Electronic Supplementary Material (ESI) for RSC Advances.


17th November 2021: This Supplementary Information file replaces that originally published on 3rd February 2021. Figure S12 on pages 13-14, which shows the raw data, 100-fold upscaled data and data filtered at 20 kHz for the single nucleotide translocations, has been added and a protocol for the analysis of the raw data on page 15 has also been added.

# Single nucleotide detection using bilayer $MoS_2$ nanopores with high efficiency

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## Supplementary

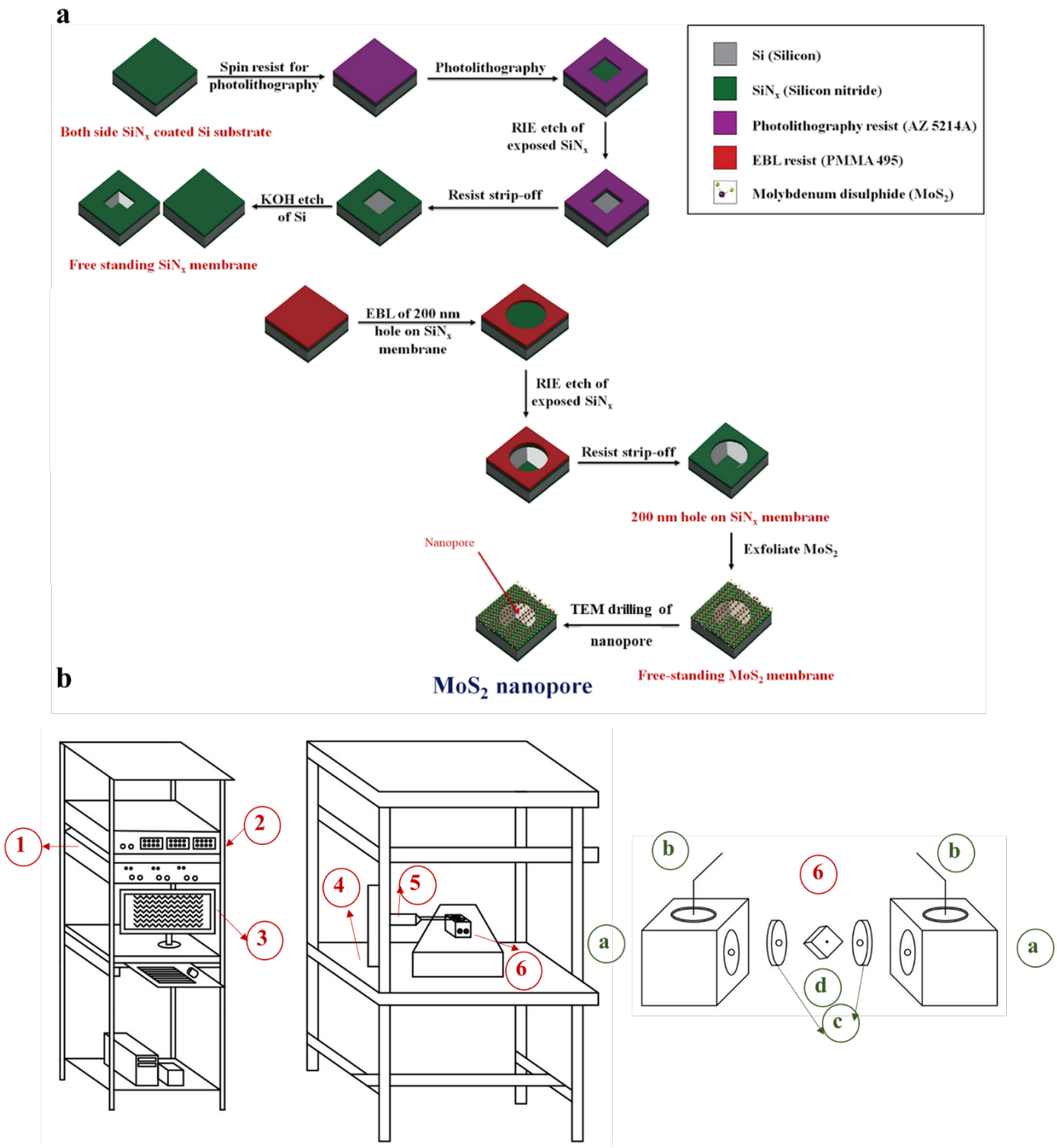
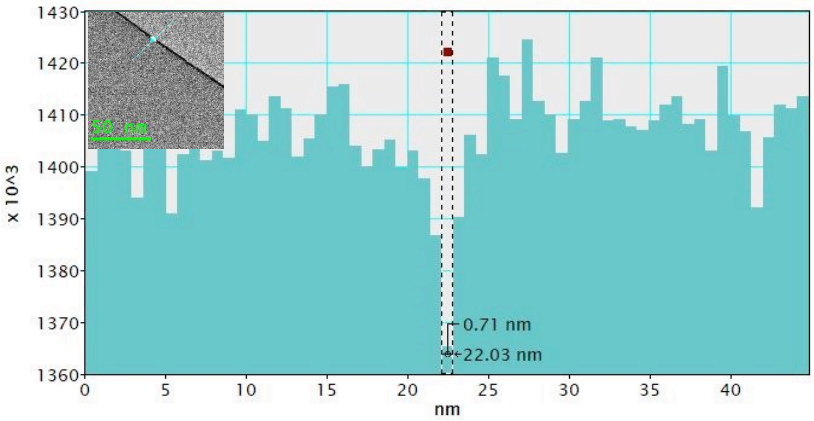
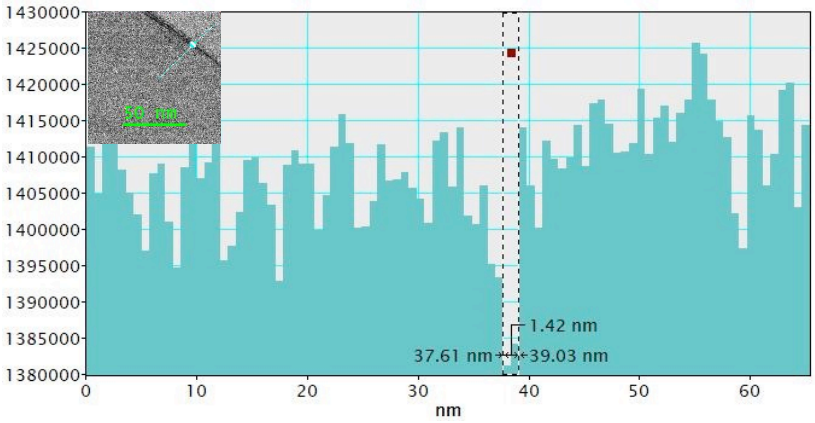



**Figure S1.** (**a)** $MoS_2$ nanopore fabrication steps and **(b)** Schematic representation of custom-designed cell assembly for sensing [1. Amplifier, 2. Digitizer, 3. User interface, 4. Faraday cage, 5. Amplifier head, 6. Cell set up (a. Teflon cell containing KCl, b. Ag/AgCl electrodes, Gaskets to hold membrane, d. Membrane bearing nanopore)].

