Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Detection of myxovirus resistance protein A (MxA) in paper-based immunoassays with surface enhanced Raman spectroscopy with hollow Ag/Au nanoshells

Lorenzo Russo[1], Maria Sánchez-Purrà[2], Cristina Rodriguez-Quijada[2], Brianna M. Leonardo[3], Victor Puntes[1,4,5], and Kimberly Hamad-Schifferli[2]**

[1] Catalan Institute of Nanoscience and Nanotechnology (ICN2), CSIC and BIST, Campus UAB, Bellaterra, 08193 Barcelona, Spain

[2]Department of Engineering, University of Massachusetts, Boston, 100 Morrissey Blvd., Boston, MA 02115

[3]Department of Biology, University of Massachusetts, Boston, 100 Morrissey Blvd., Boston, MA 02115

[3] Vall d'Hebron Institut de Recerca (VHIR), 08035, Barcelona.

[4]Institució Catalana de Recerca i Estudis Avançats (ICREA), ICN2, E-08193 Bellaterra (Barcelona), Spain.

*Email: victor.puntes@icn2.cat, kim.hamad@umb.edu

Figure S1 - TEM bright field micrograph of monodisperse hollow AuAg NSs with a mean diameter of 75 nm ± 7 nm.

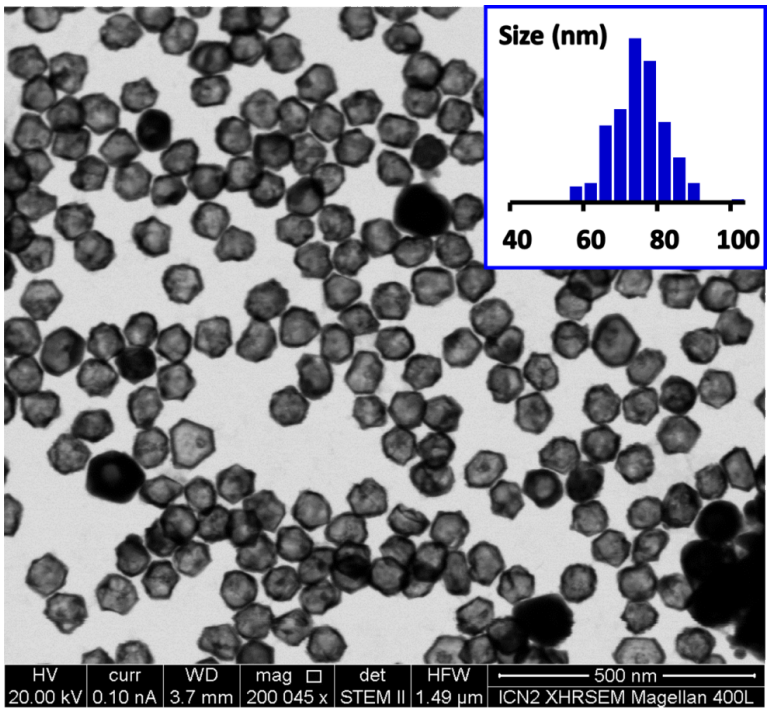


Figure S2 – *BCA assay is carried out to estimate the amount of anti-HuIgG immobilized onto AuAg NSs surface. Firstly, a calibration curve is built using antibody solutions at concentrations spanning between 0 and 800 ug/mL (Figure S2, black solid dots). The assay is then run in triplicates over AuAg NSs conjugated with antibody solutions at increasing concentration (0 to 200 μg/ml) after purification from the excess antibody, removed with centrifugation (Figure S2, blue circles). The intensity obtained do not present a significant trend, while instead showing a constant mean value of 0.423 ± 0.008 a.u. for all the different antibody concentrations used. Indeed, the measurement does not provide any measurable evidence of the different amount of antibody attached on AuAg NSs surfaces, due also to the limited sensitivity of the assay in the range of concentrations investigated. Besides, the constant absorbance value is probably caused by the presence of PVP onto the particles surface, since BCA assay is used to quantify proteins thanks to its ability to detect clusters amine residues. The presence of PVP, even at low concentrations, provides a relatively high amine content which seems to interfere with the colorimetric test. The assay is run also over the conjugation supernatant to double check the validity of the test (Figure S2, grey circles). The intensities obtained follow a linear trend, even though they seem to overestimate the antibody content compared to the calibration curve. This is probably due to the presence of some PVP, released from AuAg NSs surface during the purification process. When in fact the supernatant intensity values of each sample are corrected subtracting the value of the purified conjugate at the corresponding antibody concentration, the resulting intensity reflect very well the trend of the calibration curve (Figure S2, red solid dots).*

