Supporting Information for

High Resolution Live Cell Raman Imaging Using Subcellular Organelle-Targeting SERS-Sensitive Gold Nanoparticles with Highly Narrow Intra-Nanogap

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This pdf file includes:

Materials, methods, and data (HR-TEM, UV-Visible spectra, Raman Images, Fluorescence Images)

Figures. S1–S9

I. General

The gold nanoparticles were purchased from Ted Pella, Inc. (Redding, CA, USA). All other chemical reagents ($HAuCl_4 \bullet 3H_2O$, poly (N-vinyl-2-pyrrolidone) (*Mw*, 40,000; K value, 29–32), $NH_2OH \bullet HCl$, dithiothreitol) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as received without further purification. Thiolated single-stranded DNA were purchased from IDT Inc. (Coralville, IA, USA) and reduced by using dithiothreitol (0.1 M) in a phosphate buffer (0.17 M, pH = 8.0). The reduced oligonucleotides were then purified using NAP-5 column (Sephadex G-25 medium, DNA grade). NANOpure H_2O (>18.0 MΩ) was used for all of the experiments. The formvar/carbon coated copper grid (Ted Pella, Inc.) and high-resolution transmission electron microscopy (JEM-2010, JEOL, Tokyo, Japan) were used for the TEM analysis. CellMask™ (C10045), MitoTracker® (M7514), DPAI dihydrochloride (D1306), and live/dead assay kit (R37601) were purchased from Invitrogen (NY, USA).

II. Instrumental setups for confocal Raman microscopy[1, 2]

A custom-built NIR confocal Raman microscopy system was used, which was equipped with 785 nm wavelength Ti: Sapphire laser (3900S, Spectra-Physics) and beam was filtered by a band pass filter (BPF, LL01-785-12.5, Semrock) and redirected to the dual axes galvanometer mirrors. High-speed XY scanning was performed by the galvanometer mirrors (CT-6210, Cambridge Technology). A 1.2 NA water immersion objective lens with high NIR transmission (Olympus UPLSAPO60XWIR 60X/1.20) was used to both focus the laser light onto the sample and to collect the back-scattered light. A piezo actuator combined with a differential micrometer (DRV517, Thorlabs) was used to perform the coarse and fine adjustments, respectively, of the sample focus. A flip mirror was placed after the tube lens so that the sample focal plane from the incoherent transmission source can be observed using a video camera with 67X magnification. The back-scattered Raman light from the sample passes through two dichroic mirrors (DM1: Semrock LPD01-785RU-25, DM2: Semrock LPD01-785RU-25×36×1.1) and was collected by a multi-mode fiber (Thorlabs M14L01). The collected signal was delivered to the spectrograph (Holospec f/1.8i, Kaiser Optical Systems) and detected by a thermoelectric-cooled, back-illuminated and deep depleted CCD (PIXIS: 100BR_eXcelon, Princeton Instruments). LabView 8.6 software (National Instruments), data acquisition board (PCI-6251, National Instruments) and MATLAB 2013 software (Mathworks) were used to control the system, acquire the data, and analyze the data.

III. Preparation of the DNA-modified AuNP (A_{10} spacer)

A fast salt aging method was used to prepare DNA-modified AuNPs.[3] Freshly reduced thiolated ssDNA (760 μL of 4.3 μM: 3′-HS-$(CH_2)_3$-A_{10}-PEG_9-AAACTCTTTGCGCAC-5′) were mixed with citrate-AuNPs (1 mL, 1.0 nM), respectively, and then incubated for 30 min at room temperature. The solution was then adjusted to obtain a final phosphate concentration of 10 mM (pH 7.4) with 100 mM PB (176 μL) and a final concentration of 0.1% (wt/vol) sodium dodecyl sulfate (SDS) with 10% SDS solution (1.9 μL). The solution was further incubated on an orbital shaker for 30 min and NaCl concentration was increased up to 0.3 M by adding four aliquots of total volume of 2 M NaCl solution (0.05 M twice, 0.1 M twice) at every 30 min (48.5 μL, 48.5 μL, 97 μL, 97 μL). Brief (5 min) heating in a water bath (60 °C) was applied at every salt addition steps to maximize the loading density of ssDNA on AuNP surface. After the salt-aging procedures, AuNP and DNA mixture was gently vortexed for overnight at room temperature. The solution was centrifuged (12,000 rpm, 15 min), the supernatant was removed, and the precipitate was redispersed in distilled water (DW; twice). The particle concentration was determined by use of an UV-visible spectrophotometer. DNA-AuNP in DW was stable for more than 6 months at room temperature.

