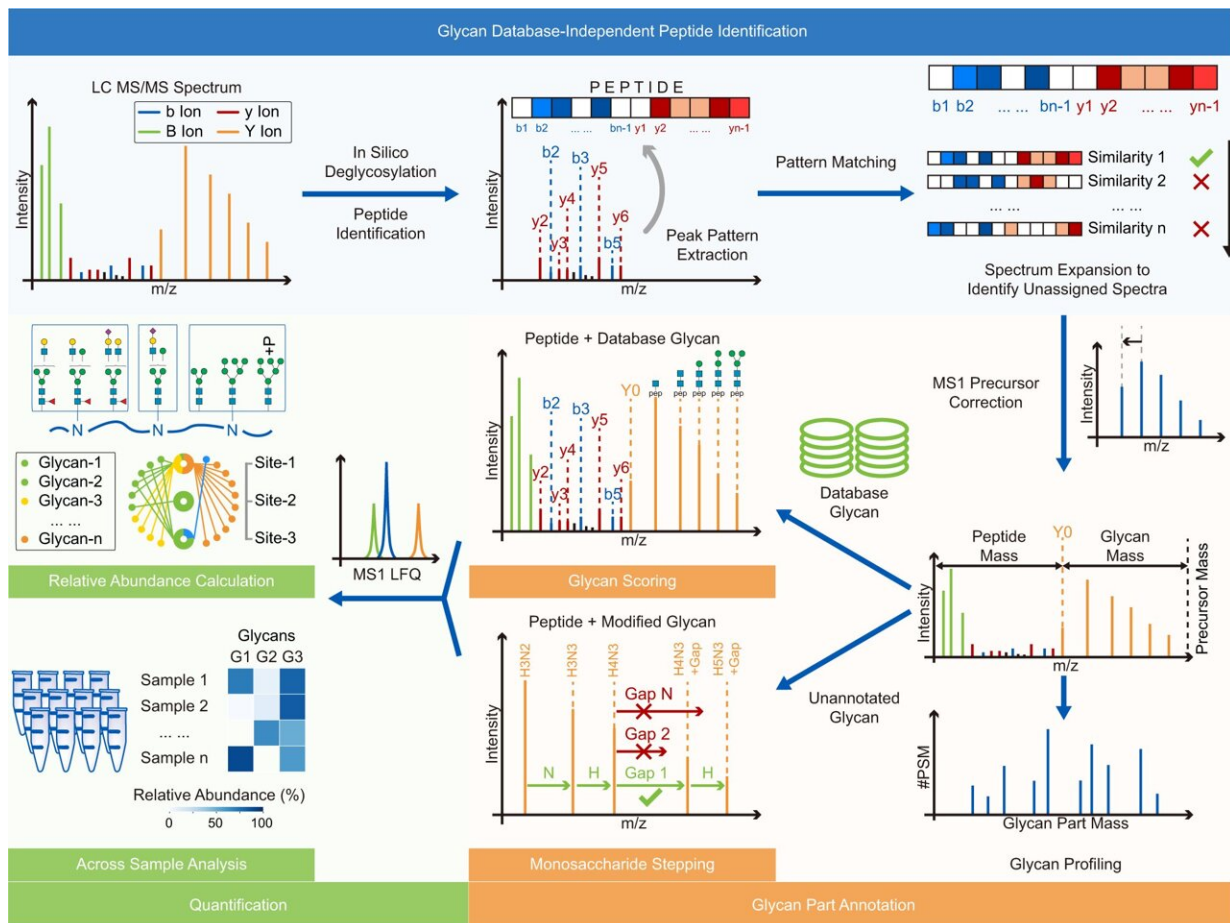# New software tool allows sensitive deciphering of protein glycosylation

April 8 2022, by Li Yuan

Workflow of Glyco-Decipher. Glyco-Decipher contains three modules: (1) Glycan database-independent peptide matching. The in silico deglycosylated spectra are searched against the protein database without setting any glycans as modifications, which determines the peptide backbone for glycopeptide spectra with rich peptide fragment ions. Then, the fragmentation patterns of the peptide backbones are extracted and utilized to match spectra that remain unannotated.

This step, termed "spectrum expansion", enables the identification of peptide backbones of glycopeptide spectra with poor peptide fragmentation. (2) Glycan annotation. The mass of the glycan part is precisely derived by the mass difference between the precursor and the peptide backbone. The mass profile of glycans in a system is constructed without the use of glycan databases. For glycan annotation, the experimental B/Y ions in glycopeptide spectra are first matched to their theoretical fragment ions of database glycans to identify glycans. For glycans that do not match any database entries, Glyco-Decipher performs monosaccharide stepping to reveal the composition of modified glycans and potential modification moiety on them. (3) Quantification. The quantification module based on the elution profiles of glycopeptides is embedded in Glyco-Decipher and allows the computation of the abundance distributions of site-specific glycans. Credit: *Nature Communications* (2022). DOI: 10.1038/s41467-022-29530-y

Glycosylation is a common post-translational modification (PTM) of proteins and plays a crucial role in many biological processes. Many disease biomarkers are glycosylated proteins.

Mass spectrometry-based intact glycopeptide identification can provide information on glycosite and the attached glycan. However, the interpretation of acquired glycopeptide spectra is still challenging.

Recently, a research team led by Prof. Ye Mingliang from the Dalian Institute of Chemical Physics (DICP) of the Chinese Academy of Sciences (CAS) has developed a new glycoproteomics software tool, Glyco-Decipher, for the sensitive interpretation of N-glycopeptide mass spectra at high confidence.

This study was published in *Nature Communications* on April 7.

The researchers found that the fragmentation pattern of the peptide

backbone of glycopeptides was not affected by the attached glycans or precursor charge states. And the high similarity of the fragmentation pattern of glycopeptides with identical peptide backbones was utilized to interpret glycopeptide spectra with poor peptide fragmentation.

In addition, the scheme of peptide identification in Glyco-Decipher was glycan database-independent, which enabled the discovery of unexpected glycans that were not present in databases. With the developed monosaccharide stepping method, Glyco-Decipher was able to unveil unexpected glycans with modification moieties in complex systems.

Moreover, the quantification module embedded in the software tool extracted elution profiles of glycopeptides, and thus enabled abundance analysis and functional studies of site-specific glycans.

"Glyco-Decipher is overall a very worthy software tool for glycoproteomics," commented one of the reviewers.

More information: Zheng Fang et al, Glyco-Decipher enables glycan database-independent peptide matching and in-depth characterization of site-specific N-glycosylation, *Nature Communications* (2022). DOI: 10.1038/s41467-022-29530-y

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