Measuring the Electric Charge and Zeta Potential of Nanometer-Sized Objects Using Pyramidal-Shaped Nanopores

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Abstract: Nanometer-scale pores are capable of detecting the size and concentration of nanometer-sized analytes at low concentrations upon analyzing their translocation through the pore, in small volumes and over a short time without labeling. Here, we present a simple method to measure the zeta-potential of nano-objects using a nanopore. Zeta-potential i.e., a quantity that represents electrical charge in nanocolloids, is an important property in manufacturing of pharmaceuticals, inks, foams, cosmetic and food; it is also imperative to understand basic properties of complex dispersions including blood, living organisms and their interaction with the environment. The characterization methods for zeta-potential are limited. Using the nanopore technique the zeta-potential and the charge of nanoparticles can be measured

independent of other parameters, such as particle size. This simple method is based on measuring the duration of the translocation of analytes through a nanopore as a function of applied voltage. A simple analytical model has been developed to extract the zeta-potential. This method is able to detect and differentiate nanometer-sized objects of similar size; it also enables the direct and precise quantitative measurement of their zeta-potential. We have applied this method to different nanometer-sized particles and compared the results with values measured by commercially available tools.

Key words: nanopore, zeta potential, nanoparticle, particle charge, particle size, electrophoretic mobility

Nanometer-sized objects in liquids are important materials in many respects. These include biological macromolecules [1, 2], such as nucleic acids and proteins, which are dispersed in living cells; organelles, such as vesicles, lysosomes and centrioles; and viruses. Moreover, synthetic polymers, such as polyethylene glycol (PEG) and polymethyl methacrylate (PMMA), suspended in a liquid have numerous industrial applications [3]. Furthermore, nanoparticle colloids, such as gold nanoparticles, have important applications in biosensors [4], photovoltaics [5], cosmetics [6] and nanomedicine [7]. Despite the numerous applications and the importance of these nanometer-sized floating objects, their detection and the techniques used to characterize them are relatively limited. For instance, the available techniques used to characterize nucleic acids are usually too time consuming and expensive in many applications [8]. Virus detection techniques require high concentrations, which is impossible during the early diagnosis of many diseases. Transmission electron microscopy (TEM) and different mass spectroscopy techniques are accurate and reliable tools to measure size and charge; however, they cannot characterize particles in their liquid environment and are expensive and time consuming. Dynamic light scattering (DLS) [9] can be used to measure the size and zeta potential of these particles. However, it requires a relatively large volume and cannot function at high or low analyte concentrations in addition to other

limitations similar to centrifugation [10], field flow fractionation [11], hydrodynamic fractionation [12], chromatography [13], gel electrophoresis [14]; and electro acoustic methods [15] and micro electrophoresis combined with nanoparticle tracking analysis can only work with particles that are large enough to be visible using optical microscopy, and problems such as electrode polarization and the introduction of gas bubbles may be encountered [16].

Nanometer-sized pores in a membrane (figure 1) have previously been introduced as a promising tool for the detection of nanometer-sized objects dispersed in a liquid at concentrations lower than 10^7 particles per milliliter, with times as short as a few seconds and volumes as small as a few microliters without any labeling or major sample preparation [17-20]. The ability of the nanopore technique to differentiate between various nanoparticles has traditionally been based on the discrimination of particles according to their size. Thus, its applications have been limited to analytes with detectable size differences. However, different particles composed of different materials with different properties can have similar sizes, and in complex samples, such as biological samples, many such particles exist [20]. Thus, we cannot rely only on the size of the particles to differentiate and detect particles. In addition, particle size is determined by measuring the amplitude of the ionic current's change during particle translocation through a nanopore; this amplitude is a function of other parameters, including the ionic concentration of the solution, particle material and the applied voltage, in addition to the size of the analyte [21-23]. Therefore, we advocate the use of translocation duration and even better, its derivative for measuring the zeta potential.

