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Increased Dwell Time and Occurrence of dsDNA Translocation Events Through Solid State Nanopores by LiCl Concentration Gradients

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Abstract:

Solid-state nanopore based biosensors are cost effective, high-throughput engines for single molecule detection of biomolecules with the added benefit of size modification. Progress in the translation of the science into a viable diagnostic tool is impeded by inadequate sensitivity of data acquisition systems in detection of fast DNA translocations through the pore. To combat this, slowing the transport of DNA through the nanopore by use of various media or by altering experimental parameters is common. Applying a concentration gradient of KCl in the experimental ionic solution has been shown to effectively prolong dwell times as well as increase the capture rate of DNA by the nanopore.

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Our previous work has corroborated the ability of LiCl ionic solution to slow down the transport of dsDNA through the nanopore by up to 10-fold through cation-DNA interactions. However, this drastically reduced the event occurrence frequency, thus hindering the efficacy of this system as a reliable biosensor downstream. Here, we present the use of a concentration gradient of lithium chloride ionic solution to increase the event frequency of single molecule dsDNA translocation through a solid state nanopore. By using 0.5 M/3 M LiCl on the cis/trans chambers respectively, average dwell times experienced up to a 3-fold increase when compared to experiments run in symmetric 1 M LiCl. Additionally, experiments using the 0.5 M/3 M displayed a greater than 10-fold increase in event frequency, confirming the capture propensity of the asymmetric conditions.

Color online: See article online to view Figs. 1–4 in color.

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Keywords: Asymmetric Salt Concentration Gradient, Controlled Dielectric Breakdown, DNA Detection, Slowed-down DNA translocation, Solid-State Nanopore

1. Introduction

Nanopore technology has the potential for detecting and characterizing biomolecules including DNA [1-3], RNA and proteins [4, 5], viruses [6], and polysaccharides [7]. As biomolecules pass through the nanopore, they cause ionic current blockages, which can be analyzed to characterize physical and chemical properties of the biomolecule.[1, 8-10] Although both biological and solid-state nanopores are effective in biomolecular detection, solid-state nanopores have the added benefit of increased durability and size tuning.[8, 10]. The major challenge facing solid-state nanopores lies within the fabrication process.[11] Nanopores are typically developed using beams of high energy particles either with a

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transmission electron microscope (TEM), or an ion beam sculpting tool.[12] This equipment is expensive, requires an experienced operator, and is often inefficient as nanopores can be easily destroyed in the process. These drawbacks make nanopores impractical to researchers. An alternative, cost effective, and more efficient fabrication method for solid-state nanopore creation is controlled dielectric breakdown, which uses sustained high voltage across a membrane in ionic solution to form a nanopore.[13] Using this method, nanopores can be formed with sub-nm precision by monitoring the induced leakage current of the membrane.[13, 14]

Modern cancer treatments benefit from early detection. DNA methylation at the 5-carbon position of the cytosine nucleotide has been shown to correspond to pre-cancerous genes including p16INKa, p15INK4b, RASSF1A, MLH1, GSTP1, CDH1, APC, and DAPK1.[15-23] Therefore, identification of methylation profiles within a DNA sequence can be a useful tool for early-stage cancer diagnostics.[15] Furthermore, solid-state nanopore devices have been shown to be able to accurately detect methylation on double stranded DNA (dsDNA).[15-17] The inherent challenge facing nanopore technology for single molecule detection is the intrinsically fast DNA translocation velocities.[8, 24, 25] In many cases, the dwell time of DNA through the pore is so short, that nucleotides cannot be properly discriminated, and characteristics, such as a methylated cytosine, may go unidentified. Therefore, in order to enhance the practicality of solid-state nanopores as a biosensor, it is necessary to impede translocation velocity enough so that individual nucleotides can be fully examined and characterized. As shown in Kowalczyk et al., ionic solution comprised of lithium chloride (LiCl) significantly decreased translocation velocities of DNA transport through solid-state nanopores.[26] In the presence of monovalent cations in solution, such as K^+, Na^+, and Li^+, the cations covalently interact with the negatively charged phosphate backbone. This makes the DNA bulky and the partial negative charge of the DNA backbone is slightly reduced, thus increasing the difficulty of motion.[26-28] When compared to more commonly used salts, such as KCl, LiCl electrolyte solution has been shown to have

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a greater binding affinity to dsDNA and thereby reduce velocity of DNA translocation by up to 10-fold.[26, 27] However, previous studies have shown a lack of DNA translocation occurrence when using LiCl electrolyte solutions in exchange for the reduction of DNA velocity.[14]The frequency of observable translocation events was shown to decrease at low applied biased voltages (200 mV) and at increased concentrations of LiCl (3 M). Ample observable DNA translocation events are crucial to developing significance in the data and reliability in the assay. Therefore, there is a need for a method to increase the event occurrence while still maintaining the prolonged dwell times that have been observed in experiments with LiCl. Efforts to accomplish this have been investigated using various methods, including plasmon induced local heating, which saw a 10-fold increase in observed events with low powered lasers.[11]

DNA translocation speeds have been shown to be modifiable through the use of varying salt concentrations on both the cis and trans experimental compartments.[29] Asymmetric concentration of KCl on either side of the nanopore has also been shown to increase the capture rate of DNA.[29, 30] Having a lower concentration of salt in the cis reservoir increases the electric field within the pore as well as increasing the capture rate on the cis side without shortening mean translocation times.[29] These findings can perhaps be applied to other monovalent cation salt ionic solutions towards the increase of capture rate and ultimately the increase of DNA translocation occurrence.

