Supplementary Information

Single-Stranded DNA Translocation Recordings Through Solid-State Nanopores on Glass Chips at 10-MHz Measurement Bandwidth

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1. Spurious event detection

Assuming Poisson statistics, the average rate of false events (λ_f) is given by:[1]

$$\lambda_f = kBe^{-\frac{1}{2}\left(\frac{\emptyset}{I_{BASELINE}^{rms}}\right)^2},$$

where k is a constant in the range of 0.849 to 1.25, B is the bandwidth, $\emptyset$ is the detection threshold, and $I_{BASELINE}^{rms}$ is the root-mean-squared noise of the baseline.[1] At 10-MHz bandwidth and with $5I_{BASELINE}^{rms}$ thresholding, λ_f is 37.2 sec^{-1}. With pores having more than 1000 translocation events per second, the false event rate is smaller than 4%.

2. Distribution of events with respect to dwell time for measured pores

Pore	Diameter d_{eff} (nm)	DNA length (nts)	Below 2µs (%)	2µs-10µs (%)	10µs-100µs (%)	Over 100µs (%)	τ_{dwell} (µs)
A	1.5	200	24	25	33	18	1.2
B	1.5	200	20	39	38	2	3.0
C	1.4	200	25	22	31	22	0.8
D	1.4	200	55	13	16	16	0.4
E	1.7	80	40	37	20	3	1.0
F	1.7	200	32	49	16	2	2.0
G	1.9	90	17	18	50	15	1.7
H	1.7	200	48	45	7	0	1.1
I	1.7	90	34	43	16	7	1.4
J	2.0	90	46	50	4	0	0.8
K	2.0	90	85	13	1	1	0.5
L	2.1	90	68	24	5	3	0.7
M	1.7	90	33	41	23	3	1.9

