Single elementary charge fluctuations on nanoparticles in aqueous solution

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Abstract

10 100 years ago, in 1923, the Nobel prize in physics was awarded for the measurement of the unit charge. 11 In addition to a profound impact on contemporary physics, this discovery has reshaped our understanding of charge-based interactions in chemistry and biology, ranging from oxidation and 12 13 ionization, to protein folding and metabolism. In a liquid, the discrete nature of the electric charge 14 becomes prominent at the nanoscale, when a charge carrier is exchanged between a molecule or a 15 nanoparticle and the surrounding medium. However, our ability to observe the dynamics of such interactions at the level of a single elementary charge is limited, due to the abundance of ions in water. 16 17 Here, we report on the observation of single binding-unbinding events with elementary charge 18 resolution at the surface of a nanoparticle suspended in water. Discrete steps in the electrical charge are 19 revealed by analyzing the motion of optically trapped nanoparticles under influence of an applied 20 sinusoidal electric field. The measurements are sufficiently fast and long to observe individual 21 (dis)charging events which occur on average every 3 s. Our results offer prospective routes for studying 22 the dynamics of diverse chemical and biological phenomena at the nanoscale with elementary charge 23 resolution.

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25 Keywords: single elementary charge, nanoparticles, surface charge, optical trapping, electrophoresis.

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Table of contents graphic

30 The electric charge of an object is a discrete quantity that can be modified by exchanging charge 31 carriers. At the macroscale, charge exchanges in water involve large numbers of charging and discharging 32 events, which blur the discrete nature. However, at the nanoscale, the discrete nature of the electrical charge 33 can emerge, and the binding/unbinding of individual charge carriers, such as electrons or ions, plays a key role. Ionization and oxidation, for example, involve free radicals¹, which can pose a threat to human health² or 34 the environment³. These free radicals can bind to macromolecules such as DNA⁴ in the cell nucleus, or to 35 36 polymer chains⁵ in plastics, causing damage and aging^{5,6}. Another important example of charge addition/removal is post-translational modification (PTM) of proteins, such as (de)phosphorylation⁷ and 37 (de)acetylation⁸, which orchestrate vital cellular functions, including enzyme activity^{7,8}, gene expression⁸ and 38 protein folding^{9,10}. Therefore, it is clear that the ability to monitor the variation of the electrical charge of a 39 nanoparticle or a molecule, with elementary charge resolution, will offer great insight into the mechanisms of 40 41 water-based reactions at the nanoscale.

42 To date, there are several options to estimate the charge of nanoobjects in water. The most common 43 technique is electrophoretic light scattering (ELS), which measures electrophoretic mobility and can estimate

the charge of particles and biomolecules averaged over a large ensemble¹¹. Particle-tracking systems based on 1 microscopy-imaging^{12,13} or laser-scanning^{14,15} offer an alternative route to study the electric charge by 2 3 observing the electrophoretic motion. Although these instruments have proven to be reliable and provide detailed information, none of them have been able to evaluate the charge with single elementary charge 4 5 resolution in water. There are a few methods which reflect the discrete nature of the electrical charge on the nanoscale¹⁶⁻¹⁸, but they have important restrictions. One method is capillary electrophoresis¹⁹, which 6 determines the average electrophoretic mobility of charged molecules that are transported in capillary 7 channels²⁰. This approach allows to reveal discrete charge ladders of proteins or ligands¹⁶, but requires a large 8 9 population of identical molecules and is based on the effective charge averaged out over an extended time 10 interval. Another method involves label-free microscopy imaging of single nanoobjects confined inside nanostructured electrostatic traps²¹. This approach allows to estimate the charge of nanoparticles¹⁷ and 11 molecules¹⁸ with high precision (around one elementary charge) but the acquisition rate is not sufficient to 12 capture distinct charging events¹⁸. Hence, despite several recent advancements, the detection of individual 13 14 (un)binding events involving a single elementary charge in water remains an outstanding challenge.

