Fast, label-free force spectroscopy of histone-DNA interactions in individual nucleosomes using nanopores

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SI 1. Force estimation in a 3 nm diameter pore

DNA is subjected to an effective driving force (F_{EDF}) during translocation through a nanopore. An externally applied electric field across the pore exerts a highly localized electrophoretic force on the negatively charged DNA, pulling it through. The same electric field acts on positively charged counterions, which screen a DNA molecule in solution, resulting in electroosmotic flow of counterions and a hydrodynamic drag force opposing the movement of the polyelectrolyte chain.[11]

F_{EDF} that accounts for both electrophoretic and electroosmotic terms has recently been measured using optical tweezers in nanopores with a wide range of diameters (8-78 nm).[8,9] The authors discovered that this force is proportional to the applied voltage with the scaling factor of 0.23 pN/mV for 8 nm diameter pores, which goes down to 0.11 pN/mV as the pore's diameter approaches 78 nm. Importantly, the voltage-to-force scaling factors are independent of the buffer ionic strength in the broad 0.2-1M KCl.

Assuming a steady velocity, zero net force is acting on DNA in a pore during a free translocation event. In

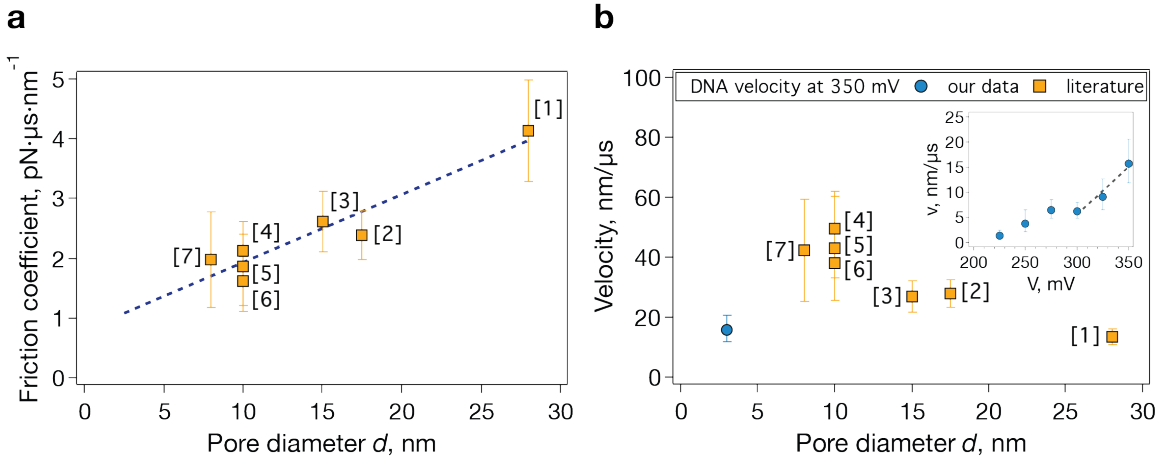

Figure S1. (a) Friction coefficient as a function of pore diameter, calculated using literature DNA translocation velocities[1-7] and absolute forces measured by Keyser *et al.*[8,9] Dashed line shows extrapolation of the data to smaller diameter nanopores. (b) Literature and our DNA translocation velocities (scaled if necessary to velocities at 350 mV). Inset shows a plot of our measured free DNA velocity vs. voltage for our 3 nm diameter pore (population 1 in Figure 2 of main text), with a linear fit in the range 300 – 350 mV.

case of sufficiently large nanopores, in which DNA-pore wall interactions can be neglected, F_{EDF} is completely balanced by the viscous drag given by the equation $F_{EDF} = F_{VD} = \zeta \cdot v$, where ζ is a friction coefficient and v is the translocation velocity. In Figure S1a, we plot $\zeta = F_{EDF}/v$ as a function of the pore diameter in the range 8-28 nm using literature DNA translocation velocities[1-7] and F_{EDF} measured by Keyser.[8,9] The apparent linear dependence of the friction coefficient on the nanopore diameter can be extrapolated to smaller diameter pores, yielding for a 3 nm pore ζ = 1.13 pN·μs·nm^{-1}. This value represents a good estimate of the friction

coefficient for DNA translocation events, in which the process is not dominated by specific DNA-nanopore interactions. We compare the translocation velocity of DNA at 350 mV through 3 nm (population 1 events) to the DNA translocation velocities through larger pores, which were scaled accordingly to correspond to velocities at 350 mV. Figure 1b demonstrates that our experimental DNA translocation velocity at 350 mV is well on par with the literature velocity values. DNA translocation velocities at 300 and 325 mV (Table S1) are not vastly different than larger diameter pores (2 to 4-fold faster on average). Moreover, the translocation velocity of DNA scales linearly with voltage in the range 300-350 mV (Figure S1b inset), allowing us to make this approximation.

