SERS nanotags for Folate Receptor α detection at the single cell level: discrimination of overexpressing cells and potential for live cell applications

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Supplementary Information

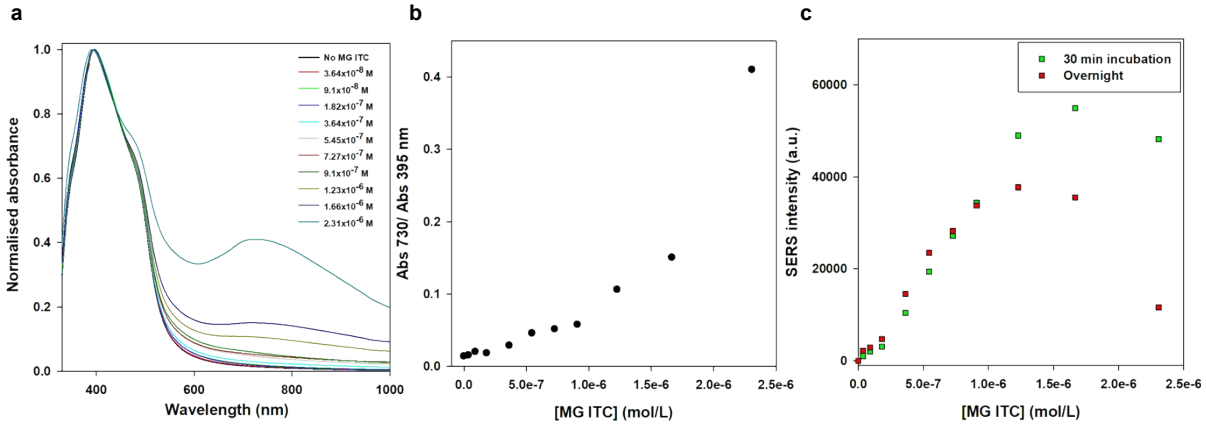

Figure 1. (a) Normalised UV-Vis spectra of Au@Ag nanoparticles with increasing concentration of MGITC. Spectra were normalised to 1 at 395 nm to emphasize the relative increase of absorbance at higher wavelength due to aggregation. (b) Aggregation was characterised by plotting the ratio of absorbance at 730 nm and at 395 nm as a function of the concentration of MGITC. (c) SERS intensity of the principal band (1616 cm^{-1}) at the corresponding MGITC concentration measured after 30 min of incubation (green) and after 24 hours (red). This plot can then be used to select the MGITC concentration that will guarantee the highest SERS signal and sufficient stability, simultaneously. Concentrations of MGITC that induce significant loss of SERS intensity (above 10^{-6} mol/L) overnight correlate well with sudden increase in the ratio of absorbance at 730 and at 395 nm.

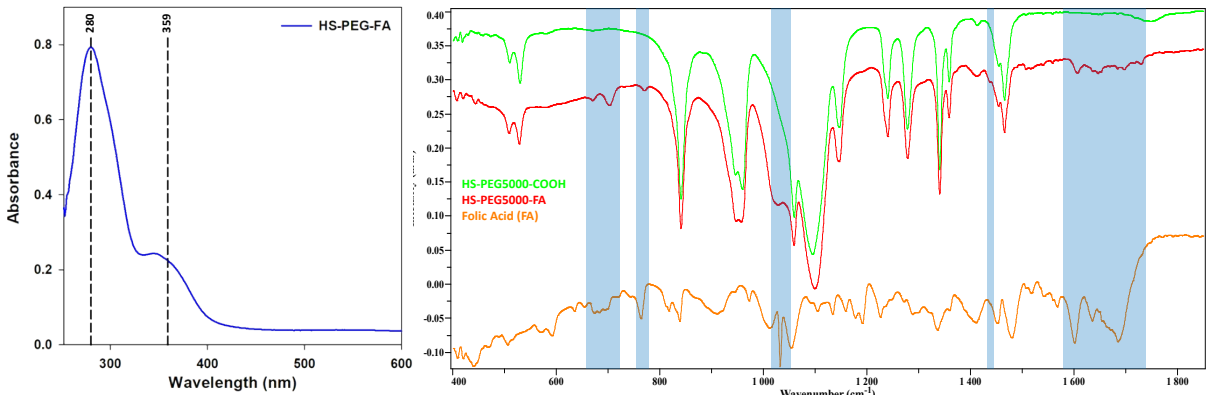

Figure 2. (Left) UV-Vis spectrum of HS-PEG-FA at $5x10^{-5}$ M in water measured through a PMMA cuvette (1 cm optical path). The absorption bands at 280 and 359 nm confirm the presence of folic acid at the end of the PEG chains. (Right) Attenuated Total Reflection infrared spectra of reference HS-PEG-COOH (green) and of the commercial HS-PEG-FA (red) along pure folic acid (orange). Spectral regions where features from folic acid are observed in the IR spectrum of HS-PEG-FA and not in the HS-PEG-COOH spectrum are highlighted, confirming that folic acid is present at the end chain of the PEG linker.

