The temporal resolution and single-molecule manipulation of a solid-state nanopore by pressure and voltage

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Abstract
The translocation of DNA molecules through nanopores has attracted wide interest for single-molecule detection. However, the multiple roles of electric fields fundamentally constrain the deceleration and motion control of DNA translocation. In this paper, we show how a single anchored DNA molecule can be manipulated for repeated capture using a transmembrane pressure gradient. Continuously and slowly changing the magnitude of the pressure provided two opposite directions for the force field inside a nanopore, and we observed an anchored DNA molecule entering the nanopore throughout the process from tentative to total entry. The use of both voltage and pressure across a nanopore provides an alternative method to capture, detect and manipulate a DNA molecule at the single-molecule level.

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Keywords: solid-state nanopore, single-molecule manipulation, DNA, pressure, time resolution

(Some figures may appear in colour only in the online journal)

1. Introduction
The ability to use single nanopores to detect individual molecules emerged and developed over the last two decades [1]. Utilizing the ionic current blockage caused by biomolecule translocation through nanopores, we can obtain information about the structure and dynamics of biomolecules [2]; this method has advantages such as high resolution, low cost, minimal sample damage, sequential direct reading, and excellent environmental compatibility and does not always require sample pretreatment. Nanopores are widely used in the detection of proteins [3–7], DNA-protein complexes [8, 9], RNA [10], polymers [11, 12], metal ions [13] etc. The development of a new generation of gene sequencing methods based on solid-state nanopores, a mature single-molecule detection technology, generated significant interest [14–16].

However, in the field of ultraprecise measurements, this technology still has many limitations, such as its time resolution, spatial resolution, signal-to-noise ratio, and high-throughput [17–19]. The temporal resolution is a particular concern, and some studies focus on decreasing the translocation speed of DNA [20–23]. In fact, the conformational fluctuations and Brownian motion of DNA molecules in the nanopore reduce the accuracy of the sequencing polynucleotides [24, 25]. Recently, several strategies have been investigated to limit and manipulate DNA movement by the use of a strongly confining nanopore [26, 27] or surface charge manipulation at different locations in the nanopore. However, we know little about the DNA dynamics before or after the translocation through nanopores in the voltage-driven method [28–31]. Inspired from the biological nanopores [32–34], the
translocation speed of targeted proteins can be slowed by coating nanopores with a fluid lipid bilayer. And the lipid coatings can also prevent pores from clogging [35]. Although optical and magnetic tweezers [36, 37] constrain nearly half of the degrees of freedom of DNA motion by fixing one end, the experimental setup is complex and difficult to implement.

In this paper, we aim to reduce the DNA translocation speed and to control the DNA motion to improve the temporal resolution of solid-state nanopores. Reversing the direction of the pressure and the voltage bias compared to those in our previous study [38] decreased the DNA translocation speed 125%–200%, and short double-stranded (ds) DNA (∼1.2 kb) was detected with an effectively extended signal bandwidth. However, further retarding the DNA translocation led to a capture rate problem. By repeatedly interrogating the same anchored DNA molecule via the voltage-driven counter-pressure (V–P) method and the pressure-driven counter-voltage (P–V) method, we can greatly improve the accuracy of measurements and select a certain geometric conformation of DNA in the nanopore, which is also a subject worthy of study in the field of biophysics [26, 39–41]. Furthermore, we observed the progressive capture dynamics of an anchored DNA molecule entering a nanopore, which could not be studied previously because the location of the molecule was unknown until it translocated through the nanopore. Combined with the effective driving force profile simulated by the proposed cross-section model [38], the results provided a comprehensive understanding of the conformation and dynamics of DNA translocation through nanopores. Finally, the proposed ‘molecular ping-pong’ experiment of a single DNA molecule based on the V–P and P–V methods not only can reduce the error rate of gene detection through multiple repeated measurements but also can be used for the detection of all charged molecules and particles.

