Supporting Information

Surface-Enhanced Raman Scattering for HSP 70A mRNA Detection in the Live Cells Using Silica Nanoparticles and DNA-Modified Gold Nanoparticles

Ju Eun Cho[a] *and Dong-Kwon Lim* [a, b, c,*]

[a]KU-KIST Graduate School of Converging Science and Technology, Korea University, 145 Anam-ro, Seongbuk-gu, Seoul 02841, Republic of Korea

[b]Department of Integrative Energy Engineering, College of Engineering, Korea University, 145 Anam-ro, Seongbuk-gu, Seoul 02841, Republic of Korea.

[c]Brain Science Institute, Korea Institute of Science and Technology (KIST), Hwarang-ro 14-gil, Seongbuk-gu, Seoul 02792, Republic of Korea.

*Correspondence: dklim@korea.ac.kr

Materials

Silica nanoparticles (SiNPs, 50 nm) were purchased from Microsphere Nanospheres (Cold Spring, NY, USA). Citrate-capped gold nanoparticles (AuNPs, 50 nm) were purchased from BBI solution (Madison, WI, USA). Reagents including 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC), N-hydroxysulfosuccinimide (NHS), sodium phosphate dibasic anhydride (NA_2HPO_4), sodium phosphate monobasic dihydrate (NaH_2PO_4), sodium chloride (NaCl), DL-dithiothreitol (DTT) and sodium dodecyl sulfate were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Target oligonucleotide (HSP 70A mRNA), amine-modified oligonucleotides for SiNPs, thiol-modified oligonucleotide for AuNPs and Cy5 modified oligonucleotide were obtained from Integrated DNA Technologies (IDT Inc., Coralville, IA, USA). DNA purification columns (NAP-5) were purchased from GE Healthcare Life Sciences (Chicago, IL, USA). Dulbecco's Modified Eagle's medium (DMEM), phenol red-free medium (DMEM), and fetal bovine serum (FBS) were purchased from HyClone (Waltham, MA, USA). Dulbecco's phosphate-buffered saline (DPBS; 0.137 M) was purchased from Mediatech Inc. (Manassas, VA, USA). Trypsin and antibiotics were purchased from Gibco (Grand Island, NY, USA).

Instruments

The extinction spectra of nanoparticles were acquired using a UV-Vis spectrometer (SCINCO, South Korea). Transmission electron microscopy (TEM) was used for size characterization. Fluorescence intensity was measured using a Cytation 3 Cell Imaging Multi-Mode Reader (BioTek Inc., Winooski, USA). Raman spectra of DNA-AuNPs were acquired using a Raman microscope (WEVE, South Korea) with a 40× objective lens (Olympus, Tokyo, Japan) and a 785-nm laser (61.2 mW, 1 s exposure time). With a confocal motorized pinhole (100 μm) directed to a spectrometer (FEX-MD, WEVE, South Korea) (600 g mm^{-1} grating)

and to the spectroscope CCD (DV401A- BVF, Belfast, Northern Ireland) all the Raman signals were obtained. Bright-field, dark-field, and fluorescence images were captured using a 40× air objective lens (Olympus, DP80, Tokyo, Japan) and EzScan (WEVE, South Korea) software.

Table S1. Sequence information of target mRNA, flare and oligonucleotide.

Name	Sequence	T_m (°C)
Target	5'-TGT CGC AGC AGC TCC TCA GGC TAG-3'	**64.6**
Flare	5'-(Cy5) TGT CGC AGC AGC CCT ACT-3'	**59.2**
Oligonucleotide for SiNPs	5'-CTA GCC TGA GGA GCT GCT GCG ACA-A_{10}-NH_2-3'	**64.6**
Oligonucleotide for AuNPs	5'-ATC AGT AGT AGG GCT GCT GCG ACA-A_{10}-SH-3'	**61.8**

Table S2. Raman band assignment. [1]

Wavenumber (cm^{-1})	Raman band assignment
540–600	Amide VI
605	Cytochrome
625–800	Amide IV, V
753	Cytochromes
–800	Phosphoric acid
1000	Phenylalanine
1130	Cytochrome
1230–1300	Amide III
1314	Cytochrome
1451	CH_2
1480–1540	Amide II
1585	Cytochromes
–1660	C=C, C=O, Amide I

