Manipulation of DNA transport through solid-state nanopores by atomic force microscopy

Wei Si, Haojie Yang, Gensheng Wu, Chang Chen and Meng Yu

1 School of Mechanical Engineering, Southeast University, Nanjing 211189, People’s Republic of China
2 Jiangsu Key Laboratory for Design and Manufacture of Micro-Nano Biomedical Instruments, Southeast University, Nanjing 211189, People’s Republic of China
3 School of Mechanical and Electronic Engineering, Nanjing Forestry University, Nanjing 210037, People’s Republic of China

E-mail: wei.si@seu.edu.cn

Keywords: DNA, nanomanipulation, slow down, pulling force, ionic current

Abstract

To realize DNA sequencing by a solid-state nanopore, the translocation speed of DNA through the nanopore should be slowed down to obtain the temporal resolution. In this study, we make a nanopore sensing system integrated with an atomic force microscope to control DNA transport through solid-state nanopores. The speed of DNA bound to the probe tip through the nanopore can be controlled by manipulating AFM probe tip, the ionic current as well as the force exerted on DNA strand while it is translocating through the nanopore could be simultaneously measured by the integrated system. The velocity of DNA molecules could be slowed down to \( \sim 100 \, \text{nm} \, \text{s}^{-1} \), much less than 1 \( \text{nt} \, \text{m} \, \text{s}^{-1} \), which fully meets the requirement for nanopore DNA sequencing. When the probe tip is moving toward or away from the nanopore, obvious current steps associated with force steps are observed during the capturing and releasing processes of DNA strands. All-atom molecular dynamics simulation further validates the observed correlation between the ionic current and dragging force when the DNA is moving through the nanopore.

1. Introduction

Nanopore technology has garnered broad interest as a sensitive, low-cost and high-throughput single molecule sensor. Since Kastanowicz, et al firstly demonstrated the successful translocation of DNA or RNA through an \( \alpha \)-hemolysin (\( \alpha \)-HL) nanopore in 1996 [1], nanopores have been widely used in probing and analyzing single molecules, such as nanoparticles [2], RNAs [3], DNAs [4–6], proteins [7, 8] and protein/DNA complexes [9, 10]. In a typical nanopore sensing system, there is a membrane which separates two chambers filled with electrolyte solution, and a nanoscale hole on the membrane acts as the only connection between two chambers. As a bias voltage is applied across the nanopore, the main potential drop will occur inside the nanopore [11], then the charged analytes will be electrophoretically driven through the nanopore [12] because of the electric force exerted on them. Since the analytes usually have different shape [13], dimension [5, 14, 15], charged state [2] as well as the strong interaction between the molecules and nanopore wall [16–18], the current blockade caused by their transport through the nanopore will vary in the amplitude, dwell time and the shape of the blocked current trace, which has been validated by our recent works [7, 8, 19]. The information contained in the current signals then could be potentially extracted to characterize and differentiate target single molecules. In particular, due to the distinctive properties of nucleotides, nanopore based DNA sequencing could be possibly realized, which offers a fast and cheap gene identification compared to other sequencing technologies. With the development of nanopore technology for about two decades, many researchers have focused on applying the nanopore sensor for sequencing DNA. Up to now, it has been reported that single nucleotide can be successfully characterized and identified by using biological nanopores, such as a phi29 DNA polymerase [20], \( \alpha \)-HL [21] and Mycobacterium smegmatis porin A (MspA) [22]. Besides biological nanopores, recently we also showed that the single nucleotides could be discriminated by a solid-state Si\(_3\)N\(_4\) nanopore once the
Figure 1. Manipulation of DNA transport through a solid-state nanopore by AFM probe tip. (a) The schematic illustration of the experimental setup. The green substrate and grey membrane indicate the silicon and silicon nitride materials. The sinking areas indicate the markers, thinned area and nanopore, respectively. (b) SEM image of the markers, thinned area and nanopore fabricated by FIB, the markers are 15 μm long, 2 μm wide and 30 nm deep. (c) Enlarged SEM image of the 750 nm thinned area with a 25 nm nanopore at the center. (d) A SEM image of the pristine AFM probe tip used in this study. (e) AFM topography of the markers, thinned area and nanopore in a 20 μm scanned area. (f) AFM topography of the 1 μm scanned area including the thinned area and nanopore. (g) A SEM image of the AFM probe tip binding with HS-dsDNA.

