

Supplementary information

Characterizing Nanoparticles Mass Distributions Using Charge-Independent Nano-resonator Mass Spectrometry

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S1. Repeatability study

Together with the data displayed in Figure 1, repeatability studies have been conducted for sphere-like gold NPs presented in this work. The following tables report the Gaussian parameters (mean and standard deviation) in different experiments.

Table S1.1 – Repeatability for the gold 15 nm nanoparticle sample.

Experiment ID	Number of events	μ [MDa]	σ [MDa]
1	398	18.7	5.2
2	1298	18.6	5.9
3	884	17.4	5.5
4	857	17.5	5.3

Table S1.2 – Repeatability for the gold 30 nm nanoparticle sample.

Experiment ID	Number of events	μ [MDa]	σ [MDa]
1	92	155.0	23.6
2	74	153.8	24.4
3	63	150.5	27.0
4	225	158.0	26.6
5	198	160	27.2

S2. Silica density characterization

The following section describes the calculations underlying the data that are presented in **Figure 2** of the paper. The raw data are displayed in

Table S2.1 – Repeatability for the silica 50 nm nanoparticle sample.

Experiment ID	Number of events	μ [MDa]	σ [MDa]
1	154	91.6	13.1
2	94	88.8	10.4
3	75	88.4	8.9
4	102	85.1	10.9
5	70	86.4	10.4
6	194	87.6	11.7
7	48	90.3	10.5
8	73	85.4	6.3

Table S.1 and **S2.2** respectively. It provides results of silica nanoparticles mass analysis (NEMS-MS) and diameter analysis, which have been measured with two different techniques (TEM and SEM).

Table S2.1 – Repeatability for the silica 50 nm nanoparticle sample.

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3	75	88.4	8.9
4	102	85.1	10.9
5	70	86.4	10.4
6	194	87.6	11.7
7	48	90.3	10.5
8	73	85.4	6.3

Table S2.2 – Raw data used to compute the density of the silica nanoparticles.

	Mean value	Uncertainty	Upper limits	Lower limits
Mass [MDa]	87.9	1.6	89.5	86.3
TEM size [nm]	51.9	1.2	53.1	50.7
SEM size [nm]	53.8	0.8	54.6	53

Based on these values, the two values of the density can be derived. The first one has computed thanks to the TEM size measurement, the second one is used using SEM data (cf. **Table S2.3**)

Table S2.3 – Density calculations and their associated upper and lower limits.

Mean value	Upper limits	Lower limits
TEM		

Density [g/cm ³]	1.99	-	-
Mass error [g/cm ³]	-	2.03	1.96
Total error [g/cm ³]	-	2.18	1.83
SEM			
Density [g/cm ³]	1.79	-	-
Mass error [g/cm ³]	-	1.82	1.76
Total error [g/cm ³]	-	1.91	1.68

The density is computed as the ratio between the NEMS-MS measured mass and the volume computed using the diameter (SEM or TEM, respectively) assuming that the particles are spherical. The contribution of the mass to the error in density is computed using the following relationship:

$$\begin{cases} \rho_{mass\ error, upper} = \frac{m_{upper}}{4/3\pi r_{mean}^3} \\ \rho_{mass\ error, lower} = \frac{m_{lower}}{4/3\pi r_{mean}^3} \end{cases} \quad (S1)$$

And the total error is computed as :

$$\begin{cases} \rho_{total\ error, upper} = \frac{m_{upper}}{4/3\pi r_{lower}^3} \\ \rho_{total\ error, lower} = \frac{m_{lower}}{4/3\pi r_{upper}^3} \end{cases} \quad (S2)$$

S3. NTP (NanoTetraPods) mass calculation

The volume and mass of NTP were calculated by the following formulas:

$$V_{NTP} = V_{core} + 4V_{arm} = V_{core} + 4 \times \left[\frac{1}{3}\pi(h-r)(R^2 + r^2 + Rr) + \frac{1}{2} \times \frac{4}{3}\pi r^3 \right] \quad (S3)$$

The core is the cuboctahedron, which is between the sphere and cube. The volume of the core is about 527 ~ 1006 nm³, and the volume of an NTP is 3803.9 ~ 4283.2 nm³.

$$m_{NTP} = \rho_{Au} \times V_{NTP} = 19.32 \text{ g/cm}^3 \times V_{NTP} \quad (S4)$$

The mass of an NTP is about 44.3 ~ 49.8 MDa. The volume and mass of other shape nanoparticles were calculated similarly, and the result was summarized in Table 2.

