Confident Characterization of N- and O-Linked Glycopeptides Using Electron-Based Dissociation on a timsOmni Platform

Hongxia Bai[1]; Ioanna Barla[2]; Anastasios Grigoriadis[2]; Kristina Marx[3]; Gadi Armony[4]; Florian Busch[5]; Athanasios Smyrnakis[6]; Greg Chopiuk[7]; Guillaume Tremintin[8]; Dimitris Papanastasiou[6]; Matthew Willetts[9]

[1]Bruker, Billerica, MA; [2]Fasmatech Science and Technology, Athens, Greece; [3]Bruker Daltonics GmbH & Co.KG, Bremen, Germany; [4]Bruker Nederland B.V., Leiderdorp, Netherlands; [5]Bruker Switzerland AG, Fällanden, Switzerland; [6]Fasmatech Science & Technology, Athens, Greece; [7]Bruker Ltd, Milton, Canada, Toronto, ON; [8]Bruker Daltonics, San Jose, CA; [9]Bruker Scientific LLC, Billerica, MA

Learn more about 4D-Multiomics

Introduction

Glycopeptide characterization requires fragmentation methods that support both confident site localization and detailed glycan structural analysis. The timsOmni implementation of trapped electron-based dissociation (tExD) provides highly tunable electron-driven fragmentation, enabling rich and complementary MS/MS spectra across glycopeptide classes. This enables preservation of glycan-bearing backbone ions for localization of O-linked glycopeptides while also generating rich glycan fragmentation for structural analysis of N-linked species. Here, we evaluate tExD and hybrid workflows for confident characterization of N- and O-linked glycopeptides.

Methods

Synthetic N- and O-linked glycopeptide standards and glycoprotein digests were analyzed in DDA-PASEF mode with targeted inclusion lists. Precursors of different charge states were evaluated across ECD, EID, ElciD, and ECciD fragmentation. Data were processed using FragPipe (MSFragger-Glyco, MS-Shepherd, O-Pair) with Byonic for complementary validation, and spectra were manually annotated in OmniScape, Glypy, and GlycoWorkbench.

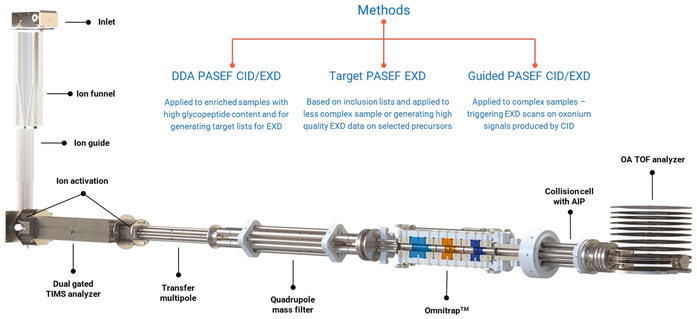

Fig. 1 Glycoproteomics workflow on the timsOmni platform. Glycopeptides are separated by nanoElute LC, ion-mobility separated and accumulated via TIMS, and fragmented in the Omnitrap using selectable electron-based dissociation modes (ECD, hECD, EID, ECID, and hybrid EXciD/ECciD). This architecture enables flexible, charge-state-dependent optimization of fragmentation for both N- and O-linked glycopeptide characterization. Three different workflows are supported.

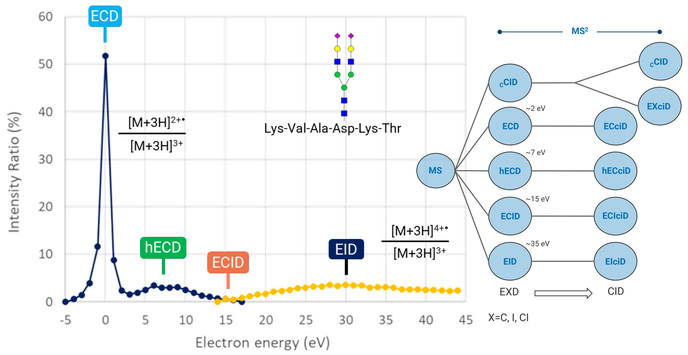

Fig. 2 Tunable electron energy enables different fragmentation mechanisms.

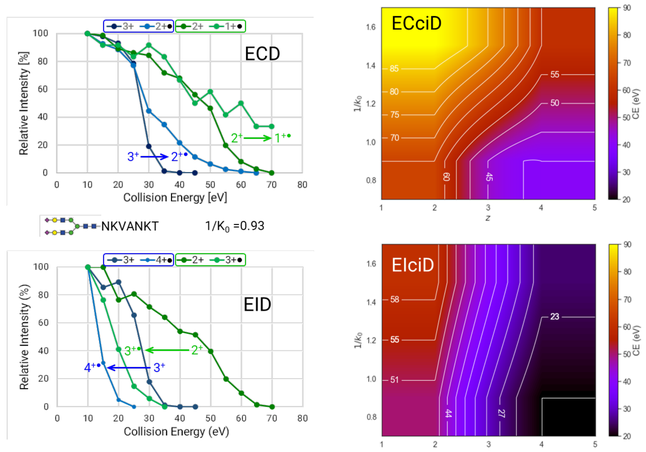

Fig. 3 Different precursor charge states require distinct assisted collision energies for efficient fragmentation of radical species.

