Electronic Supplementary Information for

SERS-Based Particle Tracking and Molecular Imaging in Live Cells: Toward the Monitoring of Intracellular Dynamics

Jongwoo Kim,[a,†] Sang Hwan Nam,[a,†] Dong-Kwon Lim,[b,*] and Yung Doug Suh[c,d,*]

[a]Center for Convergent Research of Emerging Virus Infection, Korea Research Institute of Chemical Technology, Daejeon 34114, South Korea.

[b]KU-KIST Graduate School of Converging Science and Technology, Korea University, Seoul 02841, South Korea.

[c]Laboratory for Advanced Molecular Probing (LAMP), Korea Research Institute of Chemical Technology, Daejeon 34114, South Korea.

[d]School of Chemical Engineering, Sungkyunkwan University, Suwon 16419, South Korea.

[†]These authors contributed equally to this work.

Corresponding Authors

[*] E-mail (D.-K.L.): dklim@korea.ac.kr

[*] E-mail (Y.D.S.): ydsuh@krict.re.kr

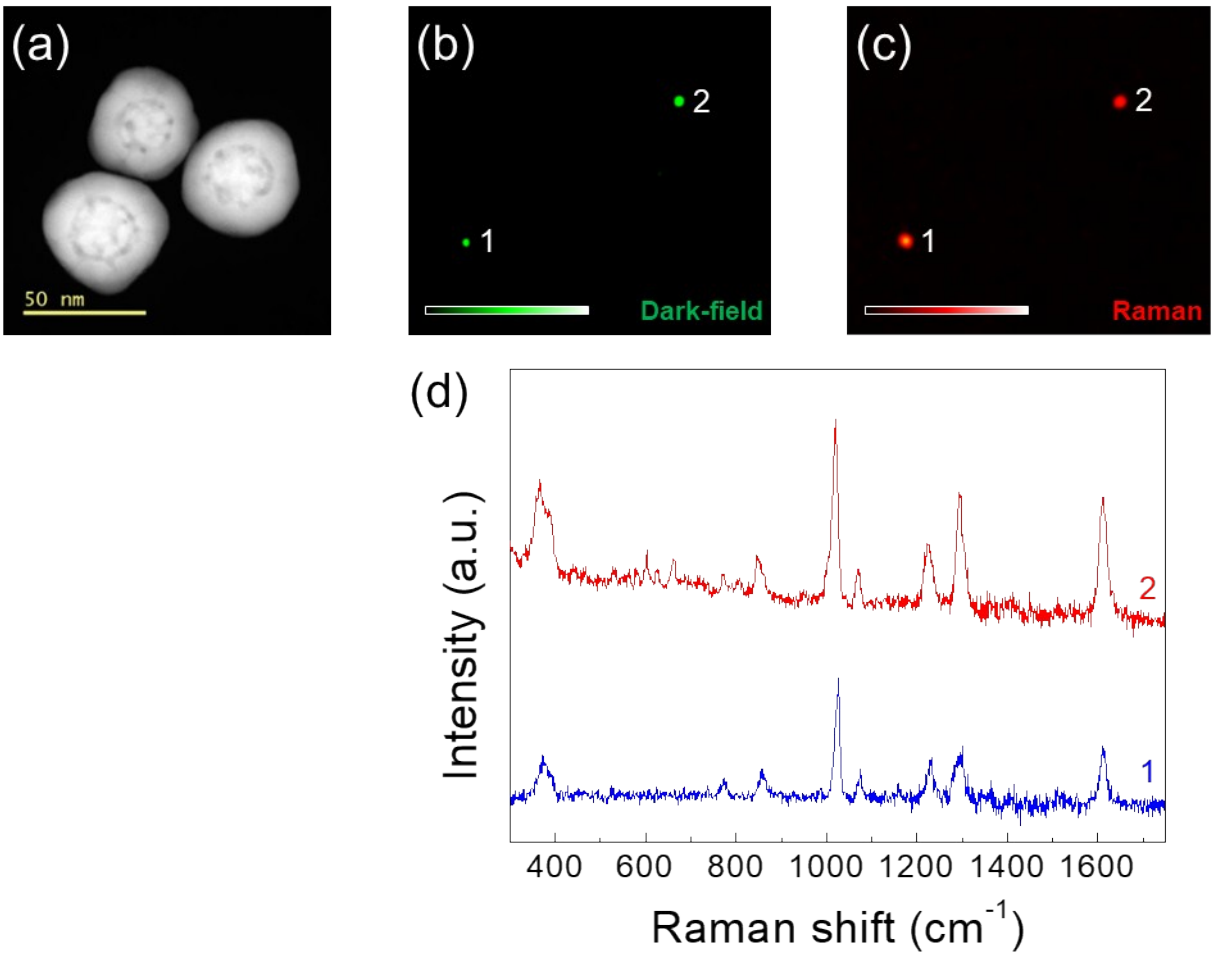

Figure S1. Characterization of gold nanogap nanobridged particles (Au-NNPs) used for wide-field Raman spectroscopic imaging of live cells. (a) A high-voltage electron microscope image of Au-NNPs. (b) Single-particle-level dark-field and (c) wide-field SERS images of Au-NNPs labeled with 4,4'-dipyridyl (44DP), and (d) their corresponding surface-enhanced Raman spectra with excitation of 785 nm. The scale bars in (b), and (c) represent 50 μm.

