

# ENTROPIC TRAPPING OF A SINGLY CHARGED MOLECULE IN SOLUTION

## SUPPORTING INFORMATION

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### SECTION I: BROWNIAN DYNAMICS SIMULATIONS IN 2D AND 3D

In order to quantitatively relate the average measured escape time,  $t_{\text{esc}}$  of a particle to the depth of the potential well,  $W$ , we performed Brownian Dynamics (BD) simulations of the escape process as described previously [1, 2]. We first determine the full three-dimensional distribution of electrical potential in a single trap by solving the nonlinear Poisson-Boltzmann equation in the fluidic nanostructure. A molecule of charge  $q_{\text{eff}}$ , noted as  $q$  from hereafter, sampling the free energy landscape, experiences both thermal fluctuations in 3 dimensions and a force given by  $-\nabla q\psi(\mathbf{r})$  at any point  $\mathbf{r}$  in the landscape. Here  $\psi(\mathbf{r})$  is the local the electrical potential at  $\mathbf{r}$  in the absence of the particle [3].

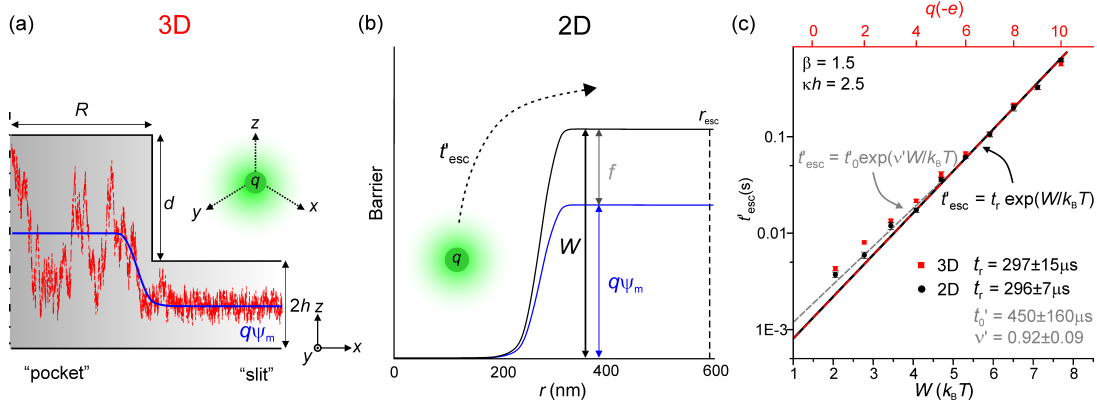


Figure S1: (a) Axisymmetric representation of the trapping nanostructure.  $2h$  is the slit height,  $d$  is the pocket depth and  $R$  the pocket radius = 300 nm. Red dashed trace indicates a representative  $z$ -trajectory of a particle of charge  $q$  sampling the 3D space of the trap.  $\psi_m$  is the electrostatic midplane potential in the slit region. (b) In the equivalent 2D simulation, the total well depth  $W$  (in black) experienced by the trapped molecule is  $W = q\psi_m + f$ , where  $f$  is the additional entropic contribution due to axial fluctuation of the molecule.  $r$  is a radial coordinate referenced to the center of the nanostructure. The escape boundary,  $r_{\text{esc}}$  (black dashed line), which must be crossed for an escape event to occur, is placed 300 nm away from the physical boundary of the nanostructure,  $r_{\text{esc}} = R + 300$  nm [2].  $t'_{\text{esc}}$  denotes the escape time based on instantaneous particle position. (c) Comparison of escape times based on instantaneous particle positions,  $t'_{\text{esc}}$  for both 3D and 2D approaches. In the regime of  $W = 5 - 8k_B T$ , the behaviour can be fit with the form  $t'_{\text{esc}} = t_r \exp(W/k_B T)$  according to Kramers' theory (overlapping black and dashed red lines). Here  $t_r$  is the position relaxation time of the particle which depends on the particle hydrodynamic radius,  $r_H$ , and is a fit parameter [1, 2]. The simulation results shown correspond to parameter values:  $r_H = 4.5$  nm,  $\beta = (2h + d)/2h = 1.5$  and  $\kappa h = 2.5$ , where  $\kappa$  is the inverse Debye length. There is no significant difference between a full 3D simulation (red squares) and a 2D simulation (black circles) in the regime of  $W > 5k_B T$ , as the fit parameter  $t_r$  is almost identical in the two cases. Error bars are smaller than the symbols. For  $W < 5k_B T$  the data is better represented by a fit of the form  $t'_0 \exp(\nu' W/k_B T)$  (dashed grey line), where  $\nu' < 1$  and  $t'_0 \sim 1.4t_r$ , discussed later in Section II.

