Supporting Information

Detection of individual proteins bound along DNA using solid state nanopores

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1. Nanopore characterization of anti-DNA antibodies

Anti-DNA Antibodies (HYB331-01) were diluted into 1M KCl TE pH8 solution to a final concentration of 312 nM. This solution was measured with a 20 nm diameter SiN nanopore. Figure S1 shows traces taken at +100 mV and -100mV applied voltage, demonstrating that the antibodies are positively charged at pH8. Figure S2 shows a scatter density plot from a dataset of 8815 antibody translocation events at +100 mV and 40 kHz bandwidth. The events produced by the antibodies have a most probable translocation time of 13 µs and a most probable current blockade of 210 pA. These short translocation times are at the edge of the temporal resolution for this technique, as discussed in detail in a previous publication[1].

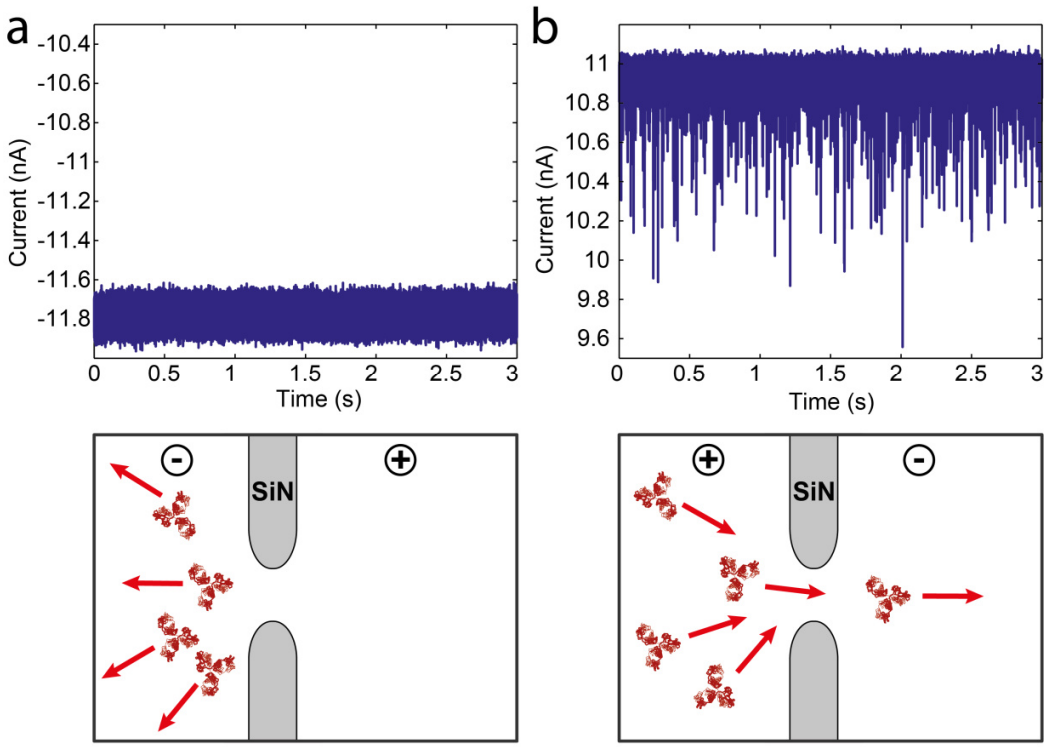

Figure S1 – Current traces at **a)** -100 mV and **b)** +100mV applied voltage for 312 nM IgG2a mouse antibodies translocating through a 20 nm pore. The large difference in event rates between the two polarities indicates that these antibodies are positively charged. Below each trace is a schematic representation of each situation.

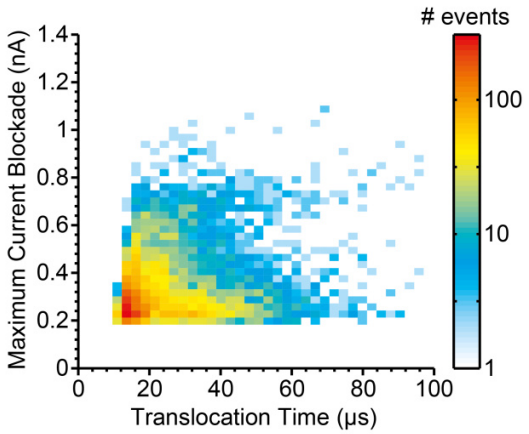

Figure S2 – Translocation data from an 8815-event dataset of antibodies translocating through a 20 nm pore at 100 mV and 40 kHz bandwidth. Scatter density plot showing the maximum current blockades and translocation times. The distributions peak at around 210 pA and 13 µs.