nanopore is thin and tiny enough [23]. Other solid-state nanopores, especially nanopores in 2D membrane materials (graphene [24] or MoS2 [25]) also demonstrated the ability to differentiate single bases from each other. Unfortunately, there still remains a challenge that DNA translocates through the nanopores so fast that the accuracy of the sequencing cannot be guaranteed. By now researchers have made great efforts and performed varieties of researches to decrease DNA translocation velocity through nanopores, including decreasing the bias voltage [26], decreasing the nanopore diameter [27], lowering the solution temperature [28], increasing the solution viscosity [29], changing the membrane materials [16] and surface charge densities [4, 17], functionalizing nanopores with a self-assembled monolayer (SAM) [18] that can enhance the interaction between DNA and nanopore surface, applying pressure gradients [30] against the electric force on DNA molecule, replacing the cations of the solution to Li+ [31] or Mg2+ [32] that can bind DNA stronger and reduce the net charge of the DNA strand. However, the methods listed above still can not reach the required speed (in milliseconds per base) for nanopore DNA sequencing. Thus a more straightforward manipulation of DNA transport is desired. Many proof-of-principle experiments, such as incorporation of enzymes to nanopores [22, 33, 34], integrating tuning fork based feedback force sensor and a nanopositioning system [35], using optical tweezers [36] and magnetic tweezers [37, 38], have been proved to slow down DNA transport speed and even reverse its moving direction. An obvious advantage of integrating optical tweezers and magnetic tweezers with nanopore devices is that it monitors both the ionic current and the force exerted on the DNA strand in real time, which provides adequate resolution to identify single DNA bases as a ssDNA strand is pulling through a nanopore. Atomic force microscopy is integrated with a piezo actuator containing a feedback loop, which promises the high-precision movement in the x, y and z directions. Thus it has been widely used to scan the interested surface and get the topography of that surface. Besides, it is also successfully applied to measure the interaction force between two contact or noncontact samples as well as the friction force when displacement happens relatively between two samples. As it has the high-resolution feedback control as well as the force measurement system, it is a good candidate to be used in manipulating DNA strand through a nanopore with a speed low enough for single DNA base identification [39], though the application is less reported by now.

Motivated to control the DNA transport speed, we designed a measuring system which integrates a silicon nitride nanopore with atomic force microscopy (AFM) as shown in figure 1(a). In this paper, we will focus on investigating the feasibility of controlling the DNA transport dynamics through a silicon nitride nanopore with an atomic force microscopy probe tip. The probe tip was firstly bonded with HS-DNA, then we used AFM probe tip that binds a DNA strand to control DNA translocation through silicon nitride nanopores. By detecting the ionic current as well as the force simultaneously while DNA is manipulated to translocate through the nanopore, the successful DNA capture events can be detected. And the DNA translocation speed can be decreased to ~100 nm s⁻¹, indicating that the direct manipulation technology could potentially accelerate the development of third-generation DNA sequencing based on nanopore technology.

