

Electrokinetic-Noise-Assisted Barrier Crossing in a Nanofluidic Environment

Shayan Lameh, Tim Zhao, and Derek Stein*

Department of Physics, Brown University, Providence, Rhode Island 02912, USA

We experimentally and theoretically studied the dynamics of DNA driven by artificial electrokinetic noise back and forth between neighboring nanopits inside a nanofluidic device. The dynamics are consistent with a noise-assisted barrier crossing process, exhibiting a rapid increase in the hopping rate with noise level beyond a threshold. A simple Arrhenius model with a fixed energy barrier of several $k_B T$ describes the hopping dynamics well at low noise levels, but to accurately describe the dynamics over a wider range, we developed a numerical model that additionally accounts for the lateral extent of the free energy landscape. The experimental and numerical methods reported here significantly expand the range of noise levels and timescales over which activated DNA hopping processes can be controlled and investigated.

I. INTRODUCTION

Nearly 100 years have passed since Kramers started work on reaction-rates [1] that led to a general theory of barrier crossing processes [2]. Kramers' theory now serves as a model for phenomena ranging from chemical reactions [3], to major shifts of Earth's climate [4–6], to crashes in the stock market [7, 8]. The theory relates the noise in a system (i.e., its intrinsic fluctuations) to the rate K that the system overcomes an energetic barrier. Barrier crossing phenomena in fluidic environments, including molecular sieving [9–11], giant acceleration of diffusion [12–14], and escape of trapped molecules from confinement [15–18], are normally driven by fundamental thermal fluctuations. Such phenomena are interesting and potentially technologically useful, but the boiling point of the liquid severely limits the range over which the thermal noise can be varied experimentally. Kramers' theory, which applies equally well to non-thermal noise sources, illuminates ways around that limitation.

We recently reported a method for injecting a controllable level of electrokinetic noise into a fluidic device and thereby amplify the Brownian motion of DNA polymers [19]. Here, we use electrokinetic noise to control the rate of DNA hopping over an entropic barrier in a nanofluidic device. We have used electrokinetic noise to explore noise levels and hopping rates that would require thermal temperatures exceeding 2000 K to achieve. We also present a numerical model of the DNA dynamics which accounts for the shape and extent of the free energy landscape, and which sheds light on timescales that are either too short or too long to be measured experimentally.

Nanofluidic devices confine ultra-small volumes of fluid and molecules in geometries that can be arbitrarily defined by nanofabrication. The nanofluidics field began with pioneering work by the groups of Austin and then Craighead [15, 20, 21], who sought to create artificial gels for separating DNA molecules by length.

The confining environment of nanofluidic systems enables one to probe statistical [22–24] and dynamical properties [25–28] of single molecules. Many unique DNA transport phenomena, including size-dependent DNA mobility in pressure-driven fluid flows [29], field-dependent electrophoretic mobility [30], giant acceleration of diffusion [14], and entrophoresis [31], also arise from interactions between a polymer coil and its confining environment. By defining topographic features within a nanofluidic device, it is in fact possible to sculpt the entropic landscape experienced by confined molecules nearly arbitrarily [11, 32–35]. This ability makes nanofluidics well suited to create the free energy landscape illustrated in Fig. 1 to study noise-assisted barrier crossing.

Figure 1b illustrates a device with two neighboring nanopits that creates a free energy landscape for DNA with two entropic traps. The free energy of DNA is relatively low inside a nanopit because it can explore more configurations and increase its configurational entropy there. The height of the free energy barrier between the nanopits is determined by the confining dimensions, in particular the height of the constriction separating the nanopits [34, 36].