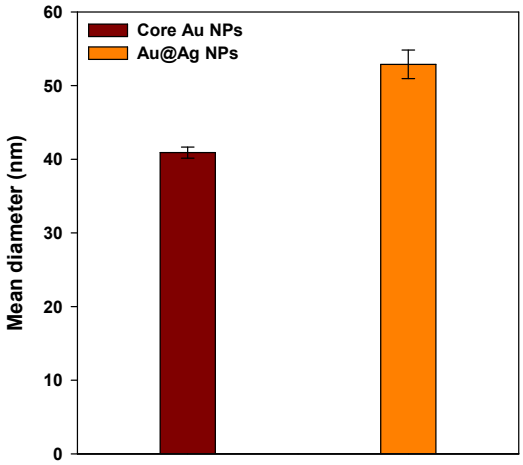

Figure 3. Hydrodynamic diameter (nm) of the core gold nanoparticles (red) used for synthesis of Au@Ag nanoparticles (orange). The DLS measurement confirms the deposition of a shell of about 7.5 nm at the surface of the gold core.

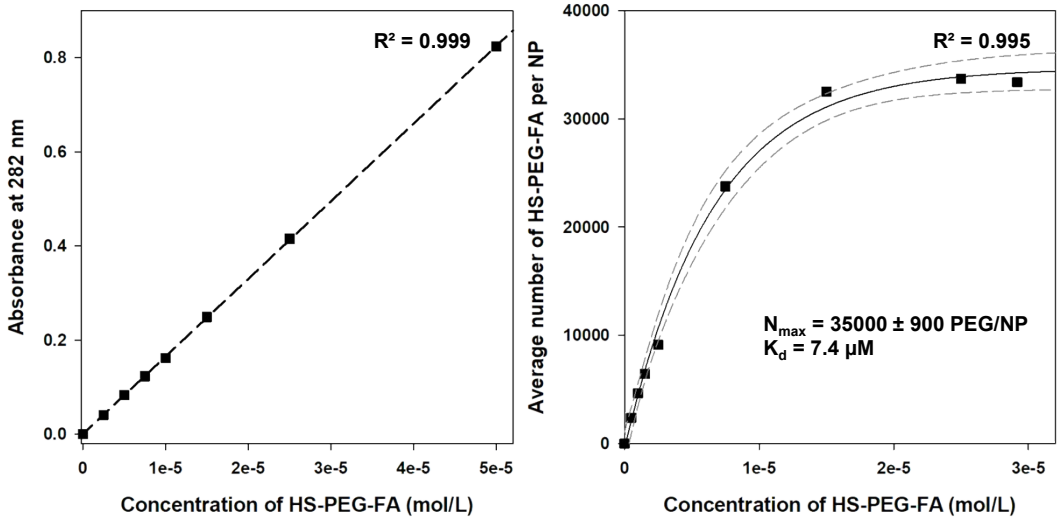

Figure 4. (Left) Calibration curve of the absorbance measured at 282 nm for standard solutions of HS-PEG-FA in water. Measurements were performed through PMMA cuvettes of 1 cm optical path. (Right) Average number of HS-PEG-FA chains grafted per nanoparticle as a function of the initial concentration of HS-PEG-FA. Measurements were performed by incubating known concentration of HS-PEG-FA with fixed number of nanoparticles. After 2 hours of incubation with the polymer solution, NPs were centrifuged at 13500 RPM during 15 min. Then, the absorbance at 282 nm of the supernatant was measured and compared to the calibration curve to compute the average number of chains immobilized on the NPs. The blank was the supernatant of nanoparticles not incubated with the polymer solution.

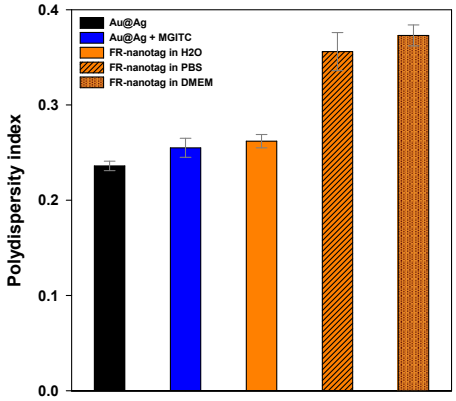

Figure 5. Polydispersity index of the FR-nanotags in water, PBS buffer and DMEM cell culture medium.