IV. Preparation of the DNA-modified AuNP (G_{10} spacer, C_{10} spacer, T_{10} spacer)

We followed the same procedures above listed for the preparation of DNA-AuNP using oligonucleotide sequences; 3′-HS-$(CH_2)_3$-G_{10}-PEG_9-AAACTCTTTGCGCAC-5′ for G_{10} spacer DNA-AuNP, 3′-HS-$(CH_2)_3$-C_{10}-PEG_9-AAACTCTTTGCGCAC-5′ for C_{10} spacer DNA-AuNP, 3′-HS-$(CH_2)_3$-T_{10}-PEG_9-AAACTCTTTGCGCAC-5′ for T_{10} spacer DNA-AuNP.

V. Preparation of Raman-dye coded Au-NNPs

For the preparations of Raman-dye (MB, 44DP, and AB) coded Au-NNPs, the DNA-modified AuNP (20 nm) was prepared using thiolated oligonucleotide (3′-HS-$(CH_2)_3$-T_{10}-PEG_9-AAACTCTTTGCGCAC-5′) with standard salt aging method.[4] To prepare Raman-dye coded Au-NNPs, first, 1.0 mL of DNA-AuNP (1.0 nM) were incubated with 200 μL of MB

(10^{-4} M) solution, 200 μL of 44DP (0.1 M) solution, and 100 μL of AB (5 mg/mL) solution, respectively, for 1 week with gentle shakings at room temperature. After removal of excess amount of Raman-dye (MB, 44DP, AB) by repeated centrifugations (2 times, 12,000 rpm/15 min), Raman-dye loaded DNA-AuNP solution (1.0 mL, OD 1.0) were mixed with 100 μL of 100 mM PB (pH 7.4) and 16.5 μL of 2.0 M NaCl to make 0.3 M PBS conditions and 1% PVP (500 μL) solution were added subsequently, then reacted with $HAuCl_4$ precursor (500 μL) in the presence of hydroxylamine solutions (500 μL). After shaking 20 sec, then the reaction mixture was allowed to stand for 1.0 h at room temperatures. The solution was centrifuged to remove supernatant (3,000 rcf/15 min) and then redispersed in distilled water (1.0 mL).

VI. PEGylation and peptide modifications of Raman dye-coded Au-NNPs

All Raman dye-coded Au-NNPs were functionalized with mPEG thiol (5 kDa, 10^3 times molar excess) and RGD peptide (10^4 times molar excess, RGDRGDRGDRGDPGC, *Mw* 1588.63) (GenScript, NJ, USA)) in the presence of 0.1% sodium dodecyl sulfate (SDS) solution for 12 h at room temperature. For cytoplasm-targeting Au-NNPs, MB-coded Au-NNP was used after PEGylation and RGD modification. For mitochondria-targeting Au-NNPs, the 44DP-coded Au-NNP was used after modifying with additional peptide that can target to the mitochondria (denoted MLS, 10^4 times molar excess, MLALLGWWWFFSRKKC, *Mw* 2072.55) (GenScript, NJ, USA). For nucleus-targeting Au-NNPs, the AB-coded Au-NNP was used after modifying with nuclear-targeting peptide (denoted NLS, 10^4 times molar excess, CGGGPKKKRKVGG, *Mw* 1271.54) (GenScript, NJ, USA).[5]

VII. Cell cultures (HSC-3 cell)

Human oral cancer cells (HSC-3) were chosen due to the well-established interactions of nanoparticles and HSC-3 cell. HSC-3 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (FBS) and 1% antibiotics. HSC-3 cells were plated onto 60 mm petri dishes modified to incorporate a quartz bottom (043210-KJ, Alfa Aesar). The cells were allowed to adhere to the quartz bottomed plates for at least 24 hours before imaging. SERS-active nanoparticle solutions were added to the petri dishes before the Raman measurements.

