Published in final edited form as:

J Phys Chem Lett. 2018 August 16; 9(16): 4686–4694. doi:10.1021/acs.jpcllett.8b01755.

The Dynamics of a Molecular Plug Docked onto a Solid-State Nanopore

Xin Shi¹, Qiao Li¹, Rui Gao¹, Wei Si^{2,3}, Shao-Chuang Liu¹, Aleksei Aksimentiev², and Yi-Tao Long¹

¹Key Laboratory for Advanced Materials, School of Chemistry & Molecular Engineering, East China University of Science and Technology, Shanghai 200237, P.R. China, ytlong@ecust.edu.

²Department of Physics and Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana–Champaign, 1110 W Green St, Urbana, IL 61801, USA

³Jiangsu Key Laboratory for Design and Manufacture of Micro-Nano Biomedical Instruments and School of Mechanical Engineering, Southeast University, Nanjing 210096, P. R. China

Abstract

Docking of a protein-DNA complex onto a nanopore can provide ample observation time for a detailed inspection of the complex, enabling collection of biophysical data for detection, identification, and characterization of the biomolecules. While docking of a protein-DNA complex onto a biological nanopore has enabled analytic applications of nanopores including DNA sequencing, the application of the same principle to solid-state nanopores is tempered by poor understanding of the docking process. Here, we elucidate the behaviour of individual protein-DNA complexes docked onto a solid-state nanopore by monitoring the nanopore ionic current. Repeat docking of monovalent streptavidin-DNA complexes is found to produce ionic current blockades that fluctuate between discrete levels within the same current blockade. We elucidate the roles of the protein plug and the DNA tether in the docking process, finding the docking configurations to determine the multitude of the current blockade levels whereas the frequency of the current level switching to be determined by the interactions between the molecules and the solid-state membrane. Finally, we prove the feasibility of using the nanopore docking principle for single molecule sensing using solid-state nanopores by detecting conformational changes of a tethered DNA molecule from a random coil to an i-motif states.

Introduction

Nanopore-based sensors derive elegance and beauty from their simple principle of operation: the physical blockade by the analyte of the ion transport across the pore reports on the analyte's size. These simple ionic current measurements on single biomolecules have been employed in the past two decades for a broad range of biomolecular studies, such as DNA/RNA sensing¹, protein identification^{2–3}, resolving conformational changes of biopolymers^{4–7} and bioinspired intelligent materials^{8–9}, going far beyond the initial idea of DNA sequencing^{10–11}. Since the emergence of nanopore-based techniques, the fast translocation of molecules has, however, hampered detailed studies of biomolecules using nanopores and has limited the amount of information that can be extracted from the measurements^{12–13}.

To drastically increase the residence time of the biomolecule in the sensor, different approaches have been developed. Several methods have focused on slowing down molecular transport to increase resolution^{14–16}. Some other techniques have focused on stalling the translocating molecules. Bringing the translocation process to a full stop not only increases the signal acquisition time, but also allows, in principle, for the observation of intrinsic dynamics of the biomolecule. Successful stalling has been achieved using optical tweezers^{17–18}, magnetic tweezers¹⁹, and the atomic force microscope^{20–21}, but these techniques are arduous to operate, and lack the throughput required for sensing.

A more fruitful approach, however, is to dock the biomolecule of interest onto the nanopore using a molecular plug, where the plug, typically a large protein, is too big to pass through the nanopore. Whilst the plug attempts to translocate through the pore and is effectively docked, the analyte is suspended in the sensing region of the nanopore, ready for inspection. This technique has been employed with remarkable success for biological nanopores sensing^{22–23}, in particular for commercial DNA sequencing where the protein plug is a DNA threading motor^{24–25}, and for general characterization of protein²⁶ and DNA²⁷ dynamics.

In the case of solid-state nanopores, however, studies utilizing the docking sensing scheme are fewer in number. Thus, previous experiment work used the protein docking scheme to study protein-DNA interactions^{28–31}. Alternative sensing strategies have been theoretically proposed but haven't yet been experimentally realized, including protein recognition³², drug optimization³³, and monitoring the conformational changes of biomolecules³⁴. One of the limiting factors is the excessive (in comparison to biological nanopores) noise in the ionic current blockades produced by the interaction of the docked molecules with solid-state nanopores. Thus, determining the molecular origin of the ionic current noise and the behavior of the docked biomolecules is a necessary first step toward applications of the docking scheme in molecular sensing using solid-state nanopores.