A common nanopore flow cell device was designed as a chip with a nanopore placed between two flow cell reservoirs as shown in figure 1(a). The two chambers refer to cis and trans chambers, which are applied negative and positive voltage bias, respectively. The nanopore wetting was carried out underwater with a process developed by our group; this process exposed the membrane directly to the aqueous solution so that static charges could not easily accumulate, improving the success rate of the wetting process. All electrical measurements were carried out inside a dark Faraday cage with external circuitry coupled to the electrolyte reservoirs with Ag/AgCl electrodes. The ionic current data were obtained by an Axopatch 200B patch-clamp amplifier (Molecular Devices, Sunnyvale, CA), filtered by an 8-pole 40 kHz low-pass Bessel filter and digitized at 250 kilosamples s⁻¹. An adjustable counter pressure was introduced by connecting one side of the airtight flow cell to a nitrogen tank and leaving the other side open to the atmosphere. The pressure values (six significant digits) were measured by a digital pressure meter with a nominal precision of 0.5% (approximately 0.01 atm). The digitized ionic current signals were processed using custom MATLAB code (The MathWorks, Natick, MA) that fits each event to a series of sharp current steps modified by the transfer function of the experimental low-pass filter.

The constant conductance of the current–voltage measurements and the stability of the baseline current were checked to ensure the accessibility of the nanopore. The 1/f noise of the power spectral density curve was also characterized to ensure the cleanliness of the nanopore. After checking for high stability and low noise, we added the DNA samples to the cis chamber, then the voltage bias was applied to pass DNA molecules through the nanopore to the trans chamber. An abrupt and total change in the ionic current implied the absorption of a single DNA molecule. Then, the cells (5 μL volume) were flushed thoroughly by 25 μL of a 1.6 M KCl (pH 9), 10 mM Tris and 1 mM EDTA buffer. The power spectral density curve was checked again to exclude the situation of a nanopore blocked by contaminants.

Axisymmetric and cross-section models were introduced to simulate a solid nanopore. The Poisson–Boltzmann equations combined with the Navier–Stokes equations were used in the axisymmetric model to simulate force profiles applied to the DNA inside and outside the nanopore. The distribution of the effective driving force in the cross-sectional area of the nanopore was simulated in the cross-section models as shown in figure 1(d).

2. Experimental details

First, a 2 μm thermal silica layer was thermally oxidized on both sides of a silicon substrate (〈100〉 surface, P-type doping resistivity of 120 Ω cm, 500 μm thickness, 4 inch diameter) as a buffer layer, which provided mechanical strength and low capacitance with the use of thin nitride films (<20 nm). Low-pressure chemical vapor deposition was used to grow 300–500 nm low-stress amorphous silicon nitride on both sides of the buffer layer. Next, a nanopore chip with a 2 μm free-standing ‘minimembrane’ of silicon nitride was fabricated using a series of micro-nano processing methods, including photolithography, KOH etching of silicon, dicing, phosphoric acid thinning of silicon nitride, and FIB etching (FEI Strata DB 235) for groove preparation, hydrofluoric acid etching of silicon oxide, and transmission electron microscope (TEM) (Tecnai F30)-guided nanopore drilling. Figures 1(b) and (c) shows an optical microscope image of a 20 μm × 20 μm open window in the SiO₂ membrane (figure 1(b)) and a TEM image of a 10 nm diameter nanopore (figure 1(c)), respectively.

3. Results and discussion

Figure 2 shows the density histogram for 10 kbp dsDNA translocation events driven by a 100 mV bias voltage and a series of counter pressures (0.5, 1.0, and 1.5 atm). We carried out the experiments in 1 M KCl solution maintained at pH 9.0 by a 10 mM Tris and 1 mM EDTA buffer and determined the average translocation time of the unfolding events at different counter pressures. T/T₀ data points were collected and marked as red circles in figure 2(e), where T and T₀ stand for...
Figure 1. (a) Schematic figure of the experimental setup for the $V$–$P$ method of retarding DNA translocation. (b) Optical image of a $20 \mu m \times 20 \mu m$ open window in the SiO$_2$ membrane. (c) TEM image of 9 nm diameter solid-state nanopore. (d) The distribution of the effective driving force along the cross section of the nanopore.

