Enhancing Biological Discovery with High-Sensitivity Ion Activation on timsOmni: A Flexible Analytical Platform

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Introduction

Deciphering the molecular intricacies of biological systems demands analytical tools that offer both high sensitivity and broad dynamic range for protein characterization. The newly developed timsOmni instrument features a sophisticated ion manipulation system that supports multiple ion activation and dissociation methods (MS^n, eXd), making it particularly well-suited for top-down proteomics. This enables comprehensive structural and functional analysis of intact proteins and proteoforms. Although optimized for advanced applications, timsOmni is built upon the timsUltra AIP platform, originally designed for bottom-up proteomics, and delivers exceptional sensitivity ideal for single-cell and other low-input workflows. This study explores the shotgun proteomics performance of timsOmni, utilizing the omnitrap module in pass-through configuration.

Methods

To evaluate the proteomics performance of the timsOmni instrument, tryptic digests of the human K562 cell line (Promega) were diluted in 0.1% formic acid containing 0.015% n-Dodecyl-β-D-Maltoside (DDM). A triple proteome mixture was prepared in-house by combining individual digests from HeLa cells, Yeast, and E. coli. Samples were separated using a ProteoElute nanoHPLC system (Bruker) equipped with PepSep Advanced columns (75 µm ID, 25 cm length) maintained at 50°C. The system was coupled to a CaptiveSpray Ultra 2 source with a 10 µm ID emitter. A linear gradient from 5% to 35% acetonitrile in 0.1% formic acid over 22 minutes at a flow rate of 250 nL/min was applied for all sample types. Data were acquired in dia-PASEF mode using a 166ms TIMS separation spanning 0.64–1.45 Vs/cm².

The dia-PASEF method employed five isolation windows, with the quadrupole isolation position stepped twice per TIMS scan. The data files were processed in Spectronaut (v20.2).

Results

- timsOmni in pass-through mode enables deep proteome coverage, identifying >5,000 protein groups from single-cell–level injections (250 pg) and up to 7,470 protein groups from 20 ng.
- Controlled quantitative experiments (CQE) combining human, yeast, and *E. coli* proteomes yielded >11,650 protein groups from 25 ng injections, with 75% quantified at CV < 10%.
- Species-resolved quantification was robust, identifying 3,091 yeast and 2,034 *E. coli* protein groups, confirming accurate differentiation of abundance changes across mixed-proteome samples.
- Overall, timsOmni demonstrates high quantitative accuracy and sensitivity, making it well-suited for detecting proteome-wide abundance changes across diverse biological sample complexities.

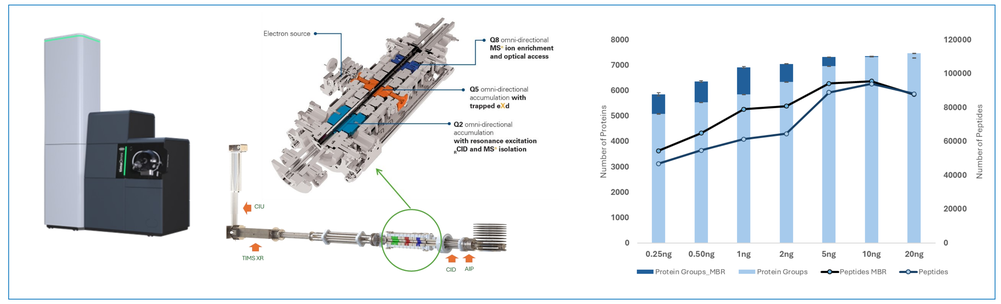

Figure 1
Proteomics performance of the timsOmni. In-depth protein identification using library-free Spectronaut data processing with and without matching for a dilution series ranging from 250pg K562 to 20ng on a 22 min gradient.

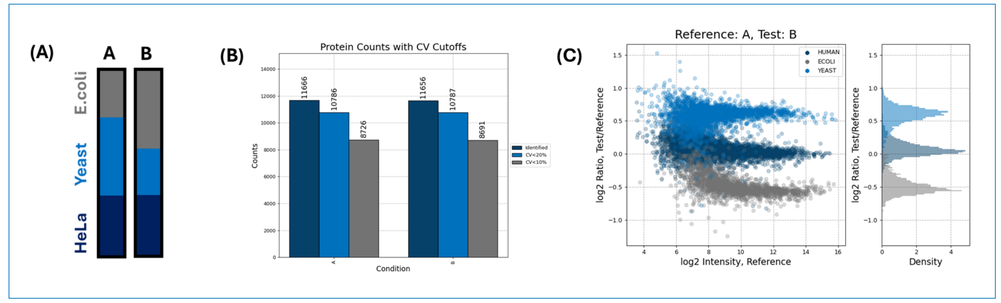

Figure 2:
Determination of the quantitation performance.
(A) Sample composition
(B) Number of total protein groups identified and quantified
(C) Measured log2 abundance ratios for each of the organism spiked-in.