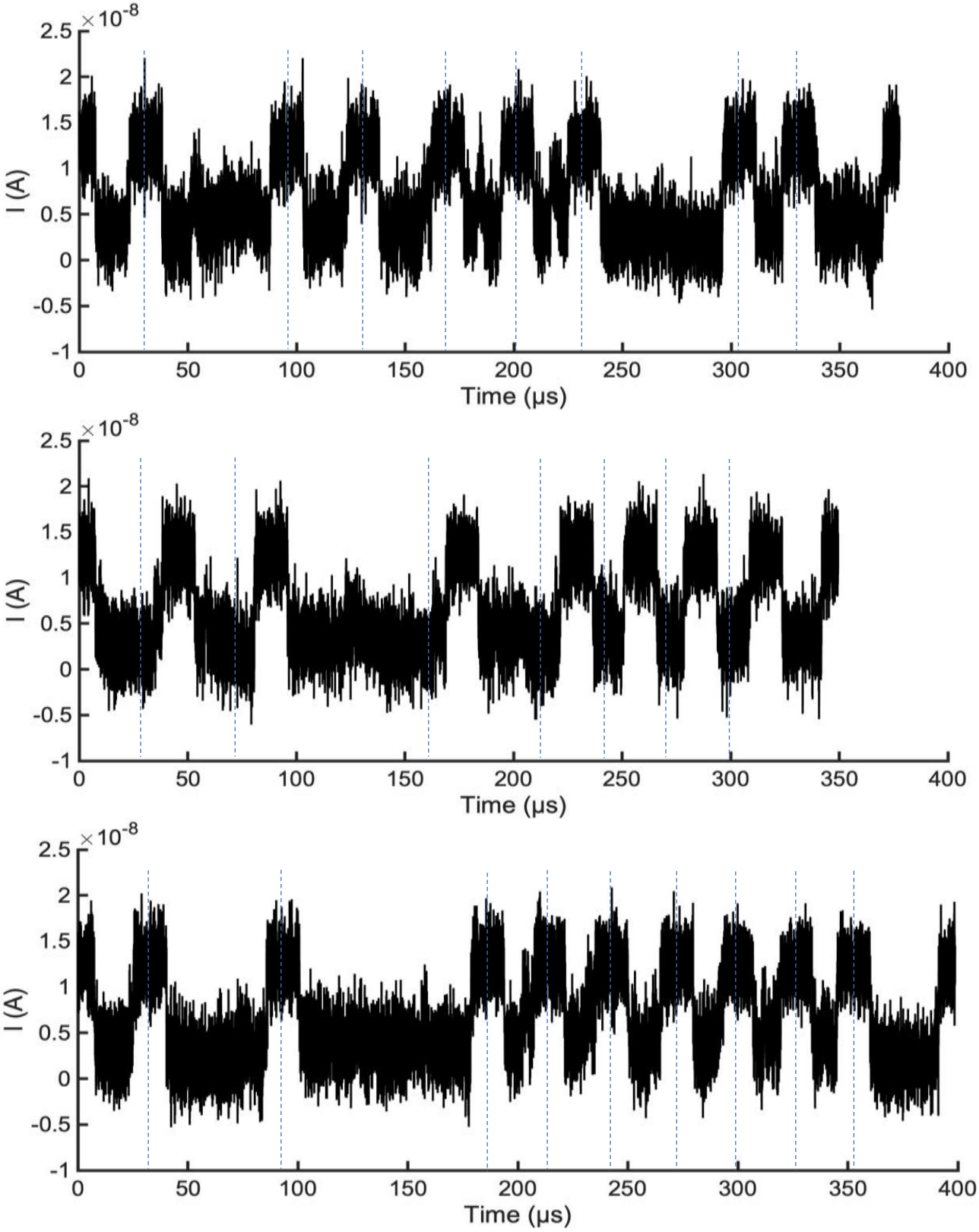
Table SI2. Distributions of events of dwell times binned for illustration in several time ranges: below 2 µs, from 2 µs to 10 µs, from 10 µs to 100 µs, and above 100 µs. The data is analyzed from data filtered to a 2-MHz bandwidth. While the characteristic dwell time τ_{dwell} is mean of the dwell-time distribution, the table provides more detailed information on the actual distribution of dwell times. For example, we can see that, despite τ_{dwell} being 1.2 µs for pore A, 33% of the events are still longer than 10 µs. The smaller pores and longer ssDNA tend to result in higher percentage of longer events. We do observe a difference in the characteristic translocation time of ssDNA molecules that differ in length for pores similar in dimensions. For example, in Pore E & Pore F, d_{eff} = 1.7 nm for both pores and the thickness, t_{eff}, for Pore E is 3.3 nm and for Pore F is 2.6 nm, respectively. The molecules used in Pore E are 200 nts ssDNA, and Pore F are 90 nts ssDNA. The characteristic translocation dwell time for Pore E was 1 µs and for Pore F was 2 µs, which scales roughly with the ssDNA length used in the two experiments.

3. Comparison of translocation experiments of ssDNA at 2-MHz and 10-MHz bandwidth

Pore J	**10 MHz**	**2 MHz**
$< \Delta I >$ (nA)	6.8	3.1
τ_{dwell} (μs)	0.7	1.1
Event counts per minute	25,000	37,000

Table SI3. Comparison between 10-MHz data and 2-MHz data for the same traces for Pore J at 700 mV with event thresholding at $5I_{BASELINE}^{rms}$. The observed ΔI is higher due to both some events being attenuated at 2 MHz, and also some shallower event not surpassing threshold at 10 MHz. The characteristic dwell time is shorter at 10 MHz showing that there are shorter events being detected when we increase the measurement bandwidth. The event counts per minute is reduced at 10 MHz because fewer events surpass the threshold due to the higher noise level.