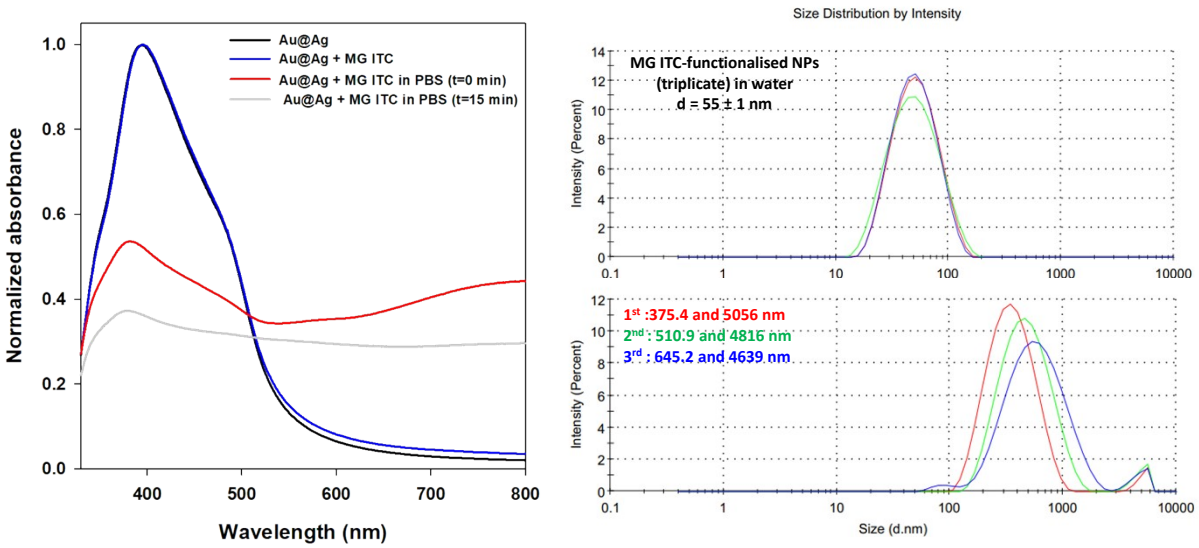

Figure 6. UV-Vis spectrum of MGITC-functionalised NPs in water (blue) and in PBS directly after incubation (red) and after 15 min (grey). The drop in the absorbance at the position of the maximum of absorbance (395 nm) and the increase of absorption at higher wavelength clearly reveals a strong aggregation. The aggregation is time dependant as illustrated by the change in the UV-Vis spectra measured at different times. The aggregation was confirmed by DLS measurement. The average diameter increases to 375.4 nm during the first cycle of measurement (just after incubation in PBS), but keeps shifting toward higher diameter during the DLS measurement (three successive measurements, shift to 510 then 645 nm). DLS data also reflects the time dependant nature of aggregation.