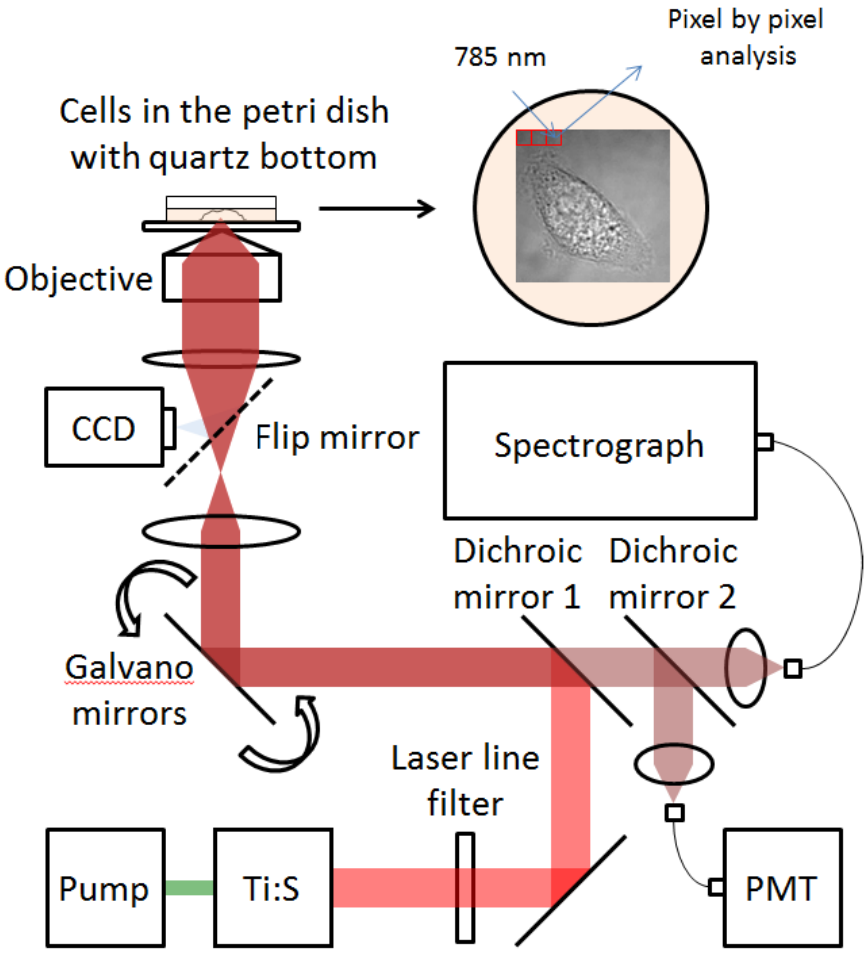

Figure S1. Description of the instrumental setup of a custom-built NIR confocal Raman microscopy system used for high resolution live cell Raman imaging.