2. AFM characterization of anti-DNA antibodies bound to 2.2kbp DNA

We imaged antibody-DNA complexes using AFM to verify binding and observe the number of bound antibodies. A solution of 2.97 nM anti-DNA antibodies, 0.7 nM 2.2kbp DNA in 10 mM NaCl, 1 mM Tris pH 8, and 10 mM MgCl was incubated on freshly cleaved Mica for 5 minutes and imaged using tapping mode AFM with an Olympus AC160TS tip. We observed, on average, two antibodies bound on each 2.2 kbp molecule at this low-salt condition. Note that the nanopore measurements used much longer 48.5 kbp DNA molecules and much higher antibody concentrations to compensate for the reduced affinity in high salt conditions.

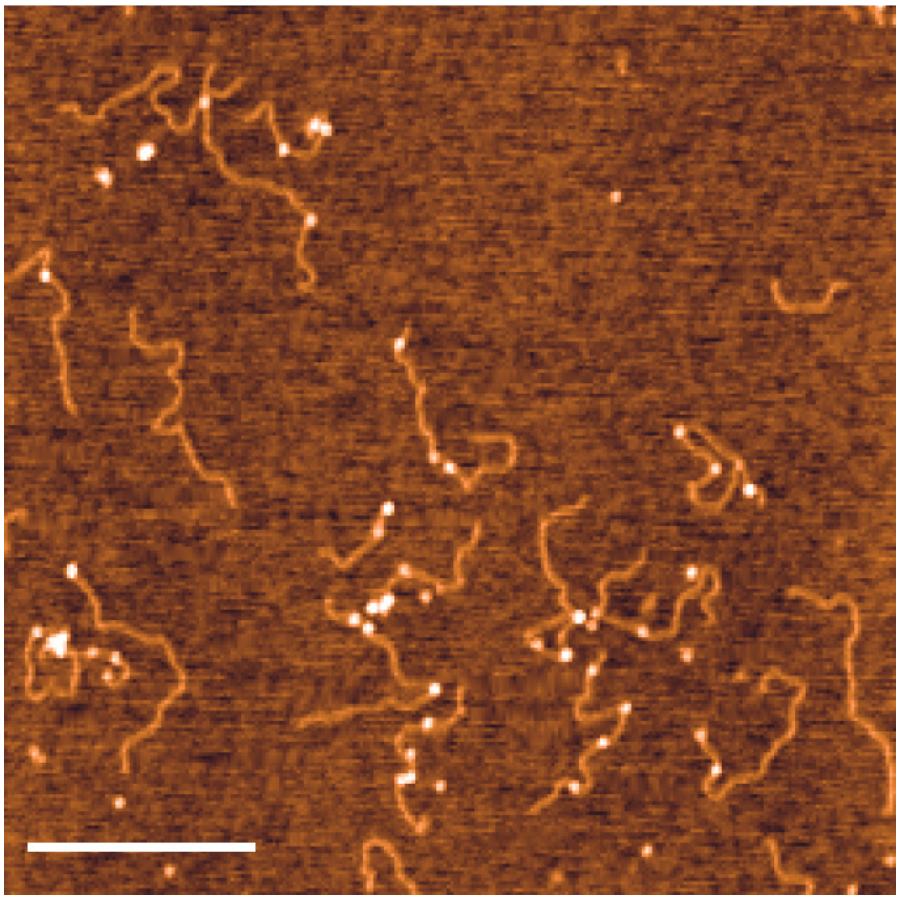

Figure S3 – Typical tapping-mode AFM scan of 2.2kb DNA molecules with bound antibodies on a mica surface. The scale bar represents 500 nm.

3. Filtering effects

The effect of the filtering must be taken into account when analyzing the spikes. The temporal distortion point of the Gaussian low pass filter used (given by $0.66/f_c$) has a value of 16.5 µs for the 40 kHz bandwidth used, so spikes with a duration time smaller than 16.5 µs will be distorted by the filtering. Since the type of filter and the cut off frequency are known, we can estimate the original undistorted duration of any given spike from the observed filtered spike duration, as shown in Fig. S5 for the 100 mV data at 40kHz and Fig. S8 for the 25 mV data at 20kHz. The same procedure can be used to determine how much the amplitude of an ideal rectangular pulse will be reduced given some initial amplitude, as shown in Fig. S6.

