Supporting Information

Investigation of the stability of chemisorbed and physisorbed labelled nanoparticles for SE(R)RS measurements in cells

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Experimental:

Cell Culture:

CHO and MG-63 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, Inyitrogen Ltd) without L-glutamine which was supplemented with fatal bovine serum (FBS, Invitrogen Ltd) to a final concentration of 10%, and L-glutamine to a final concentration of 4 mM. The cells were incubated in a humidified environment with 5% CO_2 and at 37 °C in an incubator. For harvesting, old media was removed and cells were washed with PBS solution (8.1 mM Na_2HPO_4, 1.2 mM KH_2PO_4, 138 mM NaCl, 2.7 mM KCL, pH 7.4). Cells were detached from the culture flask with a trypsin/EDTA solution (Invitrogen Ltd), some of the cells were used for the Raman measurements. The concentration of cells used for Raman experiments was in the range of 4 million cells per mL.

Inserting labelled nanoparticles into the cells:

Nanoparticles were delivered into live cells by passive uptake using a previously published method[1]. In short, 0.5 ml of *ca.* 4 million cells/mL cell solution (or 2 million cells) was added to a Nunclon^{TM} flask which has a surface area of 25 cm^2 to grow cells, followed by the addition of 5.0 ml of DMEM culture media. The cell suspension was mixed thoroughly and then 100 µl of 63 nm (mean diameter) functionalised silver colloid (home-made according to ref 2) particles were added to the above mixture . Again, the above cell and colloidal mixture

was shaken for 5-10 minutes prior to transfer to the incubator for a set amount of time. For labelling the nanoparticles, typically 10-50 µl of 50-100 µM thiols (in ethanol) was added into a 1 mL of colloid solution to be adsorbed to the nanoparticles for several minutes and the same amounts (volumes) discussed above were introduced to the cell solution. For dye labelling, 50-100 µL of 1-10 µM was added to 1 mL of colloidal solution. Also, the concentrations used are more than sufficient to form a monolayer coverage (typically 10^{-9} M concentrations of analyte molecules are sufficient for monolayer coverage, which is dependent on the size of the molecules being adsorbed).

Stability study of labels in media solution:

For the stability studies, 200 µL of functionalised nanoparticles were added into 10 mL of media solution (DMEM). The mixture was then agitated and subsequently, 1.5 mL of the above mixture was pipetted out at 0, 2, 4, 8, 16, and 24 hours time intervals and then they were centrifuged at 8000 rpm for 5 minutes, the supernatant (~ 1.4 mL) was removed and the pelleted colloid was re-suspended in 0.1 mL of the DMEM (remaining volume) and was interrogated under the Raman microscope.

Intracellular stability studies:

Nanoparticles were inserted into the cells by endocytosis[3]. In this work, cells were cultured with nanosensors for 3 hours to allow sufficient uptake to occur. For the SE(R)RS measurements of labelled intracellular nanoparticles, cells were washed vigorously 4 times (10 minutes) with PBS solution (pH 7.4) before harvesting to ensure any nanoparticles stuck to the cell membrane were washed out. Then, the harvested cells were centrifuged at 1000 rpm for 3 minutes, the supernatant (media) was removed, then the pelleted cells were washed again 3 times with PBS and finally re-suspended in PBS. A small aliquot of cells was transferred to a thin glass coverslip with an attached collar to allow a sufficient reservoir of liquid to be contained, and the cells were allowed to settle (adhere) for few minutes in the

incubator. Once the cells were sufficiently immobilised on the glass surface, the reservoir was topped up with fresh PBS solution periodically.

Confocal Raman microspectroscopy measurements:

All Raman spectra were acquired with a LabRam inverted microscope spectrometer, manufactured by Jobin Yvon Ltd. The spectrometer was equipped with multiple laser sources at wavelengths of 533 nm (70 mW) and 633 nm (He–Ne laser, 20 mW), and 830 nm (diode laser) near IR laser, true confocal optics, a holographic transmission grating, and a charge coupled device (CCD) detector with 1,024×256 pixels. The instrument included a precision motorized X–Y-Z sample stage for automated mapping at spatial resolution down to less than 1 μm in the x,y plane and ~ 2 μm depth resolution and extensive software support (LabSpec 5) for data processing. In this study, a 100 x objective lens (NA = 1.25), was used (U Plan FL, Nikon, Japan). This objective lens was mounted on a PI-721.10 piezo actuator (Physik Instrumente, Germany) for automatic focussing of the microscope objective at different depths in the Z direction enabling 3D mapping. A grating with 600 grooves mm^{-1}, a confocal aperture of 300 μm and an entrance slit of 150 μm were selected for the experiments. The Raman spectrometer wavelength range was calibrated using the centre frequency of the silicon band from a silicon sample (520.2 cm^{-1}). Using these conditions, a typical acquisition time of 0.5- 5 s was used to collect SERS spectra from cells. For stability studies in the media solution, a 10 x objective lens was used (U Plan FL, Nikon, Japan) and in each case, 10 spectra from different (from 200 μm spacing) spots were collected, and the averaged spectra were obtained. The confocal aperture was fully open to acquire maximum signals. The 'Auto' baseline correction method in LabSpec 5 (polynomial line fitting) was used to remove the unwanted baseline from the spectra. For intracellular stability studies, 10 cells were randomly selected and 10 spectra were collected in each cell (2μm spacing) giving 100 spectra for each time and the average intensity was calculated. Standard deviation was used to obtain the error

