Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

SERS detection and boron delivery to cancer cells using carborane labelled nanoparticles

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Experimental Section

Materials: Dithiobis(succinimidyl)propionate (DTSP) was purchased from Aldrich. 1-thiocarborane[1] and EG_3SH[2] were prepared according literature procedures. A 2 mM solution of DTSP in DMF and 2 mM solutions CBT and of EG_3SH in MeOH were prepared. Silver NPs (~25 nm in diameter) were synthesized with modified Lee and Meisel protocol[3]. Briefly, a solution of $AgNO_3$ (50.0 ml, 1.0 mM) in 18.2 MΩ deionized water was heated to boil under reflex. A 1.0 ml of 51.0 mM sodium citrate solution was added to the boiling $AgNO_3$ solution. The colour of the solution slowly turned into grayish-yellow. The solution was refluxed at boiling condition for another 60 minutes. The Ag sol was cooled to room temperature before storage at 4°C.

Functionalization of Nanoparticles: To 1 mL of Ag particles (~10^{13}/mL) in a glass vial (plastic tubes resulted in particle precipitation during the ligand exchange process) was added 1 μL of 2mM DTSP, 6 μL of 2mM CBT and 6 μL of 2mM EG_3SH. The resulting solution was left stirring for 4 hours. To this solution, 50 μL of the secondary antibody solution (Affinipure goat anti-rabbit IgG (H+L) 2.4 mg/mL (Jackson Immunoresearch)) was added and the resulting solution mixed by pipetting it slowly several times. This solution was left in the fridge at 4°C overnight (16 hrs). After 16 hrs, the solution was warmed to rt and 30 μL of 30% bovine serum albumin (BSA) (Sigma) was added. This solution was mixed with a pipette and left to stand for 30 min. at rt. After 30 min., the solution was transferred to a 1.5 mL Eppendorf micro-centrifuge tube and spun at 13.4 k rpm for 20 min to pellet the particles. The supernatant was then removed and the particles resuspended in 500 μL of PBS. Attempts to pellet and resuspend particles either without BSA blocking or prior to antibody treatment were not successful. The resulting solutions were determined to be approximately 1 nM by UV-Vis and were stored at 4°C. Samples were stable for over one month under these conditions. NP solutions could be collected after use and used at least 5 times before a decrease in effectiveness of labelling was observed.

Cell Culture and Sample Preparation: Huh7.5 cells (ATCC, Manassas, VA) were grown in Dulbecco's modified Eagle's medium (Invitrogen, Burlington, ON) supplemented with 10% fetal bovine serum (FBS) (NorthBio, Toronto, ON) under standard culture conditions (37 °C, 5% CO_2) and were plated onto silica wafers in a 12-well plate. After 24 hrs, cells were fixed and rinsed with PBS. To this, anti-EGFR antibody (AB) (EGFR (1005) rabbit polyclonal IgG (Santa Cruz Biotechnology) 0.2 mg/mL, 4 uL used per mL PBS) was added, and the cells stored in the fridge with the AB for 24 hrs at 4 °C. Cells were then rinsed 3 times with PBS and then the 1mL NP solution in PBS was used to cover the cells after the final wash. This cells were then stored again for 24 hrs at 4 °C with the NPs. After 24 hrs, the NP solution was recovered, and cells were again rinsed with 3 times with PBS. The cells were then stored at 4 °C in PBS until imaged.

Raman Imaging and Spectroscopy: Raman spectroscopy and microscopy were acquired with a commercial microRaman system (LabRAM HR, Horiba Jobin Yvon) equipped with a software controlled XYZ stage and a thermal-electric cooled CCD detector. In typical SERS experiments, samples were excited with 632.8 nm radiation at a power density of ~ 10^4 W/cm^2. Incident radiation was coupled into an Olympus BX51 optical microscope and focused to ~ 1 μm diameter spot through a 100X objective. The same objective also collects the retro-reflected radiation and guides it to a notch filter which removes the Rayleigh radiation. In the Raman mapping experiments, a fine set of grid points within an area of interest is defined in the software and imaged by raster the sample under the tightly focused laser beam. At each of the grid point, a full Raman spectrum was acquired. For figure 2, the SERS images were generated with 1 second acquisition time (2 accumulations) with a

power density of 10^4 W/cm^2. The multiple accumulations are necessary for the spike removal algorithm to function. Upon completion of the mapping, Raman intensity map of the B-H vibrational mode is regenerated by fitting and removing the associated background for each spectrum in the predefined spatial grid. The B-H intensity is displayed as a thermal map as shown in Fig. 2a. This is achieved by the Labspec 5.25 software (Horiba Jobin Yvon). The solid CBT Raman spectrum (Fig. 1, red trace) was acquired by 632.8 nm irradiation at a power density of ~ 10^5 W/cm^2.