15 In this work, we aim to resolve and monitor individual (dis)charging events on the surface of a 16 nanoparticle immersed in water with single elementary charge resolution. The approach is based on the optical trapping of a nanoparticle combined with electrophoresis in an alternating electric field. Previously, we have 17 used a combination of microscopy-based particle tracking²² and optical tweezers²³ to measure discrete charge 18 fluctuations on microparticles suspended in nonpolar media. This technique was able to reveal (dis)charging 19 events with a frequency of 2 Hz measured for a single particle during 3000 s²³. However, these studies have 20 limited applicability due to the use of nonpolar liquids, such as oil and dodecane. Chemical and biomolecular 21 22 reactions in water-based solutions are more relevant, but the polar nature of water makes the detection of 23 individual charges much more difficult.

24 The polar nature of a solvent carries important implications for the stability of electrical charges. Nonpolar media have low dielectric constants ε and large Bjerrum lengths λ_B ($\varepsilon = 2$ and $\lambda_B \approx 28$ nm for 25 dodecane) which leads to strong electrostatic interactions over large distances²⁴. This typically results in low 26 concentrations of free charges and a low frequency of charging/discharging events, which makes it easier to 27 detect these events for a given mobility measurement frequency^{22,23}. Polar liquids have higher dielectric 28 constants, shorter Bjerrum lengths ($\varepsilon = 80$ and $\lambda_B \approx 0.7$ nm for water) and, hence, a much higher concentration 29 of charge carriers in solution²⁵. Therefore, we can expect that the exchange of charges between a particle and 30 the surrounding medium occurs at a much higher frequency, compared to nonpolar solvents. In addition, 31 electrophoretic measurements in water become more complicated by electrochemical reactions that occur at 32 the water/electrode surfaces, by the generation of electroosmotic flow¹² near charged surfaces, and by 33 screening of the applied electric field at low frequencies²⁶. The high concentration of free charges in water 34 corresponds to a high electrical conductivity, leading to Joule heating of the sample²⁷ when a voltage is applied. 35 36 All these factors need to be carefully considered when aiming at high resolution electrophoretic measurements in an aqueous medium. Compared to previous results in non-polar media^{22,23} we will use particles with 10 37 times smaller diameter and electric fields with 10 times higher frequency. The smaller size reduces the particle 38 39 charge, which facilitates the observation of quantization. It is more difficult to keep smaller particles in an 40 optical trap when an oscillating field is applied, but it helps to use a field with higher frequency. The aim of 41 this work is to unlock the full potential of the electrophoretic mobility approach, to shed light on the dynamics 42 of diverse charge-based interactions in polar liquids.

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Results

As a test model for our studies we use fluorescent polystyrene (PS) nanoparticles suspended in deionized (DI) water. The general concept of our experiment is schematically sketched in **Fig. 1** while the detailed information can be found in **Methods**. In brief, we use the previously developed optical tweezers setup²⁸ to trap a single nanoparticle with diameter d_{NP} and surface charge q between two in-plane interdigitated indium tin oxide (ITO) electrodes. The electrodes have a width $w = 60 \ \mu\text{m}$ and a gap distance $d_{gap} = 80 \ \mu\text{m}$

while the height of the flow cell is $h = 50 \mu m$ (Supplementary Fig. S1a). By applying an AC voltage with 1 2 amplitude V_{AC} over the electrodes (Fig. 2a), we generate a sinusoidal electric field E with a frequency $f_E = 10$ 3 kHz which makes charged nanoparticle oscillate laterally (along the y-direction) in the optical trap. The particle 4 is trapped in the center of the electrode gap, where the electric field lines are parallel to the y-axis 5 (Supplementary Fig. S2b), about 5-10 µm above the bottom glass surface, to avoid spherical aberrations of the laser beam. The position of the nanoparticle is traced by collecting scattered light with a quadrant 6 photodetector (QPD) and calibrated by analyzing the power spectrum²⁹ (Supplementary Fig. S3c). Fig. 2b 7 shows an example of the calibrated y-position of the nanoparticle, which undergoes a periodic oscillation 8 9 induced by the applied electric field. The nanoparticle is visualized by fluorescence microscopy to verify that 10 it remains the only particle in the optical trap. In case a second particle is captured by the optical trap, both 11 particles escape from the trap and the experiment is terminated.