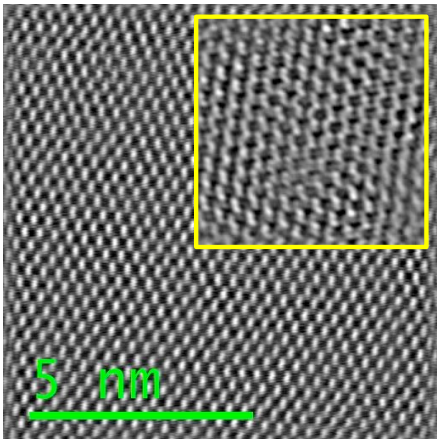
(a)


1430
1420
1410
1400
1390
1380
1370
1360
x 10^3
0.71 nm
22.03 nm
0
5
10
15
20
25
30
35
40
nm

(b)


1430000
1425000
1420000
1415000
1410000
1405000
1400000
1395000
1390000
1385000
1380000
1.42 nm
37.61 nm
39.03 nm
0
10
20
30
40
50
60
nm

(c)


5 nm

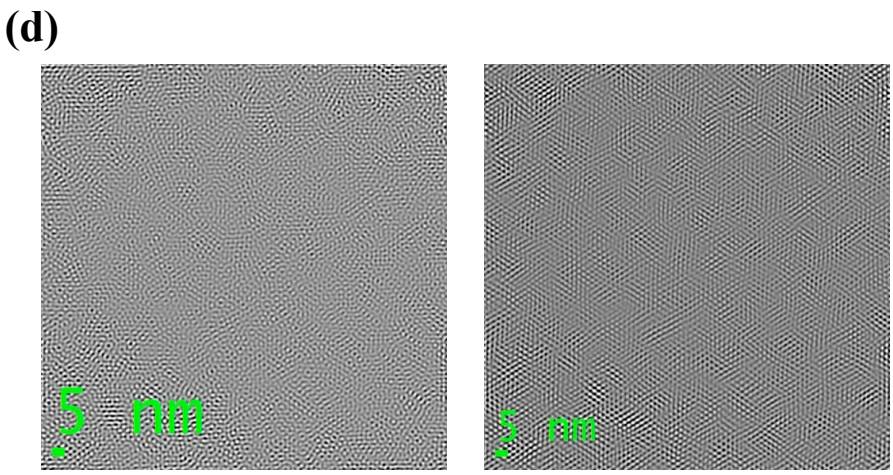
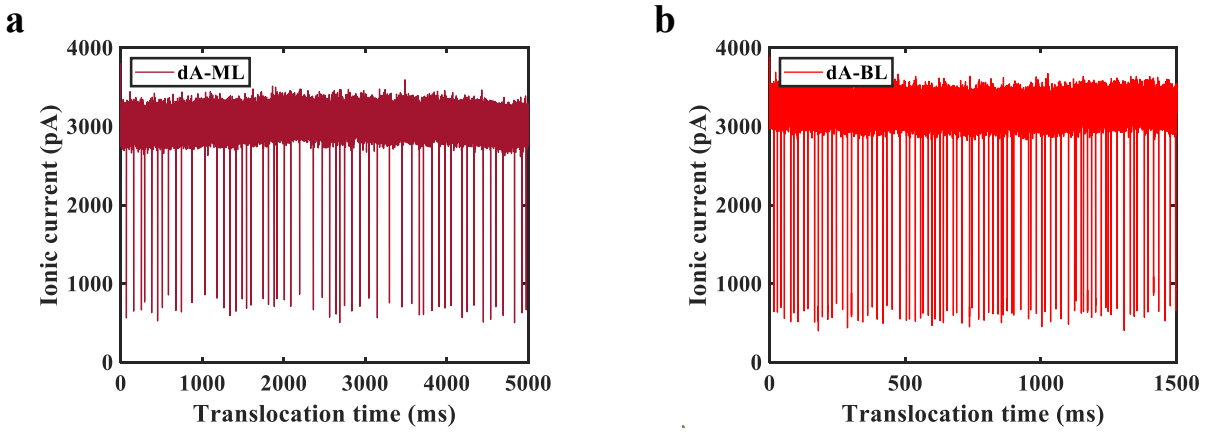



**Figure S2. (a)** TEM line profile of ML $MoS_2$ with an inset raster plot of the same region showing the layer thickness of 0.71 nm, characteristic to a ML $MoS_2$, **(b)** TEM line profile of BL $MoS_2$ with the inset showing its raster plot, showing the layer thickness of 1.42 nm, characteristic to a BL $MoS_2$, TEM raster profiles of **(c)** ML $MoS_2$ and **(d)** (left) AB stacked BL $MoS_2$ and (right) AA' stacked BL $MoS_2$.