Scanning Electron Microscopy: Scanning electron microscopy was performed on cells plated on Si substrate with a Hitachi S-4700 field-emission scanning electron microscope. Samples were imaged with an acceleration voltage of 3 KeV and at a working distance of 12 mm. The high resolution SEM image of the whole cell shown in Fig. 2a was generated from 17 individual high resolution SEM images, each acquired at a 7000X magnification. Stitching was performed manually in the image processing software.

Two-Photon Luminescence Imaging: The two-photon luminescence microscopy system uses a single femtosecond Ti:sapphire oscillator (Spectra Physics Tsunami operating at 80 MHz) as the excitation source (800 nm, 15 mW average power). A modified Olympus Fluoview 300 laser scanning system and IX71 inverted microscope was used to carry out the imaging. A 40X 1.15 NA UAPO water immersion lens with a cover slip collection was used as the objective. Light was directed to photomultiplier tubes (PMT) (Hamamatsu R3896). Two-photon luminescence images were collected on fixed cell samples in PBS in 4.2cm^2 Lab-Tek Chambers Slide System (NUNC, Rochester, NY). Bright filed images were collected simultaneously in order to define the border outlines of cells.

Additional Imaging Data:

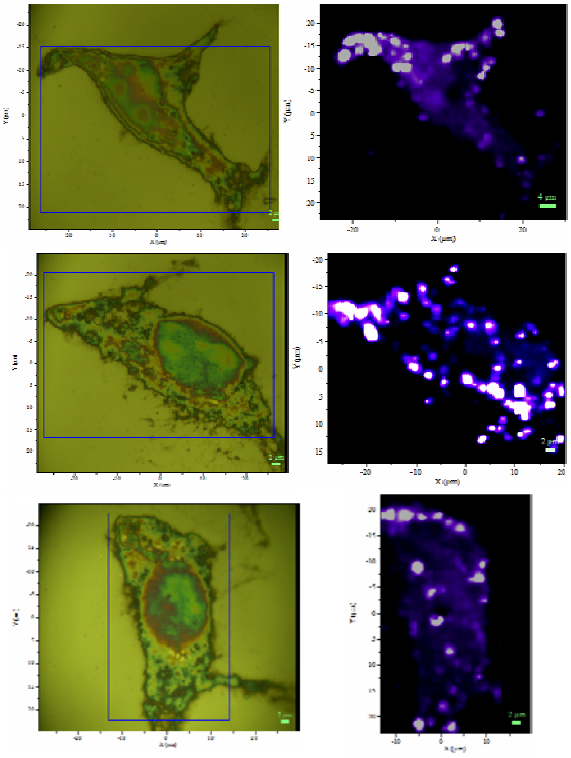

Figure S1. Additional SERS images of MDA-MB-435s cells with corresponding bright-field images to correlate the signal to the cell. Huh 7.5 cells were selected for the main body text as washing salt from them was more efficient in order to produce a high quality SEM image for overlaying with a SERS spectrum.