13 Fig. 1. Schematic illustration of the experiment. An 14 optically trapped nanoparticle with diameter $d_{NP} = 100$ nm 15 and surface charge q oscillates along the y-direction in 16 water under influence of a sinusoidally varying electric 17 field *E* with frequency $f_E = 10$ kHz. The electric field is 18 generated by a voltage V_{AC} applied over two indium tin 19 oxide (ITO) electrodes, separated by a gap $d_{gap} = 80 \ \mu m$. 20 The water is confined between two glass cover slips 21 assembled into a flow cell chamber with height $h = 50 \mu m$. 22 The dimensions in the illustration are not to scale.



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24 Our experimental design is reminiscent of Millikan's classical oil drop experiment³⁰ because it deals 25 with similar forces. Water molecules collide with the particle, resulting in a random force leading to Brownian 26 motion. There is an electric force proportional to the electric field E and to the charge q of the nanoparticle. 27 For nanoparticles in water, the sum of the above two forces, together with the hydrodynamic drag force and the optical trapping force should be equal to zero, because inertial forces are negligible 29. At low frequencies, 28 29 well below the corner frequency $f_c = 200$ Hz (Supplementary Fig. S3c), the random and optical trapping forces dominate. For the experiment we choose the frequency of the applied electric field $f_E = 10$ kHz which 30 31 is well above the corner frequency and allows neglecting the optical trapping force. At lower frequencies, the 32 amplitude of the motion is so large that the particle has a large chance to escape the trap. The position of the 33 particle is sampled with frequency $f_s = 100$ kHz, which is high enough to reveal the oscillatory motion, as 34 illustrated in Fig. 2b. By setting the sum of the described forces (electrical, thermal, optical and drag forces) 35 equal to zero, we derive the one-dimensional (along y) Langevin equation of motion (Methods), which yields 36 the following condition for resolving the elementary charge with the given measurement setup 37 (Supplementary Information):

$$SNR = \frac{eE_0}{\sqrt{12\pi k_B T \eta d_{NP} f_q}} > 1 \tag{1}$$

38 where $e = 1.602 \times 10^{-19}$ C is the elementary charge, E_0 is the amplitude of the electric field, k_B is Boltzmann's 39 constant, *T* is the temperature, $\eta = 1$ mPa s⁻¹ is the viscosity of water, and f_q is the rate at which we estimate 40 the charge (with the time interval $\Delta t_q = 1/f_q$). The expression (1) represents the signal-to-noise ratio (SNR) 41 between two motion components: the motion induced by the *E*-field which serves as the "signal", and the 42 Brownian motion which acts as the "noise". It should be possible to measure the charge of the nanoparticle 43 with single elementary charge resolution if the SNR is higher than one.

44 To reach the required SNR in (1), the charge sampling rate, the electric field amplitude and the particle 45 diameter can be modified. In other methods^{18,31} the SNR is increased by choosing a low value for f_q which 46 averages out the Brownian motion over a long time interval, however, this requires that the charge of the particle remains unchanged during that interval. In this work, we aim at monitoring elementary (dis)charging events and therefore the acquisition rate f_q should be higher than the rate of these events. To compensate for the relatively high value of f_q , we apply a strong electric field E_0 to keep the SNR in eq. (1) larger than unity. Using a particle with a smaller diameter d_{NP} should lead to a lower average charge, increase the SNR, and slow down the rate of binding/unbinding events at the particle surface. There is however a lower limit for the diameter of particles that can be trapped by optical tweezers, as it requires sufficient polarizability of the manipulated object³². In this work, we use nanoparticles with 100 nm diameter, which enables stable optical trapping, with trap stiffness of about 1 pN μ m⁻¹ (Methods).