We simulate a particle trajectory by iteratively solving the discretized overdamped Langevin equation, which reads as follows in one dimension:

$$\mathbf{x}(t + \delta t) = \mathbf{x}(t) - \mu \nabla q \psi(\mathbf{x}(t)) + \sqrt{2D\delta t} \mathbf{w}(t) \quad (\text{S1})$$

Here,  $\mathbf{x}(t)$  represents the instantaneous position of the molecule at time,  $t$  in one dimension, and  $D$  is the molecule's diffusion coefficient which depends on its hydrodynamic radius,  $r_H$ , and the viscosity of the medium,  $\eta$  as  $D = k_B T / 6\pi\eta r_H$ .  $\mathbf{w}(t)$  represents a displacement due to the random thermal force acting on the particle that satisfies  $\langle \mathbf{w}(t) \rangle = \mathbf{0}$  and  $\langle \mathbf{w}(t) \mathbf{w}^\top(t') \rangle = \mathbf{I}$  if  $|t - t'| \leq \delta t$ ,  $\mathbf{0}$  otherwise. Further  $\mu = \delta t / 6\pi\eta r_H$ , where the simulation time-step,  $\delta t = 10 \mu\text{s}$  is much larger than the momentum relaxation time  $\sim 10 \text{ ns}$  for a typical molecule.

At  $t = 0$ , the particle is located at  $x = y = 0$  and  $z = (2h + d)/2$ , where  $2h$  is the slit height and  $d$  the nanostructure depth. We use Eq.S1 to propagate the instantaneous position of the particle,  $\mathbf{x}(t)$ , forward in time for each spatial dimension, until its radial position  $r = \sqrt{x^2 + y^2}$  exceeds an arbitrary radial escape threshold,  $r_{\text{esc}}$  [2] (Fig.S1(b)). The time needed to reach  $r_{\text{esc}}$  from the bottom of the well corresponds to an escape event, or hop,  $\Delta t$ . Once the molecule has escaped, the simulation restarts and another escape trajectory is computed. The durations of the escape events are exponentially distributed and we typically average over a number of hops,  $N \sim 10^3$ , to obtain the average escape time  $t'_{\text{esc}}$ . Note that this analysis is performed without time-averaging of the spatial position and the simulation is repeated for different values of  $q$ .

We then compare the results of  $t'_{\text{esc}}$  vs  $q$  from a full 3D simulation, which is computationally very demanding, with an equivalent 2D simulation (Fig.S1(c)), in which a molecule of charge  $q$  is only permitted motion in  $(x, y)$ . Here the molecule is forced to sample a two-dimensional energy manifold whose value at any point  $(x, y)$  is given by the minimum electrostatic energy in  $z$  at every radial coordinate. Importantly in order to simulate 3D behavior using an equivalent 2D problem we add to the depth of the well, which would otherwise be simply  $q\psi_m$ , the fluctuation contribution  $f$ . Thus in the 2D simulations we use a well depth given by  $W = q\psi_m + f$ , where  $f$  is the fluctuation contribution calculated for each case as described in the main text (Fig.S1(b)). Identical to the 3D case, the molecule is considered to have escaped when it samples the region outside the radially symmetric boundary given by  $r_{\text{esc}}$ . We find excellent agreement (r.m.s. within 2%) between the average escape times,  $t'_{\text{esc}}$  computed using the two approaches in the regime of  $W \geq 5k_B T$ , where most of our experiments are performed. A representative molecular trajectory in  $z$  depicted in Fig.S1(a) clearly reveals that the axial fluctuation of the molecule in the “slit” are of much smaller amplitude than in the “pocket” region. This difference gives rise to the configurational entropy contribution to the total trap depth.

We point out that in order to relate experimentally measured escape times,  $t_{\text{esc}}$  to measured well depths, we perform the 2D BD simulation analysis using time-averaged co-ordinates  $\langle r \rangle_{t_{\text{exp}}}$ , rather than instantaneous positions  $r$ , as previously described [2]. The duration of an exposure time,  $t_{\text{exp}}$  is typically 5 ms in our experiments. Further, we examine the motion process in a 2D landscape of traps, corresponding to the experimental situation. This is because neighbouring wells in the landscape effectively act as absorbing boundaries for molecules and need to be included in order to accurately reconstruct the escape process [2].