Figure 1. The nanopore device. a) Three-dimensional schematic representation of the nanopore device. A silicon membrane separates two microfluidic chambers composed of poly(methyl methacrylate) (PMMA) and sealed by poly dimethyl siloxane (PDMS). A pyramidal nanopore in the membrane connects the two chambers. Nanometer-sized objects are electrophoretically driven from the front chamber, through the nanopore and into the back chamber. The white blades represent the Ag/AgCl electrodes that are used to apply the voltage to the liquid. b) Transmission electron microscopy (TEM) cross section image of a 40 nm nanopore (for TEM sample preparation purposes the nanopore is filled with silicon dioxide). The scale bar is 10 nm. c) Scanning electron microscopy (SEM) image of a 120 x 120 nm nanopore. The nanopore is the white square at center of the image where is the end of the pyramidal etch pit that has resulted from KOH wet etching. The scale bar is 200 nm.

Nevertheless, measuring the zeta potential of colloids is required in many applications, such as protein purification [2], production of paint and minerals, clay and drilling fluids, ceramics, pharmaceuticals, and paper as well as in ore processing, water and wastewater coagulation, and biosensors [24]. The zeta potential is defined as the electrical potential between the inner Helmholtz layer near a particle's surface and the bulk liquid in which a particle is suspended. It is a parameter that represents the charge of a particle. Detection of electrophoretic mobility (which is a parameter related to the zeta potential) was predicted [17] and translocation duration of polystyrene particles through a 3 μm long multi wall hollow carbon nanotube was related to their mobility in the specific salt concentration and voltage [25-27]. Solid-state nanopores are subject of intense research as promising analytical tools [28], measurement of zeta potential using them is not realized yet. In long and narrow cylindrical pores, or with flexible and long chain-like analytes, there is a significant interaction between the translocating analyte and the pore walls that can introduce deviation from the electrophoretic motion [29, 30]. Furthermore, due to microscopic differences between the two flow cells on the two sides of the membrane, as well as the electrodes, there is usually few millivolts offset voltage at zero current. Moreover, a minimum voltage is needed for translocation, to overcome the entropic and energy barrier of the nanopore and make the translocation probable [30-33]. Consequently, the translocation velocity-voltage curve generally has an offset and is not going through the origin [30]. Moreover, low signal to noise ratio in thick membranes [34] and lack of a physically meaningful definition of translocation duration [39] were preventing zeta potential measurement by nanopore. Here, we present a reliable method that can be used to measure the zeta potential and, consequently, the charge of nanometer-sized objects using a nanopore. This is done using a sharp-edged pyramidal-shaped nanopore to minimize the pore-particle interaction and provide an electrophoresis dominated translocation, while it gives a better field confinement as well [35], by using a physically meaningful definition and a precise measurement of translocation duration, and by elimination of the voltage offset by defining the mobility as derivative of the velocity-electric field curve

instead of ratio of the velocity to the field. Using this approach, several different nanometer sized object have been analyzed in different conditions and the results were compared with commercial methods. This method not only provides a fast and precise measurement of zeta potential of any nanoparticle mixture even in low concentrations and small volumes; at different salt concentrations, particle sizes and particle materials; this method provides perspectives for reliable detection and differentiation between various particles including biological particles of similar size based on voltage dependence of translocation duration, using a nanopore.

Operation principles

The nanopore devices are composed of two microfluidic chambers separated by a membrane with a nanopore that connects the two chambers (figure 1). A liquid fills these two chambers, one of which contains the nanometer-sized analytes. By applying a voltage between these two chambers, an ionic current flows from one chamber, through the nanopore, and into the other chamber. Furthermore, an electric field is created and act on the particles near and inside the nanopore. This force can drive the nanoparticles from one chamber, through the nanopore and into the other chamber. The temporary presence of a nanoparticle in the nanopore changes the electrical resistance of the nanopore that can be detected as a temporal change in the ionic current between the two chambers. Earlier theoretical investigations based on the calculation of electrophoretic, electro-osmotic and drag forces have showed the electrophoretic force to be the dominant force [36, 37]. In addition to the calculation of these forces based on the measured dimensions, electrical properties and surface charges of our devices [38, 39], we have also determined the electrothermal, gravitational and Brownian forces [40] using a continuum model (see the supplementary information).