Here, we characterize the behavior of individual protein-DNA complexes trapped in a solid-state nanopore by real-time monitoring of the ionic current. A DNA-tethered monovalent streptavidin - our model molecular plug - is captured in a sub-5-nm silicon nitride (SiN_x) nanopore. We choose monovalent streptavidin^{35–36} as our model molecular plug instead of other plugs, such as nanobeads or wildtype streptavidin, to prevent attachment of multiple DNA strands to a single plug. Furthermore, this one-to-one DNA-protein complex is a good model of more complex molecular systems used for DNA sequencing and other sensing schemes. As we show below, docking of such DNA-protein complexes onto solid-state nanopores produces prolonged and complex ionic current blockades. We explore the roles of both the protein plug and the DNA tether in modulating the blockade ionic current, characterize the voltage dependence of the docking process, and demonstrate the feasibility of using the docking scheme for biosensing.

Results and discussion

Docked molecules produce surprisingly complex and prolonged current blockades.

Figure 1a displays the schematic of our nanopore experiments. First, solid-state nanopores smaller than 5 nm in diameter were fabricated in 10 nm thick free-standing silicon nitride

(SiN_x, Norcada, Canada) membranes by controlled dielectric breakdown using a custom current amplifier³⁷. The nanopore size is critical as the pores need to be large enough to accommodate a DNA tether, but small enough to prevent translocation of the protein plug. The streptavidin is around 5–8 nm in diameter and the dsDNA molecules are around 2.2 nm in diameter. Therefore, nanopores with 3 to 5 nm in diameters were used in this study. The detailed description of the nanopore fabrication procedures is provided in Supplementary Information (SI), Section S1.

Negatively charged DNA-protein complexes can be electrophoretically captured by the nanopores. By applying a +100 mV bias to the trans chamber of our setup, the DNA tethers are guided toward the nanopore to insert the complex in a well-defined orientation (Figure 1b). Upon capture, the highly negatively charged DNA tether enters the pore first but the translocation of the complex is stalled by the larger streptavidin plug. This event produces a clear sustained blockade of the ionic current (Figure 1c), indicating successful docking of the complex. The blockade typically lasts several seconds in our experiments, before being released from the nanopore by a reversal of the voltage, typically to a value in the –150 to –200 mV range (Figure 1b). We apply a square wave in the transmembrane voltage to dock and release the molecular plug automatically. Experimental details of the reversible streptavidin-DNA docking are described in the SI, Section S2.

Next, we investigated the ionic current traces produced by the docking of streptavidin-DNA complexes. For these experiments, we used either 90-bp dsDNA (for sequence, see SI, Section S2) or poly(dC)₉₀ single-stranded DNA tethers. At pH 8, a dsDNA molecule has a persistence length of 50 nm whereas poly(dC)₉₀ assumes a random coil conformation. Figure 2 shows typical ionic current traces and the corresponding all-points histograms produced by docking of streptavidin-dsDNA and streptavidin-ssDNA complexes. After an initial characteristic drop of the ionic current, which indicates docking, the blockade current 1) fluctuates with significantly high amplitude (>5 nS), and 2) appears to attain distinct levels (Figure 2a), in striking contrast to current fluctuations produced by noise. Interestingly, we observe this behavior for both dsDNA- and ssDNA-streptavidin complexes (Figure 2a and c). In contrast, such a behavior was rarely observed in experiments carried out using biological nanopores^{23, 38}, for example, docking streptavidin-poly(dC)₉₀ on an α -hemolysin nanopore²⁵. All-points histograms from 200 of such docking events, Figures 2b and 2d, indicate that the blockade levels are qualitatively conserved between different docking events and persist during the course of the experiment. Interestingly, qualitatively similar but quantitatively different blockade current patterns are observed in repeat experiments carried out using different nanopores, see SI, Section S3. Under voltage higher than 200 mV, ruptures of streptavidin-biotin bonds start appearing as reported in literature. Examples of the rupture are shown in SI, Figure S6. During our nanopore experiments, we monitor the open pore current before and after each docking event to make sure that the nanopore does not contain any leftover residues, which might increase the open pore current noise. We thus conclude that the fluctuations of the ionic current upon docking report on the dynamic of the docked molecules on solid-state nanopores.

The origin of the large amplitude fluctuation of the blockade current.

We first investigate the role of the protein plugs in the solid-state nanopore docking system. The streptavidin is lodged in the entrance-access region of the nanopore, the half space directly above of the nanopore that controls about one quarter of the total resistance in our case²¹. Considering the size of the streptavidin plug (~8 nm), it can significantly alter the total measured conductance. Moreover, because of the flexibility of the protein and the shape of the nanopores, a part of the protein can still fit into the nanopore and hence the motion of the protein, partially in the access region and inside the nanopore, can affect the current blockade after the protein-DNA complex' docking.