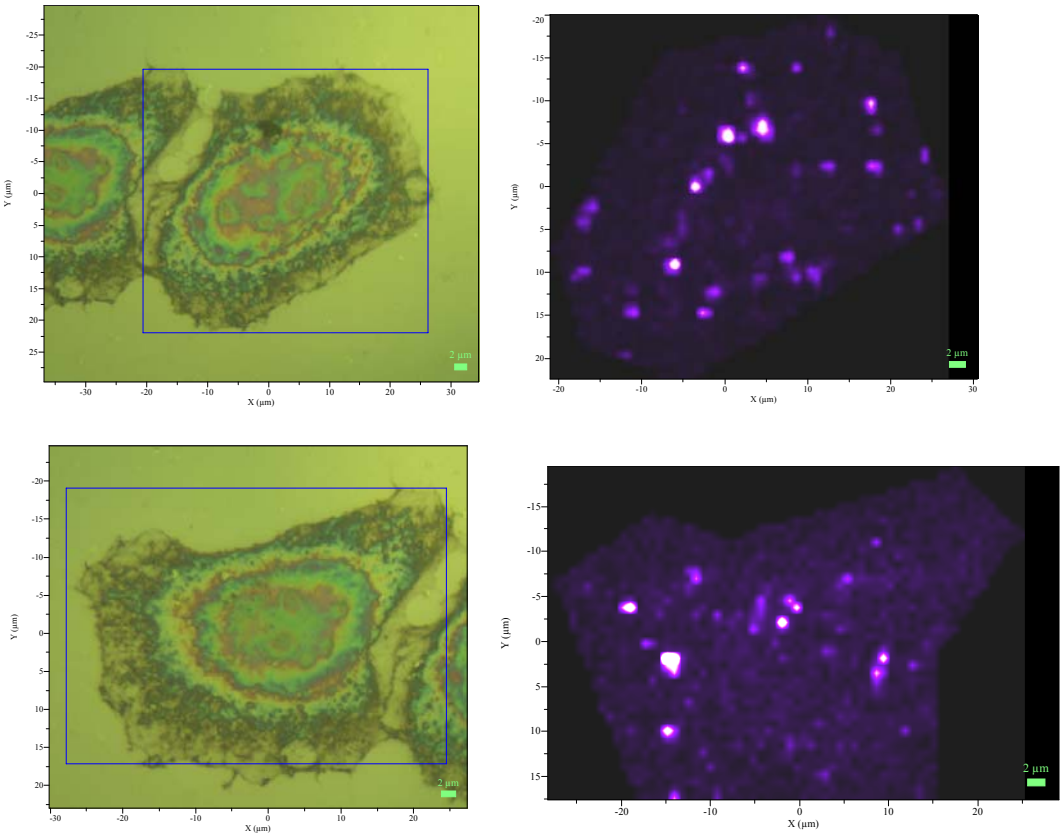

Figure S2. Additional SERS images of Huh7.5 cells but using a different anti-EGFR antibody, here using a monocloncal anti-EGFR antibody (Sigma, clone 29.1).

