Electronic Supplementary Information

One-Step Synthesis of Silver Nanoshell with Bumps for Highly Sensitive Near-IR SERS Nanoprobes

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Experimental Details

***Materials*:** Tetraethylorthosilicate (TEOS), 3-mercaptopropyl trimethoxysilane (MPTS), ethylene glycol (EG), poly(vinyl pyrrolidone) (PVP, Mw ~40,000), silver nitrate ($AgNO_3$, 99.99+%), octylamine (OA), dimethylsulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 4-fluorothiophenol (4-FBT), 4-aminobenzenthiol (4-ABT), 4-chlorobenzenethiol (4-CBT), and 4-bromobenzenethiol (4-BBT) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. Methoxy poly(ethylene glycol) sulfhydryl (mPEG-SH) (Mw 5,000) was purchased from Sunbio (Anyang, Korea). Ammonium hydroxide (NH_4OH, 27%) and ethanol (98%) were purchased from Daejung (Busan, Korea). Deionized (DI) water was used for all the experiments.

***Preparation of bumpy Ag nanoshell (AgNS)*:** Tetraethylorthosilicate (TEOS, 1.6 mL) was dissolved in 40 mL of absolute ethanol, followed by addition of a 3 mL portion of aqueous ammonium hydroxide (27%). The resulting mixture was vigorously stirred using a magnetic bar for 20 h at 25 ^oC. The synthesized silica nanoparticles (NPs) were centrifuged and washed with ethanol several times to remove the excess reagents. These silica NPs were then

functionalized with the thiol group. Silica NPs (300 mg) were dispersed in 6 mL of ethanol containing 300 μL of MPTS and 60 μL of aqueous ammonium hydroxide (27%). The mixture was stirred for 12 h at 25 ^{o}C. The resulting MPTS-treated silica NPs were centrifuged and washed with ethanol several times. A 5 mg portion of PVP was mixed with 25 mL of ethylene glycol, followed by the addition of various amounts of MPTS-treated silica NPs. Then, a 25 mL portion of $AgNO_3$ solution (in ethylene glycol) was added to the silica NP solution and thoroughly mixed (final concentration of $AgNO_3$ was 3.5 mM). A 41.3 μL portion of octylamine (5 mM) was then rapidly added to the above solution. The resulting dispersion was stirred for 1 h at 25 ^{o}C. Afterwards, the particles were centrifuged and washed with ethanol several times for purification.

***Preparation of PEGylated Bumpy AgNS*:** A 1 mL portion of Raman label compound (2 mM in ethanol) was added to 1 mg of AgNS. The resulting dispersion was shaken for 1 h at 25 ^{o}C. The AgNSs, bearing adsorbed Raman label compound at their surface, were centrifuged and washed with ethanol several times. In order to improve the biocompatibility of AgNSs, their surface was grafted with PEG. A 1mL portion of mPEG-SH (2 mM in ethanol) was mixed with 1 mg of AgNS bearing Raman labels for 1 h, followed by centrifugation several times and resuspension in DI water.

***Cytotoxicity Study of NIR-SERS probe*:** A549 cells (adenocarcinomic human alveolar basal epithelial cells) were maintained in F-12 medium containing 10% fetal bovine serum (FBS) and 1% penicilline/streptomycin at 37 ^{o}C and 5% CO_2. After incubation for 18 h at 37 °C, the cellular toxicity was examined using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. A 20 μL MTT solution (5 mg/mL) was added to the wells where cells exist, and the cells were incubated for 4 h at 37 ^{o}C. The cells were treated

with 100 µL of dimethylsulfoxide and the absorbance at 540 nm was quantified using an ELISA reader (BioRad, Hercules, CA, USA).

***In Vivo Toxicity Studies of NIR-SERS Probe*:** A 400 µl of NIR-SERS probes (0.5 nM) dispersed in PBS (pH 7.0) containing 3% BSA were intravenously administered to the three mice with 50 mg kg^{-1} NIR-SERS probe. The other three mice were injected with 400 µl PBS and this group was treated as a control. Mice were anesthetized via an intraperitoneal injection of ketamine (50 mg kg^{-1}) and xylazine (2.5 mg kg^{-1}). NIR-SERS Probes were then injected in the tail vein. All mice were sacrificed at the same time after 3 day from NIR-SERS probe injection and SERS spectra were measured at major organ such as liver, spleen, kidney and heart in order to confirm SERS probe deposition at organ site. Then, 1.5 mL of whole blood was collected by heart puncture; 0.5 mL blood was collected in 10% EDTA for complete blood count (CBC) and the remaining 1 mL whole blood was collected and serum was separated for biochemistry panel assay. The test includes examining the level of various enzymes such as alanine aminotransferase (ALT), γ-glutamyl transferase (GGT), alkaline phosphatase (ALP), and protein such as albumin and bilirubin.