* derek.stein@brown.edu

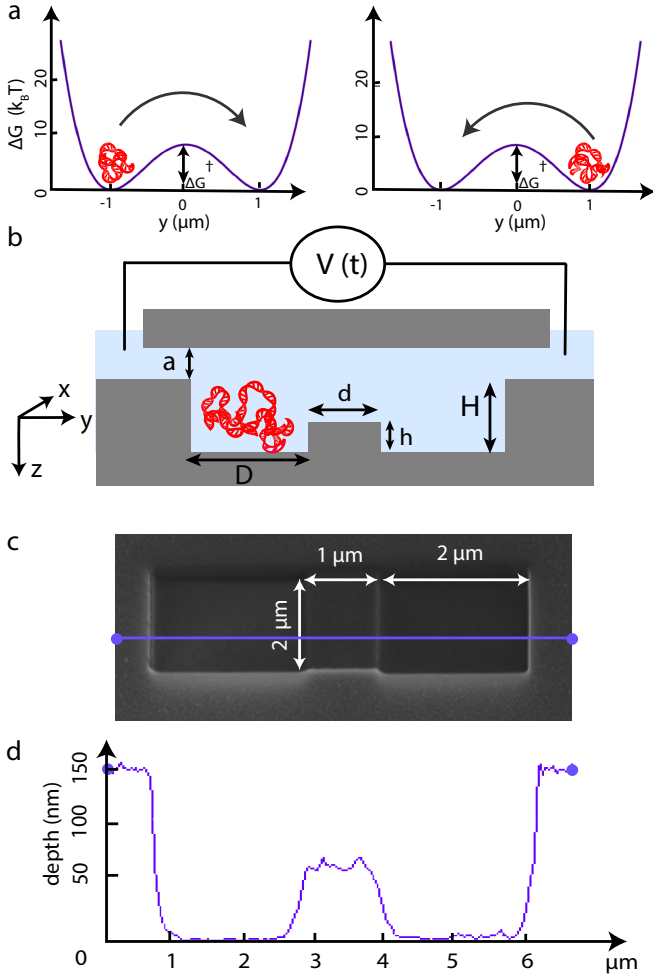


FIG. 1. Noise-assisted hopping. a) Schematic of DNA hopping between two free energy minima separated by a barrier of height ΔG^\ddagger . b) Nanofluidic device cross section with two nanopits that create entropic traps for a DNA molecule. The sketch shows the voltage noise source and the dimensions H , h , D , d , and a . c) Scanning electron micrograph of nanopits viewed from above. d) Height profile measured across a pair of nanopits as indicated by the blue line in c).

II. METHODS

A nanofluidic device was fabricated on a glass chip with a nanoslit of height $a = 75$ nm, length $L = 3.5$ mm, and width of $160 \mu m$ (Fig. 1b). Two U-shaped microchannels, each $0.5 \mu m$ deep, connected the nanoslit to two pairs of reservoirs. We used standard techniques described previously [14, 33, 34] to pattern and etch the nanoslit and microchannels into the device. To create a pair of pits, a focused ion beam (FIB) machine first milled a $2 \mu m$ by $5 \mu m$ trench within the slit, and then milled deeper in two square regions at both ends of the rectangle. Figure 1c) shows a scanning electron microscope (SEM) image of a typical double pit. The square

pits with width $D = 2 \mu m$ are separated by an obstacle with width $d = 1 \mu m$. Figure 1d) shows the profile of the double pit scanned by an atomic force microscope. The pits are $H = 150$ nm deep and the obstacle between them rises a height $h = 50$ nm above that. The purpose of the raised obstacle was to create an entropic barrier in the free energy landscape for confined DNA that molecules needed to overcome in order to move to the neighboring pit. A surface roughness with a peak-to-peak range of approximately 10 nm, as measured by atomic force microscopy, is the main source of experimental uncertainty in theoretical estimates of the free energy barrier; we parametrize the uncertainty in h and H with the 3.5 nm RMS roughness.

λ DNA (48.5 kbp, New England Biolabs) was fluorescently stained with YOYO-I dye at a 10: 1 base-pair-to-dye ratio. The DNA was suspended in 20 mM Tris-EDTA buffer that was titrated to pH 8.0 using HCl, and 4% v/v β -mercaptoethanol was added to suppress photobleaching. The suspension was diluted to a final DNA concentration of $0.2 \mu g/mL$ before being introduced into the nanofluidic device.

We imaged the DNA using an optical microscope (Nikon TE2000-U) with a $60\times$ water immersion objective lens with a numerical aperture of 1.20, a digital camera (Andor iXon). A 120 W metal-halide lamp (EXFO X-Cite 120) supplied ultraviolet light for fluorescence imaging. A physical shutter (Uniblitz Model VCM-D1) was used to limit the exposure time and thereby mitigate photo-bleaching of the stained DNA molecules. Videos of 100 images were recorded with a 50 ms exposure time and intervals of $\Delta t = 500$ ms between frames. We confirmed that the optical images of the double-pit structures showed no measurable distortion when compared with atomic force microscopy profiles of the same devices.