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Fig. 2. Electrophoretic measurements performed with a low electric field for nanoparticles with $d_{NP} = 100$ nm. The graphs show examples of the applied voltage V_{AC} as a function of time (**a**) for the generation of the electric field ($E_0 = 0.16$ MV m⁻¹) and the measured time trace of the nanoparticle's *y*-position (**b**) during 5 ms. **c**. Estimated charge number as a function of time for five different nanoparticles labeled (i), (ii), (iii), (iv) and (v). The green open circles show the data measured with charge sampling rate $f_q = 5$ Hz (averaged over 200 ms) while the red filled circles represent the sliding average with window size n = 10. The error bars in the legend indicate the noise level of the measured and averaged data.

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18 We first use the above method for nanoparticles in relatively low electric fields. We employ an electrostatic calculation^{33–35} to estimate the amplitude of the electric field in the center of the gap between two 19 ITO electrodes (Methods) as 0.16 MV m⁻¹ for an applied voltage of 20 V (Supplementary Fig. S2). For the 20 21 applied 10 kHz sinusoidal voltage (Fig. 2a), the drift component of the nanoparticle motion is expected to be 22 also sinusoidal (Fig. 2b). The acquired y-position trace is split into time windows of 200 ms, and a discrete 23 Fourier transform (DFT) is applied to each window to obtain the component of the electrophoretic motion that 24 is in phase with the field. Its amplitude is proportional to the electrical mobility and the charge of the 25 nanoparticle (Methods). Fig. 2c shows how the obtained charge varies over time for five nanoparticles labeled 26 (i), (ii), (iii), (iv) and (v), with the green open circles representing the charge averaged over a 200 ms interval, 27 and the red dots representing the sliding average with a bin size of ten. These measurements illustrate that nanoparticles have different surface charges, ranging from -3e to -138e. The latter value corresponds to an 28 electrophoretic mobility of -2.34 10⁻⁸ m² V⁻¹ s⁻¹ (Methods), which is in the range measured by the Zetasizer 29 with average -4.1 10⁻⁸ m² V⁻¹ s⁻¹ (Supplementary Fig. S4). Although the SNR is about 3 for the applied low 30 31 electric field, we are unable to identify transitions of one elementary charge. One reason may be that the charge 32 exchanges are so frequent, that in many 200 ms intervals the charge does not remain constant. Another reason 33 may be that a variable number of counter ions travel together with the particle and reduce the effective mobility. 34 To resolve single elementary charge transitions, stronger electric fields are used to move the trapped

nanoparticles. We first use an electric field amplitude of 1.6 MV m⁻¹ and determine the charge averaged over time intervals of only 50 ms. For these parameters, the estimated SNR is 15, and discrete steps in the measured charge are indeed observed as shown in **Fig. 3**. In the graphs the charge is measured for two nanoparticles (vi) and (vii), with a low electrical charge (around -4*e*). The charge histogram in **Fig. 3b** demonstrates that the

values are strongly clustered around evenly spaced levels. The nanoparticles (viii) and (ix) show even tighter 1 2 clustering around these levels (Fig. 3b) and there is a higher contrast between signal and noise (Fig. 3a) due 3 to the use of a higher electric field of 5.6 MV m⁻¹ with a charge sampling rate of 50 Hz. The resulting SNR is about 35 for these traces and enables the detection of very short charge binding and unbinding events with 4 5 high resolution. For instance, nanoparticle (viii) shows back and forth transitions around 20 s and around 22 s 6 while nanoparticle (ix) shows a back and forth transition around 18 s, which might not be identified if a lower 7 charge sampling rates or a lower SNR was used. The vast majority of the measured values (left axis, Fig. 3a) 8 are within 10-15% of the value e, which proves that each step corresponds to a multiple of the elementary 9 charge. By analyzing the error residues (Methods), we determine the scaling factor k_s as 0.93 (Supplementary 10 Fig. S5), 0.84, 0.88 and 0.9 for the nanoparticles (vi), (vii), (viii) and (ix), respectively. Multiplication with 11 this factor rescales the axis to center the maxima of the histogram around multiples of e (right axis, Fig. 3a).