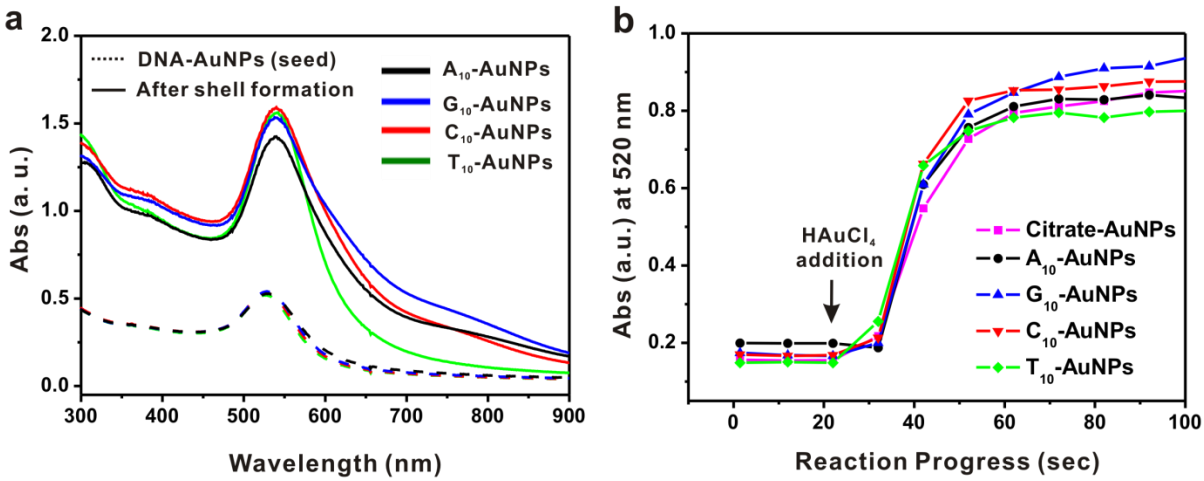

Figure S2. (**a**) The UV-Vis spectra of DNA-AuNPs and Au-NNPs obtained from A_{10}, G_{10}, C_{10}, and T_{10} spacer, respectively. (**b**) The reaction progress of shell formation reactions when used seed particle solutions such as citrate-AuNP, A_{10}-AuNP, G_{10}-AuNP, C_{10}-AuNP, and T_{10}-AuNP, respectively.

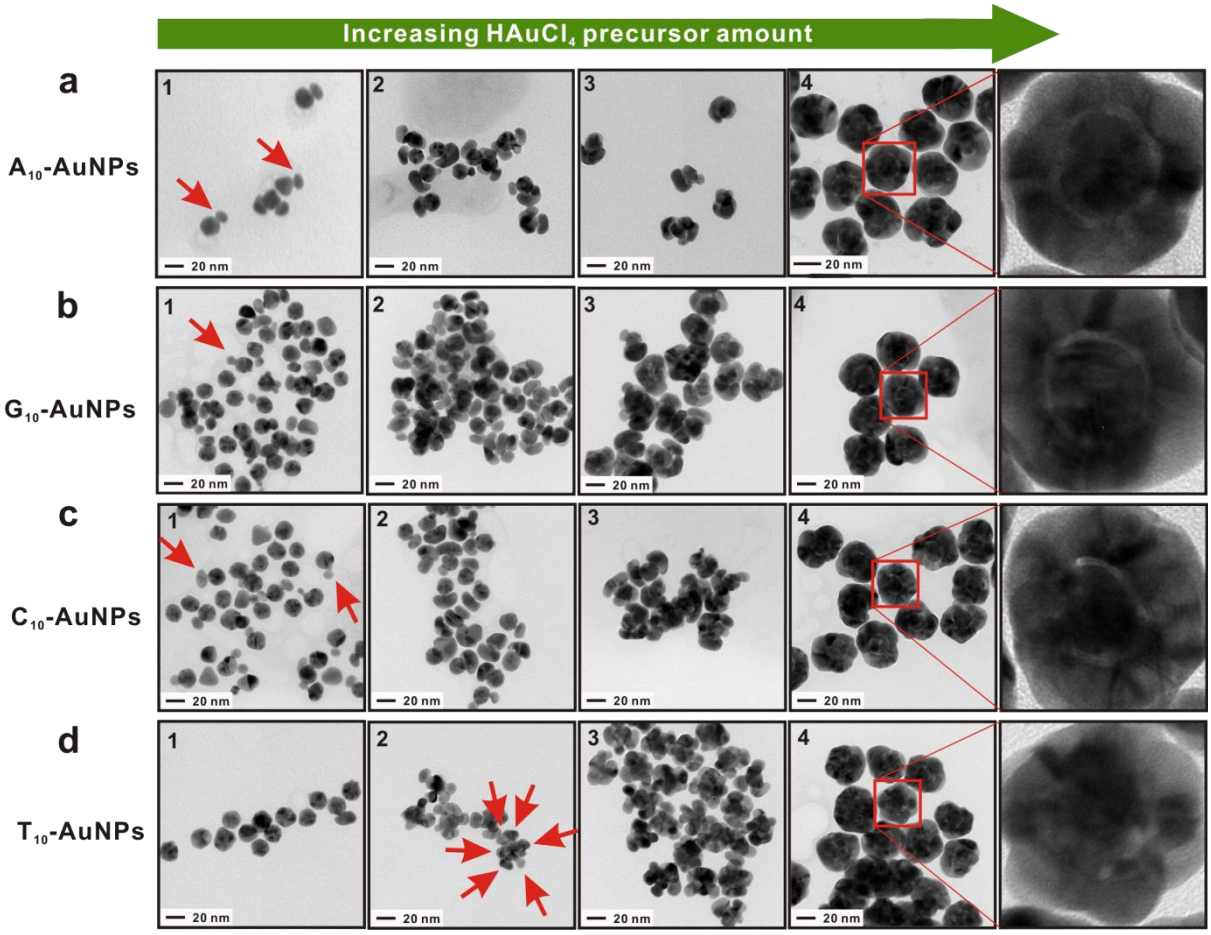

Figure S3. TEM-images of intermediate structures during the shell formation reactions with increased amount of $HAuCl_4$ solutions in the presence of (**a**) A_{10}-AuNPs, (**b**) G_{10}-AuNPs, (**c**) C_{10}-AuNPs, and (**d**) T_{10}-AuNPs. (Red arrows indicate the newly produced small budding particles on the core AuNP).