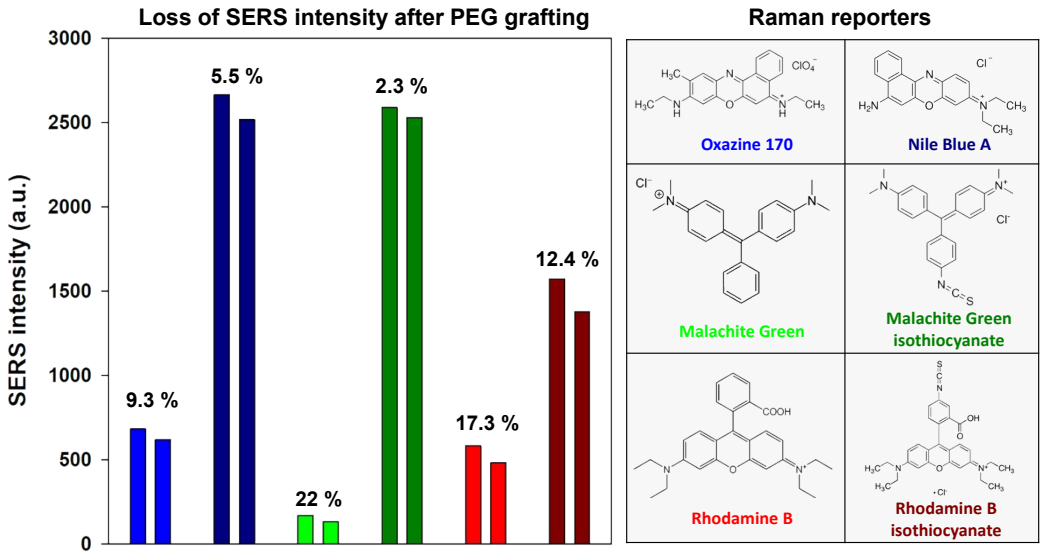

Figure 7. Absolute SERS intensity measured for six different Raman reporters before and after grafting of the HS-PEG-FA linker. The percentage value indicates the relative loss of intensity for each reporter after adding an excess of HS-PEG-FA. Measurements were performed under 647 nm irradiation with a dye concentration of 9×10^{-7} M and NPs concentration of 2.5×10^{11} NP/mL. For intensity evaluation, the most intense band of each dye was used: the band at 592 cm^{-1} for Oxazine 170, the band at 595.5 cm^{-1} for Nile Blue A, the band 1614 cm^{-1} for Malachite Green, the band at 1616 cm^{-1} for Malachite Green isothiocyanate, the band at 1653 cm^{-1} for Rhodamine B and the band at 1653 cm^{-1} for Rhodamine B isothiocyanate.

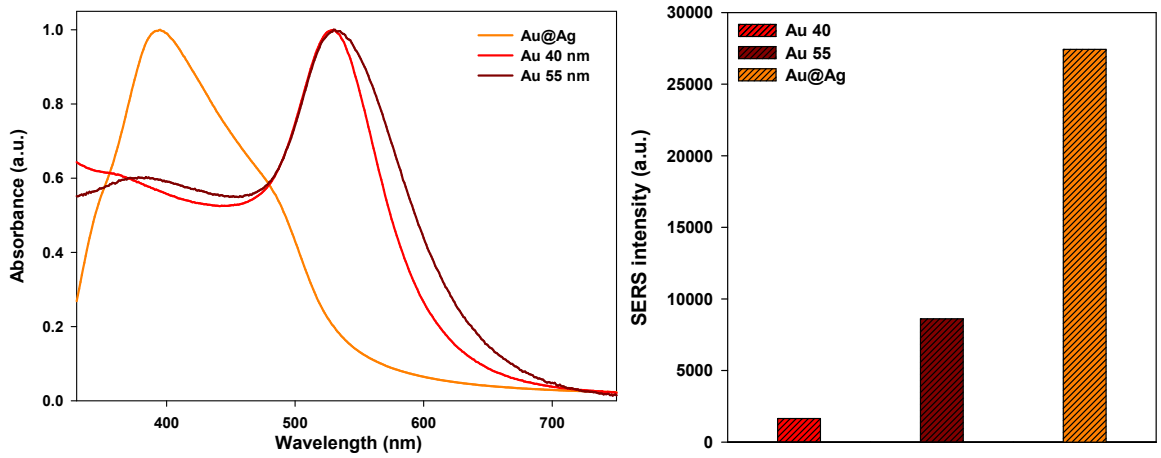

Figure 8. (Left) Normalized UV-Visible spectrum of 40 nm gold NPs (red), 55 nm gold NPs (dark red) and Au@Ag NPs (orange). (Right) Comparison of the absolute SERS intensity of the 1616 cm^{-1} band of MGITC at 638 nm obtained with 40 nm gold NPs (red), 55 nm gold NPs (dark red) and Au@Ag NPs (orange). NPs concentration was $2.5x10^{11}$ NPs/mL and MGITC concentration was $9x10^{-7}$ M in all samples.

