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Department of Chemistry

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LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY BASED GLYCOPROTEOMICS

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Liquid chromatography-mass spectrometry (LC-MS) based glycoproteomics has long been recognized as a powerful technique for characterizing glycoproteins. Multiple methods have been developed to fragment glycans or glycopeptides to generate fingerprint tandem mass spectra (MS^2) for glycan and glycopeptide identification. However, no single fragmentation method in tandem mass spectrometry can produce unbiased glycan and peptide product ions that are suitable for comprehensive glycoproteome characterization. In this seminar, I will review four analytical methods that have been developed for glycoproteomics. The conventional high-energy collision dissociation (HCD) method fragments both the glycan and peptide backbone, which often results in the loss of information regarding the attachment sites of glycans on the peptide sequence. Ravi Chand Bollineni et al. demonstrated that stepped high-energy collision dissociation (sceHCD) outperformed the HCD in generating MS^2 spectra at both the glycan and peptide levels.[1] Electron transfer dissociation (ETD) only fragments the peptide backbone while leaving the glycan intact, and therefore, cannot confidently detect glycan structures. QingYu and colleagues introduced a modified electron-transfer/higher-energy collision dissociation (EThcD) fragmentation method to generate MS^2 spectra to include fragments from both ETD and HCD in a single spectrum. This combination provides sufficient information to identify glycan structures, peptide sequences, and glycosylation sites.[2] Qinjingwen Cao and colleagues discovered glycosylated and novel neuropeptides from the crustacean nervous system using an enrichment-free approach, taking advantage of signature product ion-triggered EThcD (HCD-pd-EThcD) where HCD scans generate signature ions that indicate glycan attachment to peptides, followed by an EThcD step on the same peptide fragment to generate complete information for characterizing N-linked and O-linked glycopeptides.[3] Furthermore, pre-enrichment of glycopeptides coupled with HCD-pd-EThcD have been used to map N-glycosylation in therapeutic monoclonal antibodies (mAbs), showing that this approach could significantly reduce sample complexity, thus facilitating glycosylation analysis the HCD-pd- EThcD method.[4]

References:

1. Bollineni, Ravi Chand, et al. "Large-scale intact glycopeptide identification by Mascot database search." *Scientific Reports* 8.1 (2018): 2117.
2. Yu, Qing, et al. "Electron-transfer/higher-energy collision dissociation (EThcD)-enabled intact glycopeptide/glycoproteome characterization." *Journal of the American Society for Mass Spectrometry* 28.9 (2017): 1751-1764.
3. Cao, Qinjingwen, et al. "Signature-ion-triggered mass spectrometry approach enabled discovery of N-and O-linked glycosylated neuropeptides in the crustacean nervous system." *Journal of proteome research* 19.2 (2019): 634-643.

Li, Menglin, et al. "Efficient HCD-pd-EThcD approach for N-glycan mapping of therapeutic antibodies at intact glycopeptide level." *Analytica Chimica Acta* 1189 (2022): 339232