GproDIA enables data-independent acquisition glycoproteomics with comprehensive statistical control

Large-scale profiling of intact glycopeptides is critical but challenging in glycoproteomics. Data independent acquisition (DIA) is an emerging technology with deep proteome coverage and accurate quantitative capability in proteomics studies, but is still in the early stage of development in the field of glycoproteomics. We propose GproDIA, a framework that applies the concept of peptide-centric DIA analysis to proteome-wide characterization of intact glycopeptides with comprehensive statistical control by a two-dimentional false discovery rate approach and a glycoform inference algorithm, enabling accurate identification of intact glycopeptides using wide isolation windows. We further utilize a semi-empirical spectrum prediction strategy to expand the coverage of spectral libraries of glycopeptides. We hbenchmark our method for N-glycopeptide profiling on DIA data of yeast and human serum samples, demonstrating that DIA with GproDIA outperforms the data-dependent acquisition-based methods for glycoproteomics in terms of capacity and data completeness of identification, as well as accuracy and precision of quantification. We expect that this work can provide a powerful tool for glycoproteomic studies.



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