Electronic supporting information for **Single-Particle ICP-MS with Online Microdroplet Calibration: Toward Matrix Independent Nanoparticle Sizing**

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Table S1: Overview of the different calibrant and matrices solutions used in experiments. Cs was used as tracer element to monitor droplet delivery in most cases. “MDG” refers to micro-droplet generator.

| Sampling position variation – Figure 2 |
|---------------------------------------|
| **Solution introduced via MDG**        | **Solution introduced via PN and SP** |
| 69 µm diameter microdroplets           | 80 nm Au NPs diluted in ultra-high purity (UHP) water |
| 100 ng g⁻¹ Au and Cs prepared in 1% HCl and 1% HNO₃ | |

| Nebulizer gas flow variation – Figure 3 |
|----------------------------------------|
| **Solution introduced via MDG**        | **Solution introduced via PN and SP** |
| 70 µm diameter microdroplets           | 80 nm Au NPs diluted in UHP water and spiked into 10 ng g⁻¹ dissolved isotopically enriched ¹⁰⁹Ag |
| 200 ng g⁻¹ Ag prepared in 2% HNO₃     | |

| Acid-related matrix effects – Figure 4 |
|----------------------------------------|
| **Solution introduced via MDG**        | **Solution introduced via PN and SP** |
| 69 µm diameter microdroplets           | SiO₂ shelled Au NPs diluted in: |
| 100 ng g⁻¹ Au and Cs in 1% HCl and 1% HNO₃ | - 1-10 (v/v)% HNO₃ |
|                                          | - 1-10 (v/v)% HCl |

| Single-element matrix effects: Accounting for space-charge effects – Figure 5 |
|---------------------------------------------------------------------------|
| **Solution introduced via MDG**                                           | **Solution introduced via PN and SP** |
| 60 µm diameter microdroplets                                              | 80 nm Au NPs diluted in: |
| 100 ng g⁻¹ of Ag, Cs, Ce, Th and U in 1% HNO₃                            | - ultra-high purity (UHP) water |
| 80 nm Au NPs diluted in:                                                   | - 4.8·10⁻⁶ mM, 4.4·10⁻⁵ mM, 4.8·10⁻⁴ mM, 4.0·10⁻³ mM, 4.8·10⁻² mM, 4.8·10⁻¹ mM, and 4.8·10⁰ mM of Li, Mg, Lu, and Pb respectively |
| In a subsequent measurement, 10 ng g⁻¹ dissolved Ag was prepared in UHP, 4.8·10⁻⁶ mM, 4.4·10⁻⁵ mM, 4.8·10⁻⁴ mM, 4.0·10⁻³ mM, 4.8·10⁻² mM, 4.8·10⁻¹ mM, and 4.8·10⁰ mM of Li, Mg, Lu, and Pb respectively. Results are displayed in Figure S6. |

| Phosphate Buffered Saline matrix effects – Figure 6                      |
|-------------------------------------------------------------------------|
| **Solution introduced via MDG**                                         | **Solution introduced via PN and SP** |
| 65 µm diameter microdroplets                                             | 80 nm Au NPs diluted in: |
| Multi-element solution containing 100 ng g⁻¹ of Mg, Al, Cr, Mn, Fe, Ni, Co, Cu, Zn, Ga, Rb, Sr, Mo, Ag, In, Sn, Sb, Cs, Ba, La, W, Pt, Au, Pb, Bi, Th, U in 2% HCl and 1% HNO₃ | - ultra-high purity (UHP) water |
|                                                                         | - PBS diluted by factors of 2, 5, 10 and 100 |
Synthesis and characterization of the SiO2 shell around the 80 nm Au gold nanoshperes

Synthesis: The following procedure was adapted from Graf et al.\textsuperscript{1} for coating larger Au NPs. All reaction steps for coating the gold nanoparticles (Au-NP) with silica (SiO\textsubscript{2}) were carried out under exclusion of light. Before any manipulation, the Au NPs batch was dispersed with ultrasound for 5 min. First, the Au NPs were functionalized with polyvinyl pyrrolidine (PVP). 25.6 mg of PVP (M\textsubscript{w} 10’000 g/mol, Sigma Aldrich) were added to 1 mL ultrapure water (Milli-Q) and dissolved by ultrasonification for 15 min. This provides enough stock solution for about 100 coating reactions. Per coating reaction, 8.3 µL of the PVP stock were mixed under stirring with 1.1 mL Au-NP stock (79.7 ± 7 nm, 9.7·10\textsuperscript{9} particles/mL, NanoComposix) and 0.733 mL ultrapure water. The reaction vessel was shaken at 600 rpm for 24 h. The particles were then transferred to ethanol through centrifugation at 2000 rpm for 40 min. The supernatant was eliminated and 0.3 mL of a solution of 4.2%vol Ammonia (25% in water, VWR) in ethanol were added. The particles were re-suspended by ultrasonification for 10 min. Immediately after, 2 µL of 1.37% tetraethyl orthosilicate (TEOS) in ethanol were added under stirring. The amount of TEOS was calculated to yield a desired coating thickness, namely 30 nm. The reaction was stirred with a magnet at 600 rpm for 12 h. After successful coating, the SiO\textsubscript{2}-shelled Au NPs were transferred from ethanol to ultrapure water by centrifugation at 5000 rpm for 5 min, followed by 2 consecutive washing steps in ultrapure water.