4. Event characteristics from Pore B with dwell times > 10 µs



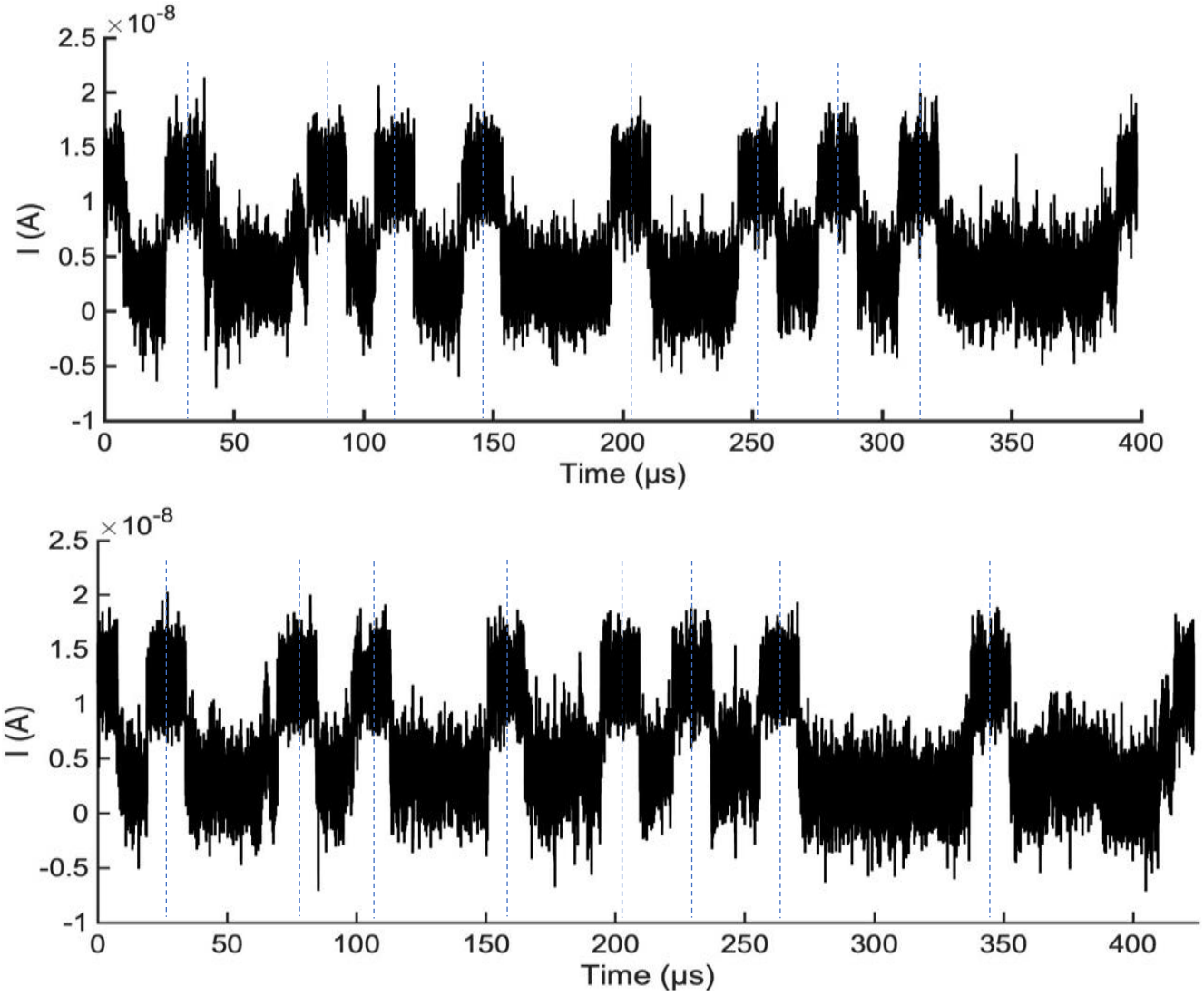


Figure SI4. Concatenated events from Pore B of which dwell time > 10 µs. Within one second of 10 MHz data trace, 47 out of 155 events have dwell time > 10 µs. We display all 47 events above as a concatenated event trace. The blue dashed line represent points where the individual events have been pasted together one next to the other.

