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Detection of Methylation on dsDNA using Nanopores in MoS₂ Membrane

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Abstract

Methylation at the 5-carbon position of the cytosine nucleotide base in DNA has been shown to be a precursor to carcinogenesis. Early detection of methylation and intervention could drastically increase the effectiveness of therapy and reduce the cancer mortality rate. Current methods for detecting methylation involve bisulfite genomic sequencing, which are cumbersome and demand a large sample size of bodily fluid to yield accurate results. Hence, more efficient and cost effective methods are desired. Building off our previous work, we present a novel nanopore-based assay using a nanopore in a MoS₂ membrane, and the methyl-binding protein (MBP), MBD1x, to detect methylation on dsDNA. We show that the dsDNA translocation was effectively slowed down using an asymmetric concentration of buffer and explore the possibility of profiling the position of methylcytosines on the DNA strands as they translocate through the 2D membrane. Our findings advance us one step closer towards the possible use of nanopore sensing technology in medical applications such as cancer detection.

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Keywords

nanopore; molybdenum disulfide; methylated DNA; cancer detection; methyl-binding protein; hypermethylation; hypomethylation

Cancer research is often centered around early detection and finding tumors before they metastasize. DNA methylation, defined as the addition or removal of a methyl group at the 5-position of the cytosine nucleotide, has been established as an early event in carcinogenesis^{1–3} with many promoter genes affected by aberrant methylation being linked to tumor formation.^{4–6} In addition, high-throughput methylation analysis has unveiled that aberrant DNA methylation is correlated to both premalignant and malignant neoplasia.^{7–10} Consequently, methylation pattern analysis in DNA can play a very critical role in the diagnosis of precancerous and early-stage cancer. However, current methods of analyzing genome-wide methylation rely heavily on bisulfite genomic sequencing.¹¹ This method requires a large sample volume due to DNA degradation during the bisulfite conversion and exhibits low PCR efficiency.^{5, 12–13} Previous studies have reported the feasibility of detecting cancer by methylation pattern analysis from genomic extracts of human bodily fluids such as plasma, serum, urine, and stool.^{5, 14–15} However, only a minuscule amount of methylated DNA can be obtained from body fluids.¹³ As a result, most conventional methylation assays are not suitable for detecting the extremely low level of methylated DNA in bodily fluids. This presents a need for a less labor intensive and direct method to characterize methylation. Our previous work has successfully investigated the possibility of nanopore-based devices for detection of hypermethylation, coarse quantification of methylation sites, and coarse profiling of single dyad methylation pattern. Thus, we believe that the nanopore technology holds significant promise for detection of methylation for precancerous and early-stage cancer detection.^{16–17}

Nanopore technology is a cost-effective, high-throughput platform that could assist in various medical applications such as immunoisolation bio capsules, drug delivery devices, and targeted biorecognition platforms.¹⁸ Solid-state nanopores are favorable because they can operate in various liquid media and pH conditions as well as their production being scalable and compatible with other detection techniques^{19–21} and other nanofabrication techniques.^{22–23} However, certain obstacles, such as controlling translocation time and discriminating between bases and proteins, introduce complications that limit commercial use of the solid-state nanopore. In particular, they tend to exhibit relatively lower single molecule detection sensitivity due to the thickness of conventional membranes and inconsistent surface charge distribution.^{22, 24–25} Although solid state nanopores yield low ionic current signal-to-noise ratios, graphene nanopores, in theory, can exhibit favorable detection sensitivity when compared to other solid-state nanopore such as SiN_x.²⁶ In this paper, we explore translocation of naked double-stranded DNA (dsDNA) and methylated dsDNA-MBP complex through nanopores drilled in a two-dimensional molybdenum disulfide (MoS₂) membrane. In addition, to attest to its viability as an alternative to graphene nanopores, we also present experiments with a buffer of asymmetric molarity to slow down translocation of biomolecules through the pore.