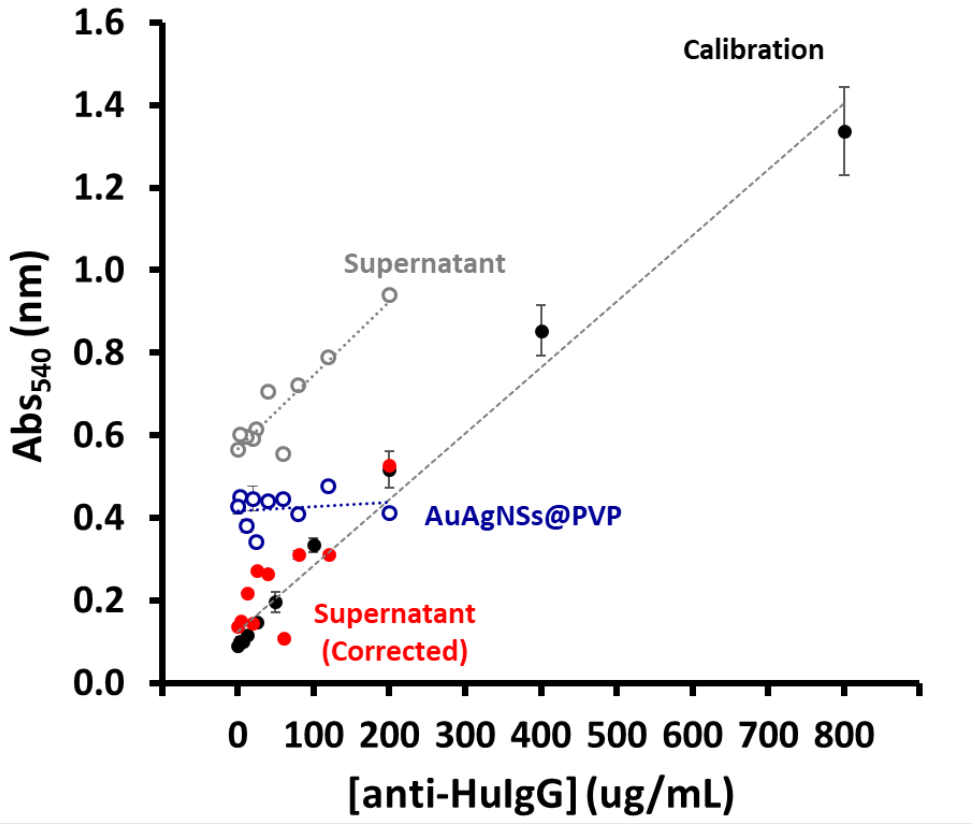


Figure S3 - SERS spectra of 4-MBA-coated AuAg NSs recorded at different concentrations of immobilized Raman reporter (red curves). In absence of either 4-MBA (grey curve) or AuAg NSs (blue curve) no signal is observed.