5. Blockade characteristics for translocation events of poly(dC)$_{30}$poly(dA)$_{30}$poly(dC)$_{30}$ ssDNA homopolymers

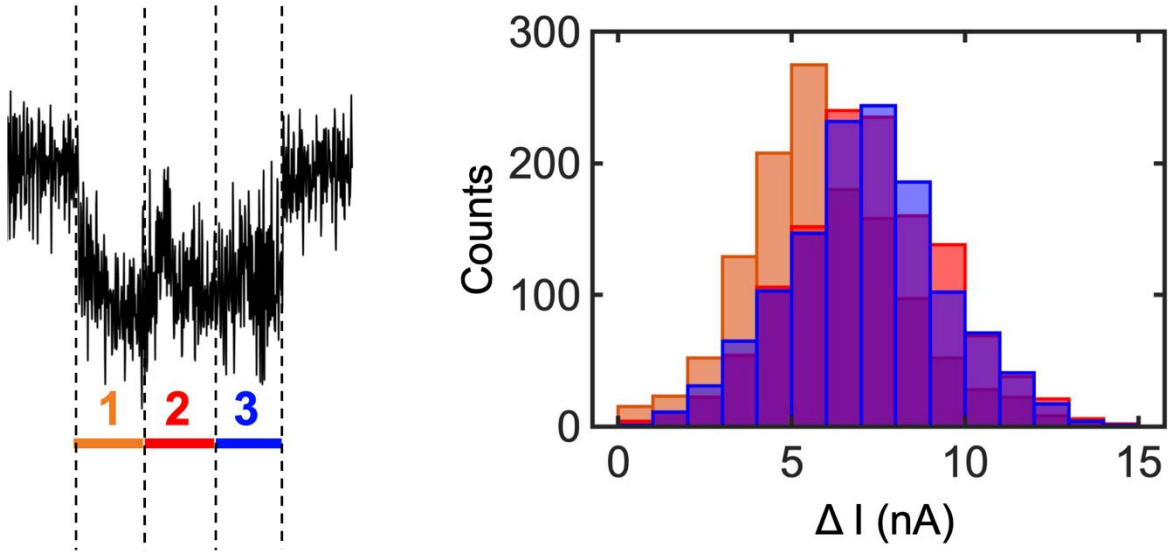
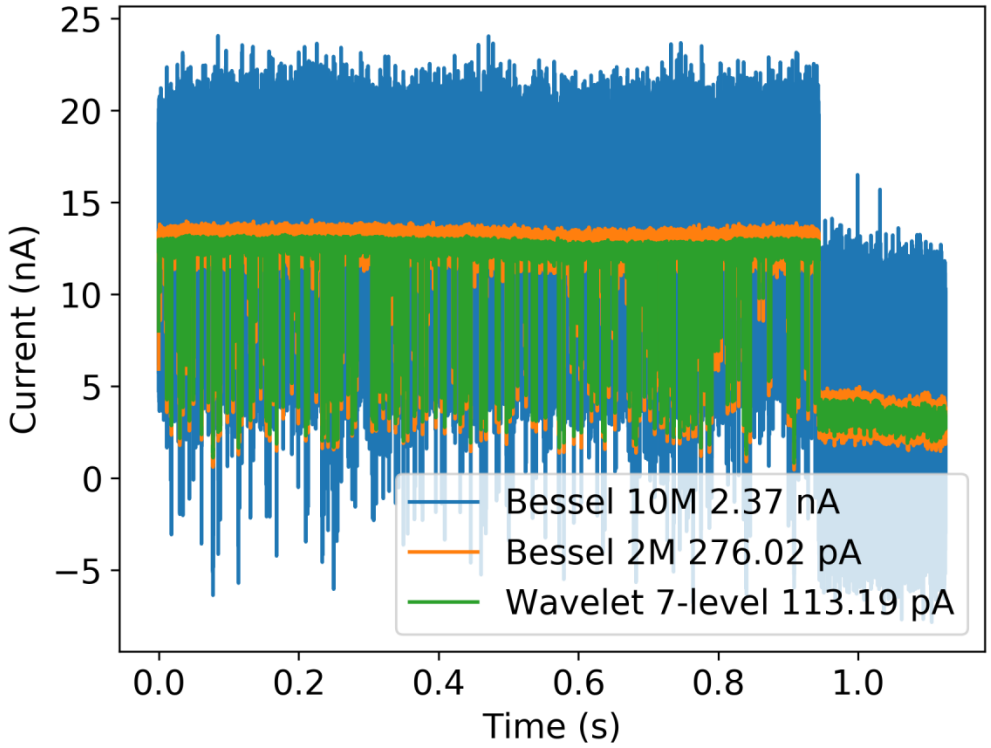