Our theoretical studies confirm the dominance of the electrophoretic force when the largest dimension of the nanoparticle is sufficiently smaller than the pore size, nanoparticle are nearly spherical, with a

sharp pyramidal nanopore and thin electric double layer (EDL) compared to the pore size. Thus, the electrophoretic and drag forces determine the translocation kinetics, and we can adopt the concept of electrophoretic mobility. Accordingly, we can define the translocation mobility as:

$$\mu_{tr} = \frac{\partial \bar{v}}{\partial E} \quad (1)$$

where $\bar{v} = \frac{l}{t_{tr}}$ is the average translocation speed, $E = \frac{V}{l}$ is the average electric field in the nanopore, V is the applied voltage, t_{tr} is the measured translocation duration and l is the effective length of device's sensing zone, which is a calibration constant that depends on the device geometry and solution conductivity. Although this length can be experimentally measured by measuring the translocation duration of an analyte with a known zeta potential as a function of the applied voltage, our simulations show the possibility of calculating it with sufficient precision (see supplementary information). Previously, there were a few slightly different approaches for measuring the translocation duration, all based on thresholding; the distance between the two adjacent crossing points of a threshold level, the distance between the point that the signals leaves the baseline and the point that it returns to the baseline [23], or considering the full width half max of the spike to reduce the effects of the limited bandwidth [35, 20]. Although such definitions may reduce the effect of bandwidth, they have no physical meaning and are prone to noise within the spike. Thus, we have developed a new definition for the spike's duration [39], which is least affected by the measurement bandwidth and noise, and most importantly has a physical meaning. This definition considers the time that the center of nanoparticle enters the sensing zone as the start of the spike and the time that its center leaves the sensing zone as the ending point. A simple analytical calculation shows that these are the times at which the second derivation of the signal reaches its maximum and minimum (figure 2).

Although classical definition of electrophoretic mobility is the ratio of velocity to electric field, it is necessary to use the differential translocation mobility as the velocity-field curve is generally not going through the origin and has an offset of tens of millivolts, as mentioned earlier. In addition to elimination of voltage offset, as we will show in the next section, high precision can be achieved simply by measuring the voltage dependence of translocation duration.

Figure 2. Translocation spikes. a) A typical current recording of a nanopore and translocation spikes. When a nanoparticle translocates through the nanopore, the temporary presence of a nanoparticle within the nanopore introduces a temporal change in the electrical resistivity of the pore. Consequently, a spike appears in the ionic current. b) A close-up view of a typical translocation spike. The red arrow indicates the translocation duration (sec), and the blue arrow indicates the spike amplitude (pA).

According to Henry's solution for electrophoretic motion [41]:

$$\mu_{tr} = \frac{2\varepsilon_r \varepsilon_0 \zeta}{3\eta} f(ka) \quad (2)$$

where $\varepsilon_r \varepsilon_0$ is the solution permittivity, η is the solution viscosity, ζ is the zeta potential of the particle. $f(ka)$ is a correction factor that depends on Debye length, k , and particle's diameter, a ; and can be curve fitted by the following equation:

$$f(ka) = \frac{2}{3} - \frac{1}{2(1 + 0.072(ka)^{1.13})} \quad (3)$$

where $k = \sqrt{\frac{2q^2 z^2 n_\infty}{\varepsilon k_B T}}$, q is the elementary charge, z is the valence of the EDL's ions, n_∞ is the bulk solution concentration, k_B is the Boltzmann constant and T is the absolute temperature. For zeta potentials of approximately 100 mV or higher, we can apply O'Brien's correction factor [42], and for jelly or porous particles, which are highly conductive, we can apply Dukhin's correction factor [43]. Although these correction factors can improve measurement accuracy, in most practical cases and applications, reasonably accurate results can be obtained without them. Because $ka \gg 1$ in our nanopore devices, we can use Smoluchowski's approximation [44] and simply write:

$$\mu_{tr} = \frac{\varepsilon_r \varepsilon_0 \zeta}{\eta} \quad (4)$$

Using equation (4) and the aforementioned parameters, the following relation between the average translocation velocity, translocation mobility, zeta potential and the measured translocation duration can be obtained:

$$\bar{v} = l \left(\frac{1}{t_{tr}} \right), \quad \mu = l^2 \frac{\partial \left(\frac{1}{t_{tr}} \right)}{\partial V}, \quad \zeta = \frac{\eta l^2}{\varepsilon_r \varepsilon_0} \frac{\partial \left(\frac{1}{t_{tr}} \right)}{\partial V} \quad (5)$$

$$t_{tr} = \frac{\eta l^2}{(V + C) \zeta \varepsilon_r \varepsilon_0} \quad (6)$$

where C is an integration constant that is the voltage offset.

Knowing the zeta potential, we can use Graham's equation to calculate the surface charge density of the particles [44, 45]:

$$\sigma = \sqrt{8n_{\infty}\epsilon_r\epsilon_0K_B T} \sinh\left(\frac{zq\zeta}{2k_B T}\right) \quad (7)$$

In most of the cases in which the zeta potential is smaller than 100 mV, equation (7) can be approximated with good precision by the following linear equation:

$$\sigma = \frac{\epsilon_r\epsilon_0\zeta}{\sqrt{\frac{\epsilon_r\epsilon_0k_B T}{qzn_{\infty}}}} \quad (8)$$

Accordingly, by measuring the zeta potential, we can measure the surface charge density of a nanometer-sized object using a nanopore.

Zeta potential measurements

We applied the zeta potential measurement method to tens of different nanometer-sized objects with different sizes and zeta potentials that were composed of biological or synthetic materials. We used gold, polystyrene, and silica nanoparticles with diameters ranging from 20 to 100 nm. To have a wider range of particles with different properties and zeta potentials, the nanoparticles were capped with citrate ($\text{C}_3\text{H}_5\text{O}(\text{COO})_3^{3-}$), carboxyl (COOH), amine (NH_2), 1-mercapto-11-undecyl-tri (ethylene glycol) ($\text{SH}(\text{CH}_2)_{11}(\text{OCH}_2\text{CH}_2)_3\text{OH}$), its carboxyl derivative ($\text{SH}(\text{CH}_2)_{11}(\text{OCH}_2\text{CH}_2)_6\text{COOH}$), and deoxyribonucleic acid (DNA) linked with 5'-mercaptohexanol.

Measurements were performed using different nanopore devices measuring approximately 120 nm in width and 720 nm in length in 20 x 20 μm membranes. Most of the investigations and measurements were performed in 30 mM potassium chloride (KCl) solutions. Some of the particles that were stable at higher ionic concentrations (see the supplementary information) were investigated and measured at such concentrations as well.

In all of the experiments, the translocation duration was reduced and the amplitude of the translocation spikes was increased by increasing the applied voltage (figure 3). A typical relationship between the translocation duration and the applied voltage is shown in figure 4.a. According to the aforementioned theory, the translocation duration is inversely proportional to the applied voltage. To evaluate this relationship, the average velocity, which is the inverse of the translocation duration multiplied by the nanopore length, was calculated as a function of voltage. As shown in figure 4.b. average translocation velocity linearly changes with the cross membrane voltage as predicted by the theory.

