Supporting information:

Quantitative Differentiation of Cell Surface-Bound and Internalized Cationic Gold Nanoparticles Using Mass Spectrometry

Singyuk Hou,† Kristen N. Sikora,† Rui Tang, Yuanchang Liu, Yi-Wei Lee, Sung Tae Kim, Ziwen Jiang, Richard W. Vachet* and Vincent M. Rotello*

† S. Hou and K. N. Sikora contributed equally to this work.

Department of Chemistry, University of Massachusetts Amherst, 710 North Pleasant Street, Amherst, Massachusetts, 01003, USA

*rwvachet@chem.umass.edu (R.W.V.), rotello@chem.umass.edu (V.M.R.)

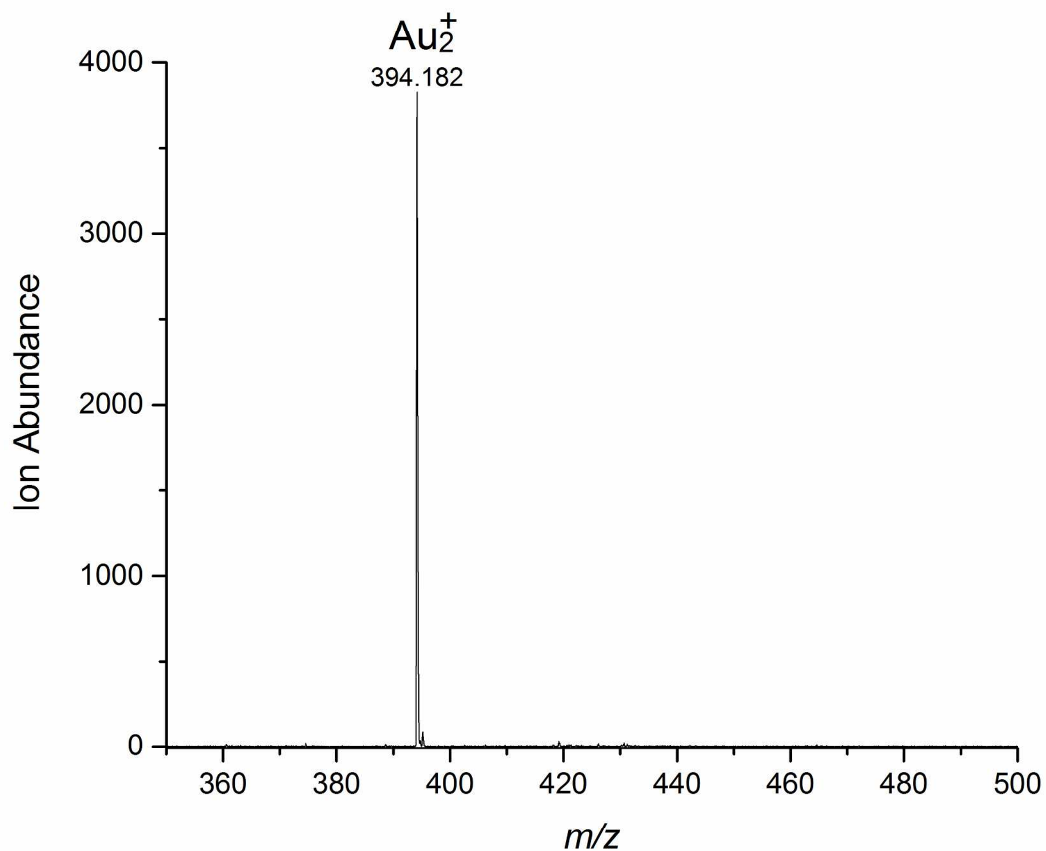

Figure S1. LDI-MS detection of AuNPs on plain slide after washing steps, 250 nM AuNP 1 media solution was incubated on poly-lysine coated glass slide for 30 minutes at 37 °C. After the incubation, the AuNP 1 solution was removed and the glass slide was washed with PBS for 5 times, AuNP 2 was then incubated for 30 minutes under the same condition and washed with PBS for one time.