Figure SI5. Illustration of one test approach to data analysis. Translocation events at 10-MHz bandwidth for Pore J at 700 mV. Left: All translocation events, regardless of duration, are divided into three equal durations. If we assume the ssDNA translocate through the nanopore at a uniform rate, we could assume Section 1 corresponds to the translocation of poly(dC)$_{30}$, Section 2 to poly(dA)$_{30}$, and Section 3 to poly(dC)$_{30}$. Right: The histogram of current amplitudes in three intervals shows that current blockades observed in Section 2 (ΔI_2 = 7.2 ± 2.3 nA) and Section 3 ((ΔI_3 = 7.0 ± 2.2 nA) are indistinguishable. Section 1 ((ΔI_1 = 6.0 ± 2.3 nA) shows a slightly lower current blockade, likely due to the contribution of the access resistance before the ssDNA actually enters the nanopore. Data with $C_{50}A_{50}C_{50}A_{50}$ shows no clearly distinguishable levels corresponding to homopolymer blocks, just as in $C_{30}A_{30}C_{30}$. For asymmetric $C_{50}A_{50}C_{50}A_{50}$, either the C-head or the A-head comes to the pore first, which further complicates the issue. Therefore, we chose $C_{30}A_{30}C_{30}$ as demonstration, yet the results for both type molecules show similar trend that distinct levels corresponding to poly(dA) and poly(dC) are not distinguishable and suggest we are detecting other molecular motions instead of the size difference between bases.

6. Wavelet denoising of nanopore recordings



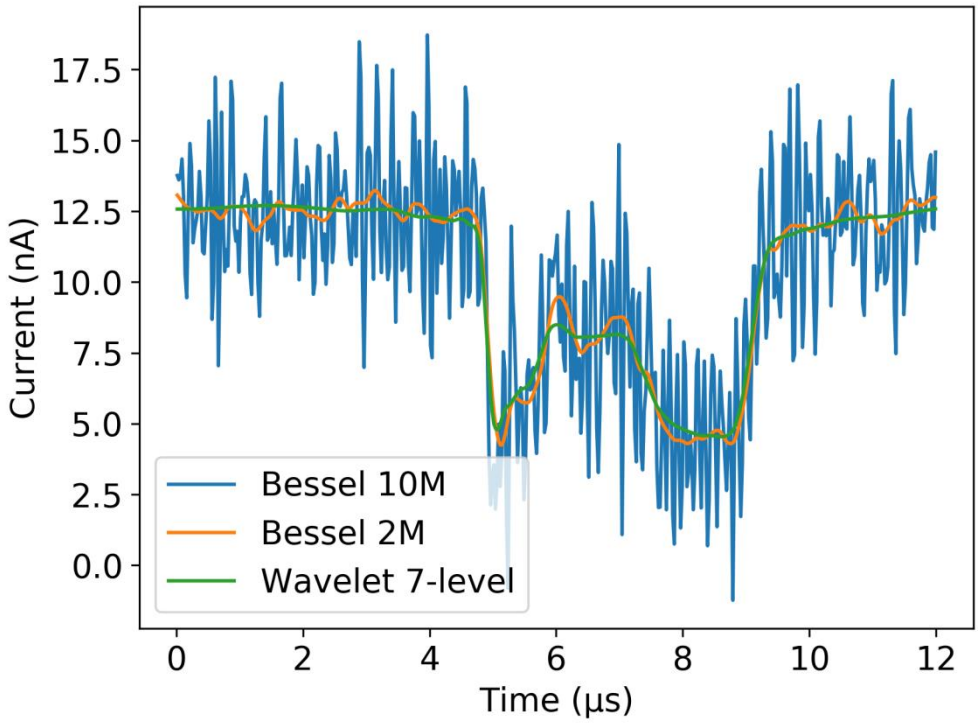


Figure SI6. Comparison of a 1-s long recording of 50A50C50A50C ssDNA through pore B filtered using a 2 MHz fourth-order Bessel filter and using a 7-level stationary wavelet (biorthogonal 1.5) transform with garrote thresholding. The lower figure shows a zoomed version of an event near the 0.7 s mark. Wavelet denoising preserves temporal features at the same level as the 2 MHz filter but with reduced noise levels. Our implementation of the denoising technique is best suited for large amplitude signals and did not reveal any additional information regarding base discrimination within an event.

