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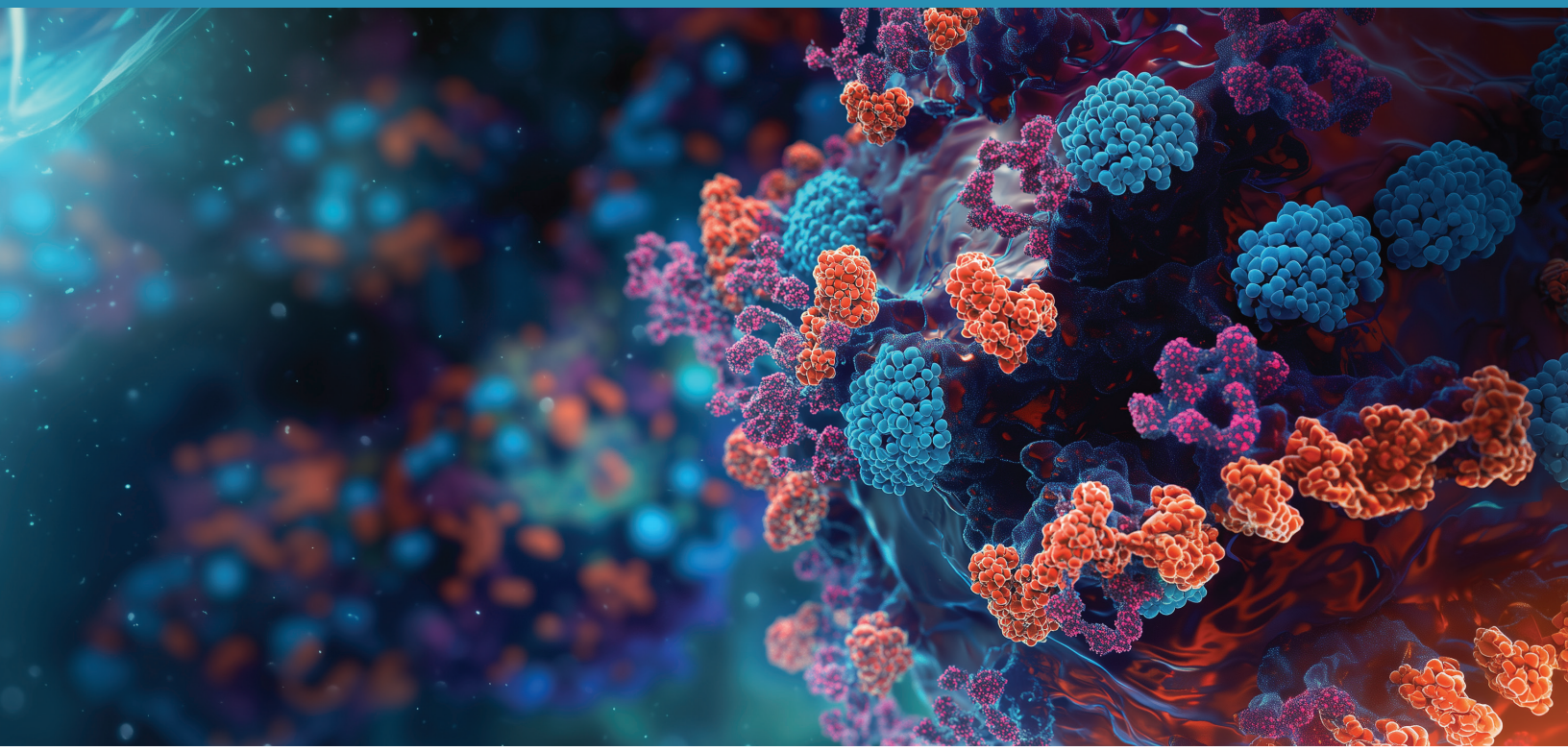