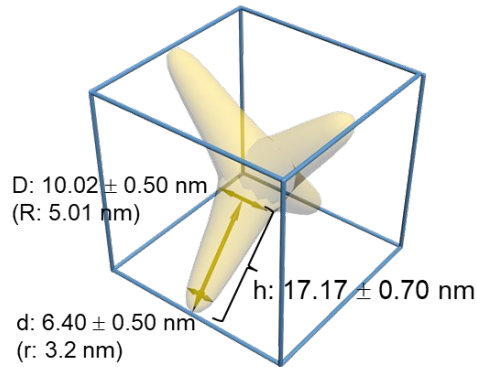


Figure S3 – Model of individual NTPs according to TEM images, the dimensions based on TEM result of more than 100 NTPs.

S4. Measurement of the mass of grafted PEG on NTPs by NMR

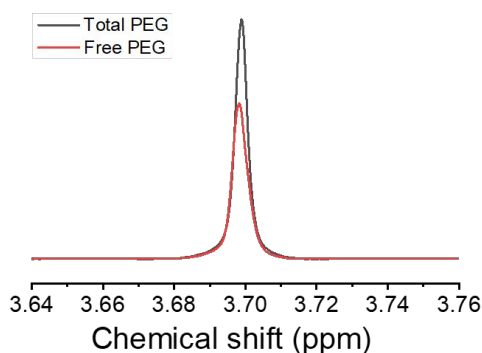


Figure S4 – ^1H NMR spectra of total PEG (total PEG added to the NTPs) and free PEG (PEG in the supernatant after grafting).

In order to get the grafting number of PEG on an NTP, we measured the ^1H NMR spectra of PEG in supernatant according to the method reported before¹ [1]. Specifically, 0.50 mg/mL PEG-SH was added into NTP solution to graft PEG on NTPs. Then, the sample was centrifugated to completely remove the NTPs with grafted PEG from the solution and left the free PEG in the supernatant. We measured the ^1H NMR spectra of 0.50 mg/mL PEG and free PEG, and the free PEG concentration was obtained by calculating integral areas at 3.7 ppm in NMR spectra. The grafted PEG concentration was obtained by the difference between 0.50 mg/mL PEG and free PEG. The grafting number of PEG on an NTP was calculated by dividing the PEG concentration by NTP concentration. The specific values are shown in Table S1. The mass of PEG on an NTP was obtained by the following formula:

$$m(\text{PEG}) = \frac{M(\text{PEG})}{N_A} \times n(\text{PEG}/\text{NTP}) = 9.8 \text{ MDa} \quad (\text{S5})$$

Table S4 – Calculation of grafted PEG on an NTP by NMR.

	Integral area (37 ppm)	Concentration (PEG)	Concentration (Grafted PEG)	Concentration (NTPs)	Grafting number
Total	1.56826	100 μM	-	-	-
Free	1.16722	74.43 μM	-	-	-
Grafting		-	17.24 μM	8.92 nM	1954 \pm 25 NTP ⁻¹

S5. Measurement of NTP mass by ICP-MS

We measured the ICP-MS of NTPs with a concentration of about 5×10^{11} NTPs/mL (the same concentration for NEMS-MS measurement), and the ICP-MS result shows the Au atom concentration is about 0.0403 ± 0.0008 g/L. The mass of NTP was obtained by the following formula:

$$m_{NTP} = \frac{c(Au \text{ atom})}{c(NTP)} = 48.5 \pm 1.0 \text{ MDa} \quad (\text{S6})$$

S6. NEMS-MS architecture

NEMS-MS is based on particle mass measurement using a NEMS sensor (*cf.* Section o) which is actuated under vacuum. Thus, the architecture aims to deliver an aerosol of solid particles from the source (electrospray ionization in this work) to the sensor in such a way that the losses and focalization of the particles are reduced as much as possible.