2. Experimental method and molecular dynamics simulation details

2.1. Experimental method

Figure 1(a) shows the schematic diagram of our experimental setup. The 120 nm-thick silicon nitride membrane was deposited on silicon wafers (2 mm × 2 mm) by low-pressure chemical-vapor deposition (LPCVD). Electron-beam
lithography followed by reactive ion etching (RIE) was used to open a window (160 μm × 160 μm) in the silicon wafer and expose the low-stress silicon nitride membrane. To make the nanopore as thin as possible, a small region (a circle with diameter of 750 nm) of the freestanding silicon nitride membrane was firstly reduced to ~10 nm by focused ion beam (FIB, FEI Helios 600i). Subsequently, a nanopore was drilled by FIB operated at 1.1 pA and 30 kV. Figure 1(c) shows an example of the SEM image of a 25 nm nanopore fabricated in a 750 nm thinned area. Since the 750 nm-diameter thinned area cannot be clearly seen in the CCD camera integrated in the AFM apparatus, it is difficult for us to quickly locate the nanopore position. To solve this problem and save searching time, two vertical rectangular markers, which are 15 μm long, 2 μm wide and 30 nm deep, were also fabricated around the thinned area by FIB, figure 1(b). Then the AFM probe tip could be conveniently moved to the location of the nanopore with the markers as a reference. In this work, the 3570-bp long double-stranded DNA (dsDNA) was chosen and synthesized by Polymerase Chain Reaction (PCR) amplification with one end modified by thiol group. For PCR amplification, 5′SH(CH3)2-CAGCACAAACCCAAAATGAGC-3′ and 5′-CGTAACCTGTGGGATCACC-3′ were automatically determined as the sense and antisense primers by software Primer Premier 6 for the target of best successful rate of amplification. Two ssDNA strands, unwound by heating the bacteriophage λ-DNA in the solution to 95 degrees, were used as the templates to incorporate with the sense and antisense primers. After PCR amplification for 30 rounds, the modified 3570-bp long dsDNA with a thiol group at one end was successfully synthesized, which has been validated by agarose gel electrophoresis, Supporting Information (SI) figure S1 is available online at stacks.iop.org/MRX/7/095404/mmedia. The synthesized dsDNA was diluted in the TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) to a final concentration of 1 μM for storage. AFM probe tips coated with Gr (5 nm thick) and Au (50 nm thick) thin films, as shown in figure 1(d), were chosen due to their advantage of application in tip functionalization and force measurements. The tips were in turn immersed into fresh Piranha solution, citrate buffer (pH 3.0), HEPES buffer (pH 10.0), ethanol solution and deionized water for 10 min, respectively. It should be noted that, before each immersion, the tips should be rinsed by deionized water at least for 3 times to make sure the tips were clean without any contaminations. Then the AFM probe tips and thiol modified dsDNA solution were incubated for 16 h at room temperature and the tips were found to be covalently bonded with thiol modified dsDNA finally (as shown in figure 1(g)). The deep colour wirelike structures occurred on the probe tip after incubation indicated that the synthesized dsDNAs were indeed bonded to the Au probe tip, further evidence for the silicon nitride membrane was also observed and shown in SI figure S2. Besides, in our experiments, effect of KCl concentration (300 mM and 1 M) on the probability of HS-dsDNA bonded to AFM probe tip was also investigated, SI figure S3. It is found that there is a bit more DNA strands attached to the AFM probe tip in 1 M KCl solution than 300 mM KCl solution which is consistent with the results found by Zhang et al [40]. Here it should be noted the DNA strands prefer to binding to the tip substrate but not the tip. Only a few DNA strands could be successfully attached to the tip area. As only the attached DNAs on the tip area could be beneficial for our manipulation experiment and not all the attached DNA strands could be successfully captured by the nanopore, finally we choose to perform experiments using the 1 M KCl solution after comprehensive consideration of the DNA capture probability and signal-to-noise ratio. Though the DNAs were observed to successfully attached to the probe surface, they were distributed sparsely and less DNAs were found to bond to the very tip of the probe. To make the manipulation of DNA through the nanopore efficiently, it should be better if the DNA can be precisely attached to the very tip of the AFM probe. A straightforward way to control the position where the thiol modified DNA should be attached is to determine the area and location of the gold coated on the probe tip. Thus it is suggested to reduce the coating area on the probe tip and only coat gold on the very tip though it is really challenging and may take a very long time to successfully attach the thiol modified DNA due to the large persistence length of the dsDNA used in our experiments. Prior to using, the clean nanopores were irradiated in an oxygen plasma environment for 30 s to make the nanopores easily filled with electrolyte solution. The nanopores were mounted in a Poly(methylmethacrylate) (PMMA) fluidic cell which is designed to combine the nanopore sensor with the AFM (MFP 3D, Asylum Research, Santa Barbara CA) scanning system. The electrolyte solution contains 1 M KCl and was buffered at pH 8.0 by 10 mM Tris-HCl and 1 mM EDTA. The ionic current was measured at 200 kHz with low-pass filtering at 10 kHz using a resistive feedback amplifier (EPC10, HEKA Elektronik, Rheinland-Pfalz, Germany).

As described in our previous work [41], to quickly locate the position of the nanopore, the AFM probe tip was clamped into the AFM cantilever holder and immersed into the buffer solution, the membrane was first scanned by the functionalized AFM probe tip to locate the position of the nanopore with remarkable marks. All AFM images were formed by a 256 × 256 matrix of pixels with a 90° scanning angle in tapping (AC) mode, other experiments working in contact mode if necessary will be specified. Figure 1(e) shows the topography of a 20 μm × 20 μm area (including the two perpendicular markers, thinned area and nanopore) scanned by the probe tip. Subsequently, a 1 μm × 1 μm area with a nanopore at the center was further scanned by AFM probe tip. Figure 1(f) shows an example of the AFM 3D topography of the 1 μm scanned area, in which, the dimension as well as the location of the nanopore were clearly depicted. For the DNA manipulation experiment, the functionalized probe tip was manipulated to approach the nanopore and retract subsequently with a desired velocity, the threshold force is 20 nN for reversing the direction of the AFM probe tip. By manipulating the AFM
probe tip, DNA transport speed and direction could be well controlled. Simultaneously, the current blockage of DNA residing inside the nanopore as well as the force exerted on the DNA strand were also successfully detected, which could be potentially used to discriminate the DNA nucleotides in the near future.

