The identification of species is a fundamental problem in analytical chemistry and biology. Mass spectrometers identify species by their molecular mass with extremely high sensitivity ($< 10^{-24}$ g). However, its application is usually limited to light analytes ($< 10^{-19}$ g). Here we demonstrate that by using nanomechanical resonators, heavier analytes can be identified by their mass and stiffness. The method is demonstrated with spherical gold nanoparticles and whole intact *E. coli* bacteria delivered by electrospray ionization to microcantilever resonators placed in low vacuum at 0.1 torr. We develop a theoretical procedure for obtaining the mass, position and stiffness of the analytes arriving the resonator from the adsorption-induced eigenfrequency jumps. These results demonstrate the enormous potential of this technology for identification of large biological complexes near their native conformation, a goal that is beyond the capabilities of conventional mass spectrometers.
mass spectrometry (MS) measures the mass-to-charge ratio of molecular species from 100 Da to 100 kDa with extremely high accuracy\(^1\). However, the performance largely degrades on the measurement of heavier species such as nanostructures and biological assemblies. In these cases, the charged analytes are successively fragmented by several dissociation methods causing a complex mass spectrum pattern that is not readily convertible into the original analyte. Nanomechanical resonators, such as singly and doubly clamped beams at the micro- and nanoscale, have recently emerged for measuring the mass of analytes with outstanding sensitivity\(^3\)–\(^7\), \(\sim\) Da, and high dynamic range, enabling the measurement of nanostructures and large macromolecular complexes\(^8\). The physical principle is that the resonance frequency of the resonator is a sensitive function of its mass. The smaller the device, the more susceptible is the resonant frequency to minuscule added masses\(^9\),\(^10\). The unparalleled attributes of nanomechanical resonators have motivated a new paradigm of MS, referred to as nanomechanical spectrometry that enables the measurement of the mass of entire analytes with no need of charge state characterization\(^9\)–\(^11\). In nanomechanical MS, the species are usually introduced by electrospray ionization (ESI) from the fluid phase into vacuum and are subsequently guided by ion optics to the resonator in high vacuum (<10\(^{-5}\) torr)\(^9\). Alternatively, matrix-assisted laser desorption ionization can be used for delivering the sample to the resonator\(^10\). As each analyte adsorbs on the mechanical resonator, abrupt resonance frequency downshifts are observed that are proportional to the ratio of the analyte mass to the device mass with a proportionality constant that depends on the adsorption position. The nanomechanical signature is insensitive to the charge of the adsorbate, simplifying the analysis of the data\(^11\). Deconvolution of the mass and adsorption position along the resonant beam requires the simultaneous measurement of at least two vibration modes\(^10\),\(^12\),\(^13\).

We recently developed a theory on the mechanical coupling between biological particles and resonant beams that predicts the analyte stiffness may significantly influence the resonance frequency shifts on particle adsorption\(^14\). This effect is particularly relevant when the particle thickness is comparable to the beam thickness and it depends on the elastic modulus and geometry of the particle, as well as on the interfacial adhesion between the particle and the resonator. However, this theoretical prediction has not been experimentally confirmed in nanomechanical spectrometry assays. In general, the stiffness effect is smaller and it can be easily hidden by the mass effect. Theoretical methods for extracting the mass of the particles landing on the resonator thus neglect the stiffness. This simplification also reduces the complexity of the inverse problem for deriving the particle mass and position from the resonance frequency jumps.

In this study, we perform nanomechanical spectrometry of 100 nm-sized gold nanoparticles (GNPs) and *Escherichia coli* DH5\(\alpha\) cells using microcantilever resonators. We develop theoretical methods that enable the determination of the stiffness, mass and position of the analytes arriving the microcantilever from the resonance frequency jumps. Ignoring the effect of the stiffness leads to an underestimation of the mass of \(\sim 10\%\) for the used microcantilevers. More importantly, we estimate the Young’s modulus of the *E. coli* cells, which is consistent with the value obtained by atomic force microscopy (AFM).

