Supplementary Information

# Visualization, Quantification and Coordination of $Ag^+$ Ions Released from Silver Nanoparticles in Hepatocytes.

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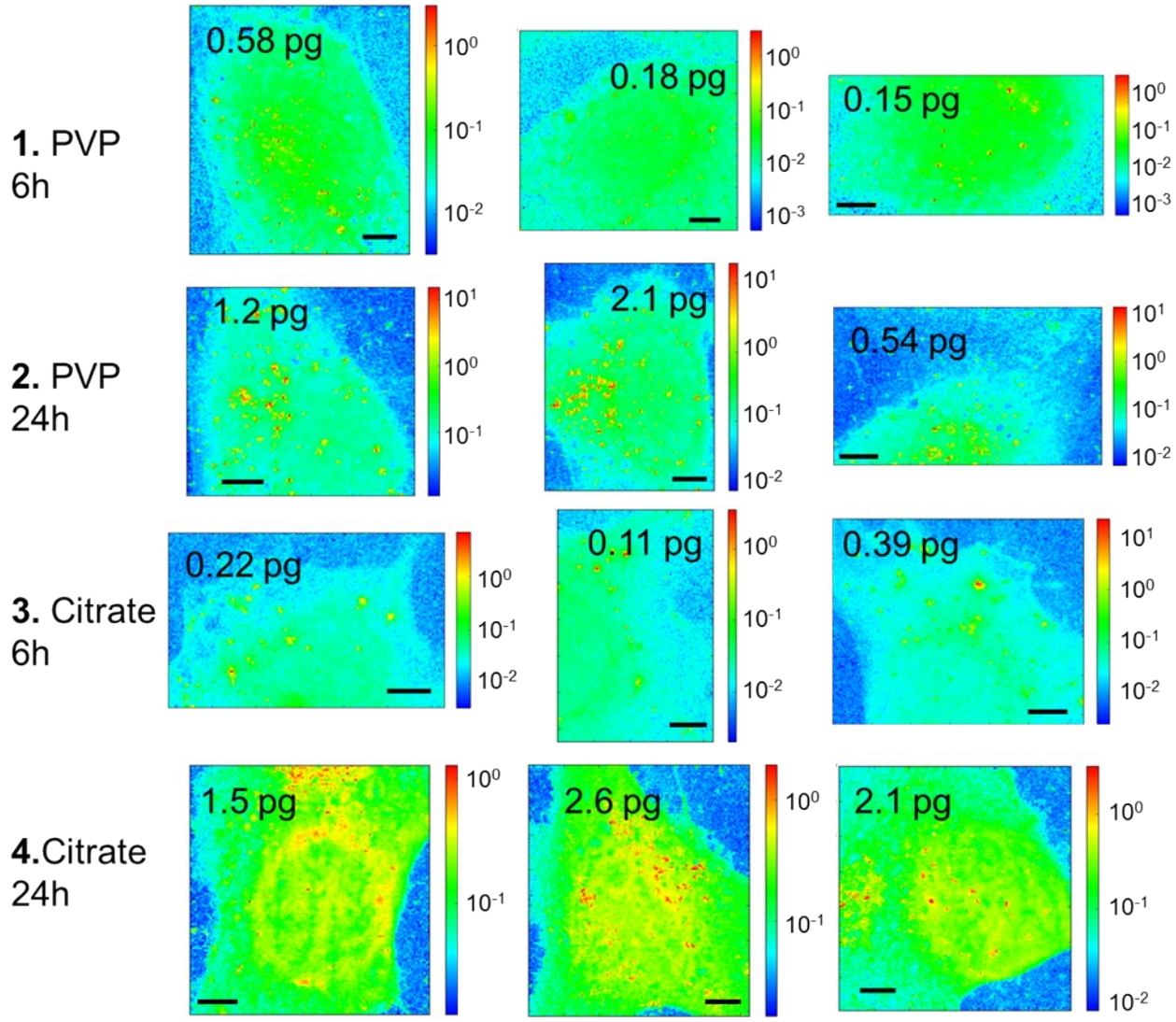


**Figure S1.** Silver areal densities in HepG2 cells, extracted from hyperspectral XRF data and represented in false-colour in logarithmic scale. Cells were exposed to PVP-coated AgNPs for 6 h (row 1) or 24 h (row 2), or to citrate-coated AgNPs for 6 h (row 3) or 24 h (row 4). All three cells measured per condition are shown (one per column). Areal densities are expressed in μg/cm$^2$; pixel size is 250x250 nm$^2$. Scale bars = 5 μm. The value in pg reported on each map is the total Ag content in the map area, obtained by integrating the areal density over all pixels.