2.2. Molecular dynamics simulations
All simulations in this work were performed using molecular dynamics program NAMD2 [42] with a 2 fs time step. Periodic boundary conditions were applied along the x, y and z directions, respectively. The silicon nitride membrane (2 nm thick) and DNA molecule (10 bp) were constructed using the method reported previously [43]. The CHARMM36 force field [44] was used to describe the silicon nitride membrane, DNA, TIP3P water and ions. RATTLE [45] and SETTLE [46] algorithms were applied to describe covalent bonds that involved hydrogen atoms in proteins and water molecules. The CUFIX corrections were applied to improve description of charge—charge interactions [47, 48]. Particle-Mesh Ewald (PME) [49] algorithm was used to evaluate the long-range electrostatic interaction on a 1 Å-spaced grid; the full electrostatic calculation was performed every three timesteps. van der Waals interactions were evaluated using a smooth 10 Å cutoff. The system was solvated using VMD’s Solvate plugin. Water molecules overlapping with proteins were removed. Potassium and chloride ions were added to neutralize the system and bring the ion concentration to 1 M (consistent with that used in our experiments). The final system contained approximately 98,000 atoms. Each system was minimized for 1200 steps using the conjugate gradient method, then equilibrated for 12 ns in the constant number of atoms, pressure and temperature (NPT) ensemble. During the equilibration, both the nanopore and DNA were restrained to their initial position with spring constant of 20 kcal/(mol·Å²) and 2 kcal/(mol·Å²), respectively. For production simulation under an external electric field, the center of mass (CoM) of the two nucleotides close to the nanopore entrance was tethered to a harmonic spring of 10.0 kcal/(mol·Å²) spring constant. The other end of the spring was moved along the z-axis with a speed of 0.6 nm ns⁻¹. A 1000 mV bias voltage was applied across the nanopore, inducing an electric force which is opposite to the pulling direction. The Nose-Hoover Langevin piston pressure control [50, 51] was used to maintain the pressure of the system at 1 atm by adjusting the system’s dimension. After that, the constant velocity steered molecular dynamics simulation was further performed for 18 ns in the constant number of atoms, volume and temperature (NVT) ensemble with the volume determined by the dimension averaged using the last 6 ns equilibration trajectory. Langevin thermostat was applied to all the heavy atoms of the system with a damping coefficient of 0.1 ps⁻¹. The electric field was applied along z direction to induce the movement of ions. All the trajectories were viewed and analyzed by using VMD [52] and Tcl scripts.

3. Results and discussion
Once the nanopore location was determined, the AFM probe tip was transferred above the nanopore. The working mode of the probe tip was quickly switched to the contact mode and the tip was gradually moved toward the nanopore with a preset velocity (100 nm s⁻¹). When the force between the tip and the nanopore reached the threshold value (20 nN), the tip was moved back to its initial position. During the whole process, both the ionic currents and the pulling forces were monitored. Figure 2(a) shows proof-of-principle examples of the DNA capturing and releasing process controlled by the AFM probe tip. At the beginning, the AFM probe tip was moved slowly toward the nanopore, but the DNA was still outside the capture area and the current was not influenced by the DNA strand, see region A in figure 2(b). As DNA was captured by the nanopore, an obvious drop was observed in both the current and force trace, see region B in figure 2(b). When the probe tip continued moving down while the tip was still outside the capture area of the nanopore, both the ionic current and force kept almost constant, see region C in figure 2(b). As the probe tip was getting closer to the nanopore, it would affect the nanopore access resistance [41] and cause a deeper current blockade and force drop. While the probe tip was very close to interact with the nanopore, the force will turn from attractive to repulsive force, characterized as a force jumping in the force measurement, see region D in figure 2(b). Similar results but for the inverse DNA translocation process were also observed as the probe tip was moving away from the nanopore, see regions E to H in figure 2(b). However, when DNA was not attached to the very tip of the probe tip, sometimes the tip would firstly enter the capture area of the nanopore and the attached DNA strand was then captured during the probe tip manipulation. In this condition, the current will mainly affected by the probe tip itself at first, which caused a gradually decreased current. This result was indeed consistent with what we have already found in our previous work [41]. After the tip was close enough to the nanopore, the attached DNA was then captured (during the tip moving toward the nanopore) by the nanopore and then released (during the tip moving away from the nanopore) from the nanopore, which induced corresponding current variations. Undeniably, the probe tip would more or less affected the ionic current, which would introduce inaccuracies. Since the current change is smooth when it is affected by the
Moving the probe tip no matter the probe tip is moving toward or away from the nanopore, while the current variation when a DNA strand is captured is much more obvious as the DNA capturing process happens very fast (figure 2(c)), it would not affect the characterization of the current induced by the probe tip or the captured DNA strand. In figure 2(c), the current trace was indeed affected by both probe tip moving and DNA capturing, for example, the current steps were not that horizontal when DNA strands were captured and the current trace in each step has a downward (during the tip moving toward the nanopore) and upward (during the tip moving away from the nanopore) trend, which is caused by the influence of the moving probe tip with a constant velocity. Though the probe tip would affect the ionic current, obvious current steps could be observed due to the residence of the captured DNA.

