Electronic Supplementary Material (ESI) for Analyst.

Supporting information

Graphene Oxide-Encoded Ag Nanoshells with Single-Particle Detection Sensitivity towards Cancer Cell Imaging based on SERRS

DaBin Yim,[a] Homan Kang,[b] Su-Ji Jeon,[a] Hye-In Kim,[a] Jin-Kyoung Yang,[c] Tae Wook Kang,[a] Sangyeop Lee,[d] Jaebum Choo,[d] Yoon-Sik Lee,[b,c] Jin Woong Kim[e,f,*] and Jong-Ho Kim[a,*]

[a] Department of Chemical Engineering, Hanyang University, Ansan 426-791, Republic of Korea

[b] Interdisciplinary Program in Nano-Science and Technology, Seoul National University, Seoul, 151-744, Republic of Korea

[c] School of Chemical and Biological Engineering, Seoul National University, Seoul, 151-744, Republic of Korea

[d] Department of Bionano Engineering, Hanyang University, Ansan 426-791, Republic of Korea

[e] Department of Applied Chemistry, Hanyang University, Ansan, 426-791, Republic of Korea

[f] Department of Bionano Technology, Hanyang University, Ansan 426-791, Republic of Korea

* To whom correspondence should be addressed: kjh75@hanyang.ac.kr and kjwoong@hanyang.ac.kr

Materials.

Graphite flake, sodium nitrate ($NaNO_3$), potassium permanganate ($KMnO_4$), sodium hydroxide (NaOH), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), tetraethyl orthosillicate (TEOS), polyvinylpyrrolidone (PVP), silver nitrate ($AgNO_3$), (3-mercaptopropyl)trimethoxysilane (MPTS), octylamine, (3-aminopropyl)trimethoxysilane (APTS), 3-mercaptopropionic acid (MPA), 4,7,10-trioxa-1,13-tridecanediamine (TEG), ethylenediamine (EDA) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without purification. Hydrochloric acid (HCl), hydrogen peroxide (H_2O_2), sulfuric acid (H_2SO_4) and ethanol (EtOH) were purchased from Dae-Jung Chemicals (Busan, Korea). Absolute ethanol was purchased from Carlo Erba Reagents (Milan, Italy) and dimethyl sulfoxide (DMSO) to kill the cells was purchased from AMRESCO (Solon, OH, USA). Centrifugal device (Amicon Ultra-15, MWCO: 10,000) and dialysis membrane (spectra/Por®, MWCO: 6-8,000) were provided by Millipore (Billerica, MA, USA) and Spectrum® Laboratories (Rancho Dominguez, CA, USA) respectively. MCF-7 cells were provided by Korean Cell Line Bank (Seoul, Korea). Roswell Park Memorial Institute (RPMI) 1640 (1X) with L-glutamine and 25 mM HEPES, PBS buffer, 50 U ml^{-1} penicillin, heat inactivated fetal bovine serum (FBS) and 0.25% trypsin-0.53% mM EDTA (0.25% TE) were purchased from Gibco® (Carlsbad, CA, USA). Cell Counting Kit-8 (CCK-8) was purchased from Dojindo (Japan).

Characterization

The UV/Vis absorption spectra of GO and its derivatives were measured using a UV/Vis spectrometer (UV-2600, Shimadzu, Japan). The morphology and size of GO and TEG-GO were characterized by atomic force microscope (AFM, XE-100, Park systems, Korea). For

AFM analysis of GO, a silicon wafer was treated with a 20 μL of APTS in 2 mL of EtOH at 35 oC for 90 min to introduce a positive charge. Then, the APTS-treated silicon wafer was dipped into the GO solution for 15 min, and washed with water several times. TEG-GO was placed on a bare silicon wafer. The FT-IR spectra for GO and its derivatives were determined by Attenuated Total Reflectance (ATR, Nicolet, Thermo Scientific Inc.).

The images of SiNPs, AgNSs, GO-SiNPs and GONSs were acquired by field emission transmission electron microscope (FE-TEM 200kV with EDS, JEM-2100F, JEOL, Germany), and GO-SiNPs and GONSs were analyzed by field emission scanning electron microscope (FE-SEM, JSM-6700F, JEOL, Germany). For TEM analysis, a 10 μg/mL of each nanomaterial (SiNPs, AgNSs, GO-SiNPs or GONSs) was dropped on a TEM grid, and dried under atmosphere. For SEM analysis in single particle of SERRS measurement, a slide glass was marked with signs indicating a position and washed with EtOH twice. Then, GO-SiNPs and GONSs (10 μg/mL) in EtOH were dropped on the slide glass with a position sign. The size distribution of SiNPs and AgNSs and their surface charge were measured by zeta-potential & particle size analyzer (ELS-Z, Otsuka electronics co., Japan).

The Raman spectra were obtained by a Raman microscope (RM 1000, Renishaw, UK) and a confocal Raman microscope (Alpha 300R+, WITec, Germany). For Raman measurement, the same procedure as the one for SEM analysis was applied. A slide glass was marked with signs indicating a position and washed with EtOH twice. Then, GO-SiNPs and GONSs (10 μg/mL) in EtOH were dropped on the slide glass with position signs. The Raman images of GO-SiNPs and GONSs were taken under a 0.2 mW power of laser (633 nm) for 1 sec.