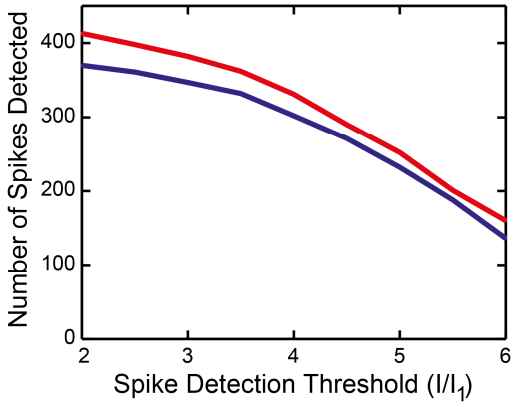

Figure S4 – The total number of spikes in a dataset detected as a function of the spike detection threshold for two independent experiments with anti-DNA antibodies and lambda DNA in 1M KCl at 100 mV in 20 nm pores.

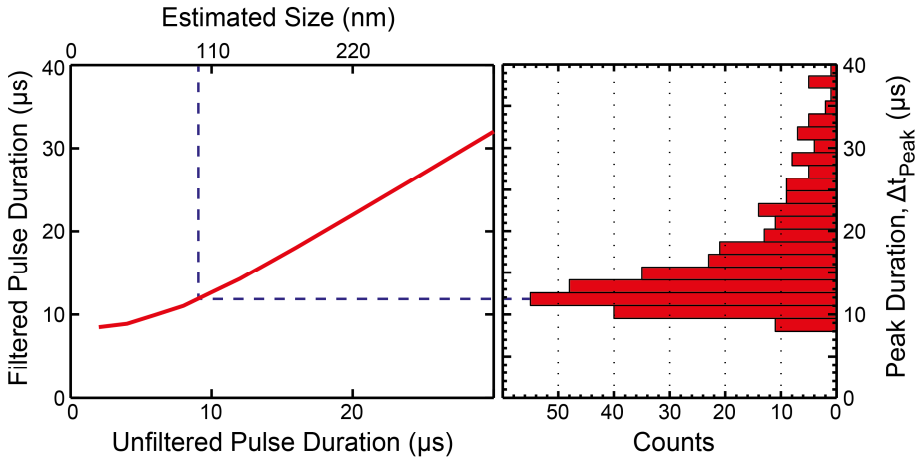

Figure S5 – **left)** The filtered pulse duration as a function of the unfiltered pulse duration for a 40Khz Gaussian low pass filter. Top axis shows the spatial size of the spikes, estimated using the mean translocation velocity of these events (11 nm/μs at 100 mV in 1MKCl and a 20 nm pore). **right)** A histogram of the observed peak duration values for spikes (Fig. 3c) found within DNA events at 100 mV and 40kHz bandwidth. Using this approach we are able to resolve features of about 100 nm.

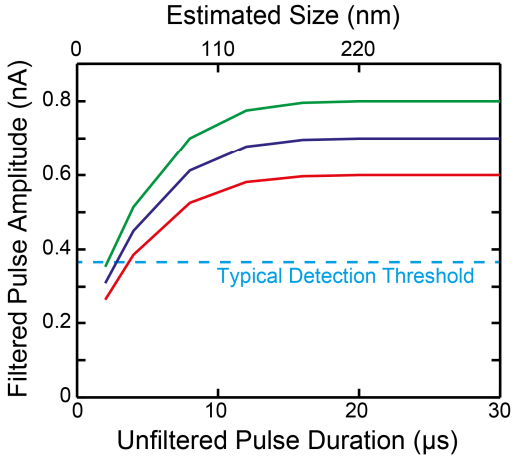

Figure S6 – The distortion of pulse amplitudes given some initial (unfiltered) pulse amplitude for a 40kHz Gaussian low pass filter. The three lines represent unfiltered pulse amplitudes of 0.6 (red), 0.7 (blue), and 0.8 nA (green). The horizontal line represents the typical spike detection threshold at 100 mV. The point at which the filter would reduce the amplitude of the spikes below the detection threshold clearly lies below the temporal resolution of our system.