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14 Fig. 3. Electrophoretic measurements of nanoparticles with diameter of 100 nm conducted with high sinusoidal 15 electric field. a. The measured (left axis) and corrected (right axis) electrical charge as a function of time for four different 16 nanoparticles: (vi) and (vii) are measured with charge sampling rate $f_q = 20$ Hz under electric field $E_0 = 1.6$ MV m⁻¹ while 17 (viii) and (ix) are obtained with $f_q = 50$ Hz under $E_0 = 5.6$ MV m⁻¹. The green open circles show the measured data, the red 18 dots indicate the sliding average over 10 measured points, the black solid line indicates the estimated charge as a multiple 19 of the unit charge, together with the (dis)charging transitions. The error bars in the legend show the noise level of the 20 measured and averaged data, and the green and red arrows indicate the charging and discharging direction, respectively. 21 b. Corresponding histograms for the averaged charge for the same nanoparticles (vi), (vii), (viii) and (ix), showing distinct 22 peaks at multiples of the elementary charge as indicated on the right axis.

There are several factors that may cause the underestimation of the measured charge, including uncertainties in the particle diameter and the amplitude of the electrical field. The latter may be due to ions in

water that screen the electric field. For DI water with conductivity $\sigma = 0.055 \ \mu\text{S cm}^{-1}$ and electric permittivity 1 2 $\varepsilon = 700 \text{ pF m}^{-1}$, the characteristic frequency for screening²³ is around $\sigma/\varepsilon \sim 7.8 \text{ kHz}$. This value is slightly lower than the 10 kHz that is applied in our experiments and, hence, screening will cause some decrease in the 3 actual field amplitude. In addition, the presence of ions may induce electroosmotic flow^{12,13} (Methods), which 4 can contribute up to 15% to the electrophoretic mobility of the particle for the given values of the frequency 5 and distance from the surface (Supplementary Fig. S6). Another factor could be the presence of counter 6 7 charges in a diffuse double layer around the particle. However, the fact that the measured charges are close to 8 multiples of the unit charge indicates that this effect is limited. The double layer may be removed in the strong 9 electric fields. We also noticed that nanoparticles with a higher charge (nanoparticles (i)-(iv) in the Fig. 2c), 10 which could be studied at lower electric field amplitudes, tend to migrate and stick to the electrodes (Supplementary Fig. S6) soon after a high electric field is applied. The measurement of highly charged 11 12 particles (more than 10e) is also hindered by the fact that they escape the optical trap under high electric fields (above 1 MV m⁻¹). Higher laser power in the optical trap or tethering of the particles to the surface may help 13 14 to compensate strong electric forces exerted on the nanoparticles with high surface charge. Despite these 15 difficulties, our measurements reveal individual (un)binding events of the elementary charge at the surface of 16 low charged nanoparticles in water.

18 Fig. 4. Analysis of the charging/discharging dynamics. 19 Measured dwell times before charging (green dots) or 20 discharging (red dots), extracted from the time traces in 21 Fig. 3 for nanoparticles (vi), (vii), (viii) and (ix), as a function 22 of the charge number. The green and red open circles 23 indicate the average dwell time for a given charge number 24 (green circles for charging and red circles for discharging). 25 The solid lines provide an exponential fit for the open 26 circles. The arrows indicate the charging (green) and 27 discharging (red) directions.

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31 The demonstrated ability to monitor discrete charging events occurring at the water/solid interface 32 offer potential avenues to study the statistics of charge-exchange processes and single-molecule interactions. 33 As an example, we present a statistical analysis of the acquired traces based on a step-finding algorithm³⁶ 34 (black lines in Fig. 3a) to detect (dis)charging events and determine the dwell time τ before each event. Fig. 4 35 presents the extracted dwell times for charging (green symbols) and discharging (red symbols) events as a function of the charge number, for the nanoparticle traces (vi)-(ix) shown in Fig. 3a. The dwell time before 36 37 charging or discharging events respectively increases and decreases, with increasing number of elementary 38 charges. This indicates there is a negative feedback, which tends to stabilize the charge around an average 39 value. The fitting curves cross around -4e, which corresponds to the average of the measured charges. From 40 the histograms in Fig. 3b it is clear that the four particles are not identical and each one will have its own (dis)charging statistics. Unfortunately the number of events in each time trace is too limited to perform a 41 42 statistical analysis for each particle separately. The overall average dwell time for the nanoparticles with 43 diameter 100 nm in DI water is 2.75 s, which corresponds to an average charge exchange rate of 0.36 Hz. The 44 typical charge of our particles is larger than that of the microparticles in dodecane (diameter 1 µm) measured with electron charge resolution²³, although their size is smaller. This illustrates that the typical charge increases 45 when the solvent is more polar. It is expected that the charging rate will be further increased for solvents with 46 47 higher ionic strength. In our study we use deionized water, which contains two types of ions H^+ and OH^- . We 48 attribute the charging/discharging process to the binding/unbinding of H⁺ or OH⁻ ions to particular sites on the