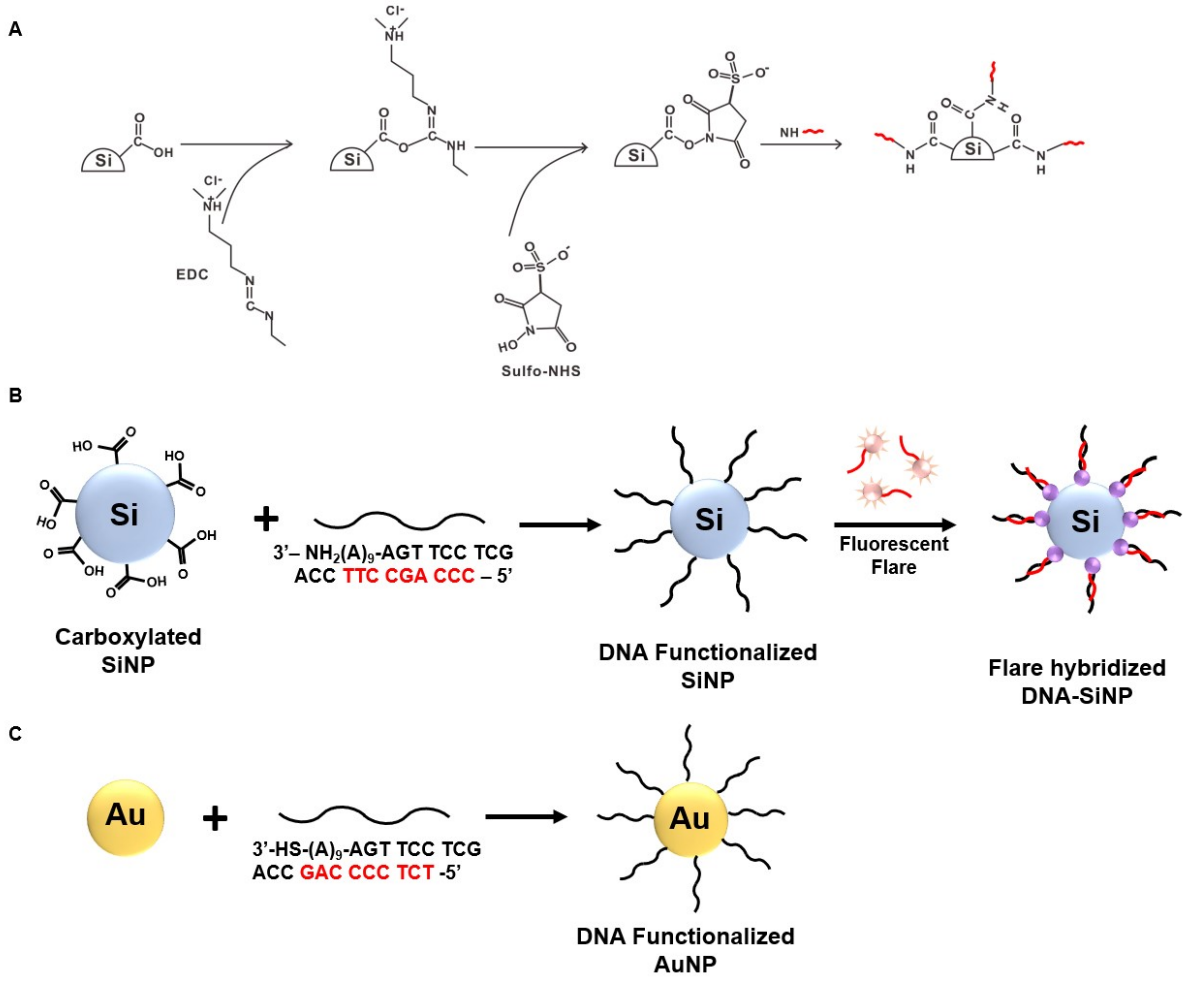

Figure S1. Schematic illustration of nanoparticles preparation. (A) Schematic representation of EDC/NHS coupling. (B) Preparation of flare hybridized DNA-SiNPs. (C) Conjugation of DNA with AuNPs.

