Charge, Diffusion, and Current Fluctuations of Single-Stranded DNA Trapped in an MspA Nanopore

Stephen J. Fleming,1 Bo Lu,1 and Jene A. Golovchenko1,2,*
1Department of Physics and 2School of Engineering and Applied Sciences, Harvard University, Cambridge, Massachusetts

ABSTRACT We report effective charges and diffusion constants of several different single-stranded DNA oligonucleotides trapped in an MspA nanopore. Nucleotide identity is found to have a substantial influence on effective charges and diffusion constants. These quantities are determined from escape time experiments for a DNA molecule attached to a NeutrAvidin molecule that, unlike the DNA, does not pass through the pore. Correlations are reported between oligonucleotide effective charges and current blockages, and between their diffusion constants and DNA-induced current blockage fluctuations. We also report an unanticipated source of current fluctuations that reflects a discrete blockage current level structure. We posit that this is associated with interactions between the NeutrAvidin molecule and the MspA nanopore.

INTRODUCTION

This work demonstrates how electrical measurements on a Mycobacterium smegmatis porin A (MspA) nanopore, in which protein-terminated single-stranded DNA (ssDNA) is trapped, can reveal fundamental and oligonucleotide-specific aspects of the interacting system of molecules. MspA is a pore-forming membrane protein that is well suited for single-molecule DNA experiments due to its geometry and stability (1–3). The narrowest constriction of MspA is ~1.2 nm in diameter, barely large enough for ssDNA to pass through. When a single MspA pore is established in a lipid membrane and a voltage bias is applied across it, a detectable ionic current flows through the pore from the surrounding electrolyte solution. Nanopore DNA experiments measure the blockage of this ionic current when a DNA molecule is threaded through the pore. Fig. 1A illustrates how a charged ssDNA molecule with a molecular stop attached can be threaded through and trapped in a nanopore by applying a voltage bias across the pore. In previous work, molecular stops such as an enzyme (4–8), bound protein (9–13), or a DNA double strand (2,14,15) have been used to assist in DNA trapping. The magnitude of the ssDNA-induced ionic current blockage depends on the sequence of ssDNA nucleotides near the pore’s constriction (4,8,16,17). It has been determined that 4–5 nucleotides contribute to the measured current blockage at any given time (9). This is significantly larger than the two nucleotides that can be simultaneously accommodated within MspA’s narrowest constriction. A factor accounting for averaging over neighboring nucleotide contributions is the thermal motion of the ssDNA in the pore (10).

Measurement of mean ionic current as ssDNA is advanced through the nanopore was first proposed (18) and later successfully used (6,8,16) to distinguish different nucleotides in nanopore sequencing. Current fluctuations during this process play a major role in the accuracy of the method, but they are not yet well studied or understood. Here, from measurements of nanopore currents, we determine effective charges, diffusion constants, current blockages, and ionic current fluctuations for ssDNA-NeutrAvidin complexes trapped in an MspA pore. We find that these experimental quantities show significant sensitivity to nucleotide identity.

Two different kinds of current fluctuations are observed. For small voltage bias (<100 mV) across the pore, Gaussian white noise is dominant. The magnitude of this noise depends on nucleotide identity. At high voltage bias (>100 mV), the current also contains telegraphlike current fluctuations between different current levels. We posit that these fluctuations are caused by the NeutrAvidin molecular stop interacting with the MspA pore rather than by the DNA in the pore’s constriction.

In addition, we identify nucleotide-dependent correlations between effective charges and current blockages, as well as between current fluctuations and diffusion constants.
These correlations provide insight into the physical processes involved.

Recently, we presented a preliminary study focused on extracting the effective charge and diffusion constant of the single DNA oligonucleotide poly(dA) trapped in an MspA pore (10). A first-passage, drift-diffusion model was used to extract these constants. Here we significantly extend this study to a range of DNA oligonucleotides, and determine for the first time, to our knowledge, the intrinsic nanopore current fluctuations for each case. See the literature (10,11,15,19–22) for earlier related work.