RESULTS AND DISCUSSION

Fabrication and Current-Voltage Signature of the Nanopores in MoS₂

Solid-state nanopores can be used as inexpensive and high-performance biosensors that are capable of the single molecule detection of a wide variety of analytes of medical interest, ranging from small molecules to post-translationally modified proteins.²⁷ Specifically, the nanopore biosensing platform has become especially attractive in the realm of DNA sequencing.^{22, 28} Nanopores use the principle of ionic current spectroscopy to electrically distinguish the unique current blockage signatures of each nucleotide base.^{16, 29} Theoretically, the graphene nanogap of 1.6 nm would read the transverse conductance of the translocating DNA and could lead to an error-free read-out.³⁰ Atomically small graphene nanopores, closely resembling the diameter of dsDNA, have a high sensitivity to infinitesimal changes in the outer diameter of the translocating DNA.³¹ These nanopores can resolve nanoscale-spaced molecular structures with a resolution of less than 0.6 nm along the length of the molecule. However, the unique density of the states of graphene and the absorption of water molecules are factors that could increase error in graphene nanopore readings.³⁰ more importantly, graphene nanopores have a strong hydrophobic interaction with ssDNA, which causes the DNA to attach to the graphene membrane and impedes translocation.³² To overcome this, the surface of the graphene membrane often must be treated with agents such as 16-mercaptohexadecanoic acid in 8:2 toluene/ethanol to demote DNA adhesion and promote translocation.^{24, 33–34}

Alternative materials have been explored to eliminate the need for additional surface treatment protocols.²² Molybdenum disulfide is a novel atomically thin material that has been recognized as a possible alternative to graphene. As shown in Gaur et. al., MoS₂ membranes grown below 900 °C exhibit high surface energy and semi-crystalline structure that is associated with a decreased hydrophobicity and increased wetting.³⁵ The less hydrophobic surface nature of the MoS₂ grown under optimal conditions allows for successful threading of dsDNA of different lengths and conformations, displaying superior yield.²⁴ MoS₂ nanopores also exhibit a lower failure rate at high ionic strength solutions,²⁴ and show four distinct ionic current signals for four homo-nucleobases.²² Unlike graphene, the semiconducting bandgap of MoS₂ is independent of the width of the nano-ribbon and can allow for detection of changes in potential induced in the liquid environment due to the translocation of the DNA.

Figure 1a illustrates the layout of the MoS₂ nanopore structure that we have fabricated. The free standing MoS₂ monolayer was situated on a 12mm × 12mm substrate structure consisting of 300 nm thick SiN_x and 20 nm thick Al₂O₃ that were deposited using PECVD (plasma enhanced chemical vapor deposition) and ALD (atomic layer deposition). An 80-micron hole was opened on the backside of the silicon substrate using DRIE (deep reactive ion etching), and a concentric ~500 nm hole was subsequently opened through the Al₂O₃ using a FIB (focused ion beam) (See the Supporting Information for the detailed process). The MoS₂ membrane was grown using CVD (chemical vapor deposition) and transferred to the device, as described in Supporting Information, and the MoS₂ nanopore was subsequently drilled using TEM (transmission electron microscopy) as described in Methods

Section. Figure 1b shows TEM image of a typical ~ 6 nm MoS₂ nanopore. Nanopores for this experiment were created between 4 nm and 6.5 nm in diameter, and the current-voltage characteristic of the MoS₂ nanopores were measured in both 1 M KCl and 0.6 M KCl as shown in figure 1c. Figure 1d shows a TEM image of free standing MoS₂ membrane over the FIB hole that confirms no defects were present on the membrane before drilling of the nanopore on the MoS₂ membrane. Also, high-resolution transmission electron microscopy (HRTEM) showed a honeycomb-like image that is unique to MoS₂, thus confirming that the MoS₂ membrane was properly grown by the CVD method, as shown in figure 1e. Additional characterizations were performed to analyze the quality of MoS₂ membrane using Raman Spectroscopy, as described in Supporting Information. The thickness of the MoS₂ membrane was examined by atomic force microscopy (AFM) as shown in Supporting Information. The height profile indicates that the thickness of the CVD grown MoS₂ membrane corresponds to the thickness of a single MoS₂ layer.

