## Model

A model originally developed for conically shaped synthetic nanopores[S1] was modified to calculate the change in resistance during the translocation of a particle through a nanopipette orifice.

## Calculation of effective length of a nanopipette

For a nanopore, the radii of the two openings have been used to define the geometry, while for a nanopipette, the angle $\theta$ is observable rather than the second radius, as shown in Fig. S1.

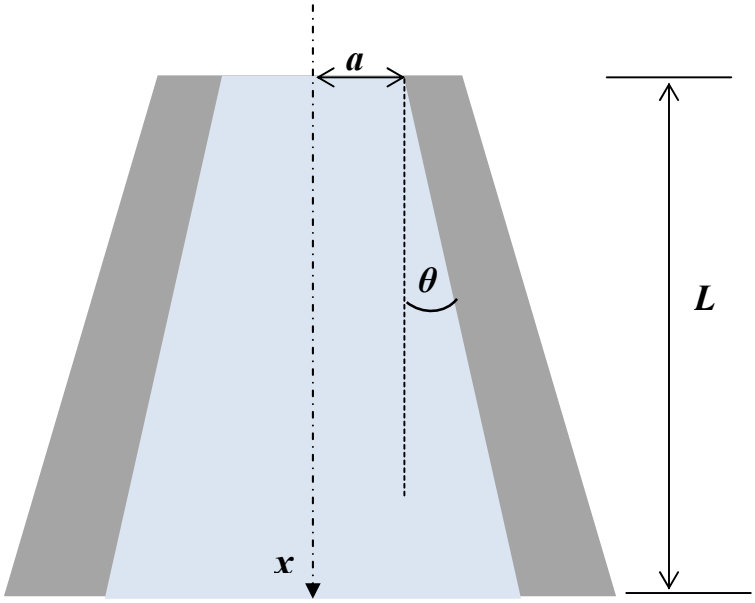


**Figure S1.** Schematic representation of a nanopipette.

The total resistance of the inner solution can be evaluated for the total length $L$ (the distance between the pipette opening and the reference electrode inserted into the pipette), $L>>a$ :

$$R_{\text{int}} = \int_0^L \frac{1}{\kappa} \frac{dx}{\pi(a + x\tan\theta)^2} = \frac{L}{\pi\kappa a(a + L\tan\theta)} \approx \frac{1}{\pi\kappa a \tan\theta} \qquad \text{(S1)}$$

The solution resistance within the length $l$ from the orifice can also be evaluated as

$$R_l = \frac{l}{\pi\kappa a(a + l\tan\theta)} \tag{S2}$$

or in the dimensionless form

$$\frac{R_l}{R_{\text{int}}} = \frac{\frac{l}{a}\tan\theta}{1 + \frac{l}{a}\tan\theta} \tag{S3}$$

The plot of $R_l/R_{int}$ versus normalized length ($l/a$) is shown in Fig.S2. The effective length $l_{eff}$ of a nanopipette can be defined as the length of its tapered shaft adjacent to the tip that produces most (e.g., 90%) of the resistance. It shows that $l_{eff}$ depends strongly on pipette angle.