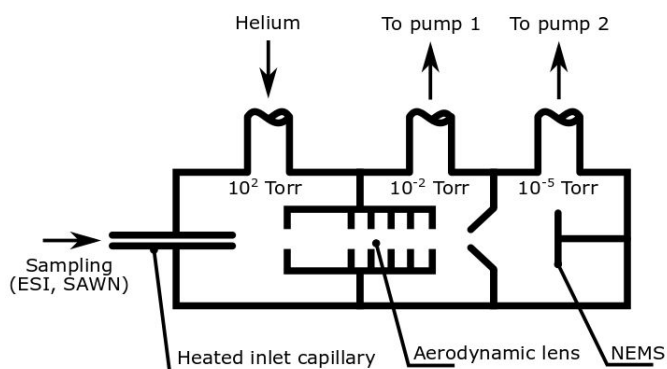


Figure S6 – Schematic of the NEMS-MS architecture, featuring its operating pressures.

Thus, after being generated by the ESI, the aerosol is sucked into the vacuum chambers through a heated inlet capillary. The purpose of this step is to remove the liquid phase out of the solid particles surface. The aerosol is then focused by an aerodynamic lens (*cf.* Section S7) that produces a narrow particle beam. A skimmer is used to maintain a lower pressure in the NEMS chamber, where the nanoparticles land on the NEMS beams surface. The turbomolecular pumps used to pump the lens and NEMS chambers down have a speed of 450 l/s and 250 l/s respectively. The throughput of the system is estimated to be approximately 1-2 Torr.l/s.

S7. Aerodynamic focusing

The geometric dimensions of the aerodynamic lens system used to focus nanoparticles on the sensor is described hereunder in **Figure** and **Table S3**. The upstream and downstream pressures are respectively 10^2 Torr and 10^{-2} Torr . The performance of the lens have been characterized using polystyrene nanoparticles in a previous work².

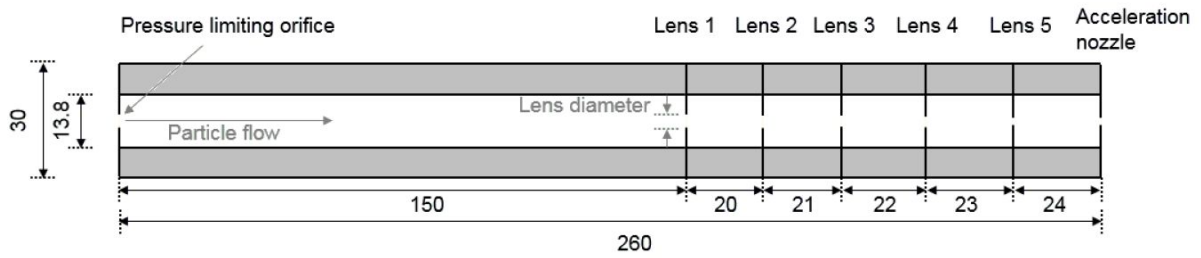


Figure S7 – Schematic of the aerodynamic lens used to focus nanoparticles on the NEMS sensor.

Table S3 – Dimensions of the lens.

	Pressure limiting orifice	Lens 1	Lens 2	Lens 3	Lens 4	Lens 5	Acceleration nozzle
Diameter [mm]	0.3	3.45	3.25	3.02	2.72	1.97	1.9
Spacer L [mm]	150	20	21	22	23	24	-
Tube D [mm]	13.8	13.8	13.8	13.8	13.8	13.8	-

S8. NEMS particle sensor

A typical NEMS used in this study is shown by **Figure S**. It shows only one NEMS among the 20 devices that compose the array of the sensor. The length of every devices slightly differed, ranging from 7.61 μm to 10 μm (See **Table S8**), resulting in different resonance frequencies for each resonator ($f \propto l^{-2}$). This design feature allows to produce a frequency-addressed NEMS array (5x4), which increases the capture cross section without degrading the mass resolution. The resonance frequencies for the first and second flexural mode range from 26 to 40 MHz and 75 to 120 MHz respectively.³

Beam length (L)	Beam width (w)	Beam thickness (t)	Anchor/gauge distance (L_1)	Gauge length (b)	Gauge width (w_1)
7.61-10 μm	300 nm	160 nm	305-400 nm	400 nm	80 nm

Table S8 : Typical dimensions of the nano-resonator devices used in the present study. Ranges pertain to the variable dimensions of individual devices in an array of 20 frequency-addressed resonators.

