Experimental study of excessively-long translocation time of single DNA through sub-5 nanometer solid-state nanopores

Xin Zhu*, Xiaojie Li, Chaoming Gu, Yang Liu

School of Microelectronics, Zhejiang University, Hangzhou, China

*Corresponding author: eexzhu@zju.edu.cn

Abstract. Excessively-long translocation events of single DNAs are experimentally observed using a small nanopore. Solid-state Nano pores on SiNx membranes with pore diameters less than 5nm are fabricated via Transmission Electron Microscopy. The translocation testing system is set up based on patch-clamp and Lab-on-Chip, and translocation experiments of Lambda DNAs are conducted. Stable current traces and single molecular translocation events are achieved. Statistical analysis under various cross-membrane voltages shows typical characteristics of SiNx Nano pores, including event rates, threshold voltages, and noise power-spectrum-density. Particularly, excessively-long dwell time (>100ms) events through <5 nm nanopore are observed and attributed to the interaction between DNAs and pore walls. This characteristic is compared against the basic current-blockage model as well as that of a 10 nm nanopore control experiment, demonstrating additional blockage effects.

1. Introduction

The solid-state nanopore is regarded as the most promising technique to fulfill low cost and high throughput DNA sequencing [1]. Solid-state nanopore usually is fabricated via membrane material including SiNx [2], MoS2 [3], Graphene [4], etc. The fundamental physics of nanopore-base bio-sensor is utilizing the electric field to drag charged bio-molecule through the nanopore (nanopore device immersed in the salt solution). When a bio-molecule translocates through the nanopore, ion current of nanopore will be blocked, producing a current blockage signal. The physical dimension information of analyte is then transformed into the electric signal for further analysis [1-4]. For such a current blockage signal, two basic physical parameters are crucial: dwell time and blockage level. Dwell time represents the time interval of bio-molecule threading through the nanopore, which is related to solution concentration, nanopore dimension, ion types. Dwell time varies because the mobility of bio-molecule will change when binding to certain sort of ions or interacting with nanopore wall. However, the dwell time of nanopore-device is usually around 10us to 10ms [5]. When applying a voltage across nanopore, as effective resistance of nanopore is much larger than solution resistance, the voltage will mostly drop near nanopore, leading to the large electric field around nanopore. Biomolecule such as DNA holds enormous amounts of charge (2e/0.1nm). Under a significant electric field, the velocity of molecule translocating through the nanopore is quite fast, namely short dwell time [6]. Too short translocation time might demand higher bandwidth of testing circuit, leading to the higher overall cost of the system [1]. The second physical parameter that matters is the current blockage level, which represents the diameter information of analyte. The current blockage is proportional to the square of the analyte dimension [2].
\[
\Delta I = I_0 \times \left( \frac{D_n}{D_a} \right)^2
\]

\[\Delta I\] is the current blockage, \(I_0\) is base current, \(D_a\) is the effective diameter of the analyte, \(D_n\) is the effective diameter of the nanopore. It’s noteworthy that current blockage is a quite complex parameter. Apart from volume-blockage effect aforementioned, interaction of the molecule with nanopore wall or membrane, as well as access resistance modulation caused by molecule will affect blockage level significantly. Length of DNA molecule, the diameter of the nanopore, material properties of the membrane, solution type and concentration will also affect the final blockage level \([1, 5]\). DNA molecules might exhibit complex physical configuration under static electric field circumstances due to the chain structure nature of the molecule (>1kb). Dekker et al \([2, 7]\) showed the possibility of detecting different configurations using a solid-state nanopore. We observed excessively long dwell time utilizing sub-5nm nanopore, shed light on solving the too fast translocation problem of the solid-state nanopore, and discuss underlying physics of the interaction between DNA and small nanopore, and additional blockage current effect in this report.

2. Fabrication of device and testing system

We drilled nanopore on a free-standing SiN\textsubscript{x} membrane (purchased from Norcada) through TEM. The thickness of the membrane is around 10nm, and the final diameter of nanopore can be well controlled by electron beam dose time and strength \([8]\), the size of which is usually around 5-100nm. Nanopore device we use in this report is a 5nm device, as shown in Fig.1 (scale bar=5nm). After fabrication, we packaged the chip on a PCB with the aid of PDMS, and then clamped and sealed the device by silicone O-ring between two custom-made PTFE reservoirs. Buffered salt solution (LiCl, 10mM Tris-EDTA, PH =8) was added to two reservoirs \([9]\). As the O-ring provides Giga-Ohm resistance seal between two reservoirs is nanopore. Two Ag/AgCl reference electrodes were used for ground electrode and patch-clamp electrode respectively. Signal (several nA) was at first amplified by patch-clamp and transferred to an analog-digital converter and finally shown on PC in real-time. The schematic of the whole testing apparatus is shown in Fig.2. After getting a stable ion current, we added 48kbp Lambda DNA (Sigma-Aldrich) into reservoirs. When DNA translocates through the nanopore, we will observe current blockage signals, and each signal represents one translocation event. After recording several minutes of the current trace, a certain amount of translocation events were collected for further analysis. We use Translyser software written by Matlab to recognize and analyze translocation events \([10]\).