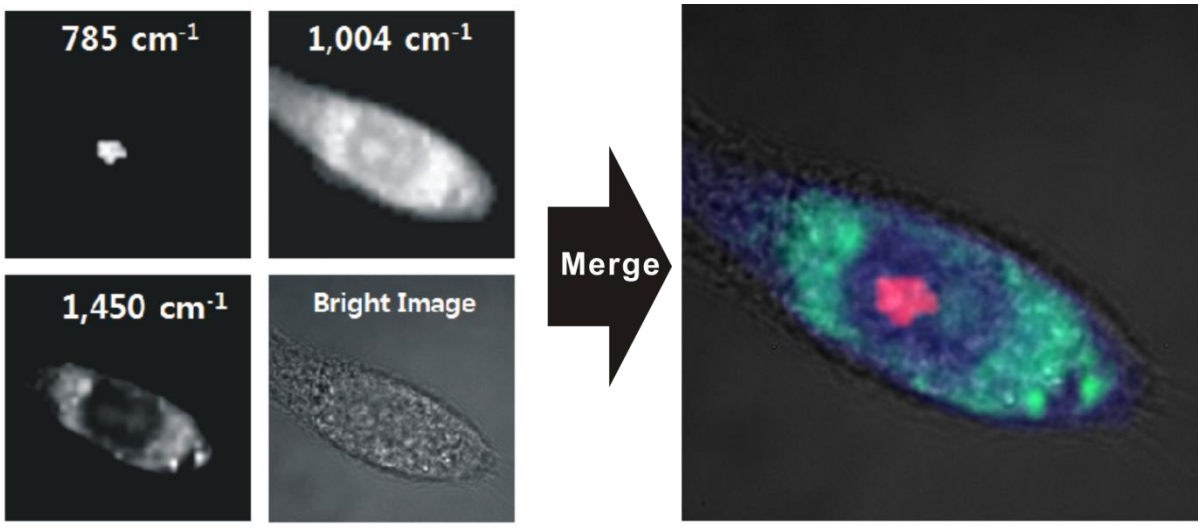

Figure S4. Label free Raman images of HSC-3 cell using the Raman shift at 785 cm^{-1}, 1,004 cm^{-1}, 1,450 cm^{-1} and overlapped bright field images of color coded Raman image (red for 785 cm^{-1}, green for 1,004 cm^{-1}, and blue for 1,450 cm^{-1}.

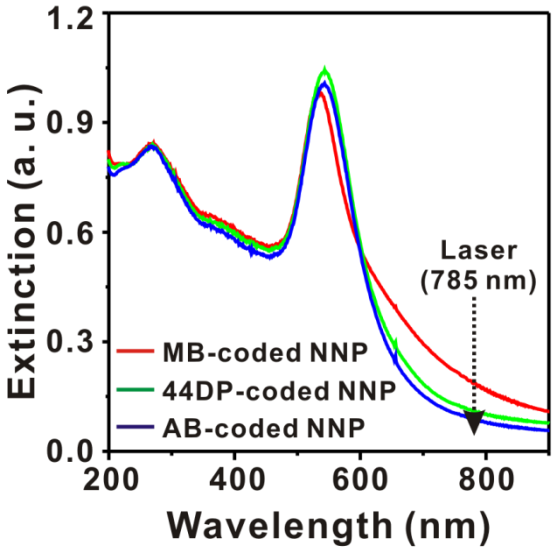

Figure S5. The UV-Vis spectra of PEGylated and targeting peptide modified Au-NNPs. (MB-coded Au-NNP (red line), 44DP-coded Au-NNP (green line), AB-coded Au-NNP (blue line)).