Detection of 10 kb double-stranded DNA

To examine the feasibility of translocating dsDNA through a MoS₂ nanopore, random sequences of 10 kbps dsDNA were introduced in a 6.5 nm MoS₂ nanopore. The dsDNA sequences were transported through the MoS₂ nanopore at various voltages in the buffer solution of 0.6M KCl at pH of 7.4 containing 10 mM Tris and 1 mM EDTA. Representative data traces of 10 kbps dsDNA transports at 500 mV, 700 mV, and 1000 mV are shown in figure 2a, 2b, and 2c. Distinct downward current blockages were observed in each of the data traces, undeniably confirming that the dsDNA transported through the MoS₂ nanopore. Figure 2d shows a scatter plot of all 10 kbps transports events at the displayed voltages. The data trace of 700 mV displayed higher dsDNA transport occurrence than that of the data trace of 500 mV, and the 1000 mV data trace showed more than that of the 700 mV data trace as expected. The higher occurrence of the dsDNA transport with increasing voltage is in agreement with previous observations of dsDNA transports in SiN_x.¹⁶ To further investigate the dsDNA transport through the MoS₂ nanopore, the current blockages and the translocation duration were analyzed. To obtain current blockage values of dsDNA transports, a histogram built with blocked current data produced by dsDNA transports was fitted with a Gaussian function as shown in figure 2e. The current blockages of 10 kb dsDNA through a 6.5 nm MoS₂ nanopore were 1.27 ± 0.24 nA at 500 mV, 1.7 ± 0.41 nA at 700 mV, and 2.35 ± 0.43 nA at 1000 mV. The amplitudes of current blockages were increased at higher biased voltages as shown in figure 2g, in agreement with the trends of dsDNA transport observed in other nanopores.^{36–37} Figure 2f shows the transport duration values of the dsDNA which were obtained by fitting an exponential decay function to the dsDNA transport dwell time histograms. As expected, accelerated transport velocity was observed at higher biased voltages. The transport durations were 188 ± 17 μ s at 500 mV, 118 ± 8 μ s at 700 mV, and 83 ± 6 μ s at 1000 mV as shown in figure 2h. As observed in dsDNA transport in other material nanopores,^{16, 36–37} higher voltage generally yielded a stronger blockage current, but shorter transport duration times. Findings with 10 kbps dsDNA transports through the 6.5 nm MoS₂ nanopore were in concordance with observations in previous studies. Molecular dynamic (MD) simulations also demonstrated larger current blockages and reduced transport duration by transporting dsDNA through a MoS₂ nanopore at the higher voltages as shown in Supporting Information, thus validating our experimental

findings. Previous studies have indicated the challenges of dsDNA transport through graphene nanopores due to the affinity of graphene surface to the DNA causing the DNA strand to adhere to the surface of the nanopore membrane.³⁶ Such difficulty was not observed on dsDNA transports through the MoS₂ nanopore in our studies as it was much less difficult to wet the nanopore membrane. However, a higher biased voltage, greater than 800 mV, can enlarge the open pore in the MoS₂ nanopore³⁸ as was evident in a previous study after recording DNA transports at 1000 mV. Also, the enlarging of the MoS₂ nanopore can be seen in figure 2b and 2c, where occasional upward spikes and increased noise level at 700 mV and 1000 mV were demonstrated. We have tested the current-voltage characteristic before and after applying 1000 mV, and the result indicate that the MoS₂ nanopore can be enlarged through electrochemical reaction (supporting information).