4. Translocation of DNA-antibody mixtures at 25 mV

Typical example events of DNA+antibody translocation events at 25 mV can be seen in Fig. S7. The observed translocation duration of the spikes, and their estimated size, taking into account the distortion introduced by the filter, is shown in Fig. S8. Fig. S9 shows histograms of the normalized position of the spikes, the spike amplitude, and the number of peaks per event. The mean number of spikes per event increases from a value of 1.5 at 100 mV to 2.5 at 25 mV. This small difference, compared to the large increase in the resolution, suggests that there are only a few antibodies bound to each DNA molecule in high salt.

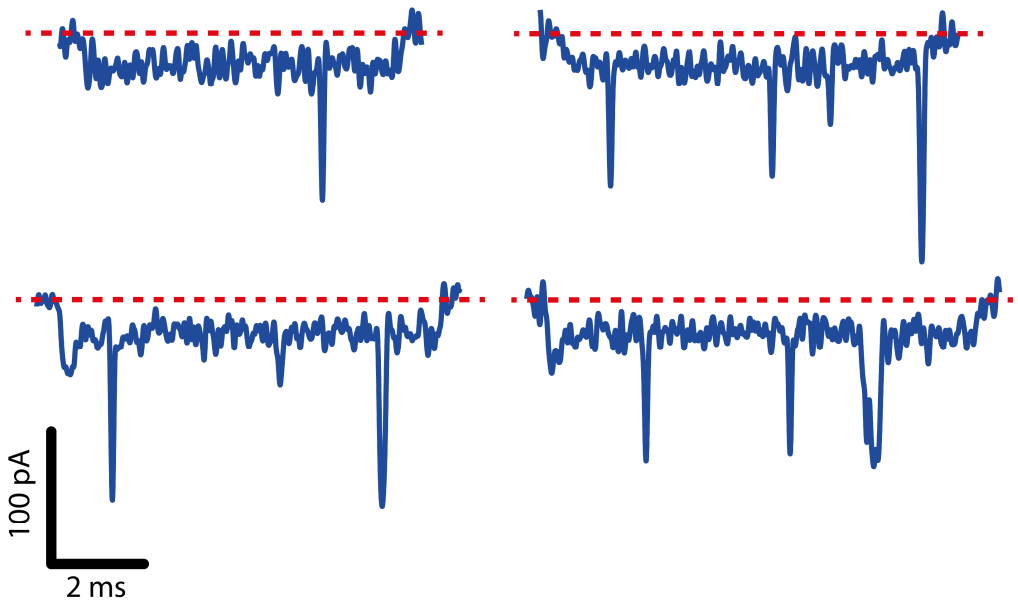

Figure S7 – Four example translocation events of DNA and antibodies at 25 mV and 5kHz bandwidth.

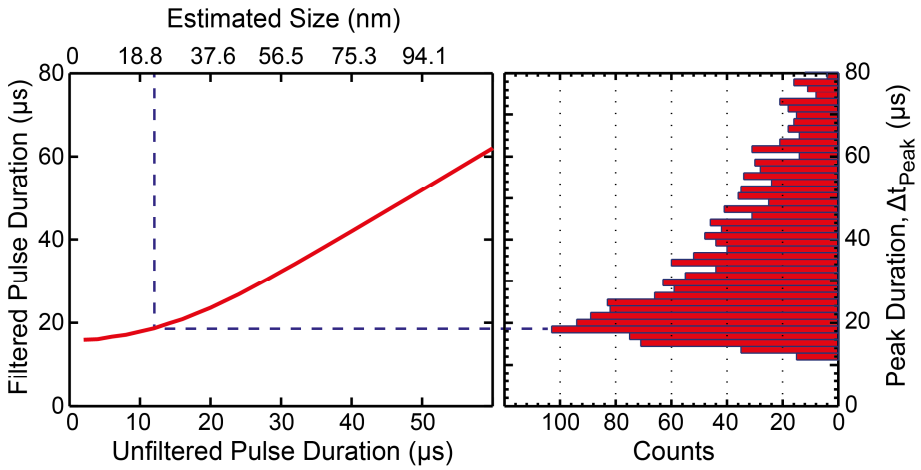

Figure S8 – left) The filtered pulse duration as a function of the unfiltered pulse duration for a 20kHz Gaussian low pass filter. Top axis shows the spatial size of the spikes, estimated using the mean translocation velocity of these events (1.88 nm/µs at 25 mV in 1MKCl and a 20 nm pore). **right)** A histogram of the observed peak duration values for spikes found within DNA events at 25 mV and 20kHz bandwidth. Using this approach we are able to resolve features below 25 nm.

