INTRODUCTION

Peptide-level analyses coupled with amino acid resolution is important to elucidate how proteins influence health and disease. Peptide-centric methodologies using liquid chromatography-tandem mass spectrometry (LC-MS/MS) are widely used to detect native and modified proteins. While LC-MS/MS stands as the dominant bottom-up proteomics technique, accessing LC-MS/MS spectra remains challenging. Due to the large capital expenditure and space required for LC-MS/MS, core facilities are often required for processing and analyzing protein samples. In addition, unexpected fragmentation patterns can preclude peptide detection, requiring additional interrogation of peptides for a more complete analysis. To address these hurdles, we demonstrate the ability of Quantum-Si’s Platinum™️ next-generation benchtop protein sequencing approach to identify proteins in mixtures and discern endogenous modifications from instrument-related effects with single molecule resolution, complementing existing LC-MS/MS techniques. This dynamic protein sequencing approach employs a mixture of dye-labeled N-terminal amino acid recognizers (NAARs) and aminopeptidases to probe digested peptides. The order of recognizer binding and kinetic properties of recognition segments are analyzed to determine peptide sequence and associated proteins.

METHODS

Here, we identify protein mixtures consisting of therapeutically relevant growth factors, cytokines, and secreted proteins. Moreover, kinetic signatures from NAARs not only reveal peptides that escape MS/MS mapping due to factors such as peptide length and pyroglutamate formation, but also enable differentiation between Asparagine (Asn) and deamidated proteins. These findings underscore Platinum as an alternative and complementary technique to LC-MS/MS for protein identification, peptide-level mapping, and monitoring critical quality attributes (CQAs) during product development.

RESULTS AND DISCUSSION

Platinum reveals protein components in mixtures

Platinum distinguishes Asn-containing and deamidated CDNF peptides

Platinum sequences an IL6 peptide that escaped MS matching

Deamidation alters the structure and function of biomolecules

REFERENCE

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