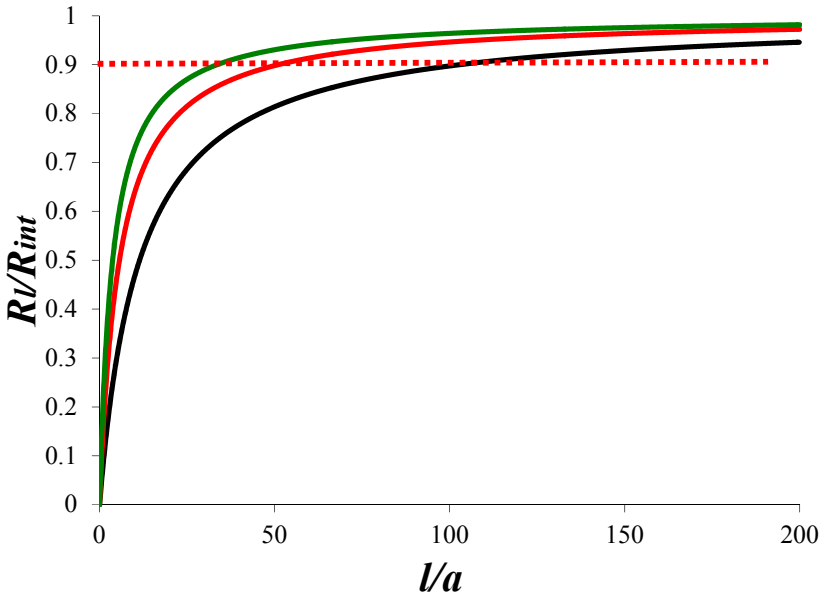


**Figure S2**. Resistance profile inside a solution-filled conical pipette as a function of pipette angle. $\theta$ = 15° (green), 10° (red), and 5° (black).

**Calculating the current change caused by the translocation of a spherical particle through the pipette tip**.

The total pipette resistance comprises two components, i.e., the resistances of the internal and external solutions:

$$R = R_{\text{int}} + R_{ext} \tag{S4}$$

The resistance of external solution is[S2]

$$R_{ext} = \frac{1}{4\kappa a} \tag{S5}$$

As a particle enters the pipette, the displacement of the electrolyte solution from the narrow shaft results in the increase in resistance of inner solution (the effect on resistance of outer solution is negligible). Two stages of the translocation process are shown in Fig.S3. The change in resistance occurs within the layer of solution containing the particle (or its part that has already entered the pipette). The resistance of this layer, $R_{par}$, can be calculated and then compared to its original resistance, $R_{ori}$, to find the change caused by the particle penetration.

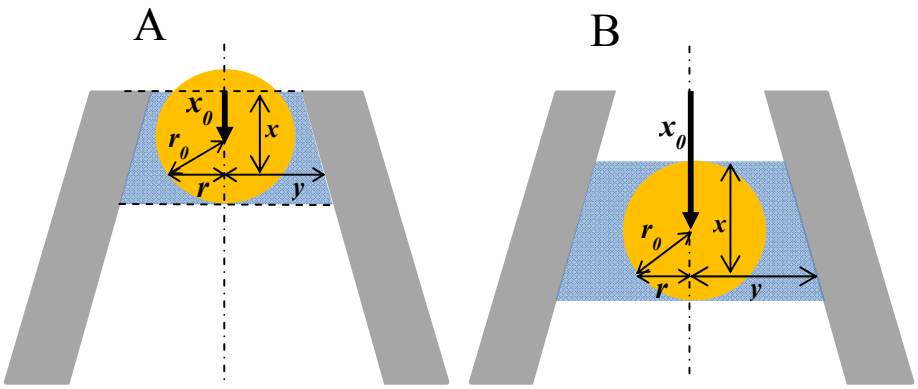


**Figure S3.** Schematics of the translocation process. (A) The particle is entering the pipette. The penetration depth, $x_0$ (i.e., the coordinate of the center of the particle with respect to the orifice) is within the range $-r_0 \le x_0 \le r_0$, where $r_0$ is the particle radius. (B) The particle is entirely inside the pipette; $x_0 \ge r_0$

To calculate $R_{par}$ for the first stage (partial penetration; Fig. S3A), consider a donut-shaped layer of the thickness *dx* at a distance *x* from the orifice, $0 \le x \le x_0 + r_0$. The outer radius of the donut, *y* is:

$$y = a + x\tan\theta \tag{S6}$$

The resistance of the layer of solution containing the particle (blue layer in Fig. S3) is

$$R_{par} = \int_0^{x_0+r_0} \frac{1}{\kappa}\frac{dx}{\pi\left(y^2 - r^2\right)} \tag{S7}$$

