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

Portable And Convenient Identification Of Enzymatic Activity By Direct Detection Of Cleaved Peptides Using A Nanopore Sequencing Device

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Rapid, sensitive and specific detection of enzyme cleavage of proteins in a portable device can bolster diagnostic capabilities in the field. Typically, detection of proteolytic activity relies on enzyme-linked immunosorbent assays (ELISA) or mass spectrometry (MS)-based assays. However, MS methods require expensive and technically complex equipment that is not field-portable, and ELISA suffers from inadequate sensitivity. Nanopore technology provides an alternative platform for the sensitive detection of enzymatic products at the single-molecule level.

Solid-state nanopore sensors are typically used in DNA sequencing applications but have also demonstrated the capacity to detect proteins and peptides. As target molecules under a constant applied potential enter the nanopore, the ionic current is modulated. This modulation of current results in a specific current signature that can be used to identify enzymatic products. We have developed a strategy to detect enzyme activity by using a commercially available nanopore sequencing device from Oxford Nanopore Technologies to differentiate the translocation current signatures between cleaved and uncleaved peptide substrates.

However, the translocation speed of peptides through solid-state nanopores poses a significant challenge to distinguishing between cleaved and uncleaved peptide substrates. To address this issue, we have altered numerous experimental parameters to enhance the difference in the typical current signature observed for each peptide form. This included altering the pH and ionic strength of the running buffer and exploring different peptide substrate forms. The addition of an N-terminal polyanionic tail to the peptide substrate, and acquisition using pH 8 running buffer containing 0.5 M KCl and 25 mM HEPES buffer showed the greatest reproducibility and the most minimal current feature overlap between the uncleaved and cleaved substrate forms. Additional experimental workflows have been explored, such as affinity pull-down of the uncleaved peptide substrate to simplify sample complexity. Enzymatic activity has been demonstrated and validated using MALDI-MS, and quantitative measurements to determine the limits of detection are underway.

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