7. Simple model for ideal poly(dC)$_{30}$poly(dA)$_{30}$poly(dC)$_{30}$ ssDNA homopolymer translocation through nanopore

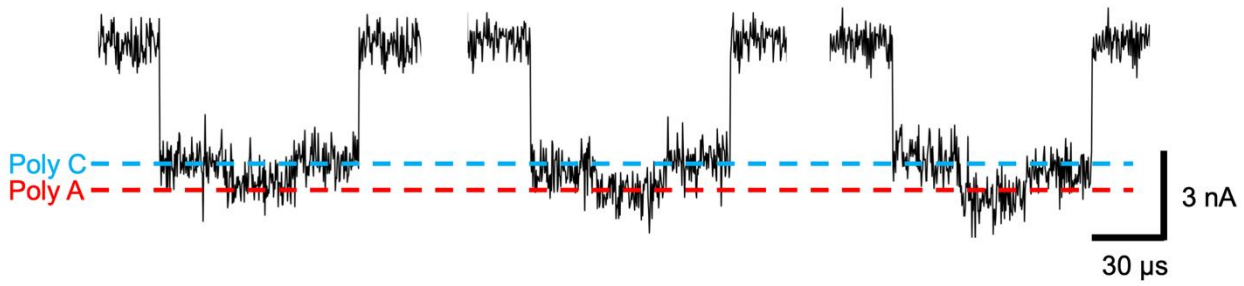


Figure SI7. Simulated sample poly(dC)$_{30}$poly(dA)$_{30}$poly(dC)$_{30}$ ssDNA homopolymer translocation events with experimental numbers from *Venta et al*,[2] where poly(dC)$_{30}$ produces $\Delta I = 4.2 \pm 0.1$ nA, and poly(dA)$_{30}$, $\Delta I = 5.1 \pm 0.4$ nA. The open pore current is 10 nA, and the noise, $I_{BASELINE}^{rms}$ is 500 pA$_{rms}$ at 1-MHz bandwidth. The model assumes a homogeneous translocation speed, and a dwell time of 0.8 µs per nucleotide. It also assumes no effect on the current traces from any modulation in the conductance of the access region.

8. Effect of temperature and salt solution on ssDNA translocation dynamics

Pore C	**25 °C**	**4 °C**
I (nA)	14.8	8.8
$< \Delta I >$ (nA)	8.5	4.3
τ_{dwell} (μs)	0.8	1.0

Table SI8. Experiments with Pore C conducted at approximately 4 °C and at 25 °C (room temperature) at 500 mV in 3MKCl at 2-MHz bandwidth. The translocation dwell time increases from 0.8 to 1.0 μs at lower temperature, but the SNR is sacrificed due to reduced ΔI from 8.5 to 4.3 nA. The translocation dwell time only slightly increased at this lower temperature, but we sacrificed the SNR due to reduced I and ΔI.[3]

We also performed experiments with 1M and 3 M LiCl solution. These solutions were observed to slow down translocation by a factor of 10 in 19.8-nm SiN pores.[4] With the same experimental conditions in these studies but with much smaller nanopores, we did not detect any translocation events.

References

(1) Rosenstein, J. K.; Shepard, K. L. Temporal Resolution of Nanopore Sensor Recordings. *Proc. Annu. Int. Conf. IEEE Eng. Med. Biol. Soc. EMBS* **2013**, 4110-4113.

(2) Venta, K.; Shemer, G.; Puster, M.; Rodríguez-Manzo, J. A.; Balan, A.; Rosenstein, J. K.; Shepard, K.; Drndić, M. Differentiation of Short, Single-Stranded DNA Homopolymers in Solid-State Nanopores. *ACS Nano* **2013**, *7*, 4629-4636.

(3) Fologea, D.; Uplinger, J.; Thomas, B.; McNabb, D. S.; Li, J. Slowing DNA Translocation in a Solid-State Nanopore. *Nano Lett.* **2005**, 5, 1734-1737.

(4) Kowalczyk, S. W.; Wells, D. B.; Aksimentiev, A.; and Dekker, C. Slowing Down DNA Translocation Through a Nanopore in Lithium Chloride. *Nano Lett*. **2012**, *12*, 1038-1044.