Finally, we applied a recently described technique to inject electrokinetic noise in the nanofluidic device and study its effects on the DNA dynamics [19]. Briefly, a digital-to-analog voltage controller (National Instruments) applied a time-dependent noise voltage $V(t)$ across the nanoslit. $V(t)$ had Gaussian fluctuations with an adjustable standard deviation, σ_e , zero mean, and a finite bandwidth Δf . The resulting electric field induced a fluctuating force in the y direction on confined molecules, as illustrated in Fig. 1 b). The applied voltage is related to the resulting DNA velocity by the electrophoretic mobility, $\mu = v/V(t)$, which accounts for the electrophoretic DNA motion through the fluid and the electroosmotic flow of fluid within the channel.

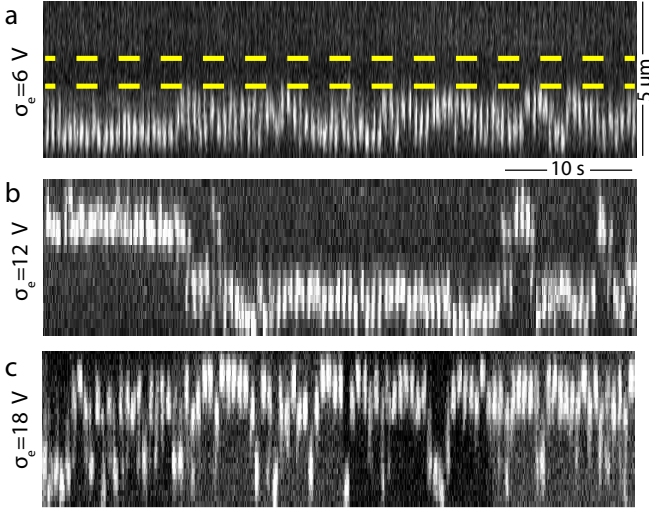


FIG. 2. Montage of time-resliced fluorescence images of a λ -DNA molecule hopping between neighboring nanopits with (a) $\sigma_e = 6$ V, (b) $\sigma_e = 12$ V, and (c) $\sigma_e = 18$ V applied across the fluidic device. (a) shows the boundaries between the pits and the central obstacle. The outer boundaries of the pits reach the upper and lower boundaries of the image. The contrast and brightness of the images were adjusted for clarity.

Figure 2 shows representative λ DNA trajectories under the influence of different levels of applied noise. With a low noise level of $\sigma_e = 6$ V, a molecule remained trapped within a single pit for the 50 s duration of the measurement (Fig. 2a). With $\sigma_e = 12$ V, an identical molecule hopped between the two pits about 7 times in total, with apparently random intervals of time between each hop (Fig. 2b). $\sigma_e = 18$ V resulted in about 20 hops in the same time interval (Fig. 2c). We note that we only tracked molecules that were at least $20 \mu\text{m}$ away from the edges of the $160 \mu\text{m}$ slit in order to avoid possible edge effects.

The data in Fig. 2b shows that the DNA molecule occasionally straddled two pits simultaneously for more than a single frame of the video. This indicates that DNA could require more time than the 0.5 s interval between images to overcome the central obstacle, especially for low values of σ_e . We also note that in Fig. 2c the molecule occasionally hops back and forth between the two pits with every frame. This suggests that the crossing time may be significantly shorter than the 0.5 s interval between frames when σ_e is large. Taken together, the observations emphasize that in our measurements the DNA cannot move the distance between pits instantaneously, nor can the camera resolve the fastest crossing rates. To build a theoretical understanding of the dynamics, we developed a numerical model that takes these important physical factors into account.