**Results**

**Nanomechanical spectrometer system.** Figure 1a schematically depicts our prototype of nanomechanical spectrometer that comprises three stages with decreasing pressure. At the first stage, an ESI unit is used to generate mostly desolvated charged species at ambient pressure. The charged species are immediately attracted by the second stage, a heated metallic capillary at 200 °C and vacuum pressure \(\approx 10\) torr placed at 5 mm below the ESI needle. The elevated temperature aids to fully desolvate the microdroplets and to prevent analyte loss by sticking to the internal wall of the capillary. The charged particles at the exit of the heated capillary are attracted by a skimmer with a 100 μm-wide orifice that connects with the third stage, a low vacuum chamber (\(\approx 0.1\) torr) in which the nanomechanical resonator is placed a few centimetres below the hole.

![Figure 1](image.png)

**Figure 1 | Nanomechanical spectrometer.** (a) Schematic of the experimental set-up. It comprises three chambers with decreasing pressure from top to bottom: the ESI chamber at ambient pressure, the heated capillary chamber at 10 torr and the resonator chamber at 0.1 torr. The charged species are driven to the nanomechanical resonator through the pressure gradient. (b) SEM image of a 100 nm-thick silicon nitride microcantilever used in the experiments with GNPs. (c) Schematic of the optical beam deflection method used for measuring the cantilever vibration. The cantilever is excited by means of a piezoelectric actuator (piezo) beneath the cantilever chip. Digital PLLs are used to track in real time the resonance frequency of the first flexural vibration modes. A, current-to-voltage amplifier; \(f_n\) = resonance frequency of \(n\)th mode; QPD, quadrant photodetector; \(v_n\), AC voltage signal at frequency \(f_n\), sent by the PLL to the piezoelectric actuator.
resonator (Fig. 1b) is driven by a piezoelectric actuator placed underneath and the vibration is detected by the optical beam deflection method\textsuperscript{15,16} (Fig. 1c). It is noteworthy to emphasize that the vacuum pressure of the resonator chamber is at least four orders of magnitude higher than in previous nanomechanical spectrometers. This moderately limits the attainable frequency resolution due to the fact that the quality factor is lower than in high vacuum\textsuperscript{17}. However, it allows to place the nanomechanical resonator close to the ion source (at 18 cm in comparison with ~2 m required in previous set-ups), which eliminates the need of ion guide optics. The proximity between the ion source and the nanomechanical detector is advantageous to achieve high capture efficiencies (one of the main limitations of both conventional\textsuperscript{18} and nanomechanical\textsuperscript{9–11} MS). In the case of GNP experiments, the ion beam at the detector chamber has a flow rate of ~4 particles per second, a cross-section diameter of 600 µm at the resonator’s position and a low divergence of ~0.2° (Supplementary Fig. 1 and Supplementary Discussion). The flow rate at the detector chamber represents ~0.24% of the particles emitted by the ESI needle. If we multiply this number by the ratio of the resonator’s plan view area to the ion beam cross-sectional area, we obtain a capture efficiency of ~10~^5. Larger efficiencies approaching 100% can be readily envisaged by using two-dimensional arrays of cantilevers\textsuperscript{13} combined with nano-ESI ionization\textsuperscript{19}.

**Theory of nanomechanical spectrometry.** During the steady-state motion of the cantilever, the average rates of kinetic energy and potential energy must be equal. This equality provides the resonance frequencies of the system and allows an easy understanding of the effect of particle adsorption. When a mass with no stiffness is added to the resonator, the frequency of the resonator must decrease, to keep constant the average rate of kinetic energy. Conversely, the adsorption of a massless particle with stiffness induces an increase of the potential energy of the system due to the energy cost associated to the bending of the particle and thus the frequency must increase, to keep the balance between the average rates of kinetic and potential energies. The mass effect on the resonance frequency increases when adsorption takes place at regions along the beam with higher amplitude (higher kinetic energy), whereas the stiffness effect increases in the regions where the beam undergoes higher changes of curvature during vibration (higher potential energy). Figure 2a,b schematically illustrate the effect of adsorbate adsorption on the resonance frequency of a cantilever. The fractional change of the resonance frequency for the rth vibration mode when an analyte lands on the beam can be expressed as the sum of the mass and stiffness contributions\textsuperscript{14,20,}