Cell Internalization and Cell-tracking In Vivo: A549 cells (6×10^5) in a Lab-tek glass chamber slide (Nalge Nunc International) were incubated for 24 h and mixed with the as-prepared NIR-SERS probe solution for 12 h at 37 ^oC. After washing the slide several times with PBS solution, the cells were fixed with paraformaldehyde (4%) for 10 min at 37 ^oC. The chamber slides were washed and dried for Raman measurements. For cell-tracking in vivo with SERS spectroscopy, the A549 cells (6×10^5) were incubated with the NIR-SERS $probe_{[4FBT]}$ (0.1 nM in PBS) for 18 h at 37 ^oC. After trypsinization, the harvested A549 cells containing the NIR-SERS $probe_{[4FBT]}$ (in 100 µL PBS) were subcutaneously implanted into a

gluteal region of a 8-week-old male nude mouse. This study was approved by the Seoul National University Institutional Animal Care and Use Committee (IACUC No. SNU-130423-2).

***Discrete Dipole Approximation (DDA) Calculation for Local Field Distribution of Bumpy AgNS*:** The electric field near AgNSs was calculated by the DDSCAT 7.1 package.[1] In our simulation, isotropic dipoles are evenly placed in a Ag shell at dipole–dipole distances of 3 nm. These nanoshells were 27 nm in thickness and 204 nm in diameter, and silica core was 150 nm in diameter. The bumpy nanostructures were assumed half sphere structures with 21 nm in radius. The bumpy AgNS has ~250000 dipoles. The incident radiation is along the *z* direction, with a y direction polarization. E-field distribution was calculated as $/E/E_0/^2$, where E is magnitude of the scattered electromagnetic field and E_0 is magnitude of the incident electromagnetic field.

***Raman Instrument*:** Raman measurement was conducted using a confocal microscope Raman system (LabRam 300, JY-Horiba, Edison, NJ, USA) equipped with an optical microscope (Olympus, Tokyo, Japan) and optical fiber coupled portable-Raman system (B&W TEK, i-Raman). In the micro Raman system, Raman scattering signals were collected in a back-scattering geometry and detected using a spectrometer equipped with a thermo-electrically cooled (−70 ^{o}C) CCD detector. The excitation laser was focused and the Raman signals were collected using a 100× objective lens (NA 0.90, Olympus). In the portable-Raman system, the Raman system is equipped with a diode laser emitting at 785 nm. The diffraction grating limits the spectral range to ~3200–175 cm^{-1} with a spectral resolution of 3 cm^{-1}. The maximal output power of the diode laser at the source is 300 mW.

***SERS Measurement*:** For single particle SERS mearsurement, the Raman label-adsorbed AgNS suspension (0.1 mg mL^{-1} in ethanol) was dropped on a patterned slide glass, and SERS spectra was measured by point-by-point mapping with a 1 μm step size. The mapping experiments were carried out using a ×100 objective lens (NA 0.90) with 785 nm photo-excitation of 28 μW laser power and a 10 s acquisition time. After the SERS measurement, the same area of Raman mapping was observed using field emission-scanning electron microscopy (JSM-6701F, JEOL, Tokyo, Japan) to ensure that there were only single-particles. For *in vivo* SERS measurement, each male mouse was anesthetized via an intraperitoneal injection of ketamine (50 mg kg^{-1}) and xylazine (2.5 mg kg^{-1}). SERS spectra were obtained from NIR-SERS $probe_{[4FBT]}$ labeled A549 cells injected site using the portable-Raman system with a 785 nm excitation of 90 mW laser power and 30 s acquisition time.