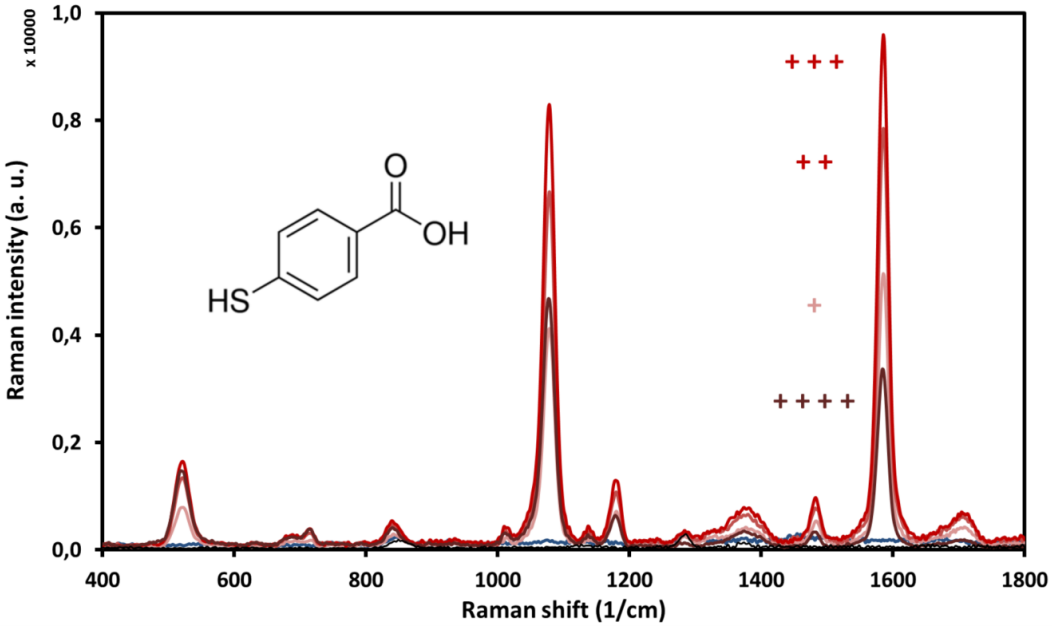


Figure S4 – Comparison SERS signal enhancements between the different reporter-encoded nanotags. **A:** Raman spectral intensities of plain reporter molecules (grey line) were compared to intensities of the Ag NPs (orange), AuAg NShells (blue) and Au NStars (red) under the same measuring conditions (laser excitation at 785 nm, 2 coadditions of 5 sec each). **B:** Enhancement factor calculated for the three reporter-encoded nanotags using the following equation:

$$EF = \frac{I_{SERS}}{I_{Raman}} \times \frac{N_{Raman}}{N_{SERS}} \times \frac{P_{Raman}}{P_{SERS}} \times \frac{t_{Raman}}{t_{SERS}}$$

where ***I*** is the peak intensity, ***N*** is the number of contributing reporter molecules, ***P*** is the laser power, ***t*** is the integration time, and subscripts ***Raman*** and ***SERS*** stand for measurements performed on the reporter molecule or on the nanotag, respectively. **C:** Re-scale of **A** for better comparison of AuAg NShells (blue) and Au NStars (red) SERS enhancement (Raman spectral intensities of plain 4-MBA molecules (grey line) are shown for reference). **D:** Bright field images of the measured area for Ag NPs, AuAg NShells and Au NStars drop-casted onto nitrocellulose.