Expanding the usage of the timsOmni for glycopeptide analysis

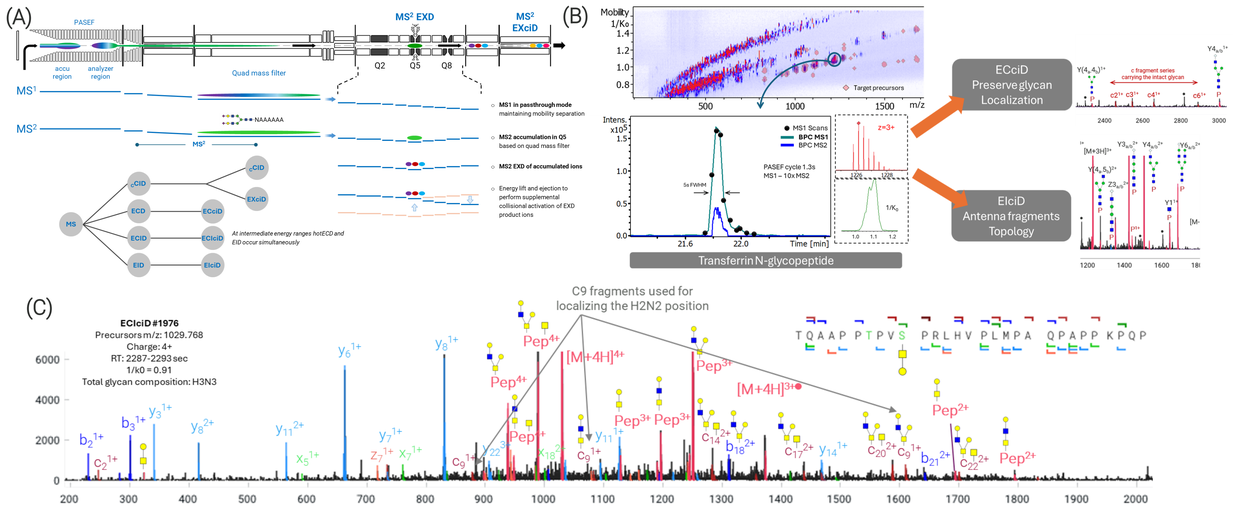

Figure 3: ExD-PASEF for Glycoprotoemics. (A) Workflow of ExD-PASEF illustrating different electron-based fragmentation modes regulated by electron energy. (B) Ion mobility-guided precursor selection and targeted analysis using collision-assisted electron capture dissociation (ECciD) and collision-assisted electron ionization dissociation (EIciD). (C) Representative ECciD spectrum showing a glycan-retaining c_9 fragment ion, enabling confident O-glycosite localization.

- The timsOmni platform enables multiple electron-based and hybrid fragmentation strategies, including ECD, EID, ECciD, and EIciD, within PASEF-enabled workflows.
- Hybrid collision-assisted ExD methods generate complementary fragmentation, producing c- and z-type peptide ions while retaining glycan moieties on backbone fragments.
- Ion mobility separation reduces spectral complexity, improving precursor isolation and fragmentation specificity for glycopeptide analysis.

Summary

The timsOmni system integrates MS^n and ExD capabilities, enabling confident structural characterization of complex biomolecules such as glycopeptides. By providing glycan-retaining fragment ions for accurate glycosite localization, it overcomes key limitations of conventional CID workflows. At the same time, timsOmni maintains top-tier sensitivity and quantitative precision for complex sample analysis. These results demonstrate its suitability for both in-depth structural proteomics and routine, high-throughput biological investigations, making it a valuable platform for biomarker discovery and functional proteomics.

Conclusion

- timsOmni combines MS^n and ExD capabilities to enable confident structure elucidation while maintaining high sensitivity and quantitative performance for complex biological samples.

timsOmni

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