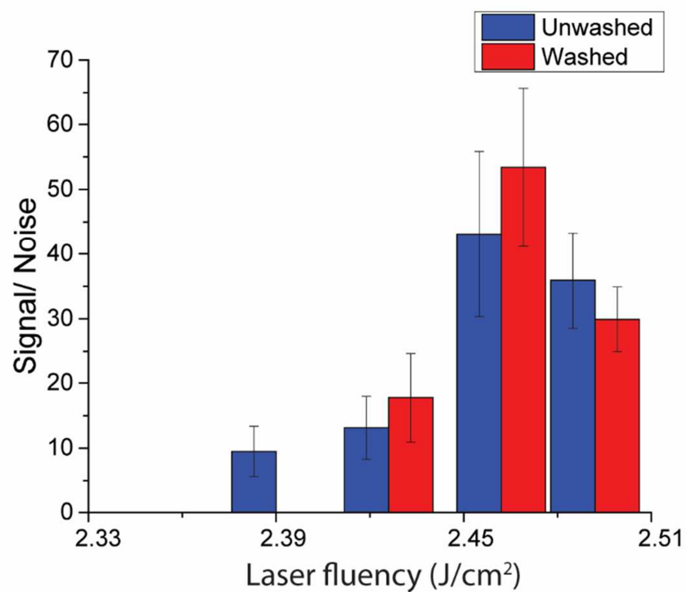

Figure S2. LDI-MS of AuNP 2 on cell monolayers before and after washing. 250 nM AuNP 2 was incubated with the cell monolayer for 60 minutes in serum free media at 37 ºC. After incubation, the cell monolayer was either washed four times (washed) or one time (unwashed) before LDI-MS analysis.

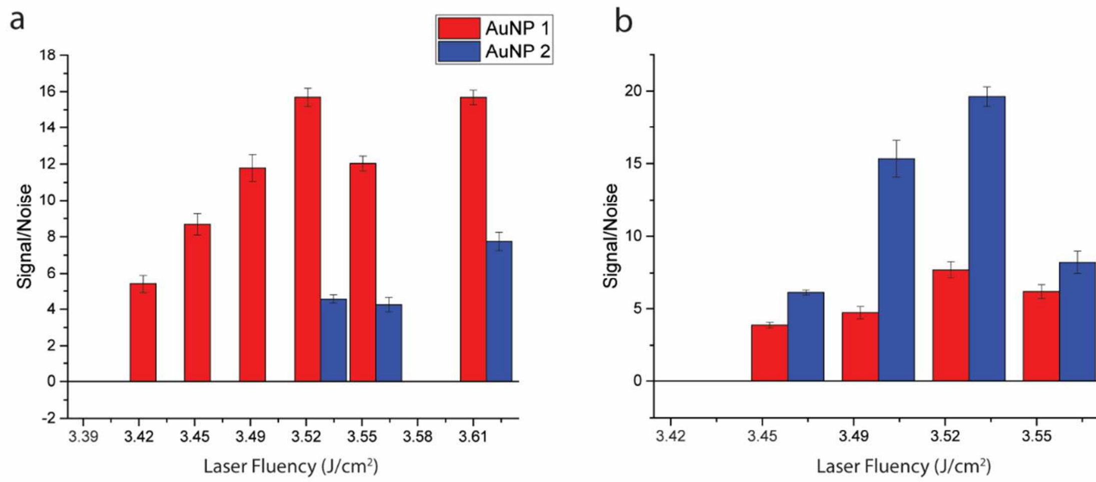

Figure S3. a) LDI-MS of HeLa cell monolayer after sequential incubation by AuNP 2 and AuNP 1. b) LDI-MS of HeLa cells monolayers treated with 3mg/ml NaN_3 and 50 nM 2-deoxyglucose in DMEM for 30 minute prior to sequential AuNP incubation.