Since force data was measured for larger, 8-28 nm diameter nanopores, three assumptions were made for force estimation: (1) specific DNA-nanopore wall interactions are insignificant at high unraveling forces (300-350 mV); (2) pulling force is identical on free and nucleosome-bound DNA; (3) All of the DNA pulling force within the pore acts to disrupt the DNA-histone core interactions.

We list below in detail our **three assumptions** made for force calculation in a 3 nm diameter pore:

Assumption 1. We assume that in the voltage regime where DNA moves at a high velocity (i.e., at 300-350 mV), DNA/wall interactions are not the dominant DNA slowing mechanism, and transport is drift-limited. In this regime, the force is calculated as $F_{EDF} = F_{VD} = \zeta \cdot v$ with ζ equal to 1.13 pN·μs·nm^{-1}. Then, $F_{EDF}^{300mV} = 7$ pN, $F_{EDF}^{325mV} = 10.3$ pN, and $F_{EDF}^{350mV} = 17.8$ pN.

Interestingly, the DNA translocation velocities at 275 and 300 mV are almost the same (Table S1) and, thus, $F_{EDF}^{275mV} \approx F_{EDF}^{300mV}$, yet we observe no ~50-100 μs long nucleosome collision events (population 2) at 275 mV. This can be explained by a low nucleosome capture rate at 275 mV (*see* ***SI 6***). Indeed, at 350 mV the fraction of nucleosome-related events comprises ~30%, whereas at 300 mV the fraction of the events lasting longer than 50 μs drops to only ~7%. At lower voltages, 225 and 250 mV, DNA translocates through 3 nm diameter pore an order of magnitude slower than through larger pores. This suggests that the assumption of no DNA-pore wall interactions is inappropriate for these voltages.

Table S1. Summary of DNA translocation times, velocities, and effective forces (population 1).

Voltage, mV	t_d, μs	Velocity v, nm/μs	F_{EDF}[b], pN
225	51.3 (-23/+41.8)	1.4 (-0.6/+1.1)	NA[c]
250	19.1 (-8.2/+14.2)	3.7 (-1.6/+2.8)	NA[c]
275	11 (-2.7/+3.5)	6.4 (-1.6/+2.1)	7.3 (-1.8/+2.3)
300	11.4 (-2.5/+3.2)	6.2 (-1.4/+1.7)	7.0 (-1.5/+2.0)
325[a]	7.8 (-2.2/+3.1)	9.1 (-2.6/+3.6)	10.3 (-2.9/+4.1)
350[a]	4.5 (-1.1/+1.4)	15.7 (-3.8/+4.9)	17.8 (-4.3/+5.5)

[a]A digital 300 kHz low-pass filter was applied to the 325 and 350 mV data in order to facilitate the detection of timescales below 5 μs for free DNA translocations.

[b]F_{EDF} was calculated as $F_{EDF} = \zeta \cdot v$ with ζ equal to 1.13 pN·μs·nm^{-1}.

[c]Our assumption of no DNA-nanopore wall interactions becomes infeasible at these voltages.

Assumption 2. The force F_{EDF} which pulls DNA during nucleosome unwrapping is the same as F_{EDF} driving DNA through the pore during a translocation event (Table S1). Since we assumed that interactions between DNA and nanopore walls are insignificant at 300-350 mV voltages, F_{EDF} acting on the DNA in the pore is mainly determined by the electrophoretic and electroosmotic terms. Using COMSOL, finite-element simulation software, we solved the Poisson-Nernst-Planck equations to quantify the electric field inside the nanopore with both DNA and the DNA-histone complex (Figure S2). Our simulations suggest that the presence of the model nucleosome next to the pore does not affect the electric field inside the pore, and therefore, the DNA-pulling force is similar in both cases.