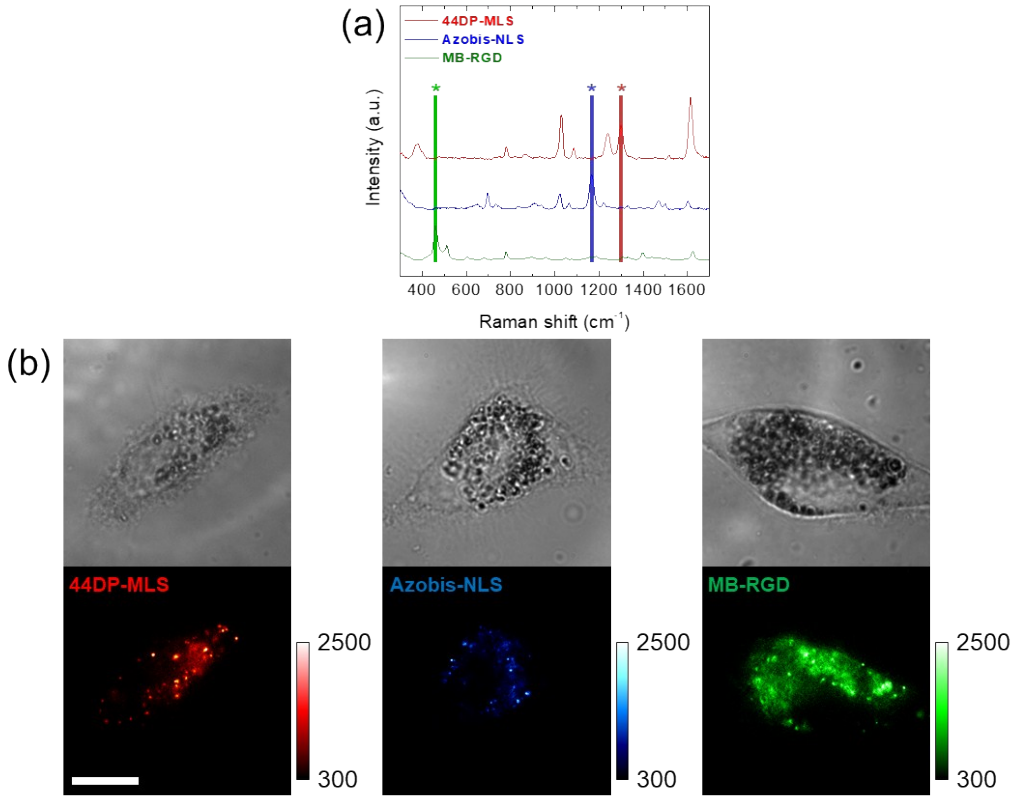

Figure S2. Wide-field Raman imaging and spectroscopy of live cells based on different labeling of Raman reporter–targeting sequence pairs. (a) SERS spectra of Au-NNPs functionalized with methoxy-polyethylene glycol (mPEG) thiol, cell-penetrating peptide (with sequence of RGDRGDRGDRGDPGC), and additional peptides that can target subcellular organelles. Three types of Raman reporter–targeting sequence pairs: (1) 4,4′-dipyridyl (44DP)–mitochondria localization signal (MLS; MLALLGWWWFFSRKKC), (2) 4,4′-azobis(pyridine) (Azobis)–nuclear localization signal (NLS; CGGGPKKKRKVGG), and (3) methylene blue (MB)–cell-penetrating peptide. Since bands of the Raman spectra are narrow, we selected one specific band in each Raman reporter without interference from the bands of other Raman reporters. The selected band of each reporter for labeled SERS imaging is marked with an asterisk (*). The filled box on each spectrum represents the position and bandwidth (0.75 nm) of the liquid crystal tunable filter (LCTF) for the wide-field Raman imaging. (b) (top) Bright-field images and (bottom) wide-field Raman images of single HeLa cells incubated with the Au-NNPs of the Raman reporter-targeting sequence pairs. The resolution of the SERS images was 512 × 512 pixels, and their exposure time was 1 s. The scale bars represent 20 μm.