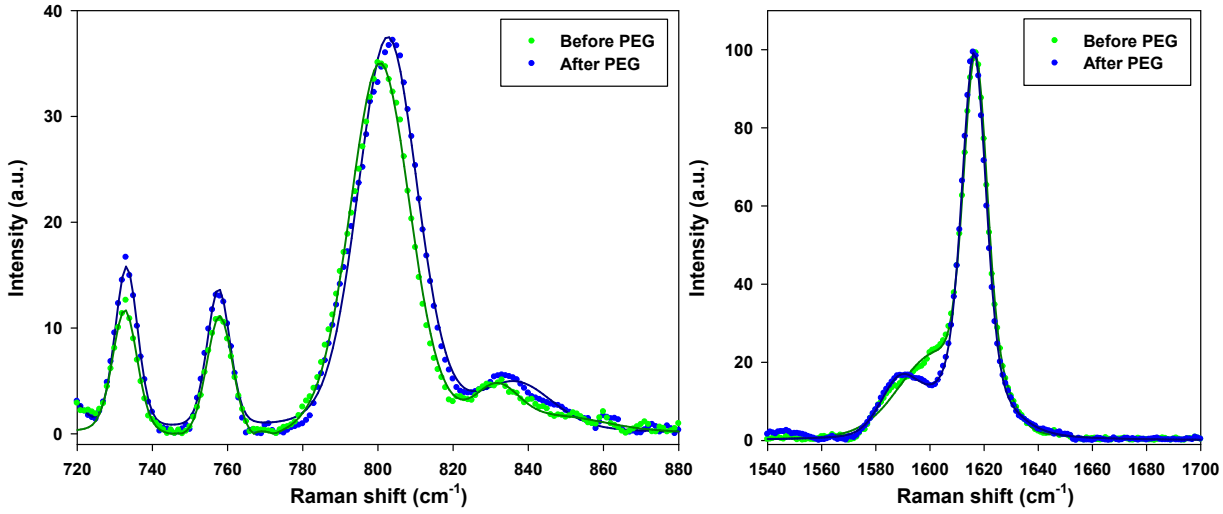

Figure 9. Modifications observed in the SERS spectrum of MGITC at 638 nm after functionalisation with HS-PEG-FA. Fitting of the bands of interest was performed in Labspec using a convolution of Gaussian and Lorentzian functions. For example, the bands located at 1596 and 1616 cm^{-1} undergo a significant change after functionalisation. The band at 1596 cm^{-1}, that initially appears as a shouldering, shifts to 1590 cm^{-1} while the band at 1616 cm^{-1} remains at the same position. Similarly, the band located at 801 cm^{-1} shifts to 803 cm^{-1} after functionalisation, also indicating the slight change of conformation of MGITC at the surface of the NPs.

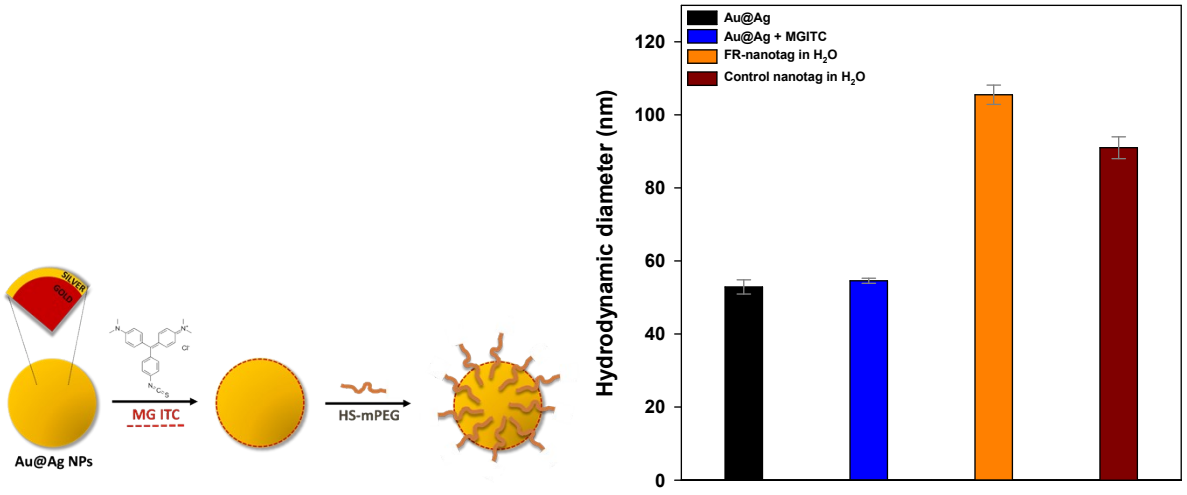

Figure 10. (Left) Schematic representation of the "control" nanotag. (Right) Hydrodynamic diameter of the "control" nanotag and comparison with the FR-nanotag. The increase in diameter compared to bare Au@Ag NPs confirms the deposition of the HS-mPEG at the surface of the nanoparticles. The diameter of the control nanotag in water is slightly smaller (91 ± 3 nm) than the FR-nanotag (105 ± 3 nm) but the overall size remains comparable. The stability in different media was also comparable to the FR-nanotag (data not shown).