bars. A 100 x objective lens was used (U Plan FL, Nikon, Japan) for this study. For all data collection, the 633 nm laser line was used and the power at the sample was *ca.* 3 mW.

Stability Raman data:

The following two figures show stability data for the remaining dyes (SI-1) and thiol (SI-2) in the cell culture media.

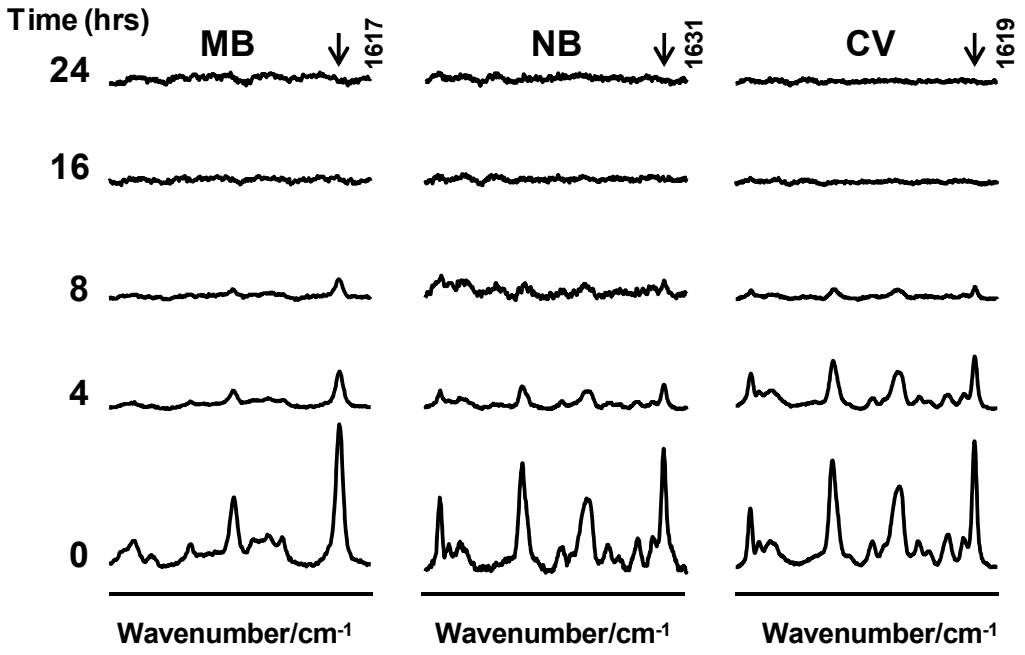

Fig SI-1: SERRS signals from physisorbed labels (MB, NB and CV) diminish rapidly over time in cell culture media.

