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Fast, Label-Free Force Spectroscopy of Histone–DNA Interactions in Individual Nucleosomes Using Nanopores

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Supporting Information

ABSTRACT: Herein we report a novel approach for fast, label-free probing of DNA–histone interactions in individual nucleosomes. We use solid-state nanopores to unravel individual DNA/histone complexes for the first time and find that the unraveling time depends on the applied electrophoretic force, and our results are in line with previous studies that employ optical tweezers. Our approach for studying nucleosomal interactions can greatly accelerate the understanding of fundamental mechanisms by which transcription, replication, and repair processes in a cell are modulated through DNA–histone interactions, as well as in diagnosis of diseases with abnormal patterns of DNA and histone modifications.

Eukaryotic DNA is packaged into nucleosomes, each comprised of ~147 base pairs of DNA wrapped 1.7 turns around histone octamers.[1] Nucleosome organization inherently limits the accessibility of regulatory proteins to genes, which serves as a sophisticated mechanism to control transcription, replication, and repair processes in a cell.[2] While it is known that dynamic modulation of nucleosomal structures is achieved via epigenetic modifications of histone proteins and DNA[3,4] and by ATP-dependent remodelers,[5] the mechanisms by which these enzyme-assisted modifications affect intra-nucleosomal interactions remain elusive.

Methods for fast, label-free measurements of DNA–histone interactions at the single nucleosome level can greatly accelerate our understanding of the factors that modulate nucleosome stability. While valuable insight has been obtained by Forster resonance energy transfer (FRET),[6−8] atomic force microscopy,[9] and optical tweezers,[10−14] all of these methods require time-consuming sample labeling and/or surface immobilization. Recently, Soni and co-workers reported the use of nanopores for studying chromatin substructures such as histone monomers, tetramers, and octamers.[15] While other DNA/protein systems have been studied using solid-state nanopores,[16−18] in this paper we employ force spectroscopy for studying nucleosomal structures by choosing a pore size that applies an unraveling force to the wound DNA molecule.

Figure 1a displays the schematic of our solid-state nanopore setup. A nanopore in an ultrathin silicon nitride membrane connects two chambers filled with an ionic buffer solution (0.265 M KCl, 0.0825 M NaCl, 1 mM EDTA, 10 mM tris buffered to pH 7.9) that is isotonic with the eukaryotic cell nucleus.[19] Voltage applied across the membrane creates a steady-state trans-membrane ion current flux that sculpts a highly localized (<10 nm) electrophoretic force gradient within the pore (see Supporting Information (SI)). Entry and exit of DNA from the pore are signaled by a characteristic ion current spike of measurable duration, t_d, and mean ion blockade fraction, $\Delta I/I_{open}$ (see SI). Mononucleosomes used in this study were reconstituted *in vitro* with 208-bp control DNA provided

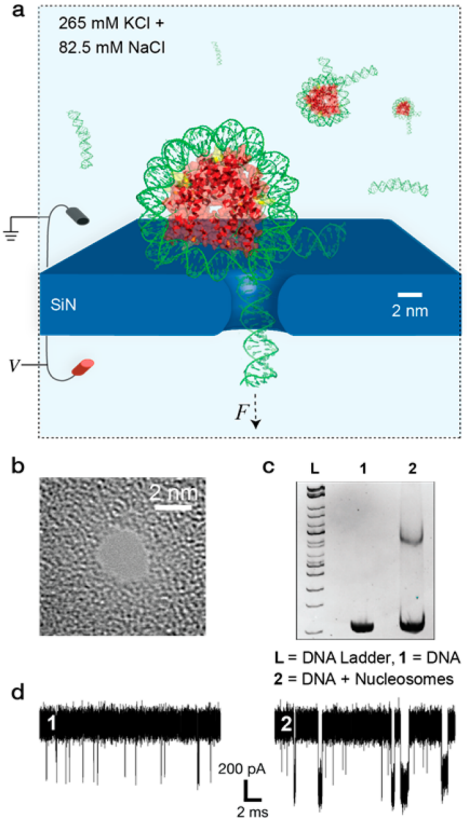

Figure 1. (a) Scheme of a nanopore setup for measuring DNA–histone interactions. (b) A transmission electron microscope (TEM) image of the ~3 nm silicon nitride nanopore used in the experiment. (c) Gel validation of nucleosome assembly (6% PAGE). (d) Current traces for 100 nM DNA (208-bp) and DNA/Nucleosome samples at 325 mV.