To verify the above conjecture, we calculated the current blockade for different orientations and positions of a streptavidin protein at the entrance of the pore using a previously described theoretical method³⁴. As shown in Figure 3, the streptavidin molecule was held along the axis of a nanopore, 2.2 nm above the nanopore constriction. The ionic current was calculated under a bias of 150 mV while the streptavidin molecule was rotated about the *x*, *y* and *z* axes, with the center of mass of the protein fixed. The angle of rotation of the protein was randomly chosen from 0° to 360°. Figure 3b shows that conductance variation caused by the changes in the streptavidin's orientation produces a ~0.5 nA (3.3 nS) magnitude variation of the blockade current. Moreover, when the orientation of the protein is fixed, a translation perpendicular to the membrane by roughly the size of the protein gives another ~0.5 nA of current variation (see SI, Figure S7). These results strongly imply that the motion of protein in the access region of the nanopore can induce ~7 nS conductance variation, which is of the same order as the variation of the blockade current observed in our experiments. Details of the calculation are provided in SI, Section S5.

Next, we discuss if the large amplitude fluctuation of the blockade current could be produced by the DNA tether inside the nanopores. First, we note that different blockade current levels can be caused by different folding states of dsDNA in the nanopore, as have been shown in previous works⁴. However, these levels, observed in unobstructed translocations, typically indicate folded states of a long (> 1 μ m) dsDNA molecule and are observed upon capture of long molecules into relatively large (typically >10 nm in diameter) nanopores. The short dsDNA tethers (< 30 nm, shorter than the persistence length of dsDNA) used in our work are unlikely to enter the small pore in a folded fashion and will be pulled taut by the electric field in the nanopore after docking. Such short dsDNA tethers are thus not expected to show dynamic behavior, which would indicate a highly unfavorable trans-side pore re-entry of the DNA molecule. Moreover, similar current levels are observed for the more flexible ssDNA tethers, which have a much smaller persistence length than dsDNA and hence should not display any stable secondary structures³⁹. Discarding conformational changes of the tethers as the origin of the current fluctuations, we note that trapping dsDNA in different orientations with respect to the nanopore can produce different magnitude blockade currents⁴⁰. However, considering the small diameter of the nanopores used in our experiments, the difference contributed by the orientations of dsDNA should be considerably less than in 10 to 20-nm diameter nanopores. Furthermore, this argument cannot explain large amplitude fluctuations observed in our ssDNA experiments. Thus, we identify changes in the docking configurations of the complex, mainly produced by re-

orientation of the protein plug, as the main cause of the large amplitude fluctuations of the ionic current.

While above analysis could explain why we observe large fluctuations in the residual current, it does not immediately elucidate the cause of the characteristic dynamic of the docked molecule, including the relatively free motion of the docked complex and the prolonged current states. As for the mobility of the molecules, one explanation would be that the transition is driven purely by the Brownian motion of the biomolecules. Such motion is ubiquitous in all molecular systems at room temperature. Furthermore, local orientations of a typical protein undergoing stochastic rotational diffusion are expected to be uncorrelated at the time scale of microseconds³⁴, introducing high-frequency noise in the ionic current recording rather than sporadic discrete transitions between the current levels.

Another physical force that can displace a docked complex is the viscous drag of the electroosmotic flow. Such electroosmotic forces can be substantial. In the case of dsDNA, the viscous drag force compensates $\frac{3}{4}$ of the pull force of the electric field^{17, 41}. In the case of weakly charged proteins, the viscous drag dominates over electrostatics and determines the direction of nanopore transport⁴². Even though the electro-osmotic flow quickly decays away from the pore, the weakly charged streptavidin has a much larger cross section than the DNA tether and will experience a much larger drag⁴². Thus, the net force acting on the complex can be much reduced, allowing the molecular plug to be slightly released from the pore and reorient before being pressed onto the membrane again.

Both Brownian motion and electroosmotic forces cannot, however, explain long and sporadic trapping of the docked complexes. Previous molecular dynamics simulations have showed that non-specific interactions between DNA³² or a protein⁴³ with the nanopore surface can transiently arrest nanopore translocation, a phenomenon that has been also observed in experiment⁴⁴. Such non-specific adhesion can both stabilize a docked conformation and reduce the effective force on the complex³², allowing the complex to adjust its conformation. Thus, a spontaneous rearrangement of the docked protein-DNA complex might be related to a stick-and-slip nanopore transport governed by surface interactions.

Modulation of the dynamics of docked molecules by transmembrane voltage.

To demonstrate the voltage response of the docked molecules, we monitor the blockade ionic current from the same molecules under different voltages. The monovalent streptavidin-poly(dC)₉₀ complex was docked onto a solid-state nanopore as described above, under a driving voltage of 100 mV. After docking, the bias was decreased to 75 and then to 50 mV, or increased to 150 mV and then to 200 mV, respectively in each experiment. Then, the voltage was flipped to -200 mV to release the molecule and clear the nanopore, making it ready for the next docking event. Figure 4a and b show typical current traces recorded in these experiments.