PEAKS GlycanFinder 2.5: Launching a New Era for Glycoproteomics and Glycomics

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Abstract

Protein glycosylation is one of the most common post translational modifications (PTMs). It plays a crucial role in many biological processes and changes in glycosylation can signal disease. Thus, studying protein glycosylation is important for our understanding of the proteome and for discovering potential drug targets. However, due to glycan heterogeneity and complexity of data, glycosylation is challenging to study using mass spectrometry methods. Specifically, the main challenges come from diverse glycan structures, which vary from mono to complex oligosaccharides, as well as the existence of multiple glycans per peptides and proteins. In addition, mass spectra are complicated by the presence of both peptide backbone and glycan fragments. PEAKS GlycanFinder provides a unique approach to tackle these challenges and offers an accurate and sensitive comprehensive solution for qualitative and quantitative glycoproteomics and glycomics.

Introduction

In the glycopeptide search, PEAKS GlycanFinder employes a peptide-first search approach, followed by a glycan-based approach, and finally deep learning-based glycan de novo sequencing [1]. This ensures that no spectra are left out due to poor glycan or peptide backbone fragmentation. Adequate fragmentation along the peptide backbone and glycan structure is a challenge in glycoproteomics and thus, fragmentation optimization is important in glycoproteomics studies. Using a publicly available dataset (PXD052616) [2], it was shown that PEAKS GlycanFinder 2.5 can analyze glycoproteomics data at different CID fragmentation energies by capturing multiple fragment ion types and improve identifications compared to PEAKS GlycanFinder 2.0 results reported in the publication.

In addition, released glycan analysis by mass spectrometry offers a complementary technique to glycoproteomics. PEAKS GlycanFinder allows for quick and easy analysis considering both the intact glycan mass and fragmentation evidence, without the need for manual analysis. Using a publicly available dataset (PXD038501) [3], PEAKS GlycanFinder 2.5 was used to quantify the change in glycan levels in ECC cells over time following a treatment with a 12-plex sugar-labelling technique developed by the Li lab [2]. PEAKS GlycanFinder 2.5 reported a similar trend in glycan levels over time, which is in agreement with the publication report.

Table 1. PEAKS GlycanFinder search parameters for dataset PXD052616

Precursor mass tolerance	10 ppm
Fragment mass tolerance	0.02 Da
Glycan fragment mass tolerance	20 ppm
Enzyme	Trypsin (Specific), 2 missed cleavages
Fixed PTMs	Carbamidomethylation (C)
Variable PTMs	Oxidation (M), acetylation (N-terminal)
Protein Database	Uniprot Human (Reviewed) (20427 entries)
Glycan Database	Built-in N-linked (1867 entries)

Methods

For the glycoproteomics analysis, a benchmarking dataset from PXD052616 was used. Briefly, a pooled glycopeptide sample from human serum was denatured, reduced, alkylated, digested by trypsin, and desalted prior to LC-MS/MS analysis using a ZenoTOF 7600. MS2 were acquired in DDA mode. Collision energy (CE) values from 20 to 100 as well as dynamic CE were used. In the publication, the data were analyzed using PEAKS GlycanFinder 2.0, PEAKS Studio, and FragPipe. Here, the datasets were re-analyzed in PEAKS GlycanFinder 2.5 using the parameters shown in Table 1.

For the released glycan analysis, a dataset from PXD038501 was used. ECC1 cells were cultured and divided into four groups (A, B, C, D). Groups A and C were treated with DMSO (control), and groups B and D were treated with Atovaquone, an antimicrobial medication. The treatment time was 48 h (A and B) or 72 h (C and D). After treatment, cells were trypsinized, harvested, lysed, and proteins were extracted. The protein concentration was determined. Then, the groups were divided into three equal aliquots. Glycans were released and labelled.

LC-MS/MS was performed using a Q Exactive HF Hybrid Quadrupole-Orbitrap. The source was operated in positive ion mode and MS2 spectra were acquired in DDA mode. The data was re-analyzed using PEAKS GlycanFinder 2.5 using the parameters shown in the table.