**MATERIALS AND METHODS**

The specific MspA protein mutant used in these experiments is the G75S/G77S/L88N/D90N/D91N/D93N/D118R/Q126R/D134R/K139E mutant of wild-type MspA, the same pore used in Lu et al. (10).

Single-channel recordings of MspA in a lipid bilayer were carried out as previously described in the literature (18). Briefly, a lipid bilayer of 1,2-diphtyranoyl-sn-glycero-3-phosphocholine (DPhPC; Avanti Polar Lipids, Alabaster, AL) was established across a 30 μm diameter aperture in Teflon, separating two Teflon reservoirs containing 1 M KCl and 10 mM HEPES at pH 8.0. 1-hexadecene was used as the lipid solvent to form an annulus connecting the bilayer to the Teflon support. A single pore was characterized by a return to the full open-pore conductance, which is essential for these measurements, and was ensured by measuring the ionic current as a function of voltage (details in Fig. S2 in the Supporting Material). The correct orientation of the pore is determined for the first time, to our knowledge, the intrinsic nanopore current fluctuations for each case. See the literature (10,11,15,19–22) for earlier related work.

Single-channel recordings of MspA in a lipid bilayer were carried out as previously described in the literature (18). Briefly, a lipid bilayer of 1,2-diphtyranoyl-sn-glycero-3-phosphocholine (DPhPC; Avanti Polar Lipids, Alabaster, AL) was established across a 30 μm diameter aperture in Teflon, separating two Teflon reservoirs containing 1 M KCl and 10 mM HEPES at pH 8.0. 1-hexadecene was used as the lipid solvent to form an annulus connecting the bilayer to the Teflon support. A single pore was characterized by a return to the full open-pore conductance, which is essential for these measurements, and was ensured by measuring the ionic current as a function of voltage (details in Fig. S2 in the Supporting Material). The correct orientation of the pore is determined for the first time, to our knowledge, the intrinsic nanopore current fluctuations for each case. See the literature (10,11,15,19–22) for earlier related work.

RESULTS AND DISCUSSION

Here ssDNA-NeutrAvidin escape time experiments are described for (dA)_{27}, (dC)_{27}, (dT)_{27}, and (dGdA)_{13}(dG) ssDNA oligonucleotides (all 27 nucleotides long) escaping from an MspA pore. Effective charges and diffusion constants are extracted from these experiments for each oligonucleotide. Ionic blockade currents and their fluctuations are measured. Correlations are pointed out between the effective charges and the ionic current blockages, and between the diffusion constants and the blockade current fluctuations. We also present and discuss an unanticipated contribution to current fluctuations at high voltage bias.

**DNA escape times**

Fig. 1 A contains a schematic illustration of the experiment. The ssDNA, shown in red, is linked via a 3′ biotin to a NeutrAvidin protein shown in gray. This
ssDNA-NeutrAvidin complex is captured in the MspA nanopore from solution by applying a 160 mV bias across the pore via electrodes in the surrounding electrolyte. This orients the ssDNA in the pore in the same direction used in nanopore sequencing experiments, 5′ first (16). In Fig. 1 B, typical current traces are shown for the open pore current and for currents observed after ssDNA oligonucleotides have been trapped in the pore, blocking ionic current. Poly(dA), poly(dC), and poly(dGdA) have slightly different current blockage levels, while poly(dT) exhibits a far greater blockage. Note that poly(dGdA) has a noisier current trace than the other oligonucleotides.

The effective charges and diffusion constants are deduced from escape-time experiments illustrated in Fig. 1 C. The ssDNA-NeutrAvidin complex is first attracted, captured, and trapped in the MspA pore at 160 mV bias. The bias is then reduced to a value such that the diffusion process leads to an eventual escape of the ssDNA back out through the pore on a timescale that can be easily measured (here 100 μs to 5 s). Fig. 1 C, bottom, shows one such event for a single poly(dT) molecule held at 65 mV. The escape time is the time interval between the voltage bias reduction and the return of the nanopore current to its open pore value (at the reduced bias), which occurs when the ssDNA leaves the MspA pore constriction. For the case shown, the escape time is 680 ms. The escape time depends on the bias voltage and the statistical diffusion process, so the escape times will not be the same even for identical single-molecule experiments. With larger bias voltages applied during the escape process, the escape time is expected to increase.