Under 100 mV, the blockade current shows similar fluctuations to those shown in Figure 2. The all-point-histograms of the blockade current exhibit a main peak at ~ 6 nS (~ 1 G), along with smaller peaks at higher ~ 10 G values. When the bias was reduced, the changes in the

current histograms were subtle. From the three examples shown in Figure 4a and 20 other examples shown in the SI (Figure S8), no significant change in the location of the main G peak (the blockade value) was observed. As for other peaks, the changes in the relative population can be observed in some cases. For example, the histogram for molecule #3 (SI Figure S8), the peak at ~ 10 nS decreased with voltage, while molecule #6 showed an increased population at the same value under 50 mV. Overall, the pattern of current fluctuations remained constant as the voltage was decreased from 100 mV to 50 mV.

As we increased the voltage, the difference in the fluctuating patterns became more obvious, however, the fluctuations were not eliminated by the increased electrophoretic force. Although an increase or a decrease of population at different blockade levels was observed in some cases, such as trace #5 in Figure 4b, in the majority of the examples (see Figure S8), a dramatic shift of G peak position was rarely seen. A similar experiment was then performed using a streptavidin-dsDNA complex, Figure 4c and Figure S9. Consistent with the outcome of the ssDNA tether experiments, increasing the voltage effected the current fluctuations, however, the location of the peaks barely changed. More importantly, a higher voltage did not eliminate the current fluctuations.

To evaluate the influence of the voltage on the lifetime of the current states, we plot, in the SI Figure S11, the lifetime of the most populated state versus G for 100 dockings of streptavidin-poly(dC)₉₀ molecules under 100 mV, 150 mV, and 200 mV, corresponding to the examples shown in Figure 4b. From the scatter plots and corresponding histograms, the systematic effect of the voltage magnitude can be discerned, although the pattern of current fluctuation does change. However, increasing the voltage does not necessarily prolong or shorten the lifetime of certain states, resulting in the similar lifetime distributions under different voltages. Furthermore, we also observed that the changes in the fluctuation patterns do not necessarily happen right after changing the voltage (SI, Figure S12), which indicates that such pattern transitions are not solely caused by the voltage. This interpretation of the measurements is consistent with the results of molecular dynamics simulations³² that showed the DNA-surface interaction to play a major role in stabilizing the motion of the docked molecules, compensating the effective force on the complex, and producing a finite, voltage-independent lifetime.

Modulation of the dynamics of docked molecules by DNA conformation.

To further demonstrate the role of the DNA tethers in governing the docking dynamics, we show below that the motion of a docked complex can be altered or even stabilized by the DNA tethers and that such modulation can be used for probing biomolecular conformations. In this experiment, the DNA tether was changed into a short C-rich ssDNA strand from the human telomere⁴⁵ which can be folded into an intercalated motif (i-motif)³⁹. Such a tetrameric structure is formed by intercalation of hemiprotonated cytosine-cytosine basepairs and is expected to occupy a larger volume in the nanopores, at an acidic pH (see SI section S8), than an unstructured ssDNA at alkaline pH. Such i-motif structures have been studied extensively in the context of nanomachines and biosensors⁴⁶ for their ability to reversibly fold and unfold by simply altering the pH. Very recently, such structures have also been

observed in human cells and can be recognized by certain human antibody fragments with high selectivity and affinity⁴⁷.

We performed docking experiments using a single-stranded 22-nt C-rich DNA of a d(CCCTAA)₃ CCCT sequence attached to the monovalent streptavidin. Typical current traces and corresponding all-points histograms are shown in Figure 5. Under alkaline conditions (pH 8, Figure 5a), current fluctuations, similar to those recorded for the poly(dC)₉₀ and dsDNA tethers, can be observed after the docking, albeit at slightly higher frequency. The current levels are much less pronounced, as illustrated by the broad peaks in the all-points histograms. This can be caused by the shorter DNA strand, barely spanning the length of the pore, destabilizing the whole complex in the nanopore, incurring more severe wobbling, blurring the current levels. Next, we changed the buffer pH from 8 to 5. Typical traces of docking events are shown in the Figure 5b. First, we note that the residual current shows a lot less fluctuations or transition between levels and appears much more stable.

Second, the blockade is larger, increasing from ~10 nS at pH 8 to ~20 nS at pH 5. Finally, narrow peaks can (again) be resolved in the current histograms, with a wide distribution in G varying from ~13 nS to ~25 nS, significantly wider than the blockades in the alkaline solution (Figure 5c).