## SECTION II: ESTIMATING UNCERTAINTIES IN THE CHARGE MEASUREMENT ON WEAKLY CHARGED MOLECULES

The measurement error,  $x_e$  on a quantity  $x$ , which is a function of the variables  $f, g, h$ , etc., each with uncertainties  $f_e, g_e, h_e$ , etc., can be expressed as follows:

$$x_e = \sqrt{\left(\frac{\partial x}{\partial f}\right)^2 f_e^2 + \left(\frac{\partial x}{\partial g}\right)^2 g_e^2 + \left(\frac{\partial x}{\partial h}\right)^2 h_e^2 + \dots} \quad (\text{S2})$$

In the regime of  $W \geq 5k_B T$ , the relationship between measured escape time,  $t_{\text{esc}}$ , and molecule effective charge, noted here as  $q$ , is well described by Kramers' theory and is given by:

$$q\psi_m + f = k_B T \ln \left( \frac{t_{\text{esc}}}{t_r} \right) \quad (\text{S3})$$

However the experiments shown in Fig.4, in which we measure the effective charge of 3 dye molecules, were performed in the regime of  $W < 5k_B T$ , where the dependence of  $t_{\text{esc}}$  on  $W$  departs from that given in Eq.S3.

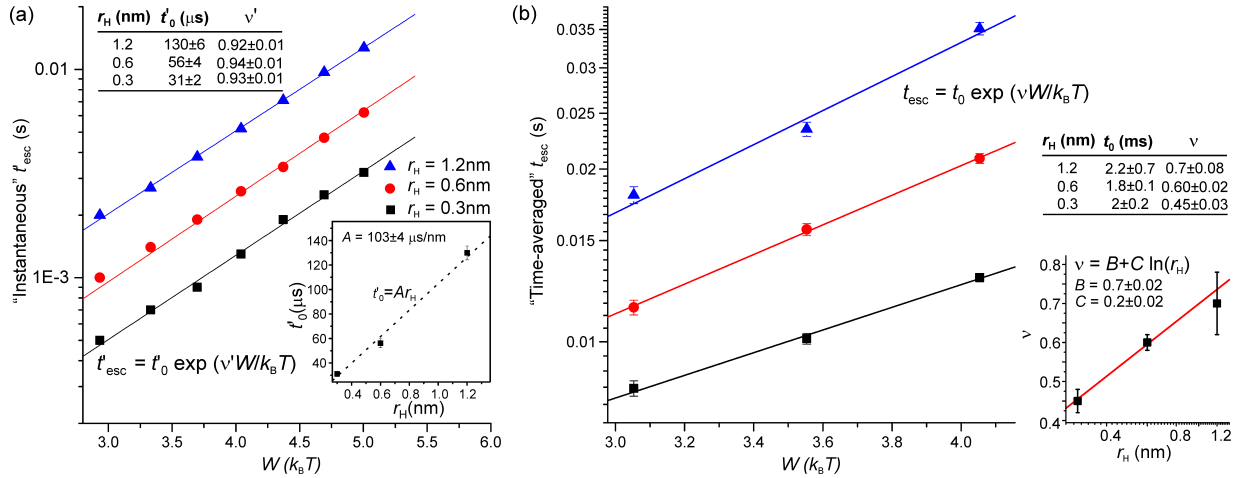


Figure S2: (a) The plot presents simulated  $t'_{\text{esc}}$  in the low  $W$  regime ( $W \leq 5k_B T$ ) for molecules of  $r_H$  ranging from 0.3 to 1.2 nm, analyzing the instantaneous coordinates of an object escaping from a single well. The fits shown are of the form  $t'_0 \exp(\nu' W / k_B T)$ . (b) Simulated  $t_{\text{esc}}$  using time-averaged coordinates of molecules sampling a landscape of traps. In general, for a given combination of  $W$  and  $r_H$ ,  $t_{\text{esc}}$  is substantially larger in this case. The inset table shows the values of the fit parameter  $t_0$  and  $\nu$  for different  $r_H$ . Inset plot shows  $\nu$  vs  $r_H$ , fit by a logarithmic form,  $\nu = B + C \ln(r_H)$ .