where $r$ is the inner radius of the donut defined as

$$r^2 = r_0^2 - (x - x_0)^2 \tag{S8}$$

After the integration, Eq. S7 yields

$$R_{par} = \frac{1}{\kappa\pi}\frac{2}{\sqrt{4AC - B^2}}\left\{\arctan\left[\frac{2A(x_0 + r_0) + B}{\sqrt{4AC - B^2}}\right] - \arctan\left(\frac{B}{\sqrt{4AC - B^2}}\right)\right\} \tag{S9}$$

where

$$A = \tan^2\theta + 1 \tag{S10}$$

$$B = 2(a\tan\theta - x_0) \tag{S11}$$

$$C = a^2 + x_0^2 - r_0^2 \tag{S12}$$

The resistance of the same layer without a particle was:

$$R_{ori} = \frac{x_0 + r_0}{\pi\kappa a[a + (x_0 + r_0)\tan\theta]} \tag{S13}$$

and the change in resistance is:

$$\Delta R = R_{par} - R_{ori} \tag{S14}$$

For the complete particle penetration (Fig. 3B), $0 \le x \le 2r_0$ and other variables are

$$y = a + (x_0 - r_0 + x)\tan\theta \tag{S15}$$

$$r^2 = r_0^2 - (r_0 - x)^2 \tag{S16}$$

$$R_{par} = \int_0^{2r_0} \frac{1}{\kappa}\frac{dx}{\pi(y^2 - r^2)}$$

$$= \frac{1}{\kappa\pi}\frac{2}{\sqrt{4AC - B^2}}\left[\arctan\left(\frac{4Ar_0 + B}{\sqrt{4AC - B^2}}\right) - \arctan\left(\frac{B}{\sqrt{4AC - B^2}}\right)\right] \tag{S17}$$

$$A = \tan^2\theta + 1 \tag{S18}$$

$$B = 2\left[\tan^2\theta(x_0 - r_0) + a\tan\theta - r_0\right] \tag{S19}$$

$$C = [a + \tan\theta(x_0 - r_0)]^2 \tag{S20}$$

The original resistance of the layer, which now contains the particle, was:

$$R_{ori} = \frac{2r_0}{\pi\kappa[a + (x_0 - r_0)\tan\theta][a + (x_0 + r_0)\tan\theta]} \tag{S21}$$

For both steps, the current change can be calculated as

$$\Delta i / i = [1/R - 1/(R + \Delta R)]/(1/R) = \Delta R/(R + \Delta R) \tag{S22}$$

Eq. S22 was used to calculate the effects of the pipette radius and angle (Fig. 6A and 6B in the main text).

**Fitting experimental current pulses to the theory**

Consider a one-dimensional motion (along the *x* axis) of a particle entering the pipette so that the particle center moves through the center of the orifice. We calculate a theoretical *i* vs *t* curve assuming that the transport inside the pipette is dominated by electrophoresis. The steady-state electrophoretic velocity of a particle in the electric field *E* is:[S3]

$$u = \mu E \tag{S23}$$

where $\mu$ is the mobility of the particle. The field, *E*, can be evaluated from the potential profile near the pipette orifice. If $V_0$ is the voltage applied between the internal and external reference electrodes, the potential at a certain point outside the pipette ( $x_0 < 0$; $x_0 = 0$ corresponds to the orifice) can be calculated by Newman's model as following:[S4]

$$V_{ext} = V_0 \times \frac{R_{ext}}{R} \times \left[1 - \frac{2}{\pi}\tan^{-1}\left(\frac{-x_0}{a}\right)\right] \tag{S26}$$

Eq. (S26) is an approximation because in Newman's model the potential is uniform over the disk surface (i.e., the pipette orifice in our model). However, the differences between the results obtained using Eq. (26) and those simulated using COMSOL Multiphysics were relatively small.