\[
\Omega_n = \frac{1}{2} \lambda_m \left\{ -\psi_m^2(\xi_0) + 3 C_\text{c} \kappa_m^2(\xi_0) \right\}
\]

where \(\psi_m\) is the mode shape, \(\xi_0\) is the longitudinal adsorption position normalized to the length of the beam, \(\lambda_m = m_m / m_b\) is the mass ratio of the adsorbate to the beam, \(\kappa_m = \psi_m^\prime(\xi_0) / (\int_0^1 \psi_m^\prime(\xi)^2 d\xi)^{1/2}\) is the normalized curvature of the mode shape and \(C_\text{c}\) is referred to here as adsorbate stiffness factor given by,

\[
C_\text{c} = \varepsilon \left( \frac{c_m}{c_b} \right)^2
\]

where \(c = \sqrt{E/\rho}\) is the one-dimensional speed of sound in the solid, \(E\) is the Young’s modulus and \(\rho\) is the density, and the subscripts \(a\) and \(b\) refer to the adsorbate and the cantilever, respectively. The dimensionless parameter \(\varepsilon\) includes (i) the linear and quadratic terms of the relative increase of flexural rigidity that become significant for relatively thick and stiff adsorbates\textsuperscript{21}, and (ii) the ratio between the effective volume of the adsorbate where the strain is confined and the actual volume of the adsorbate\textsuperscript{14}. This last term is smaller than unity, as the bending stress exerted by the beam on the adsorbate is partly screened by the free surface of the adsorbate. The parameter \(\varepsilon\) depends on the shape of the adsorbate and the contact area between the adsorbate and the cantilever, which depends on the adhesion energy, effective elastic modulus and size of the adsorbate\textsuperscript{14}. In general, \(\varepsilon\) must be numerically computed by the finite element method (FEM). Figure 2c shows FEM simulations of the bending strain distribution in the beam and in a quasi-spherical adsorbate for different contact areas. As predicted by Euler–Bernoulli beam theory, the strain in the beam increases linearly along the thickness direction. However, this behaviour is not observed in the adsorbate due to the screening effect of the free surface. The simulations show how the mechanical coupling between the beam and the adsorbate increases with the contact area.

**GNP measurements and inverse problem algorithm.** We start our experiments by analysing whether the stiffness of 100 nm-sized GNPs can induce a detectable effect on the resonance frequency of nanomechanical resonators. This is perhaps the most challenging test for our method, as the
mechanical coupling between the nanoparticles and the microcantilever is rather small due to the reduced contact area and the large surface-area-to-volume ratio of the nanoparticles. We use low-stress silicon nitride microcantilevers with nominal dimensions of $50 \times 15 \times 0.1 \mu m$ (Fig. 1b). The resonance frequencies of the first three flexural vibration modes are of $\sim 50$, 320 and 860 kHz, respectively (Supplementary Fig. 2), and are simultaneously tracked by three digital phase-locked loops (PLLs). The measurement of at least three vibration modes is required to decouple the effect of the adsorption position, mass and stiffness.

Figure 3a shows a real-time record of the fractional changes of the first three resonance frequencies during ESI of the GNPs. Simultaneous and sporadic jumps in the frequencies are observed with an average rate of two events per minute. The distribution of the GNPs on the cantilever after the experiment was characterized by scanning electron microscopy (SEM) (Fig. 3b). The distribution of the GNPs on the cantilever was rather small due to the reduced contact area and the large surface-area-to-volume ratio of the nanoparticles.