The proof-of-principle current and force signals demonstrated in figure 2(b) are obtained by assuming (1) there is only one DNA strand captured by the nanopore and (2) no interaction would happen between the DNA and the nanopore. However, a lot of factors will affect the controlled DNA translocation process and make the signals obtained from experiments more complicated. For example, in figure 2(c), the ionic current trace as well as the force trace versus time were plotted when the DNAs were controlled to translocate through the nanopore. Interestingly, during the approaching process of the probe tip to the nanopore, three current steps were observed. These results frequently occurred in many of our experiments. It is reasonable and understandable as there will be usually more than one DNA strand attached to the probe tip, which have been validated by the SEM characterization shown in figure 1(g). Besides, the nanopore is tens of nanometers in diameter which is large enough to accommodate multiple strands of DNA. Thus, we speculate that the first step of current drop was caused by one DNA captured by the nanopore while the second step of current drop was caused by another DNA captured by the nanopore (the previous captured DNA was still residing inside the nanopore). The third step of the current drop was then caused by the third DNA captured by the nanopore.

Figure 2. Ionic current and force signals measured during the entire capturing and releasing processes of DNA through a solid-state nanopore by AFM probe tip. (a) The schematic illustration of DNA translocation process controlled by the functionalized AFM probe tip. The pink arrow indicates the moving direction of the probe tip. The dashed circle represents the capture area of the nanopore. Each independent panel (A-H) qualitatively shows the main configurations of the probe tip bonded with DNA molecule that is moving close to and away from the nanopore. (b) Typical current as well as force traces during DNA capturing process by the nanopore and releasing process from the nanopore. The variations of the current and force signals shown in the intervals between two dashed lines were indeed induced by different status and position (A-H) of the probe tip bonded with HS-DNA which is depicted in panel a. (c) Simultaneous direct measurements of the current and force as DNA molecules were captured by and released from a nanopore. The current was measured in 1 M KCl solution at pH 8.0 with 1000 mV bias voltage applied. Multiple current drops were observed when DNA molecules were captured by the nanopore, and multiple current enhancements were also found during the releasing process of DNA molecules from the nanopore. Similar results but for force signals as the AFM probe tip approached (the red line) and lifted (the blue line) from the nanopore surface were also shown. Enlarged plots of the forces from 18.5 s to 19.4 s and from 20.5 s to 21.5 s were also shown as the inserts for clarity.
captured DNA was pulled out from the nanopore two current increasing steps were found due to the successive releasing of the DNA strands. Interestingly, force signals were found to be highly related to current signals as shown in figure 2(c). Once the first and second DNA strand was captured by and/or released from the nanopore, simultaneous force steps were also found. The more the DNA strands were captured, the more the ionic current would be blocked and the more the pulling force would be required. This result actually further confirmed the current steps were indeed induced by the DNA strands captured by the nanopore.

After we performed the experiments frequently using methods described above, as shown in figures 3(a)–(b) we further analyzed the data by plotting the histograms of current drop amplitude and current recovery amplitude, which were fitted by Gaussian equations. The extracted current drop amplitude from the Gaussian equation was 0.52 nA, which is similar to the current recovery amplitude of 0.57 nA. This result indicates the captured and released DNA strands were the same, further validating the current blockades were indeed caused by the residence of DNAs inside the nanopore. It should be noted that the total count of current drop events was
a little bit smaller than that of current recovery events, the reason of this phenomenon was that some DNAs were captured when the AFM probe tip was just entering the capture area of the nanopore, thus the current drop was not obvious and was neglected. Besides the current blockades, the capture distance and release distance of DNA were also measured and analyzed. The capture distance is defined as the distance between the position of the AFM probe tip where a DNA strand was just captured by the nanopore and the location of the membrane surface, while the release distance is defined as the distance between the location of the membrane surface and the position of the AFM probe tip where all DNA strands were finally released from the nanopore. By analyzing the distributions of capture and release distance of DNA, shown in the figures 3(c)–(d), it is found that many capture distances were much smaller than the release distances, the reason is that when the AFM probe tip was close to the nanopore most of the DNAs attached to the AFM probe tip were in a cluster [40], however when the DNAs were released from the nanopore the DNA was stretched by the electric field force inducing a longer distance measured. Besides, as we can see most of the capture and release distances of DNA were smaller than 200 nm, which is much smaller than the length of 3570 bp dsDNA (∼1000 nm). It is because most of DNAs were bonded to the bevels of the AFM probe tip but not the steeples of the probe tip, see figure 1(g). Besides the current blockades and the interaction distance, we again analyzed the distributions of force drop amplitude and force recovery amplitude, which were also fitted by Gaussian equations shown in the figures 3(e)–(f). The extracted force drop amplitude from the Gaussian equation was 0.34 nN during the capture process of DNA, which is also close to the force recovery amplitude of 0.27 nN due to the similar electric force exerted on the DNA stands.