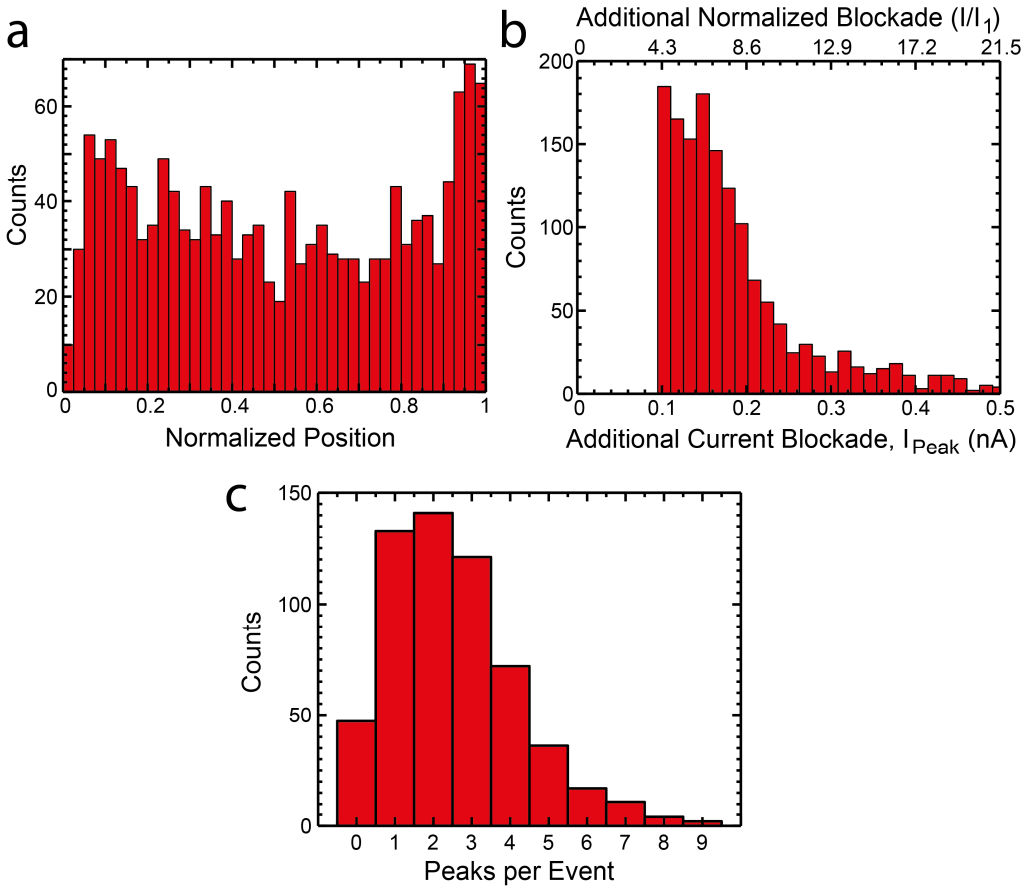

Figure S9 – **a)** The normalized position of spikes observed on DNA at 25 mV in 1MKCl and a 20 nm pore. **b)** The additional amplitude of the spikes. **c)** The number of spikes observed per event, with a mean of 2.5.

5. Antibody-DNA binding in high salt

We investigated the time dependence of the percentage of events with spikes over the ~30 minute duration of the experiments and found no signs of significant DNA-antibody complex dissociation. This stability suggests the antibody-DNA binding is at equilibrium in the high salt concentrations (1M KCl) used. Since DNA-protein interactions tend to be electrostatic in nature, exposure to high ionic concentrations such as those used here (1M KCl – a standard condition for nanopore experiments), typically lead to significant reductions in the DNA-protein binding affinity constants, with many proteins dissociating after exposure to high salt. Figure S10 shows the percentage of events with spikes as a function of time for the first 30 minutes of two independent experiments. No significant reduction is observed, indicating that the antibody-DNA complex has reached an equilibrium in the high salt conditions.

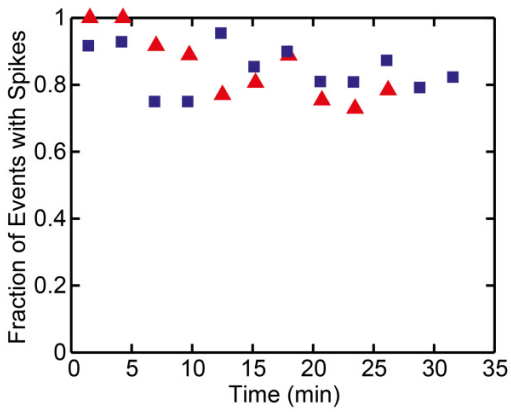

Figure S10 – Time dependence of the fraction of events with spikes detected at 100 mV for the first 30 min after the antibody-DNA solution was diluted into high (1M KCl) salt. No significant dissociation is observed on this timescale.