Results

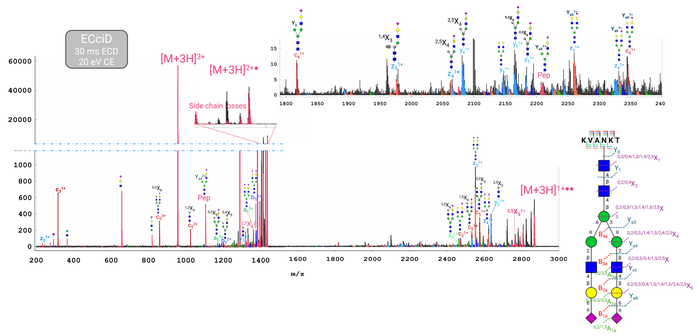

Fig. 4 Extensive sequence coverage and glycan-retaining fragmentation of an N-linked glycopeptide achieved by ECciD.

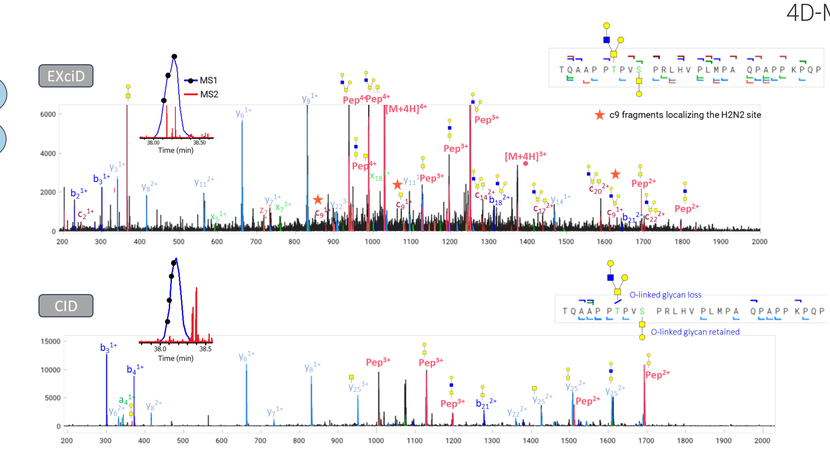

Fig. 5 ECciD enables confident localization of O-linked glycosylation sites through glycan-retaining c and z• fragments, whereas CID predominantly results in glycan loss.

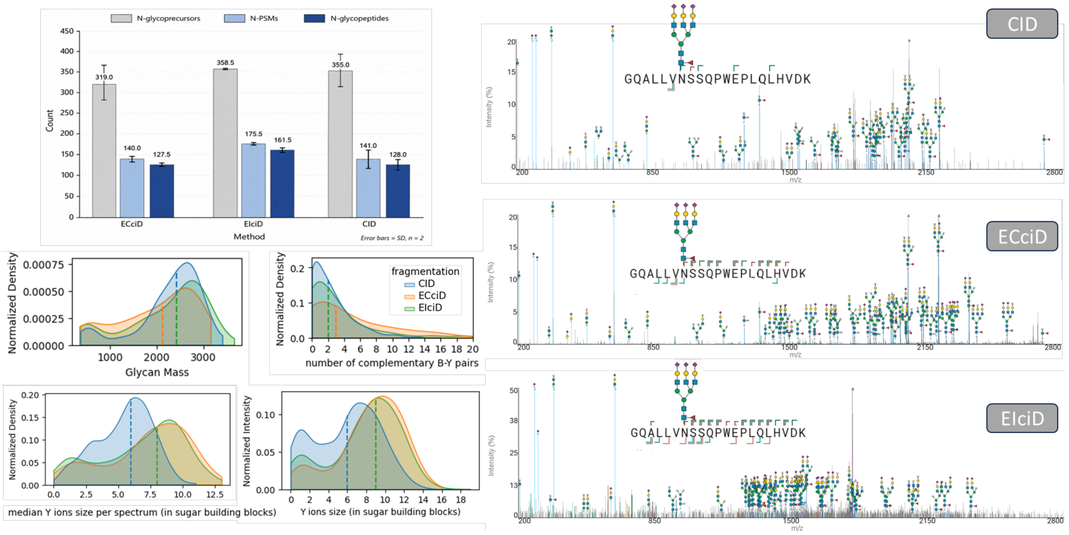

Fig. 6 ECciD and ElciD yield comparable glycoPSMs, glycopeptide precursors, and unique glycopeptide identifications in targeted DDA-PASEF experiments with an inclusion list. However, ECciD generates more high-mass Y ions for structural elucidation and provides enhanced peptide backbone fragmentation.

Summary

tExD workflows on the timsOmni enable confident characterization of N- and O-linked glycopeptides by combining glycan preservation with extensive backbone fragmentation. Fragmentation can be tuned by precursor charge state, supporting flexible, high-throughput analyses. ECciD and ElciD show comparable identification performance, with ECciD providing enhanced structural detail.

Conclusion

- tExD preserves glycans while enabling backbone fragmentation. Confident localization for N- and O-glycopeptides
- ECciD provides richer structural information (more high-mass Y ions, improved coverage)
- Comparable identifications across ECciD and ElciD
- Flexible, high-throughput workflows

Technology

© 2026 Bruker

Authors are employees of Bruker and may hold financial interests in the company.

For Research Use Only. Not for use in diagnostic procedures.

Innovation with Integrity