The analysis of data begins by selecting the time-correlated frequency jumps that fulfill the Allan deviation and (3) the joint probability density function. Mathematically, this is equivalent to finding the values that minimize the following functional

$$ F = \left( \Omega(\xi_0, \lambda_m, C_1) - M \right) \Sigma^{-1} \left( \Omega(\xi_0, \lambda_m, C_1) - M \right)^T $$

We apply the inverse problem method to the time-correlated frequency jumps, $(\Omega_1, \Omega_2, \Omega_3)$, obtained for each GNP landing on the microcantilever to obtain the position, mass and stiffness of the nanoparticles. The derived nanoparticle positions were in good agreement with the positions obtained by dark-field microscopy characterization of the cantilever (Supplementary Fig. 4). Figure 4a shows the two-dimensional probability distribution of the values of the mass ratio and stiffness factor $(\lambda_m, C_1)$ for 120 events. It is noteworthy that as long as the vibration mode shapes are known, the calculation of these parameters does not require previous knowledge on the cantilever properties (dimensions, density and Young’s modulus). We assume that the vibration mode shapes are described by Euler–Bernoulli beam theory. We find that the mean and s.d. of these orthogonal parameters $(\lambda_m, C_1)$ are $(39.8 \pm 13.2$ p.p.m., $0.031 \pm 0.023)$. To obtain accurate data on the mass and stiffness of the nanoparticles, the density and Young’s modulus of the cantilever must be calibrated. We harness the well-known effect of the hydrodynamic forces on the frequency response of cantilevers immersed in viscous fluids, to derive the spring constant and mass of the cantilever. SEM was used to measure the exact dimensions of the frequency response of cantilevers immersed in viscous fluids, to derive the spring constant and mass of the cantilever. SEM was used to measure the exact dimensions of the frequency response of cantilevers immersed in viscous fluids, to derive the spring constant and mass of the cantilever. SEM was used to measure the exact dimensions of the frequency response of cantilevers immersed in viscous fluids, to derive the spring constant and mass of the cantilever.
Mass and stiffness spectrometry of whole intact bacteria. We apply the nanomechanical spectrometry technique for accurate and high-throughput measurement of the mass and stiffness of individual bacterial cells. The relevance of this application is multifold. First, characterization of microorganisms is essential for rapid diagnosis and targeted treatment of infection. Most of the techniques study the average properties of populations, ignoring the large heterogeneities between individual cells. Our technology enables high-throughput analysis of individual microorganisms based on two orthogonal coordinates, the dry mass and the stiffness. Both parameters provide insights on how the structural conformation, pathological properties and mechanical properties are related to each other.

Dry mass is generally estimated by electron microscopy analysis or optical interferometry; however, these measurements are indirect and require assumptions about the material properties. On the other hand, the most widely used method to measure the mechanical properties of biological entities has been so far nanoindentation with the cantilever/tip assembly of an AFM. However, the AFM throughput is still a limiting factor. Here we use our nanomechanical spectrometer to characterize E. coli DH5α cells.

Figure 5a shows a SEM image of a bacterium delivered by ESI onto a microcantilever. AFM and SEM morphological analysis of the bacterial cells that arrive to the cantilevers provides a length of 1.87 ± 0.52 μm and a diameter of 0.49 ± 0.05 μm, in close agreement with the dimensions of the native structure. In these experiments, we use silicon nitride cantilevers with nominal length of 200 μm, width of 20 μm and thickness of 0.560 μm. In this case, the resonance frequencies of the first four flexural vibration modes of the cantilever were tracked by PLLs. The Allan deviations at the used acquisition time (20 ms) are typically 0.82, 0.10, 0.02 and 0.03 p.p.m., respectively (Supplementary Fig. 8). As each bacterium lands on the microcantilever, quasi-instantaneous jumps on the eigenfrequencies are produced (Fig. 5b). We apply our inverse problem algorithm to the correlated fractional frequency jumps to obtain the mass ratio and stiffness factor of the bacterial particles for a total of 189 events (Fig. 5c).
following the same procedure used for the nanoparticles (Supplementary Discussion). The obtained dry mass of the bacterial cells is of $318 \pm 95$ fg that agrees with the values of dry mass estimated by transmission electron microscopy and suspended microchannel resonators\textsuperscript{26,27} (Fig. 5d). When the effect of the stiffness is not accounted, the obtained mass values decrease $\sim 10\%$. The broad distribution in mass comes from the heterogeneity in the cell population.