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with the EpiMark nucleosome assembly kit (New England Biolabs, Ipswich, MA), and their formation was confirmed by gel shift analysis (Figure 1c; see SI). Nucleosome concentrations were kept above 100 nM during experiments to prevent nucleosome dissociation.[20] For nucleosome unraveling via DNA pulling, ~3 nm diameter pores were chosen (Figure 1b). Electrophoretic force captures a DNA end into the pore until the nucleosome is unwrapped, and the unwrapping time is measured for many individual complexes in the solution. Measurement time resolution of 4−5 μs was achieved by collecting raw current samples at a 4.2 MHz sampling rate and low-pass filtering the data using a digital 200 kHz low-pass filter (Chimera Instruments).[21] Offline data analysis was then performed using OpenNanopore, an open-source MATLAB software.[22]

Representative current traces at 325 mV reveal a striking difference between free DNA (sample **1**) and DNA + nucleosomes (sample **2**) (Figure 1d; see SI). A distinct population of events with considerably longer dwell times is observed for sample **2** at V > 300 mV. We hypothesize that these events correspond to nucleosome unraveling. To support this, we have analyzed >6000 events for each voltage in the range 225−350 mV for the DNA/nucleosome sample. Surprisingly, during the course of our experiments our pores did not foul with nucleosomes, which may be due to the larger size of histones with respect to our 3 nm pore.

Results for sample **2** are summarized in Figure 2, in which we plot two-dimensional color maps of normalized $\Delta I/I_{open}$ and t_d for different applied voltages. Above each scatter plot we show log-normal dwell time histograms fit to multi-Gaussian distributions. Three distinct populations, labeled as Populations **1**−**3**, are attributed to free DNA translocation, nucleosome collisions, and nucleosome unraveling events, respectively. A minor, fast population seen for V ≤ 225 mV (t_d ~5 μs, $\Delta I/I_{open}$ ~0.6) is attributed to signal artifacts due to fast DNA translocation.

Our assignment of Population **1** as free DNA translocations is supported by the systematic decrease in dwell times with voltage, with log(t_d) regularly declining from 1.71 to 0.65 (corresponding to t_d of 51.3 and 4.5 μs). Increased broadening of $\Delta I/I$ in the maps for V > 250 mV is due to the coincidence of our signal durations with the minimum time resolution (~5 μs). While in similar experiments with pure DNA only Population **1** was observed, Populations **2** and **3** only appeared for the nucleosome sample. Population **2** appears at V = 300 mV and gradually disappears at larger voltages. Two observations suggest that events in Population **2** represent nucleosome collisions with the pore: (1) Mean t_d values do not decrease with increasing force (78 μs at 300 mV vs 85 μs at 325 mV), and (2) the rate of events in Population **2** decreases with voltage. Population **3**, which becomes pronounced at voltages 325 mV and higher, is attributed to nucleosome unraveling by the pore. This is supported by an increase in the frequency of events in Population **3** with voltage and a decrease in mean event duration (1.29 ms for 325 mV, 1.07 ms for 340 mV, 0.96 ms for 350 mV, 0.78 ms for 360 mV, and 0.71 ms for 370 mV; see SI).

To provide further rationale for our hypothesis, we have estimated the force acting on the nucleosomal DNA in the nanopore and compared it to the literature value of the total rupture force required for complete disruption of DNA from the histone octamers (see SI for a detailed description of our assumptions). To provide an estimate of force, we have