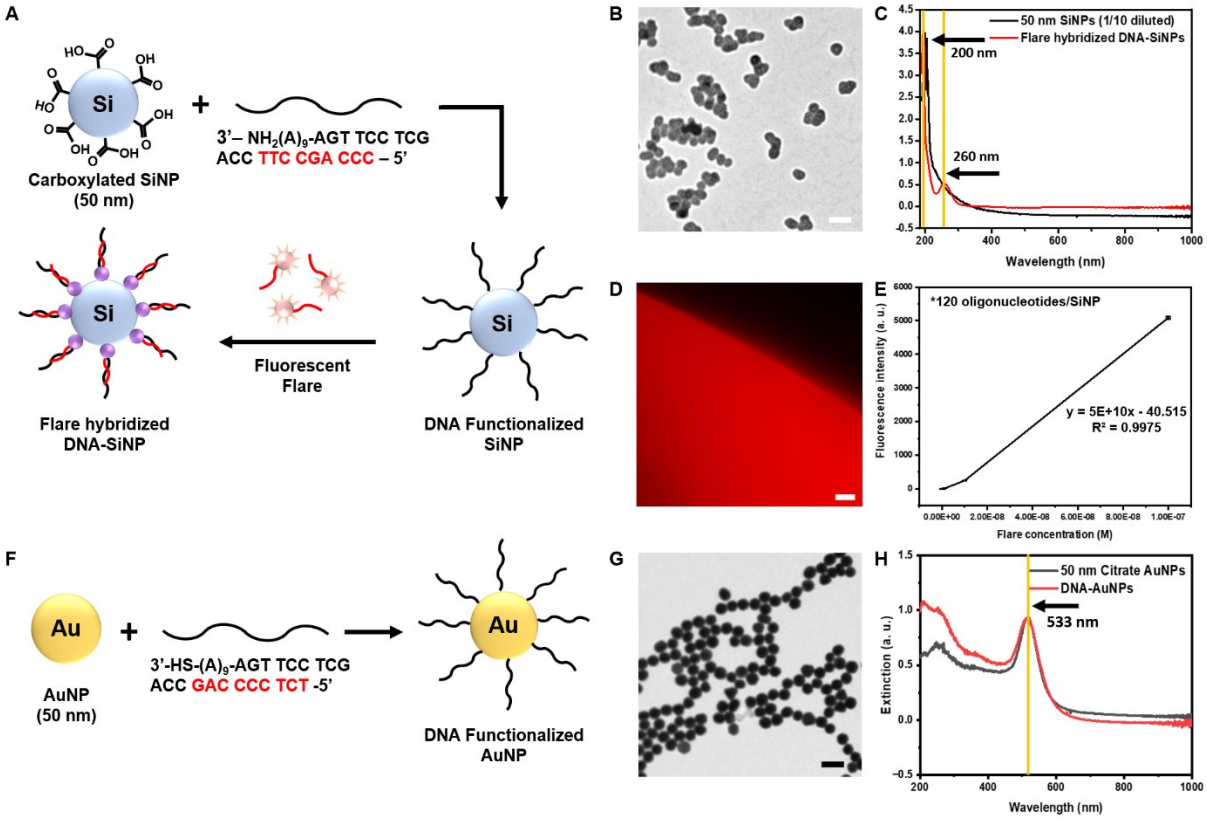

Figure S2. Characterization of nanoparticles. (A) Scheme for the preparation of flare hybridized DNA-SiNPs. (B) TEM image of the SiNPs (scale bar = 100 nm). (C) UV-Vis spectrum of SiNPs and Flare hybridized DNA-SiNPs. (D) Fluorescence image of the flare hybridized DNA-SiNPs (scale bar = 20 μm). (E) The number of oligonucleotides conjugated per SiNP. (F) Scheme for DNA conjugation with AuNPs. (G) TEM image of AuNPs (scale bar = 100 nm). (H) UV-Vis spectra of AuNPs and DNA-AuNPs.