Escape time distributions are obtained from repeated (many hundreds to approximately a thousand) single-molecule events for each oligonucleotide at each of eight clamping voltages. Escape time distributions for each of the four oligonucleotides at 60 mV bias are shown in Fig. 2 A. The first-passage model we use predicts an exponentially decaying distribution of escape times. The distribution of escape times in Fig. 2 A agrees with this prediction, except at long times where there are very few events. The origin of the infrequent, nonexponentially distributed events with long escape times has been discussed in previous work (11,22,23), and we believe these events are likely caused by infrequent, specific ssDNA-nanopore interactions in our case, but interactions between MspA and NeutrAvidin might also contribute. The exponential distribution function is characterized by a decay constant called the "average escape time". Its value is determined by a least-square fit to each data set's short-time exponential region, as explained in the Supporting Material. The average escape times depend on both oligonucleotide identity and the applied bias voltage, and they are plotted as data points in Fig. 2 B.

**Extraction of effective charges and diffusion constants**

The dotted curves in Fig. 2 B are least-square fits of our first-passage dynamical model for the voltage dependence of the average escape time for each oligonucleotide to the data points in Fig. 2 B. The fits have been obtained for each oligonucleotide by adjusting a voltage-independent effective charge and diffusion constant, which are the only parameters in the drift-diffusion model. (Details of the first-passage model are contained in the Supporting Material.) The agreement obtained is excellent over many orders of magnitude of average escape times, as is evident from Fig. 2 B.

In the model, ssDNA is taken to be a rod of charge diffusing in one dimension through the pore. The effective charge per base, \( Q_{\text{eff}} \), is given by \( Q_{\text{eff}} = \sigma_{\text{eff}}/b_0 \), where \( \sigma_{\text{eff}} \) is the effective linear charge density of the ssDNA in MspA, and \( b_0 \) is the distance between bases, taken to be 0.5 nm (24). The effective charge per base, \( Q_{\text{eff}} \), and effective charge density, \( \sigma_{\text{eff}} \), take into account both the force from the electric field acting on the negatively charged ssDNA phosphate backbone and the opposing force of electroosmotic drag acting on the ssDNA, both of which are proportional to the applied voltage, \( V \). The electroosmotic driving force used in the model can then be written as \( F = \sigma_{\text{eff}} V \), which includes both of these forces. When

![FIGURE 2](image-url)

(4) Escape time distributions for (dA)27, (dC)27, (dT)27, and (dGdA)13(dG) oligonucleotides at a bias voltage of 60 mV. Error bars are due to counting statistics. On a logarithmic y axis, the exponential fits are the bold, straight lines. The distribution for (dT)27 extends beyond 500 ms, and the full distribution is plotted in the Supporting Material. (B) Average escape time as a function of bias voltage for each oligonucleotide. (Dashed lines) First-passage fits, whose parameters are the effective charge and diffusion constant.
The differences between different oligonucleotides are significant.

Although the ssDNA is bound to NeutrAvidin, the flexibility of ssDNA sequence effective charge, $Q_{\text{eff}}$ (electrons per base) Driving Force, $F$, at 100 mV (pN) Diffusion Constant (Multiple of $D_0$)

<table>
<thead>
<tr>
<th>ssDNA Sequence</th>
<th>$Q_{\text{eff}}$</th>
<th>$F$</th>
<th>$D$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(dT)$_{27}$</td>
<td>0.62 ± 0.02</td>
<td>19.7 ± 0.4</td>
<td>0.16 ± 0.03</td>
</tr>
<tr>
<td>(dC)$_{27}$</td>
<td>0.56 ± 0.01</td>
<td>17.9 ± 0.3</td>
<td>0.25 ± 0.05</td>
</tr>
<tr>
<td>(dA)$_{27}$</td>
<td>0.53 ± 0.02</td>
<td>17.1 ± 0.5</td>
<td>0.17 ± 0.05</td>
</tr>
<tr>
<td>(dGdA)$_{13}$ (dG)</td>
<td>0.51 ± 0.01</td>
<td>16.2 ± 0.5</td>
<td>0.13 ± 0.04</td>
</tr>
</tbody>
</table>

The differences between different oligonucleotides are significant.
explanation for broad, unresolved current distributions measured with NeutrAvidin and MspA (9).

