**GlycoTools: An environment for organizing, inspecting, and visualizing quantitative glycoproteomic experiments**

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**Abstract**

Advances in glycoproteomic profiling, enabled by hybrid-type electron transfer and stepped collisional energy dissociation methods, have enabled the confident assignment of peptide structures with intact modifying glycan compositions. However, the robust downstream software architecture for data handling is not available for the search results provided by the leading glycoproteomic search engines. Here, we provide a software environment to easily organize, inspect, and explore the search results from the Byonic search engine in an intuitive graphical user interface. As well, we identify sources of error inherent to glycoproteomic data processing, specifically, glycan localization and the identification of in-source fragment ions, and provide tools to detect and reassign or remove incorrect glycopeptide identifications.

**Introduction**

The characterization of proteins from biological samples by mass spectrometry (MS) (i.e., proteomics) has advanced to a point where one can confidently sample the comprehensive proteome (1, 2). Similarly, the identification of protein modifications, including phosphorylation, acetylation, and ubiquitination, are now routinely employed to further biological investigations (Cite a PTM review). Yet, certain protein modifications remain elusive to in-depth, global identification. Protein glycosylation has been particularly challenging due to the diversity of oligosaccharide (glycan) modifications and inadequate fragmentation of methods (3). The majority of fragments generated with vibrational methods, such as collision-included dissociation (CID) and higher-energy collisional dissociations (HCD), are of the more labile, glycosidic bonds and produce few peptide sequence informative fragments. Conversely, while electron transfer dissociation (ETD) is specific to the peptide backbone and has been beneficial for site localization of protein modifications, little information is provided about the structure of the glycan modification. Recently, hybrid-type ETD methods, such as activated ion electron transfer dissociation (AI-ETD) and ETD with supplemental activation (EThcD), have emerged as forerunners in their ability to generate sequence informative product ion spectra for both the peptide backbone and the glycan modification (4, 5).

*In silico* search algorithms for high throughput glycopeptide sequencing are essential for comprehensive glycoproteome analyses. However, the heterogeneity of glycan modifications and their inherent technical limitations makes scan assignment increasingly difficult.

**Material and Methods**

**Experimental**

**Results and discussion**