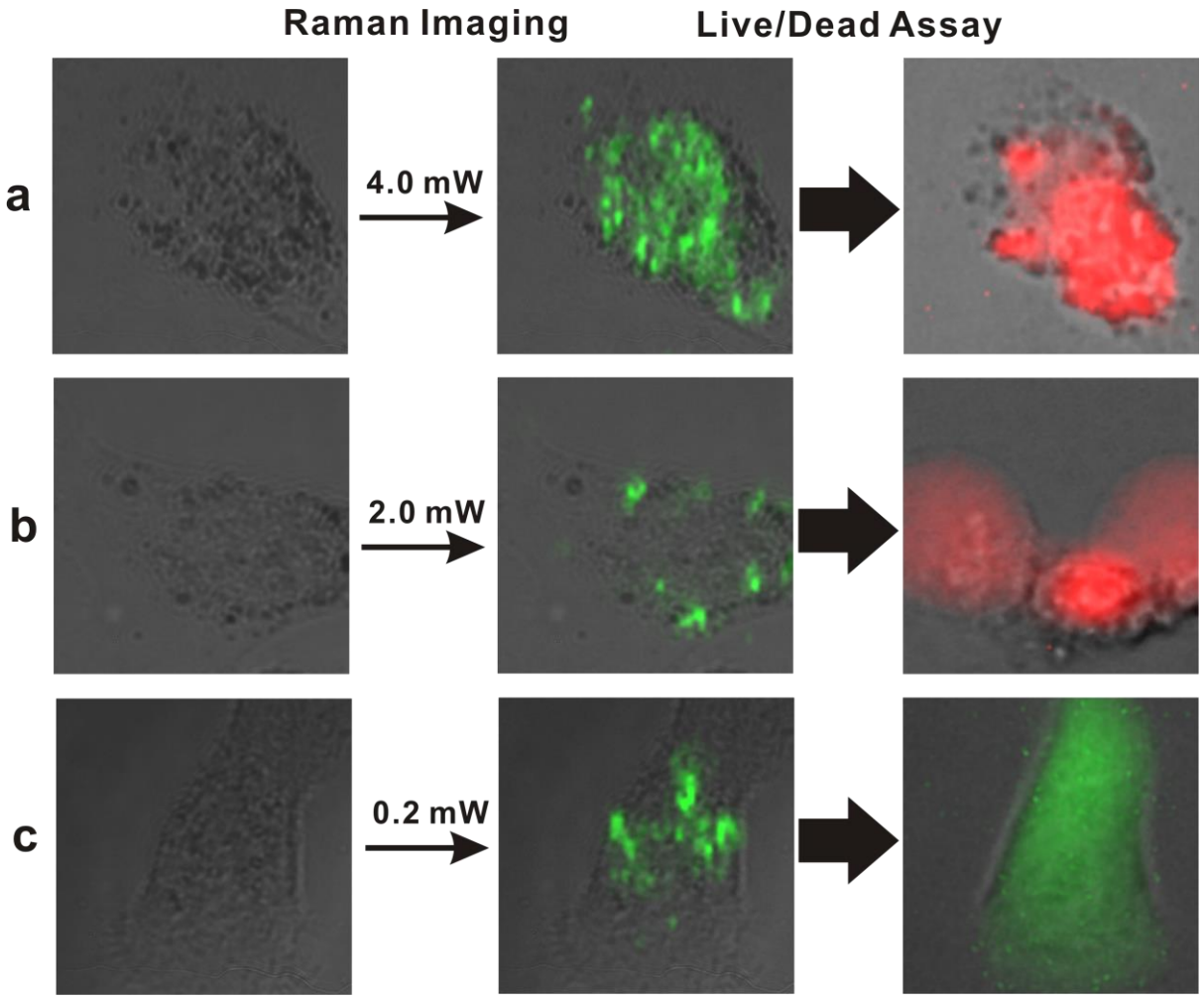

Figure S6. The incident laser power dependent Raman images and cell viabilities (First, each denoted incident laser power was applied to obtain Raman image, and then the same cell was stained with live/dead assay kit). (**a**) When applied 4.0 mW of laser power for Raman imaging, the significant change of cell morphology and the cell death (red staining) were observed. (**b**) When applied 2.0 mW of laser power, significant changes of cell morphology and the cell death also were observed. (**c**) When applied 0.2 mW of laser power, no significant change of cell morphology was observed and cell was live (stained with green).