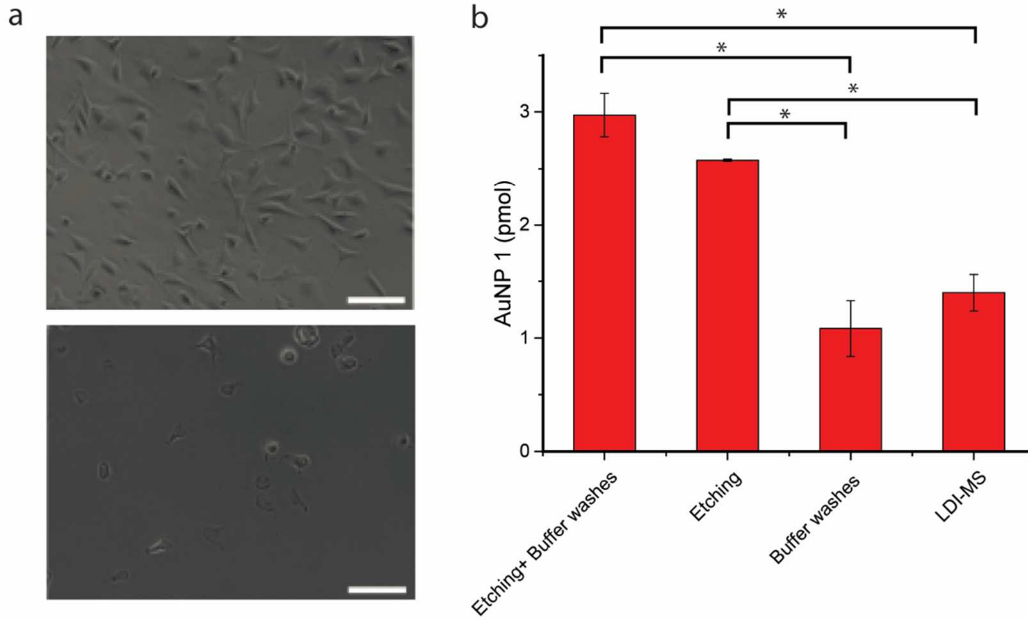

Figure S4. a) Microscopic image of HeLa cells not etched (up) and etched (down) by I_2/KI solution. Scale bars: 100µm. b) Comparisons of removal of surface-bound AuNPs via etching and buffer washing measured by ICP-MS. LDI-MS measurement of cell-surface adhered AuNP was added for further comparison. All the experiments were done with HeLa cells incubated with AuNP 1 for 30 minutes. The gold amount measured by ICP-MS in the etched and non-etched samples were converted to AuNP amount through division by 48.62 ng gold/ pmol AuNP. One way-ANOVA ($P<0.05$) was performed, n=3, all error bars represent standard deviation. Stars above the bars indicate significance, whereas no stars suggest not significantly different.