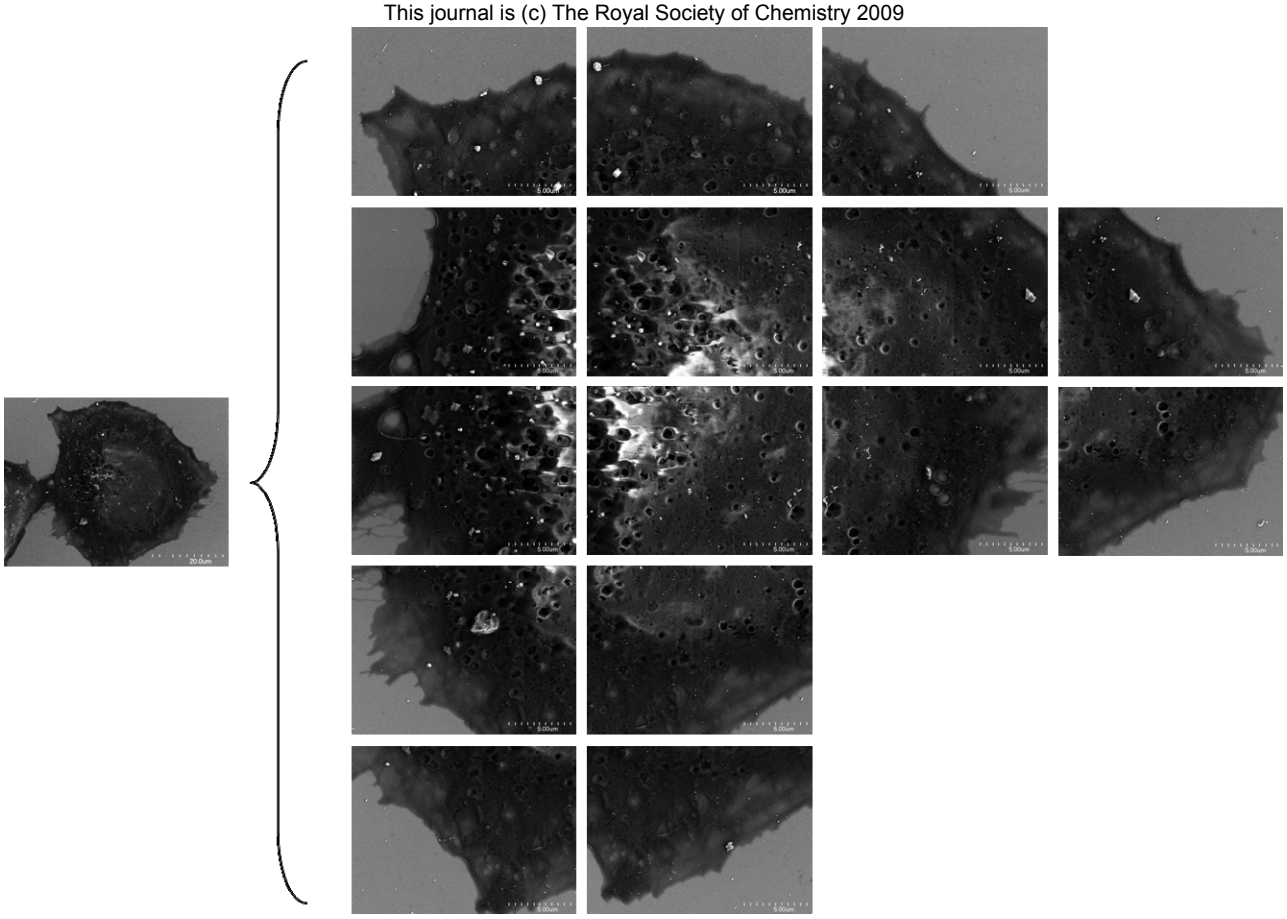

Figure S3. 15 High resolution SEM images that were used to compile the total cell high resolution SEM image seen in Figure 2 of the manuscript.

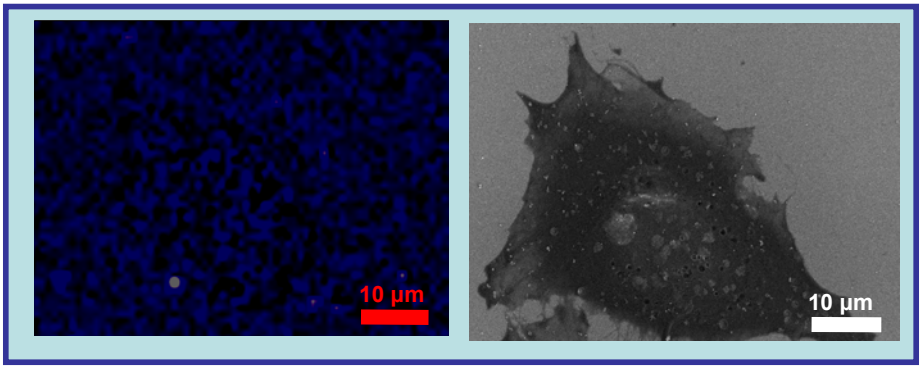

Figure S4. SERS intensity map (left) and SEM image (right) of a cell treated with functionalized NPs but no primary antibody showing that these particles are specific for cells labelled with the anti-EGFR antibody.

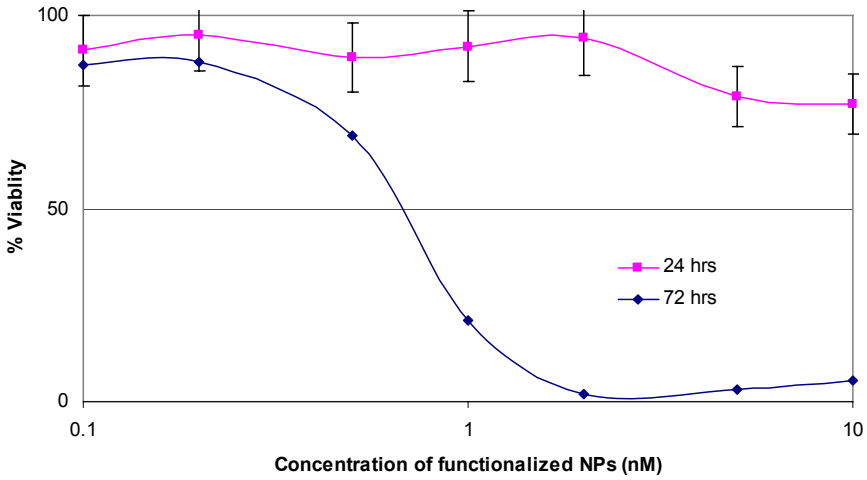

Figure S5. MTT assay performed on Huh7.5 cells with treatment of functionalized NPs spanning in concentration from 10 to 0.1 nM. Cells were incubated with the NPs for 24 hrs or 72 hrs before the addition of MTT. Details for this protocol have been previously published.[4] The NPs were not found to be toxic over this concentration at 24 hrs; however, concentrations of 1nM and above were toxic over 72 hrs.

References:

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(3) Lee, P. C.; Meisel, D. *J. Phys. Chem.* **1982,** *86*, 3391-3395.
(4) Kennedy, D. C.; Lyn, R. K.; Pezacki, J. P. *J. Am. Chem. Soc.* **2009,** *131*, 2444-2445.