Table 2. PEAKS GlycanFinder search parameters for dataset PXD038501

Precursor mass tolerance	10 ppm	
Glycan fragment mass tolerance	20 ppm	
Fixed PTMs	Sugar-label 12plex	
Glycan Database	Built-in N-linked (1867 entries)	
Reporter Ion Groups	Group A (48 h control)	115a 115b
	Group B (48 h control)	116a 116b 116c
	Group C (72 h control)	117a 117b 117c
	Group D (72 h control)	118a 118b 118c 118d

Results

Glycopeptide identification at different CID collision energies.

PEAKS GlycanFinder reports glycopeptide identifications at compositional and structure glycan levels as well as it provides protein coverage view for easy visualization of site-specific glycans.

Figure 1. The glycopeptide coverage of Human Alpha-1-acid glycoprotein. The pie chart shows the glycan profiling at site N56, where each glycan is presented as a percentage of the total based on glycopeptide area.

The site-specific glycopeptide abundance can easily be viewed in PEAKS GlycanFinder 2.5 by clicking on an amino acid in the Protein Coverage View. The pie chart shows the percentage of a particular site on a protein modified by a particular glycan based on its feature area.

Glycopeptide identifications are scored based on overall glycopeptide fragmentation including glycan and peptide fragments. An S-score is used to evaluate the evidence for the top-scoring structure candidate, and it is calculated as normalized gap between the best and second-best glycan candidate structure. An S-score of 100% indicates there are no other candidates and an S-score of 0% indicates that two or more structure candidates are equally likely. An AScore is also provided as a measure of the positional confidence of the glycan modification on the peptide. A higher AScore indicates higher confidence in the glycan site.

