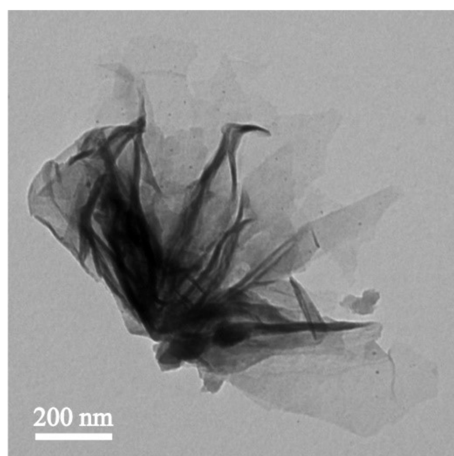

Fig. S1 TEM image of GDY sheet.

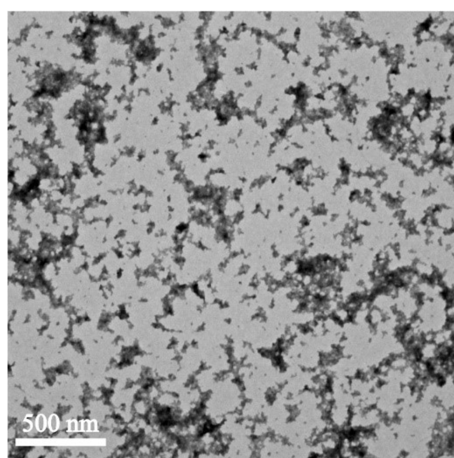

Fig. S2 TEM image of GDY fragments.

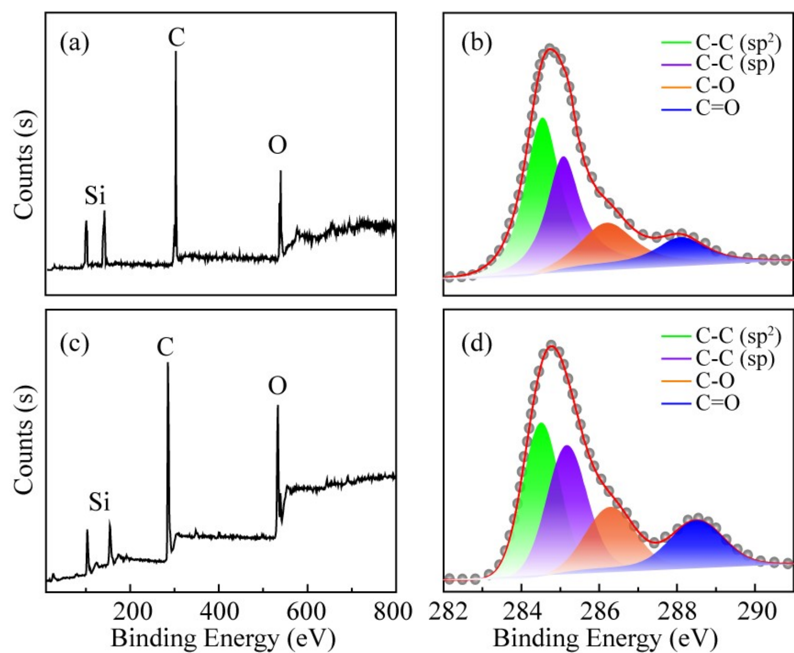

Fig. S3 (a, b) XPS survey scan and C1s spectra of GDY sheet. (c, d) XPS survey scan and C1s spectra of GDY fragments.

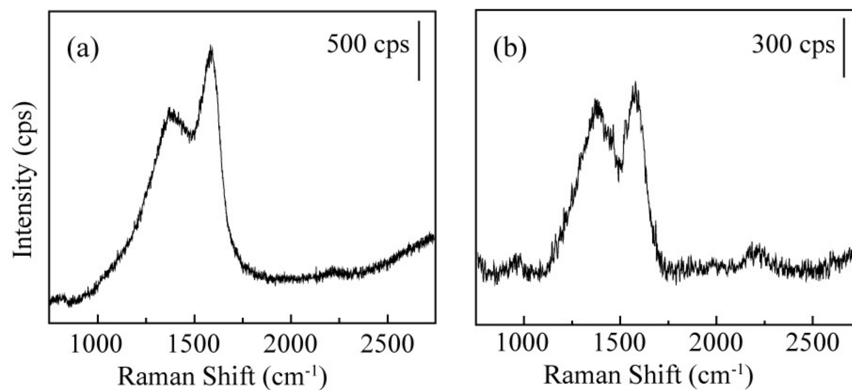

Fig. S4 Raman spectra of GDY sheet (a) and GDY fragments (b).