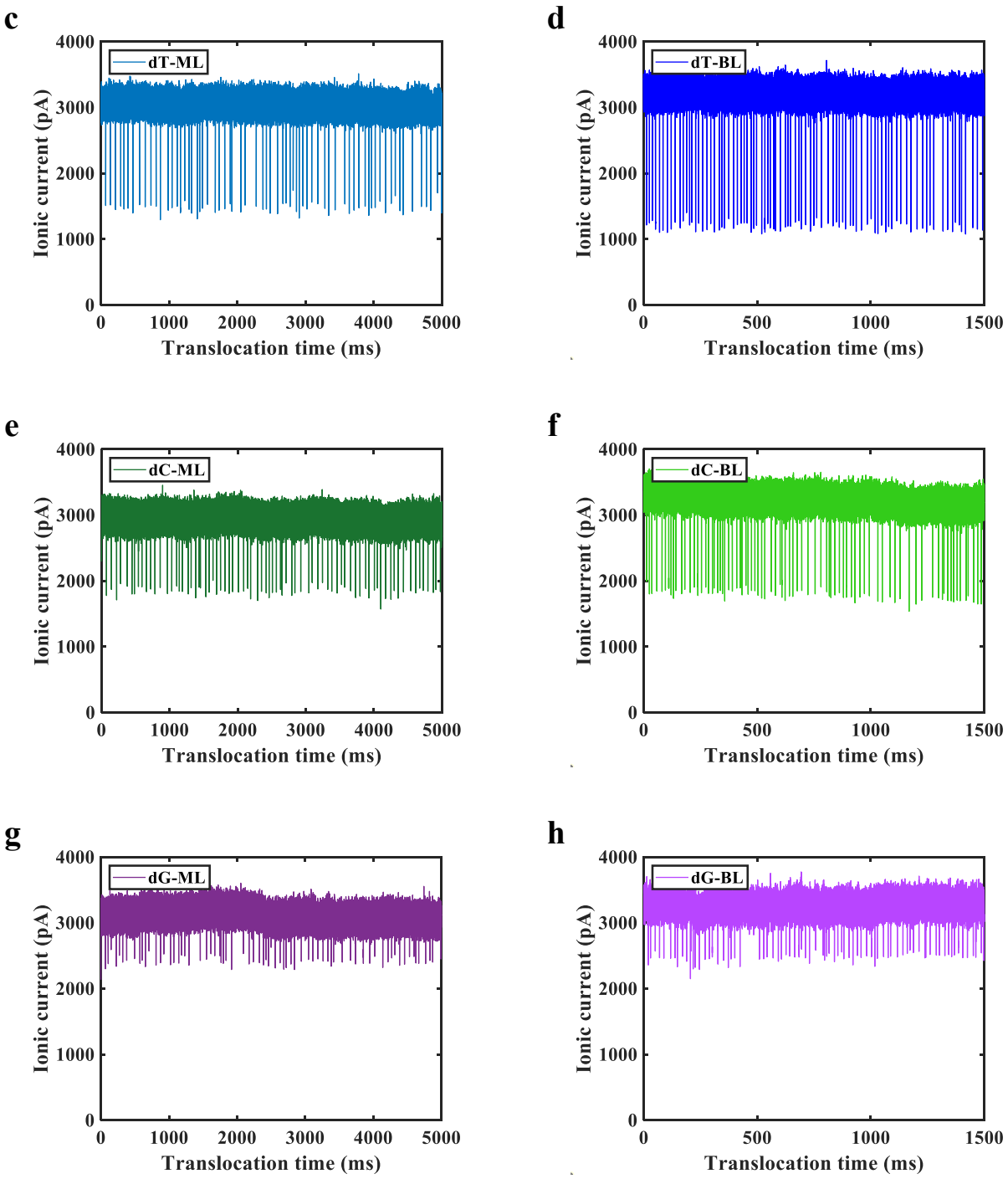



**Figure S3:** Recorded data for single nucleotide (for each of dA, dT, dC and dG) translocation for 5 sec and 1.5 secs through ML $MoS_2$ and BL $MoS_2$ nanopores respectively.

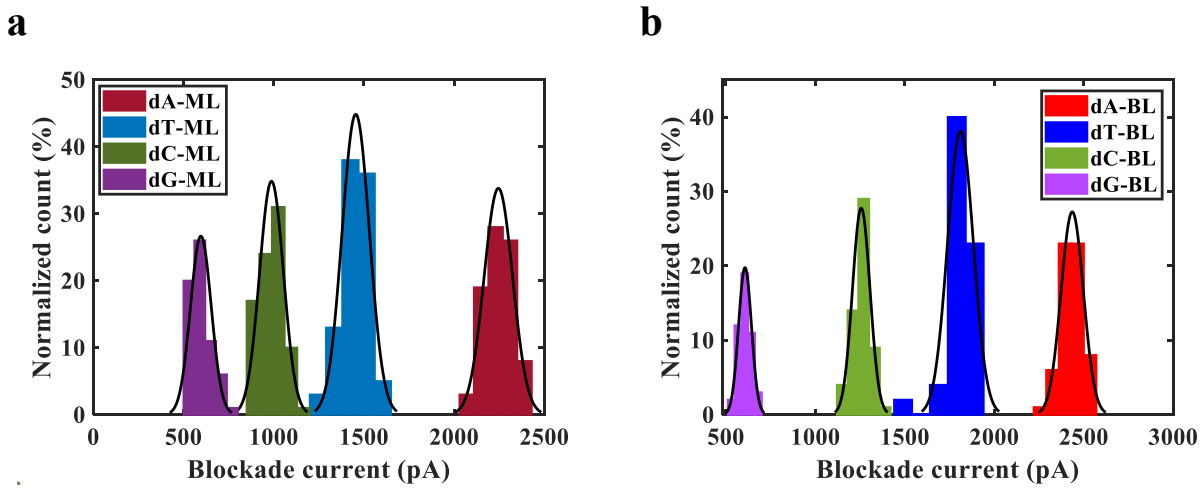
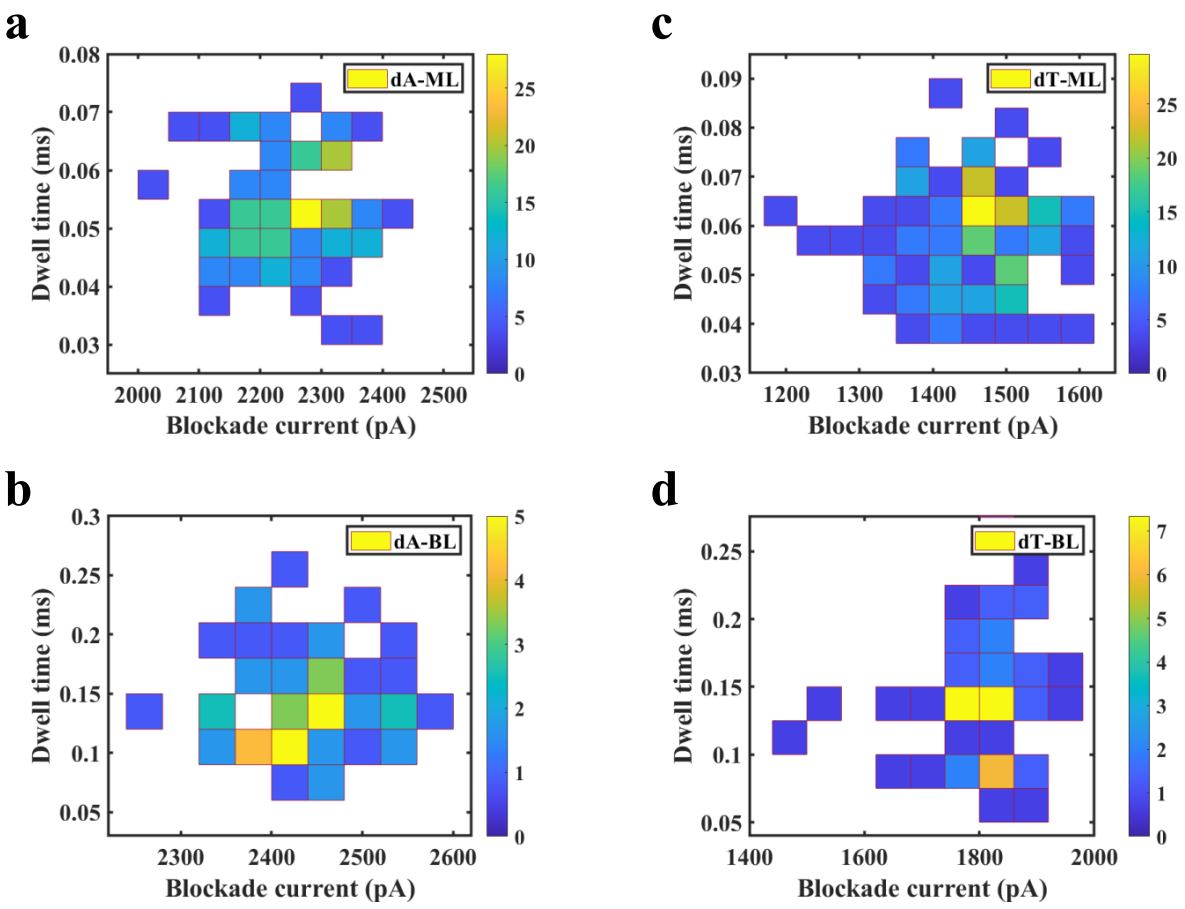