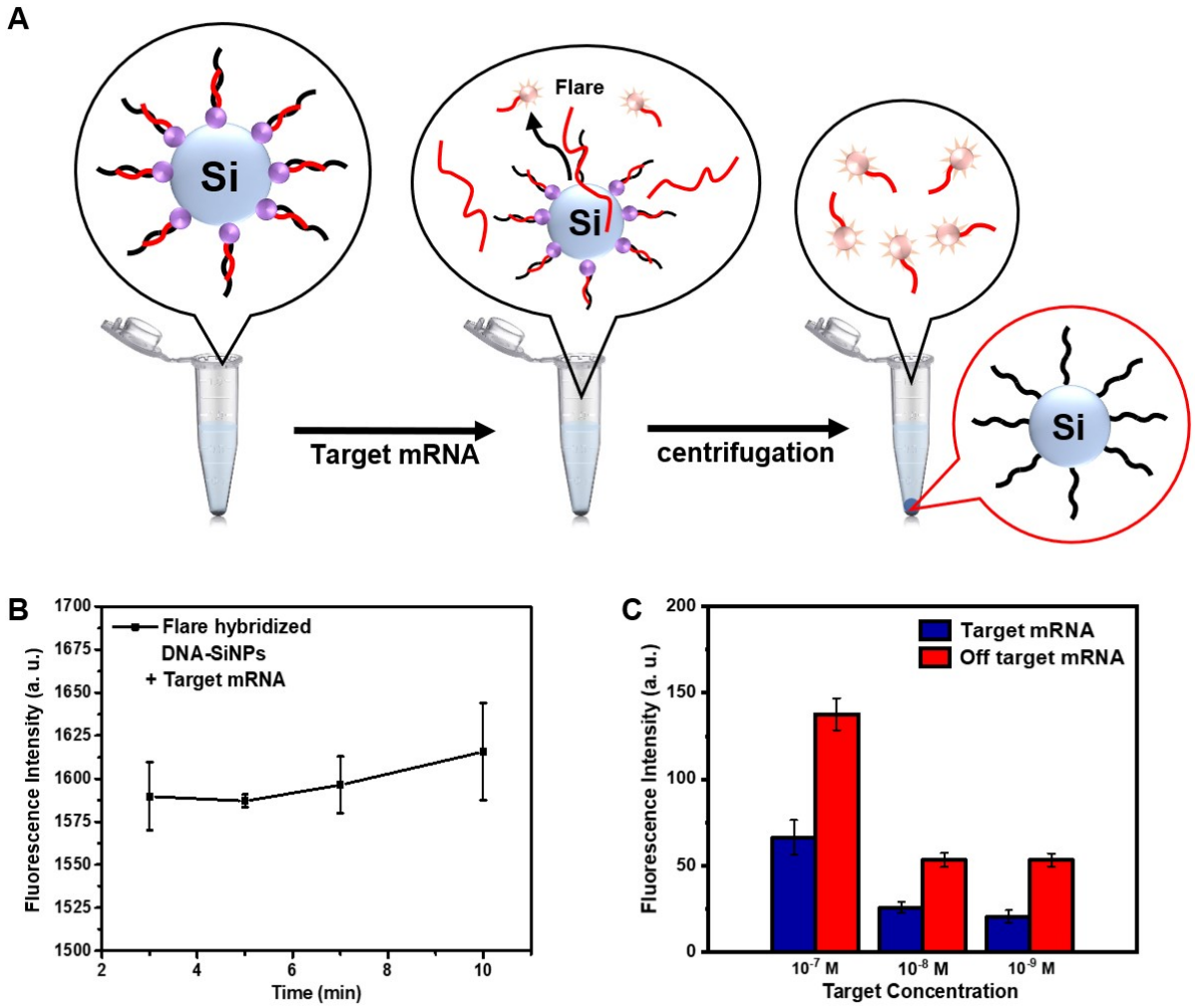

Figure S3. Test tube-based study of flare hybridized DNA-SiNPs. (A) Schematic illustration of procedure. (B) Fluorescence changes in the flare hybridized DNA-SiNPs following the addition of the target mRNA. (C) Selectivity of flare hybridized DNA-SiNPs to the target.