In this study, we will investigate the effects of an asymmetric concentration gradient of LiCl on dsDNA transport in hopes of increasing event occurrence and slowing down DNA transport through a solid-state nanopore.

2. Materials and Methods

2.1 Dielectric Membranes

Membranes used were 10-nm-thick SiN_x deposited on a 200 μm SiO_2 substrate with a 0.05 x 0.05 mm window. The membranes were purchased from Norcada (Alberta, Canada).

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2.2 Experimental Apparatus

The SiN_x membrane was contained within a precision-machined, two-part acrylic chamber, with its face normal to the horizontal direction. The membrane itself, which sits on the flat plane of a silicon substrate chip, rests in between two silicon O-rings at the center of the chamber.

2.3 Nanopore Fabrication

The nanopores were fabricated via controlled dielectric breakdown in 1 M symmetric LiCl ionic solution. The fabrication solution consisted of 1 M LiCl, 10 mM Tris and 1 mM EDTA, titrated at pH 7.2. Impurities were removed from the solution by filtering it through a 25 mm PES filter with 0.2 μm pore and the solution was degassed. The integrity of the membrane was tested by applying a biased voltage field of 1 V using an Axopatch 200B amplifier. Insulating behavior indicated that the membrane was not damaged and ready for fabrication. A Keithley 6487 PicoAmmeter/Voltage Source was used during fabrication to provide a sustained voltage ranging from 7 V-9 V. The voltage was applied until a predetermined leakage current was observed, indicating that a nanopore of a desired size had been drilled. Once the nanopore was formed, a series of 0-2 V square wave pulses were applied to the membrane to refine the pore. A MATLAB script was used to control the Keithley device and facilitate this process.

2.4 DNA Preparation and Experiments

Individual fragments of 3 kbps dsDNA were purchased from ThermoFisher Scientific. DNA was added to the cis side of the experimental apparatus with a final concentration of 10 nM. Filtered and degassed 0.5 M, LiCl, 1 M LiCl, 1.5 M LiCl, and 3 M LiCl buffered with 10 mM Tris, 1 mM EDTA, and titrated to pH 7.2 were used for DNA experiments. Asymmetric concentration of 0.5 M cis/3 M trans, 1 M cis/3 M trans, and 1.5 M cis/3 M trans were used for the asymmetric experiments. Experiments were conducted by applying biased voltage of 200-600 mV in increments of 100 mV and acquiring data at each level for 5 minutes per individual run. When DNA translocation was prevented due to air bubbles, or other

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obstruction, electrowetting was used to "wet" the pore. Cyclic voltage pulsations ramping from -1 V to 1 V were applied by an Axopatch 200B Amplifier to increase the hydrophilicity of the nanopore. Once the blockage was removed, DNA translocation was restored, and the experiment was continued.

2.5 Data Acquisition and Instrumentation

For all experiments, an Axopatch 200B Amplifier with a built in 8-pole Bessel filter and Digidata 1550B from Molecular Devices were used for their high quality, good signal-to-noise ratio single channel measurements. These devices were used with Clampex data acquisition software for recording ionic current traces and data analysis. Data was recorded at a 100 kHz sampling rate and a lowpass Bessel filter of 10 kHz. Clampfit data analysis module was used for analyzing translocation events. The dwell time was calculated by analyzing the duration of I_B events and fitting that data with a single exponential curve. To determine the event frequency, the reciprocal of the time between I_0 events was analyzed. Exponential function was used to fit the I_0 events. The Current blockage amplitude was also analyzed using Clampfit, and a current blockage histogram was fitted to a Gaussian function to get the average and peak values of current blockages.

3. Results and Discussion

3.1 Nanopore Fabrication

Nanopores in this study were fabricated via controlled dielectric breakdown in 1 M LiCl ionic solution. The process for pore creation and characterization is summarized in Supplementary Information (Figure S1) and follows techniques demonstrated in previous works. [13, 14] The conductance model used to determine pore size was $G = \sigma\left[\frac{4t}{\pi d^2} + \frac{1}{d}\right]^{-1}$, where G represents the average conductance of the pore, σ is the conductivity of the ionic solution, t represents membrane thickness, and d denotes pore diameter.[31]

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3.2 Increased Event Occurrence Frequency by Application of a LiCl Gradient