6. Estimated antibody current blockade

The current blockade produced by the antibody translocating through the pore can be estimated using the excluded volume technique as in a number of previous studies[2-4]. The expression used is[4]

$$\Delta I = \frac{\gamma \sigma V_a V_{excluded}}{\left(h_p + \frac{\pi d_p}{4}\right)^2} f(d_p, d_m) \qquad \text{Eq. 1}$$

where γ is the shape factor taken as 1.5 (even though these antibodies clearly are not perfect spheres[4]), σ is the bulk buffer conductivity (10.5 S/m for 1M KCl), V_a is the applied voltage set as 100 mV, $V_{excluded}$ is the volume of the antibody (taken as 347 nm^3)[4], h_p is the effective thickness of the pore set to 8.6 nm based on previous work[5], d_p is the diameter of the pore set to 20 nm, and f is a correction factor taken to be 1. Based on these values we would expect a current blockade of around 0.92 nA, well above what we observe in both the free antibody translocation (0.21 nA) and in the DNA-bound antibody case (0.5 nA). These observations can be explained by the fact that the majority of translocation times in both of these cases are below the filtering distortion frequency (22 µs for 30 kHz and 16.6 µs for 40 kHz) as discussed in detail previously[1].

7. Translocation of DNA + F_{ab} fragments

Figure S11 shows the percentage of events with spikes as a function of the spike detection threshold for an experiment with F_{ab} fragments and lambda DNA, as well as a DNA only control experiment, both in 10 nm pores. No additional population of spikes is observed upon the addition of F_{ab} fragments. Using Eq. 1 in Supplementary Section S6, we would expect a blockade of around 0.6 nA (or 3.3 I/I_1) at 100 mV in a 10 nm pore, which is not convincingly observed.

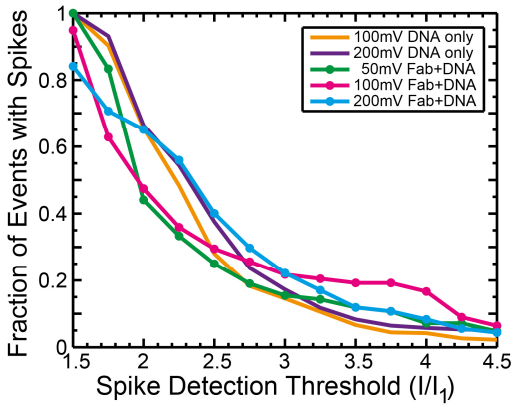

Figure S11 – The fraction of events with spikes as a function of the spike detection threshold for two experiments with F_{ab} fragments and lambda DNA (green, red, and blue) as well as a control experiment with only lambda DNA (orange and violet) in a 10 nm pore. No F_{ab} population is observed.

Additionally, it is well known that dissociation constants for F_{ab} fragments are larger than the dissociation constants of the full IgG antibody. This is simply due to the fact that the full length IgG antibody contains twice as many binding sites. If one of the F_{ab} regions in the full length IgG unbinds from the DNA, the other F_{ab} region can remain bound, allowing the unbound Fab region to rebind due to the high effective local concentration of the DNA ligand. If the K_D of the antibody is significantly reduced in high salt then we expect the K_D of the F_{ab} fragment would be even much higher, meaning that very few F_{ab} fragments are bound to the DNA.

8. Long blockade antibody-DNA events

Among the events observed, occasionally we observe some events with a very long duration event, which can be attributed to long-lasting antibody-pore interaction events (sticking). Figure S12 provides some examples.