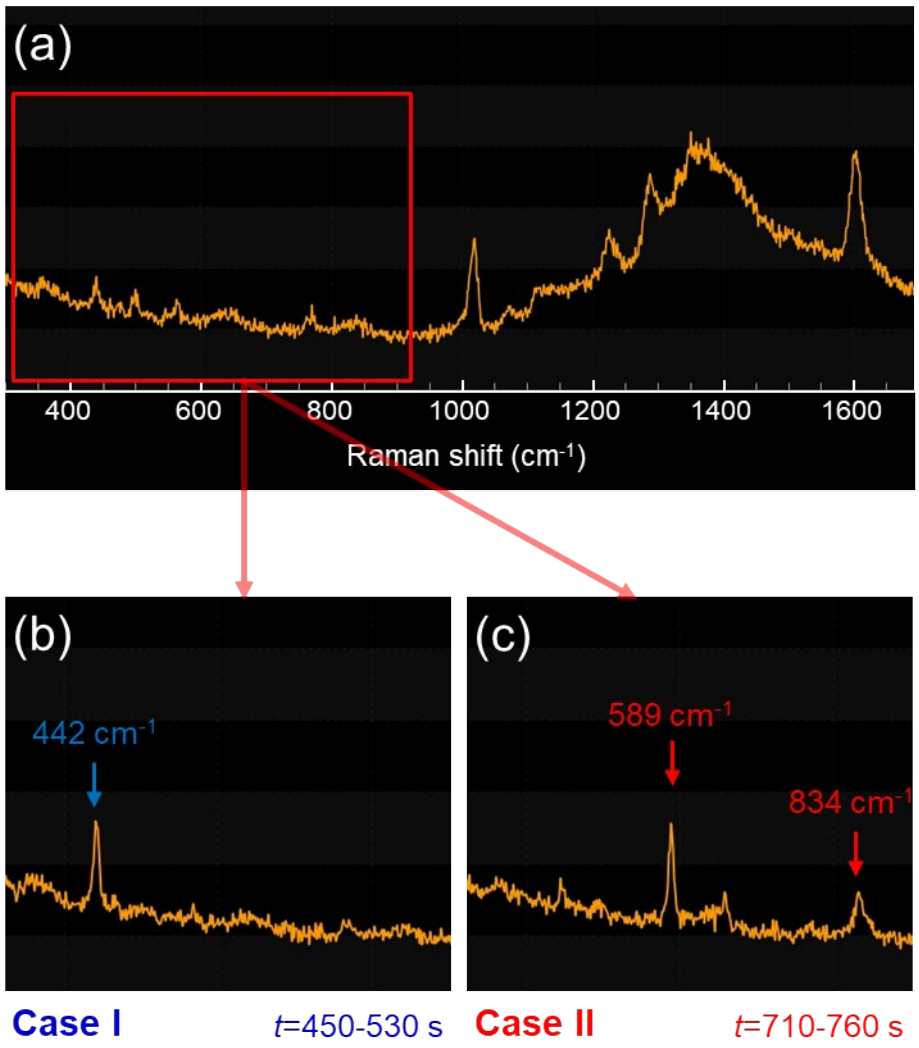

Figure S3. (a) Snapshot of a time-resolved full Raman spectrum of a live HeLa cell incubated with 4,4'-dipyridyl-coded gold nanobridged nanogap particles. For an accompanying video, see Video S2 in the Supporting Information. (b-c) Snapshots of time-resolved Raman spectra showing a small Stokes shift presented in Videos S3 and S4 in the Supporting Information, respectively. (b) and (c) show different Raman bands.