Our previous work has shown the efficacy of LiCl ionic solution in increasing dwell time of the dsDNA molecule within the nanopore.[14] This is especially apparent in high concentrations of LiCl, such as 3 M. However, the frequency of event occurrence for these experiments was very low and required multiple data acquisition sessions to obtain sufficient data points. At lower voltages, such as 100 mV to 200 mV and with the same concentration of DNA as shown in other successful experiments (10 nM), DNA translocation events are not detectable as shown in supplementary information (Figure S2). To improve on this and increase the overall practicality of this biosensing device, experiments were conducted with asymmetric salt concentration on either side of the nanopore. Asymmetric salt gradients have been shown to allow for the detection of low concentration of analyte, with miRNA reportedly being detected in concentrations as low as 0.1 pM [32], and Hg^{2+} molecules at concentrations as low as 7 nM [33]. This increase in sensitivity accounts for an improvement of 2 orders of magnitude without adding complexity or cost to the system.[33] Regarding event occurrence, KCl has been shown to yield more frequent DNA translocation events than LiCl at the same applied biased voltage. This can be attributed a higher capture range for dsDNA in KCl than in LiCl, a direct result of higher DNA mobility for KCl than in LiCl.[26, 34] The mechanism by which DNA finds itself “captured” by the nanopore before subsequently translocating through follows a set of chronological steps including the coiling of the polymer, diffusive motion, and capture once the DNA reaches a critical distance.[26, 29, 35] The capture range represents the distance at which the DNA is irreversibly captured by the electrical field of the solid-state nanopore and transported through the pore.[35-38] 2 M LiCl and 1 M KCl have been shown to have a capture range of 150 nm and 1000 nm respectively.[11] The DNA molecules in LiCl ionic solution experience random drift due to diffusive forces being greater than the DNA molecules in KCl.[35, 36] Because of this, not only is the capture range a great deal shorter in LiCl than KCl, but the DNA in LiCl ionic solution takes longer to reach the capture range as well.

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One possible explanation to the capture range in LiCl being shorter than KCl is because Li^+ has a higher binding affinity to DNA than K^+.[26] On average, the amount of time that lithium ions remain bound to DNA in a 4 M LiCl system is 50 ps. In a 4 M KCl system, potassium ions bind for an average of only 10 ps.[26] DNA in KCl experience less hydrostatic drag because it has fewer ions bound to the molecule. Consequently, the DNA in KCl have a higher velocity, and a larger capture range because a smaller electrical field is needed to remove the K^+ ion. The Li^+ ion, on the other hand, has to overcome higher hydrostatic drag and needs a high electrical field to remove the Li^+ compared to K^+.[36-38] This is beneficial when slowing down translocations, but it also decreases the capture rate and the efficacy of using this salt for bio sensing applications.

For this study, long dsDNA was inserted into the cis chamber of the experimental apparatus in which a LiCl concentration gradient was applied with the lower concentration of salt being used in cis and the larger concentration used in trans as shown in Figure 1A. Interestingly, when obtaining an I-V relationship from nanopores in asymmetric ionic solution, the curve was found to be parallel to the I-V curve from the same pore in symmetric conditions (1M/1M LiCl), but with a marginal offset. This occurrence is shown in Figure 1B for a 5.0 nm pore in a 1 M/3 M (cis/trans) salt gradient. Upon introducing an asymmetric salt gradient, there is automatically ion flow because of the tendency of ions to move down their concentration gradient. This explains why there is current reading even with 0 V applied. Similar occurrences are provided in Figure S2 in supplementary information.

For the study, a 5.0 nm pore was used with a concentration gradient of 1 M/3 M LiCl (cis/trans), a 6.0 nm pore was used with a gradient of 1.5 M/3 M LiCl, and a 10.9 nm pore was used with a gradient of 0.5 M/3 M. Nanopores of 5.8 nm and 6.4 nm in symmetric 1 M LiCl and 3 M LiCl respectively were used as controls to compare the augmentation in event occurrence frequency. 3 kbps dsDNA was inserted into the cis chamber of the experimental apparatus at a concentration of 10 nM. Transport events were recorded at biased applied voltage levels ranging from 200 mV to 600 mV in increments of 100 mV. Qualitatively, the

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application of salt gradients increased mean translocation event occurrence when compared to the 1 M LiCl and 3 M LiCl controls that were tested in symmetric experimental conditions as seen in Figure 1C. Events were found to occur about 5-times more frequently in 0.5 M/3 M LiCl than in 1 M LiCl and over 50-times more frequently than in 3 M LiCl as shown in Figure 1D. The increase in event occurrence frequency was quantified by analyzing the duration of I_0 (open pore current baseline) between transport events and fitting this data with an exponential decay function. The frequency was obtained by applying the reciprocal of the mean I_0 durations for each experiment. The comparison of event frequencies among each condition is plotted in Figure 2. As shown, all nanopores exhibited the same trend of increasing transport events with increasing applied voltage. Each experiment performed in asymmetric conditions yielded more frequent events than the controls. 0.5 M/3 M LiCl had the highest occurrence rate of dsDNA translocations at $27.78 \pm 0.69\ s^{-1}$ at 300 mV. A summary of all the event frequencies for every condition at applied biased voltages of 200 mV, 300 mV, 400 mV, 500 mV, and 600 mV are provided in Table 1. As the trend suggests, we can expect to see further increases in event frequency as we apply an even larger gradient of salt. This is significant because it would allow for more data points at lower biased voltages. Application of low voltages may be crucial for relatively sensitive biomolecules, or for particles with high charge density as they are more susceptible to changes in their properties if exposed to higher voltages.[39, 40]

3.3 Slowed Translocation of dsDNA by use of LiCl Gradient

The utilization of salt gradients was also studied in terms of further slowing transport of dsDNA through the nanopore. The interionic effects of cations in solution have been found to increase at higher concentrations, thereby increasing the resistivity of the ionic solution.[41] Interionic effects occur when ions are submerged in an ionic space with a net charge opposite of the ion's charge.[42] Thus, the ionic solution diminishes the mobility of the ions within through a drag force. The magnitude of the effect is proportional to the concentration of the ionic solution. A weak electrolyte solution generally promotes weak

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effects whereas a strong electrolyte solution promotes strong effects. Upon saturation with counterions, the net charge on the DNA molecule will be decreased and therefore alter the speed of transport.