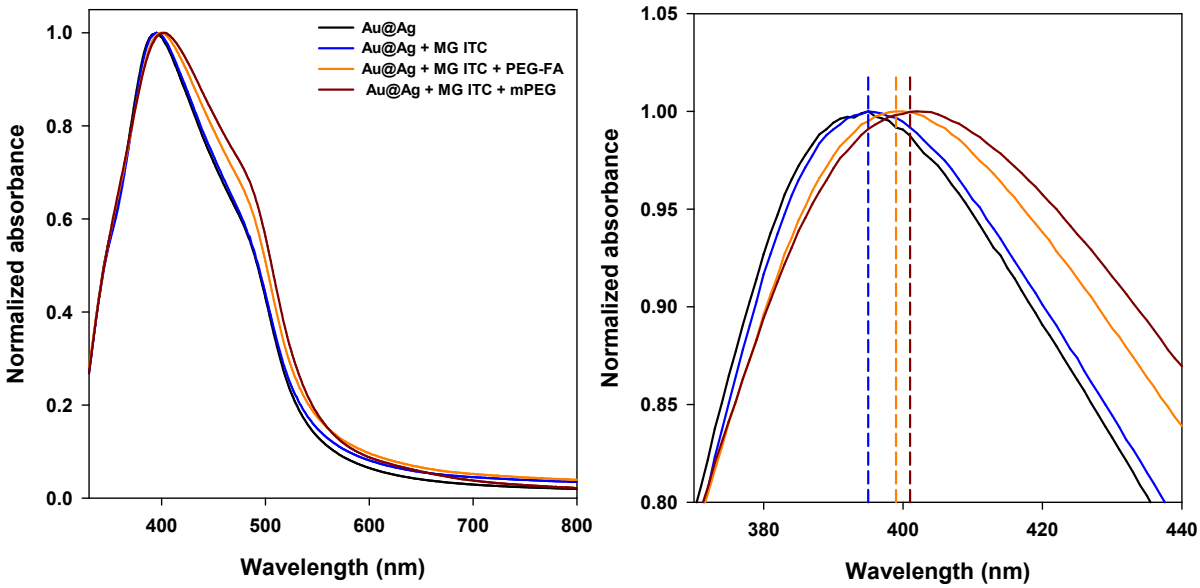

Figure 11. UV-Vis spectrum of the "control" nanotag overlaid to the UV-Vis spectra obtained during the synthesis of the FR-nanotag. A red shift of the absorbance maximum and a band broadening are also observed, confirming the functionalisation of the surface with HS-mPEG.

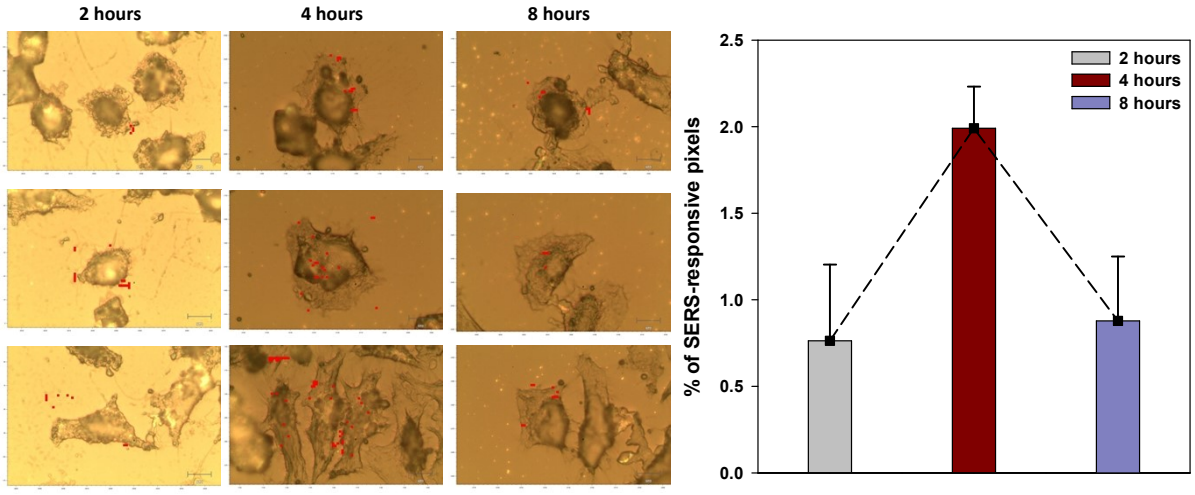

Figure 12. Influence of the incubation time on the percentage of SERS-responsive pixels. Note that these maps were constructed by taking only into account the pixels with a sufficient fit coefficient (> 0.4) after DCLS using the reference spectrum of MGITC. The % of SERS-responsive pixels is lower at 8 hours than at 4 hours. We observed after 8 hours the appearance of many Raman bands that are not characteristic from MGITC but are rather associated with intrinsic biological molecules. Our hypothesis is that after this long incubation period, the structure of the nanotag was altered, giving the possibility to intrinsic biomolecules to reach the metallic surface of the NPs and undergo surface enhancement, therefore contributing to the measured spectra. Note that in the analysis conditions selected in this study (633 nm, 1 s acquisition, 8 mW), no classical Raman signal was detected when mapping cells. We considered that any detected signal during the mapping was due to surface enhancement effects. Four hours was therefore selected as the optimal incubation time.