To further investigate the translocation dynamics of DNA when it was pulled through a solid-state nanopore, steered molecular dynamics simulation was performed and the constant velocity was applied to the two nucleotides close to the nanopore, the system setup is shown in figure 4(a). As the DNA was residing inside the nanopore (see the bottom panel in figure 4(b)), the ionic current was blocked (see the top panel in figure 4(b)). Because the nanopore dimension is comparable to the diameter of the DNA strand, an additional force will be required to facilitate the successful translocation of DNA through the nanopore, see the middle panel in figure 4(b). Then an obvious correlation between the blocked ionic current and the pulling force was observed just as what we have found in our experiments. All the theoretical observations were consistent with our experiments described above.

Figure 4. The steered molecule dynamics simulation of DNA transport through the nanopore. (a) Schematic illustration of the system setup. The nanopore is shown by grey molecular surface representation and is cut by half for revealing the nanopore with diameter of 2 nm. The 10 bp dsDNA is shown by NewCartoon representation colored by the type of nucleotides. The ionic solution is shown as an ice blue transparent surface. And the potassium and chloride ions are shown as orange and green spheres, respectively. (b) The ionic current (above), the pulling force (middle) and the center of mass (below) of DNA versus time during the pulling process of DNA through the nanopore. All the data were sampled at 2.4 ps intervals and block averaged in 0.12 ns blocks.
4. Conclusions

In this study, we successfully fabricated a nanopore-sensing system integrated with an AFM probe tip to control DNA motion. The HS-dsDNA made by the PCR experiment bonded to the AFM probe tip can be easily captured and released from the solid-state nanopore through manipulating the AFM probe tip. The speed of DNA in the solid-state nanopore can be controlled as slow as 100 nm/s, meeting the requirement for the nanopore based DNA sequencing. By using the integrated system, we could simultaneously measure the current and force signals as DNA molecules were translocating through the nanopore. Besides, the experiment can easily detect the same DNA strand for several times to increase the sensing accuracy of single molecules. By applying the all-atom molecule dynamics simulations, we further investigated the translocation dynamics of DNA when it was pulled through the nanopore. It is found that the current would be blocked and the pulling force would have an obvious change when the DNA was pulled through the nanopore, which are consistent with our experimental findings. The integrated nanopore system with AFM and patch clamp has been proved to be a success in measuring the ionic current blockades as well as the pulling forces in this work, though it is still challenging for the nanopore based DNA sequencing due to the dsDNA and thick Si3N4 membrane used in this work. In the future work we will focus on characterizing the single nucleotide of a single-stranded DNA by applying the integrated system to measure the ionic currents as well as the forces using thin membranes, such as graphene and MoS2. We believe the work performed here will facilitate the development of DNA sequencing using solid-state nanopores in the near future.

Acknowledgments

The authors thank the financial support from the National Natural Science Foundation of China (Grant No. 51905097) and the Natural Science Foundation of Jiangsu Province (Grant No. BK20190358). The authors also acknowledge supercomputer time at Beijing Computing Center.