Transport is also influenced by forces near the wall of the channel. At this interface, the ion distribution is strongly influenced by the size of the ions.[43] The continuum flow theory based on the Navier-Stokes equations assumes that the state variables (i.e. density) do not significantly change over intermolecular distances. Additionally, shear stress can be related to strain with a linear consecutive relationship. Nevertheless, fluctuations have been observed in fluid density close to the surface in molecular dynamics simulations and experiments.[43] Consequently, the shear viscosity near the nanopore wall dramatically increases which will leads to slower translocation times.[43] A study reported in Wanunu et al. showed that salt gradients on either end of the nanopore can also increase nanopore sensitivity and mean translocation time of DNA transport.[29, 44] The use of asymmetric salt conditions allows for positive K^+ ions to be pushed in the trans-to-cis direction more efficiently than negatively charged Cl^- ions moving in the cis-to-trans direction.[29]

Nanopores of 5.8 nm and 6.4 nm in symmetric 1 M LiCl and 3 M LiCl respectively were used as controls to compare the change in dwell time. Transport events were again recorded at biased applied voltage levels ranging from 200 mV to 600 mV in increments of 100 mV. Figure 3A shows samples of raw data traces for the specified conditions at an applied voltage of 300 mV. Here, the discrepancy in event frequency can be seen between the conditions. Figure 3B shows individual events from the data traces for each condition to illustrate in more detail the degree by which the dwell time is increasing. Qualitatively, there is a significant increase in dwell time observed between 1 M LiCl and 0.5 M/3 M LiCl for an applied biased voltage of 300 mV. To quantify the magnitude of the increase, the dwell times were fitted with an exponential decay function as shown in the histogram in Figure 3C. Figure 4 shows a trend between all pores that features a decrease in dwell time as the applied voltage increases. There is about a 3-fold increase in mean translocation time when

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comparing 0.5 M/3 M LiCl to 1 M LiCl. The other asymmetric conditions (1 M/3 M and 1.5 M/3 M) exhibit marginal increases as well when compared to 1 M LiCl. However, when compared to the pore in symmetric 3 M LiCl, there was no observable increase in dwell time in any of the asymmetric cases. A possible explanation for this might be that the effect of the cation binding affinity to the dsDNA backbone, which makes the molecule bulkier, overshadows the effect of the double layer formation that restricts the DNA motion at this high of a concentration. The opposite seems to be true at lower concentration of lithium chloride, such as 1 M. A summary of all the recorded dwell times for every condition at applied biased voltages of 200 mV, 300 mV, 400 mV, 500 mV, and 600 mV is provided in Table 2.

4. Concluding remarks

Our previous studies have demonstrated the adeptness of LiCl ionic solution in prolonging the dwell time of dsDNA within the solid-state nanopore.[14] However, it was also shown that the DNA transport event occurrence was reduced, most likely due to Lithium's binding affinity to the DNA.[14] Here, we showed the effects of applying a 2-fold, 3-fold, and 6-fold LiCl concentration gradient on dsDNA translocation experiments. When compared to experiments carried out in symmetric 1 M LiCl, the experiments conducted in asymmetric conditions resulted in more prolonged dwell times, with the most significant increase, about 2-fold, observed in 0.5 M/3 M LiCl. This was about 4 times longer than dwell times observed in 1 M KCl in previous studies. Conversely, when compared to 3 M LiCl experiments, the observed dwell times in asymmetric experiments were shorter. However, the unbalanced experimental conditions yielded an overall increase in event occurrence. For instance, 0.5 M/3 M LiCl showed a 5 time and 54 time increase in event occurrence when compared to symmetric 1 M LiCl and 3 M LiCl respectively. This increase in event frequency increases the data generated per run and consequently gives way to more reliable statistical analysis in far less time. Obtaining sufficient data to yield conclusive results in less time and while retrieving small samples from the patient speaks to the practicality of the solid-state

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nanopore towards biosensing applications. The benefit of increased event occurrence retains the inclusion of asymmetric salt conditions in the realm of experimental parameters, even though the observed dwell time in the 6-fold concentration gradient (0.5 M/3 M LiCl) is still faster than in the symmetric 3 M LiCl. Other groups have applied up to a 20-fold concentration gradient increase to increase nanopore sensitivity and capture rate and ultimately enhance detection of miniscule concentrations of DNA. Perhaps a larger gradient will also result in further prolonged dwell time of the DNA molecule inside the nanopore when compared to the smaller gradients. Gradients of 10-times or greater could possibly approach the dwell times observed in symmetric 3 M LiCl, although none of our data supports that a larger gradient would surpass the temporal resolution of the symmetric 3 M LiCl.