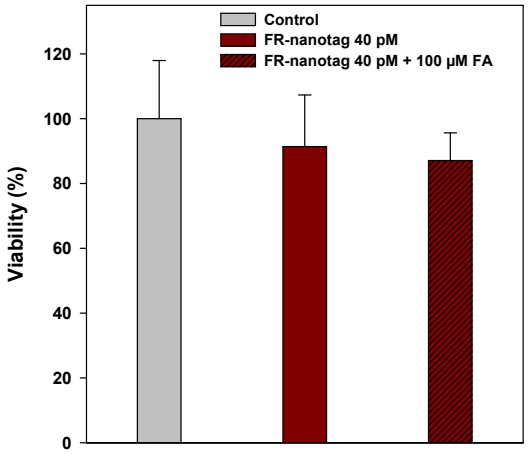

Figure 13. Viability of HeLa cells in various conditions. 100.000 HeLa cells were seeded on glass coverslips for 24 hours in DMEM medium before removing the media and incubation with fresh media containing the nanotags. Viability of the cells was measured by counting cells after Trypan Blue labelling and counting on a haematocytometer. Each condition was applied for 4 hours at 37°C and under 5% CO_2 atmosphere. Cells cultured in DMEM medium only are denoted as "control" and their viability is set to 100 % for comparison of others conditions.

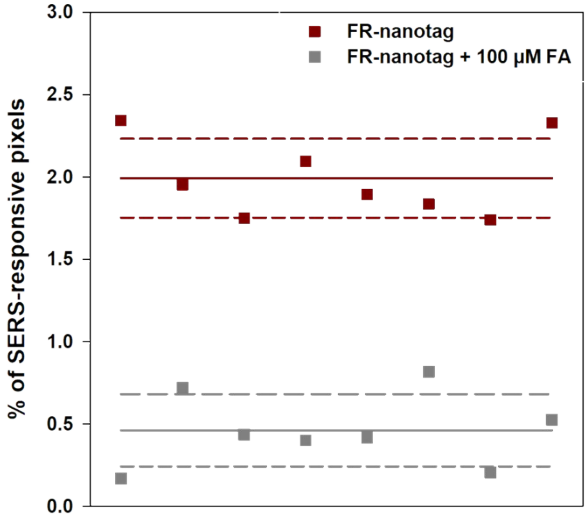

Figure 14. Consistency of the % of SERS-responsive pixels among different HeLa cells obtained in the presence of the nanotags at 40 pM (red squares) and in the presence of the nanotag at 40 pM + 100 µM folic acid (grey squares). Plain and dashed lines represent the mean value ± standard deviation, respectively.

