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The Ioniser: Searching for additional glycoproteins

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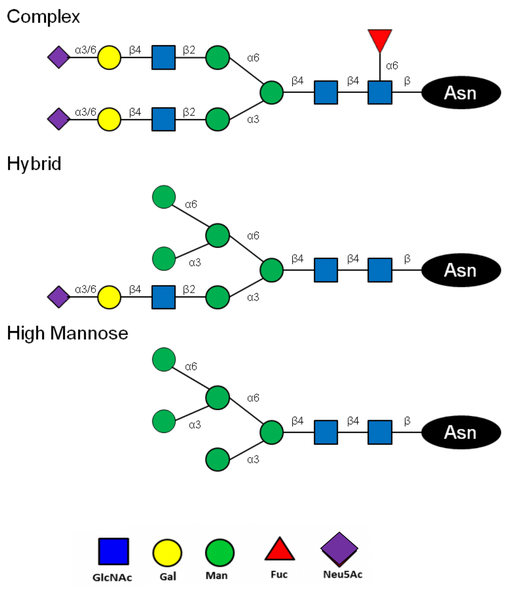
Introduction

The characterization of glycosylated proteins is a challenging task in the proteomics field as they are commonly presented by multiple glycoforms.

The Ioniser assists in identifying potentially novel glycoforms, without the need for prior knowledge of the existing glycostructures for a given peptide.



It processes and filters large amounts of mass-to-charge (m/z) ratio and abundance data allowing the user to identify additional glycosylated proteins based on user-specified parameters.

Future work

Currently, the Ioniser implements the most basic functionality required to find additional glycoforms, and there are several features we wish to implement in the future. It would, for example, be advantageous to link the ExPASy web-based tool GlycoMod and the Ioniser. GlycoMod finds possible compositions of a glycan structure from its experimentally determined mass. This would allow the user to immediately see a list of proposed compositions, without the need to switch tools and re-enter parameters.

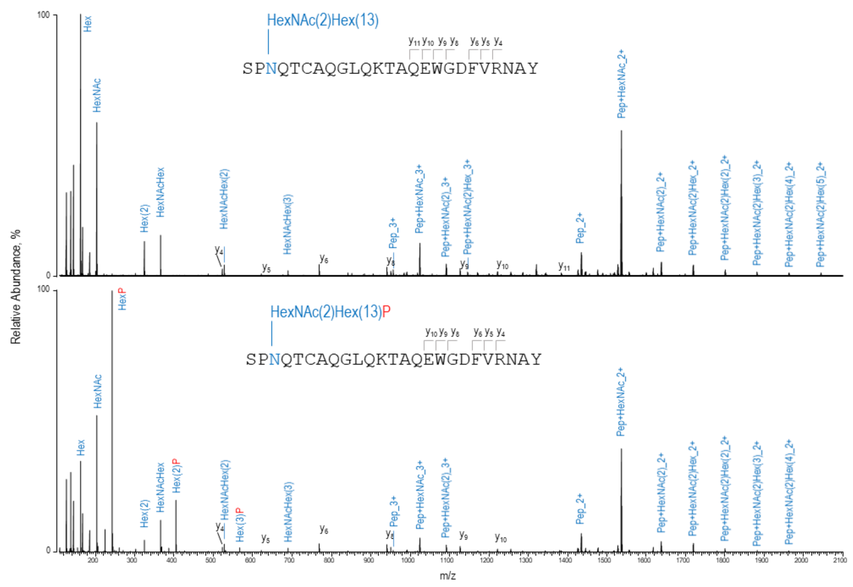
The method

Following a LC-MS (Liquid chromatography – tandem mass spectrometry) experiment, an mgf file is generated containing several different precursor peptides, and a list of each of their fragments m/z and intensity.

A Byonic™ search is used to identify major glycopeptides (their sites and most abundant glycoforms), leading to identification of the characteristic ions and their m/z values for each glycopeptide of interest. The identified glycopeptide specific ions are provided to the Ioniser to facilitate identification of other potential glycoforms for a given peptide.

When selected, the theoretical doubly and/or triply charged masses ($[M+H]^{+2}$ and $[M+H]^{+3}$) of a peptide are calculated and treated as any other value that is being searched. This includes having a tolerance based on ppm and looking for isotopes before selecting the highest intensity.

The results can be further filtered using the ‘zero sum’ option, which allows for the in/exclusion of precursor peptides where the sum of the intensities for glycans and peptides respectively equals zero. When applicable it will also include doubly and triply charged peptides.



Examples of MS/MS spectra for an *N*-glycopeptide.

References

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https://www.proteinmetrics.com/products/byonic/

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