We simulated a point particle in a bistable potential with a fixed energy barrier, as illustrated in Fig. 1a. The dynamics are described by a Langevin equation for an

overdamped Brownian particle at position y

$$\dot{y} = -\frac{dG(y)}{dy} \frac{1}{\zeta} + \sqrt{\frac{2D_{\text{eff}}}{dt}} dW_t, \quad (1)$$

where D_{eff} is the effective diffusion coefficient under σ_e , ζ is the drag coefficient, and W_t is a standard Wiener process with $\langle dW_t^2 \rangle = dt$. As we reported previously [19], D_{eff} is related to σ_e^2 and the thermal diffusivity, D_0 , by $D_{\text{eff}} = D_0 + \alpha\sigma_e^2$, where $\alpha = \mu^2/4\Delta fL^2$. D_0 depends on the confinement of the nanofluidic device. However, D_0 is expected to vary only modestly between the different parts of the double-pit structure: When a molecule is above the central barrier, the effective slit height is 175 nm and $D_0 \approx 0.08 \mu\text{m}^2/\text{s}$, and when the molecule is in either pit, the effective slit height is 225 nm and $D_0 \approx 0.11 \mu\text{m}^2/\text{s}$. For the purpose of simplifying our numerical model calculations, we implemented a uniform diffusivity. The value $D_0 \approx 0.09 \mu\text{m}^2/\text{s}$ we used corresponds to the diffusivity of λ DNA in a slit of intermediate height $H = 200$ nm, based on its bulk radius of gyration $R_g = 0.69 \mu\text{m}$, its bulk self-diffusion coefficient $D_{\text{bulk}} = 0.46 \mu\text{m}^2/\text{s}$ [37], and the previously measured relationship between D_0 , D_{bulk} , R_g , and H for DNA confined in a nanoslit [38]. It is reasonable to base estimates of DNA diffusivity in our device on theory and measurements of DNA in slits, because the in-plane radius of gyration for λ DNA confined in a 200 nm deep slit is about $0.7 \mu\text{m}$ [39], hence a molecule at the center of a $2 \mu\text{m} \times 2 \mu\text{m}$ square pit should not significantly interact with the pit edges. The double-well potential $\Delta G(y) = -c_2 y^2 + c_4 y^4$ is sketched in Fig. 1a. The parameters $c_2 = 12.2 k_B T (\mu\text{m})^{-2}$ and $c_4 = 6.1 k_B T (\mu\text{m})^{-4}$ describe a free energy landscape with a barrier of height $\Delta G^\dagger = 6.2 \pm 0.4 k_B T$ between the two minima; we obtained that value of ΔG^\dagger from a theoretical scaling model of the confinement free energy that was previously validated [33, 36, 40–42] (See Supplemental Material at [URL] for details of the calculations). The minima of the free energy landscape are located at $y = \pm 1 \mu\text{m}$, which is where we estimate the λ DNA coil begins to interact with the physical obstacle. We simulated trajectories by solving Eq. 1 using Euler's method in Python with a time step of $dt = 1$ ms.

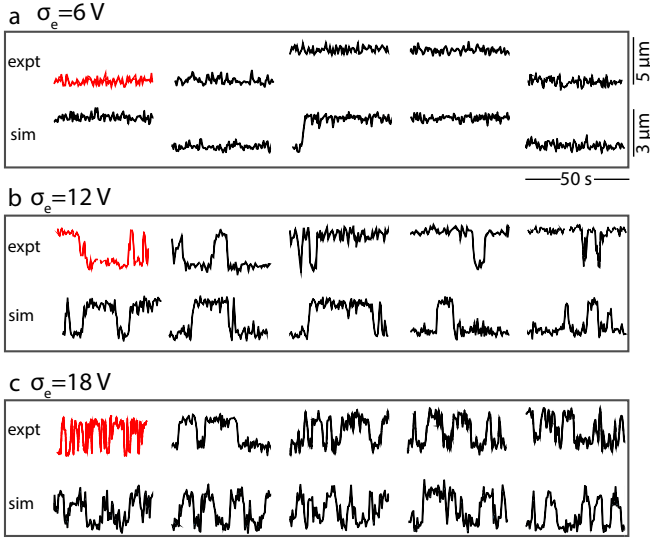


FIG. 3. Representative trajectories of DNA molecules in the y direction are presented from measurements and simulations with a) $\sigma_e = 6$ V, b) $\sigma_e = 12$ V, and c) $\sigma_e = 18$ V. Vertical scale bars are $5 \mu\text{m}$ for experimental traces and $3 \mu\text{m}$ for simulations. Horizontal scale bars are 50 s. The red trajectories correspond to the molecules represented in Fig 2. Simulated trajectories were sampled every $\Delta t = 500$ ms, matching the experiments.