We attribute the difference in the observed behavior at different pH to both the structure of the ssDNA tether and the charges of the protein-ssDNA complex and the surface. First, in the more acidic solution, ssDNA is expected to fold into an i-motif structure⁴⁸. The i-motif structure has a larger cross-section than unstructured ssDNA and thus is expected to block a larger fraction of the nanopore current, in accordance with the observed current blockade increase of ~10 nS from pH 8 to pH 5. Second, the more compact structure of the i-motif makes the whole complex less mobile when docked, because of the restricted movement of the bulkier DNA inside the pore, resulting in more stable current levels, as observed. Moreover, the isoelectric points of DNA, streptavidin and the SiN_x surface are all around ~4–5^{42, 49}, hence, the pH 5 condition reduces the charges of all constituents, which is expected to give the complexes more freedom of mobility. A control experiment (SI, section S7, Figure S13 and Figure S14), performed with docking of streptavidin-dsDNA at both pH 8 and pH 5, did not show a significant change in the current blockade patterns, especially the current is not stabilized by the reduced pH. We thus conclude that the significant changes in the blockade current patterns demonstrates utility of the docking experiments for single-molecule observations of conformational transitions.

Conclusion

In summary, we investigated the docking of protein-DNA complexes on solid-state nanopores experimentally, where the DNA serves as a tether to drag the complex into the pore whereas the protein, monovalent streptavidin in our case, acts as a molecular plug halting the translocation of the complex. The ionic current blockades exhibit complex patterns and behavior, with defined and prolonged current levels, for both dsDNA and ssDNA tethers. We attribute this diversity of the blockade current to the movement of the protein while being docked at the nanopore entrance. Streptavidin docked in different orientations and configurations can indeed produce large fluctuations in the current

blockades, as we show using theoretical calculation, and that long-lasting levels arise from preferred configurations of the docked complex, stabilized mainly by interactions of DNA with the nanopore surface.

Whereas fluctuations of the blockade current generally hinder the use of the molecular plug concept for detection of real time single-molecule dynamics, they nevertheless can be exploited as a signal transducer. To demonstrate this, we observed the formation of an i-motif secondary structure in a short C-rich ssDNA strand at pH 5 in the blockade current behavior of docked protein-ssDNA complexes. The blockade current at pH 5 is deeper, much more stable, and shows fewer transitions than the same molecule docked at pH 8, which we attribute to the bulkier i-motif tether, blocking a larger fraction of the pore and restricting the movement of the complex. We thus have shown that this system can be used for sensing single-molecule conformational changes.

Several approaches are possible for stabilization of protein dockings and prevention of the large-scale protein motion, including chemical locking of the protein on the solid-state membrane, tweaking experimental conditions to reduce the electroosmotic flow and surface interaction. Furthermore, such a docking scheme will also provide ample read-out time for other sensing strategies besides the ionic current, such as molecular spectroscopy or tunneling current probes, which present the experimentalists with the detailed information about the chemical constituents of the captured molecules.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We would like to acknowledge Prof. Cees Dekker and his group for the valuable discussions. We thank Mr. Daniel Vershueren for the discussions, comments and help during the preparation of the manuscript. We greatly thank Prof. Mark Howarth and his group for providing the monovalent streptavidin. This research was supported by the National Natural Science Foundation of China (21421004, 21327807), the Programme of Introducing Talents of Discipline to Universities (B16017), United States National Institutes of Health (R01-HG007406 and P41-GM104601), and China Scholarship Council (CSC201506090040 for W.S and CSC201606740021 for X. S.).

References

1. Wang F; Zahid OK; Swain BE; Parsonage D; Hollis T; Harvey S; Perrino FW; Kohli RM; Taylor EW; Hall AR, Solid-State Nanopore Analysis of Diverse DNA Base Modifications Using a Modular Enzymatic Labeling Process. *Nano Lett.* 2017, 17 (11), 7110–7116. [PubMed: 28967259]
2. Yuskov EC; Bruhn BR; Eggenberger OM; Houghtaling J; Rollings RC; Walsh NC; Nandivada S; Pindrus M; Hall AR; Sept D, Real-time shape approximation and fingerprinting of single proteins using a nanopore. *Nat. Nanotechnol* 2016.
3. Bell NA; Keyser UF, Digitally encoded DNA nanostructures for multiplexed, single-molecule protein sensing with nanopores. *Nat. Nanotechnol* 2016, 11 (7), 645–51. [PubMed: 27043197]
4. Li J; Gershow M; Stein D; Brandin E; Golovchenko JA, DNA molecules and configurations in a solid-state nanopore microscope. *Nat. Mater* 2003, 2 (9), 611–5. [PubMed: 12942073]
5. Waduge P; Hu R; Bandarkar P; Yamazaki H; Cressiot B; Zhao Q; Whitford PC; Wanunu M, Nanopore-Based Measurements of Protein Size, Fluctuations, and Conformational Changes. *ACS Nano* 2017, 11 (6), 5706–5716. [PubMed: 28471644]