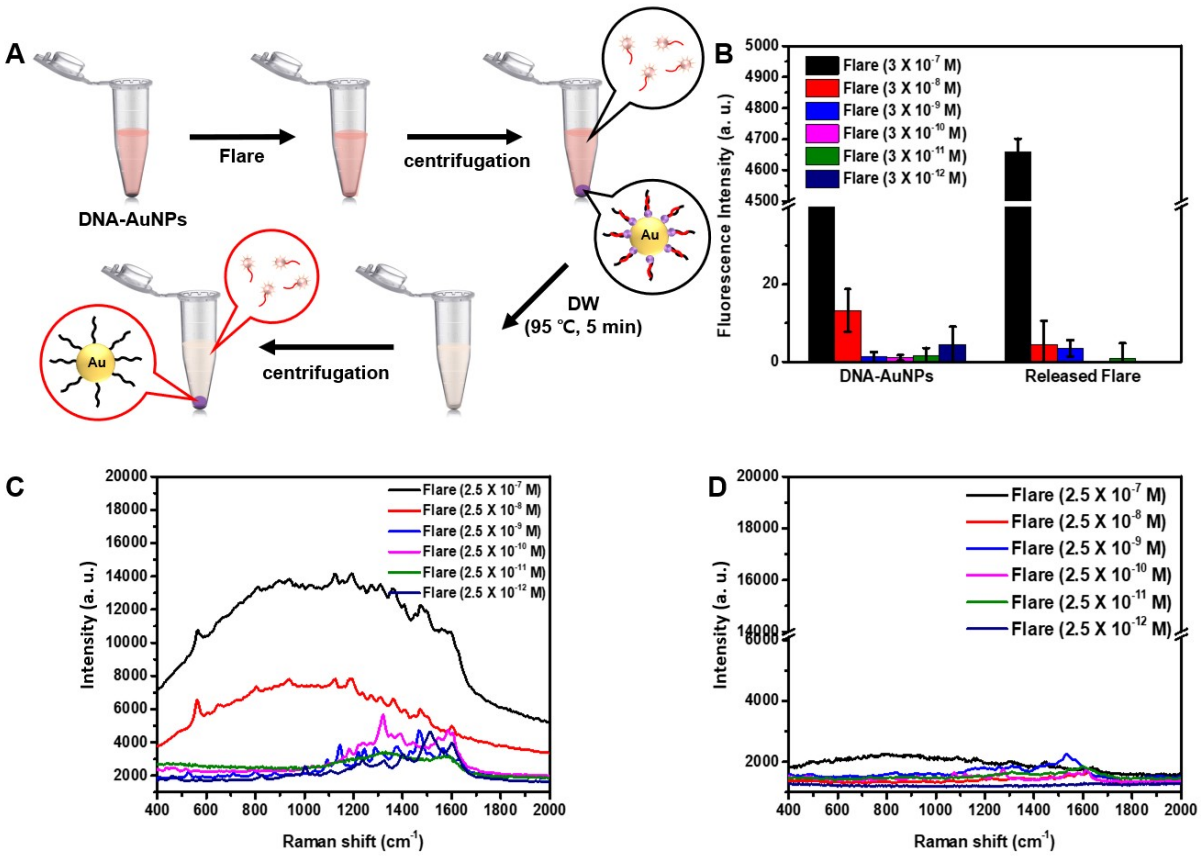

Figure S4. Test tube-based study of DNA-AuNPs. (A) Schematic illustration of procedure. (B) Fluorescence intensity of DNA-AuNPs and released flares after reaction with different flare concentrations. (C) Raman signals of flare-hybridized DNA-AuNPs depending on flare concentration. (D) Raman signals of DNA-AuNPs after flare release.