Figure 3 compares experimental and simulated DNA trajectories over 50 s intervals at different σ_e . Custom image analysis software [14, 19, 33] obtained the DNA center-of-mass (COM) location every $\Delta t = 500$ ms from the video recordings. The simulated trajectories presented in Fig. 3 were also sampled every $\Delta t = 500$ ms, although the trajectories were computed using a time step of $dt = 1$ ms. The measured and simulated trajectories corresponding to the same noise intensity are both qualitatively and quantitatively similar, with the understanding that an exact correspondence of stochastic trajectories is not to be expected: With $\sigma_e = 6$ V, none of the 5 measured DNA trajectories showed a hop, while only a single hop occurred within the 5 simulated trajectories. As σ_e increased to 12 V, the frequency of hops increased to about 5 per 50 s interval for both the measured and the simulated trajectories. At $\sigma_e = 18$ V, the hops occurred so frequently in both measurements and simulations that it became difficult to distinguish individual hops by eye.

To systematize our analysis of noise-assisted hopping, we define a hop by the following rule: A successful hop occurs when the COM escapes the boundary of one pit and subsequently enters the boundary of the neighbouring pit. The relevant boundaries are illustrated as yellow dashed lines in Fig. 2a.

III. RESULTS

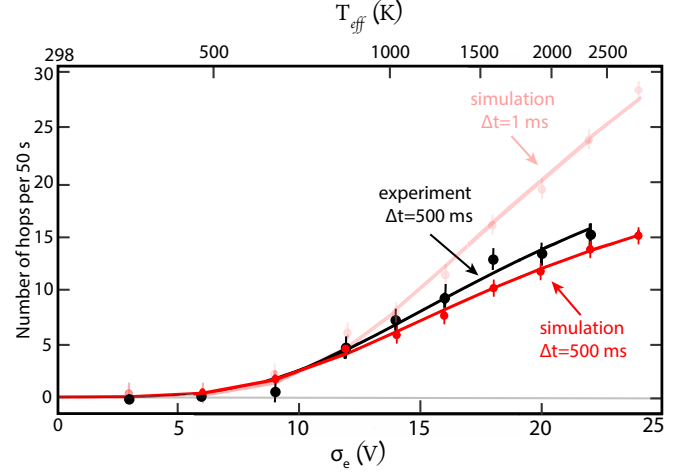


FIG. 4. σ_e -dependence of the mean number of DNA hops between nanopits in a 50 s interval. Black dots are mean experimental hops from at least 40 DNA molecules. Dark red dots represent mean hops from 100 simulated trajectories sampled at $\Delta t = 500$ ms. Light red dots are mean hops from the same simulated trajectories but sampled at $\Delta t = 1$ ms. Error bars are the standard error. Solid curves are fits of Eq. 3 to the data, obtaining $K_0 = 0.64 \text{ s}^{-1}$ and $\Delta G^\ddagger = 7.7 k_B T$ for the experiments, $K_0 = 0.54 \text{ s}^{-1}$ and $\Delta G^\ddagger = 7.4 k_B T$ for the simulations with $\Delta t = 500$ ms, and $K_0 = 1.23 \text{ s}^{-1}$ and $\Delta G^\ddagger = 10.1 k_B T$ for the simulations with $\Delta t = 1$ ms.

Figure 4 shows relationships between the hopping rate and σ_e . In measurements, DNA molecules were stably pinned in one pit or the other for low values of σ_e . The hopping rate increased rapidly from 0.01 s^{-1} to 0.26 s^{-1} as σ_e increased from 9 V to 18 V, beyond which value the hopping rate began to saturate. The results from the simulated trajectories closely followed the measured results when the sampling rates matched ($\Delta t = 500$ ms). However, when the same simulated trajectories were sampled every $\Delta t = 1$ ms, the number of hops detected increased more rapidly beyond $\sigma_e = 9$ V; the hopping rate reached 0.47 s^{-1} at $\sigma_e = 22$ V, as compared with 0.27 s^{-1} with $\Delta t = 500$ ms. An explanation for this discrepancy is that some back-and-forth hops that complete in less than 500 ms are undetectable in trajectories sampled at $\Delta t = 500$ ms, whereas they can be observed with $\Delta t = 1$ ms. The discrepancy is low at low σ_e because rapid hops are less likely to occur.