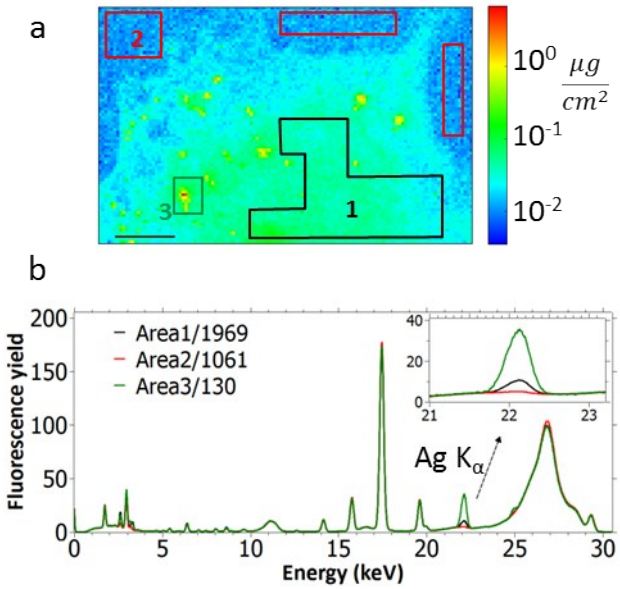


**Figure S2.** (a) Quantitative Ag distribution in an HepG2 hepatocyte exposed for 6 h to citrate-coated AgNPs. On the XRF maps three regions are chosen, corresponding to putative dissolved $Ag^+$ ions (Area 1), extracellular background (Area 2) and putative AgNP aggregates (Area 3). Scale bar = 5 μm. (b) Sum XRF spectra relative to the three regions, normalized by the number of pixels in each area; the inset shows a magnification of the Ag $K_\alpha$ emission spectral region.

The three chosen regions (panel a) provide the average spectra reported in panel b, which clearly show differences in the relative intensity of the Ag $K_\alpha$ emission line. In the area where the estimated Ag concentration per pixel is close to the detection limit (area 1, black curve), an Ag signal is clearly visible, and significantly higher than the background (panel b, inset), although much lower than the sum signal of a few pixels surrounding a putative AgNP aggregate (region 3, green curve).

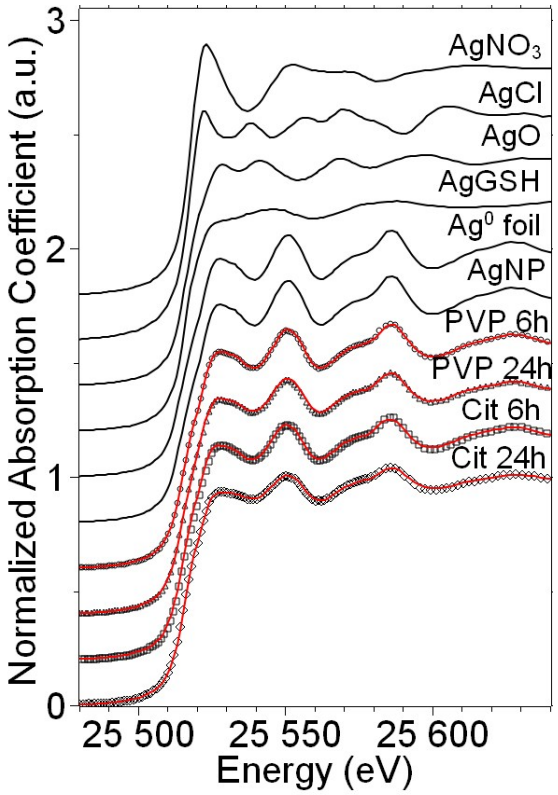


**Figure S3.** Experimental Ag K-edge XANES spectra of reference compounds (black curves) and of HepG2 cells exposed to AgNPs (open symbols). Best fitting curves (red) of cellular samples based on Linear Combination Fitting (LCF). Cells were exposed to PVP-coated AgNPs for 6 h (circles) or for 24 h (triangles), or to citrate-coated AgNPs for 6 h (squares) or 24 h (diamonds).

**Table S1.** Quantitative real time polymerase chain reaction primer sequences

| Gene | Forward primer sequence | Reverse primer sequence |
|---|---|---|
| HPRT | ATGGACAGGACTGGACGTCTTGCT | TTGAGCACACAGAGGGCTACAATG |
| GAPDH | ATGGGGAAGGTGAAGGTCG | GGGGTCATTGATGGCAACAATA |
| MET1X | GCTTCTCCTTGCCTCGAA | TGACGTCCCTTTGCAGATG |
| GCLM | CCTCCTGCTGTGTGATGCCAC | CGTGCGCTTGAATGTCAGGAATGC |