Figure 2. Voltage-driven DNA translocation with counter pressure. (a)–(c) The density histogram of translocation events for 10 kbp dsDNA driven by a 100 mV bias voltage and $-0.5$, $-1.0$, and $-1.5$ atm external counter pressures, respectively. (d) The current trace from the $V$–$P$ retarding experiment corresponds to $-0.5$ (navy), $-1.0$ (green), and $-1.5$ atm (red) counter pressures. (e) The fit curves for the dependence of the event duration on the counter pressure based on the assumption that 1 atm corresponds to a 30 mV voltage. Red circles are experimental data points, which are 1.08, 1.19 and 1.36 for $-0.5$ atm, $-1.0$ atm and $-1.5$ atm counter pressure, respectively.
the average event duration under a certain counter pressure and under no pressure, respectively. The event duration increased as a function of counter pressure for counter pressures less than 1.8 atm. The statistical distribution of translocation events is presented as the crescent-shaped structures in the two-dimensional current blockage-event duration histogram in figures 2(a)–(c), which show that all the DNA folding and unfolding event durations were prolonged when we applied counter pressure. The average event duration of unfolding events increased from 305 to 385 $\mu$s at the same current blockage, indicating that a high signal-to-noise ratio was maintained. We assume that the relation between $T$ and the counter pressure force can be simplified as

$$\frac{T}{T_0} = \frac{F_0}{F_0 - F_p}. \quad (1)$$

$F_0$ is the voltage driving force, which is 100 mV induced electrostatic force in our experiment. $F_p$ is the applied counter pressure force. Based on the experimental condition of 1 M KCl, pH = 9 and a 9 nm diameter nanopore, we fit our
experimental data points to equation (1) in the blue solid curve region. The capture rate of the DNA translocation events drastically decreased as the pressure magnitude increased, as shown in the typical current trace in figure 2(d). We could barely detect any translocation events at pressures greater than 1.8 atm. When we applied 2–3 atm counter pressures, the translocation times were expected to be 5–10 times longer, but this increase could not be observed because the capture rate had decreased to zero.

To make the counter pressure force more effective, we performed this experiment at a higher salt concentration, 1.6 M KCl, to decrease the effective charge density of DNA. The histogram of the translocation events for 3.3 and 1 kbp dsDNA is shown in figure S1, available online at stacks.iop.org/NANO/29/495501/mmedia. At a 200 mV driving bias voltage, we applied a 2 atm counter pressure, and barely ~100 translocation events were detected for 1 kbp DNA, even with a very high DNA molar concentration (nM), in 2 h of recording. The prolonged events could not be more than 2 times longer than the original events (T/T₀ < 2), which should be the upper limit for prolonging the event duration by increasing the counter pressure.

The explanation for the capture rate decreasing to nearly zero while the event duration increased to only 200% instead of an infinite time can be found in the capture radius model [42]. As the distance from the DNA to the nanopore (d) is much larger than the radius of the nanopore (r), the electric field around the nanopore is E(r) = Vd²/8r², making the drift velocity \( ν_{\text{drift}} = μVd²/8r² \), the diffusion velocity of DNA located away from the nanopore is \( ν_{\text{diffusion}} = D/r \). We define the region where the drift velocity is greater than the diffusion velocity as the capture region, and the capture radius can be presented as \( R_{\text{capture}} = \Delta Nμd²/8LD \), where \( L \) represents the length of the nanopore, \( D \) represents the DNA diffusion coefficient, \( ΔV \) represents the voltage applied to the electrodes, and \( μ \) represents the DNA electrophoretic mobility. The counter pressure flow field is constrained by the no-slip boundary conditions near the nanopore surface, which reach the minimum value at one Debye length (0.3 nm for 1 M salt concentration [43, 44]) away from the nanopore walls. The boundary conditions do not restrict the electric field. The combination of the two forces is shown as figure 1(d). Therefore, when the pressure-induced force increased to balance the voltage driving force within the capture radius region, DNA would not be captured from the cis side because of the overwhelming pressure outside the capture radius region opposite to the direction of the DNA translocation.

