

MS Fragmentation for Glycans — HCD ETD EThcD — Hands-on

Gain practical intuition for how glycans and glycopeptides fragment in the mass spectrometer. This module explains oxonium ions, glycosidic vs cross ring cleavages and how HCD, ETD and EThcD complement each other for site specific glyco analysis and confident structural assignments.

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Session 1

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Fundamentals of Glycan & Glycopeptide MS/MS

Ionization and charge state behavior of glycans and glycopeptides

[ESI overview](#) [positive vs negative mode](#) [adduct formation](#)

Glycosidic vs cross ring cleavages: concept and nomenclature

[Domon Costello B / Y ions](#) [A / X cross ring ions](#)

peptide b / y ions recap

Instrument types and fragmentation options for glyco work

ion trap and Orbitrap beam type CID vs HCD ETD / EThcD capability

Session 2

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HCD Spectra, Oxonium Ions & Cross Ring Cleavage

HCD behavior of glycans and glycopeptides

stepwise glycosidic loss energy and charge effects in source decay awareness

Diagnostic oxonium ions and their interpretation

HexNAc and Hex ions Neu5Ac related ions fucose and sulfation hints

Cross ring ions for linkage and branching clues

2,4 A and 0,2 A examples distinguishing isomers limits of information content

Session 3

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ETD / EThcD for Site Specific Glycoproteomics

Principles of ETD fragmentation

electron transfer concept c / z ion series preserving labile glycans

EThcD and hybrid methods for combined information

ETD plus supplemental HCD peptide backbone plus glycan fragments benefits for site localization

Choosing fragmentation strategy for different questions

released glycan vs glycopeptide focus **discovery vs targeted assays** **instrument time constraints**

Session 4

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Mini Capstone: Spectrum Interpretation & Annotation

Hands on interpretation of provided HCD and EThcD spectra

stepwise assignment of oxonium, glycan and peptide ions

Building small annotation tables for key spectra

m/z, intensity and ion type **linking to proposed structures** **evidence level notes**

Deliverables and documentation

annotated spectrum images **ion assignment tables**
short interpretation summary