Figure 3. Scatter plots of translocation events obtained at different voltages. On the bottom and on the left axis, the Gaussian fits to the histograms of the distributions are depicted. The sample that was used is a colloid of citrated gold nanoparticles with a diameter of 20 nm and $\zeta = -49$ mV; the diameter and zeta potential of the particles are normally distributed with standard deviations of approximately 10% of their means. Measurements were performed using a 120 nm nanopore; the nanoparticle concentration was 7×10^{10} particles/ml, and the solution was 30 mM KCl.

Figure 4. Voltage dependence of the translocation kinetics. a) Translocation duration as a function of the applied voltage for $\text{SH}(\text{CH}_2)_{11}\text{PEO}_6\text{COOH}$ coated gold nanoparticles with 50 nm diameter and $\zeta = -38$ mV; the diameters and zeta potentials of the particles are normally distributed with standard deviations of approximately 10% of their mean values. Measurements were performed using a 120 nm nanopore; the nanoparticle concentration was 7×10^{10} particles/ml, and the solution was 30 mM KCl. The error bars show the standard errors of the population of the recorded translocation spikes. b) The average translocation velocity as a function of the applied voltage calculated by dividing the nanopore length by the translocation duration, and removing the voltage offsets (see supplementary information).

As mentioned previously, the velocity-voltage curves may shift from one device to another or from one sample to another; however, the slopes of these curves are not affected by these shifts. Thus, the slope represents a more precise and reliable parameter for the measurements. Translocation mobility can be calculated by multiplying the slope of the velocity-voltage curve by a constant number, as described by equation (5). A linear relationship between the translocation mobility and the zeta potential measured by DLS has been found (see supplementary figure 6) which is in agreement with the theory. As predicted by the theory, the translocation mobility is independent of nanoparticle size (see supplementary figure

Finally, equation (5) was used to extract the zeta potential of the nanoparticles having their mobility and the results are compared with the zeta potential of the same particles measured under similar conditions by DLS (figure 5). As shown in this figure, the nanopore technique provides a simple and advantageous method of measuring the zeta potential of nanometer-sized objects. According to equations (7) and (8), knowing the zeta potential of a nanoparticle gives the particle's surface charge.

Figure 5. Zeta potential of the nanoparticles measured by the nanopore device versus the zeta potential of the same particles measured by a commercial DLS system. Error bars show the distribution in the population; the measurement precision is on the order of millivolts. Blue line shows the translocation mobility as predicted by the theory and dotted lines are showing the approximated dispersion due to the dispersion in zeta potential of different particles.

Conclusion

We have introduced a novel method for measuring the zeta potential and, consequently, the surface charge of nanometer-sized objects suspended in a liquid. Because this method is based on a nanopore technique, it does not require labeling and functions with optically active and inactive as well as opaque

and transparent samples; it requires only very small sample volumes and concentrations and no prior knowledge of analytes. This simple method not only enables the precise measurement of the zeta potential of nanometer-sized analytes using a nanopore technique, it also allows for the detection of and differentiation between analytes based on surface charge. Measuring the voltage dependence of the translocation spikes also enhances the precision, reliability and repeatability of size measurements and reduces the need for calibration according to size. Hereby, the nanopore technique has been improved to provide an inexpensive charge and size spectroscopy technique capable of characterizing analytes in their natural liquid environments in small amounts and over a short period of time.

Materials and methods

Device fabrication

We have previously described the fabrication process of similar pores [45]. Briefly, a pit was etched through the back side of silicon on insulator wafer (SOI, 720 nm Si on 1 μm buried SiO_2 on 700 μm Si) by potassium hydroxide (KOH) wet etching to produce a 20 x 20 μm membrane on the front side of the wafer that consists of 720 nm Si on 1 μm SiO_2 . A nanopore patterned by an electron beam lithography (EBL) process [46] and etched away through the 720 nm thick of the front side's silicon by anisotropic KOH wet etching, followed by buffered hydrofluoric acid (BHF) wet etching to remove the 1 μm buried oxide and open the nanopore. The device was packaged between two microfluidic flow cells, which provide inlets and outlets for fluids, visual access, and Ag/AgCl electrodes for electrical contacts.

