

## Supporting Information

### Experimental

*Microfluidic device preparation:* SU-8 masters were generated using soft lithographic techniques, as previously reported (21,22). Microfluidic devices were cast in polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning) against these masters using an elastomer to curing agent ratio of 10:1 (w/w) and thermally cured for 2 h at 70 °C. After release, PDMS structures were irreversibly bonded to glass coverslips using plasma oxidation for 30 s and a power of 50 W. Devices were sterilized/cleaned immediately prior to use by immersion for 10 min in a sonic bath containing methanol, followed by washing in sterile culture media. Gas-tight syringes (Hamilton) were fitted to 50  $\mu\text{m}$  inner diameter PEEK (poly-ether-ether-ketone) tubing (VICI Precision Sampling, Inc.) and controlled using microsyringe pumps (Harvard Apparatus).

*Raman measurement:* All Raman spectra were acquired with a LabRam inverted microscope spectrometer, (Jobin Yvon Ltd). The spectrometer was equipped with a Torus single-frequency DPSS 532 nm continuous wave laser (Laser Quantum), true confocal optics (with  $\sim 2\text{ }\mu\text{m}$  depth resolution (z)), a holographic transmission grating and a Synapse CCD detector (1,024 $\times$ 256 pixels, Horiba Jobin-Yvon). The instrument includes a precision motorized x,y,z sample stage for automated mapping at spatial resolution of 0.5  $\mu\text{m}$  in the x,y plane and an extensive software support (LabSpec 5) for data processing.

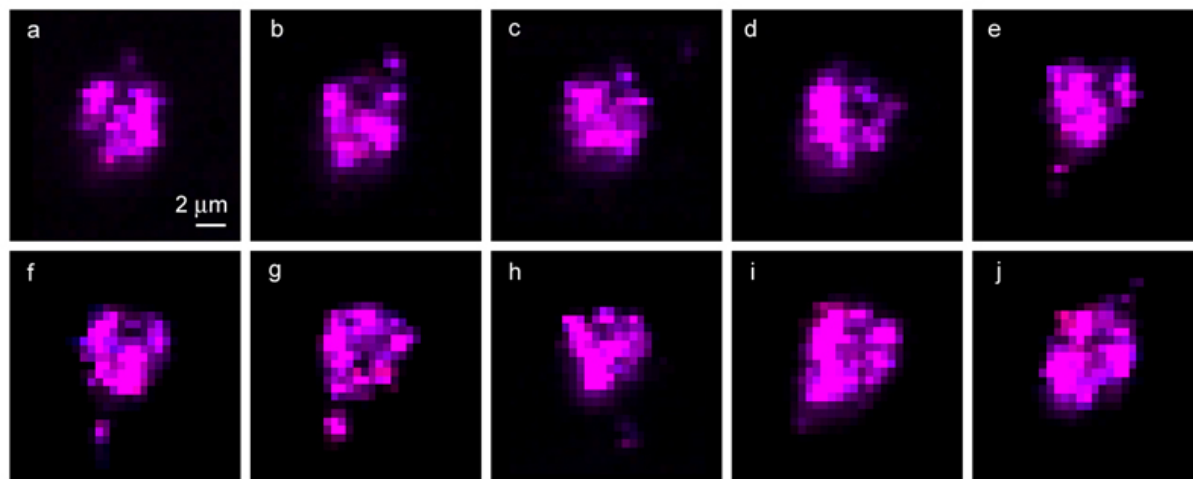
In this study, a 100x (N.A. = 0.75) PL Fluotar objective lens (Leica, Germany). a grating (with 600 grooves per mm), a confocal aperture of 100  $\mu\text{m}$  and an entrance slit of 150  $\mu\text{m}$  were used for all of the experiments. The Raman spectrometer wavelength range was calibrated using the centre frequency of the silicon band from a native silicon sample (520.2  $\text{cm}^{-1}$ ).

## Materials and Methods

*Cell Culture:* CHO cells were obtained from ATCC/LGC Promochem (ATCC® No. CCL-61™, LGC Promochem, UK). The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen Ltd ) without L-glutamine which was supplemented with foetal bovine serum (FBS, Invitrogen Ltd) to a final concentration of 10%, and L-glutamine to a final concentration of 4 mM. Cells (~0.5 ml of ca. 4 million cells/mL) were seeded in Nunclon™ flasks before 5.0 ml of DMEM culture media was added. They were incubated in a humidified environment at 5% CO<sub>2</sub> and at 37 °C. At harvesting, media was removed and cells were washed with PBS solution (8.1 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 138 mM NaCl, 2.7 mM KCL, pH 7.4), before being detached with ~0.5 ml trypsin-EDTA solution (Invitrogen). To stop the trypsin reaction, ~2 ml of DMEM media was added.

*Cellular uptake of nanoparticles and acquisition of SERS data:* Cells were harvested and labelled colloid was added to the cell suspension (~ 100 µl ml silver colloid solution per 1 ml of cells (at ~4 million cells/ml). Typically 100 µl of 10 mM of either 4-mercaptobenzoic acid (4-MBA) or 2-mercaptopyridine (2-MPy) in ethanol was added into a 1 mL of colloid solution, which was added to the cell suspension which was incubated for ~12 hours, to allow passive uptake of the colloid by the cells. The cells were first washed in PBS and centrifuged at 1000 rpm for 5 minutes to pellet the cells. This was repeated, and the resulting pellet was resuspended in cell culture media. Cells were transferred to the microfluidic device by aspirating directly from the centrifugation pellet into the PEEK tubing (50 µM ID, 360 µM OD) which was subsequently inserted into the device inlet port. Fluid flow rates of 50 nl min<sup>-1</sup> were sufficient to load, trap, and perfuse cells for the duration of the experiment. The device was mounted onto the Raman microscope stage. The microscope was focused such that the z-position was stored and held constant throughout the experiment. A region equivalent to that defined by the walls of a single cell trap, Figure 1, was selected using the LabSpec5 software, and represented an area of 18 µm<sup>2</sup>. The Raman spectra were acquired with a laser power: ~10 mW (at source), a mapping increment: 0.5 µm x 0.5 µm pixels, and an acquisition time per pixel: 0.2 s over a spectral range of 700 – 1800 cm<sup>-1</sup>.

## Supplementary Data



**Figure 4 (Supporting Information):** Time-course SERS mapping of a trapped live cell containing 4-MBA-coated nanoparticles trapped on a microfluidic device for a period of ~3 hours. Each image is the Raman map (pink pixels) revealing the location and distribution of nanoparticles inside the cell. In each case, the map has been centred with respect to the centre of the cell as observed in the brightfield images as shown in Figure 4.