Calculation of the SERS enhancement factor: SERS enhancement factors (EF) for the NIR SERS $probes_{[4FBT]}$ can be calculated using the following equation:

$$EF = (I_{SERS} \times N_{normal})/(I_{normal} \times N_{SERS}) \quad \text{(eq. S1)},$$

where I_{SERS} and I_{normal} are the intensities of the bands from SERS and normal Raman scattering, and N_{normal} and N_{SERS} are the numbers of 4-FBT molecules in a pure form and self-assembled on the AgNS. The peak at 1075 cm^{-1} for 4-FBT was used to estimate the EF. Raman signal intensities were measured for both single-particles and neat 4-FBT, using an identical level of laser power for the EF calculation. The probing volume (18.8 μm^3) was approximated as a cylinder form with a diameter of 2 μm and a height of 6 μm for the normal Raman measurements. N_{SERS} was calculated by geometrical estimation of the particle's surface area (AgNS is assumed to be a complete spherical shape, r = 125 nm) and a molecular footprint of 4-FBT (0.383 nm^2/molecule), assuming the 4-FBTmolecules formed a complete monolayer.[3]

References

1. B. T. Draine, P. J. Flatau, 2010, “User Guide to the Discrete Dipole Approximation Code DDSCAT 7.1”, http:// http://arxiv.org/abs/1002.1505

2. W. M. Haynes, *Handbook of chemistry and physics*, CRC, 91st edn 2010–2011

3. P. Jiang, K. Deng, D. Fichou, S. S. Xie, A. Nion, C. Wang, *Langmuir* **2009**, 25, 5012.

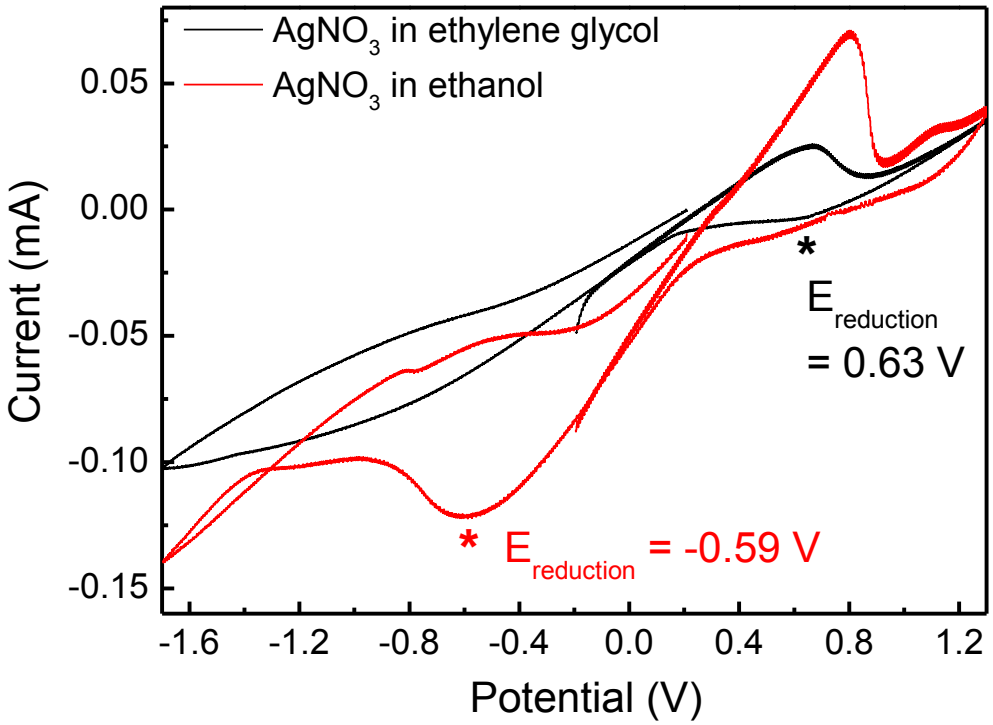

Figure S1. Cyclic voltammograms for comparison of reduction potential between $AgNO_3$ in ethylene glycol and in ethanol. Experimental condition; 10mM concentration of $AgNO_3$, Au working electrode, Pt counter electrode, Ag/AgCl reference electrode, scan range from −1.7 to 1.3 V, scan rate of 50 mV s^{-1}.