Measurement setups

We have used an Axopatch 200B (Axon Instruments) patch clamp amplifier to apply the desired voltage and measure the current. A MiniDigi (Molecular Devices) was used for digitization and a pCLAMP 10 (Molecular Devices) was used for data acquisition. All measurements were performed in a Faraday cage on an antivibration table in a dark room.

Sample preparation

Commercially obtained citrated, carboxyl-capped and amine-capped gold nanoparticles (Ted Pella), silica nanoparticles (Keiser Biotech) and polystyrene nanoparticles (Interfacial Dynamics) with concentrations ranging from 5×10^9 particles/ml to 7×10^{11} particles/ml were diluted 5 to 200 times in the desired KCl aqueous solutions. The thiol- and DNA-capping processes and the investigation of the stability of these functionalized particles are described elsewhere [47]. We used 30 mM KCl for all of the particles. In addition, for highly stable particles, such as thiolated particles, the experiment was repeated at higher ionic concentrations. Some of the measurements were also repeated at lower ionic concentrations.

Signal processing

All signals were low-pass filtered by a 10 kHz 4-pole Bessel analog built-in filter in the amplifier. We developed a software specifically for nanopore signal processing [39]. This software first reduces the noise in the signal by a 5 kHz 8-pole Gaussian low-pass filter and using Bior3.9 wavelet for wavelet denoising. Then, it removes the baseline by fitting a two component exponential. Thereupon, it detects any fluctuation which is more than six times larger than noise RMS and uses a selection algorithm to find the translocation signals. Finally, it uses the local maxima and minima of the second derivative of the signal to find the starting and ending point of each spike; and reports all the parameters related to each spike in a tab-delimited file.

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Supporting information available

Size measurement, Stability study of the nanoparticles, Calculating the forces acting in a nanopore, Simulating the current spikes, Mobility-size and mobility-zeta potential relations. This material is available free of charge *via* the Internet at <http://pubs.acs.org>.

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Supplementary information

Size measurement

It has been previously shown that the amplitudes of the translocation spikes can be used to calculate the sizes of the translocating analytes [1]. We observed a linear relationship between the spike amplitude and the applied voltage (supplementary figure 1). This demonstrates the fact that in the working region of the nanopore device, a nanopore-nanoparticle complex acts as a linear resistor. We defined the translocation resistance as the inverse slope of the spike amplitude-voltage curve, which is an advantageous parameter in measuring analyte size. Amplitude-voltage curves may shift from one device to another or even from one sample to another. These shifts are caused by the offset voltage between the two sides of the nanopore that originates from the inequalities between the two sides, variations in the surface of the nanopore and variations in the properties of the suspending liquid. These shifts reduce the precision of size measurements using the spike amplitude and introducing the need for frequent calibration of the device. This voltage offset can be approximated by measuring the voltage at which the ionic current between the two chambers is zero. However, it is easier and more precise to use translocation resistance instead of spike amplitude to measure the analyte size. Because the translocation resistance is not affected by these shifts and is independent of the applied voltage, it can be used as a more precise and reliable parameter for measuring the sizes of nanoparticles. The spike amplitude and the translocation resistance are functions of the solution composition, nanoparticle charge and material composition [2-5]. Consequently, it is necessary for size measurements to calibrate the device with nanoparticles with known diameters that are composed of a material similar to the analyte material and that are suspended in similar solutions.

Supplementary figure 1. The amplitudes of the translocation spikes as a function of the applied voltage. Measurements were performed with a 120 nm nanopore in 300 mM KCl. The samples were citrated gold nanoparticles with different sizes and zeta potentials. The standard deviation of the size and zeta potential of the particle population is approximately 10% of the corresponding mean values. The error bars show the standard errors of the population of the recorded translocation spikes.