For a point inside the pipette ( $x_0 > 0$), the voltage drop can be evaluated as

$$V_{\text{int}} = V_0 \times \frac{R_{ext} + R_{x_0}}{R} \tag{S27}$$

where $R_{x_0}$ is the resistance of the electrolyte solution between the orifice and the point $x_0$, $R$ is the total resistance from Eq. S4 and $R_{ext}$ is the external resistance from Eq. S5.

$$R_{x_0} = \frac{x_0}{\pi\kappa a(a + x_0 \tan\theta)} \tag{S28}$$

Combining Eq. S27 with Eqs. S1, S4, S5 and S28, one obtains:

$$V_{\text{int}} = V_0 \times \frac{\frac{\pi}{4} + \frac{x_0}{a + x_0 \tan\theta}}{\frac{1}{\tan\theta} + \frac{\pi}{4}} \tag{S29}$$

The local electric field is:

$$E_{ext} = \frac{dV_{ext}}{dx_0} = \frac{V_0}{\frac{1}{\tan\theta} + \frac{\pi}{4}} \times \frac{1}{2a\left[1 + (x_0 / a)^2\right]} \tag{S30}$$

or

$$E_{\text{int}} = \frac{dV_{\text{int}}}{dx_0} = \frac{V_0}{\frac{1}{\tan\theta} + \frac{\pi}{4}} \times \frac{1}{a[1 + (x_0 / a)\tan\theta]^2} \tag{S31}$$

Therefore, the local velocity is

$$u_{ext} = \mu \times \frac{V_0}{\frac{1}{\tan\theta} + \frac{\pi}{4}} \times \frac{1}{2a\left[1 + (x_0 / a)^2\right]} \tag{S32}$$

or

$$u_{\text{int}} = \mu \times \frac{V_0}{\frac{1}{\tan\theta} + \frac{\pi}{4}} \times \frac{1}{a[1 + (x_0 / a)\tan\theta]^2} \tag{S33}$$

The time, $t$, required for the travel between the point $-r_0$ (where the front edge of the particle touches the pipette orifice) and a point $x_0$ in the external solution is

$$t_{ext} = \int_{-r_0}^{x_0} \frac{dx_0}{u_{ext}} = \frac{1}{\mu} \frac{\frac{1}{\tan\theta} + \frac{\pi}{4}}{V_0} \times 2a^2 \left\{ \frac{x_0}{a} + \frac{r_0}{a} + \frac{1}{3}\left[ \left(\frac{x_0}{a}\right)^3 + \left(\frac{r_0}{a}\right)^3 \right] \right\} \tag{S34}$$

If the point $x_0$ is inside the pipette, the corresponding time is

$$t_{\text{int}} = t_0 + \int_0^{x_0} \frac{dx_0}{u_{\text{int}}} = t_0 + \frac{1}{\mu} \frac{\frac{1}{\tan\theta} + \frac{\pi}{4}}{V_0} \times \frac{(a + x_0 \tan\theta)^3 - a^3}{3a \tan\theta} \quad \text{(S35)}$$

while $t_0$ is the time spent between $-r_0$ to 0.

Using Eqs. S34 and S35, the current vs. position plots (e.g., Figs 6A and 6B) can be converted to the current vs time dependence. An example of fitting an experimental pulse to the theoretical *i* vs. *t* curve is shown in Fig. 10B. If the electroosmotic flow is significant and opposite to the electrophoretic direction, the effective mobility value extracted from the fit should be approximately equal to the difference of the electrophoretic and electroosmotic mobilities.[S5]

S1. E. A. Heins, Z. S. Siwy, L.A. Baker, C. R. Martin, *Nano Lett.* 2005, **5**, 1824.

S2. J. Newman, K. E, Thomas-Alyea, *Electrochemical Systems*, John Wiley & Sons: Hoboken, 2004, p. 424.

S3. D. C. Harris, *Quantitative Chemical Analysis*, W.H. Freeman and Company, 6th edition, 2002, p. 657.

S4. J. Newman, *J. Electrochem. Soc*. 1966, **113**,501.

S5. M. Firnkes, D. Pedone, J. Knezevic, M. Döblinger, U. Rant, *Nano Lett.* 2010, **10**, 2162.