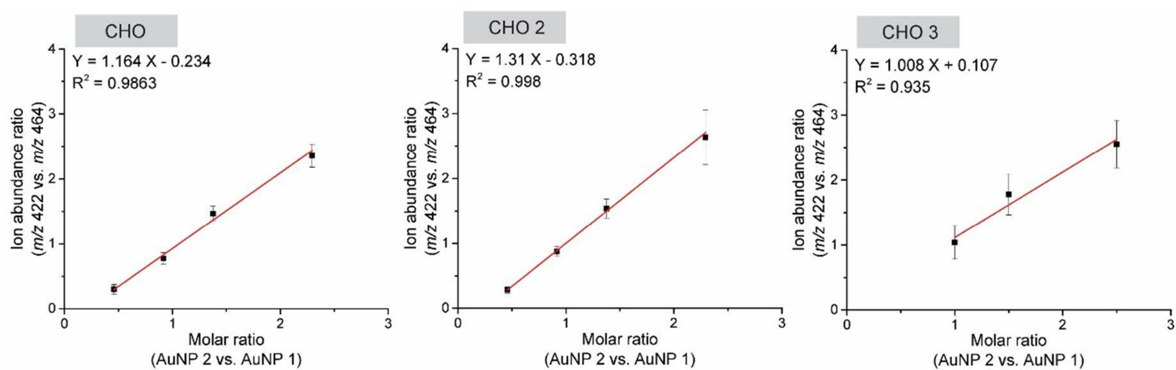

Figure S5. LDI-MS calibration curves for AuNP 2 on the cell surface. HeLa, CHO, CHO 2 and CHO 3 cells were cultured on ITO-glass slide. Increasing concentrations of AuNP 2 were mixed with AuNP 1 (internal standard) and incubated with cells as described in the text. Cells on the glass slide were detected using 38.6 μJ to only detect AuNPs on cell surface. Molecular ions of both AuNPs were plotted against molar ratios.