Interestingly, the use of asymmetric experimental condition of ionic salt solution had a deleterious effect on signal to noise ratio of the nanopore sensing as well as the overall health of the pore. Although the desired slower translocation times and increased event occurrences were observed, these findings were coupled with fluctuating baselines and obstruction of the pore that often impeded further DNA translocation. A possible explanation for this can be the contribution of hydrodynamic slip that occurs as a result of an amalgam of experimental conditions (i.e. asymmetric LiCl solution concentration) and the inherent hydrophobic properties of the silicon nitride dielectric membranes that was used for the experiments.[45, 46] The LiCl ionic solution seemingly augmented this occurrence further and created more hydrophobic air pockets within the pore. Air bubbles in the nanopore can “dewet” the pore and prevent passage of ionic solution and molecules.[46] Such a phenomenon is reversible through various techniques including electrowetting,[1, 47] which employs a cyclic voltage pulse to enhance the hydrophilicity of the pore albeit while also marginally increasing your pore diameter in the process.[1] Electrowetting allows for continued data acquisition, although introduces the need for constant monitoring of the data acquisition session to interfere when a hydrophobic blockage occurs. This hinders some of

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the autonomy that is desired in nanopore biosensors. More work has to be done to enhance the surface and inner channel wettability of the dielectric membrane in order to consider asymmetric LiCl as a viable medium for DNA translocation experiments, although the results for increased dwell time and capture efficiency are promising.

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Table 1 Translocation Event Occurrence Frequency

Conditions	Est. Pore Size (nm)	$f_{200mV}(s^{-1})$	$f_{300mV}(s^{-1})$	$f_{400mV}(s^{-1})$	$f_{500mV}(s^{-1})$	$f_{600mV}(s^{-1})$
1 M LiCl	5.8		1.86 ± 0.42	2.48 ± 0.77	4.75 ± 2.00	7.16 ± 3.20
3 M LiCl	6.4		0.53 ± 0.04	0.59 ± 0.05	2.0 ± 0.16	2.67 ± 0.11
1 M/3 M LiCl	5.0	6.34 ± 0.67	8.68 ± 0.96	15.92 ± 0.47	12.48 ± 2.86	19.65 ± 2.00
0.5 M/3 M LiCl	10.9	16.66 ± 1.09	27.78 ± 0.69	39.17 ± 0.55	38.16 ± 0.75	53.65 ± 1.15
1.5 M/3 M LiCl	6.0	5.78 ± 0.13	8.19 ± 0.47	9.46 ± 0.43	11.07 ± 0.85	11.59 ± 1.30

Table 2 Transport Duration

Conditions	Est. Pore Size (nm)	$\tau_{200\ mV}$(ms)	$\tau_{300\ mV}$(ms)	$\tau_{400\ mV}$(ms)	$\tau_{500\ mV}$(ms)	$\tau_{600\ mV}$(ms)
1 M LiCl	5.8	0.136 ± 0.054	0.089 ± 0.020	0.059 ± 0.021	0.040 ± 0.002	0.037 ± 0.007
3 M LiCl	6.4		0.970 ± 0.160	0.560 ± 0.050	0.380 ± 0.070	0.260 ± 0.020
1 M/3 M LiCl	5.0	0.228 ± 0.030	0.120 ± 0.050	0.092 ± 0.006	0.058 ± 0.002	0.051 ± 0.003
0.5 M/3 M LiCl	10.9	0.314 ± 0.030	0.219 ± 0.017	0.160 ± 0.020	0.111 ± 0.008	0.071 ± 0.002
1.5 M/3 M LiCl	6.0	0.203 ± 0.030	0.0947 ± 0.005	0.068 ± 0.009	0.046 ± 0.003	0.410 ± 0.001

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Figure Captions

Figure 1 Overview of the dsDNA translocation experiments in LiCl salt gradients. A) Schematic representation of the experimental conditions. Here, the lower concentration of LiCl is denoted by the lighter blue in the cis chamber. The higher concentration is denoted by the darker blue in the trans chamber. dsDNA is shown in the cis chamber and transporting through the nanopore with Li^+ ions attached to the backbone (inset). B) I-V relationship of a 5.0 nm nanopore in both symmetric 1 M LiCl and asymmetric 1 M/3 M LiCl is plotted. Characteristic offset in I-V relationship of a pore in asymmetric salt conditions due to ion flow down the salt gradient is shown. The curve maintains linearity and is parallel to the I-V relationship in symmetric conditions. C) Representative data trace samples of dsDNA translocation event occurrences recorded at 300 mV in 5.8 nm and 6.4 nm pores in symmetric 1 M LiCl and 3 M LiCl respectively as well as a 10.9 nm pore in asymmetric 0.5 M/3 M LiCl. An increase in event frequency is observed. D) Histogram created by fitting the data for I_0 duration for the 0.5 M/3 M LiCl, 1 M LiCl, and 3 M LiCl conditions with an exponential decay function. Reciprocal of mean I_0 duration (the time in between the conclusion of one event and the start of the next) is used to calculate event frequency. Difference in time between observed events in provided for symmetric 1 M LiCl, 3 M LiCl and for 0.5 M/3 M LiCl (inset). The time between events is reduced by about 5-fold in the asymmetric conditions.