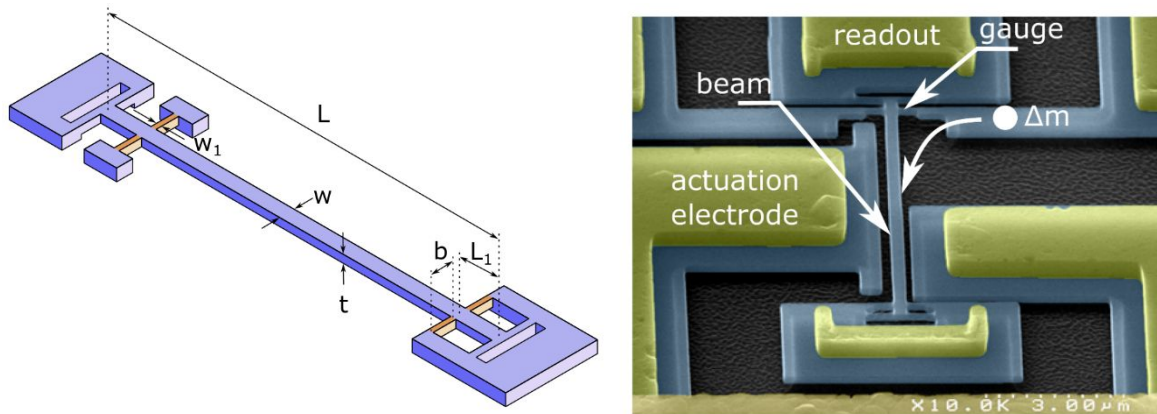


Figure S8 – Left panel: Sketch of the nano-resonator devices discussed in the present work (not to scale). The elongated doubly clamped beam oscillates laterally (in the x/y plane); two piezoresistive nanogauges (in orange, top) placed in a bridge configuration capture these oscillations. The other end of the beam is not electrically contacted but the mechanical design preserves mode symmetry. Right panel: False color SEM micrograph of one resonator in the 20 NEMS array showing the sensing element (beam), actuation electrodes and readout gauges.

The beams are actuated electrostatically and the signal is sensed thanks to the piezoresistive nanogauges as shown in **Figure S**. In order to perform frequency shifts measurements, each structure has to be actuated at its resonance frequency and is locked sequentially in time with

a Phase Locked Loop (PLL). The two modes of each of the 20 NEMS are measured simultaneously for 10 ms. Since 10 ms are spent for every beam, they are locked every 200 ms (20 NEMS x 10 ms).

References

- (1) Lu, J.; Xue, Y.; Shi, R.; Kang, J.; Zhao, C. Y.; Zhang, N. N.; Wang, C. Y.; Lu, Z. Y.; Liu, K. A Non-Sacrificial Method for the Quantification of Poly(Ethylene Glycol) Grafting Density on Gold Nanoparticles for Applications in Nanomedicine. *Chem. Sci.* **2019**, *10* (7), 2067–2074. <https://doi.org/10.1039/C8SC02847H>.
- (2) Dominguez-Medina, S.; Fostner, S.; Defoort, M.; Sansa, M.; Stark, A. K.; Halim, M. A.; Vernhes, E.; Gely, M.; Jourdan, G.; Alava, T.; Boulanger, P.; Masselon, C.; Hentz, S. Neutral Mass Spectrometry of Virus Capsids above 100 Megadaltons with Nanomechanical Resonators. *Science (80-.)*. **2018**, *362* (6417), 918–922. <https://doi.org/10.1126/science.aat6457>.
- (3) Sage, E.; Sansa, M.; Fostner, S.; Defoort, M.; Gély, M.; Naik, A. K.; Morel, R.; Duraffourg, L.; Roukes, M. L.; Alava, T.; Jourdan, G.; Colinet, E.; Masselon, C.; Brenac, A.; Hentz, S. Single-Particle Mass Spectrometry with Arrays of Frequency-Addressed Nanomechanical Resonators. *Nat. Commun.* **2018**, *9* (1), 1–8. <https://doi.org/10.1038/s41467-018-05783-4>.