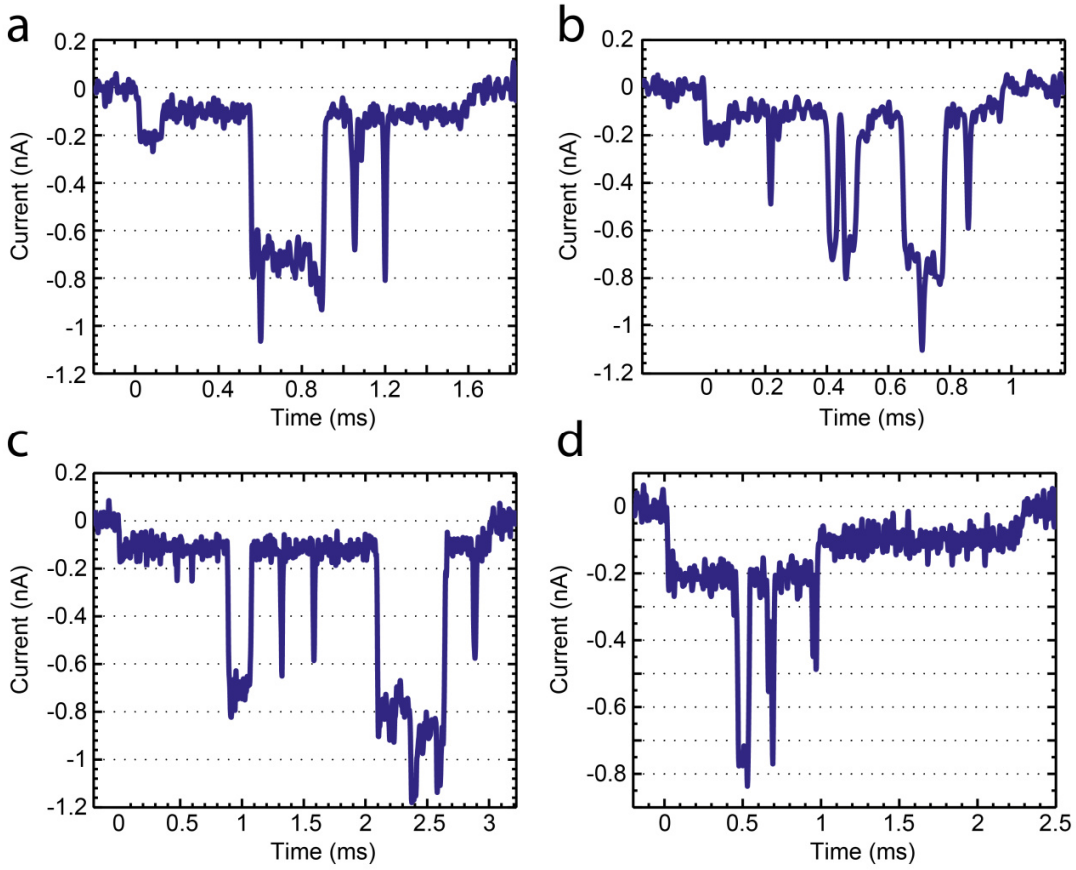

Figure S12 – Four example events with long duration blockades present within the DNA event. These long blockades are attributed to sticking between the antibody-DNA complex and the pore.

9. Antibody+DNA translocations in a 35 nm pore

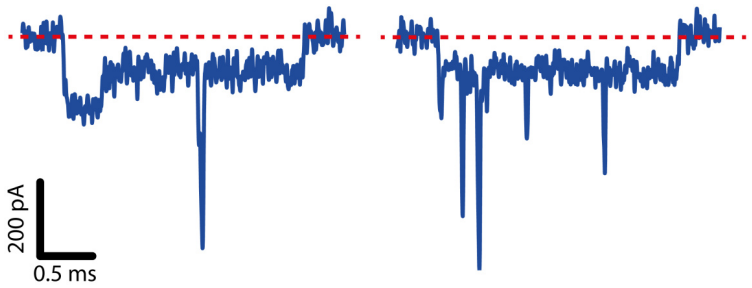

Figure S13 - Two example translocation events of DNA and antibodies at 100 mV in a 35 nm pore.

10. Additional discussion

What effect do antibodies have on the local translocation velocity?

Antibodies are positively charged, which leads to an electrophoretic force in the opposite direction as the DNA translocation. Furthermore, the antibodies also increase the drag force. Could these factors cause the local translocation velocity to slow down significantly? We begin by noting that in the high salt conditions used, the charge of the antibody is heavily screened. We can estimate the net charge of the antibody and its counter-ion cloud using[6]

$$\frac{Q_{eff}}{Q} = \frac{1}{1 + \kappa R_g} \qquad \text{Eq. 2}$$

where κ is the inverse Debye screening length and R_g is the radius of gyration. Using values of κ = 3.28 nm^{-1} for 1M KCl and R_g = 5.45 nm of the IgG2a antibody[7], we find that the high salt screens the charge to just 5.2% of its original value. Capillary electrophoresis experiments[8] reveal that these antibodies have a electrophoretic mobility of about $3.27 \cdot 10^{-8}$ $m^2/V/s$ in 0.1M sodium phosphate at pH7.9, which corresponds to a bare charge of +143e or an effective charge of +8e in 1M KCl. Taking a 20 nm length for the segment of DNA (60 bp) within the pore and using the measured effective linear charge density of 0.5 e/bp[9], the effective charge of the DNA segment in the pore is -30e, i.e. almost four times higher than the charge of the antibody. Relative to the very highly charged DNA, the antibodies' electrophoretic contribution is thus small.