6. An N; Fleming AM; Middleton EG; Burrows CJ, Single-molecule investigation of G-quadruplex folds of the human telomere sequence in a protein nanocavity. *Proc. Natl. Acad. Sci. U.S.A.* 2014, 111 (40), 14325–31. [PubMed: 25225404]
7. Li J; Fologea D; Rollings R; Ledden B, Characterization of protein unfolding with solid-state nanopores. *Protein and Peptide Letters* 2014, 21 (3), 256–65. [PubMed: 24370259]
8. Wen L; Hou X; Tian Y; Nie FQ; Song Y; Zhai J; Jiang L, Bioinspired smart gating of nanochannels toward photoelectric-conversion systems. *Adv. Mater* 2010, 22 (9), 1021–1024. [PubMed: 20217833]
9. Li P; Kong XY; Xie G; Xiao K; Zhang Z; Wen L; Jiang L, Adenosine-Activated Nanochannels Inspired by G-Protein-Coupled Receptors. *Small* 2016, 12 (14), 1854–1858. [PubMed: 26915491]
10. Branton D; Deamer DW; Marziali A; Bayley H; Benner SA; Butler T; Di Ventra M; Garaj S; Hibbs A; Huang X; Jovanovich SB; Krstic PS; Lindsay S; Ling XS; Mastrangelo CH; Meller A; Oliver JS; Pershin YV; Ramsey JM; Riehn R; Soni GV; Tabard-Cossa V; Wanunu M; Wiggin M; Schloss JA, The potential and challenges of nanopore sequencing. *Nat. Biotechnol* 2008, 26 (10), 1146–53. [PubMed: 18846088]
11. Schneider GF; Dekker C, DNA sequencing with nanopores. *Nat. Biotechnol* 2012, 30 (4), 326–8. [PubMed: 22491281]
12. Storm AJ; Storm C; Chen JH; Zandbergen H; Joanny JF; Dekker C, Fast DNA translocation through a solid-state nanopore. *Nano Lett.* 2005, 5 (7), 1193–1197. [PubMed: 16178209]
13. Plesa C; Kowalczyk SW; Zinsmeister R; Grosberg AY; Rabin Y; Dekker C, Fast translocation of proteins through solid state nanopores. *Nano Lett.* 2013, 13 (2), 658–663. [PubMed: 23343345]
14. Lu B; Hoogerheide DP; Zhao Q; Zhang HB; Zhipeng TP; Yu DP; Golovchenko JA, Pressure-Controlled Motion of Single Polymers through Solid-State Nanopores. *Nano Lett.* 2013, 13 (7), 3048–3052. [PubMed: 23802688]
15. Squires AH; Hersey JS; Grinstaff MW; Meller A, A nanopore–nanofiber mesh biosensor to control DNA translocation. *J. Am. Chem. Soc* 2013, 135 (44), 16304–16307. [PubMed: 24143914]
16. Yusko EC; Johnson JM; Majd S; Prangkio P; Rollings RC; Li JL; Yang J; Mayer M, Controlling protein translocation through nanopores with bio-inspired fluid walls. *Nat. Nanotechnol* 2011, 6 (4), 253–260. [PubMed: 21336266]
17. Keyser UF; Koeleman BN; Van Dorp S; Krapf D; Smeets RMM; Lemay SG; Dekker NH; Dekker C, Direct force measurements on DNA in a solid-state nanopore. *Nat. Phys* 2006, 2 (7), 473–477.
18. van Dorp S; Keyser UF; Dekker NH; Dekker C; Lemay SG, Origin of the electrophoretic force on DNA in solid-state nanopores. *Nat. Phys* 2009, 5 (5), 347–351.
19. Peng HB; Ling XSS, Reverse DNA translocation through a solid-state nanopore by magnetic tweezers. *Nanotechnology* 2009, 20 (18), 185101. [PubMed: 19420602]
20. King GM; Golovchenko JA, Probing nanotube-nanopore interactions. *Phys. Rev. Lett* 2005, 95 (21), 216103. [PubMed: 16384162]
21. Hyun C; Rollings R; Li JL, Probing Access Resistance of Solid-State Nanopores with a Scanning-Probe Microscope Tip. *Small* 2012, 8 (3), 385–392. [PubMed: 22393313]
22. Sanchez-Quesada J; Saghatelian A; Cheley S; Bayley H; Ghadiri MR, Single DNA rotaxanes of a transmembrane pore protein. *Angew. Chem. Int. Ed* 2004, 43 (23), 3063–3067.
23. Hornblower B; Coombs A; Whitaker RD; Kolomeisky A; Picone SJ; Meller A; Akeson M, Single-molecule analysis of DNA-protein complexes using nanopores. *Nat. Methods* 2007, 4 (4), 315–317. [PubMed: 17339846]
24. Manrao EA; Derrington IM; Laszlo AH; Langford KW; Hopper MK; Gillgren N; Pavlenok M; Niederweis M; Gundlach JH, Reading DNA at single-nucleotide resolution with a mutant MspA nanopore and phi29 DNA polymerase. *Nat. Biotechnol* 2012, 30 (4), 349–53. [PubMed: 22446694]
25. Stoddart D; Heron AJ; Mikhailova E; Maglia G; Bayley H, Single-nucleotide discrimination in immobilized DNA oligonucleotides with a biological nanopore. *Proceedings of the National Academy of Sciences* 2009, 106 (19), 7702–7707.
26. Van Meervelt V; Soskine M; Singh S; Schuurman-Wolters GK; Wijma HJ; Poolman B; Maglia G, Real-time conformational changes and controlled orientation of native proteins inside a protein nanoreactor. *J. Am. Chem. Soc* 2017, 139 (51), 18640–18646. [PubMed: 29206456]