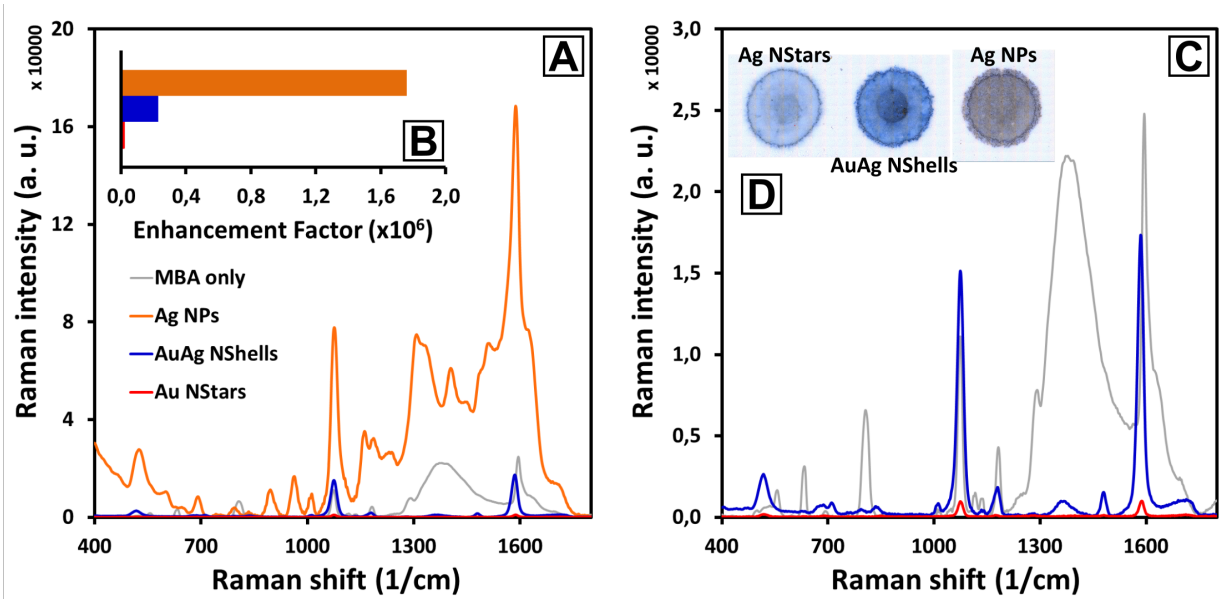


Figure S5 – HuIgG LFA using Ag NPs as labelled SERS nanotag. Ag NPs of ≈ 75 nm coated with 4-MBA (0.04 mM) and functionalized with anti-HuIgG (40 µg/mL) were used in the immunoassay strip against HuIgG (concentrations ranging from 2.5 ng/mL to 2.5 µg/mL). SERS read-out of the test-line was performed inmediately after the test was run (orange), 30 (light brown) and 75 days (dark brown).