Although DNA molecules can be captured and slowed by the V–P method, the mechanism underlying the transport of the molecules to the nanopore and the conformation of the molecules remain unknown because once a DNA molecule moves outside the pore, no information can be obtained about its position and conformation. Therefore, we randomly adhered a single 3 kbp dsDNA molecule to the cis side of a SiN membrane, as described in the experimental method section. A 1.6 M salt concentration was selected because the rate of DNA adsorption on the membrane surface increases with the salt concentration. The schematic of the experimental process is shown in figure 3(a). The baseline current decreased when the unfolded DNA blocked the current, indicating that the anchored DNA entered the nanopore. Then, the baseline current remained steady and did not change again until a certain large counter pressure (~2.5 atm) was applied (figure 3(c)). The periodic fluctuation of the baseline current cannot be attributed to a contaminated nanopore, as shown by the comparison of the power spectrum in figure S4. When the pressure was greater than 2.52 atm, the current baseline jumped to the original normal level, indicating that the DNA was pushed out. With the slow release of the counter pressure, the DNA remained outside the nanopore for some time but was drawn into the nanopore again as the pressure gradually dropped below 2.2 atm. We repeated this operation ten times every fifteen seconds by increasing the counter pressure to ~2.5 atm to push the DNA out of the pore and then decreasing the pressure to ~2.2 atm to trap the DNA in the nanopore. The manipulation values of counter pressure...
are labeled in red (entry) and blue (exit) along the baseline current. And they were consistent. To further prove the above reasoning, we selected a series of driving bias voltages from 100 to 80 mV. As shown in figure 4(a), figure S2 and table S1, the same single DNA V–P manipulation experiment at 5 mV voltage corresponds to 0.1–0.2 atm pressure.

The mechanism behind this experiment is as follows. The electrical driving force could overcome the pressure flow force inside and outside the nanopore for pressures smaller than 2.2 atm, so the DNA molecule would be pushed into the nanopore from cis side. When the counter pressure became greater than 2.55 atm, the drag force induced by this pressure could overcome the electrical driving force inside and outside the nanopore, which repulsed the anchored DNA, moving it outside the nanopore. Nevertheless, for the pressures in the ‘buffer’ region between 2.2 and 2.55 atm, the pressure-induced drag force could overcome the electrical driving force outside the nanopore, so the anchored DNA outside the nanopore could not be drawn into the pore at ‘buffer’ pressures. However, inside the nanopore, the situation resembled a stalemate. Due to the parabolic force profile caused by the constraints of the no-slip boundary conditions, the pressure-induced drag force dominated only slightly in the center region, but the electrical driving force dominated most of the

Figure 5. (a) The entry process of the single anchored DNA molecule as the driving pressure increases from 1.3 to 1.9 atm. (b) Representative events corresponding to each driving pressure.
off-center region, so a trapped DNA molecule could be in a stalemate situation inside the nanopore, resulting in an ultralong time in the trapped state.

To verify the rationality of the above reasoning, we reversed the direction of the counter pressure and bias voltage to conduct the $P$–$V$ manipulation experiment, as shown in figure 3(b). By tuning the values of the driving pressure, we also manipulated the anchored DNA. When the driving pressure flow force was larger than 1.85 atm, the anchored DNA was pushed into the nanopore, and the baseline current underwent a sudden increase with the current blockage of an unfolded dsDNA molecule. When the pressure driving force was decreased to values smaller than 1.70 atm, the anchored DNA was pushed back out, and the baseline current decreased to the original level until the counter pressure was restored to 1.85 atm or larger. By linearly changing the values of the counter voltage (bias), as shown in figure 4(b), figure S3 and table S1, we also proved that the driving force of 5 mV was equivalent to 0.1 atm.