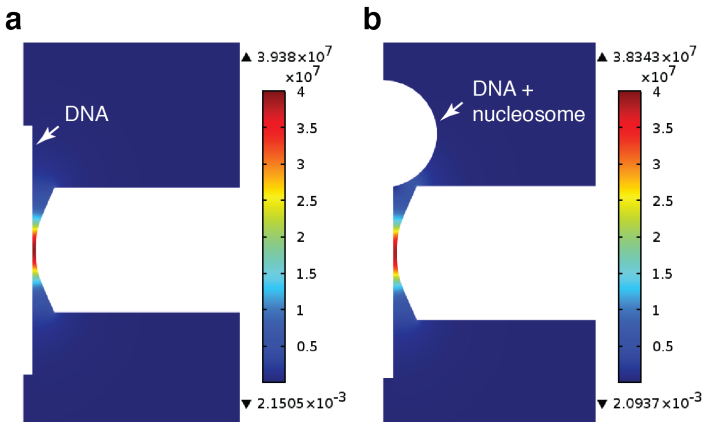

Figure S2. COMSOL simulation results of the electric field distribution in the vicinity of a 3 nm diameter pore in the presence of (a) free DNA and (b) DNA/nucleosome complex.

In the simulations, the *cis* and *trans* chambers were approximated as 5 μm tall cylinders with a 3 nm diameter, hourglass-shaped nanopore connecting the two compartments. The conditions for our simulation were an applied voltage of 300 mV, 350 mM monovalent salt conditions, no slip and zero charge boundaries. The inherent charge of DNA and DNA-histone complex were assumed to be zero for simplicity.

Assumption 3. The role of viscous drag force in balancing F_{EDF} becomes negligible during nucleosome unwrapping, and the major force that balances F_{EDF} is the DNA-histone interactions. This is reasonable because viscous drag forces that balance F_{EDF} during free DNA translocations are proportional to the DNA velocity, and vanish as DNA stalls in the pore.

SI 2. Representative current traces at different voltages

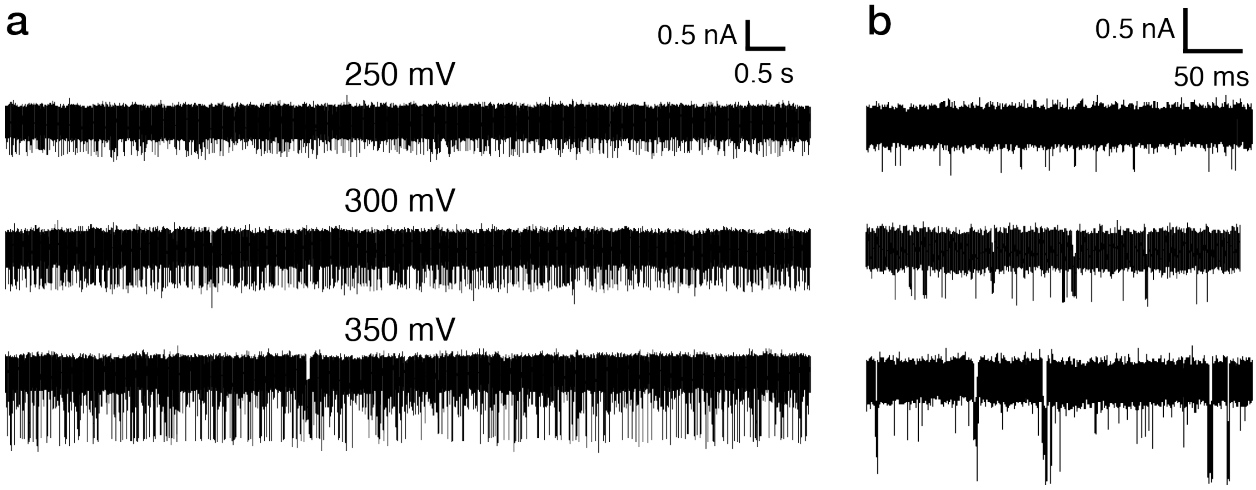

Figure S3. Representative current traces at different voltages low-pass filtered digitally to 200 kHz and presented at different time scales – the entire trace is (a) 10 s and (b) 300 ms long.