Detection of naked and Methylated dsDNA fragments

DNA methylation patterns are often correlated to tumor formation and cancer progression. Especially, previous studies reported that hypermethylation is associated with many types of cancer and potentially correlated with metastasis in many other tumor types.^{5–6, 39–41} Previous studies have also demonstrated that nanopore sensors cannot discriminate between methylated DNA and unmethylated DNA without labeling of some sorts.¹⁶ One method to utilize nanopore technology to detect methylation is to attach methyl binding proteins (MBPs) to the methylated DNA. MBPs selectively bind to the methylcytosine bases on methylated DNA, thereby confirming its presence in the sequence.^{16–17} Our previous studies have presented the selective labeling of methylated sites on dsDNA using MBPs. Herein, we used the same strategy and DNA with the same methylated pattern to show the ability of the MoS₂ nanopore to discriminate naked DNA from hypermethylated DNA (hyMethDNA). Target DNA were 90 bp sequences with 30 *CpG* sites. No methylation was added to the naked DNA and 10 methylcytosine domains were added on hypermethylated DNA to have uniformly distributed methylation sites. For our experiment, a nanopore with a 7.2 nm diameter was used for detection of the 90bp dsDNA fragments in our nanopore experimental setup of 1M KCl at pH 7.2 containing 10 mM Tris and 1 mM EDTA. We first examined the detection of 90bp naked dsDNA through a MoS₂ nanopore at 200mV to prevent the MoS₂ nanopore from damage resulting from the enlargement of the nanopore at the high biased voltage. However, translocation events under these conditions were unnoticeable. Interestingly, upon lowering the applied voltage to 100 mV, transport events for the 90bp dsDNA were observed. It was surprising to see the transport of dsDNA through MoS₂ at these low biased voltages as other membranes typically require higher voltages to reveal translocation events. Representative transport sample events of 90bp dsDNA fragments through a MoS₂ nanopore are displayed in figure 3a, 3b, and 3c for applied voltages of 50mV, 80mV, and 100mV, respectively. To date, SiN_x and graphene nanopore experiments have not reported dsDNA transport below 200mV. Possible explanations for the lack of transportation data with these commonly used membranes could be the negative surface charge of the SiN_x membrane and the hydrophobic surface affinity to graphene causing DNA to adhere. This is especially relevant for the nanopores in SiN_x membranes ranging from 10~30 nm in thickness, where a smaller pore diameter than membrane thickness results in a pore that behaves like a nanochannel, increasing the entropic barrier that the DNA molecule needs to overcome to result in translocation. Since the isoelectric point of SiN_x is

~4 and negatively charged in experimental solution at pH 7.2,⁴² the dsDNA would possibly be repelled at bias voltages under 200mV. In comparison, a pore in a thin MoS₂ 2D membrane does not repel dsDNA. The values of transport current blockage and transport duration were obtained by fitting a Gaussian function, and an exponential decay function to the blocked current histogram and transport dwell time histogram respectively as demonstrated in figure 3d and 3e. The MoS₂ nanopore of 7.2 nm in diameter could detect 90bp dsDNA, with blocked current of 282 ± 22 pA at 50 mV, 312 ± 13 pA at 80 mV, and 376 ± 16 pA at 100 mV as shown in figure 3f and as well as dwell time of 53 ± 3 μ s at 50mV, 36 ± 4 μ s at 80 mV, and 32 ± 2 μ s at 100mV as shown in figure 3g. The increase in the bias voltage to the original 200 mV did not result in noticeable transport events as demonstrated in figure 3h. However, the addition of hyMethDNA bound with MBD1x produced detectable events as shown in figure 3i. Representative sample events of hyMethDNA/MBD1x complex through the MoS₂ nanopore are presented in figure 3j. To form the complex of hyMethDNA and MBD1x, the two molecules were mixed and incubated at room temperature for 15 minutes before experiments. While recording when naked DNA was in the solution and no translocations were observed, hyMethDNA fully bound with MBD1x were introduced to the chamber. Surprisingly, the hyMethDNA with MBPs displayed a signature current blockage as shown in figure 3j and were selectively detected at 200mV. It can be inferred that the complex translocated through the pore slower than naked DNA due to the altered larger physical dimension brought on by annexing 10 MBD1x on the dsDNA fragment, in addition to the positive net charge of the MBD1x on the DNA reducing the overall charge on the complex. The isoelectric point of MBD1x is known to be 8.85,⁴³ thus it would be positively charged in the solution with pH 7.2. Not only does this serve as a confirmation of the presence of a methylcytosine base on the DNA sequence and the affinity of the MBP to the methyl group, but it also alludes to the ability of MoS₂ nanopores in detecting methylated DNA. The structure of the complex is presented in figure 3h to illustrate methylation and the presence of MBD1x in the DNA. The unmeasurable naked dsDNA transport events at 200mV (figure 3i) can be explained by translocation that was too rapid. The DNA translocation duration time was demonstrated to decrease at higher voltages in a voltage-dependent manner. Fitting with an exponential decay function (Supporting Information) shows the expected value of translocation of 90 bp dsDNA transport at 200 mV to be ~10 μ s, which is close to the data acquisition time interval of 10 μ s of the experimental setup (see methods section), and hence was likely out of the measurement range. Figure 3l shows obtained current blockage and transport durations of naked DNA and hyMethDNA/MBD1x complex along with the expected current blockage and duration of 90bp dsDNA fragments transport at 200 mV, which is unnoticeable under our experimental recording condition.