There were further deeper or higher current blockages of the baseline current for both the $V$–$P$ and $P$–$V$ manipulation methods, which could be explained by the different directions of the effective driving force verified by the cross-section model [43]. Translocation of extra DNA molecules could be excluded because the flow cells were thoroughly flushed, as the experimental section shows. One notable difference between figures 3(c) and (d) is the behavior of DNA outside the nanopore. With the $V$–$P$ manipulation method, there were no deflections along the flat baseline current. However, with the $P$–$V$ manipulation method, many deflections appeared on the baseline current. This finding can also be explained by the dominance of the pressure-induced force outside the nanopore in both manipulation methods.

To further elucidate the process of a single DNA molecule entering the nanopore when the pressure is gradually increased, we continuously and slowly changed the pressure to make the DNA gradually enter the nanopore, as shown in figure 5(a). Representative translocation events for each driving pressure from 1.3 to 1.9 atm are shown in figure 5(b). At first, no pressure was applied, and no ionic current blockage appeared on the flat baseline current. As the pressure gradually increased to 1.3 atm, some shallow and short events began to appear on the baseline current, with current blockages and event durations of approximately 50 pA and 50 $\mu$s, respectively. These events should be the ‘tentative’ DNA entries into the nanopore driven by the overwhelming pressure outside the nanopore, but inside the nanopore, the effect of voltage was almost dominant.

With further increases in the pressure to 1.6 atm, many deflections appeared on the baseline current with current blockages of 90–110 pA, which corresponds to unfolded dsDNA translocation events, and the duration of these events was approximately tens of milliseconds. The DNA had not completely entered the nanopore when the driving pressure was less than 1.7 atm. Upon further increases in the pressure to 1.8 atm, the baseline current eventually underwent a sharp deflection, with the height corresponding to the height for a single unfolded DNA current blockage. Additional upward deflections on the baseline current were observed, which we attribute to the DNA in the pore moving to the edge of the nanopore and folding. The results at higher pressures confirmed this reasoning. For the 1.9 atm driving pressure, the further upward deflections due to folding of the DNA molecule began to disappear as the region where the internal bias voltage dominated was further reduced. At a 100 mV driving voltage, the SiN nanopore has a 55 mC m$^{-2}$ surface charge density [43].

We simulated the effective driving force distribution on the whole cross section of the nanopore, as shown in figure S5, and the results could quantitatively explain the above experimental phenomena. The parameters were set at a 100 mV bias voltage, a 55 mC m$^{-2}$ surface charge density for the SiN membrane and a changeable pressure. For the 1.3 and 1.6 atm driving pressures, the total force at the center position ($x = 0$) of the nanopore is very weak (<1 pN), resulting in the anchored DNA swaying near the nanopore. For the 1.8 and 1.9 atm driving pressures, the direct proportional relationship between the pressure-dominated region area and the driving pressure completely anchored and trapped the DNA in the nanopore, and the increase in the driving pressure made it less likely for the unattached end of the DNA to fold.

4. Conclusion

Novel experiments based on the $V$–$P$ and $P$–$V$ methods were designed to manipulate the same anchored DNA molecule at the single-molecule level, and the dynamics and conformation of the DNA near the pore were investigated by repeatedly interrogating the same DNA molecule. The translocation speed of 3 kbps dsDNA was decreased 125%–200% using the $V$–$P$ method, and a short dsDNA (~1.2 kb) signal could be detected with the effectively extended signal bandwidth, although the capture ratio was very low. The entry process of the anchored DNA could be revealed by gradually tuning the driving pressure. We have shown how the effective driving force on the cross section of the nanopore can account for the experimental results. Passing the same DNA molecule through the nanopore multiple times by the $V$–$P$ and $P$–$V$ techniques will not only improve the accuracy of single-molecule measurements but also provide a new single-molecule manipulation approach worthy of study in the field of biophysics.

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