At low bias voltages, the NeutrAvidin tends to be farther away from the MspA pore more of the time due to the reduced tension in the DNA molecule (refer to Fig. 1C, top, labeled 60 mV). This reduces the likelihood of the NeutrAvidin being in the tightly clamped state, with its additional structure, and the spectrum of blockage currents becomes Gaussian. This picture is also consistent with the measured current fluctuation power spectra to be discussed shortly.

An alternative explanation for the discrete peaks in current blockage at high bias is that the ssDNA has some specific, transient binding interaction with the MspA pore that manifests at high bias. Recent molecular dynamics simulations have indicated that specific interactions between ssDNA phosphates and the pore constriction can stabilize high-tension states of the ssDNA (28). However, the simulations show that these states have lifetimes of approximately one microsecond, while the states we observe in experiments last for milliseconds.

Due to the long times that the oligonucleotides are trapped in the pore and the large number of molecules measured, it is possible to extract extremely accurate and sharp distribution functions for the average fractional blockage currents for each nucleotide. These are shown in Fig. 4A at 60 mV bias. Shown in Fig. 4B are the much broader distributions obtained at 160 mV bias for the same individual molecules. The values of $I/I_0$ we report largely agree with those obtained by others at high voltage biases (see, for example, Manrao et al. (9)) using the M1-MspA mutant, which is different from the one used in this work (see Materials and Methods).

**Correlation between current blockages and effective charges**

We now point out a relationship between effective charge and current blockage for each oligonucleotide at low bias voltage. We restrict the discussion to 60 mV bias to avoid the complicated features attributed to NeutrAvidin-MspA interactions at higher voltages.

**Fig. 5 A** shows the frequency power spectrum of current fluctuations obtained at 60 mV bias for each oligonucleotide. Notice that they are all different and substantially white $\sim$1 kHz. **Fig. 5 B** compares the power spectra for poly(dC) at biases of 60 and 160 mV. There is a significant increase in the low frequency part of the spectrum at the higher bias. The difference correlates with the current fluctuations for poly(dC) shown in Fig. 3, in particular the enhancement expected at low frequencies associated with telegraph noise.
the effective charge being related to the electroosmotic force. Increased current blockage reduces the flow of counter ions through the pore and hence reduces the electro-osmotic contribution to the total force on the ssDNA, which increases the effective charge.

**Correlation between ionic current fluctuations and diffusion constants**

Fig. 6B shows the diffusion constant for the different oligonucleotides at 60 mV bias plotted against the “mean normalized excess white noise”, which we describe here. We use the notion of “excess white noise”, which is shown in Fig. 5A on the right side vertical axis for poly(dGdA). By “excess white noise,” we mean the difference between the measured power spectral density and that expected from Johnson noise for the resistance of the pore blocked by a given oligonucleotide.

The measured current fluctuation power spectrum, or current noise, S, presumably includes three contributions: (1) thermal Johnson noise, S₁, (2) noise caused by electrolyte ion number fluctuations in the pore S_{ion}, and (3) excess current fluctuations induced by the ssDNA itself, S_{DNA}. These contributions can be written as 

\[ S = S₁ + S_{ion} + S_{DNA}. \]

For solid state pores (30), the OmpF membrane channel (31), and MspA (data not shown), the excess white noise in the open pore, S_{ion}, depends quadratically on the mean ionic current, S_{ion} \propto I^2. This relationship is the result of ion number fluctuations in the pore (32), i.e., a resistance fluctuation. We define “normalized excess white noise” as 

\[ N = (S - S₁)/I^2, \]

where \( I \) is the mean ionic current for each oligonucleotide. It follows that 

\[ N = (S_{ion} + S_{DNA})/I^2 = C + S_{DNA}/I^2, \]

where \( C \) is approximately constant and independent of nucleotide identity. We have divided by the square of the mean ionic current to show the oligonucleotide dependence of S_{DNA} in N. Averaging N over a 50–500 Hz bandwidth yields \( \bar{N} \) with dimension 1/Hz that we compare for all four oligonucleotides in Fig. 6B.