Stability of the nanoparticles

If the nanoparticles coagulate, the resulting coagulations will be larger than the individual particles. Thus, the probability of these large coagulations being translocated through the nanopore will be very small. In addition, because the nanopore is kept vertical during the measurement, the large coagulations that settle more quickly do so far away from the nanopore, at the bottom of the microfluidic chamber. However, particles that are much smaller than the nanopore may produce relatively small coagulations that can translocate through the pore and that have sedimentation time constants that may be much longer than the duration of the experiment. Thus, for a clearly understood and well-controlled experiment and to ensure that the properties of the samples do not change during an experiment, we have precisely investigated the stability of different nanoparticles. To do so, the theory of Brownian collision was used to calculate the characteristic time between two collisions [6, 7]. This time was found to be on

the order of one hour for the concentrations and particles we used, whereas the ionic current recording lasts only a few seconds, and the entire measurement lasts approximately one minute. In addition, Woodcock's theory was used to calculate the average surface-to-surface distance between the particles [7]. For the conditions used in our experiments, the distance was found to be on the order of micrometers, which is much larger than the range within which van der Waals force can act and produce coagulations. Furthermore, the stability of the samples was experimentally verified using DLS by measuring the zeta potential and size of the particles as a function of time.

Calculating the forces acting in the nanopore

To have an order-of-magnitude estimate of the forces acting on a nanoparticle in a nanopore, the dimensions of the nanopore devices and the analytes were measured by SEM and TEM, as well as the surface charge of the nanopore [8], electrical properties of the solution, analytes and the nanopore device were measured and its equivalent circuit was extracted [9]. Using this information, first, the electric field within and around the nanopore was calculated. To do so, we first calculated the conductivity of the KCl solution using the following equation:

$$\sigma_l = \Lambda \times c \approx 143 \left(S \cdot cm^2 / mol \right) \times c \quad (1)$$

where σ_l is the liquid conductivity, Λ is the molar conductivity of the KCl solution and c is the KCl concentration. In the ionic concentrations that we typically use for nanopore measurements, the thickness of the electric double layer (EDL) is less than 10 nm, whereas our nanopore is larger than 100 nm. Thus,

we are in the Smoluchowski regime and can neglect the surface conduction of the nanopore [8]. Consequently, we can numerically calculate the electric field within the nanopore device (supplementary figure 2) by integrating over the liquid's volume.

Supplementary figure 2. The calculated electric field along the axis of a 120 x 120 nm nanopore filled with 30 mM KCl when 100 mV is applied between the two sides of the nanopore. The orange shadow represents the cross section of the nanopore in order to show its position.

Knowing the electric field within the nanopore, we can calculate the forces involved, which are governed by the following equations [10]:

Drag:
$$F_{drag} = 6\pi\eta a(U - V) \quad (2)$$

Electrothermal:
$$\langle F_E \rangle = \frac{1}{2} \text{Re} \left\{ \frac{\sigma \nabla \varepsilon - \varepsilon \nabla \sigma}{\sigma} \cdot |E|^2 \right\} - \frac{1}{4} |E|^2 \nabla \varepsilon \quad (3)$$

Brownian:
$$\Delta x = \sqrt{2D\Delta t} \quad (4)$$

Gravitational:
$$F_g = v(\rho_p - \rho_l)g \quad (5)$$

Dielectrophoretic:
$$F_{DEP} = \pi a^3 \varepsilon'_l K_{Re}(\omega) \nabla E^2 \quad \text{where} \quad K_{Re}(\omega) = \text{Re} \left(\frac{\tilde{\varepsilon} - \varepsilon_l}{\tilde{\varepsilon} + 2\varepsilon_l} \right) \quad (6)$$

where η is the viscosity of the liquid, a is the nanoparticle diameter, U is the nanoparticle velocity, V is the liquid velocity, E is the electric field, D is the diffusion coefficient of the nanoparticles, Δx is the distance traveled during the time Δt due to Brownian motion, v is the nanoparticle volume, ρ_p is

the nanoparticle mass density, ρ_l is the liquid mass density, g is the gravity constant, ϵ_l is the liquid permittivity with real part ϵ'_l and $\tilde{\epsilon}$ is the effective permittivity of the nanoparticle.