In order to study this behaviour, we performed BD simulations in the range of  $W \sim 3 - 5k_B T$ , as described in Section I (Fig.S2(a)). Figure S2 compares the results of simulated escape from a single well, obtained using instantaneous positions (as in Section I), and time-averaged approach, which also includes the effects given by the trap landscape geometry. In the experiment, a molecule must reside in a given trap for  $\Delta t \geq t_{\text{exp}}$  in order to accumulate enough signal on the detector and be recognized as trapped. In the simulation, we use the same criterion on the minimum residence time when analyzing a particle position trajectory, as previously described [2]. In general, for a given well depth, the "time-averaged"  $t_{\text{esc}}$  is substantially longer than the "instantaneous"  $t'_{\text{esc}}$  (Fig.S2).

In both cases, we find - as expected - that in the low well depth regime  $t_{\text{esc}}$  depends less strongly on  $W$ , by a factor  $\nu$ . This fit parameter is  $< 1$  for  $W \leq 5k_B T$  and is equal to 1 for  $W > 5k_B T$  where the Kramers' prediction well describes the behavior (Fig.S1(c)). Fitting the simulation with a functional form given by

$$t_{\text{esc}} = t_0 \exp\left(\frac{\nu W}{k_B T}\right) = t_0 \exp\left[\frac{\nu(q\psi_m + f)}{k_B T}\right] \quad (\text{S4})$$

we find that, when operating with instantaneous positions (Fig S2(a)), the prefactor  $t'_0$ , depends linearly on the size of the molecule (inset in Fig.S2(a)), and is also slightly larger than the relaxation time,  $t_r$  at high well depths by a factor  $\sim 1.4$  (Fig.S1(c)). Upon position averaging, however, the escape time no longer depends in a simple linear fashion on  $r_H$ . The inset Table in Fig.S2(b) shows that in this case the prefactor  $t_0$  is a constant, larger in magnitude and close to  $t_{\text{exp}}$ , that no longer carries information on the radius of the molecule. The fit parameter  $\nu$  captures the weaker response of the measured timescale on well depth. This different  $t_{\text{esc}}$  vs.  $W$  behaviour is most likely due to a finite (size-dependent) return probability of the molecule during the observation time [2]. For molecule of  $r_H = 0.6\text{nm}$ , which corresponds to the size the dyes measured [4], we find that  $\nu = 0.6 \pm 0.02$ .

We now write the fractional error  $q_e/q$  according to Eq.S2 as:

$$\frac{q_e}{q} = \sqrt{\left(\frac{1}{q^2}\right) \left(\frac{\partial q}{\partial \psi_m}\right)^2 \psi_{m,e}^2 + \left(\frac{1}{q^2}\right) \left(\frac{\partial q}{\partial t_{\text{esc}}}\right)^2 t_{\text{esc},e}^2 + \left(\frac{1}{q^2}\right) \left(\frac{\partial q}{\partial r_H}\right)^2 r_{H,e}^2} \quad (\text{S5})$$

The first term on the RHS of Eq.S5, from now on denoted as  $(q_e/q)_{\psi_m}$ , represents the uncertainty in determining the electrical potential at the midplane of the slit,  $\psi_m$ . This aspect has been discussed at length in our previous work [1], and arises from the uncertainty in determining the slit height ( $2h$ ) with an accuracy better than  $h_e = 1\text{ nm}$  and is estimated at about 5% when averaging over 4-5 independent experiments (typical Debye length  $\kappa^{-1} \sim 9\text{nm}$  and  $2h \sim 70\text{nm}$ ). In a single experiment however where the height of the slit may depart from the mean value by as much as 2 or  $3h_e$ , this (single) measurement inaccuracy can be as large as 15%. For the measurement of the dye ATTO532-maleimide, performed using a larger Debye length ( $\kappa^{-1} \sim 20\text{ nm}$ ) and thus higher  $\psi_m$ , the estimated fractional uncertainty on a single measurement is calculated to be as low as 6% ( $\sim 2\%$  upon averaging).