**Figure S4**: Normalized histogram of the recorded dA, dT, dC and dG translocation events through **(a)** ML and **(b)** BL $MoS_2$ nanopores with respect to the blockades current produced for each type of analyte.


a
dA-ML
Dwell time (ms)
0.08
0.07
0.06
0.05
0.04
0.03
2000 2100 2200 2300 2400 2500
Blockade current (pA)
25
20
15
10
5
0
c
dT-ML
Dwell time (ms)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
1200 1300 1400 1500 1600
Blockade current (pA)
25
20
15
10
5
0
b
dA-BL
Dwell time (ms)
0.3
0.25
0.2
0.15
0.1
0.05
2300 2400 2500 2600
Blockade current (pA)
5
4
3
2
1
0
d
dT-BL
Dwell time (ms)
0.25
0.2
0.15
0.1
0.05
1400 1600 1800 2000
Blockade current (pA)
7
6
5
4
3
2
1
0

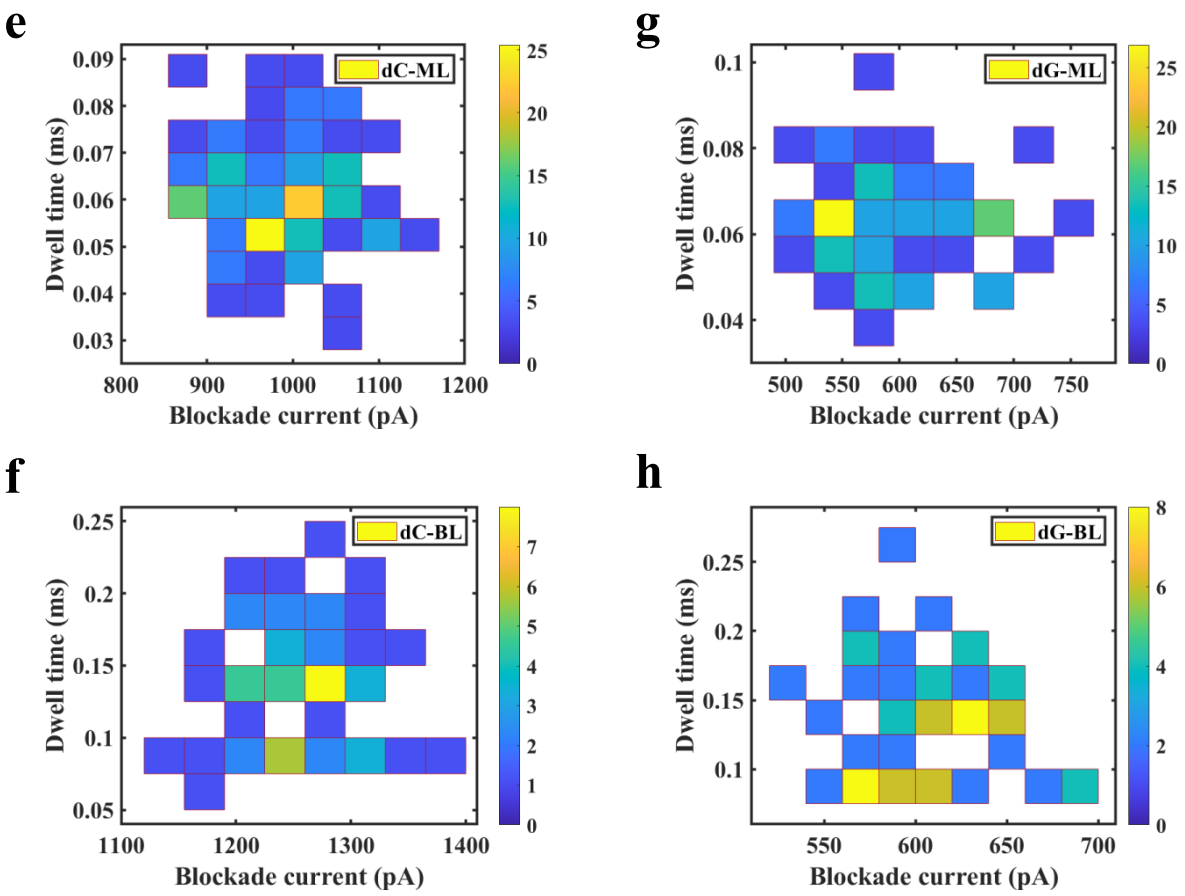



**Figure S5**: 2D flat density plots showing distribution of polynucleotide traces wrt. both blockade current and dwell time with ML pores demonstrating highest density of peaks at 0.05-0.07 ms dwell time and BL pores showing highest density of peaks at 0.06-0.14 ms.