SI 3. Concatenated sets of fit events

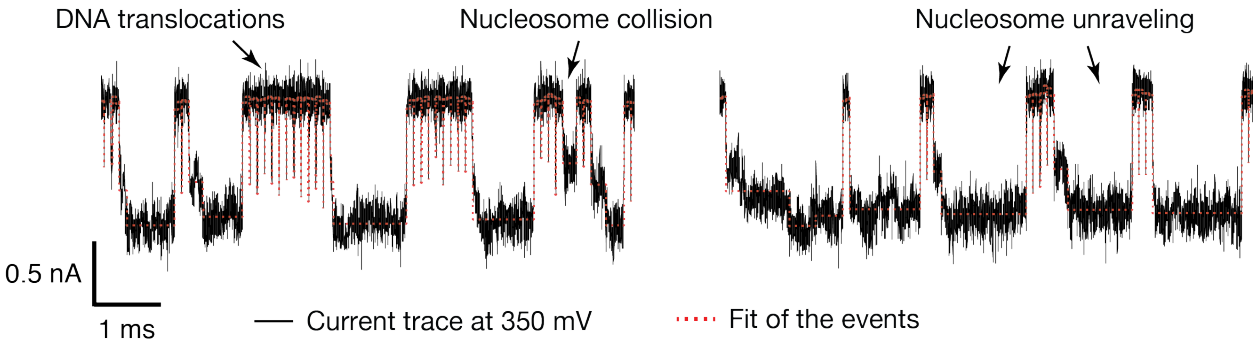

Figure S4. Examples of event fits obtained using OpenNanopore, custom MATLAB software.[10]

SI 4. Nucleosome reconstitution and details of gel shift assay

Nucleosomes were prepared from the Epimark Nucleosome Assembly Kit (NEB E5350) following the dilution assembly protocol for 25 pmol. Briefly, 5M NaCl was mixed with the provided 5s rDNA, and dimer and tetramer histone proteins for each reaction. Additional amounts of 10mM Tris were added to reduce the concentration of NaCl as per the protocol. Incubations were 30 minutes at 24°C. Once reactions were complete, nucleosome assembly was determined by gel shift of the 5s rDNA. Gel shift was done by running 10ul of reaction on a 6% polyacrylamide gel, staining the DNA with SYBR-safe (Invitrogen), and imaging with a Chemi-doc imager (Bio-rad) (Figure 1c). The '**L**' lane is a 2-log ladder from NEB (TriDye™ 2-Log DNA Ladder (0.1 - 10.0 kb) N3270S), the '**1**' lane is 1 µl of 1 µM 5s rDNA, the '**2**' lane is 10 µl of a 25 pMole assembly reaction. DNA is shifted from 200 bp to approximately 700 bp when nucleosomes are formed.

SI 5. Analysis of 325 and 350 mV data low-pass filtered to 300 kHz

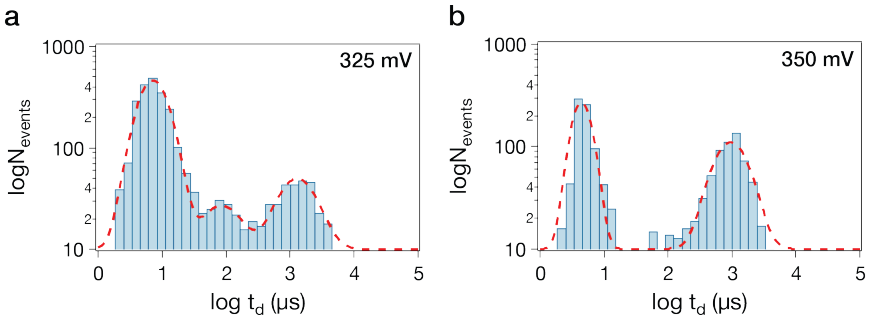

Figure S5. Dwell time histograms made from logarithmically binned durations of > 2000 events at (a) 325 and (b) 350 mV and their Gaussian fits. A digital 300 kHz low-pass filter was applied to these data in order to detect faster sub-10 μs DNA translocation events.

Table S2. Dwell times obtained from the histogram fits (st. dev. in parentheses).

Voltage, mV	t_d, μs
325	7.8 (-2.2/+3.1), 85.1 (-29.5/+47.5), 1288.3 (-653.1/+1324.5)
350	4.5 (-1.1/+1.4), 955 (-475.1/+945)

SI 6. Analysis of 310, 340, 360, and 370 mV data from a different 3 nm in diameter nanopore low-pass filtered to 300 kHz