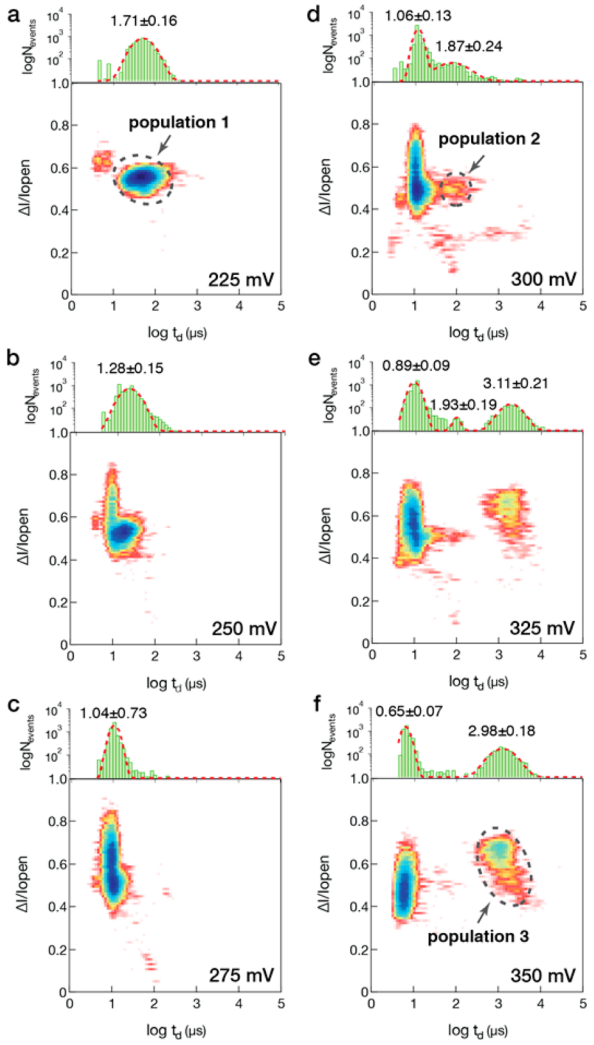

Figure 2. Scatter plots of current blocked vs dwell time in the voltage range 225−350 mV (n = 6400 for each voltage) collected from a single pore, and corresponding log(dwell time) histograms. Log-normal fits (red dashed lines) are shown, and errors are indicated (see Supporting Information).

compiled a set of reported DNA translocation times[23−29] and related the observed velocities to directly measured forces.[30,31] Our approach yields nucleosome rupturing forces of ~10 pN for V = 325 mV and ~18 pN for V = 350 mV, in good agreement with previous reports.[9,11−13] In a study by Gemmen et al.,[12] DNA−histone assemblies were stretched using optical tweezers at a constant rate, in which rupture occurred with sub-10 ms kinetics, similar to our rupturing rates. Mean nucleosome rupture forces decreased from 31 to 24 pN as monovalent salt concentrations increased from 5 to 100 mM. Extrapolation to our monovalent salt regime of 350 mM yields a nucleosome rupture force of ~6 pN, consistent with our study. We note that in contrast to most AFM and optical tweezers measurements, the loading rate in a typical nanopore experiment is unknown and can exceed that of established single-molecule techniques because of the μs-time scale loading of a protein−DNA assembly into a pore. Molecular dynamics simulations have recently provided an in-depth description of this process,[32] suggesting that direct comparison of nanopore force measurements to other single-molecule studies can be difficult. Future

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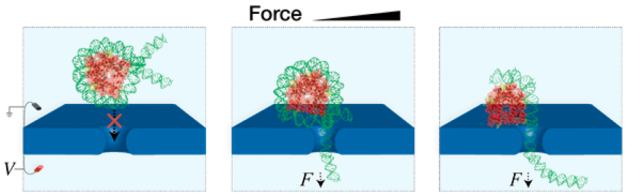

Figure 3. Scheme outlining the force-dependent interactions of nucleosomes with a nanopore.

use of active voltage control techniques can provide more precise control over loading rates.