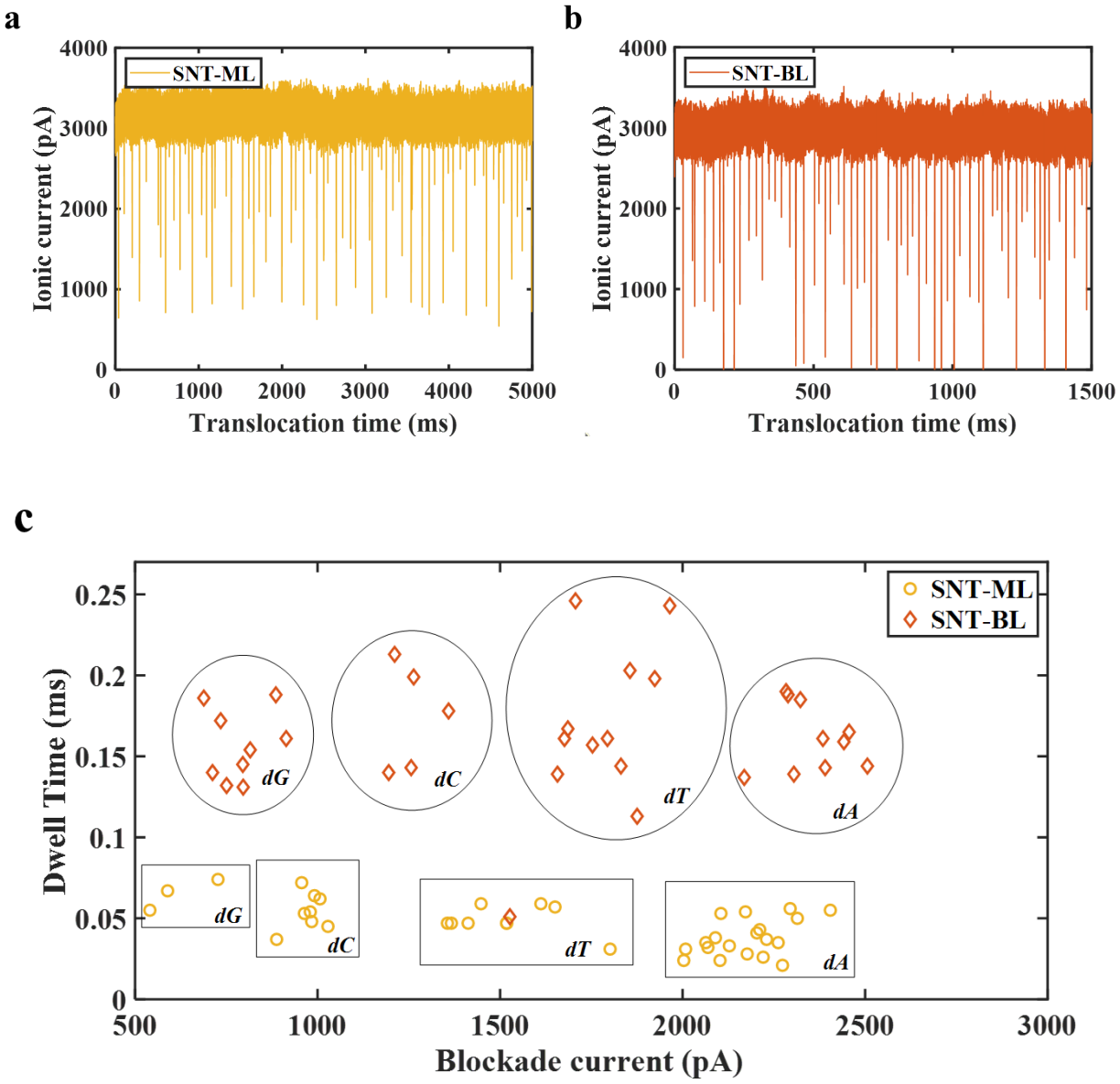



**Figure S6:** Translocation traces for a mixture of the four nucleotides through **(a)** ML and **(b)** BL $MoS_2$ nanopores and **(c)** dwell time vs blockade current plot for the same.

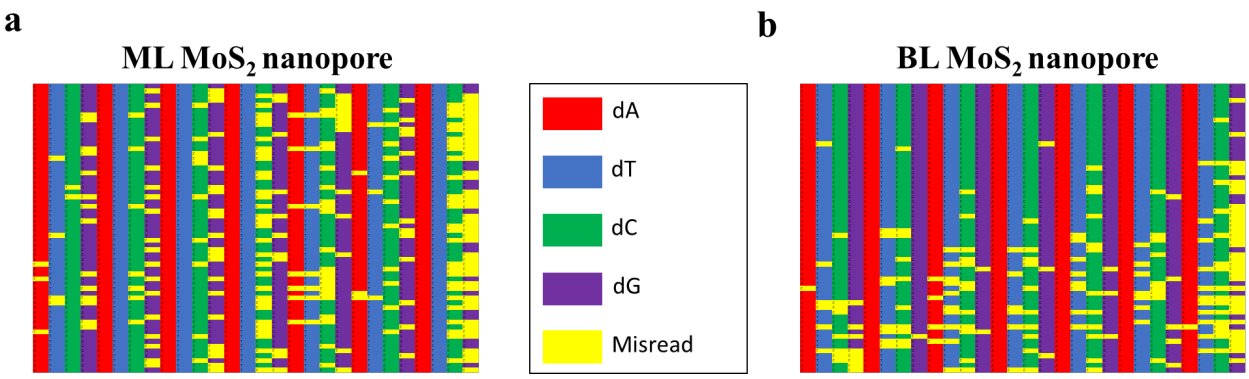


**Figure S7.** Color coded sequence for 60 ssDNA molecules (1800 nucleotides) detected through **(a)** ML and **(b)** BL $MoS_2$ nanopore: dA (red), dT (blue), dC (green), dG (violet) and undetected (yellow).