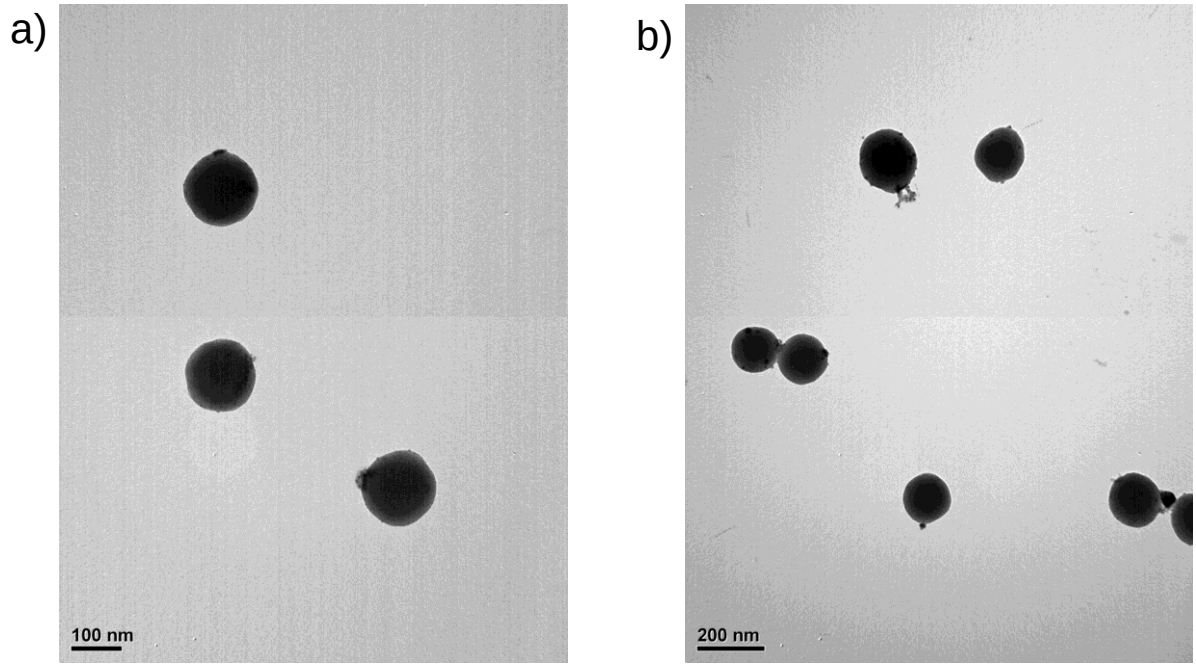

Figure S2. TEM images of silica nanoparticles after treating with $AgNO_3$ and octylamine in (a) ethanol, and (b) ethylene glycol monoethyl ether for 1h at room temperature.

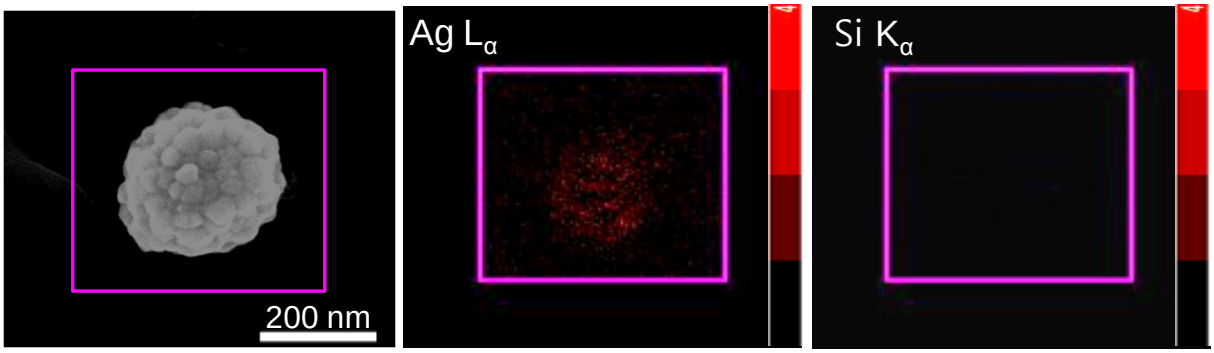

Figure S3. Elemental mapping images of bumpy AgNS by EDX analysis.

Table S1. Size and Silver shell Thickness of AgNS as a Function of Weight Ratio of $AgNO_3$ to Silica Nanoparticles [$AgNO_3$/SiNP]. (mean ± S.D., n = 30)

[$AgNO_3$/SiNP]	Size of silver shell (nm)	Shell Thickness (nm)
7.5	214 ± 16	~32
10	228 ± 16	~39
15	251 ± 16	~51
30	301 ± 17	~76