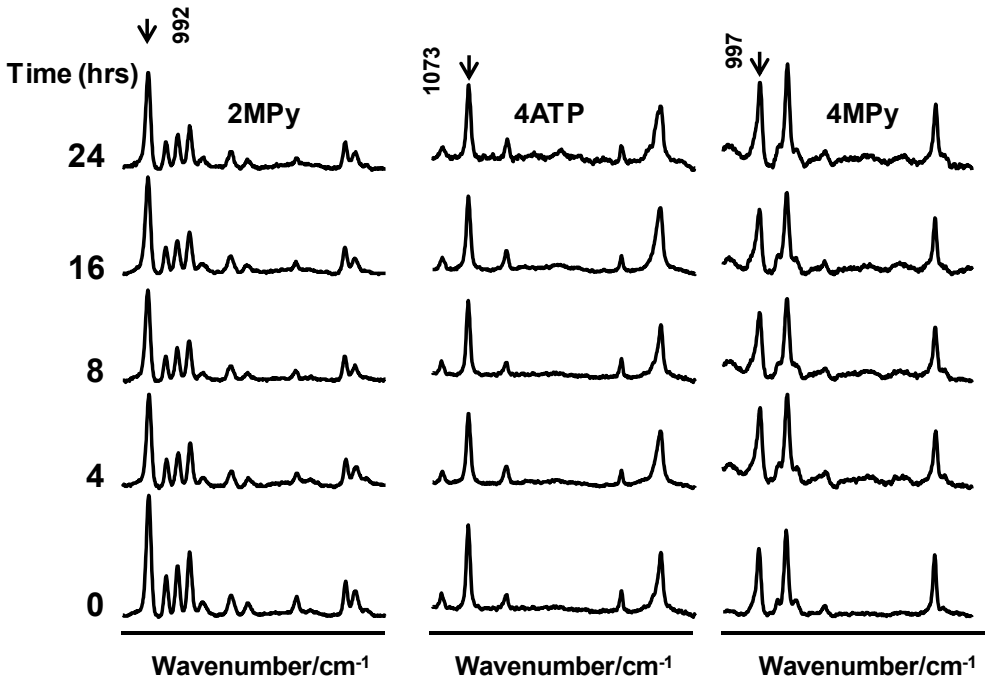

Fig SI-2: SERS signals from chemisorbed labels (2-MPy,4-ATP and 4-MPy) do not diminish over time in cell culture media.

UV-vis data:

Fig SI-3 shows the UV-Vis data obtained for the physisorbed (CV) and chemisorbed (4-MBA) molecules attached to silver colloid in cell culture media. The nanosensors were added to the cell culture media and the mixture was agitated before collecting data at 0, 2, 4, 8, and 16 hours. The band at 558 nm is due to the absorption from the cell culture media whilst the bands at 412 and 419 nm are due to nanoparticle absorption. These data show that the no significant change has occurred to the resonance band of nanoparticles, hence there is no evidence for any significant change in the aggregation state of the colloid. Therefore, we conclude that the disappearance of the signals from the physisorbed dyes is due to the detachment of dye molecules rather than the change in the aggregation state of the nanoparticles.

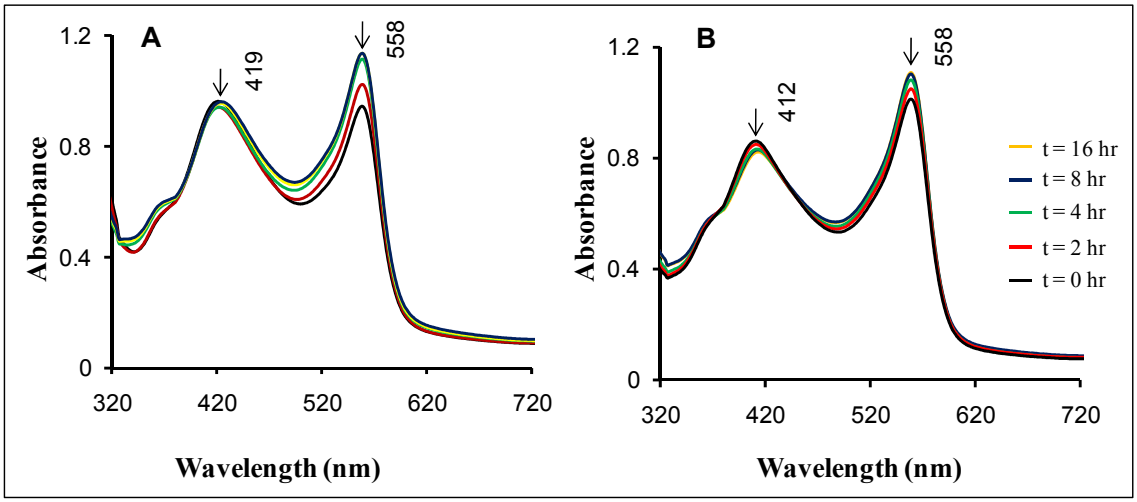

Fig SI-3: UV-vis data obtained from (A) chemisorbed molecule (4-MBA) and (B) physisorbed molecules (CV). The band at 558 nm is due to the absorption from the cell culture media and the bands at 412 and 419 nm are due the nanoparticles absorption. It is clear that the resonance bands do not change over time.

References:

1) N. M. S. Sirimuthu, C. D. Syme, J. M. Cooper, *Analytical Chemistry*, 2010, **82**, 7369.

2) N. Leopold, B. Lendl, *Journal of Physical Chemistry B*, 2003, **107**, 5723.

3) J. Kneipp, H. Kneipp, A. Rajadurai, R. W. Redmond, K. Kneipp, *Journal of Raman Spectroscopy*, 2009, **40**, 1.