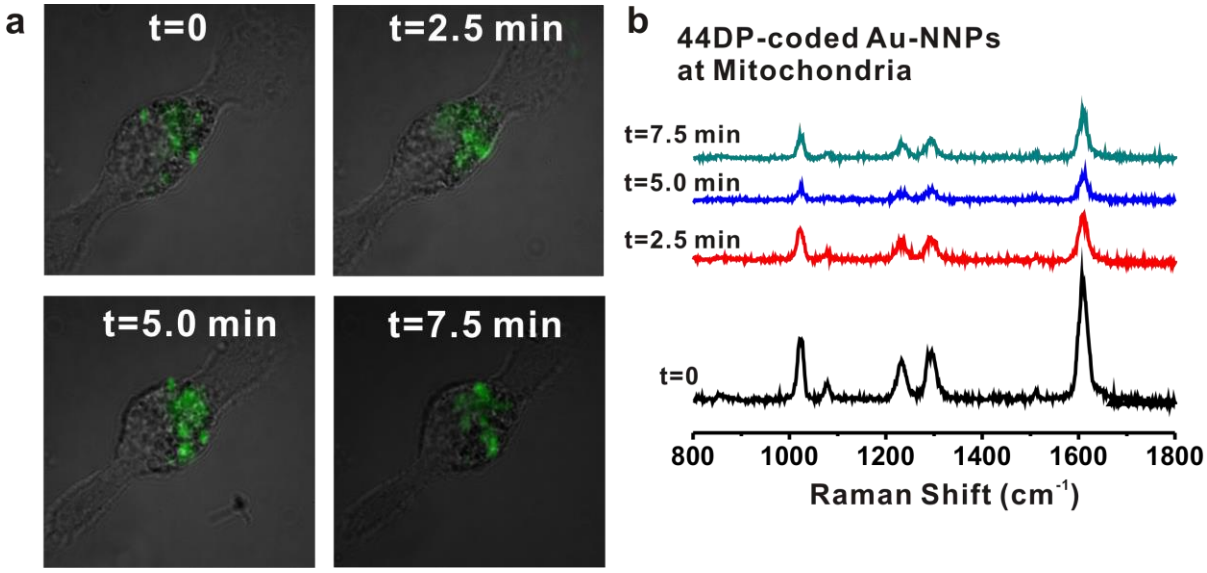

Figure S7. (**a**) Time dependent Raman images overlaid with bright field image after addition of 10 μL of potassium cyanide solution (1.0 M) into HSC-3 cell line. (**b**) Representative Raman spectra obtained from inside cells at designated time points (0, 2.5 min, 5.0 min, 7.5 min). Imaged at every 2.5 min after additions of KCN (50 × 50 pixel, 200 μW laser power density, 10 ms/pixel, 27.5 s (total imaging time)).