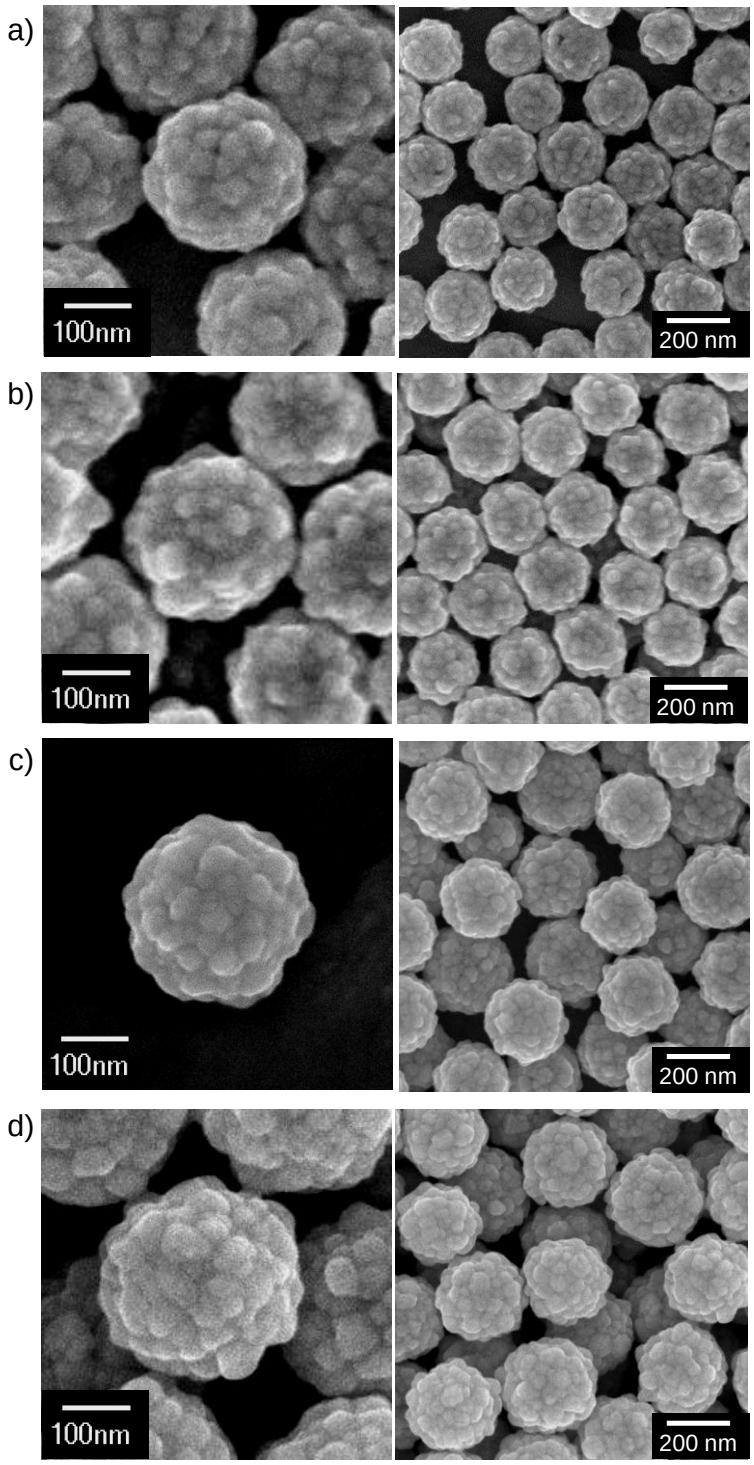

Figure S4. SEM images showing the size and surface morphology of bumpy AgNSs that were synthesized at different weight ratios of $AgNO_3$ to SiNP. High magnification (left) and low magnification (right); Weight ratios of $AgNO_3$ to SiNP are a) 7.5, b) 10, c) 15, and d) 30, respectively.

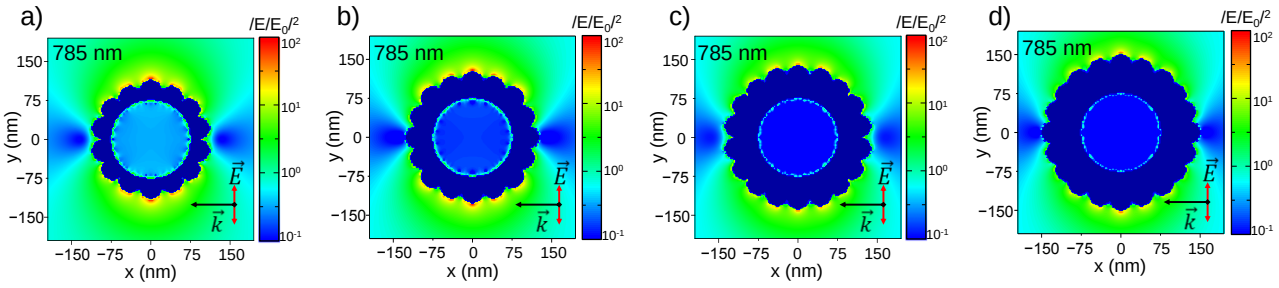

Figure S5. Calculated E-field distributions and surface E-field enhancement values of bumpy AgNSs with a) 18, b) 27, c) 39, and d) 54 nm of inner shell thicknesses under 785 nm excitation laser lines using discrete dipole approximation (DDA). Diameter of silica core is 150 nm. The outer bump-nanostructures were assumed to have half sphere structures with a 21 nm radii.