The σ_e -dependent hopping rate seen in Fig. 4 is reminiscent of a thermally activated barrier crossing process, except in this case, electrokinetic noise plays the role of temperature. We previously reported how the addition of electrokinetic noise across a nanoslit amplifies Brownian dynamics in a way that can be characterized by an effective temperature [19]

$$T_{\text{eff}} = T(1 + \alpha\sigma_e^2). \quad (2)$$

Combining Eq. 2 with the Arrhenius equation obtains a simple model of the noise-assisted hopping rate

$$K = K_0 \exp \left[- \frac{\Delta G^\ddagger}{k_B T (1 + \alpha \sigma_e^2)} \right], \quad (3)$$

where K_0 is the attempt rate and ΔG^\ddagger is the barrier height. We fit Eq. 3 to the data in Fig. 4, using K_0 and ΔG^\ddagger as fit parameters. The Arrhenius rate and the DNA hopping rate both rise rapidly beyond a certain effective temperature threshold. The fits follow each data set closely, and the measured and simulated trajectories with $\Delta t = 500$ ms obtained comparable values for K_0 and ΔG^\ddagger .

Shortcomings of the simple Arrhenius model are revealed by fits of Eq. 3 to the data in Fig. 4. Consider the simulated trajectories: Whereas the true barrier height was $\Delta G^\ddagger = 6.1 k_B T$, the fit of Eq. 3 obtained a somewhat higher value, $\Delta G^\ddagger = 7.4 k_B T$, for $\Delta t = 500$ ms, and a significantly higher value, $\Delta G^\ddagger = 10.1 k_B T$, for $\Delta t = 1$ ms. Furthermore, the fit value of K_0 was inconsistent; the value obtained with $\Delta t = 500$ ms was less than half the value obtained with $\Delta t = 1$ ms. Why does the Arrhenius model fall short? The model only considers a fixed free energy barrier between two stable states, without accounting for the distance between them or the time it takes an object to travel that distance. That approximation is reasonable for low σ_e , where the DNA molecule is strongly confined and spends most of the time inside a pit. However, for high σ_e the DNA molecule hops frequently across the free energy landscape, and the time it spends in transit between the pits becomes a significant fraction of the total time. A model must account for the finite distance between the free energy minima to accurately describe the dynamics at high σ_e .

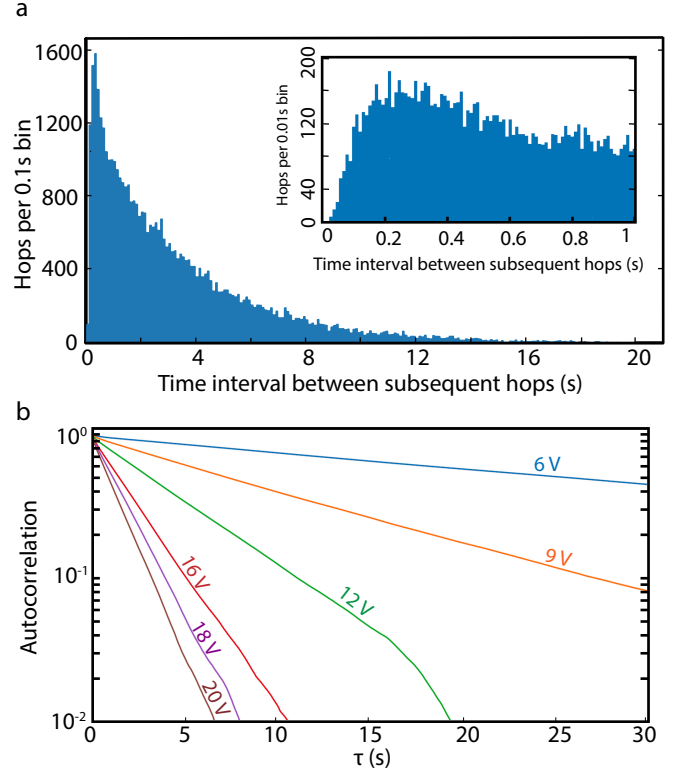


FIG. 5. Statistics of simulated hopping trajectories. a) Distribution of time intervals between subsequent hops for a 100,000 s simulated trajectory with $\sigma_e = 20$ V. Inset: Short time detail of the same distribution. b) Autocorrelation of y position with τ for simulated trajectories with $\sigma_e = 6, 9, 12, 16, 18$, and 20 V.