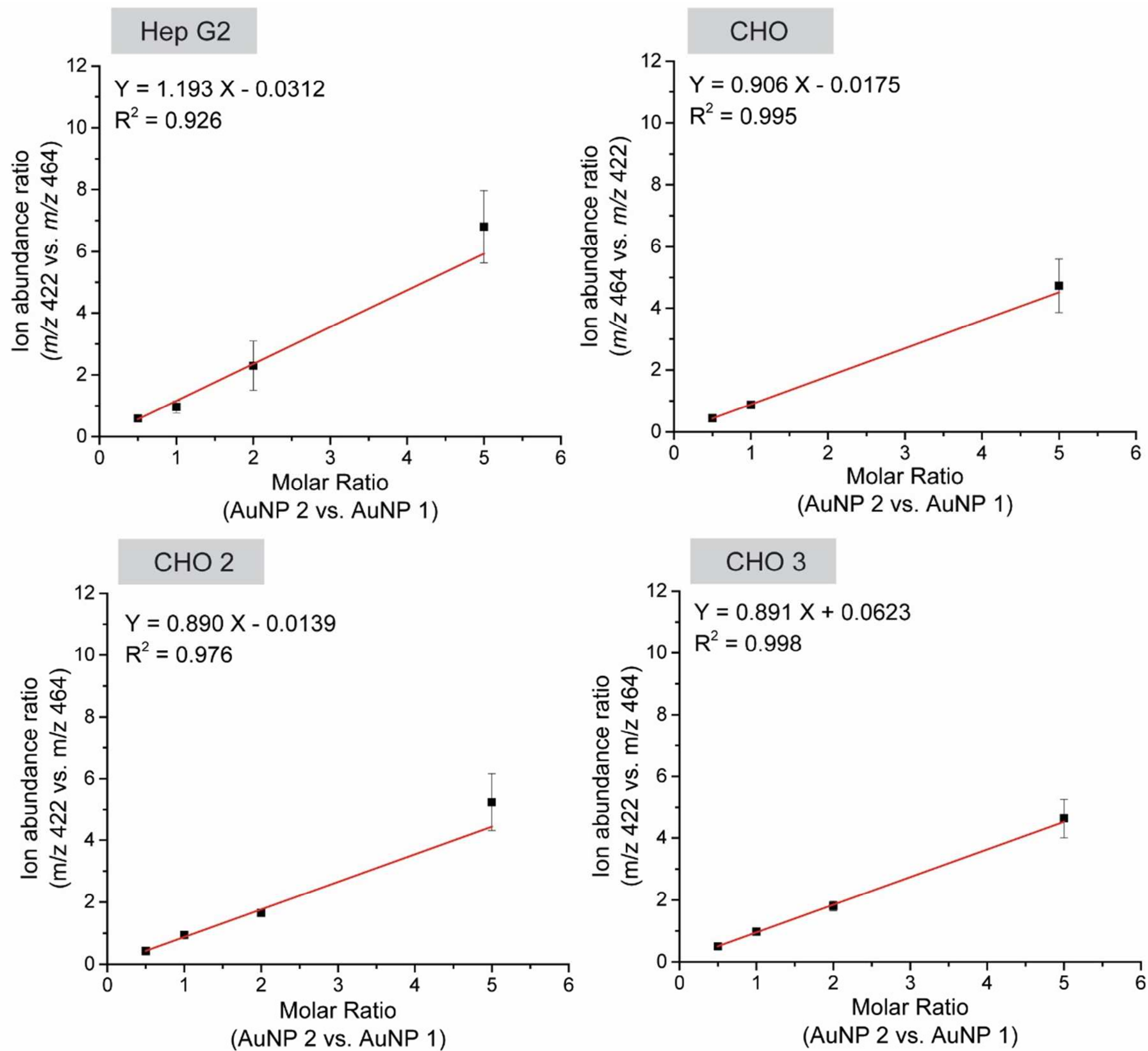

Figure S6. LDI-MS calibration curves for AuNP 2 in cell lysate. Increasing concentrations of AuNP 2 were mixed with AuNP 1 (internal standard) and spiked into cell lysate of HeLa, Hep G2, CHO, CHO 2 and CHO 3 cells. After centrifugation, the resulting pellets were deposited on a stainless steel target and analyzed with LDI-MS. Molecular ions of both AuNPs were plotted against molar ratios.