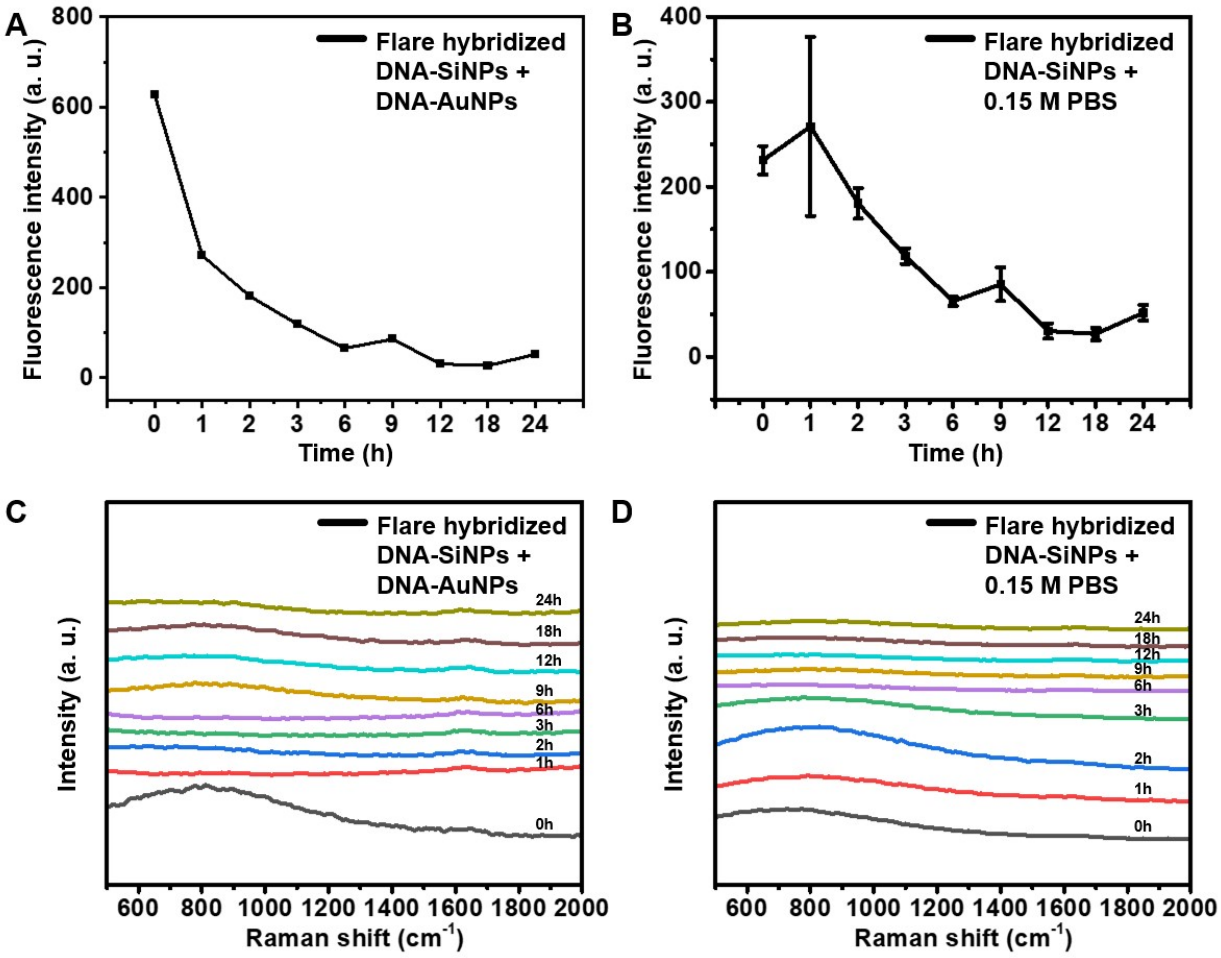

Figure S5. Control experiment (without target). (A) Fluorescence intensity change in flare-hybridized DNA-SiNP and DNA-AuNP solutions without the target. (B) Fluorescence intensity change of flare-hybridized DNA-SiNPs and 0.15 M PBS without the target. (C) Raman signals of flare-hybridized DNA-SiNP and DNA-AuNP solutions without the target. (D) Raman signals of flare-hybridized DNA-SiNPs and 0.15 M PBS without the target.