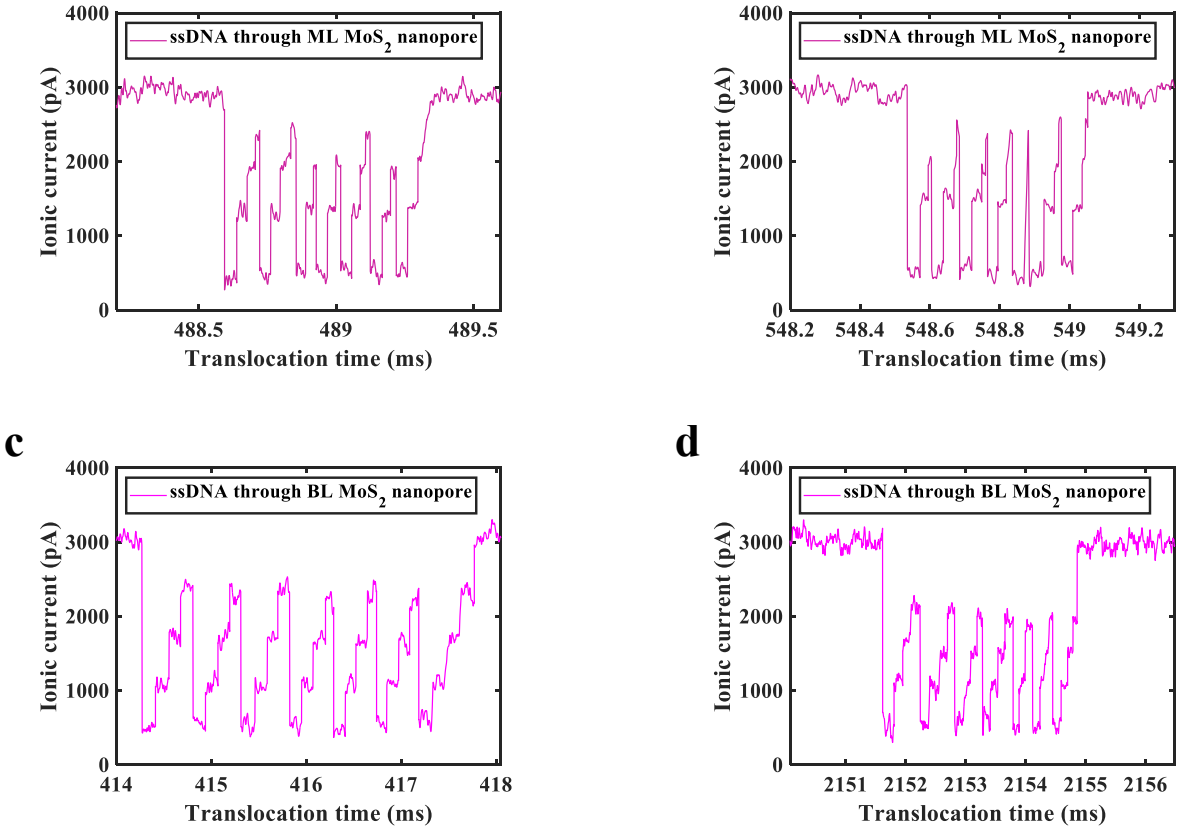



**Figure S8:** Few instances of 3'-5' sequencing of ssDNA translocation through **(a-b)** ML and (**c-d)** BL $MoS_2$ nanopores

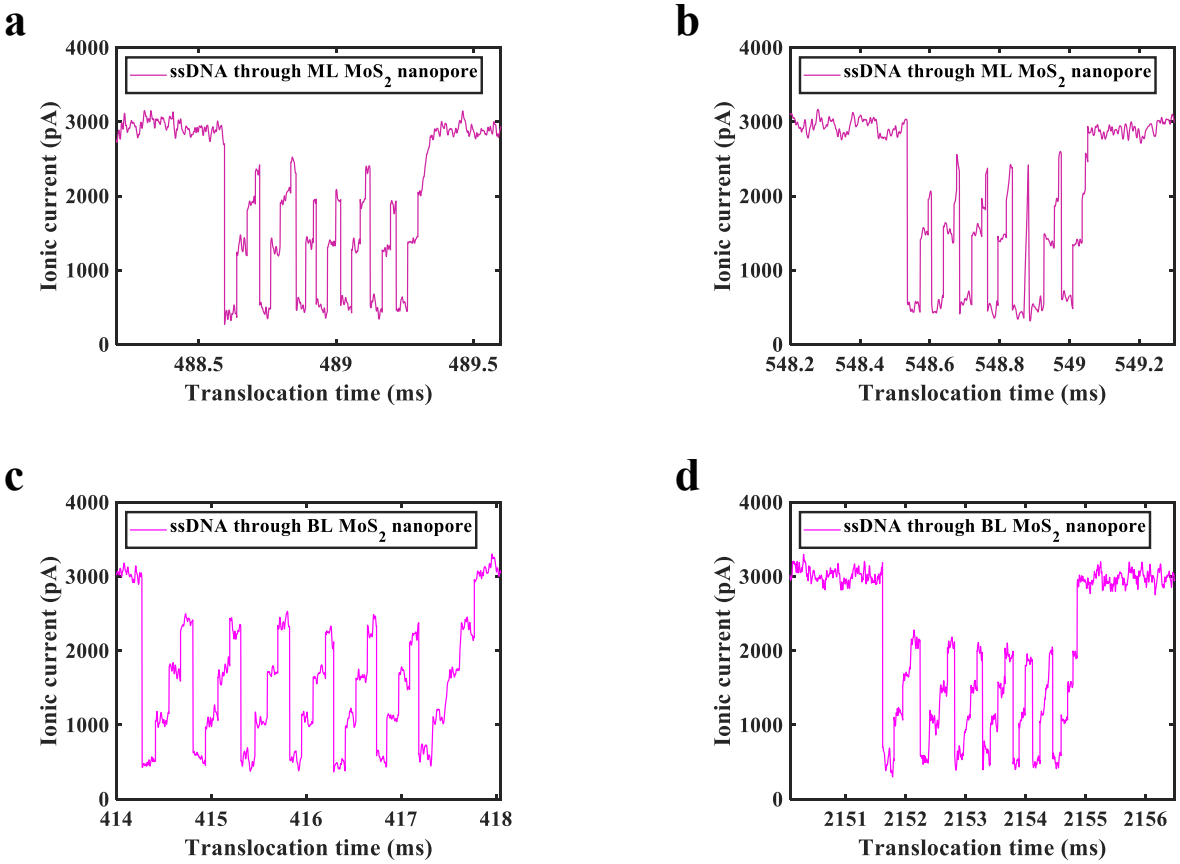
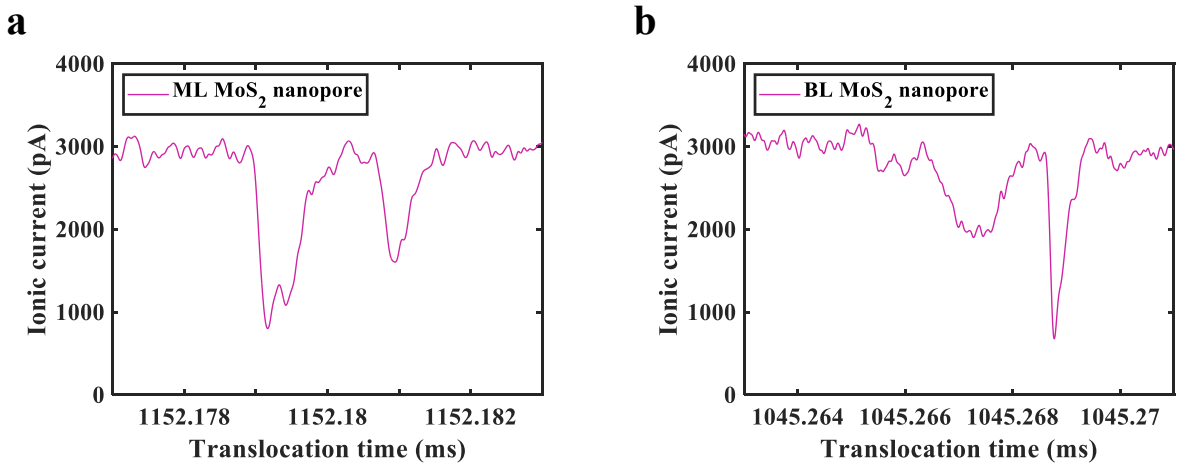



**Figure S9:** Few instances of 5'-3' sequencing of ssDNA translocation through **(a-b)** ML and (**c-d)** BL $MoS_2$ nanopores