Characterization: Following the synthesis procedure, the particles were diluted by a factor x100 in ethanol for characterization. 1.5 µL of diluted silica coated Au-NP were put on a TEM grid with graphene support film. The SEM images were acquired with a Scanning Electron Microscope (Nova NANOSEM 450). Figure SI 1 displays the commercially available Au NPs (top) and the synthesized SiO\textsubscript{2} shelled Au NPs (bottom).
Figure S1: STEM image of the synthesized SiO$_2$ shell (bottom) around the 80nm Au NPs from Nanocomposix (original NPs top).
Explanation – Sensitivity calculation

In this study, we determined the elemental sensitivity $S_{\text{Analyte}}$ (counts/g) of the ICP-MS by introducing monodisperse droplets doped with the analyte of interest into the ICP; because the solution concentration and droplet size are known, the analyte mass entering the plasma can be calculated (1). Using this sensitivity as a calibration factor, the counts recorded per NP can be converted to a mass $M_{NP}$ (2) and finally, assuming a spherical shape and the same density ($\rho$) as the bulk material, converted to a diameter $Size_{NP}$ (3).

$$S_{\text{Analyte}} = \frac{\text{Average Counts}_{\text{Analyte/droplet}}}{\text{Mass}_{\text{Analyte/droplet}}} \quad (1)$$

$$M_{NP} = \frac{\text{Counts}_{\text{NP}}}{S_{\text{Analyte}}} \quad (2)$$

$$Size_{NP} = \frac{3}{\pi \rho_{\text{Analyte}}} \sqrt{\frac{6 M_{NP}}{\pi \rho_{\text{Analyte}}}} \quad (3)$$

Sizing of 80-nm Au NPs using the above strategy is shown in Figure S2, where the size distribution on the left was calibrated using the sensitivity determined from microdroplets recorded at the start burst and on the left, from the end burst respectively. This online microdroplet calibration also provides a means to correct for analyte intensity drift.

![Figure S2](image)

**Start**: 83 ± 12 nm  
**End**: 82 ± 12 nm

*Figure S2*: Size distributions of 80 nm Au NPs calibrated with sensitivities determined from start (right) and end (left) droplet bursts.
Box and whisker plot for NP analysis

A box and whisker plot is a standardized graphical way of summarizing a set of measured data in explanatory data analysis, introduced in 1969 by the mathematician John W. Tukey. This type of visual display allows to show the spread and centre of a data set. Even though boxplots do not describe a distribution in as much detail as histogram do, they are especially useful to analyse large data sets and to compare multiple data sets between each other. In the example presented above, the signal intensity frequency distribution for nominal 60-nm Au NIST NPs (RM 8013) is presented, but the same graphical representation can be applied to diameter distributions. The histogram of signals from NIST 60-nm Au NPs is used here for illustrative purposes because the uniform size of these Au NPs allows for a normally distributed signal histogram. Note that in skewed distributions, observation points found outside the whiskers are not necessarily real outliers and that such skewed distributions will require an adjusted boxplot approach.

![Box and whisker plot with annotations](image)