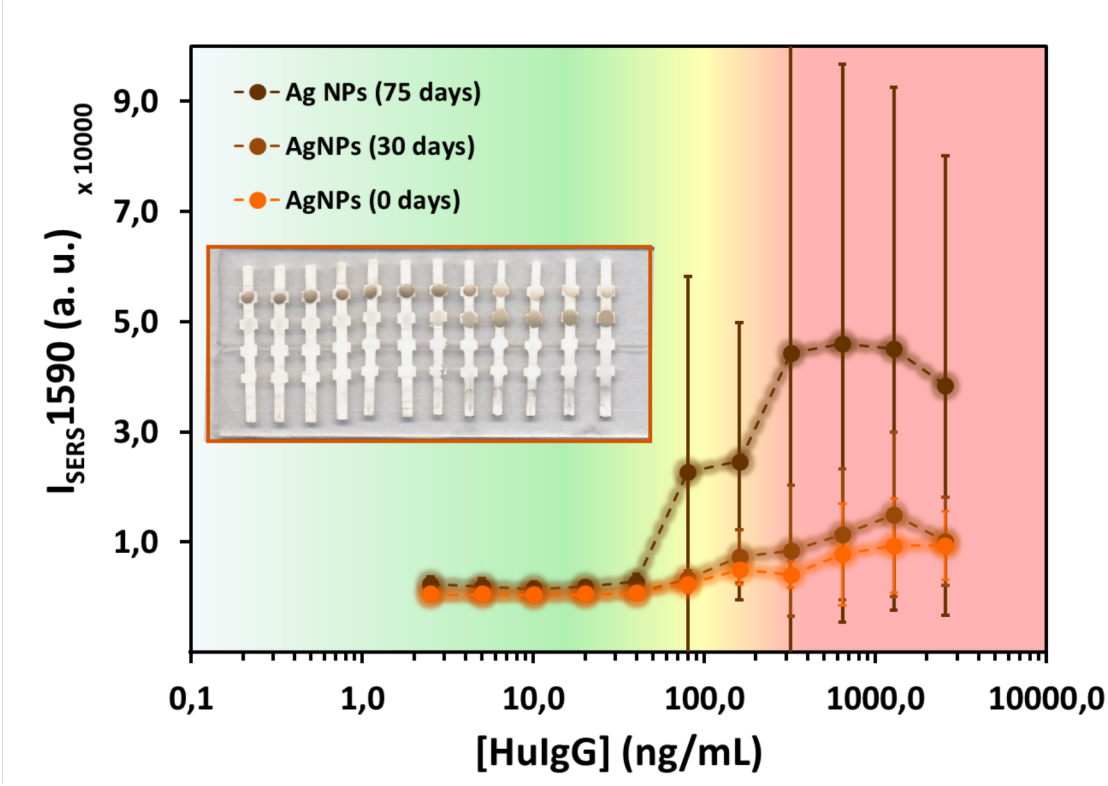


Figure S6: SERS detection of **A:** HuIgG and **B:** MxA with LFA (antigen concentrations ranging from 2.5 ng/ml to 2.5 µg/ml; "Hill" fitting curves (HuIgG: R^2 = 0.97, K_d = 105.4 nM; calculated LOD = 70.1 ng/ml. MxA: R^2 = 0.99, K_d = 64.2 nM; calculated LOD = 118.4 ng/ml).

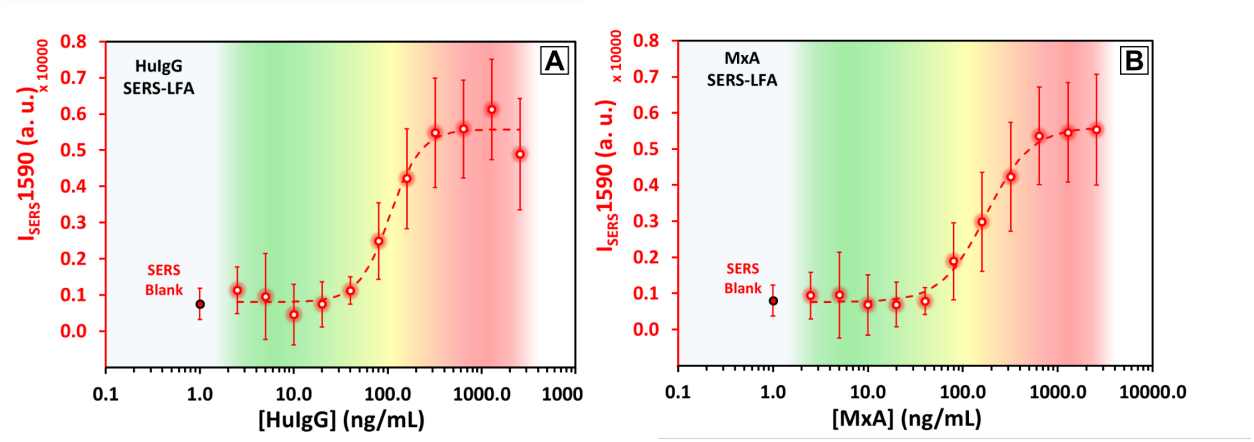


Figure S7 – Stability of nanotags in HS. The comparison between UV-Visible absorption spectra of conjugates (AuAgNSs@4-MBA@Ab) in PBS (blue curve) and HS (red curve) show that transferring the nanotags to a protein-rich medium does not affect significantly their stability. While the slight red-shift in wavelength maximum accounts for the change in the refractive index of the new medium, the relatively small decrease in intensity could be due to a negligible aggregation of the particles in the protein-rich medium.