The determination of the bacteria stiffness requires knowledge of the density. We estimate the density of the dry bacteria from the mass calculations and the volume obtained from the AFM and SEM images, being $900 \pm 120$ kg m$^{-3}$. Figure 6 shows the probability density of the estimated effective Young’s modulus of the bacteria cells (symbols). In contrast with the behaviour of the nanoparticles, the distribution of the effective Young’s modulus is broad and it exhibits a characteristic exponential decay. We relate this behaviour to the rod-like shape of the bacteria cells that induces anisotropy in the stiffness effect on the resonance frequencies. We apply a recently developed theoretical model that provides an analytical formula for the stiffness effect of rod-like analytes on the resonance frequencies of nanomechanical resonators\textsuperscript{14}. Briefly, the contact zone between the bacterium and the cantilever is a strip of length, $L_y$, and width $2a$. When the long axes of the bacterium and the cantilever are aligned, the cantilever bending stress is efficiently transmitted to a significant part of the bacterium volume with little dependence on the contact area (Fig. 6, insets at the right). The strain exponentially decays to zero near the bacterium ends with a characteristic length given by the bacterium diameter. When the long axis of the bacterium is transversally oriented to the beam length, the stress exerted by the cantilever along the contact width is significantly screened by the free surface of the bacterium (Fig. 6, insets at the left). In this case, the contact width plays a critical role and thereby the amount of bending strain within the adsorbate scales
up with the contact area. The dimensionless parameter $c$ for rod-like adsorbates is given by,

$$c(x, l, r_n, \eta) = p(x, \eta) - g_0(x, r_n) \cos \theta - g_m(x, \eta) \sin \theta - g_{1m}(x, \eta) \sin^2 \theta$$

(7)

where $x$ is the angle between the long axes of the bacterium and the beam, $l$ is the ratio between the length and the bacterium diameter, $r_n$ is the ratio between the contact’s width, 2$a$, and bacterium diameter and $\eta$ is the ratio between the bacterium diameter and the beam thickness. The functions $g_0(l, r_n, \eta)$, $g_m(l, r_n, \eta)$, and $g_{1m}(l, r_n, \eta)$ are explicitly given in the Supplementary Discussion. The effect of the bacterial cell orientation produces a probability density function that approximately follows $PDF(c) = \frac{d_0 + a1 + a2^2 + c^2}{c}$, where the constants $d_0$, $a1$, $a2$ and $c$ are obtained by Monte-Carlo simulations (Supplementary Fig. 9 and Supplementary Discussion). We fit our results to this equation (line in Fig. 6), which allows obtaining the Young’s modulus of biological systems.

In conclusion, we demonstrate that nanomechanical spectrometry can be used to characterize the mass and stiffness of intact micrometre- and nanosized analytes. Ignoring the effect of the stiffness can lead to a significant underestimation of the mass, particularly evident in the case of ultrathin cantilevers. The capability to describe the analytes that arrive to the resonator by two orthogonal coordinates, the mass and the stiffness, clearly enhances the selectivity of nanomechanical spectrometry and it opens the door to relevant biomedical applications. The important role of mechanical properties in biological processes and in pathogenic disorders is becoming increasingly clear. The technology presented here shows great promise for high-throughput characterization of the stiffness in addition to the mass of large biological complexes near their native conformation, a goal that is beyond the capabilities of conventional mass spectrometers.