Slowing down dsDNA transport and detection of a single CpG site in dsDNA fragment

Hypomethylation, the loss of methylation in DNA when compared to normal levels, is another major epigenetic modification in cancer cells. The pattern of DNA epigenetic alterations in cancer varies from the individual CpG dyad at the local level, to methylation in 1 million base pairs, to DNA demethylation during carcinogenesis. This results in loss of methylation on both strands via possible intermediates of hemimethylated dyads.⁴⁴

However, conventional methylation assays such as methylation-specific PCR is technically limited and challenging for the diagnosis of DNA hypomethylation.⁴⁵ Although our previous study demonstrated discrimination between hypomethylated DNA and individual *CpG* dyad at the center of sequences by labeling with MBP, profiling of the methylation pattern using thick SiN_x membrane was not possible.¹⁷ In that study, the single MBP was in the middle of the molecule and the electrical peak indicating the methylation location was broad. Herein, we report the methylation profile of dsDNA through a MoS₂ nanopore with a slow translocation velocity due to an asymmetric salt gradient.⁴⁶ Longer translocations can allow for high-resolution measurements to distinguish individual nucleotide bases.⁴⁷ Various strategies, from applying gel media on the cis or trans side of the membrane, to varying voltage or buffer solution concentration gradient, have been implemented to slow translocation and suspend DNA strands in the pore.^{47–48} For our experiment, we used an asymmetric buffer solution of 0.6M KCl on the cis side of the membrane and 3M KCl on the trans side. Our MoS₂ nanopore-based methylation assay demonstrates detection of a single methylation *CpG* dyad site at the end of 90bp dsDNA. We utilized the MBD1x and chose a single methylation *CpG* dyad to check the viability of MoS₂ nanopore on detection of hypomethylated DNA. The target DNA fragments were designed to have a single *CpG* dyad methylated site near to the end of the strand, 84th to 87th bases in 90 bps dsDNA (endMethDNA). Both naked DNA and the endMethDNA bound with a single MBD1x were used for translocation experiments in a 9 nm MoS₂ nanopore and both displayed measurable current blockade signatures as shown in Figure 4a. Samples of 90bp naked DNA (Figure 4a top) and MBD1x bound endMethDNA (Figure 4a bottom) are shown in Figure 4a and the corresponding scatter plot is shown in Figure 4b. The transport current blockage values of both naked DNA, -277 ± 20 pA, and complex, -600 ± 66 pA, were obtained using a Gaussian function as shown in Figure 4c. The translocation times of naked DNA and the endMethDNA/MBD1x were also obtained by fitting a Gaussian function to transport dwell time histogram as shown in Figure 4d. In comparison to dsDNA transport in symmetric 1M KCl shown in Figure 3, using asymmetric salt gradient significantly slowed down the transport duration of 90bp naked DNA. Although direct comparison is not appropriate due to the large diameter of the nanopore used for this experiment, naked DNA was successfully detected with a duration of 1.23 ± 0.21 ms at 200 mV. In comparison with the expected transport in Figure 3I, the transport was slowed down ~100-fold. The endMethDNA bound with a single MBD1x translocated through the MoS₂ nanopore at 1.39 ± 0.23 ms, which was a ~160 μ s slower translocation than the naked DNA. While we had previously found ~20-fold difference between naked DNA and locally methylated DNA through SiN_x nanopores, the similar range of translocation duration between naked DNA and endMethDNA was unexpected. However, the similar range of transport duration between naked DNA and endMethDNA/MBD1x can be explained. The negatively charged inner wall of a 10-nm thick SiN_x nanopore is likely to interact with the positively charged methyl-binding protein during transporting, whereas 2-D material MoS₂ nanopore is unlikely to interact during transporting. In addition, compared to hyMethDNA fully bound with MBD1x, endMethDNA possesses only one MBD1x. Thus the overall charge on the molecule is not perturbed. Consequently, the 90bp naked DNA and endMethDNA would not be significantly different in transport duration in the 2D MoS₂ membranes. The main factor impeding the complex transport through a MoS₂ nanopore is biased positive voltage. A demonstration of