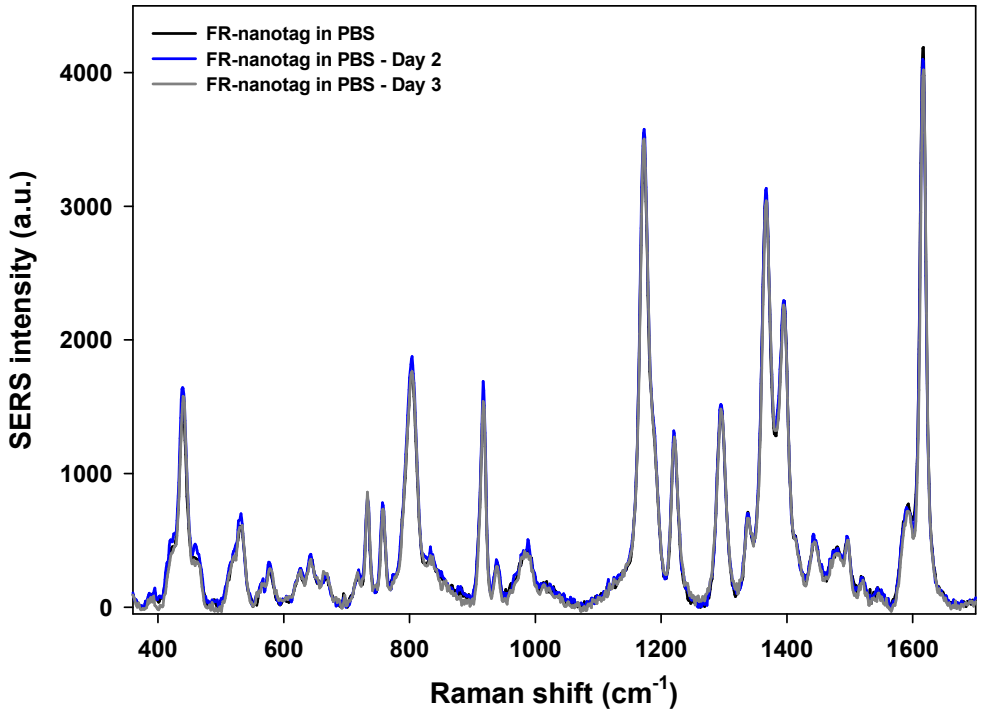

Figure 15. SERS spectra of the FR-nanotag during storage in PBS for three days. Spectra were acquired on a Snowy Range handheld Raman instrument with an irradiation wavelength of 638 nm, approximate power at the sample of 1 mW and 3 acquisitions of 1 s.

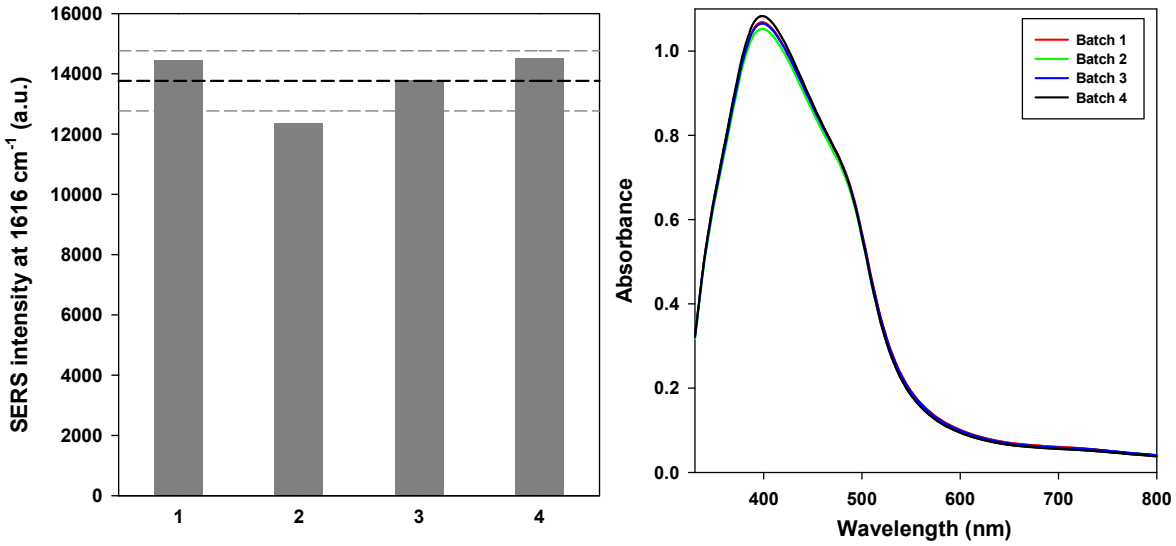

Figure 16. (Left) SERS intensity of the principal band of MGITC (1616 cm^{-1}) among four different synthesis of the nanotags (same starting batch of Au@Ag NPs). Black dashed line represents the mean intensity of the four samples and grey dashed lines represent one standard deviation. We obtain Relative Standard Deviation (RSD) of 7.25 % on the absolute SERS intensity. (Right) UV-Vis spectra of four different batches of nanotags after functionalisation by HS-PEG-FA (starting from the same batch of Au@Ag NPs). Metrics related to these spectra can be found in Table 1.

Table 1. Metrics obtained from the UV-Vis spectra measured for four different synthesis of FR-nanotags (starting from the same initial batch of Au@Ag NPs). FWHM is full width at half maximum.

	Position of the max (nm)	**Absorbance at the max**	**FWHM (nm)**
Sample 1	398	1.0682	162
Sample 2	399	1.0525	162
Sample 3	399	1.0653	161
Sample 4	398	1.0829	159
Mean	**398.5**	**1.06723**	**161**
SD	**0.57**	**0.0125**	**1.4**
RSD (%)	**0.15**	**1.17**	**0.9**