ORCID iDs

Wei Si https://orcid.org/0000-0001-7285-058X

References

[1] Kasianowicz J J, Brandin E, Branton D and Deamer D W 1996 Characterization of individual polynucleotide molecules using a membrane channel Proc. Natl Acad. Sci. USA 93 13770–3
[2] Venta K E, Zanjani M B, Ye X, Danda G, Murray C B, Lukes J R and Drndic M 2014 Gold nanorod translocations and charge measurement through solid-state nanopores Nano Lett. 14 5358–64
[3] Wanunu M, Dadosh T, Ray V, Jin J, McReynolds L and Drndic M 2010 Rapid electronic detection of probe-specific microRNAs using thin nanopore sensors Nat. Nanotechnol. 5 807–14
[4] Si W, Zhang Y, Sha J and Chen Y 2018 Controllable and reversible DNA translocation through a single-layer molybdenum disulfide nanopore Nanoscale 10 19450–8
[5] Si W, Zhang Y, Wu G S, Sha J J, Liu L and Chen Y F 2014 DNA sequencing technology based on nanopore sensors by theoretical calculations and simulations Chin. Sci. Bull. 59 4929–41
[6] Traversi F, Raillon C, Renamuer S M, Liu K, Khlybov S, Tosum M, Krasnozhon D, Kis A and Radenovic A 2013 Detecting the translocation of DNA through a nanopore using graphene nanoribbons Nat. Nanotechnol. 8 939–45
[7] Sha J, Si W, Xu B, Zhang S, Li K, Lin K, Shi H and Chen Y 2018 Identification of spherical and nonspherical proteins by a solid-state nanopore Anal. Chem. 90 13826–31
[8] Si W and Aksimentiev A 2017 Nanopore sensing of protein folding ACS Nano 11 7091–100
[9] Kong J, Bell A N A and Keyser U F 2016 Quantifying nanomolar protein concentrations using designed DNA carriers and solid-state nanopores Nano Lett. 16 3557–62
[10] Kowalczyk S W, Hall A R and Dekker C 2010 Detection of local protein structures along DNA using solid-state nanopores Nano Lett. 10 324–8
[11] Hyun C, Rollings R and Li J L 2012 Probing access resistance of solid-state nanopores with a scanning-probe microscope tip Small 8 385–92
[12] Si W, Sha J, Liu L, Qiu Y and Chen Y 2013 Effect of nanopore size on poly(dT)30 translocation through silicon nitride membrane Sci. China: Technol. Sci. 56 2398–402
[13] Yusko E C et al 2017 Real-time shape approximation and fingerprinting of single proteins using a nanopore Nat. Nanotechnol. 12 360–7
[14] Lee M H, Kumar A, Park K B, Cho S Y, Kim H M, Lim M C, Kim K Y and Kim K B 2014 A low-noise solid-state nanopore platform based on a highly insulating substrate Subr. Rep. 47448
[15] Venta K, Shemer G, Puster M, Rodriguez-Manzo J A, Balan A, Rosenstein J K, Shepard K and Drndic M 2013 Differentiation of short, single-stranded DNA homopolymers in solid-state nanopores ACS Nano 7 4629–36
[16] Banerjee S, Wilson J, Shim J, Shankla M, Corbin E A, Aksimentiev A and Bashir R 2015 Slowing DNA transport using graphene–DNA interactions Adv. Funct. Mater. 25 936–46
[17] Shankla M and Aksimentiev A 2014 Conformational transitions and stop-and-go nanopore transport of single-stranded DNA on charged graphene Nat. Commun. 5 5171
[18] Wang D, Harrer S, Luan B, Stolovitzky G, Peng H and Afzali-Ardekani A 2014 Regulating the transport of DNA through biofriendly nanochannels in a thin solid membrane Sci. Rep. 4 3985

[19] Si W, Sha J, Sun Q, He Z, Wu L, Chen C, Yu S and Chen Y 2020 Shape characterization and discrimination of single nanoparticles using solid-state nanoropes Analyst 145 1657–66

[20] Chen Y-S, Lee C-H, Hung M-Y, Pan H-A, Chioiu J-C and Huang G S 2013 DNA sequencing using electrical conductance measurements of a DNA polymerase Nat. Nanotechnol. 8 452–8

[21] Clarke J, Wu H-C, Jayasinghe L, Patel A, Reid S and Bayley H 2009 Continuous base identification for single-molecule nanopore DNA sequencing Nat. Nanotechnol. 4 265–70

[22] Manrak S A, Dermontin I M, Laslo A H, Langford K W, Hopper M K, Gillgren N, Pavlenok M, Niederwies M and Gundlach J H 2012 Reading DNA at single-nucleotide resolution with a mutant MspA nanopore and phi29 DNA polymerase Nat. Biotechnol. 30 349–53

[23] Yang H, Li Z, Si W, Lin K, Ma J, Li K, Sun L, Sha J and Chen Y 2018 Identification of single nucleotides by a tiny charged solid-state nanopore J. Phys. Chem. B 122 7929–35

[24] Qi H and Guo W L 2012 Detecting ssDNA at single-nucleotide resolution by sub-2-nanometer pore in monoatomic graphene: a molecular dynamics study Appl. Phys. Lett. 100 083106

[25] Peng J, Liu K, Bulusheva R D, Khilyov S, Dumencu D, Kis A and Radenovic A 2015 Identification of single nucleotides in MoS2 nanoropes Nat. Nanotechnol. 10 1070–6

[26] Liu Q, Wu H, Wu L, Xie X, Kong J, Ye X and Liu L 2012 Voltage-driven translocation of DNA through a high throughput conical solid-state nanopore PLoS One 7 e46014

[27] Akahori R, Haga T, Hatano T, Yanagi I, Ohura T, Hamamura H, Iwasaki T, Yoko T and Anazawa T 2014 Slowing single-stranded DNA translocation through a solid-state nanopore by decreasing the nanopore diameter Nanootechnology 25 275701

[28] Meller A, Nivon L, Brandin E, Golovchenko J and Branton D 2000 Rapid nanopore discrimination between single polynucleotides Proc. Natl. Acad. Sci. USA 97 1079–84

[29] Pologea D, Uplinger J, Thomas B, McNabb D S and Li J L 2015 Slowing DNA translocation in a solid-state nanopore Nano Lett. 5 1734–7

