QUANTUM TECHNOLOGIES, METAMATERIALS, AND THE FUTURE OF CB SENSING

Detection Of Enzymatic Activity Using Commercial-off-the-shelf (COTS) Nanopore Sequencing-based Instrument

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Many biological agents' mechanism of activity is enzymatic base removal or other forms of nucleic acid damage that ultimately lead to cell death. The detection of enzymatic activity on specific residues in biological and environmental matrices has traditionally been performed with mass-spectrometry, fluorescence, and cell-based assays which require high-resourced laboratories. There is a need for portable and scalable diagnostics that can be performed by non-experts for detecting nucleic acid damage in the field, clinic or in a low-resourced laboratory. An assay has been developed to detect enzymatic activity on a nucleic acid target using a portable nanopore-based DNA sequencer that is already in use as a COTS platform in federal laboratories and in the field. This assay uses a synthesized single-stranded DNA (ssDNA) substrate that contains multiple enzyme target residues (i.e., motifs) that can be sequenced in real-time on the COTS nanopore platform. The assay utilizes the standard sequencing preparation kit and manufacturers' software to generate substrate reads. The substrate is designed to detect the presence of active enzyme based on base modifications to the substrate. These base modifications are captured as a metric called mismatch proportions which compares the number of missing/matched bases across substrate reads. The ability of this assay to detect base modification due to enzymatic activity was tested by exposing the nucleotide substrates to a concentration range of target enzymes (0 - 475 nM) in a buffered system. Based on the mismatch proportion metric, reproducible differences in substrate modifications were observed when enzyme was present versus absent. In the buffer system, enzyme concentrations as low as 4.75 nM showed distinct enzyme activity patterns compared to the no-enzyme controls. The assay has been successfully used to detect activity across a range of enzyme concentrations in several diagnostic and environmental sample matrices. The enzyme activity assays have also been performed by a secondary laboratory. The assay enables specific, sensitive, and accurate enzyme detection and quantitation out of a laboratory and into resource-limited environments for military and first responder users.