Because of the equal ionic concentration between the two sides of our nanopore, the diffusiophoresis force is zero. Because we are working in a DC regime, the AC electroosmotic force is zero as well. The electroosmotic force is calculated according to the model described in [11]. Supplementary figure 3.a shows the driving forces acting on a nanoparticle along the nanopore's axis. As shown in this figure, the electrophoretic force is dominant, and the other acting forces are negligible.

Supplementary figure 3. Force fields and velocity that a nanoparticle experiences within a nanopore. a)

The forces acting on the nanoparticle as a function of its position on the axis of the nanopore. As shown in the figures, the force fields extend out of the nanopore, and the main driving force is the electrophoretic force. b) The nanoparticle velocity calculated as a function of its position. Calculations were performed for a 40 nm citrated gold nanoparticle with $\zeta=-40$ mV translocating through a 120 nm pyramidal nanopore covered with silicon dioxide and filled with 30 mM KCl solution. The orange shadows represent the cross section of the nanopore in order to show its position.

After calculating the total forces acting on the nanoparticle, we used Stoke's law [10] to calculate the nanoparticle's speed as a function of its position during translocation through the nanopore (supplementary figure 3.b).

Simulating the current spikes

To simulate the change in the ionic current caused by the translocation of a nanoparticle through a nanopore, we first calculated the associated Brownian motion according to Einstein's equation (eq. 1). Then, we incorporated the Brownian motion as a weighted Gaussian into the calculation of velocity and position as a function of time (supplementary figure 4.a).

Supplementary figure 4. Simulation results of the translocation of a nanoparticle through a nanopore. a)

Position of a nanoparticle translocating through a nanopore versus time (position is measured as the distance from the narrow side toward the wide opening of the nanopore). The small fluctuations in the curve are caused by the Brownian motion of the nanoparticle. The orange shadow represents the cross

section of the nanopore in order to show its position. b) Change in the ionic current caused by translocation of a nanoparticle through the nanopore as a function of time. Calculations were performed for a 40 nm citrated gold nanoparticle with $\zeta = -40$ mV that was translocated through a 120 nm pyramidal nanopore covered with silicon dioxide and filled with a 30 mM KCl solution.

We calculated the ionic current that passes through the nanopore taking into account the conduction current through the liquid, the conduction current through the electric double layers (EDL) of the nanopore and the nanoparticle. We also calculated the electrical noise of the amplifier according to the manufacturer's specifications and our measurements and added it to the simulated current. Finally, the simulated current was sampled to make the results more similar to the experimental setup (supplementary figure 4.b). Because of the lack of precise knowledge about some of the parameters, such as the conduction within EDLs and the approximations used, there is a difference between the measurement and simulation results. However, the results are in agreement with the experimental measurement within an order of magnitude precision. For instance, the calculated length of sensing zone, l , is 1.15 μm while the measured length in similar conditions is 1 μm (the length of the sensing zone can be measured by measuring the voltage dependence of translocation duration of a particle of known charge, using equation 5 of the main text).

Mobility-diameter and mobility-zeta potential relations

As predicted by theory and confirmed by the measurements (supplementary figure 5), no relation between the translocation mobility and nanoparticle size has been found and the translocation mobility varies linearly with the zeta potential (Supplementary figure 6).

Supplementary figure 5. The translocation mobilities measured by nanopore versus particle diameters measured by DLS.

Supplementary figure 6. Translocation mobility as a function of the zeta potential. Different nanoparticles measured by 120 x 120 nm nanopore devices. Solid blue line shows the translocation mobility as predicted by the theory and dotted lines are showing the theoretically estimated dispersions due to the dispersion in zeta potential of different particles. Error bars are representing standard deviation of distributions of zeta potential and translocation mobility in the samples.

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