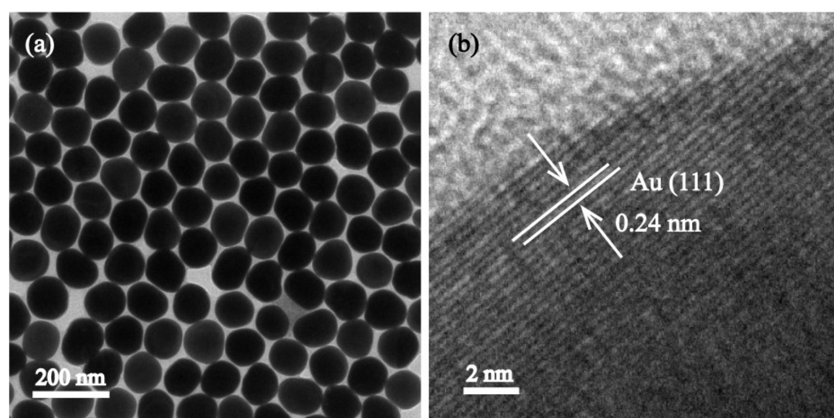

Fig. S5 TEM and HRTEM images of Au NSs.

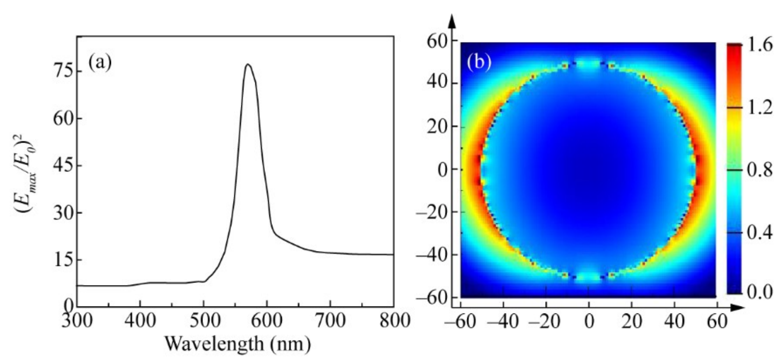

Fig. S6 FDTD simulated maximum electric field intensity $(E\text{max}/E_0)^2$ at the Au/air interface of the Au core as a function of the wavelength and the *E*–field distribution of the Au core obtained at the wavelength of 633 nm.

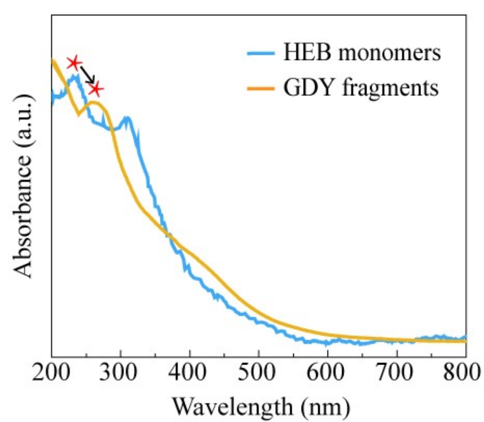

Fig. S7 UV-vis spectra of HEB monomers and GDY fragments.

