Supplementary information

SERS Tags Derived From Silver Nanoparticles And Aryl Diazonium Salts For Cell Raman Imaging

Da Li,^a Philippe Nizard,^{*a} Delphine Onidas,^a Aazdine Lamouri,^b Jean Pinson, ^b Samia Mahouche-Chergui, ^c Kelly Aubertin,^d Florence Gazeau,^d Yun Luo^{*a} and Claire Mangeney^{*a}

^{a.} Université de Paris, Lab Chim & Biochim Pharmacolog & Toxicol, UMR 8601, F-75006 Paris, (France)

^{b.} University Paris Diderot, ITODYS, UMR 7086, 75013 Paris (France)

^{c.} Université Paris-Est, ICMPE, UMR7182, 94320 Thiais (France)

^{d.} Université de Paris, MSC, CNRS UMR 7057, 75013 Paris (France)



Figure S1. TEM images recorded before and after functionalization of Ag NPs by aryl diazonium salts: (a) bare Ag NPs, (b) Ag@NO₂ NPs, (c) Ag@CCH NPs and (d) Ag@CN NPs. Insets: size distribution curves of the Ag NPs cores (black spheres), estimated from over 100 NPs, for each sample.



Figure S2. Normalized UV-vis spectra of Ag NPs before (black line) and after the step-by-step addition of multilayers for (a) Ag@NO₂@CCH@CN and (b) Ag@CCH@CN@NO₂.



Figure S3. UV-vis extinction spectra of all NPs samples over time, in pure water media or in NaCl 0.2 M and 1 M aqueous solutions. The spectra were recorded each week during 7 weeks.



Figure S4. SERS spectra of concentrated aqueous dispersions of Ag NPs (1.6 mg.mL⁻¹) modified by aryl diazonium salts bearing either NO₂, CN or CCH Raman reporter groups or multilayers. The SERS spectra were obtained with a 638 nm laser and a 100× objective lens.



Figure S5. Raman spectra of aryl diazonium salts bearing –NO₂ (D-NO₂), CN (D-CN) and CCH (D-CCH) Raman reporter groups.



Figure S6. Cytotoxicity assay of NPs on HELA cells. Cell viability assessed by Alamar Blue metabolic activity test normalized to the control non-exposed cells. 300 000 Hela-HSP 70/GFP cells were seeded in 2 ml in 6-well plates. After 24 h, medium was renewed with 2 or 20 μg.mL⁻¹ of SERS labels and the NPs were left for incubation with cells during 6 hours. Then, the medium was replaced with 1 mL of medium containing 10% of Alamar Blue.



Cytotoxicity assay of NPs on Fibroblasts

Figure S7. Cytotoxicity assay of NPs on fibroblasts. 20 000 fibroblasts per wells were cultivated in 48-well plates in 400 μ l of medium for 24 h. Medium was replaced with 200 μ l of fresh medium containing different concentrations of Ag NPs functionalized with CCH, CN or NO₂ groups and left for 24 hours. The medium was then replaced with 200 μ l of fresh medium containing 10 % of Alamar Blue.

Table S1. Hydrodynamic diameters determined by DLS

Samples	Ag NPs	Ag@NO₂	Ag@CN	Ag@CCH	Ag@NO₂@CN@CCH
D _H (nm)	32	39	43	46	51
PDI	0.24	0.31	0.30	0.30	0.31

Table S2. Description of the Supplementary Excel File Table on cytotoxicity assays

All raw data obtained from cytotoxicity assays are provided in this Table:

- *Sheets 1 to 3* display the raw data for the 3 experiments of cytotoxicity assessment by Alamar blue for Ag@CCH, Ag@CN, Ag@NO₂ and Ag@CN@NO₂@CCH on HELA Cells.

- *Sheet 4* displays the raw data for the single experiment of cytotoxicity assessment by Alamar blue for bare unmodified Ag NPs, Ag@CCH, Ag@CN and Ag@NO₂ on HELA Cells.

- **Sheet 5** displays the histogram of all cytotoxicity results for each kind of NPs (Ag NPs, Ag@CCH, Ag@CN and Ag@NO₂ and Ag@CN@NO₂@CCH) on HELA Cells.

- *Sheet 6* displays the data gathered in the Table which has been used for analysis by the software Prism of cytotoxicity experiments on HELA Cells.

- *Sheet* **7** displays the raw data for the single experiment of cytotoxicity assessment by Alamar blue for Ag@CCH, Ag@CN and Ag@NO₂ performed in 6-well plates on HELA Cells.

- *Sheets 8 and 9* display the raw data for the 2 experiments of cytotoxicity assessment by Alamar blue for Ag@CCH, Ag@CN, Ag@NO₂ and Ag@CN@NO₂@CCH on fibroblasts.