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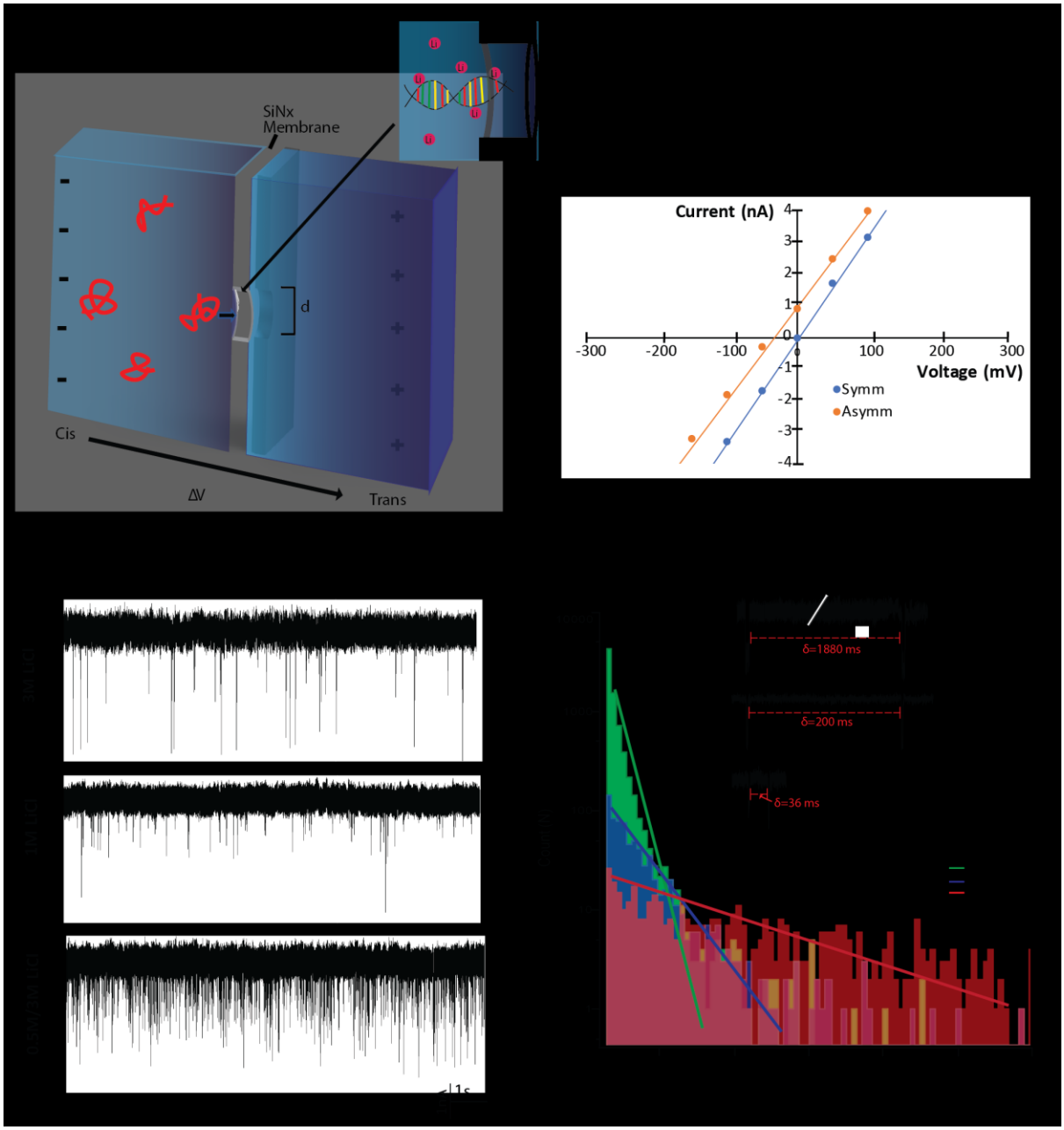

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Figure 2 Graphical representation of the event occurrence frequencies of dsDNA translocations in various LiCl gradients. Experiments run in symmetric 1 M LiCl and 3 M LiCl and asymmetric 1.5 M/3 M LiCl, 1 M/3 M LiCl, and 0.5 M/3 M LiCl are provided for comparison. Data was recorded at biased voltage levels of 200 mV, 300 mV, 400 mV, 500 mV, 600 mV. An overall increase in event occurrence is observed for experiments run in asymmetric LiCl conditions as opposed to symmetric LiCl.

