Next-Generation Protein Sequencing on Quantum-Si's Platinum™: Discernment of Protein Mixtures and Peptide Modifications

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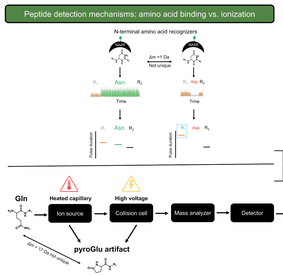
INTRODUCTION

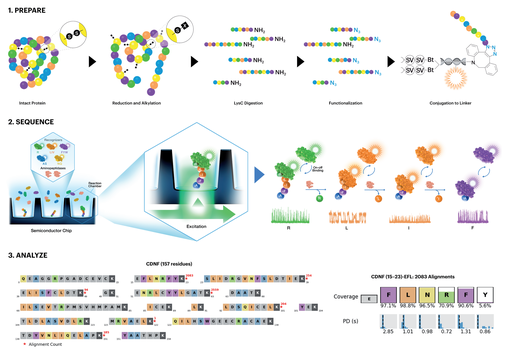
Peptide-level analysis coupled with amino acid resolution is important to elucidate how proteins influence health and disease. Peptide-centric methodologies using liquid chromatography-tandem mass spectrometry (LC-MS/MS) are widely used to detect native and modified proteins. While LC-MS/MS stands as the dominant bottom-up proteomics technique, accessing LC-MS/MS and interpreting 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 sequencer 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.

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 peptide variants. 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.



In proteomics analysis, the concern is always ***what is missed***

METHODS



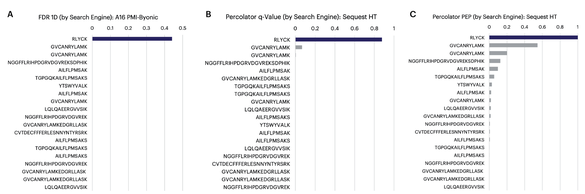
Platinum

RESULTS AND DISCUSSION

Platinum reveals protein components in mixtures

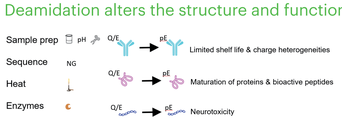
Green boxes indicate peptides sequenced on Platinum.

Platinum detects a short FGF2 peptide that escaped MS matching

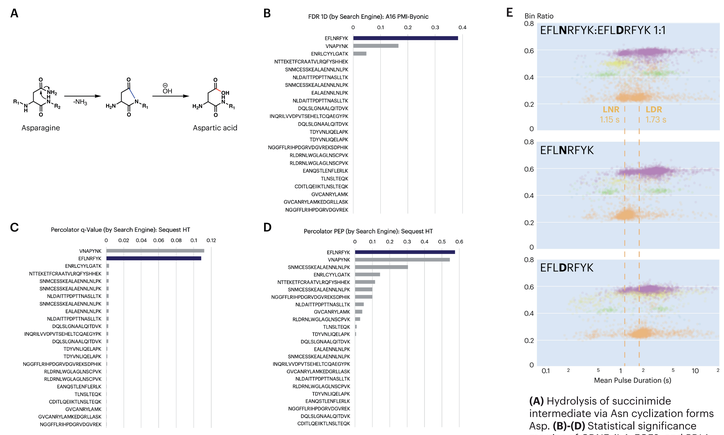


(A) Byonic has 2 types of peptide group FDR controls; protein-oblivious FDR (peptide 1D FDR) without considering the protein of origin. FDR measures the error rate associated with a collection of PSMs. **(B)** Percolator q-value represents the minimal FDR threshold for classifying a PSM. **(C)** Posterior error probability (PEP) indicates the probability the observed PSM is incorrect. A PEP of 1 indicates minimal confidence in the presence of the RLYCK peptide in the mass spectrometer during spectrum generation.

Deamidation alters the structure and function of biomolecules

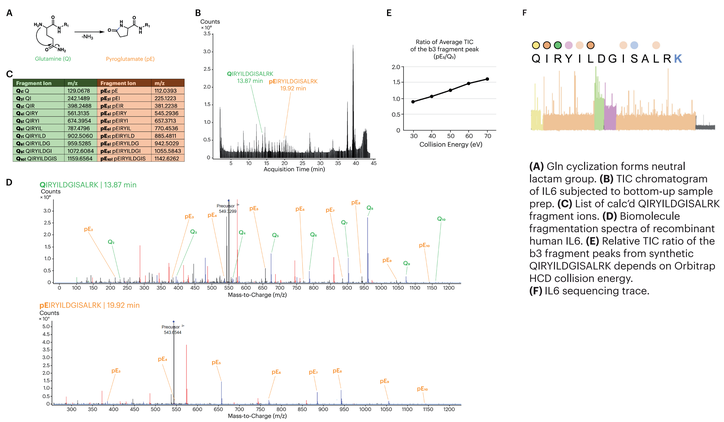


Platinum distinguishes Asn-containing and deamidated CDNF peptides



(A) Hydrolysis of succinimide intermediate via Asn cyclization forms Asp. **(B)-(D)** Statistical significance metrics of CDNF, IL4, FGF2, and PDL1 peptides. **(E)** PD vs. bin ratio (fluorescence lifetime metric) for synthetic CDNF peptide in isolation and mixture.

Platinum sequences an IL6 peptide that escaped MS matching



Fragment Ion	m/z	Fragment Ion	m/z
b_1 Q	129.0678	**$pE\text{-}y_{13}$** pE	112.0393
b_2 QI	242.1489	**$pE\text{-}y_{12}$** pEI	225.1223
b_3 QIR	398.2488	**$pE\text{-}y_{11}$** pEIR	381.2234
b_4 QIRY	561.3135	**$pE\text{-}y_{10}$** pEIRY	545.2936
b_5 QIRYI	674.3954	**$pE\text{-}y_{9}$** pEIRYI	657.3713
b_6 QIRYIL	787.4796	**$pE\text{-}y_{8}$** pEIRYIL	770.4536
b_7 QIRYILD	902.5060	**$pE\text{-}y_{7}$** pEIRYILD	885.4811
b_8 QIRYILDG	959.5285	**$pE\text{-}y_{6}$** pEIRYILDG	942.5039
b_9 QIRYILDGI	1072.6084	**$pE\text{-}y_{5}$** pEIRYILDGI	1055.5843
b_{10} QIRYILDGIS	1159.6564	**$pE\text{-}y_{4}$** pEIRYILDGIS	1142.6262

(A) Glutamine (Q) and pyroglutamate (pE) cyclization forms neutral lactam group. **(B)** TIC chromatogram of IL6 subjected to bottom-up sample prep. **(C)** List of calc'd QIRYILDGISALRK fragment ions. **(D)** Biomolecule fragmentation spectra of recombinant human IL6. **(E)** Relative TIC ratio of the b3 fragment peaks from synthetic QIRYILDGISALRK depends on Orbitrap HCD collision energy. **(F)** IL6 sequencing trace.

REFERENCE

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