atomic-scale translocation dynamics of the complex through a MoS₂ nanopore is shown in Supporting Information. To further analyze the whole data set for the current blockade and transport duration, a box-and-whisker plot was adopted. Figure 4e shows that most current blockages of naked DNA fall in -276 ± 20 pA, and endMethDNA/MBD1x showed two distinct current blockage levels; shallow at -251 ± 39 pA and deeper at -600 ± 66 pA. The shallow current blockage is at similar levels of transport current blockage to that of naked DNA, thus corresponding to transport of DNA region on the complex. The deeper current blockages are associated with transport of MBD1x on the DNA through the nanopore. The ~ 3 -fold increased current blockage by the MBP region is on the same order of magnitude as the current blockage found in previous studies as well.¹⁶ The transport duration of naked DNA and endMethDNA bound with MBD1x is shown in Figure 4f. The transport of the MBP region on the DNA took 0.138 ± 0.11 ms. This translates to 10% of the entire complex translocation over a 139 ms period. Comprehensively, the MoS₂ nanopore can detect the methylation site more precisely than SiN_x membrane. The schematic in figure 4g illustrates dsDNA and endMethDNA bound with MBD1x. Slowed translocation times were observed by varying the buffer concentration.

Methylation site profiling shown in Figure 4h revealed that translocation events with the methylated DNA/MBD1x complex ended with the terminal protein instead of leading with it. It can be inferred from this finding that the positively charged MBD1x near the nanopore is always repelled by the positively applied bias voltage and will spin to the origin while the DNA region of the complex is being pulled into the nanopore. Consequently, all the endMethDNA/MBD1x transport into the pore occurred in a fixed orientation which included the DNA entering first and the protein complex lagging behind. From this, it can be assumed that the negatively charged DNA that was used for the study was attracted by the applied positive charge and pushed towards the nanopore ahead of the region where MBD1x was attached. This was confirmed through profiling of methylation location using the MoS₂ nanopore. The occurrence of deeper current blockage was mainly observed at the end of the whole complex transport, as shown in figure 4h. The abscissa represents the length of the entire complex translocation, normalized and recalculated as 100%. The peak occurrence of deeper current blockage was obtained by fitting a Gaussian function to the occurrence histogram. The fitting value was $90.5 \pm 3\%$ which indicates the deeper current blockages mainly occur at the end of entire complex translocation. This result suggests that the position of methylation on dsDNA can be more precisely detected using nanopores in thin membranes.

Conclusions

We investigate the use of the MoS₂ nanopore for dsDNA translocation and methylation detection experiments. Detection of methylcytosine bases has been reported previously with SiN_x and graphene nanopore sensors, but MoS₂ presents several favorable and specific characteristics that could make it a more viable and robust option than the more commonly used membranes. These characteristics include the ability to produce a small nanopore on a thin membrane while retaining good signal-to-noise ratios and no need for surface treatment to reduce hydrophobic surface interactions as is necessary for graphene membranes. After determining that dsDNA translocation was possible through MoS₂, we also showed that

dsDNA translocation could be slowed by using asymmetric concentration of buffer solutions to provide higher spatial resolution. We demonstrated detection of single MBD1x proteins in a site-specific manner which could be used to distinguish the location of the methyl cytosine nucleotide base on the DNA sequence. We also hypothesized that the charge of the DNA strand coupled with the applied voltage allows one to control the orientation of the translocating DNA strand.