We hypothesize, inspired by the studies of particle-induced noise investigated by Rostovtseva and Bezrukov (33), Berezhkovskii et al. (34), and Rostovtseva et al. (35) that current fluctuations caused by ssDNA in MspA should have the form \( S_{DNA} \propto (\Delta I)^2/D \), where \( \Delta I \) is the range of current changes due to fluctuations in ssDNA conformation or position inside MspA, and D is the diffusion constant of the ssDNA in MspA. A simple physical description of telegraph noise for a fluctuating current signal explains this relation, as described in the Supporting Material. Because \( \bar{N} \) is proportional to \( S_{DNA} \), we expect \( \bar{N} \propto 1/D \). Fig. 6B reveals that, for the four oligonucleotides measured at 25°C, the correlation is plausible (black line). Additional measurements were made for poly(dT) at 10, 15, 20, 25, 30, and 35°C. These measurements are plotted in Fig. 6C, and confirm the \( \bar{N} \propto 1/D \) correlation.

Understanding the nucleotide-dependence of ionic current fluctuations when ssDNA is trapped in an MpsA nanopore could prove beneficial for sequencing experiments. For sequences with levels that are nearly indistinguishable based on blockage current alone, \( \bar{N} \) provides additional information to aid in sequence determination.

**CONCLUSIONS**

In this work, we report effective charges and diffusion constants deduced from escape time measurements of ssDNA oligonucleotides in an MspA nanopore. We show that nucleotide identity has a significant influence on these quantities, as well as on the observed current blockages and fluctuations. Correlations between the effective charges and the current blockages, as well as between the diffusion constants and the current fluctuations, are identified and found to be qualitatively in agreement with simple physical arguments.

We observe the presence of an extra source of current fluctuations when ssDNA is held in the pore at high voltages, and we argue that this is caused by the molecular stop, here NeutrAvidin, interacting with the MspA nanopore. The various contributions to the current fluctuations display different power spectral densities: the current fluctuations associated with the molecular stop occur on long timescales measurable on our current amplifier and exhibit power spectra consistent with a telegraph noise process, while the current fluctuations associated with specific nucleotides in the constriction exhibit power spectra that are “white” within our observed frequency window of 1 Hz to ~1 kHz. Clearly, avoiding current fluctuations associated with the NeutrAvidin or another molecular stop, or
attempting to obtain useful information from these fluctuations, can be important for improving accuracies in nanopore sequencing. These fluctuations can also provide microscopic information about specific interactions between the protein molecular stop and the nanopore. Nucleotide-specific current fluctuations, on the other hand, can be utilized as an additional source of information about the nucleotide sequence in the pore’s constriction.

In future work, it would be helpful to further explore and clarify the microscopic physical phenomena reported here. Heteropolymer DNA would present a different landscape of potential energies than the homopolymer oligonucleotides studied here. Homopolymer measurements shed light on the behavior of ssDNA with a relatively flat potential energy landscape, and are only a first step toward understanding the properties of heteropolymer DNA in nanopore sequencing conditions. Molecular dynamics calculations over long enough timescales would provide further insight into the atomic scale processes that influence the parameters that describe the experimental results reported here. Given the already demonstrated success of nanopore science in sequencing, and given the potential for other applications to nanoscale molecular science, such efforts would seem manifestly worthwhile.

SUPPORTING MATERIAL

Supporting Material and four figures are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(16)34282-5.

AUTHOR CONTRIBUTIONS

S.J.F. and B.L. contributed equally to this work; B.L., S.J.F., and J.A.G. designed the experiment; S.J.F. performed the measurements; B.L. and S.J.F. analyzed data; and S.J.F., J.A.G., and B.L. wrote the article.