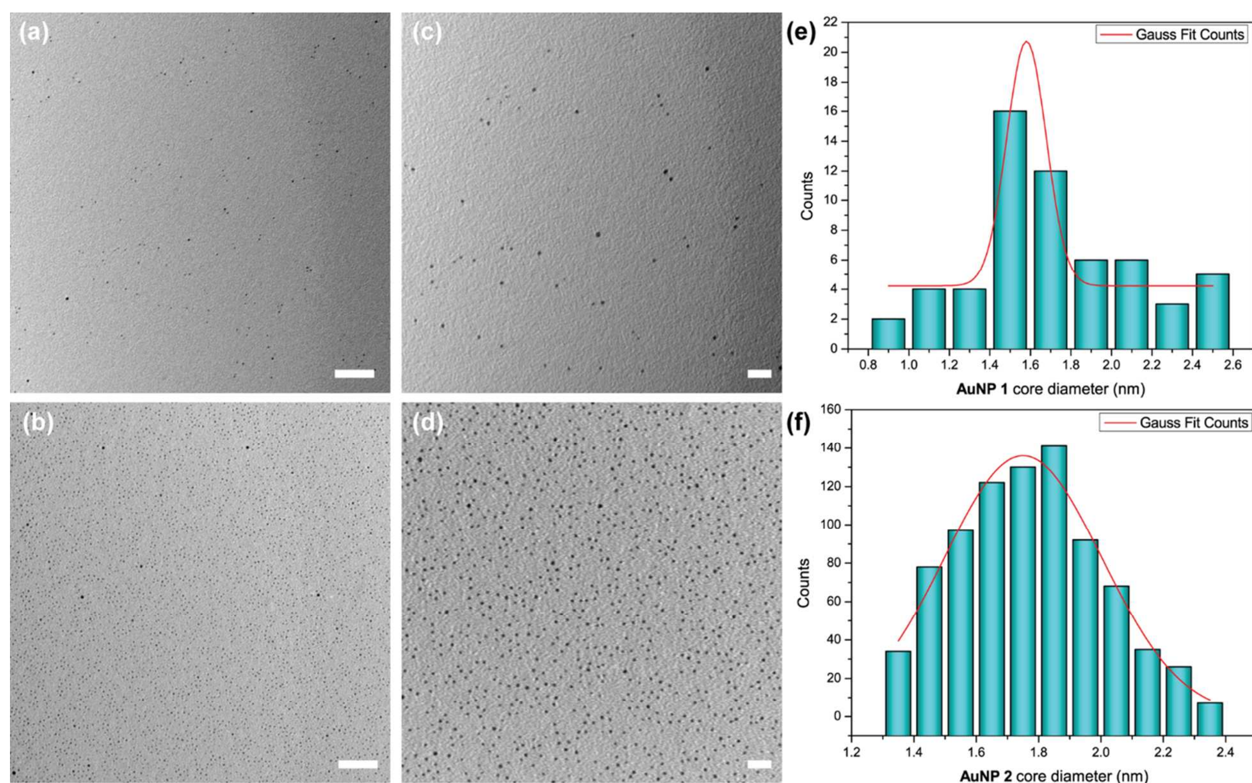

Figure S7. (a) ~ (d) TEM images of AuNPs: (a) & (c) AuNP 1; (b) & (d) AuNP 2. The white scale bar is 50 nm in (a) & (d) and 10 nm in (c) & (d). (e), (f) Core size distribution analysis for AuNPs corresponding to (c), (d), respectively: (e) AuNP 1: (1.6 ± 0.1) nm, and (f) AuNP 2: (1.7 ± 0.2) nm.

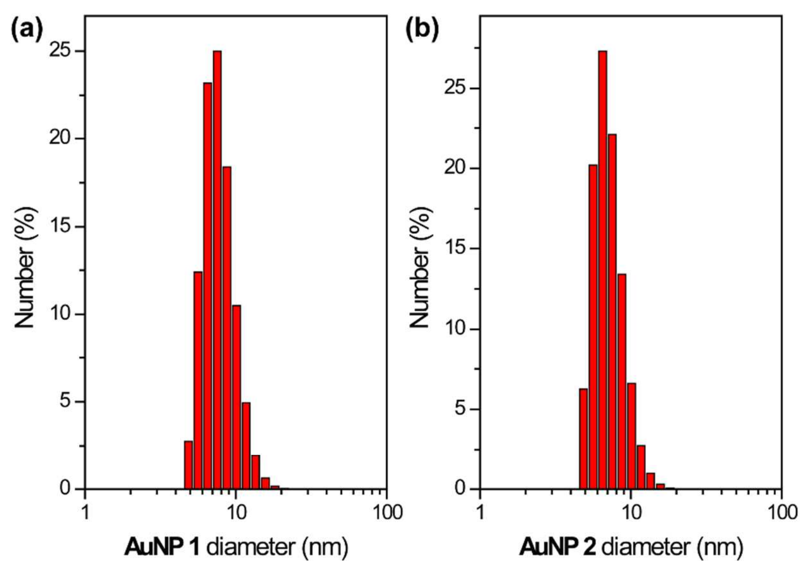

Figure S8. DLS histogram of AuNPs, demonstrating the hydrodynamic diameter distribution of nanoparticles: (a) AuNP 1 and (b) AuNP 2.

Calculation of absolute quantity of total, internalized and cell surface-bound AuNP 2 by coupling LDI-MS and ICP-MS

After incubation and addition of the internal standard (AuNP 1), the cells are lysed for ICP-MS detection. The gold amount measured from the sample is denoted by X (ng). X arises from contributions from the gold amounts of AuNP 2 (X (AuNP 2) total) and AuNP 1 (X (AuNP 1)). The LDI-MS measured molar ratio between AuNP 2 and AuNP 1 is denoted by Y. Ytotal represents the molar ratio of the total amounts of AuNP 2 to AuNP 1. With equation (1), the absolute amount of total AuNP 2 with cells can be calculated.

Equation (2) describes the absolute amount of AuNP 2 on the cell surface. Ysurface represents the molar ratio of cell surface-bound AuNP 2 to AuNP 1. By subtracting AuNP 2 on cell surface from total amount of AuNP 2 in equation (3), absolute amount for internalization can be determined.

$$\begin{cases} X\ (AuNP\ 2)_{total} = X - X\ (AuNP\ 1) \\ \dfrac{X\ (AuNP\ 2)_{total}}{X\ (AuNP\ 1)} = Y_{total} \end{cases} \quad (1)$$

$$X(AuNP\ 2)_{surface} = \frac{Y_{surface}}{Y_{total}} \times X(AuNP\ 2)_{total} \quad (2)$$

$$X(AuNP\ 2)_{internalization} = X(AuNP\ 2)_{total} - X(AuNP\ 2)_{surface} \quad (3)$$