surface of the polystyrene particle. It is well-known that protons H⁺ form hydrogen bridges with negatively-1 charged substrates such as polystyrene²⁵. These bridges have short lifetimes³⁷ (~ 10 ps) that cannot be resolved 2 with our approach, and one might expect that this would lead to an average charge that is a fraction of the unit 3 charge. The fact that we measure charge values that are multiples of the unit charge implies that hydrogen 4 5 bonds do not contribute appreciably to the average charge. In the future, these experiments may be repeated in the presence of univalent or bivalent ions, to evaluate binding events with particular ions³⁸. However, high 6 7 electric field experiments in solutions with high ionic strength are hampered by the electric current and 8 associated Joule heating. To remain below a given temperature, the application of an ac voltage should be 9 limited in time.

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Conclusion

12 In conclusion, we have demonstrated the detection of binding and unbinding of single elementary 13 charges to the surface of nanoparticles suspended in water. We measured the charge of 100 nm diameter polystyrene particles in deionized water with accuracy better than the elementary charge. During a time of 14 15 more than 30 seconds, the charge is measured every 200 ms, revealing that the (dis)charging rate is about 0.36 16 Hz. This ability to monitor individual (dis)charging events can be used to unveil a plethora of different 17 phenomena involved in diverse chemical and biomolecular interactions. Particularly, the presented method has great potential to reveal mechanisms of UV-induced photodegradation of micro- and nanoplastics³ as well as 18 19 oxidation-induced ion release of antibacterial agents. This technique can further be adapted for single-molecule bioassays³⁸ to study charge-based reactions, such as acetylation⁸, phosphorylation⁷ and DNA repair⁴. The 20 developed approach may lead to a variety of relevant applications, including methods for drug screening, 21 22 biosensing and medical diagnostics, setting high expectations for future discoveries.

Methods

25 Fabrication of microfluidic devices and sample preparation. ITO-coated coverslips (Thickness #1, 26 22x26mm², SPI Supplies) are cleaned by ultrasonication in three different solvents (1:20 RBS T 105 and DI water; acetone; isopropyl alcohol) for 15 minutes in each solution. Next, the coverslips are baked for 15 27 28 minutes on a hotplate at 150°C and spin-coated with the photoresist (AZ5214E, Micro Chemicals). The coated coverslips are soft baked on a hot plate at 115 °C for 55 seconds to ensure that no solvent remains in a 1.4 µm 29 30 thick photoresist film. The photopatterning is realized via contact lithography by exposing the samples to a parallel beam of UV light with intensity 13.7 mW/cm² for 8.5 s through a lithographic mask. The samples are 31 32 developed in a solution of 1:4 diluted developer (AZ MIF 726, Micro Chemical) for 55 s followed by etching 33 in a 9 M solution of HCl acid and eventually stripped in acetone and isopropyl alcohol for 120 s each. The 34 microfluidic device is assembled with a thin patterned coverslip with designed ITO interdigitated electrodes 35 (Supplementary Fig. S1a) that is fixed to 25x25mm² cleaned glass slide with a thickness of 1.1 mm. The 36 glasses are glued with NOA68 UV curing glue mixed with ball spacers (Supplementary Fig. S1b) to make a 37 flow cell with a height $h = 50 \,\mu\text{m}$. We obtain deionized water (18 MOhm cm) from the clean room and 38 immediately use it to conduct the experiments, avoiding exposure to air as much as possible. Fluorescent 39 polystyrene nanoparticles (Magsphere) with a size of 100 nm are diluted, centrifuged, and resuspended in DI 40 water several times to purify them from traces of salts and contaminations. The microfluidic chambers are 41 filled with an aqueous solution of diluted nanoparticles and sealed to prevent the evaporation of the liquid.