Methods

Nanomechanical spectrometer system. Supplementary Fig. 11a shows a photograph of our nanomechanical spectrometer prototype. The set-up comprises three chambers with decreasing pressure. The top chamber is at ambient pressure, its height is 12.5 cm and it contains the heated capillary. The heated capillary has an inner diameter of 400 µm and a length of 11.4 cm. The temperature is set to 200 °C and the pressure is kept by a four-stage diaphragm pump (Vacuubrand GmbH, Germany). A skimmer with an orifice of 100 µm is connected to the sample solution by a fluidic capillary inject system (Attocube Systems AG, Germany). The pressure in this chamber is kept by a two-stage oil-sealed rotary pump (Oerlikon Leybold Vacuum GmbH, Germany). The base plate of the vacuum chamber is connected just below the resonator holder, to produce a straight-down flow. The ESI needle is fabricated in polyether ether ketone and is connected to the sample solution by a fluidic capillary inject system (Supplementary Fig. 11b). The sample solution is in a 2 ml microtube (Eppendorf). The sample solution flow through the fluidic capillary to the ESI tip needle. The inner diameter of the ESI needle is 63 µm. A high voltage (4–5 kV) is applied to the solution, to form the Taylor’s cone with the subsequent production of charged micro-droplets. The channel is connected to ground and the case of the heated capillary to 20 V. A charge-coupled device camera (Dino-Lite, Taiwan) is used to image the needle tip and to ensure the correct Taylor’s cone formation. Photographs of the ESI needle before and after the application of the high voltage are shown in Supplementary Fig. 11c,d, respectively. Supplementary 11e shows the formed Taylor’s cone. The distance between the ESI needle tip and the heated capillary is 5 mm. The ESI system (Electrospray ES-3020) was purchased from IONER, Spain. The laser diode used for measuring the cantilever displacement was purchased from Schaffer Kirchhoff GmbH, Germany. The wavelength is 658 nm, the output power 100 µW, the spot diameter 4 µm and the working distance 5.4 cm. The quadrant photodetector that measures the laser beam deflection is from Hamamatsu, Japan. The photocurrent of the upper and lower halves of the quadrant photodetector is amplified by two low-noise transimpedance amplifiers (DHPCA-199 from FEMTO Messtechnik GmbH, Germany). The differential voltage signal is connected to a digital lock-in amplifier (model HF2LI-PLL from Zurich Instruments AG, Switzerland) that enables fast frequency tracking of several vibration modes by digital PLLs.

Sample preparation. The 100 nm-sized GNP (Sigma Aldrich, USA) are in citrate buffer solution. After centrifugation of a microtube with 1 ml of the GNP solution (8,000 r.p.m., 10 min, 25 °C), 950 µl of the supernatant was removed and 950 µl of Milli-Q water was added to the microtube. This process was repeated three times. In the last washing step, the GNPs were resuspended in Milli-Q water with 0.5% of Tween 20. The concentration of this GNP solution was measured by using a BioSpectrophotometer (Eppendorf). The concentration of the GNP solution used in the experiments was of 3 × 10^10 GNP s ml^-1. Cold DH5α was kindly supplied by Dr Jesús Mingorance from the Microbiology Department of Hospital La Paz (Madrid, Spain). We used the strain at its stationary phase. Thus, 10 ml of Luria-Bertani broth (Sigma Aldrich, USA) were inoculated with 50 µl of a stationary phase culture and E. coli was grown overnight at 37 °C under agitation before its use. To avoid the formation of any debris from the culture medium, the cells were harvested by centrifugation at 4,400 r.p.m. during 25 min at 20 °C and resuspended in Milli-Q water. This process was repeated three times. Finally, the cells were resuspended in 50% isopropl alcohol/Milli-Q water. The concentration was adjusted to 10^7 cell per ml.

Data availability. All data of this study are available from the authors upon reasonable request.

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Author contributions
J.T., O.M. and M.C. conceived and designed the work. O.M., E.G.-S. and J.T. designed the instrument. O.M., C.M.D. and P.M.K. carried out the experiments. J.J.R., O.M. and J.T. analysed the data and developed the inverse problem method. J.T., O.M., J.J.R., P.M.K. and M.C. wrote the manuscript with inputs from all authors. All the authors analysed the data, discussed the results and commented on the manuscript.

Additional information
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