27. Ding Y; Fleming AM; He L; Burrows CJ, Unfolding kinetics of the human telomere i-Motif under a 10 pN force imposed by the α -hemolysin nanopore identify transient folded-state lifetimes at physiological pH. *J. Am. Chem. Soc* 2015, 137 (28), 9053–9060. [PubMed: 26110559]
28. Zhao Q; Sigalov G; Dimitrov V; Dorvel B; Mirsaidov U; Sligar S; Aksimentiev A; Timp G, Detecting SNPs using a synthetic nanopore. *Nano Lett.* 2007, 7 (6), 1680–1685. [PubMed: 17500578]
29. Dorvel B; Sigalov G; Zhao Q; Comer J; Dimitrov V; Mirsaidov U; Aksimentiev A; Timp G, Analyzing the forces binding a restriction endonuclease to DNA using a synthetic nanopore. *Nucleic Acids Res.* 2009, 37 (12), 4170–4179. [PubMed: 19433506]
30. Tabard-Cossa V; Wiggin M; Trivedi D; Jetha NN; Dwyer JR; Marziali A, Single-molecule bonds characterized by solid-state nanopore force spectroscopy. *ACS Nano* 2009, 3 (10), 3009–14. [PubMed: 19751064]
31. Ivankin A; Carson S; Kinney SR; Wanunu M, Fast, label-free force spectroscopy of histone–DNA interactions in individual nucleosomes using nanopores. *J. Am. Chem. Soc* 2013, 135 (41), 15350–15352. [PubMed: 24079416]
32. Comer J; Ho A; Aksimentiev A, Toward detection of DNA-bound proteins using solid-state nanopores: Insights from computer simulations. *Electrophoresis* 2012, 33 (23), 3466–3479. [PubMed: 23147918]
33. Luan B; Huynh T; Zhou R, Nanopore-Based Sensors for Ligand–Receptor Lead Optimization. *The journal of physical chemistry letters* 2015, 6 (3), 331–337. [PubMed: 26261942]
34. Si W; Aksimentiev A, Nanopore Sensing of Protein Folding. *ACS Nano* 2017, 11 (7), 7091–7100. [PubMed: 28693322]
35. Howarth M; Chinnapen DJ; Gerrow K; Dorrestein PC; Grandy MR; Kelleher NL; El-Husseini A; Ting AY, A monovalent streptavidin with a single femtomolar biotin binding site. *Nat. Methods* 2006, 3 (4), 267–73. [PubMed: 16554831]
36. Fairhead M; Krndija D; Lowe ED; Howarth M, Plug-and-play pairing via defined divalent streptavidins. *J. Mol. Biol* 2014, 426 (1), 199–214. [PubMed: 24056174]
37. Kwok H; Briggs K; Tabard-Cossa V, Nanopore fabrication by controlled dielectric breakdown. *PLoS One* 2014, 9 (3), e92880. [PubMed: 24658537]
38. Stoddart D; Heron AJ; Mikhailova E; Maglia G; Bayley H, Single-nucleotide discrimination in immobilized DNA oligonucleotides with a biological nanopore. *Proc. Natl. Acad. Sci. U.S.A.* 2009, 106 (19), 7702–7. [PubMed: 19380741]
39. Gueron M; Leroy JL, The i-motif in nucleic acids. *Curr. Opin. Struct. Biol* 2000, 10 (3), 326–331. [PubMed: 10851195]
40. Pud S; Chao SH; Belkin M; Verschueren D; Huijben T; van Engelenburg C; Dekker C; Aksimentiev A, Mechanical Trapping of DNA in a Double-Nanopore System. *Nano Lett.* 2016, 16 (12), 8021–8028. [PubMed: 27960493]
41. Luan B; Aksimentiev A, Electro-osmotic screening of the DNA charge in a nanopore. *Phys. Rev. E* 2008, 78 (2), 021912.
42. Firmkes M; Pedone D; Knezevic J; Dobliger M; Rant U, Electrically Facilitated Translocations of Proteins through Silicon Nitride Nanopores: Conjoint and Competitive Action of Diffusion, Electrophoresis, and Electroosmosis. *Nano Lett.* 2010, 10 (6), 2162–2167. [PubMed: 20438117]
43. Carr R; Comer J; Ginsberg MD; Aksimentiev A, Modeling pressure-driven transport of proteins through a nanochannel. *IEEE transactions on nanotechnology* 2011, 10 (1), 75–82. [PubMed: 22611338]
44. Mohammad MM; Prakash S; Matouschek A; Movileanu L, Controlling a single protein in a nanopore through electrostatic traps. *J. Am. Chem. Soc* 2008, 130 (12), 4081–4088. [PubMed: 18321107]
45. Phan AT; Mergny JL, Human telomeric DNA: G-quadruplex, i-motif and Watson–Crick double helix. *Nucleic Acids Res.* 2002, 30 (21), 4618–4625. [PubMed: 12409451]
46. Wang F; Liu X; Willner I, DNA switches: from principles to applications. *Angew. Chem. Int. Ed* 2015, 54 (4), 1098–129.

