DNA methylation quantification using electro-optical sensing in solid-state nanopores

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Modifications in the methylation patterns of genomic DNA play a significant role in gene expression regulation. Most notably, aberrant methylation patterns, particularly in promoter regions, are associated with cancer development and are therefore considered to be viable cancer biomarkers. Recently, we evidenced the emergence of single-molecule techniques for DNA methylation detection, designed to replace the classical bulk methods. For example, nanopores can be used in conjunction with a large bulky group, bounded to a methylated site, such as methyl-CpG-binding domain (MBD), KZF and Streptavidin proteins. These labels, however, cannot be applied in densely methylated regions due to their bulkiness and require usage of relatively large pores hence reducing the temporal and spatial resolution of the method.

To overcome these limitations, we develop a methyltransferases (MTases) based DNA labeling approach that can target unmethylated CpG sites with high specificity. Our method involves a single-step covalent coupling of a fluorescent tag or a small bulky group directly to the unmethylated CpG sites. The DNA molecules are then quantified one by one using an “optipore” setup capable of recording single molecule fluorescence and electrical signals from a solid-state nanopore, simultaneously. The optical signal obtained by our method scales linearly with the number of unmethylated CpGs in the target sequences of each DNA molecule, permitting a highly quantitative readout of the number of unmethylated CpG in each molecule. Our method can be extended to multiple colors, that can be mapped to different MTases target sequences. Moving forward, for the proof of concept of small bulky group sensing, we also labeled DNA having one unmethylated site with Gamma Cyclodexterin using the DNA MTase M.TaqI, and we show representative two-level blockade events.

The nanopore/MTase method is highly sensitive and allows detection of hypermethylated promotors of tumor suppressor genes as well as promotors of oncogenes which are often hypomethylated in tumor DNAs. It can serve for sensing epigenetic biomarkers and potentially be used as a diagnostic tool for tumor DNA at a preliminary stage of the disease.

References

Gilboa, T.; Torfstein, C.; Juhasz, M.; Grunwald, A.; Ebenstein, Y.; Weinhold, E.; Meller, A. Single-Molecule DNA Methylation Quantification Using Electro-Optical Sensing in Solid-State Nanopores. *ACS nano* **2016,** *10,* 8861-8870.