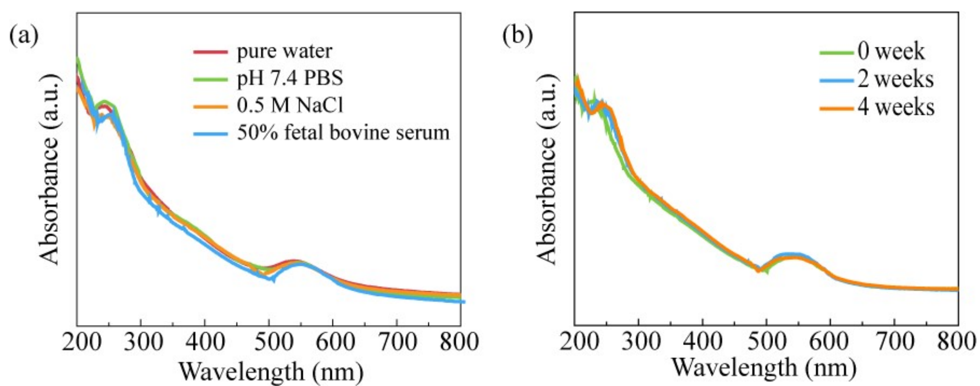

Fig. S8 (a) UV-spectra of Au@GDY suspended in water, PBS (pH 7.4), NaCl (0.5 M), and 50% fetal bovine serum. (b) UV-spectra of GDY suspension after storing for 0, 2, and 4 weeks.

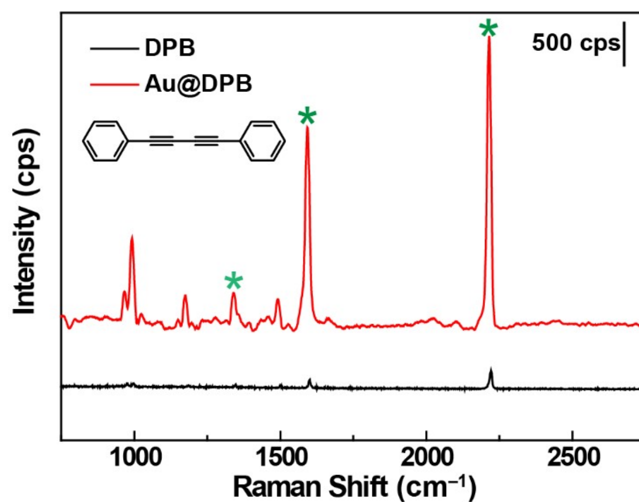

Fig. S9 SERS spectra of DPB (1 μM) at Au NSs surface. Laser: 633 nm. Power density: 2.5 mW cm^{-2}.

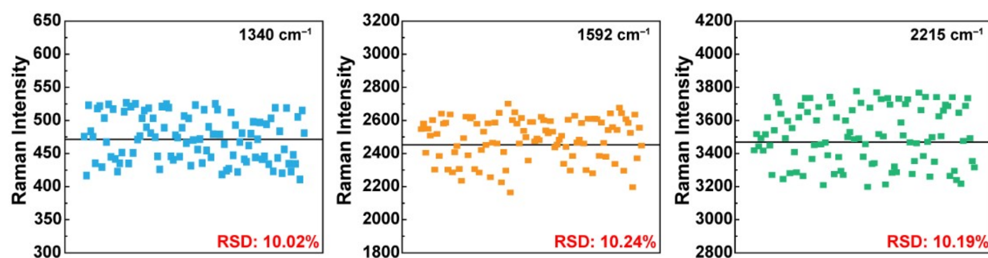

Fig. S10 Deviation in the intensity of the D, G, and Y bands in the SERS spectra of DPB (1 μM) collected at 300 random sites, respectively. Laser: 633 nm. Power density: 2.5 mW cm^{-2}.

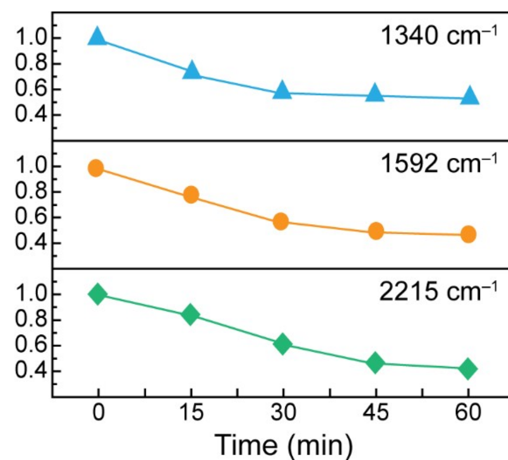

Fig. S11 Deviation in the intensity of the D, G, and Y bands in the SERS spectra of DBP (1 μM) collected from the same site within 1 h.