ACKNOWLEDGMENTS

The authors would like to thank Eric Brandin for help with preparation of the ssDNA-NeutrAvidin complexes, and Tamas Szalay for developing the Arduino analog-output functionality (MightIO board) used here for dynamic voltage bias control. In addition, the authors thank Daniel Branton and Lene Hau for advice and enlightening discussions. This research was supported by the National Human Genome Research Institute of the National Institutes of Health under award No. R01HG003703. Stephen Fleming was supported by the National Science Foundation Graduate Research Fellowship under grant no. DGE1144152.

SUPPORTING CITATIONS

Reference (36) appears in the Supporting Material.

REFERENCES


Supplemental Information

Charge, Diffusion, and Current Fluctuations of Single-Stranded DNA Trapped in an MspA Nanopore

Stephen J. Fleming, Bo Lu, and Jene A. Golovchenko
Supporting Material

A. Chi-square fitting of exponential escape time curves

A histogram of measured escape times shows that most of the events follow an exponential distribution; however, there are some extra events with long escape times. Around ten percent of the total number of events consist of these longer escape-time events, which have previously been hypothesized to be caused by DNA-pore sticking interactions (1). In order to estimate the time constant of the exponential distribution, we first estimate the time constant as $\tau_0 = \text{median}(t_{\text{esc}}) / \ln(2)$, where $\text{median}(t_{\text{esc}})$ is the median escape time. The exponential time constant is then obtained by performing a chi-square fit to a histogram of the data shorter than $2.5\tau_0$, using a bin width of $0.25\tau_0$ and error bars from Poisson counting statistics, $\sqrt{N}$.

Because the full histogram for poly(dT) does not fit in Figure 2A in the main text, we have included it here. As can be seen, the data fit an exponential for the shortest ~90% of events.

![Poly(dT) escape times at 60mV bias](image)

*Figure A1. Histogram of escape times for (dT)$_{27}$. Same data as in main text Figure 2A, but with an extended time axis so that the full distribution can be clearly seen. As mentioned, the exponential distribution (solid line) is a good fit for about ninety percent of the events.*
B. First-passage model of DNA escape

ssDNA escape from the MspA nanopore is modeled as a one-dimensional, drift-diffusion process. The probability of escape by a given time \( t \) is \( \int_0^t f_{\text{esc}}(x_0, t') dt' \), given an initial starting position \( x_0 \). This first-passage probability flux, \( f_{\text{esc}}(x_0, t) \), obeys the adjoint Smoluchowski equation, which describes drift and diffusion:

\[
\frac{\partial f_{\text{esc}}(x_0, t)}{\partial t} = \frac{F_{\text{total}}}{k_B T} \frac{\partial f_{\text{esc}}(x_0, t)}{\partial x_0} + D \frac{\partial^2 f_{\text{esc}}(x_0, t)}{\partial x_0^2}.
\]

Here \( F_{\text{total}} = F + F_{\text{entropy}} = \sigma_{\text{eff}} V + F_{\text{entropy}} \), where \( \sigma_{\text{eff}} \) is the linear effective charge density. In our simplified model, \( F_{\text{entropy}} \), the force due to entropy of the ssDNA, only takes on a nonzero value when one Kuhn length of ssDNA remains in the constriction of MspA, just before escape (1). \( D \) is the diffusion constant. Thus we have two adjustable parameters when performing a fit to the model: \( \sigma_{\text{eff}} \) and \( D \). The physical first-passage domain is the length of 14 nucleotides, the maximum number which could stick out through the constriction of MspA for 27-mer ssDNA (2). There is an absorbing boundary condition at the end of the ssDNA, as well as a reflecting boundary when the ssDNA-NeutrAvidin complex is butted up against the top of the MspA. The average escape times are obtained by integrating \( f_{\text{esc}}(x_0, t) \) over initial positions \( x_0 \) and time \( t \).

Further details can be found in our previous work (3).