In summary, we have shown here the reproducible (see SI) capture and unraveling of individual nucleosomes using a 3 nm diameter pore. Three force regimes were observed, as shown in Figure 3: Below 300 mV (<7 pN), no pronounced nucleosome-related events were observed, implying that a minimum force is required to capture a nucleosome. At 300 mV, nucleosome collisions with the pore are observed, although the force is insufficient for nucleosome unraveling. At voltages above 300 mV (>7 pN), nucleosomes are captured and unraveled by the pore. Nanopore-based measurements of histone−DNA interactions are label-free and convenient. Since samples contain a resolvable mixture of free DNA and nucleosomes, unraveling forces are easily calibrated based on free DNA velocities during the experiment. This self-calibration is important in solid-state nanopore experiments, where pore size variability can substantially affect forces within the pore. Future studies can focus on mechanisms that control transcription, replication, and repair processes in a cell through modulation of DNA−histone interactions, as well as in diagnosis of diseases with abnormal patterns of DNA and histone modifications. In addition, further work will be carried out to obtain a more accurate force determination, as well as an analytical model that accounts for DNA−pore interactions and hydrodynamics.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Detailed description of nucleosome preparation, data for higher voltages, data analysis, raw traces, and force estimation. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Luger, K.; Mader, A. W.; Richmond, R. K.; Sargent, D. F.; Richmond, T. J. *Nature* **1997**, *389*, 251.
(2) Workman, J. L.; Kingston, R. E. *Annu. Rev. Biochem.* **1998**, *67*, 545.
(3) Kouzarides, T. *Cell* **2007**, *128*, 693.
(4) Strahl, B. D.; Allis, C. D. *Nature* **2000**, *403*, 41.
(5) Saha, A.; Wittmeyer, J.; Cairns, B. R. *Nat. Rev. Mol. Cell Biol.* **2006**, *7*, 437.
(6) Choy, J. S.; Wei, S.; Lee, J. Y.; Tan, S.; Chu, S.; Lee, T. H. *J. Am. Chem. Soc.* **2010**, *132*, 1782.
(7) Gansen, A.; Toth, K.; Schwarz, N.; Langowski, J. *J. Phys. Chem. B* **2009**, *113*, 2604.
(8) Gansen, A.; Valeri, A.; Hauger, F.; Felekyan, S.; Kalinin, S.; Toth, K.; Langowski, J.; Seidel, C. A. M. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 15308.
(9) Mihardja, S.; Spakowitz, A. J.; Zhang, Y. L.; Bustamante, C. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 15871.
(10) Bennink, M. L.; Leuba, S. H.; Leno, G. H.; Zlatanova, J.; de Grooth, B. G.; Greve, J. *Nat. Struct. Biol.* **2001**, *8*, 606.
(11) Brower-Toland, B. D.; Smith, C. L.; Yeh, R. C.; Lis, J. T.; Peterson, C. L.; Wang, M. D. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 1960.
(12) Gemmen, G. J.; Sim, R.; Haushalter, K. A.; Ke, P. C.; Kadonaga, J. T.; Smith, D. E. *J. Mol. Biol.* **2005**, *351*, 89.
(13) Hall, M. A.; Shundrovsky, A.; Bai, L.; Fulbright, R. M.; Lis, J. T.; Wang, M. D. *Nat. Struct. Mol. Biol.* **2009**, *16*, 124.
(14) Shundrovsky, A.; Smith, C. L.; Lis, J. T.; Peterson, C. L.; Wang, M. D. *Nat. Struct. Mol. Biol.* **2006**, *13*, 549.
(15) Soni, G. V.; Dekker, C. *Nano Lett.* **2012**, *12*, 3180.
(16) Dorvel, B.; Sigalov, G.; Zhao, Q.; Comer, J.; Dimitrov, V.; Mirsaidov, U.; Aksimentiev, A.; Timp, G. *Nucleic Acids Res.* **2009**, *37*, 4170.
(17) Kowalczyk, S. W.; Hall, A. R.; Dekker, C. *Nano Lett.* **2010**, *10*, 324.
(18) Zhao, Q.; Sigalov, G.; Dimitrov, V.; Dorvel, B.; Mirsaidov, U.; Sligar, S.; Aksimentiev, A.; Timp, G. *Nano Lett.* **2007**, *7*, 1680.
(19) Dick, D. A. *J. Physiol.* **1978**, *284*, 37.
(20) Dyer, P. N.; Edayathumangalam, R. S.; White, C. L.; Bao, Y. H.; Chakravarthy, S.; Muthurajan, U. M.; Luger, K. *Methods Enzymol.* **2004**, *375*, 23.
(21) Rosenstein, J. K.; Wanunu, M.; Merchant, C. A.; Drndic, M.; Shepard, K. L. *Nat. Meth.* **2012**, *9*, 487.
(22) Raillon, C.; Granjon, P.; Graf, M.; Steinbock, L. J.; Radenovic, A. *Nanoscale* **2012**, *4*, 4916.
(23) Chen, P.; Gu, J. J.; Brandin, E.; Kim, Y. R.; Wang, Q.; Branton, D. *Nano Lett.* **2004**, *4*, 2293.
(24) Kowalczyk, S. W.; Wells, D. B.; Aksimentiev, A.; Dekker, C. *Nano Lett.* **2012**, *12*, 1038.
(25) Langecker, M.; Pedone, D.; Simmel, F. C.; Rant, U. *Nano Lett.* **2011**, *11*, 5002.
(26) Li, J. L.; Gershow, M.; Stein, D.; Brandin, E.; Golovchenko, J. A. *Nat. Mater.* **2003**, *2*, 611.
(27) Li, J. L.; Talaga, D. S. *J. Phys.: Condens. Matter* **2010**, *22*, 454129.
(28) Storm, A. J.; Storm, C.; Chen, J. H.; Zandbergen, H.; Joanny, J. F.; Dekker, C. *Nano Lett.* **2005**, *5*, 1193.
(29) Wanunu, M.; Sutin, J.; McNally, B.; Chow, A.; Meller, A. *Biophys. J.* **2008**, *95*, 4716.
(30) Keyser, U. F.; Koeleman, B. N.; Van Dorp, S.; Krapf, D.; Smeets, R. M. M.; Lemay, S. G.; Dekker, N. H.; Dekker, C. *Nat. Phys.* **2006**, *2*, 473.
(31) van Dorp, S.; Keyser, U. F.; Dekker, N. H.; Dekker, C.; Lemay, S. G. *Nat. Phys.* **2009**, *5*, 347.
(32) Comer, J.; Ho, A.; Aksimentiev, A. *Electrophoresis* **2012**, *33*, 3466.