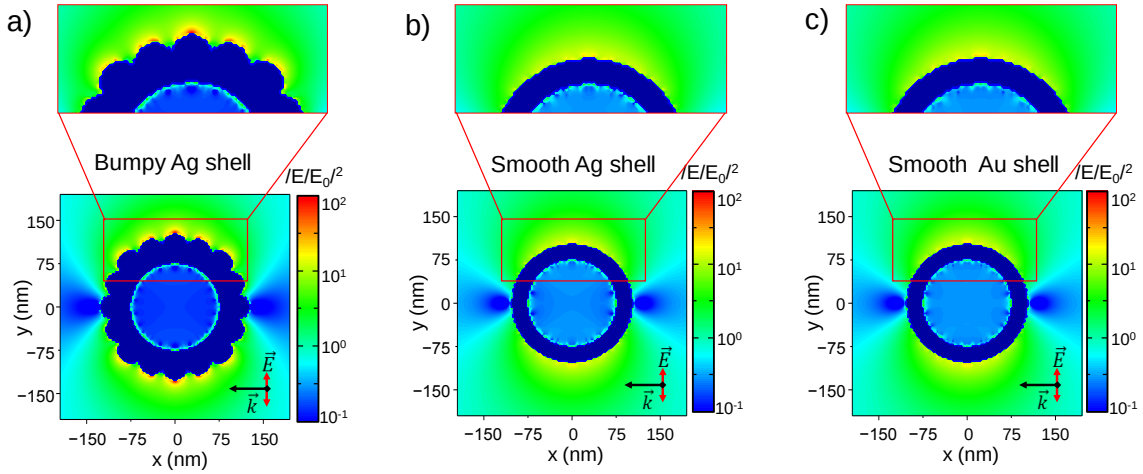

Figure S6. Calculated local electric field distribution of a) bumpy AgNS-10, b) smooth Ag shell, and c) smooth Au shell under 785 nm excitation laser lines using discrete dipole approximation (DDA). These nanoshells were 27 nm in thickness and 204 nm in diameter, and silica core was 150 nm in diameter. The bumpy nanostructures were assumed half sphere structures with 21 nm in radius.

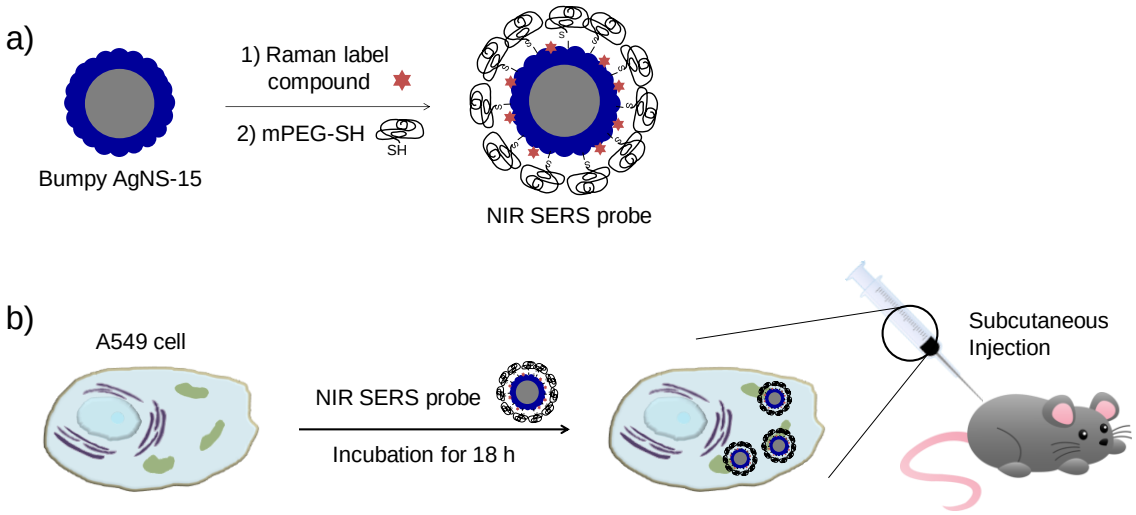

Figure S7. Schematic illustration for a) SERS coding and PEGylation of bumpy AgNS for NIR-SERS probe coded with 4-FBT, denoted as NIR-SERS probes$_{[4FBT]}$, and b) cellular uptake of NIR-SERS probes$_{[4FBT]}$ and subcutaneous injection of the cell suspension into a mouse.