C. Reproducibility of measurements

Values obtained from repeated experiments using different individual MspA pores (same mutant) are in agreement with each other. See Figure C1 for example data from poly(dC). Data obtained for other oligonucleotides are also reproducible within the measurement uncertainty.
Figure C1. Escape times for the same ssDNA-NeutrAvidin complex (poly(dC)) at the same temperature, measured on different days with different individual MspA pores (same mutant). Data are reproducible pore-to-pore to within experimental uncertainty. The same is true for all the oligonucleotides measured.

D. MspA current versus voltage, and pore orientation

As shown in Figure D1, MspA pore orientation in the membrane can be determined by examining the current versus voltage curve. MspA is introduced only on one side of the
membrane (for the schematic in Figure 1A, the left-hand side). MspA inserts into the membrane on its own, without breaking the membrane, in only one orientation: that shown in Figure 1A.

**Figure D1.** Blue circles show the MspA open pore current ($I_0$) as a function of bias voltage in 1M KCl, 10mM HEPES, at pH 8.0 and 25.0°C. The red dashed line is a linear current-voltage relation for 2.3nS conductance, shown for comparison. The blue data shows a characteristic upward bend at a large positive bias as well as characteristic gating at large negative bias. This asymmetry indicates that the pore is inserted into the membrane in the correct orientation (as depicted in Figure 1). The spikes from -130mV to -200mV are caused by pore gating, which has been averaged over to obtain the blue data points.

E. Current fluctuations and power spectra

A telegraph process associated with binary level fluctuations of current difference $\Delta I$ that have a particular characteristic time scale $\tau$ should have a Lorentzian power spectrum (4), with power spectral density as a function of frequency given by $S(f) = (\Delta I)^2 \tau / [1 + (2\pi f)^2]$. For fluctuations at frequencies of order $2\pi / \tau$ much higher than frequencies we can measure, it holds that $2\pi f \ll 1$, and so $S(f) \approx (\Delta I)^2 \tau$. In these experiments, if the fluctuation timescale $\tau$ corresponding to $\Delta I$ is set by molecular diffusion between adjacent ssDNA nucleotides, then we would expect $\tau \approx b_0^2 / D$ where $b_0$ is the length per base of ssDNA. This time scale is on the order of nanoseconds (3), and so in our experimentally accessible frequency window (1 –
10kHz), the power spectral density of current fluctuations appears to be \( S(f) \propto (\Delta I)^2 / D \), independent of frequency.

On the other hand, for the data recorded at higher voltages when NeutrAvidin – MspA interactions become more prominent, there is no reason, a priori, that we would expect the interactions between NeutrAvidin and MspA to have only one characteristic time scale. Thus we could expect to see a superposition of one or more Lorentzians in the power spectrum, leading to a falloff \( S(f) = f^{-\alpha} \) with \( 0 < \alpha \leq 2 \), depending on all the complicated protein-protein interactions that can occur. A superposition of many such processes could even give rise to a power spectrum with \( 1/f \) character.

F. Qualitatively different escape events for poly(dGdA)

Poly(dGdA) exhibits escape events that show qualitative differences and fall into two categories, “quiet” and “noisy,” shown in Figure F1. The table in Figure F1 shows that if we make the assumption that the quiet and noisy events have the same driving force, then for the noisy events we can fit the diffusion constant and another parameter, the length of the “escape domain”: the length of ssDNA that sticks out past the constriction of MspA (see Figure F1, panel C). Fit in this way, the noisy events have a far shorter escape domain, indicating that the noisy events may be caused by G-G association between or within ssDNA molecules, whose interactions prevent them from completely threading through the pore. In the main text, we limit our analysis to the quiet events, roughly one-third of the total, which correspond to ssDNA properly threaded through the constriction of MspA.
**Figure F1.** Poly(dGdA) has two different categories of escape events that can be distinguished by their noise. (A) Plot of the RMS current noise of the captured molecules at 160mV. There are clearly two categories of captured molecules. (B) The escape times as a function of voltage for the two categories of escape events, separated according to current noise. (C) Schematic of a proposed explanation for the “noisy” category of events. The mechanism causing the noise could be transient G-G binding between molecules or within the same molecule, which would lead to current noise from transient extra DNA in the vestibule of MspA, and would also lead to a shorter escape domain and faster escape times.
Supporting References