**Figure 1.** TEM figure of 5nm nanopore (scale bar: 5nm). **Figure 2.** Schematic of testing system.
3. DNA translocation experiment of the sub-5nm nanopore device

DNA molecules will maintain long-chain configuration within the solution. As DNA holds negative charges, it will move by electrophoresis and electroosmosis under external electric field \(^{[11, 12]}\). DNA molecule has to overcome an entropy barrier when threading through the nanopore. As nanopore is a comparatively small region compared with the whole device, to overcome the entropy barrier, the cross-membrane voltage has to be large enough. DNA concentration is often low enough in conventional translocation experiments, so the possibility of two or more molecules translocating through nanopore simultaneously is quite low, so we regard translocation events as single-molecule translocation events.

We conducted the single DNA molecule translocation experiment using a 5nm device, clear translocation events were obtained, as shown in Fig.3. We used 2M LiCl buffer solution and 5nM, 48kbp double-strand lambda-DNA in the experiment. At first, we recorded the current trace before injecting DNA, as shown in Fig.3a, under 500mV cross-membrane voltage, and no events were observed. Then DNA molecules were added to reservoirs, clear translocation events were observed within the current trace. Constantly translocation events were acquired under 500mV, 600mV, and 700mV regime, as shown in Fig.3b-d.

We analyze the Power Spectrum Density of current trace under various voltages, as shown in Fig.4. We can see 1/f noise rise enormously after elevating voltage. The variation might contribute to the acceleration of cations and anions under higher voltage, and more extensively interaction between DNA molecule and nanopore-wall. Although PSD analysis shows an obvious increase in noise, the signal-noise ratio (SNR) is still good enough for recognizing translocation events. By statistics of events, we can derive translocation rate under various voltages, as shown in Fig.5. The events rate increases proportionally to cross-membrane voltage as expected: higher voltage increases the ability to overcome the entropy barrier \(^{[13]}\). The physical picture of single DNA molecule translocating nanopore can be derived by interrogating the single translocation event. Fig.6 shows translocation events under various trans-membrane voltages: ion current descends at first and get to lowest level soon, and then sustains for a while and then ascend to the baseline gradually. Obvious levels of current, higher-order increase and decrease within a single event can be observed. Signals might differ from each other, and more importantly, deviate from conventional translocation events in configuration and time scale aspects. We will discuss it later in this report.
Figure 3. Translocation events under various voltages.

Figure 4. PSD spectrum under various cross-membrane voltages

Figure 5. Events rate under various cross-membrane voltages
4. Control experiment of large nanopore

To elucidate the size effect of small nanopore on dwell time and folding states of DNA, we conduct a control experiment: we use a device with the same thickness while larger diameter (10nm) for comparison. Buffer solution type and analyte are identical with the small nanopore experiment: 2M LiCl and 48kbp double-strand Lambda DNA. Fig.7a shows the current trace of 10nm device when transmembrane voltage is 500mV, and the current level is much higher than the 5nm device as expected. The threshold voltage of the 10nm device is around 100mV, compared with 500mV of 5nm device. Fig.7b shows translocation events and corresponding configuration, blockage level is from 1 to 10nA. Current blockage level is proportional to the number of DNA chain presents in nanopore simultaneous while threading through[2,7]: if DNA linearly translocates through, then the current blockage level is $\Delta I$, if there is folding at the head of DNA molecule, blockage level will double($2\Delta I$), if knots exist within DNA, blockage level will triple($3\Delta I$). We can see three scenarios aforementioned shown in Fig. 7b. Higher-order events (>6nA) represent more complex configuration while following the same underlying physics. For the 10nm device, 1st order blockage level is around 1.5nA, coincides well with 1.6nA derived from classical blockage theory, so as folding and knots scenario.

