TOXIN DIAGNOSTICS – DEVELOPMENT OF NOVEL, FIELDABLE TECHNOLOGIES TO DIAGNOSE TOXIN EXPOSURE

Direct Detection Of Peptides Following Enzymatic Cleavage Using A Field-portable Nanopore Sequencing Device

Fanny Chu Pacific Northwest National Laboratory **Sarah Jenson** Pacific Northwest National Laboratory **Kai-For Mo** Pacific Northwest National Laboratory **Matthew Turner** Pacific Northwest National Laboratory **Kristin Engbrecht** Pacific Northwest National Laboratory **David Wunschel** Pacific Northwest National Laboratory

Conventional enzyme activity detection approaches have relied on ELISA and mass spectrometry assays. However, ELISA assays suffer from insufficient sensitivity and mass spectrometry assays are typically not field-portable. Alternatively, nanopore sequencers have recently emerged as a promising rapid and sensitive tool. An additional advantage of this technology is field-portability, which can meet point-of-care diagnostic needs, such as detection of biomarkers in a clinical setting, or detecting activity of bacterial virulence factors.

However, few studies have focused on peptide detection using nanopore sequencers; these devices have conventionally been used for long nucleotide sequencing. Commercial nanopore systems can differentiate between the four nucleotide bases for read calling, but no such software is available for “reading” amino acids. Further, owing to short peptide lengths compared to oligonucleotides, their translocation through a nanopore is substantially shorter, on the order of milliseconds, whereas conventional nucleotide reads persist on the order of hundreds of milliseconds to a second, which makes peptide signal detection much more challenging.

We present a novel, field-portable strategy for enzyme activity detection using a nanopore sequencing device via direct detection of bait peptides as enzymatic cleavage products. Using a commercial-off-the-shelf (COTS) nanopore sequencing device from Oxford Nanopore Technologies, we have developed an approach to differentiate cleaved and uncleaved forms of peptide substrates for targeted enzyme detection.

To overcome software limitations of peptide detection, a data analysis pipeline using R and Python was developed to identify peptide signals acquired on the nanopore device with R9 pore chemistry. Briefly, data processing includes differentiation of baseline (i.e., when the nanopore is unoccupied) and nanopore occupation signals, and “square” feature detection with multiple filtering steps to detect translocation of peptides of interest. We capture features of interest by characterizing their signal length and R/O ratio, which is the ratio of signal depth to the baseline signal.

We examined different peptide substrate forms, substrate concentration, and mixtures of uncleaved and cleaved substrate forms to optimize for reproducible peptide translocation and substrate form differentiation. Bait peptide substrate with addition of an N-terminal polyanionic tail, loaded at 1 µM concentration, showed the greatest peptide translocation reproducibility among replicates and minimal feature overlap between the uncleaved and cleaved substrate forms. Further assay development under optimized conditions using quantitative measures of both substrate forms and demonstration of peptide cleavage product detection with the target enzyme is underway.

This approach can be applied as a rapid, field-portable tool for targeted enzyme activity detection to meet point-of-care diagnostic needs, such as biomarker detection in the clinic, and detect bacterial virulence activity.

DTRA for funding. PNNL is a multiprogram national laboratory operated by Battelle Memorial Institute for the US DOE under contract DE-AC06-76RLO.