The second term of Eq.S5, that we denote as  $(q_e/q)_{t_{\text{esc}}}$ , arises from statistical uncertainty in measuring  $t_{\text{esc}}$  in a temporally limited experiment. Given that the escape events  $\Delta t$  are exponentially distributed, the measurement error  $t_{\text{esc},e}$  on their average value,  $t_{\text{esc}}$ , is function of the number of detected hops,  $N$ , as follows:

$$t_{\text{esc},e} = \frac{t_{\text{esc}}}{\sqrt{N}} \quad (\text{S6})$$

According to Eq.S4,  $(\partial q / \partial t_{\text{esc}}) = (k_B T / \nu t_{\text{esc}} \psi_m)$ . Using Eq.S6,  $(q_e/q)_{t_{\text{esc}}}$  gives

$$\left(\frac{q_e}{q}\right)_{t_{\text{esc}}}^2 = \left(\frac{1}{q^2}\right) \left(\frac{\partial q}{\partial t_{\text{esc}}}\right)^2 t_{\text{esc},e}^2 = \left(\frac{1}{q^2}\right) \left(\frac{k_B T}{\nu t_{\text{esc}} \psi_m}\right)^2 t_{\text{esc},e}^2 = \left(\frac{k_B T}{\nu q \psi_m \sqrt{N}}\right)^2 \quad (\text{S7})$$

Note that in this analysis we assume that  $f$  in Eq.S4 is constant, since the fluctuation contribution is largely charge-independent as discussed in the main text. We finally analyze the last term of Eq.S5, denoted by  $(q_e/q)_{r_H}$ , which represents the fractional contribution of the uncertainty on hydrodynamic radius,  $r_H$  to the total single measurement charge error.

As shown above (Fig.S2(b)), in the BD time-averaged approach at low  $W$ , the fit parameter  $\nu$  in Eq.S4 captures the dependance of  $t_{\text{esc}}$  on the size of the molecule and scales with  $r_H$  in a logarithmic fashion,  $\nu = B + C \ln(r_H)$ , where  $B = 0.7 \pm 0.02$  and  $C = 0.2 \pm 0.02$ . Therefore we can write  $(q_e/q)_{r_H}$  as function of  $\nu$  as follows:

$$\left(\frac{q_e}{q}\right)_{r_H}^2 = \left(\frac{1}{q^2}\right) \left[\left(\frac{\partial q}{\partial \nu}\right) \left(\frac{\partial \nu}{\partial r_H}\right)\right]^2 r_{H,e}^2 \quad (\text{S8})$$

Using Eq.S4 this can be rearranged as:

$$\left(\frac{q_e}{q}\right)_{r_H}^2 = \left(\frac{1}{q^2}\right) \left\{ \left[ -\frac{k_B T}{\psi_m \nu^2} \ln\left(\frac{t_{\text{esc}}}{t_0}\right) \right] \left(\frac{C}{r_H}\right) \right\}^2 r_{H,e}^2 = \left[ -\frac{C k_B T}{q \psi_m \nu^2} \ln\left(\frac{t_{\text{esc}}}{t_0}\right) \right]^2 \left(\frac{r_{H,e}}{r_H}\right)^2 \quad (\text{S9})$$

The fractional error  $(r_{H,e}/r_H)$  in Eq.S9 is estimated from an independent Dual-focus Fluorescence Correlation Spectroscopy (2fFCS) measurement [2] and is typically 5%.

The following Table summarizes the fractional errors for each dye molecule, and the overall uncertainty,  $(q_e/q)$ .

Molecule	$q_{\text{str}}(-e)$	$q\psi_m(k_B T)$	$W(k_B T)$	$(q_e/q)_{t_{\text{esc}}}$	$(q_e/q)_{\psi_m}$	$(q_e/q)_{r_H}$	$q_e/q$
ATTO 542-carboxy	4	0.72	3.37±0.06	9%	5%	3.4%	11%
ATTO 532-carboxy	2	0.36	2.75±0.10	20%	5%	6.2%	22%
ATTO 532-maleimide	1	1	3.36±0.05	4%	2%	5.4%	7%

# SUPPORTING MOVIE

The video shows an ETe measurement of 60bp dsDNA molecules in a device with neighbouring slits patterned with nanostructured circular indentations of depth,  $d_1 = 130$  nm (Panel a) and  $d_2 = 330$  nm (Panel b). The slit height  $2h$  was 70nm in both cases (Figure 1).

Both panels present fluorescence microscopy images of the molecules superimposed on an SEM of the nanostructured surface. The bottom panels display durations,  $t_{\text{esc},i}$  of individual escape events,  $i$ , for each case. The molecules sampling the nanostructure lattice with the deeper indentations (right panel) show a longer average escape time. The ratio of measured timescales agrees well with the theoretical expectation.

The sampling rate in the experiment was 10Hz. The movie is slowed down by a factor 5.

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