**Figure S3:** In the above box and whisker plot, the median is represented as a line at the height of the sample median Q2. The box part comprises 50% of the data and is named the interquartile range (IQR=Q3-Q1). It is delimited by the first quartile (25%) and the third quartile (75%). The whiskers represent the minimum and maximum values in a simple case, without outliers. However, real NPs data set may display surprisingly high maximums (agglomerates) as well as low minimums (dissolved analyte). These values are plotted as individual points and are described as outliers. In NP analysis, outliers are not considered here as “bad” data points, as they may be the key to the observed phenomenon. Indeed, they may reveal crucial information regarding the state of the solution under analysis, i.e. presence of aggregates. Additional information such as the mean can be added to the box plot (here the small white box).
Figure S4. For measurement of matrix effects on Ag solution, NPs, and droplets, all were introduced simultaneously into the ICP and can be differentiated based on their isotopic mass and signal intensity differences between signals from NPs and droplets. a) Histogram of $^{107}\text{Ag}$ signal shows three distributions that are clearly separated, i.e. that from solution background, Ag NPs, and Ag in droplets. b) The histogram of $^{109}\text{Ag}$ signal shows two histograms: a signal distribution from dissolved isotopically enriched $^{109}\text{Ag}$ and the signal from dissolved $^{109}\text{Ag}$ in the droplets on top of the steady-state $^{109}\text{Ag}$ signal from sample introduced through the pneumatic nebulizer / spray chamber. Due to the lower signal intensity from the Ag NPs, $^{109}\text{Ag}$ signal from the NPs cannot be differentiated from the dissolved solution of isotopically enriched $^{109}\text{Ag}$ based on the $^{109}\text{Ag}$ signal alone. In a and b, the coloured highlights show the signal regions used to determine signals from Ag NPs, Ag in droplets, and dissolved Ag for the matrix effect study in Figure 3 of the manuscript. Zoom-in views of the histograms of the $^{107}\text{Ag}$ signals from NPs and microdroplets are given in c and d, respectively.
Figure S5: In the acid matrix study, the sizes of SiO$_2$-shelled 80 nm Au NPs were assessed. The synthesis of the SiO$_2$-shell was carried out on monodisperse nominal 80 nm Au NPs purchased from NanoComposix. a) During the analysis, a large variation in the magnitude of the NP peaks was observed in the transient time trace. As the magnitude of these peaks is proportional to the mass ionized and detected, one can assume that large agglomerates were present in the solution. The dotted line shows the 100-counts threshold (expected signal intensity for an 80 nm Au NP). b) By using microdroplets with known size and known concentration, the sensitivity can be determined for calibration, and based on our measurements, one would expect a single 80 nm Au NP to produce a signal of roughly 102 counts. c and d) Single NPs as well as large agglomerates are clearly recognizable on the SEM-images of the solution analysed. e) The typical workflow for NPs analysis is to bin the raw data into a histogram and set a threshold for low count signals originating from the background. The resulting fractions of NPs signals can then be converted to their corresponding masses and sizes. In case A (red data), all NPs signals above the low count threshold are taken into account, while in case B (blue data), an upper count threshold of 200 counts was set, so that agglomerates are not included in the size distribution. Consequently, the size histograms present two different shapes, which can be fitted with a lognormal fit in the first case, and a normal fit in the second.
Table S2. Statistics on data plotted above in Figure S5, subplot e, for case A and case B. As explained above, many agglomerates were present in the solution and these can strongly influence descriptive statistics. Indeed, when calculating the mean (black square) with the whole data set, it is heavily influenced by outliers, and results in a mean size of 102 nm for 80 nm Au NPs. However, if the data set is truncated by a realistic upper threshold (i.e. 200 counts, which would represent 2x 80 nm Au NPs), then the mean decreased down to 81 nm. The histogram then also resembles a normal distribution, where the mean equals the median and the mode. The mode is 77 nm for both cases. This number can easily be biased due to insufficient data sampling and does not describe meaningfully a multimodal distribution or its broadness. Due to the low count threshold which was the same in both cases, the same minimum size was obtained. The median (black line) is a very useful quantity for describing any datasets, where the mean is likely to be biased due to large measured diameters. By determining the median for the complete set, a size overestimation of 10% was obtained, which is still within the boundaries (80 ± 9 nm), while the mean yielded a size overestimation of 27%. Finally, a maximum size of 355-nm was determined for the complete dataset, which most likely corresponds to an agglomerate of 87x 80-nm Au NPs.

|        | N total | Diameter (nm)          |
|--------|---------|------------------------|
|        |         | Mean | Std | Mode | Minimum | 1st Quartile (Q1) | Median | 3rd Quartile (Q3) | Maximum |
| Case A) All data | 1538    | 102  | 39  | 77   | 39       | 80       | 88       | 114     | 355     |
| Case B) Upper Threshold | 998     | 81   | 10  | 77   | 39       | 76       | 83       | 87      | 101     |
Figure S6: Overview of the matrix induced change in signal intensity recorded for the NPs and microdroplets as a function of matrix concentration for Li (a), Mg (b), Lu (e) and Pb (f). In order to increase S/N for the NPs, signal from both isotopes $^{107}$Ag and $^{109}$Ag were combined. The data were normalized with respect to reference measurements with no matrix in each set and show the extent of the signal suppression for each analyte. Additionally, one can observe the trend for the dissolved silver introduced through the nebulizer in a subsequent measurement series with matching concentrations of elemental matrix (see Table S1 for details). As it was measured in a second round, without any NPs nor microdroplets, the isotope $^{107}$Ag was monitored. Subplots (c), (d), (g) and (h) show the resulting sizing using the microdroplets as calibrant in the Li, Mg, Lu and Pb-matrices respectively. The dashed green lines span the size range expected from the manufacturer. As the trend observed for the dissolved Ag introduced via PN is similar to that of the NPs; this supports the possibility for matrix-matched external calibration.
References:

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