Figure 5a shows the distribution of intervals between subsequent hops of a simulated molecule. The distribution shows an exponential decay on long time intervals (> 1 s), which is a hallmark of a noise-activated, barrier-crossing process. On short time intervals, there is a delay before the distribution exhibits a decaying character; the frequency of hopping intervals rises from zero to the peak value over the range 0 to 0.2 s. The finite time it takes a molecule to travel across the barrier explains that relative dearth of short hopping intervals.

Figure 5b plots the position autocorrelation, C_y , of a simulated particle as a function of the lag time, τ , for $\sigma_e = 6, 9, 12, 16, 18$, and 20 V. C_y decays exponentially with τ , and higher σ_e is associated with faster rate of decay. From the slopes of the curves in Fig. 5b, we find the characteristic decay times to be 35, 11, 4.6, 2.2, 1.7, and 1.4 s, ordered by increasing values of σ_e .

IV. DISCUSSION AND CONCLUSION

We have shown that it is possible to create a bi-stable fluidic device for a DNA molecule and controllably increase the DNA hopping rate between the two stable sites with artificial electrokinetic noise (Fig. 1). The resulting DNA hops correspond to thermally activated

barrier crossing at an effective temperature that is adjustable well above temperatures that DNA can survive in (Fig. 4). It is worth noticing that the actual thermal temperature was not changed; we confirmed using a thermocouple that the device temperature increased by only 0.1 C after an hour of applying the highest electrokinetic noise level, $\sigma_e = 22$ V. The curves in Fig. 4 are experimental and simulation fits of the noise-induced hopping rate between the stable states using Arrhenius equation 3 with 2 fit parameters, attempt rate K_0 and barrier height ΔG^\ddagger .

The Arrhenius model explains the hopping rate of confined DNA polymers under electrokinetic noise reasonably well at low noise levels, but Fig. 3, 4, 5 are evidence that the Arrhenius model deviates significantly at higher noise levels. For example Fig. 3 highlights the significant amount of time that the DNA molecules spend between the stable sites at high noise level, which is not accounted for in the Arrhenius model. Fig. 4 shows that different sampling rates of the same simulated trajectories produce different parameters under the Arrhenius model. The inset of Fig. 5a shows the violation of the instantaneous transition assumption under Arrhenius model. The shortcomings of an Arrhenius model can be overcome with a numerical model of Brownian particle dynamics in an extended free energy landscape that accounts for the distance between the two stable sites. Parameters of the free energy landscape used in the model can be obtained from Flory scaling theory; remarkably accurate predictions are obtained with a scaling pre-factor of 1. Additionally, the numerical simulations offered a way to study the DNA hopping dynamics on much shorter timescales (< 0.2 s) and longer timescales (> 60 s) than one can probe experimentally. We conclude that this numerical approach makes superior quantitative predictions of the dynamics and offers better theoretical insight into the noise-

assisted hopping process for DNA.

An ability to control the hopping rate of DNA across free energy barriers could open avenues for fundamental studies and biotechnological applications. For example, stochastic resonance (SR) is a general phenomenon that could be realized with a single DNA. SR refers to the collaboration of white noise with a weak time varying signal in a bistable system where an optimum noise level enhances the effect of the weak signal. To experimentally study SR for a particle over an entropic barrier, one needs access to an extremely wide range of noise levels without varying the barriers height. The indicator of SR would be to see the synchronization between a weak periodic driving force and the hopping of the particle back and forth over an entropic barrier.

There is also an opportunity to study how artificial noise influences the rates at which DNA molecules cross biologically relevant constrictions. In particular, passing DNA across barriers such as the blood-brain barrier [43–45] and endosomes [46–49] is a major obstacle in gene therapy. Electroporation is a technique of applying electric field pulses across a cell to increase the cell membrane permeability, which is a proven method of increasing the gene transfer efficiency in vivo and in vitro [50–52]. However, the strength of the electric field and the duration of exposure that cells can survive severely limits the technique; for example, tests on brain endothelial cells found the cell death grew increasingly likely as 50 ms electric field pulses increased beyond approximately 250 V/cm [44, 45]. We speculate that electrokinetic noise, whose strong electric fields have a mean of zero, may pose a relatively reduced risk to the cells, while the effect of increasing the barrier crossing rate might promote gene transfection. Fundamental research along these lines could lead to new biotechnologies and improved therapeutic methods.

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