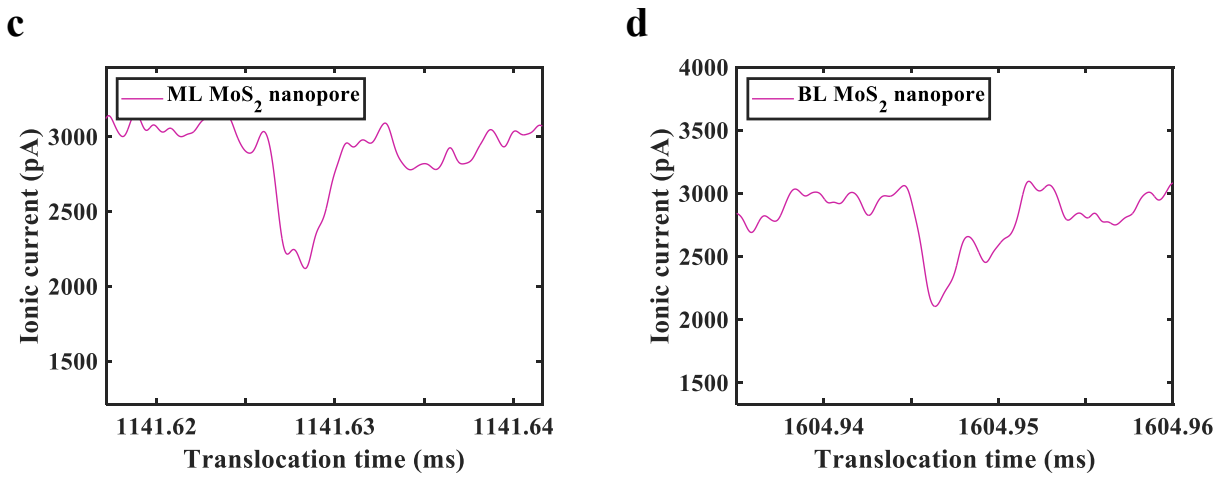



**Figure S10:** Transient ssDNA bouncing events observed for **(a)** ML $MoS_2$ and **(b)** BL $MoS_2$ nanopore and single nucleotide non-translocation interactions observed for **(c)** ML $MoS_2$ and **(d)** BL $MoS_2$ nanopore.

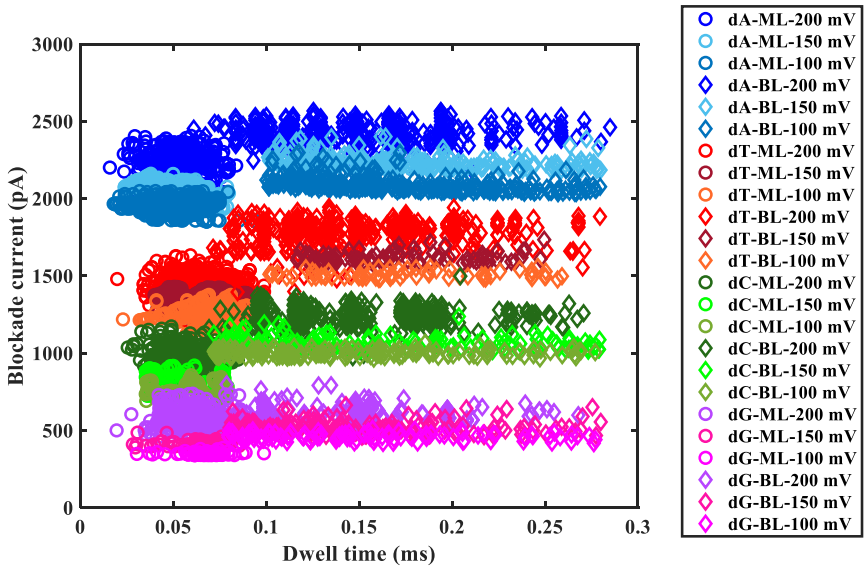
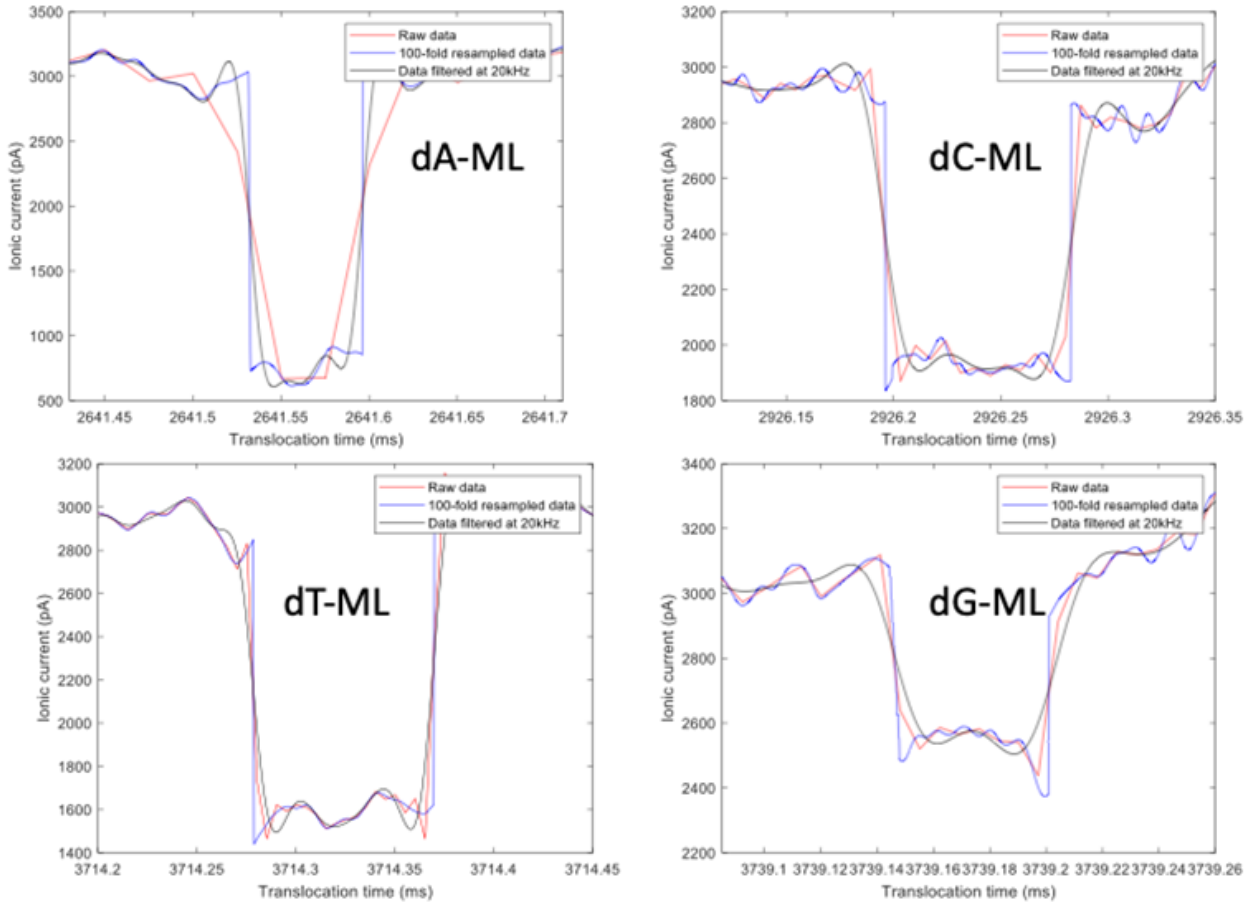



**Figure S11:** Blockade current vs dwell time scatter plot for single nucleotide translocations through ML and BL $MoS_2$ nanopores for 100 mV, 150 mV and 200 mV transmembrane bias.