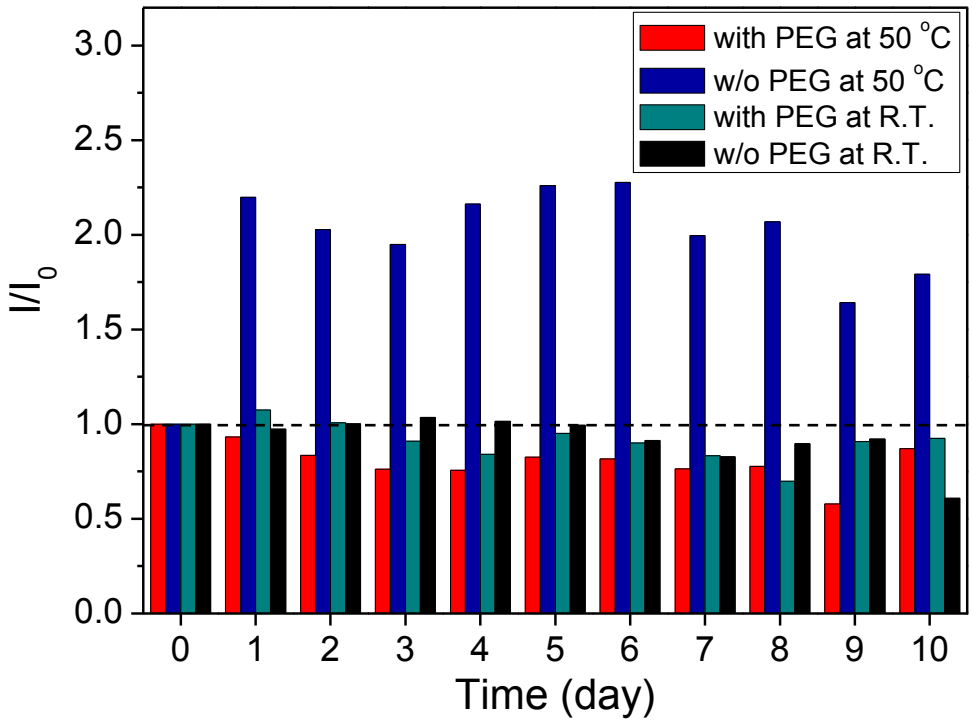

Figure S8. SERS signal intensities from various conditions of AgNSs; AgNSs$_{[4FBT]}$ stored at 50 °C with PEG ligands (red) and without PEG ligands (blue). And, AgNS$_{[4FBT]}$ stored at room-temperature with PEG ligands (dark cyan) and without PEG ligands (black).

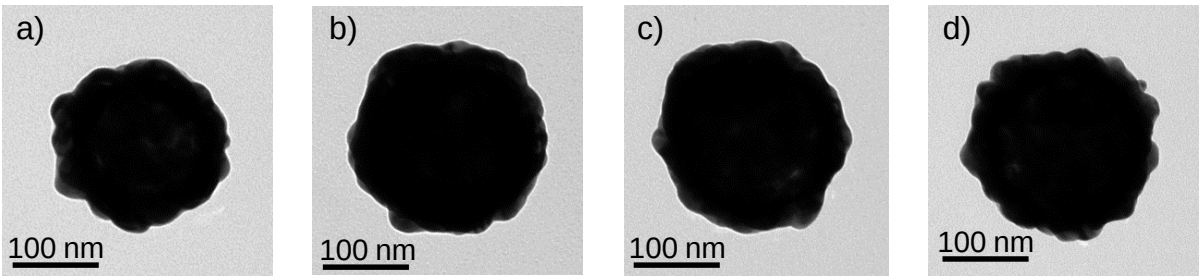

Figure S9. TEM images of AgNSs after 9 days storing; a) $AgNS_{[4FBT]}$ stored at 50 ^{o}C with PEG ligands and b) without PEG ligands. c) $AgNS_{[4FBT]}$ stored at room temperature with PEG ligands and d) without PEG ligands.

