Nanopore Diameter Impact on DNA Methylation Detection Using Methyl Binding Domain Protein Tags

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Methylation of DNA leads to epigenetic modifications like cancer, neurological diseases etc.[1] Early-stage detection of DNA methylation is difficult. Conventional approaches to detect methylated DNA are bisulfite genomic sequencing, methylation-specific PCR, etc., require long time, high cost and large sample volume. Solid state nanopore sensing has emerged as one of the potential tools to study the label free interactions between biomolecules such as DNA and proteins at the single molecule level. The target biomolecules are driven by electrophoretic force to pass through a nanometer size pore in a solid-state material membrane (Fig.1a), producing current signal modulations, which are characterized by the dwell time and current blockage amplitude of passage signals[2]. In this work, the methylation of DNA is detected by the methyl binding domain MBD2 protein, which targets 12-14 base pairs(bp) around a single binding site of methylated DNA (mDNA). This mDNA-MBD2 complex has a much larger size compared to DNA itself, thus producing significant difference in dwell time and current level blockage as compared to unbind mDNA. The binding of DNA and MBD2 complex was confirmed using AFM imaging (Fig. 1b and 1c). To understand the nanopore size impact on the differentiation of mDNA and mDNA-MBD2 complex, we detected 110 bp mDNA with and without MBD2 proteins from 6 and 11 nm pores at 100KHz recording bandwidth and a bias of 100mV and 150mV, respectively. Clearly, mDNA (diameter ~2 nm) could translocate through a 6 nm nanopore, with the translocation and collision events clearly delineated (Fig. 2a). Yet, the mDNA-MBD2 complex, with an estimated diameter of about 6-7nm, could not smoothly pass through, instead producing only collision events with current blockage levels comparable to the collision events for mDNA only (Fig. 2b). Very differently, when the mDNA-MBD2 complex was sent to pass through a 11nm pore, it produced both translocation and collision events with different peak current blockage values. Our AFM imaging and nanopore size scanning tests proved the binding between DNA and MBD2 protein, revealing the potential of nanopores in analyzing DNA-protein interactions (Fig. 2c). The nanopore sensors also have potential to detect and quantify DNA methylations towards early diagnosis of diseases.

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[1] Moore, L., Le, T. & Fan, G. DNA Methylation and Its Basic Function. Neuropsychopharmacol 38, 23–38 (2013).

[2] Xia, Pengkun, et al. "Sapphire-supported nanopores for low-noise DNA sensing." Biosensors and Bioelectronics 174 (2021): 112829.

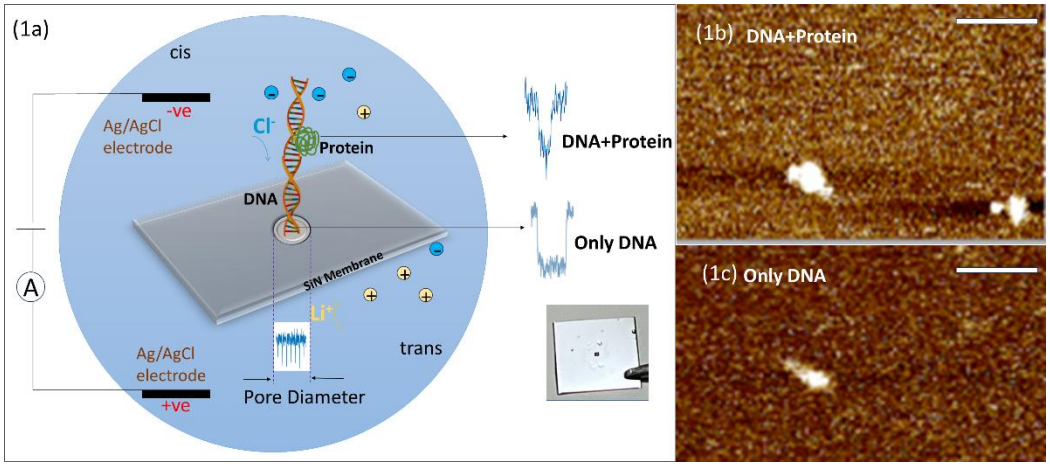

Figure 1: (1a) represents the working of nanopore sensing with DNA bind to protein and corresponding electrical signals. (1b) represents AFM Image of 110bp methylated DNA, scale Is 50 nm. (1c) represents AFM Image of 110 bp methylated DNA bind to MBD2 protein, scale Is 50 nm.

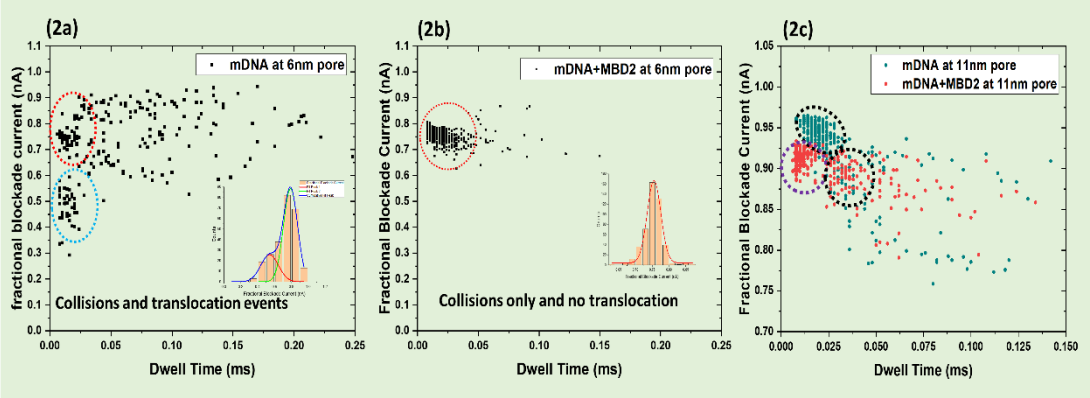

Figure 2: (2a) Scatter plot of current blockage signals of 110bp methylated DNA from a 6nm nanopore. The Insert shows the corresponding histogram for collision (red circle) and translocation (blue circle) events. (2b) Scatter plot of translocation of methylated DNA bound to MBD2 protein through a 6nm nanopore, with Insert showing the corresponding histogram of collision events. (c) Comparative scatter plot to detect 110bp methylated DNA only (green dots with black circle) shows translocation events and 110 bp methylated DNA bound to MBD2 protein (red dots with black circle) in a 11 nm nanopore shows translocation and collision events.