[30] Lu B, Hoogerheide D P, Zhao Q, Zhang H, Tang Z, Yu D and Golovchenko J A 2013 Pressure-controlled motion of single polymers through solid-state nanoropes Nano Lett. 13 3048–52

[31] Kowalczyk S W, Wells D B, Aksimentiev A and Dekker C 2012 Slowing down DNA translocation through a nanopore in lithium chloride Nano Lett. 12 1038–44

[32] Zhang Y, Liu I, Shah J, Ni Z, Yi H and Chen Y 2013 Nanopore detection of DNA molecules in magnesium chloride solutions Nanoscale Res. Lett. 8 245

[33] Cherf G M, Lieberman K R, Rashid H, Lam C E, Karplus K and Akeson M 2012 Automated forward and reverse ratcheting of DNA in a nanopore at 3-angstrom precision Nat. Biotechnol. 30 344–8

[34] Olasagasti F, Lieberman K R, Benner S, Cherf G M, Dahl J M, Deamer D W and Akeson M 2010 Replication of individual DNA molecules under electronic control using a protein nanopore Nat. Nanotechnol. 5 798–806

[35] Hyun C, Kaur H, Rollings R, Xiao M and Li J 2013 Threading immobilized DNA molecules through a solid-state nanopore at >100 mus per base rate ACS Nano 7 5892–900

[36] Keyser U F, Koelmann B N, Van Dorp S, Krapf D, Smeets R M M, Lemay S G, Dekker N H and Dekker C 2006 Direct force measurements on DNA in a solid-state nanopore Nat. Phys. 2 473–7

[37] De Vlaminck I, Henighan T, van Lenoent M T, Burnham D R and Dekker C 2012 Magnetic forces and DNA mechanics in multiplexed magnetic tweezers PLoS One 7 e41132

[38] Peng H and Ling X S 2009 Reverse DNA translocation through a solid-state nanopore by magnetic tweezers Nanotechnology 20 185101

[39] Nelson EM, Li H and Timp G 2014 Direct, concurrent measurements of the forces and currents affecting DNA in a nanopore with comparable topography ACS Nano 8 5484–93

[40] Zhang X, Servos M R and Liu J W 2012 Surface science of DNA adsorption onto citrate-capped gold nanoparticles Langmuir 28 3896–902

[41] Si W, Yang H J, Li K, Wu G S, Zhang Y, Kan Y J, Xie X, Sha J J, Liu I and Chen Y F 2017 Investigation on the interaction length and access resistance of a nanopore with an atomic force microscopy Sci. China: Technol. Sci. 60 552–60

[42] Phillips J C, Braun R, Wang W, Gumbart J, Tajkhorshid E, Villa E, Chipot C, Skeel R D, Kale I and Schulten K 2005 Scalable molecular dynamics with NAMD J. Comput. Chem. 26 1781–802

[43] Aksimentiev A 2010 Deciphering ionic current signatures of DNA transport through a nanopore Nano Lett. 10 3568–72

[44] Vanommeslaeghe K, Hatcher E, Acharya C, Kundu S, Zhong S, Shim J, Darian E, Guvench O, Lopes P, Vorobyov I and Mackerell AD 2010 CHARMM general force field: a force field for drug-like molecules compatible with the CHARMM all-atom additive biological force fields J. Comput. Chem. 31 671–90

[45] Andersen HC 1983 Rattle - a velocity version of the shake algorithm for molecular-dynamics calculations J. Comput. Phys. 52 24–34

[46] Miyamoto S and Kollman P A 1992 Settle — an analytical version of the shake and rattle algorithm for rigid water models J. Comput. Chem. 13 932–62

[47] Yoo J and Aksimentiev A 2012 Improved parametrization of Li+, Na+, K+ and Mg2+ ions for all-atom molecular dynamics simulations of nucleic acid systems J. Phys. Chem. Lett. 3 45–50

[48] Yoo J and Aksimentiev A 2016 Improved parametrization of Amine–Carboxylate and Amine–Phosphate interactions for molecular dynamics simulations using the CHARMM and AMBER force fields J. Chem. Theory Comput. 12 430–43

[49] Darden T, York D and Pedersen L 1993 Particle mesh ewald: an N-log(N) method for Ewald sums in large systems J. Chem. Phys. 98 10089

[50] Keller S, Zhang Y H, Pastor R W and Brooks B R 1995 Constant-pressure molecular-dynamics simulation – the langevin piston method J. Chem. Phys. 103 4613–21

[51] Martyna G J, Tobias D J and Klein M L 1994 Constant pressure molecular dynamics algorithms J. Chem. Phys. 101 4177–89

[52] Humphrey W, Dalke A and Schulten K 1996 VMD: visual molecular dynamics J. Mol. Graphics 14 33–8