Secondly, the additional drag due to the antibody is much smaller than the typical forces experienced by a translocating DNA molecule. We can estimate the contribution of the drag force due to the antibody using the Stokes equation

$$F_{drag-AB} = 6\pi\eta R_g v. \qquad \text{Eq. 3}$$

For the antibody we estimate this to be below 1 pN, using a viscosity of η = 0.89 mPa·s, and assuming a velocity of v = 11 mm/s based on the observed translocation times and the length of lambda DNA. This approach likely even overestimates the drag due to the antibody since it ignores the contact area between the antibody and DNA which is not exposed to the solution. For comparison the typical force on a DNA molecule in 1M KCl at 100 mV has been measured to be around 20 pN[9], much larger than the drag force introduced by the antibody.

In conclusion, while the antibody complex will have a small effect on the local velocity, this is quantitatively insufficient to slow down its translocation to the point where the antibody is visible during (non-interacting) translocation.

What is the affinity of antibodies against DNA in high salt?

The 25 mV data indicate that there are only a few antibodies bound to the DNA in high salt conditions. We attempted to make a better estimate of the dissociation constant of the antibodies binding to DNA in high salt conditions by measuring the event rate of free (unbound) antibodies. In the normal translocation polarity, only DNA and DNA-bound antibodies are pulled through the nanopore, while free antibodies are pushed away from the pore by electrophoretic force, due to their opposite charge. We measured the event rate of unbound antibodies by reversing the polarity of the electric field (in a DNA+antibody mixture experiment), finding an event rate of 66 ±5 Hz. Previous experiments using only antibodies revealed an event rate per concentration of 0.44 ±0.03 Hz/nM for these same antibodies translocating in identical conditions. We note that the observed event rate of 66 ±5 Hz is much higher than what would be expected (25 Hz) if K_D=90 nM, as in low salt, suggesting that the K_D is indeed much higher in high salt conditions. Since we expect the event rate to scale linearly with the concentration of antibody present[1], an event rate of 66 Hz very roughly corresponds to a concentration of free antibodies of 150 nM. Due to the large uncertainty in the measurements of the event rate as well as the non-linear

scaling between the event rate and K_D, we only put a lower bound on the value of K_D. Taking into account the uncertainties in the event rate measurements and using standard Michaelis-Menten kinetics we thus estimate the lower bound for the K_D of the antibody in 1M KCl as 1 μM. This lower bound is least ten times higher compared to the value in low salt. This is consistent with the observations that there was only a slight increase in the amount of spikes observed at 25 mV compared to 100 mV, despite a 4x increase in spatial resolution.

What is the probability of more than one antibody bound on a 25 nm segment of DNA?

Let us estimate the probability of having more than one antibody in a 25 nm segment of DNA, since this is the spatial resolution achieved at 25 mV. If we use a very conservative estimate of K_D = 1 μM for the antibodies binding in high salt (see above), we expect a mean of 0.0103 antibodies/nm. We can use the Poisson distribution, due to the random binding process, to estimate the probability of more than one antibody being bound on a 25 nm DNA segment, with a mean of $\lambda = 0.2575$ antibodies/segment using

$$P(X > 1) = 1 - (1 + \lambda)e^{-\lambda}. \qquad \text{Eq. 4}$$

This gives a 3% probability of finding more than one antibody in a 25 nm segment. This value is much smaller than the observed >80% of events which contain spikes, which indicates that the spikes are due to individual antibodies, not multiple closely-bound antibodies.

How many spikes can be attributed to knots?

In DNA-only experiments characterizing knotting[10], we observe that, in the same conditions as used in this study, 12% of lambda molecules contain observable knots, with 80% of those knots having amplitudes of 2 I/I_1. By using a threshold of 3.5 I/I_1 we expect that only about 2% of the events with spikes to be attributed to knots.

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