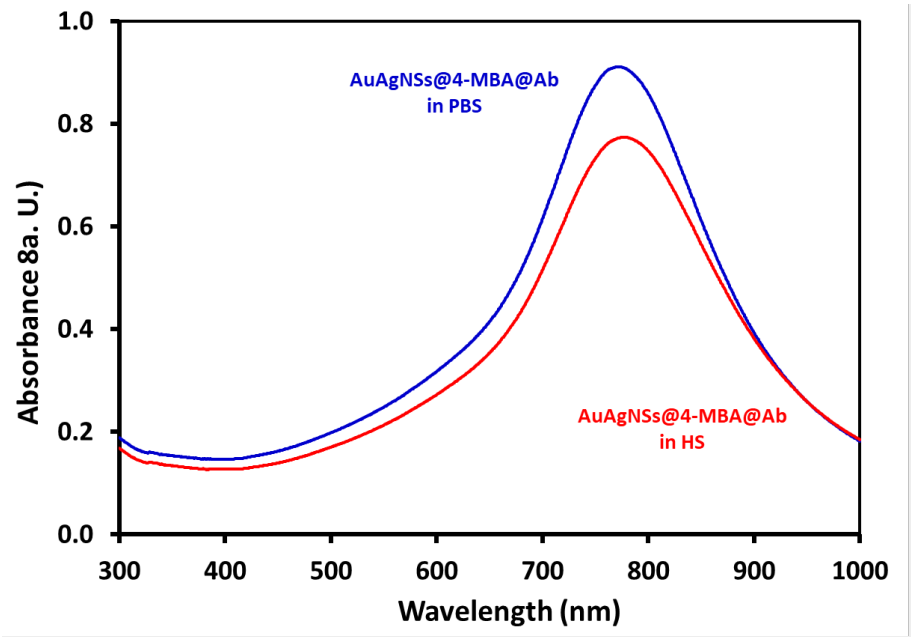
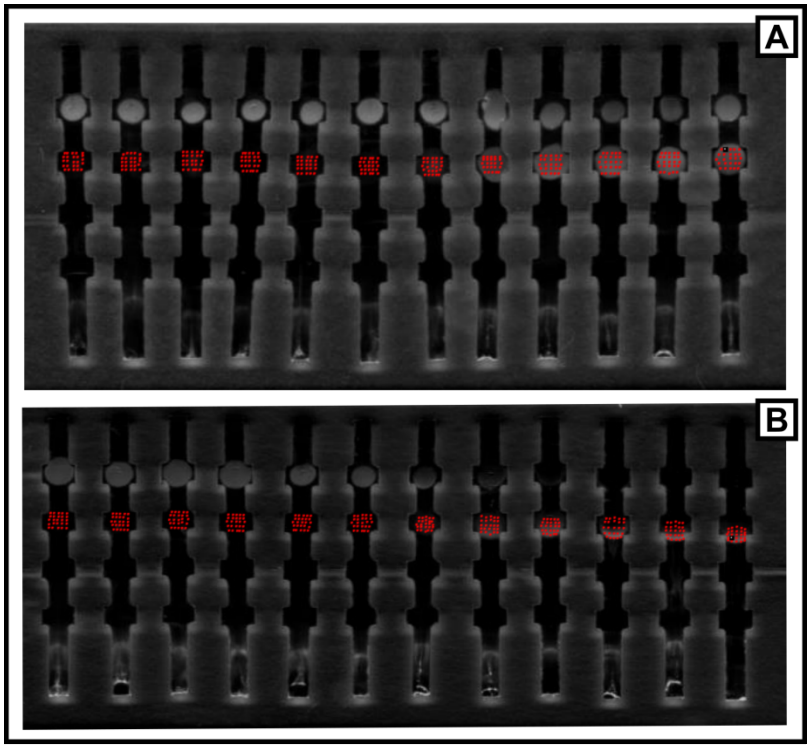


Figure S8 - Dark field image of LFA strips used for the detection of **A:** HuIgG and **B:** MxA. The visual detection of the analyte was quantified using the free software "ImageJ" by measuring 25 spots on the Test Line area of each strip (red dots). Each spot was obtained by tracing a circular area with area = 1.



s