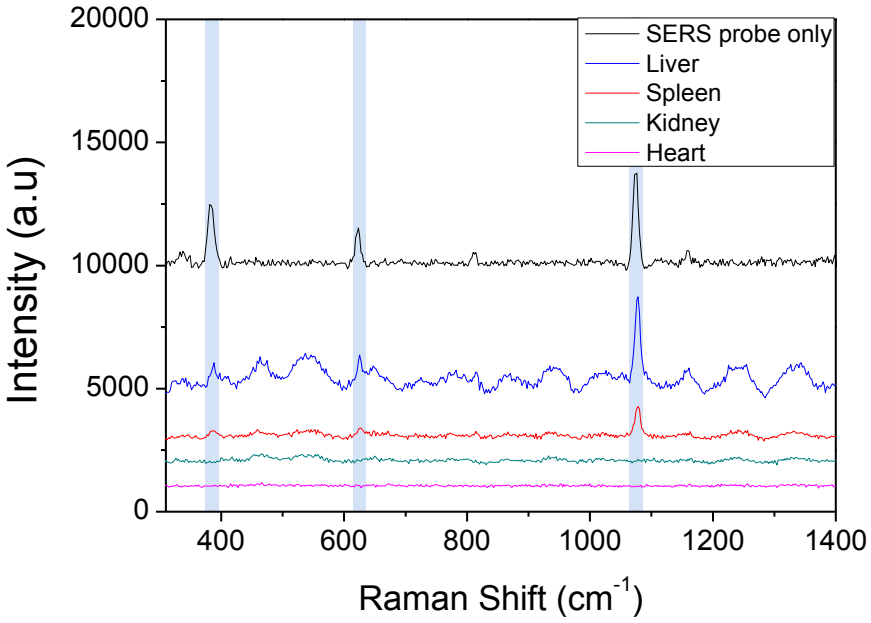

Figure S10. Representative Raman spectra from mouse major organs (liver, spleen, kidney, and heart) after tail vein injection of NIR-SERS $probe_{[4FBT]}$ (PEGylated $AgNS_{[4FBT]}$) (dose=50 mg/kg).

Table S2. Hematology Results from Animals Treated with NIR SERS Probes (mean±S.D., *n* = 3)

	White blood cells (Counts/μL)	Hemoglobin (g/dL)	Platelets (Counts/μL)
Control	4600±1800	12.0±1.2	675700±236400
50 mg/kg[a] treatment	5600±1900	10.9±0.9	564300±201800

[a]Concentration based on Ag weight in NIR-SERS Probes.

Table S3. Liver Function Results from Mice Treated with NIR-SERS Probes (mean±S.D., *n* = 3)

	Alkaline phosphatase (U/L)	Alanine aminotransferase (U/L)	Total bilirubin (mg/dL)	Albumin (g/dL)	Cholesterol (mg/dL)	γ-Glutamyl transferase (U/L)
Control	61.7±14.2	46.7±1.5	0.3±0	3.1±0.06	97.0±11.5	<5
50 mg/kg[a] treatment	81.7±5.9	49.3±2.4	0.3±0	2.7±0.15	96.3±11.6	<5

[a]Concentration based on Ag weight in NIR-SERS Probes.

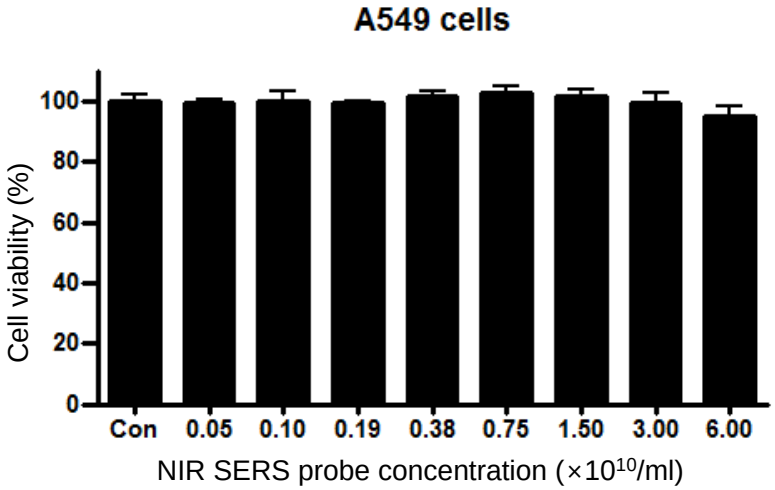

Figure S11. Cytotoxicity of NIR SERS probes using MTT assay. The MTT assay was performed against A549 cells at 37 ºC as a function of NIR SERS probe concentrations (100 μL) after incubation of NIR-SERS probes for 18 h. (mean ± S.E., n = 3)