47. Zeraati M; Langley DB; Schofield P; Moye AL; Rouet R; Hughes WE; Bryan TM; Dinger ME; Christ D, I-motif DNA structures are formed in the nuclei of human cells. *Nature Chemistry* 2018, 1.
48. Gehring K; Leroy JL; Gueron M, A tetrameric DNA structure with protonated cytosine.cytosine base pairs. *Nature* 1993, 363 (6429), 561–5. [PubMed: 8389423]
49. Hoogerheide DP; Garaj S; Golovchenko JA, Probing surface charge fluctuations with solid-state nanopores. *Phys. Rev. Lett* 2009, 102 (25), 256804. [PubMed: 19659110]

Figure 1. Schematic of the experimental setups.

a) Schematic of the protein-DNA complex docked onto a solid-state nanopore. The biotinylated double-stranded DNA or single-stranded DNA is tethered on a monovalent streptavidin. b) Example of a current trace with repeated docking events and the applied square wave voltage to capture, dock, and release the complexes. c) Schematic illustrations of the complex capture, docking, and release. At a positive bias voltage, the streptavidin-DNA complex is docked on the nanopore but cannot traverse through because the protein plug is larger than the nanopore. At a negative bias, the complex is released and the nanopore is cleared. d) A representative ionic current trace corresponding to docking and release of a streptavidin-ssDNA complex. The streptavidin-poly d(C)₉₀ complexes are docked onto the nanopore using a 100-mV bias potential and released under -200 mV. The blockade current fluctuates between several discrete levels. The details of the fluctuation current are discussed in the main text.

Figure 2. Typical current traces and corresponding all-points histograms produced by docking of dsDNA- and ssDNA- streptavidin complexes.

a) Typical current traces recorded from dsDNA- streptavidin docking experiments. The top panel in (a) shows a 4-second current trace from one docking event, followed by three zoomed-in views of representative blockade current traces (left) and the corresponding normalized (area = 1) current histograms (right). b) Collection of all-points current histograms from 200 dsDNA-streptavidin docking events. c) Typical 2-second snapshot of current traces produced by ssDNA-streptavidin docking and the corresponding normalized current histograms. d) Collection of all-points current histograms from 200 ssDNA-streptavidin docking events. Red dashed lines are guides to the eye.

Figure 3. Theoretical current blockades produced by streptavidin.

a) Current blockade fluctuation caused by the stochastic rotation of the streptavidin in the access region. The ionic current was measured under a bias of 150 mV while the streptavidin molecule was randomly rotated about the x, y and z axes, with its centre of mass (red dot in inset) fixed. b) Histogram of the current shown in (a).

Figure 4. Voltage dependence of docking dynamics.

a) Representative current traces produced by docking of three different streptavidin-poly(dC)₉₀ complexes (one in each row) under the bias voltage of 100 mV, 75 mV, and 50 mV. Right panel shows the corresponding all-points histograms. Red: 100 mV, green: 75 mV, and blue: 50 mV. b) Representative current traces produced by docking of three different streptavidin-poly(dC)₉₀ complexes (one in each row) under the bias voltage of 100 mV, 150 mV, and 200 mV. Right panel shows the corresponding all-points histograms. Red: 100 mV, green: 150 mV, and blue: 200 mV. c) Same as in panel (b) but for three different streptavidin-dsDNA complexes (one in each row). Right panel shows the corresponding all-points histograms. Red: 100 mV, green: 150 mV, and blue: 200 mV. Red dash lines are guides to the eye.

Figure 5. Effect of tether conformation on docking dynamics.

a,b) Typical current traces and corresponding all-points histograms produced by docking of a 22nt c-rich ssDNA-streptavidin complex at pH 8 (a) and pH 5 (b). Images to the right illustrate the proposed pH-dependent conformational transitions of the ssDNA tether. c) All-points histograms of the current traces obtained from docking of the c-rich ssDNA-streptavidin complexes at pH 8 and 5 along with the corresponding all-points histogram of all 100 traces at pH 8 (orange) and 100 traces at pH 5 (blue). Red dash lines are guides to the eye.