**Table T1:** T-test results conducted on ~300 nucleotides (each for dA, dT, dG and dC) and polynucleotide molecules (dA30, dT30, dC30) translocated through ML and BL $MoS_2$ nanopores.

| Analyte type | Dwell time difference (ms) | Confidence interval (ms) | p-value |
|---|---|---|---|
| dA | 0.0879 | 0.0785 - 0.0973 | 1.10E-39 (<0.05) |
| dT | 0.0853 | 0.0764 - 0.0942 | 7.65E-43 (<0.05) |
| dC | 0.0813 | 0.0720 - 0.0905 | 1.37E-36 (<0.05) |
| dG | 0.0797 | 0.0691 - 0.0902 | 2.81E-28 (<0.05) |



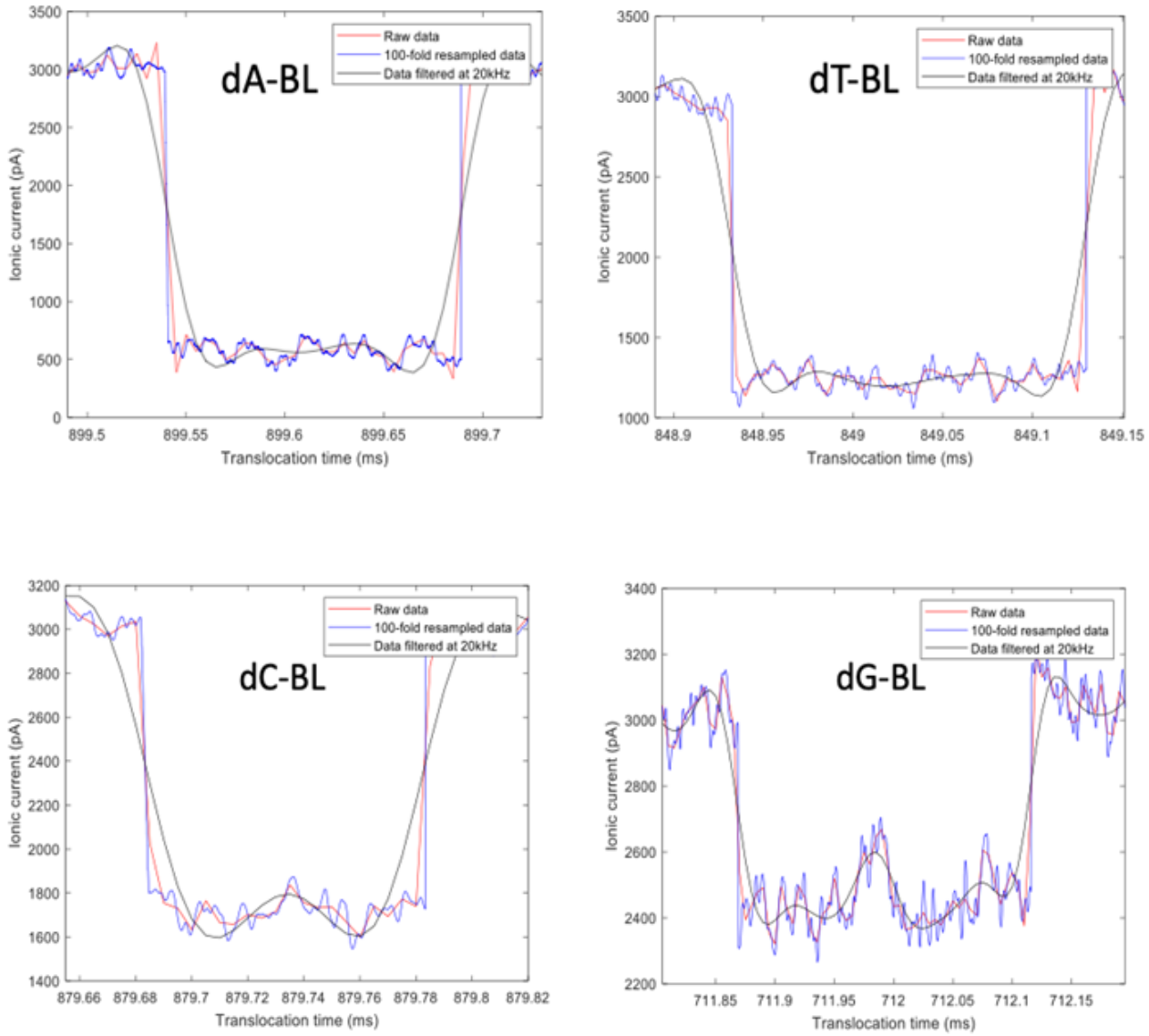



**Figure S12**: Single nucleotide translocations through the ML and BL $MoS_2$ for different nucleotides are shown. Each plot shows the raw data, 100-fold upscaled data and the data filtered at 20kHz for dA, dC, dT, dG for the ML and BL $MoS_2$. It can be observed that no additional artifacts were induced due to oversampling except obtaining crisp current drop (this is similar to the optimization used in Clampfit analysis as well for dwell time and blockade calculation). Also, the peaks demonstrate a rise time of ~30 us after filtering (which is close to the one expected for the 8-pole Bessel filter).

The following protocol was used for the analysis of our raw data acquired using Axon patch.

1. The data was recorded with Multiclamp 700 B without the in-built Bessel filter for both primary and secondary output. Data was recorded at a sampling rate of 200 kHz.
2. Raw data was processed using the following two methods
    a. Raw data was filtered using an 8-pole Bessel filter in Clampfit software. This was done to separate translocation events from interaction noise.
    b. Data was imported in MATLAB and was up sampled (100 times) to obtain accurate dwell time.
3. This analysis does not affect the dwell time or blockade value, but it does change the rise and fall time of the peaks.
4. The up sampled and filtered data were compared and events only with the dwell time > 2Tr (of the filtered data) are identified and considered for efficiency analysis.