42 Calculation of the electric field. The electric field between the ITO electrodes is calculated using the commercially available finite-element solver COMSOL Multiphysics 5.6 (Electrostatics module) according to 43 our previously published methods^{33–35}. The flow cell with interdigitated electrodes is modelled as a 2D unit 44 cell (Supplementary Fig. S2a and b) with a height h and Periodic Boundary Conditions (PBC) applied on the 45 46 left and right sides of the geometry to simulate an infinite array of electrodes separated by the gap distance 47 d_{gap} . The medium inside the cell is water with dielectric constant $\varepsilon_m = 80 \varepsilon_0$. The electrostatic potential problem $\nabla(\varepsilon \cdot \nabla V) = 0$ is solved with Neumann Boundary Conditions (NBC) used at the top side of the unit cell and 48 49 Dirichlet Boundary Conditions (DBC) for the electrodes at the bottom side. The left and right electrodes have

potentials of 20 V and 0V, respectively (Supplementary Fig. S2a). The entire geometry is meshed with a
 triangular mesh, with maximum element size h/200. The resulting electric field distribution (Supplementary
 Fig. S2b) allows to estimate the electric field in the middle between two electrodes, which slowly decays away
 from the surface (Supplementary Fig. S2c). For charge estimation analysis we use the electric field value
 calculated 10 µm above the bottom surface in the middle of the gap.

Optical setup and detection. The optical trapping combined with electrophoresis experiments are 6 based on a previously developed optical tweezers setup²⁸. The setup is based on an inverted optical microscope 7 8 (Eclipse Ti, Nikon), where an expanded continuous-wave laser beam with a wavelength $\lambda_0 = 975$ nm 9 (ITC4005, Thorlabs) is guided to an oil-immersion objective lens (100×, NA = 1.3, Plan Fluor, Nikon), which 10 creates a stable optical trap (Supplementary Fig. S3a). The fluorescent nanoparticles are excited with blue light generated by a mercury lamp (Intensilight C-HGFIE, Nikon) and visualized with a CCD camera (iXon+, 11 12 Andor). The light scattered by a trapped nanoparticle is collected with a condenser lens (NA = 0.72, CLWD, 13 Nikon) and projected onto a quadrant photodiode detector (QPD; PDQ80A, Thorlabs) to monitor its linear displacement within the trap. Supplementary Fig. 3b shows an example of the acquired signal from the QPD 14 15 as a function of time for the nanoparticle, oscillating under an external sinusoidal electric field with frequency 16 $f_E = 10$ kHz. A custom-written LabVIEW program and data acquisition board (USB-6229, National Instruments) are used to record the QPD voltage signals and to generate the applied AC voltage with a 10x or 17 100x amplifier. To calibrate the trap stiffness and to convert the QPD voltages into a y-position, we analyze 18 the power spectral density²⁹ of the trapped nanoparticle as shown with green open circles in **Supplementary** 19 20 Fig. 3c, while the green dots represent the frequency spectrum of the empty trap. By fitting a Lorentzian function (red solid line) to the power spectral density of the particle, we obtain the corner frequency $f_c = 200$ 21 Hz, which corresponds to a trap stiffness around 1 pN µm⁻¹ for a laser power of 150 mW measured at the 22 23 entrance of the objective lens.

Electrophoretic mobility measurements. The electrophoretic mobility of nanoparticles and electrical conductivity of the solution are measured by electrophoretic light scattering (ELS) using a Zetasizer Nano (Malvern Instruments). The obtained electrophoretic mobility μ is presented in **Supplementary Fig. S4** with mean value -4.1 10⁻⁸ m² V⁻¹ s⁻¹. To compare these measurements with optical trapping experiments, we calculate the electrophoretic mobility using the estimated charge:

$$\mu = \frac{q}{3\pi d_{NP}\eta} \tag{2}$$

The conducted measurements show a large variation of charges between -3e and -138e (Fig. 2c), which correspond to the electrophoretic mobilities between 0 and $-2.34 \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$, which is in the range measured by the Zetasizer (Supplementary Fig. S4).