Being able to detect aberrant methylation in a routine lab screening could help identify cancer signatures at various stages or progression. This could prove crucial regarding early intervention and therapy and ultimately lead to an increase in the rate of survival. Work remains to be accomplished in the control of translocation speeds, resolution of signature current blockades, and in profiling the location of attached MBPs. We hope that this work serves as a springboard for future MoS₂ nanopore studies.

MATERIALS AND METHODS

Supporting Substrate Fabrication

Substrates consisting of stacked layers of Al₂O₃ and SiN_x are fabricated on 300 ± 2 μm thick double-side polished <100> silicon wafers purchased from Silicon Quest International. Wafers are piranha cleaned (2:1 H₂SO₄/H₂O₂) for 20 min before deposition SiN_x. 300 nm of low-stress SiN_x is deposited (STS Mesc PECVD system) using a mixed-frequency recipe (high frequency, 6 s at 13.56 MHz, platen power of 20 W; low frequency, 2s at 380 kHz, platen power of 60 W) with precursors SiH₄ and NH₃ at flow rates of 40 and 55 sccm, respectively, at a platen temperature of 300 °C. Subsequently, 20 nm of Al₂O₃ was deposited via ALD (Cambridge Nanotech) at a platen temperature of 250 °C using tetramethyl-aluminum (TMA) and water vapor precursor. Optical lithography is used to define square windows of 80 μm on the back side of the wafer with the aid of plasma resistant Megaposit SPR-220 photoresist and an ABM Flood Exposure (model 60) tool. Then the wafer is placed inside an STS Pegasus ICP (inductively coupled plasma) DRIE and 80 μm square membranes are suspended using a Bosch etching process; 500 to 600 nm holes are then drilled in these membranes using a focused ion beam (FIB) (FEI DB235) operated at a beam current of 30 pA.

Chemical Vapor Deposition of Molybdenum Disulfide

The detailed process of growing and characterizing of the MoS₂ film using Chemical Vapor Deposition on SiO₂ and Sapphire substrates are described in previous studies.^{49–50}

Nanopore Fabrication, Chemicals, and Materials

Single nanopores of various diameters were drilled in the free standing MoS₂ membrane on the supporting substrate with condensed electron beam using a JEOL 2010F field-emission gun TEM operated at 200kV in CBD mode with focused electron probe of diameter at 1.6 nm. All custom DNA fragments were synthesized, purified and purchased from Integrated DNA Technologies (Coralville, IA). The electrical nanopore measurements were performed in 0.6 M, 1 M, and 0.6 M / 3 M KCl at pH 7.2 containing 10 mM Tris and 1 mM ethylenediaminetetraacetic acid (EDTA) for naked DNA fragments and DNA fragments

bound with MBD1x. The methylated DNA/MBD1x complexes were prepared and incubated for 15 min at room temperature ($25 \pm 2^\circ\text{C}$) immediately before the nanopore experiments.

MBD1x Protein Purification

MBD1x purification was described in a previous report.¹⁶

Nanopore Electrical Measurements

The nanopore chip was assembled in a custom-built chamber. Ethanol was then filled in reservoirs of both chambers initially to clean the device and promote wetting. Subsequently, the ethanol was flushed out with deionized water and the reservoirs are filled with desired experimental salt solutions. Ag/AgCl electrodes are immersed in reservoirs for ionic current measurements. All nanopore experiments are performed with Axopatch 200B and Digidata 1440A at room temperature ($25 \pm 2^\circ\text{C}$). Axopatch 200B was used for applying biased voltages and measuring currents through a nanopore, and data were recorded using a Digidata 1440A acquisition system. Data were low-pass filtered at 10 kHz using the built in 8-pole Bessel filter, and recorded at 100 kHz sampling rates. Clampex 10.2 was used for instrumental control, and Clampfit 10.2 was used for data analysis. All nanopore experiments are performed on an antivibration table in a dark double Faraday cage.

MD simulations

The dsDNA was created by the X3DNA program⁵¹. The atomic structure of the terminal-methylated DNA molecule/MBD1 complex was constructed by linking dsDNA fragments to the ends of DNA molecule in the reported crystal structure of the mDNA-MBD1 complex (pdb code: 1IG4)⁵².