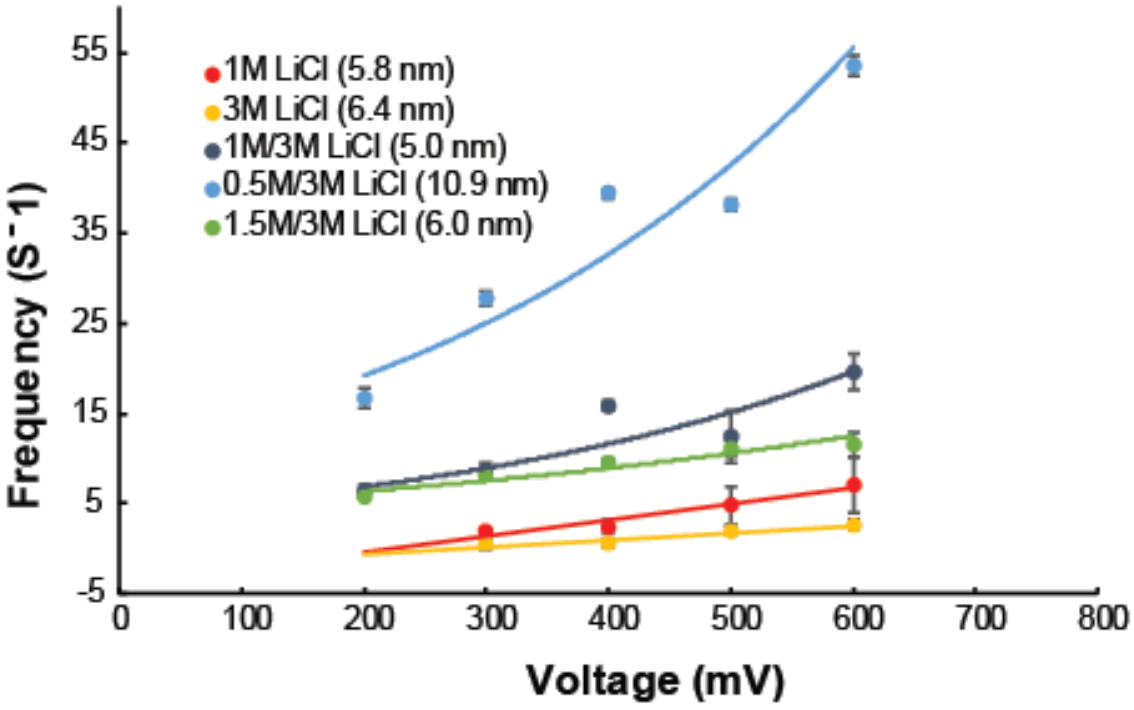

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Figure 3 Analysis of the transport duration of dsDNA translocation events in various LiCl gradients. A) Representative data traces for experiments run in 0.5 M/3 M LiCl (cis/trans), 1 M/3 M LiCl, and 1.5 M/3 M LiCl. Data traces for similar experiments in symmetric 1 M LiCl (1 M/1 M) and 3 M (3 M/3 M) is provided above as a control. Each data trace was recorded at a biased applied voltage of 300 mV. B) A detailed view of representative individual transport events for the conditions of 1M LiCl,3 M LiCl, 1.5 M/3 M LiCl, 1 M/3 M LiCl, and 0.5 M/3 M LiCl. An increase in dwell time of dsDNA molecules is observed for each experiment run under asymmetric conditions when compared to symmetric 1 M LiCl with 0.5 M/3 M LiCl exhibiting the largest increase out of the samples with a LiCl gradient. C) Histogram created by fitting the data for translocation events for the 0.5 M/3 M LiCl, 1 M/3 M LiCl, and 1.5 M/3 M LiCl conditions with an exponential decay function. A translation to the right as well as a decrease in slope is observed as one increases the LiCl gradient.

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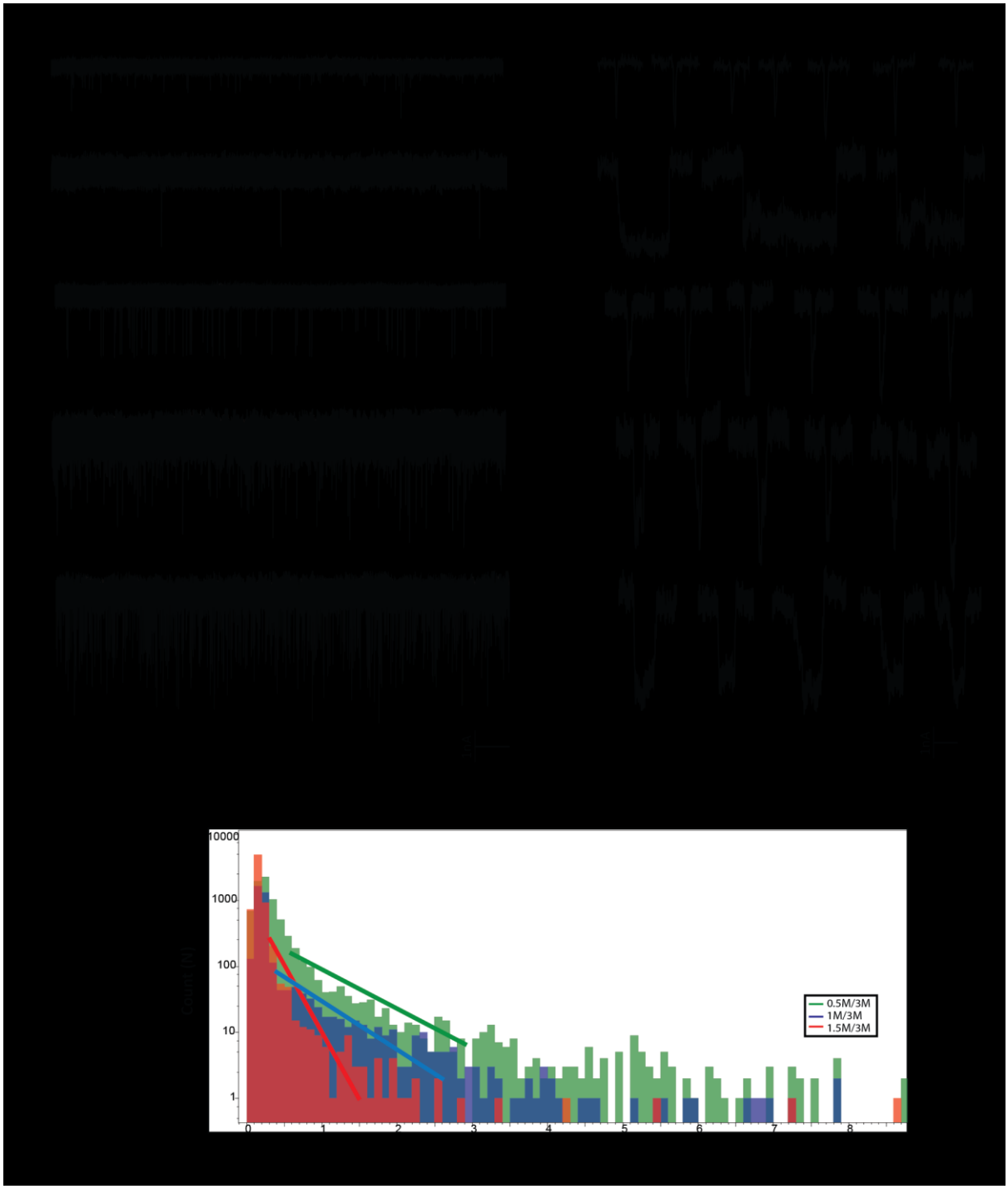