32 Data analysis for charge estimation and correction. The full analytical derivation of the applied 33 theory can be found in Supplementary Information. In short, we start from one-dimensional (for *y*-axis) 34 Langevin equation of motion for our developed experimental system, which has the following form:

$$m\ddot{y}(t) + \gamma\dot{y}(t) + \kappa y(t) = qE_0 \cos(2\pi f_E t) + \sqrt{2k_B T \gamma}\xi(t)$$
Inertial Frictional Optical Electrical Thermal
(3)

with several forces (indicated under each corresponding term) acting on the nanoparticle. In this equation $\gamma = 3\pi \eta d_{NP}$ is Stokes's drag coefficient and $\xi(t)$ is a random Gaussian process that represents Brownian fluctuations. We can transform the expression (3) into a difference equation for y_n by solving it for the chosen time interval of $1/f_q$ between t_n and t_{n+1} (**Supplementary Information**) and then apply discrete Fourier transform (DFT) on the resulting equation to obtain the expression for the charge estimation:

$$q = \frac{4\pi\gamma f_q}{E_0} \left(f_c \Re(\tilde{y}_E) + f_E \Im(\tilde{y}_E) \right)$$
⁽⁴⁾

40 where \tilde{y}_E is a DFT of y position at electric field frequency f_E . Thus, we can estimate the surface charge of 41 nanoparticles by calculating eq. (4) based on the calibrated y-position for the non-overlapping time intervals 42 with a window size of $1/f_q$ as shown in **Fig. 3a**. The standard deviation of the estimated charge based on eq. 43 (4) leads us to the condition for single elementary charge resolution shown in eq. (1). To correct the measured 44 charge, we apply the so-called R^2 -method with the following expression (**Supplementary Information**):

$$R^{2}(q) = \frac{1}{P} \sum_{p=1}^{P} \left(q_{p} - \left[\frac{q_{p}}{q} \right] q \right)^{2}$$
(5)

- 1 where q is a proposed value of an electric charge, q_p is a measured point for every p = 1, 2, ..., 600 for the time
- 2 interval of 30 s with a charge sampling frequency of 20 Hz while the square brackets indicate the rounding to
- 3 the closest integer number. Supplementary Fig. S5 shows the calculated $R^2(q)$ which has a minimum dip at a
- 4 certain value $\Delta q = 1.485 \times 10^{-19}$ C indicated with a black arrow. Using this value, we can find the best value for
- 5 the scaling factor k_s :

$$k_s = \Delta q/e \tag{6}$$

- 6 For the shown examples of the charge measurements in **Fig. 3a**, we obtain the scaling factor k_s of 0.93, 0.84, 7 0.88 and 0.9 for the nanoparticles (vi), (vii), (viii) and (ix), respectively.
- 8 Calculations of electroosmotic flow. To estimate the contribution of electroosmosis to the mobility,
 9 we apply a previously developed model^{12,13}, with the following equation:

$$u_{eo} = -\frac{\cosh(\sqrt{2\pi i f_E \rho/\eta} (z - h/2))}{\cosh(\sqrt{2\pi i f_E \rho/\eta} h/2)}$$
(7)

with water density ρ and distance from the bottom surface *z*. **Supplementary Fig. S6** shows the calculated electroosmosis factor as a function of distance *z* for three different electric field frequencies. According to the calculations, the contribution of electroosmosis to the mobility at the distance of 5-10 µm from the glass substrate and frequency of 10 kHz is within 15% only.

Associated content

16 The Supporting Information is available free of charge at . This PDF file contains: optical microscopy 17 images of the fabricated microfluidic devices; numerical calculations of the electric field; schematic 18 representation of the optical tweezers and detection; electrophoretic mobility measurement of the particles; R²-19 method to determine for the charge correction; electric field induced effects in water; analytical framework to 20 estimate the electric charge.

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