Title:

Glycopeptide characterization with MetaMorpheus

Abstract:

Mass spectrometry (MS) is the gold standard for interrogating the glycoproteome, enabling the localization of glycans to specific glycosites. Recent applications of electron-driven dissociation methods have shown promise in localizing modified O-glycosites even in multiply glycosylated peptides. Yet, standard approaches for interpreting MS/MS spectra are ill-suited to the heterogeneity of O-glycopeptides, especially for the most challenging mucin-type O-glycosylation. O-glycoproteomic analysis pipelines are needed to search for multiply O-glycosylated peptides within reasonable time frames for simple mixtures of O-glycoproteins and proteome-scale experiments.

We developed O-Pair Search identifies O-glycopeptides via an ion-indexed open modification search and localizes O-glycosites using graph theory and probability-based localization Using paired collision- and electron-based dissociation spectra. O-Pair Search reduces search times compared to current popular O-glycopeptide processing software Byonic, while defining O-glycosite localization confidence levels and generating more O-glycopeptide identifications.