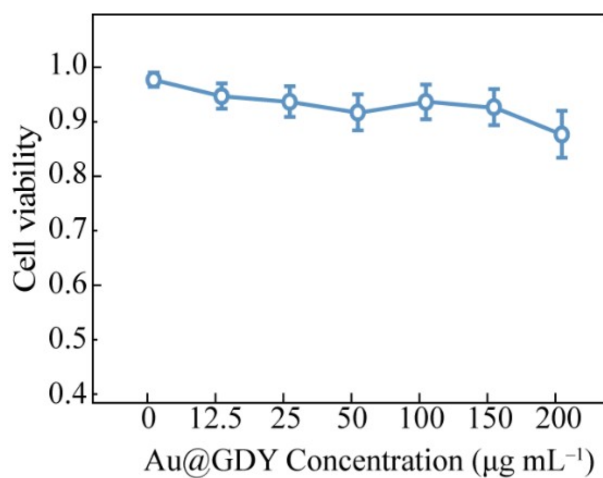

Fig. S12 Viability of MCF-7 cells incubated with Au@GDY at various concentration in the range of 0–200 μg mL^{-1} for 24 h.

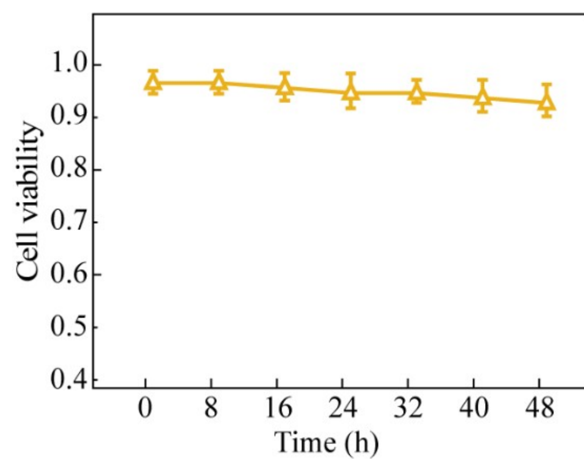

Fig. S13 Viability of MCF-7 cells incubated with 100 μg mL^{-1} of Au@GDY suspension for 0–48 h.

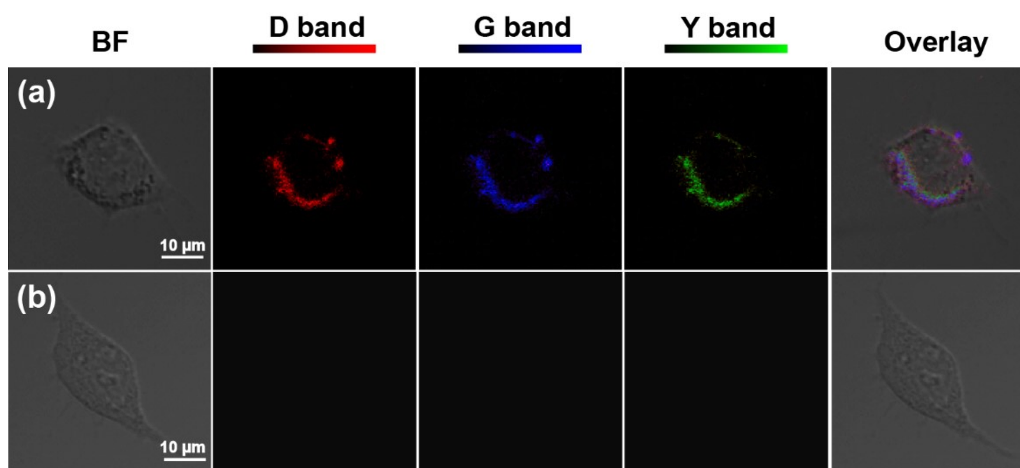

Fig. S14 Raman imaging of MCF-7 cells (a) and HepG2 cells (b) stained with Au@GDY (100 μg mL^{-1}) at 4°C. The cells were cultured with Au@GDY for 0.5 h and washed with copious PBS.