5. Interaction of DNA and small nanopore

Interaction of DNA and small nanopore can be summarized as two categories: firstly, DNA molecule interacts with nanopore wall, including adhesion effect; secondly, DNA molecule interacts with nanopore membrane surface [14, 15]. Dwell time can be prolonged utilizing interaction effects: using

![Figure 6. Blockage events under various cross-membrane voltages.](image1)

![Figure 7. Translocation experiment of the 10nm nanopore.](image2)
certain kind of ion \[16\] can decrease the mobility of DNA, special membrane materials such as HfO\(_2\) \[17\] have been found feasible for slowing down translocation process, other methods include functional nanopore \[18\] or shrinking dimension of nanopore \[14, 19, 20\]. The possibility of DNA interaction with nanopore is found to be higher in the small nanopore, leading to lower event rate and complex translocation process. Conventional theory predicts two sorts of events for small nanopore scenario: translocation and collision \[14\]. For translocation events, the small nanopore is similar to the large nanopore. As for collision event, it means DNA molecule does not thread through the nanopore, instead, hover adjacent to nanopore orifice. Collision events usually have shorter dwell time and smaller blockage levels compared with translocation events. The portion of collision events will increase while shrinking nanopore diameter, as entropy barrier ascends. For 1nm-5nm nanopore, the interaction will excessively change even under a slight derivation of nanopore diameter, while for nanopore larger than 8nm, the interaction will significantly decrease \[14\].

We can speculate the probable reason for stable and excessively long dwell time events of 5nm device by comparing performances and experiment circumstances of 5nm and 10nm device. Fig.8 shows blockage current level (averaged by the minimum value of each event) under various cross-membrane voltages. From the conventional blockage current model, we can estimate the blockage current level for the linear and folding state. For the 10nm device, as aforementioned, the experiment coincides well with the model, while for 5nm device, as shown in Fig.8, blockage level is around 5 times of linear translocation blockage level, far more than prediction. The maximum configuration allowed to thread through is the folding state as the diameter of the nanopore is 5nm (DNA chain effective diameter is 2.2nm, 4.4nm for folding state). Compared with the 10nm device, the 5nm device might produce blockage current level much higher than the conventional model, from which we deduce interaction of DNA-nanopore plays the most predominant role in blockage current. We compare 5nm and 10nm devices under the same cross-membrane voltage according to statistics of dwell time, as shown in Fig.9. For the 10nm device, dwell time distributes from 0.1ms to 10ms, while for 5nm, dwell time ranges from 50ms-150ms. Then we can verify the predominant role of DNA-nanopore interaction from the dwell time point of view.

Underlying physics of the interaction between DNA and small nanopore is complex and related to many parameters. Through comparing performances of 5nm and 10nm device, we can also speculate the most probable reason leading to abnormal long dwell time events of 5nm device. At first, it’s noteworthy that 5nm and 10nm devices are both tested under 2M LiCl. LiCl has been found that can slow down DNA motion \[16\], while dwell time is usually less than 10ms, which meets well with our 10nm device results (see Fig.9), so we can conclude that Li\(^+\) is not the predominant reason. Compared with similar work \[14, 19, 20\], the distribution of our device is much more narrow (all the events are around 50ms-150ms, nearly no events under 10ms), and long dwell time events constantly appear for a long period time (>5 min), while event rate is much low compared with literature. We can speculate that, compared with small nanopore work in former literature, the interaction between nanopore and DNA is more significant, and probably related to our relatively thin SiN membrane (~10nm, compare with 25~30nm in literature \[14\]) and longer DNA (48kbp, compared with 0.4-20kbp in literature \[14\]). Relatively thin membrane corresponds to the higher electric filed, and lower entropy barrier, so for our device, DNA molecule is more likely to translocate under the same circumstance for large nanopore. As we use longer DNA, folding, knots or higher configuration are more likely to exist, so folding state DNA molecule is more feasible to thread through and interacts with nanopore more profoundly (5nm is close to two times of dsDNA chain diameter). The aforementioned feature of our device finally leads to the fact that nearly all the translocation events have long dwell time and high blockage level, and short dwell time or low blockage level events are quite rare, while the translocation process is quite stable. Our experiment also suggests that, other than the conventional interaction regime, under specific diameter, thickness, and DNA chain length, there are additional effects than expected. From events comparison of Fig.6a and Fig.7, events of 5nm have some unusual feature: events abruptly descend and then slowly ascend. Abruptly descending part might correspond to the temporary stay of DNA molecule near the orifice of nanopore (not translocate through yet), slowly ascending part may represent real translocation
process. Complex rise and dip peak within slowly ascend process might be related to the interaction between DNA and nanopore boundary. Some parts of events of 5nm have similar configurations as 10nm, these parts might represent conventional translocation events, while longer dwell time due to more severe interaction.

![Figure 8](image1.png) ![Figure 9](image2.png)

**Figure 8.** Experiment and model blockage current of 5nm nanopore. **Figure 9.** Statistics of dwell time of 5 and 10nm nanopore.