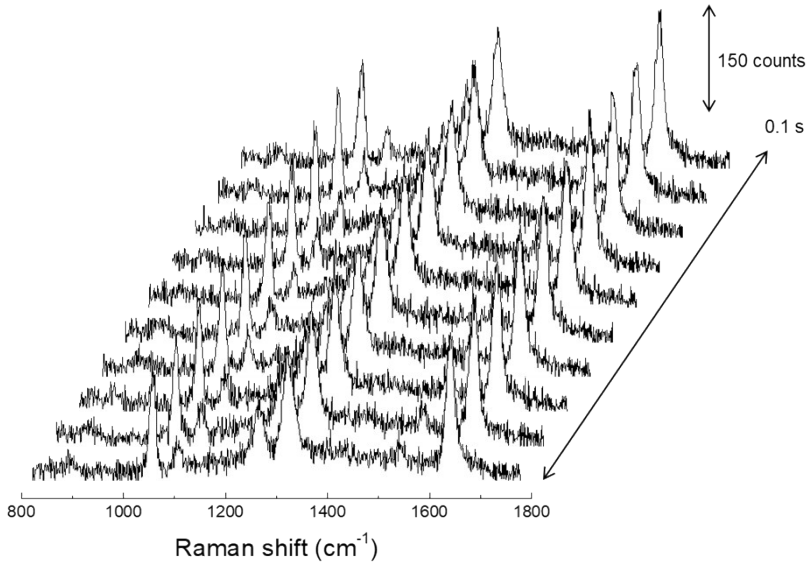

Figure S4. Representative Raman spectra of 4,4′-dipyridyl-labeled gold nanobridged nanogap particles in a HeLa cell obtained with an incident laser power density of ca. 1 kW/cm^2 and 10 ms exposure time per spectrum.

MATERIALS AND METHODS

Internalization of gold nanobridged nanopgap particles (Au-NNPs) in HeLa cells. HeLa cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM: 11995-065; Life Technologies, USA) supplemented with 10% fetal bovine serum (FBS: 16000-044; Life Technologies, USA) and 1% penicillin–streptomycin (15070-063; Life Technologies, USA). The cells were plated on 35-mm glass-bottomed dishes (100350; SPL, South Korea). For the internalization of Au-NNPs in cells, the cells were incubated with Au-NNPs for 12 h followed by washing out three times.

Wide-field Raman imaging of a live cell. A glass-bottomed dish containing cultured cells was placed in a live cell incubator (Chamlide TC; Live Cell Instruments, South Korea) on an optical microscope (IX71; Olympus, Japan). The incubator was maintained at a temperature of 36.5°C. As an excitation source, we used a diode-pumped solid state (DPSS) laser with a wavelength of 785 nm, and with output power of approximately 250 mW. The dish placed on an oil-immersion objective lens (UPLSAPO60XO2; Olympus, Japan) was uniformly irradiated for wide-field Raman imaging by a laser passed through a lens (L1 in Figure 1) and the objective lens. The power density of the laser at the sample was estimated to be 1 kW/cm^2. The light emitted from the sample longer than the laser wavelength was passed through a 785-nm long-pass dichroic beamsplitter (LPD02-785RU-25x36; Semrock, USA) and then through a 785-nm long-pass edge filter (BLP01-785R-25; Semrock, USA). A Raman image was taken using an electron-multiplying charge-coupled device (EMCCD) camera (iXon3 888; Andor Technology, UK). A liquid crystal tunable filter (LCTF: Varispec NIRR-0.75-20; PerkinElmer) with a very narrow bandwidth (0.75 nm) was placed in front of the EMCCD camera to facilitate the acquisition of images based on a single selected Raman band.

Particle tracking in a live cell. Images were taken by selecting a single Raman band of the reporter using an LCTF. The playback speed of Video S1 is normal (1×). The trajectory of a particle was obtained by using DiaTrack 3.0 software.

Simultaneous measurement of Raman spectra and images. In order to measure spectra and images simultaneously, the Raman scattered light was divided by a cover slip as a beamsplitter. Approximately 10% of the light was reflected by the cover slip and passed to a spectral CCD camera (PIXIS400BR; Princeton Instruments, USA) equipped with a spectrograph (Isoplane SCT320, Princeton Instruments, USA) after passing through a 785-nm long-pass edge filter (BLP01-785R-25; Semrock, USA) for spectral detection. The light transmitted through the cover slip was used for imaging, and an 830-nm (691 cm^{-1} with respect to 785 nm) long-pass dichroic beamsplitter (LPD02-830RU-25; Semrock, USA) was employed to divide the signals depending on the degree of Stokes shift. Since the molecules of 4,4'-dipyridyl used as a Raman reporter have strong SERS bands in the range of 1000–1600 cm^{-1}, the image obtained by the camera on the transmission side of the dichroic beamsplitter (large Stokes shift) represents the SERS image of the Raman reporter. Another camera (iXon Ultra 888; Andor Technology, UK) on the reflection side was additionally installed to obtain Raman images of intracellular cargo bands (small Stokes shift), and an LCTF was also employed to determine the position at which the Raman band at 443 cm^{-1} appeared.