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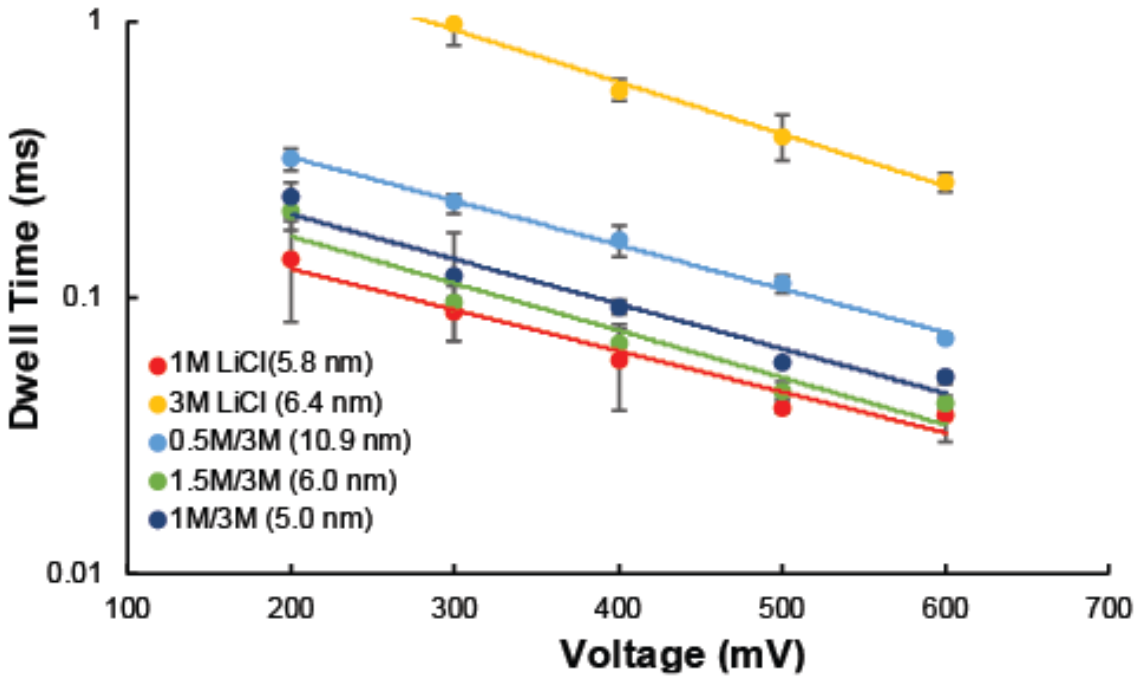
Figure 4 Graphical representation of the mean dwell times for dsDNA translocation events in various LiCl gradients. Experiments run in symmetric 1 M LiCl and 3 M LiCl and asymmetric 1.5 M/3 M LiCl, 1 M/3 M LiCl, and 0.5 M/3 M LiCl are provided for comparison. Data was recorded at biased voltage levels ranging from 200 mV to 600 mV in 100 mV increments. An increase in mean dwell times for experiments run in asymmetric conditions is observed when compared to the symmetric 1 M LiCl with 0.5 M/3 M LiCl showing the largest increase. However, experiments run in symmetric 3 M LiCl yielded longer dwell times when compared to all other conditions.



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