

Nanopore-Based Detection of Cancer Biomarker at Molecular Level

Methylation on DNA is an epigenetic modification of DNA in which methyl groups are added at the 5-carbon position of cytosine. Aberrant DNA methylation, which has been associated with carcinogenesis, can be assessed in various human biological fluids and potentially be used as biomarkers for detection of cancer at early-stage. Analytically sensitive and specific assays for methylation targeting low-abundance and fragmented DNA are needed for optimal clinical diagnosis and prognosis. We present a solid-state nanopore-based direct methylation detection assay that circumvents bisulfite conversion and PCR amplification. We used methyl-binding proteins (MBPs), which selectively label the methylated DNA. The nanopore-based assay selectively detects methylated DNA/MBP complexes through a 19 nm nanopore with significantly deeper and prolonged nanopore ionic current blocking, while unmethylated DNA molecules were not detectable due to their smaller diameter. Discrimination of hypermethylated and unmethylated DNA on 90 bp, 60 bp, and 30 bp DNA fragments was demonstrated using sub 10 nm nanopores. Hypermethylated DNA fragments fully bound with MBP are differentiated from unmethylated DNA at 2.1-fold to 6.5-fold current blockades and 4.5-fold to 23.3-fold transport durations. These nanopore assays can also detect *CpG* dyads in DNA fragments and could someday profile the position of methylated *CpG* sites on DNA fragments. Our findings advance us one step closer towards the possible use of nanopore sensing technology in medical applications such as cancer detection.