6. Conclusion

We conduct single-molecule translocation experiments using sub-5nm nanopore and observe excessively long dwell time (>100ms). By statistical analysis and control experiments of large nanopore (10nm), we find the interaction of DNA molecule and nanopore is the predominant reason for excessively long dwell time. The prolonged dwell time of the translocation process through solid-state nanopore has great meaning for next-generation real-time and high throughput DNA sequencing. The cost of needed back-end testing circuits will be decreased to a great extent. Blockage current caused by DNA-nanopore interaction might exceed twice of volume blockage, causing difficulty for configuration detection. Future optimization including functional nanopore surface [18], more hydrophilic material [17], or integration gate control device [21] might make small nanopore devices feasible for detecting complex physical configuration on the ultra-long dwell time scale. Furthermore, the underlying physics of small nanopore-DNA interaction is much more complex than expected, future research with the aid of MD simulation is necessary. The more stable fabrication process is needed for further massive production and application of devices.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (Grant Nos. 61774132, 61574126). The work of transmission electron microscopy was done at the Center of Electron Microscopy of Zhejiang University.

References

[1] D. Branton, W. Deamer, A. Marziali, The potential and challenges of nanopore sequencing, Nature Biotechnology. 26 (2008): 1146–1153.
[2] C. Plesa, D. Verschueren, S. Pud, Direct observation of DNA knots using a solid-state nanopore, Nature Nanotechnology, 153 (2016):1–6.
[3] C.M. Gu, X.J. Li, X. Zhu, Experimental study of protein translocation through MoS2 nanopores, Appl. Phys. Lett. 115 (2019): 1-6.
[4] S. Garaj, W. Hubbard, A. Reina, Graphene as a subnanometre trans-electrode membrane, Nature, 467(2010): 190–193.
[5] R.M. Smeets, U.F. Keyser, D. Krapf, Salt Dependence of Ion Transport and DNA Translocation
hrough Solid-State Nanopores, Nano Lett, 6(2006): 89–95.

[6] C.Plesa, S.W.Kowalczyk, R.Zinsmeester, Fast Translocation of Proteins through Solid State Nanopores, Nano Lett, 13(2014):658–663.

[7] X.Zhu, X.W.Wang, Z.Cao, Nanopores incorporating ITO electrodes for electrical gating of DNA at different folding states, IEEE International Electron Devices Meeting, San Francisco, 2017, pp. 26.1–26.6

[8] J.Li, J.A.Golovchenko, D.Branton, Nanoscale Ion Beam Sculpting, Nature,166 (2001): 1–11.

[9] D.Stein, M.Kruithof, C.Dekker, Surface-charge-governed ion transport in nanofluidic channels, Phys. Rev. Lett, 93(2004): 035901-4

[10] C.Plesa, C.Dekker, Data analysis methods for solid-state nanopores, Nanotechnology, 26(2015): 1–8.

[11] T.M.Squires, S.R.Quake, Microfluidics: Fluid physics at the nanoliter scale, Rev. Mod. Phys, 77 (2005):977–1026.

[12] K.H.Paik, Y.Liu, V.T.Cossa, Control of DNA capture by nanofluidic transistors, ACS Nano, 6(2012):6767–6775.

[13] M.Mihovilovic, N.Hagerty, D.Stein, Statistics of DNA Capture by a Solid-State Nanopore, Phys. Rev. Lett, 110(2013):028102–5.

[14] M.Wanunu, J.Sutin, B.Mcnally, DNA Translocation Governed by Interactions with Solid-State Nanopores, Biophysj, 95(2018):4716–4725.

[15] I.C.Yeh, G.Hummer, Nucleic acid transport through carbon nanotube membranes, PNAS, 101(2004):12177–12182.

[16] S.W.Kowalczyk, D.B.Wells, C.Dekker, Slowing down DNA Translocation through a Nanopore in Lithium Chloride, Nano Lett, 12(2012):1038–1044.

[17] J.Larkin, R.Henley, D.C.Berl, Slow DNA Transport through Nanopores in Hafnium Oxide Membranes, ACS Nano, 7(2013):10121–10128.

[18] Y.R.Kim, J.H.Min, I.H.Lee, Nanopore sensor for fast label-free detection of short double-stranded DNAs, Biosensors and Bioelectronics, 22(2007):2926–2931.

[19] J.Li, M.Gershow, D.Stein, DNA molecules and configurations in a solid-state nanopore microscope, Nat Mater, 2(2003):611–615.

[20] J.B.Heng, C.H.T.Kim, Sizing DNA Using a Nanometer-Diameter Pore, Biophysj, 87(2004):2905–2911.

[21] Y.Liu, L.Yobas, Slowing DNA Translocation in a Nanofluidic Field-Effect Transistor, ACS Nano,10 (2016):3985–3994.