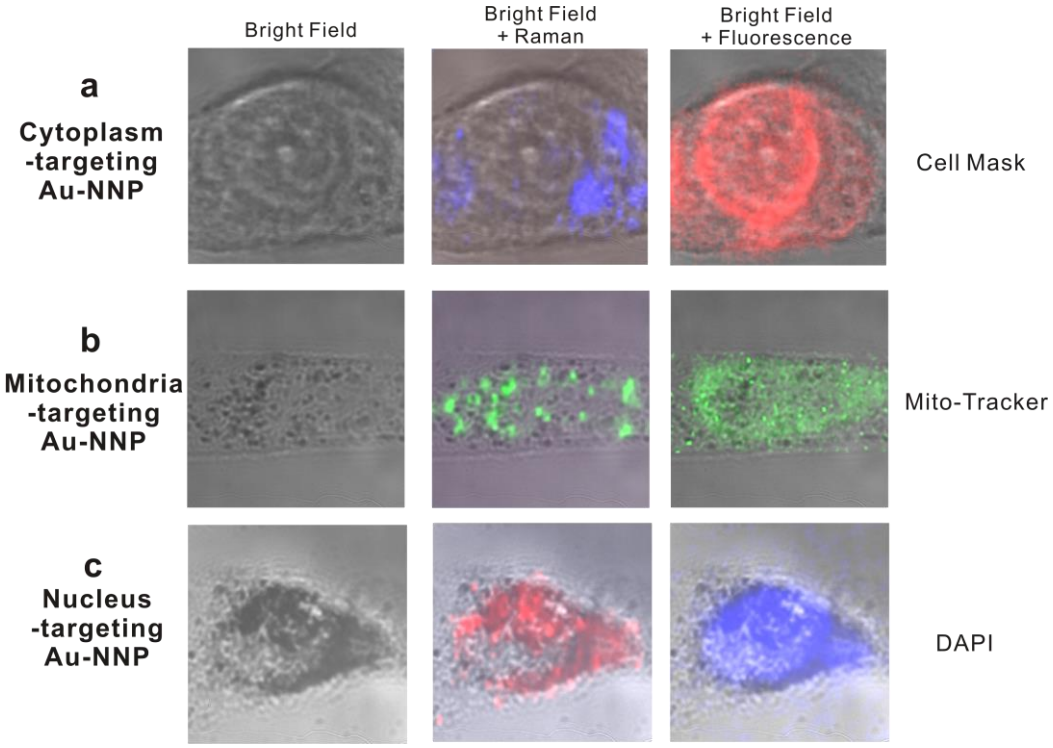

Figure S8. The co-localization of Raman-dye coded Au-NNPs and fluorescence probe. (**a**) Bright field images overlaid with Raman image (obtained with cytoplasm-targeting Au-NNPs) and fluorescence image stained with CellMask™. (**b**) Bright field images overlaid with Raman image (obtained with mitochondria-targeting Au-NNPs) and fluorescence image stained with Mitotracker®. (**c**) Bright field images overlaid with Raman image (obtained with nucleus-targeting Au-NNPs) and fluorescence image stained with DAPI.

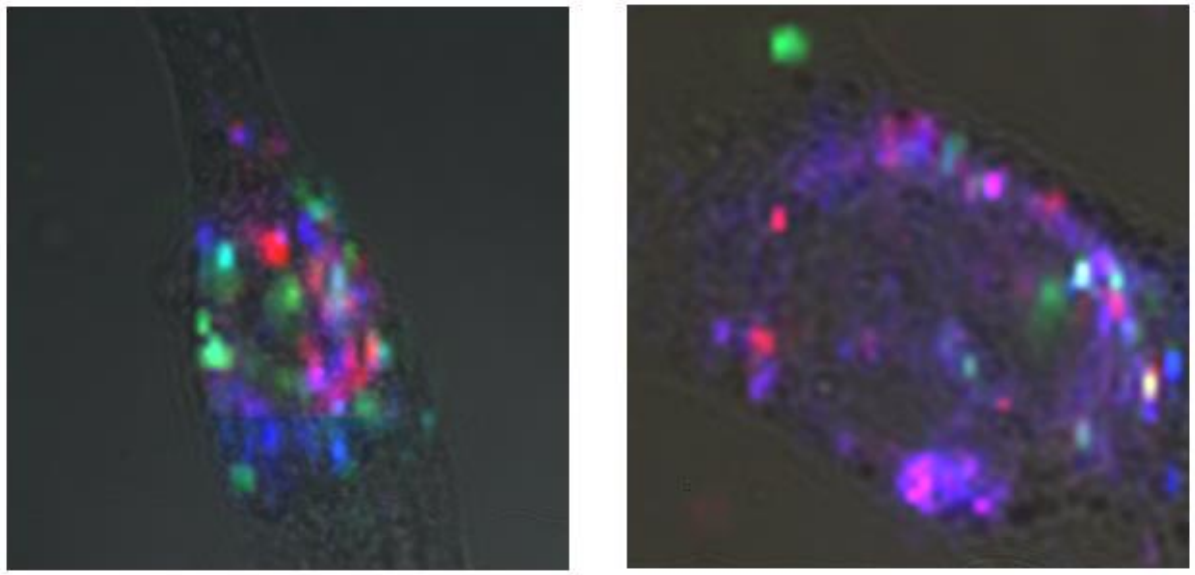

Figure S9. Multiplexed Raman images of HSC-3 cell (1.0 ml media volume) incubated 12 h with MB-coded Au-NNPs (30 μL), 44DP-coded Au-NNPs (30 μL), and AB-coded Au-NNPs (30 μL) with an analysis conditions (50 × 50 pixel, 200 μW laser power density, 10 ms/pixel, 27.5 s total imaging time for single cell).

References

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