The result of CCK-8 assay was measured by a microplate reader (MQX 200, BioTek Instruments, Winooski, VT, USA).

Preparation of Graphene Oxide (GO)

In order to synthesize GO, a modified Hummers' method was used[21]. A 0.375 g portion of $NaNO_3$ was slowly added to 17 mL of H_2SO_4. A 0.5 g portion of graphite flake was added to the solution in an ice bath (at 0 ^oC). Then, a 2.5 g of $KMnO_4$ was slowly added to the graphite flake mixture in an ice bath. After 10 min, the temperature increased to 35 ^oC, and the mixture solution was vigorously stirred further for 2 h. A 30 mL portion of distilled water (DI water) was slowly added to the reaction mixture in an ice bath. The resulting mixture was stirred further for 2h at room temperature. A 2 mL of H_2O_2 was slowly added in an ice bath for quenching the reaction, and stirred vigorously at room temperature until the gas was not generated. The mixture was washed with a 10% (vol/vol) HCl aqueous solution (250 mL) and DI water several times. To completely wash the graphite oxide flakes, the resulting graphite oxide was dissolved in water again, and a NaOH solution was added until the pH of the solution reaches 7.4, and washed more by centrifugation several times. The mixture was then filtered by a cellulose acetate membrane filter. The graphite flakes were then sonicated in water (15 mL) with 13 W for 1.5 h in an ice bath. The resulting dispersion was finally centrifuged at 10000 rpm for 1.5 h to collect a GO solution from a supernatant.

Preparation of GO-encoded SiNPs (GO-SiNPs)

In order to encapsulate SiNPs with GO, 1 mL of SiNPs (100 μg/mL, in ethanol) were treated with 2 mM of (3-aminopropyl)trimethoxysilane (APTS) for 2 h, and then the obtained particles were washed with ethanol several times. Then, 250 μL of GO (30 μg/mL) was added to the solution of APTS-SiNPs, and the resulting dispersion was gently shaken for 1 min. The mixture was centrifuged to remove GO unadsorbed on the surface of APTS-SiNPs. The process from GO adsorption to centrifugation was repeated 20 times.

Supplementary Figures:

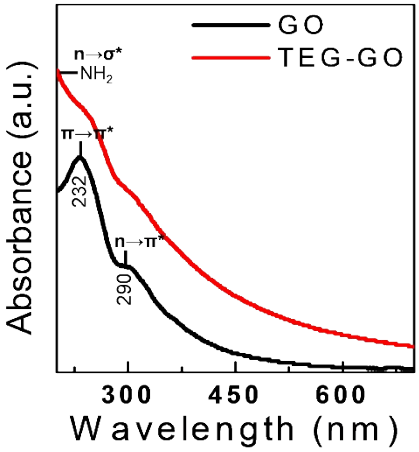

Figure S1. UV-Vis spectra of GO and TEG-GO.

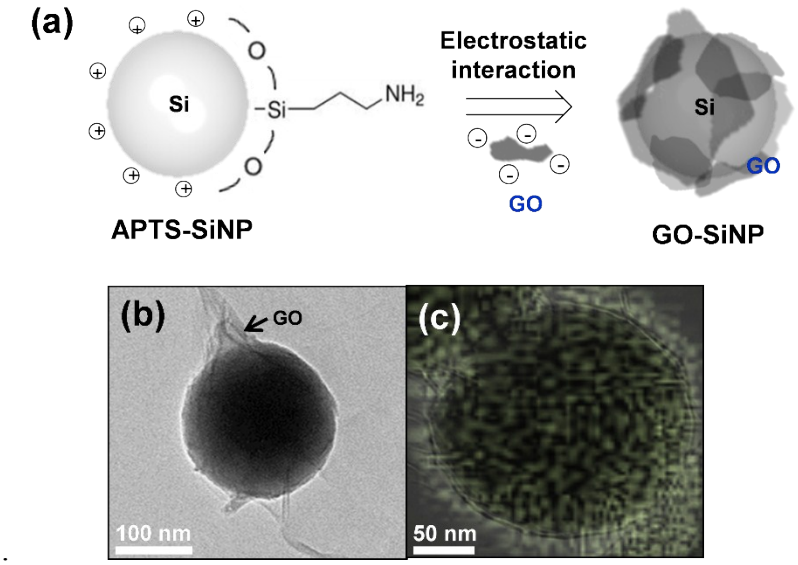

Figure S2. (a) Schematic illustration for the synthesis of GO-encapsulated silica nanoparticle (GO-SiNP), (b) TEM image and (c) carbon atomic mapping of GO-SiNP using EDX. A green color indicates carbon atoms.

$$EF = \frac{I_{SERS}}{I_{NR}} \times \frac{N_{NR}}{N_{SERS}} = \frac{I_{SERS}}{I_{NR}} \times \frac{M_{NR}}{M_{SERS}} = \frac{6211.5}{1416.8} \times \frac{25.909}{23.346}$$

$$= 4.3842 \times 1.1098 = 4.8656$$

I_{SERS} = the intensity of surface-enhanced Raman scattering
I_{NR} = the intensity of normal Raman scattering
N_{SERS} = number of molecules used in the SERS measurement
N_{NR} = number of molecules used in the normal Raman
M_{SERS} = mass of Raman reporter used in the SERS measurement
M_{NR} = mass of Raman reporter used in the normal Raman

Figure S3. Enhancement factor of GONS, compared to GO-SiNPs.