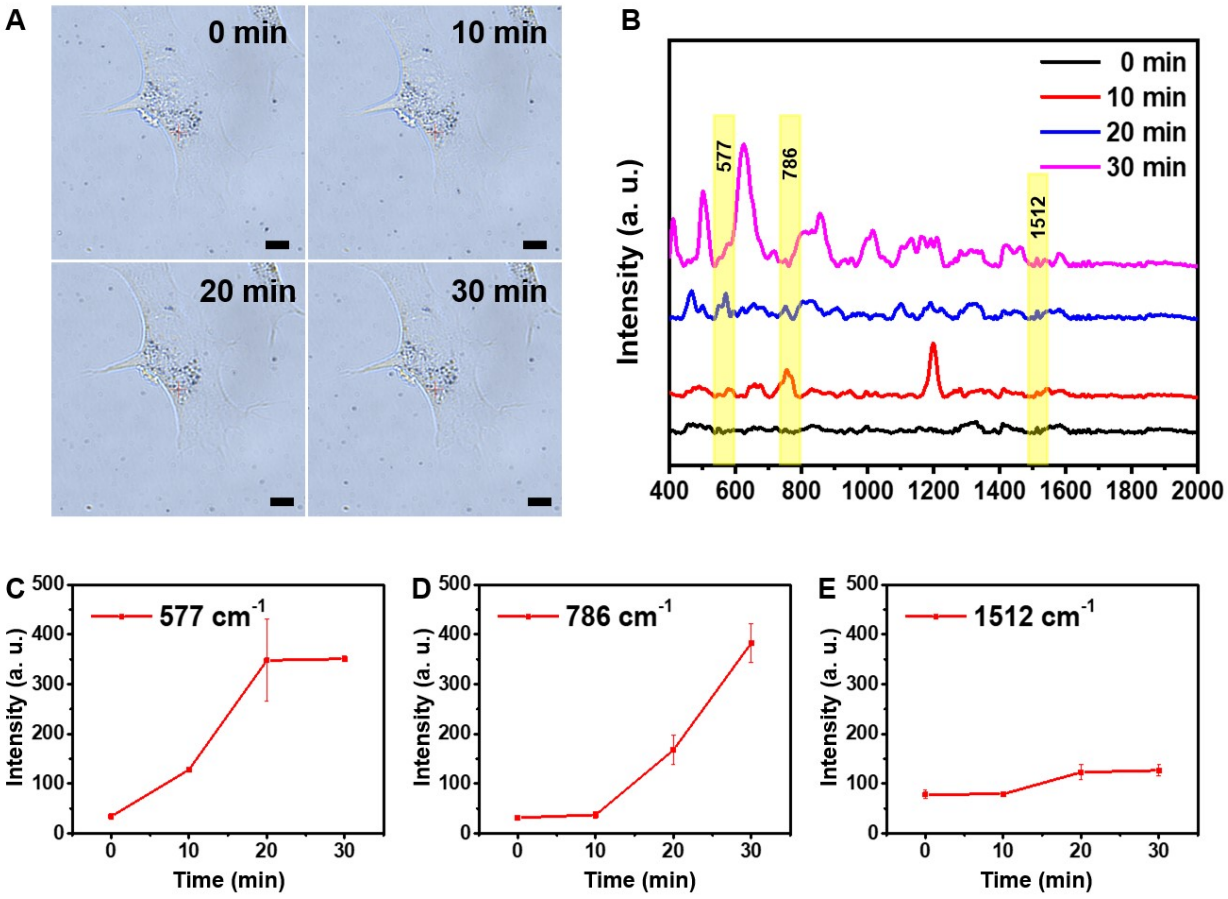

Figure S6. Raman signal changes in cell by photothermal damage. (A) Bright-field image of HeLa cells with AuNPs (scale bar = 20 μm). (B) Raman signal changes after 1 s of irradiation. (C) Raman signal intensity changes at 577 cm^{-1}, (D) 786 cm^{-1}, (E) 1512 cm^{-1} with time (0 min – 30 mn).

Reference

[1] K. Dodo, K. Fujita, M. Sodeoka, *J. Am. Chem. Soc*. 144 (2022) 19651-19667.