All molecular dynamics simulations were carried out using NAMD⁵³, with protein and DNA described by the CHARMM22 force field with CMAP corrections and the CHMARM27 force field, respectively⁵⁴. Water was modeled with the TIP3P water model⁵⁵. An integration time step of 2 fs was adopted. Long-range Coulomb interactions were computed using the particle-mesh Ewald (PME) method⁵⁶. The system was energy minimized for 5000 steps, then heated to 300 K. A 2 ns equilibration under NPT ensemble conditions was performed to equilibrate the system to a desired pressure of 1 atm, during which the temperature was maintained by applying Langevin dynamics and the pressure was maintained by a Nose-Hoover Langevin piston method⁵⁷. This equilibration step was followed by a 4 ns equilibration under NVT ensemble condition. After the equilibration, an external electric field was applied along the z direction to drive the transport of biomolecules through MoS₂ nanopores.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

a. Schematic illustrates the MoS₂ nanopore structure. b. TEM image of typical 6 nm MoS₂ nanopore. c. IV characteristic of MoS₂ nanopores in KCl solution at pH 7.2 containing 10 mM Tris and 1 mM EDTA. d. TEM image shows that MoS₂ layer covers entire FIB pore of 600nm diameter. e. HRTEM shows honeycomb-like MoS₂ membrane structure. (inlet shows zoomed-in image of yellow square).

Figure 2.

a. Data trace of 10 kbps dsDNA transport at 500 mV. b. Data trace of 10 kbps dsDNA transport at 700 mV. c. Data trace of 10 kbps dsDNA transport at 1000 mV. d. Scatter plotting of 10 kbps dsDNA transports in 6.5 nm MoS₂ nanopore at 600mM KCl solution at pH 7.4 containing 10 mM Tris and 1 mM EDTA. e. Current blockade histogram. f. Transport duration histogram. g. Current blockades of DNA transports h. Transport durations of DNA transports.

Figure 3.

a. Sample events of 90 bps dsDNA transports at 50 mV biased voltage in 9 nm MoS₂ nanopore. b. Sample events of 90 bps dsDNA transports at 80 mV biased voltage in 9 nm MoS₂ nanopore. c. Sample events of 90bps dsDNA transports at 100 mV biased voltage in 9 nm MoS₂ nanopore. d. Current blockage histogram in the function of biased voltages. e. Blocked current duration histogram in the function of biased voltages. f. Current blockade of transports. The values are obtained by fitting Gaussian function to the current blockade histogram. g. Blocked current duration of transports. The values are obtained by fitting the exponential decay function to the blocked current duration histogram. h. Current trace of 90 bp dsDNA transport at 200 mV biased voltage in 9 nm MoS₂ nanopore. Momentary transport duration of short DNA is beyond of detection limit of measurement device, consequently transports of 90 bp dsDNA were undetectible. i. Current trace of 90 bp methylated dsDNA-MBP transports at 200 mV biased voltage in 9 nm MoS₂ nanopore. j. Sample events of 90 bp methylated dsDNA-MBP transports at 200 mV in 9 nm MoS₂ nanopore. k. Still image of molecular dynamic simulation showing methylated dsDNA-MBP complex transport through MoS₂ nanopore. l. Transports data comparison between dsDNA and methylated DNA-MBP.

Figure 4.

a. Sample transport events of naked DNA (top) and terminal-methDNA/MBD1x (bottom) in asymmetric solution. b. scatter plotting of transport events. Blue dots represent naked DNA transport and red dots represent the complex. c. Current blockade histogram of naked DNA (blue) and complex (red) transport. d. Transport duration histogram of naked DNA (blue) and complex (red) events. e. Box chart of current blockades. Current blockade of naked DNA is shown in black, DNA region of complex (shallow) is in blue, and DNA/protein region (deeper) in green. f. Box chart of transport durations. Transport duration of naked DNA is shown in black, DNA region of complex (shallow) in blue, DNA/protein region

(deeper) in red, and entire complex in green. g. Schematic shows scaled dsDNA and methDNA/MBD1x complex. h. Methylation site profiling shows that most complex transports end with terminal-protein. It is hypothesized that negatively charged DNA enters the nanopore by applied positive voltage and transport ends with protein.

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