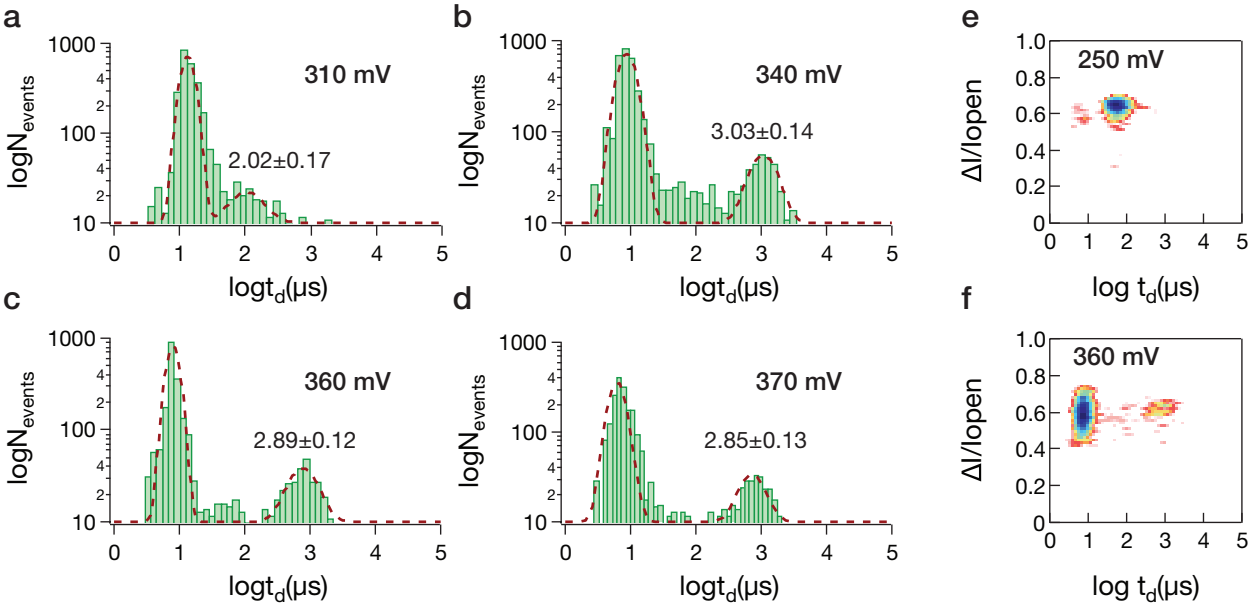

Figure S6. Dwell time histograms made from logarithmically binned durations of > 2000 events at (a) 310 (b) 340, (c) 360, (d) 370 mV and their Gaussian fits. A digital 300 kHz low-pass filter was applied to these data in order to detect faster sub-10 μs DNA translocation events. Representative scatter plots of events at low and high voltages are shown in (e) and (f), respectively.

Dwell time histograms presented in Figure S6 further indicate that the nucleosome collision peak disappears as voltage is increased, while the nucleosome-unraveling peak shifts to shorter times.

Table S3. Dwell times obtained from the histogram fits for nucleosome unraveling events (st. dev. in parentheses).

Voltage, mV	t_d, μs
340	1071 (-294.7/+408.1)
360	776 (-207/+283.3)
370	708 (-183.2/+247)

SI 7. Representative current traces at different voltages from a different 3 nm pore.

Figure S7. Representative current traces at different voltages low-pass filtered digitally to 200 kHz from a different 3 nm pore.

SI 8. Capture rate of DNA and nucleosomes as a function of voltage

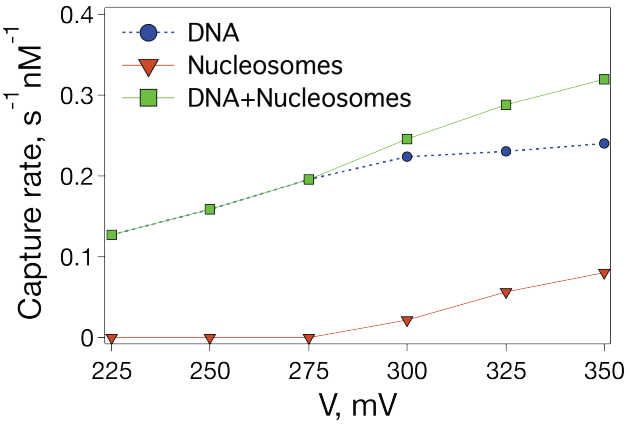

Figure S8. Capture rates of free DNA (blue) and nucleosomes (red), and combined capture rate (green) as a function of voltage.

Capture rates of DNA and nucleosomes (Figure S8) were calculated assuming that events with durations longer than 100 μs represent nucleosome capture, while faster events are DNA translocations.

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