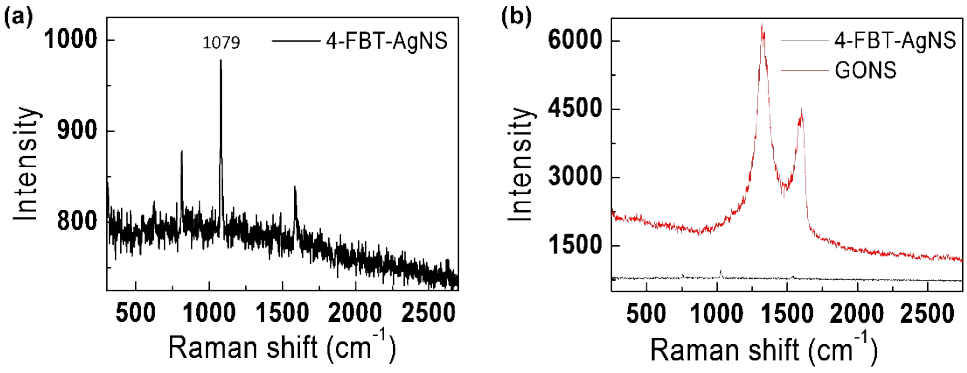

Figure S4. Raman spectra of (a) 4-FBT-AgNS and (b) GONS.

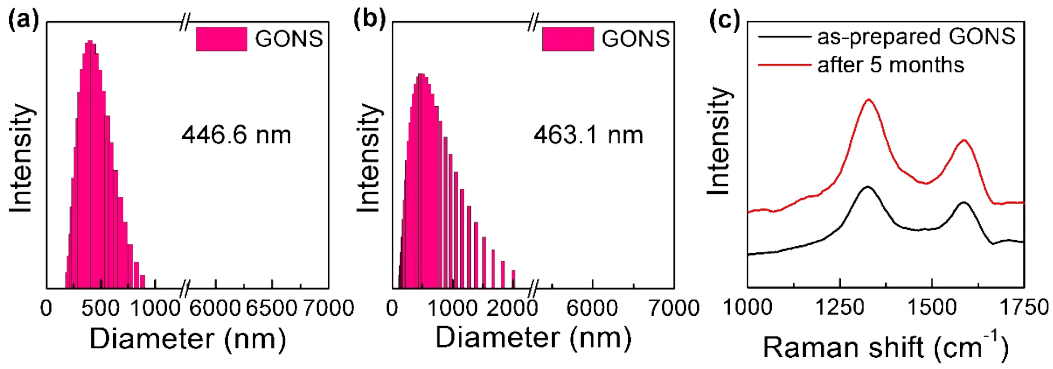

Figure S5. Stability of GONS in terms of its size and Raman signals as a function of storage time. Size distribution of (a) as-prepared GONS, (b) after 5 weeks measured by DLS. (c) Raman spectra of as-prepared GONS and the one after 5 months, showing the same pattern of peaks with similar intensity.

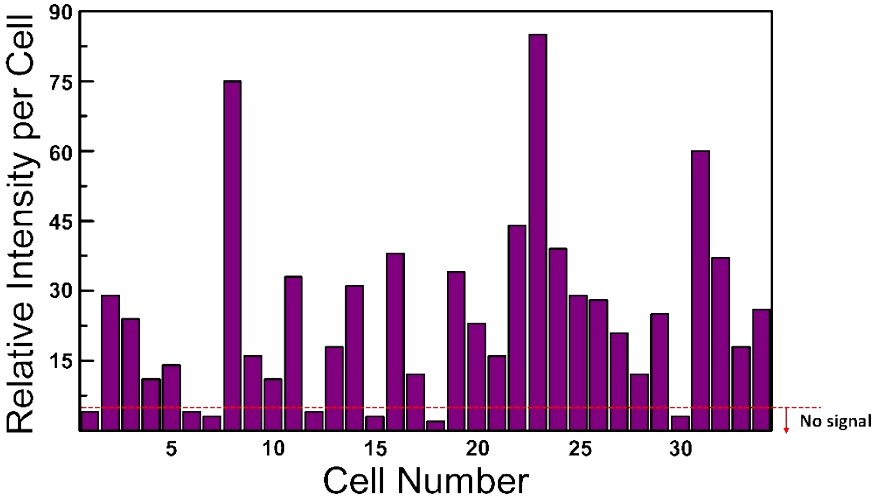

Figure S6. Ensemble SERRS signal intensity of GONSs per cell for thirty-four different MCF-7 cells treated with the GONS nanoprobes.