EFTMS2026.org 27-30 April 2026 - Rouen, France
European Fourier Transform Mass Spectrometry Workshop

DEEP LEARNING-ENHANCED CHARACTERIZATION OF (GLYCO-) PEPTIDE FRAGMENTATION ON THE ORBITRAP-OMNITRAP

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While collision-induced dissociation (CID) has long been the standard for peptide sequencing in bottom-up proteomics , the shift toward identifying and localizing complex post-translational modifications (PTMs) has created a need for more sophisticated fragmentation techniques. Methods such as ultraviolet photodissociation (UVPD), electron ionization dissociation (EID), and electron capture dissociation (ECD) offer significant advantages but remain understudied due to a historical lack of large-scale experimental data.

In this study, we characterized these alternative fragmentation techniques using an Orbitrap-Omnitrap hybrid instrument and deep learning. We performed a large-scale multienzyme proteomics experiment using human cell lysates digested with five different enzymes. This resulted in a massive dataset of over 300 LC-MS files, from which we identified approximately 1 million peptide-spectrum matches (PSMs) per activation technique using MSFragger. These identifications were used to train the Prosit deep learning algorithm to predict UVPD, EID, and ECD fragmentation patterns. The resulting model demonstrated excellent agreement (~0.9) between predicted and experimental spectra, significantly enhancing our ability to rescore and discriminate between true and false positives.

Furthermore, we evaluated the platform's performance on complex N-glycopeptides extracted from plasma. Both UVPD and EID generated extensive peptide, glycosidic, and cross-ring fragments. These techniques achieved identification efficiencies comparable to stepped collisional dissociation while providing critical additional linkage information for detailed structural characterization. Our results establish the Omnitrap as a powerful platform for comprehensive glycoproteomic analysis and underscore the vital role of enhanced computational tools in interpreting complex fragmentation spectra.

Keywords
UVPD (Ultraviolet Photodissociation), EID (Electron Ionization Dissociation), ECD (Electron Capture Dissociation), Bottom Up Proteomics, Glycoproteomics