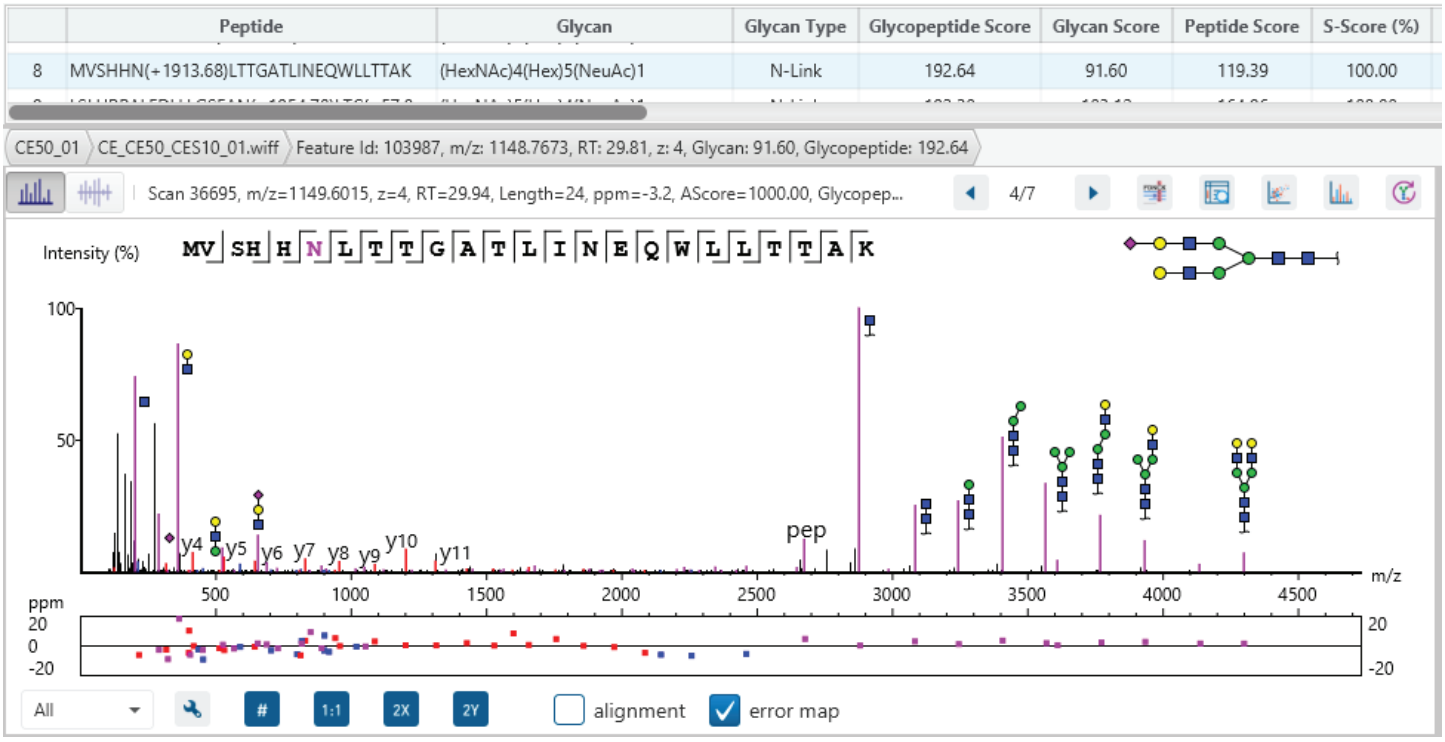

Figure 2. An example glycopeptide spectrum. Peptide fragments are shown in red and blue and glycan fragments are shown in magenta with structural evidence. The top glycan structure is displayed on the spectrum along with the peptide sequence and glycan site.

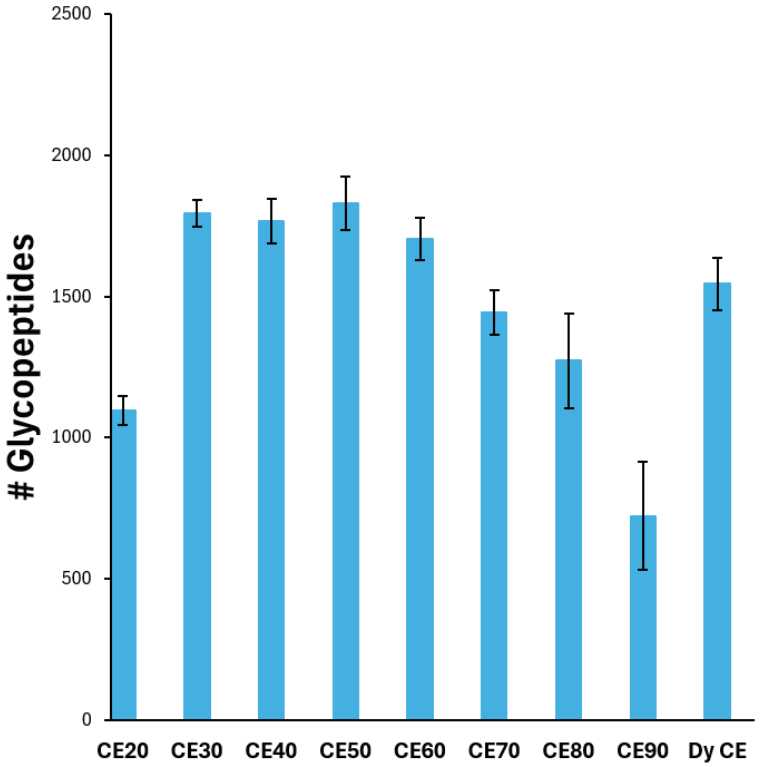

Figure 3. The number of glycopeptides identified at different CID collision energies in the dataset from PXD052616, using PEAKS GlycanFinder 2.5.

PEAKS GlycanFinder 2.5 identified a total of 10461 unique glycopeptides, a 37% increase compared to the total glycopeptides found using PEAKS GlycanFinder 2.0 (Dataset S1 from the publication). The number of glycopeptides identified followed a similar trend to that seen in the publication. However, PEAKS GlycanFinder 2.5 could identify a higher proportion of peptides from lower collision energies compared to the publication.

In addition, PEAKS GlycanFinder supports various fragmentation modes from different vendors in addition to CID and HCD fragmentation modes. For example, ETD/EthCD and EAD fragmentation modes often produce higher glycan and in particular peptide backbone fragmentation, which allows for an increased sensitivity for glycopeptide identification [4].

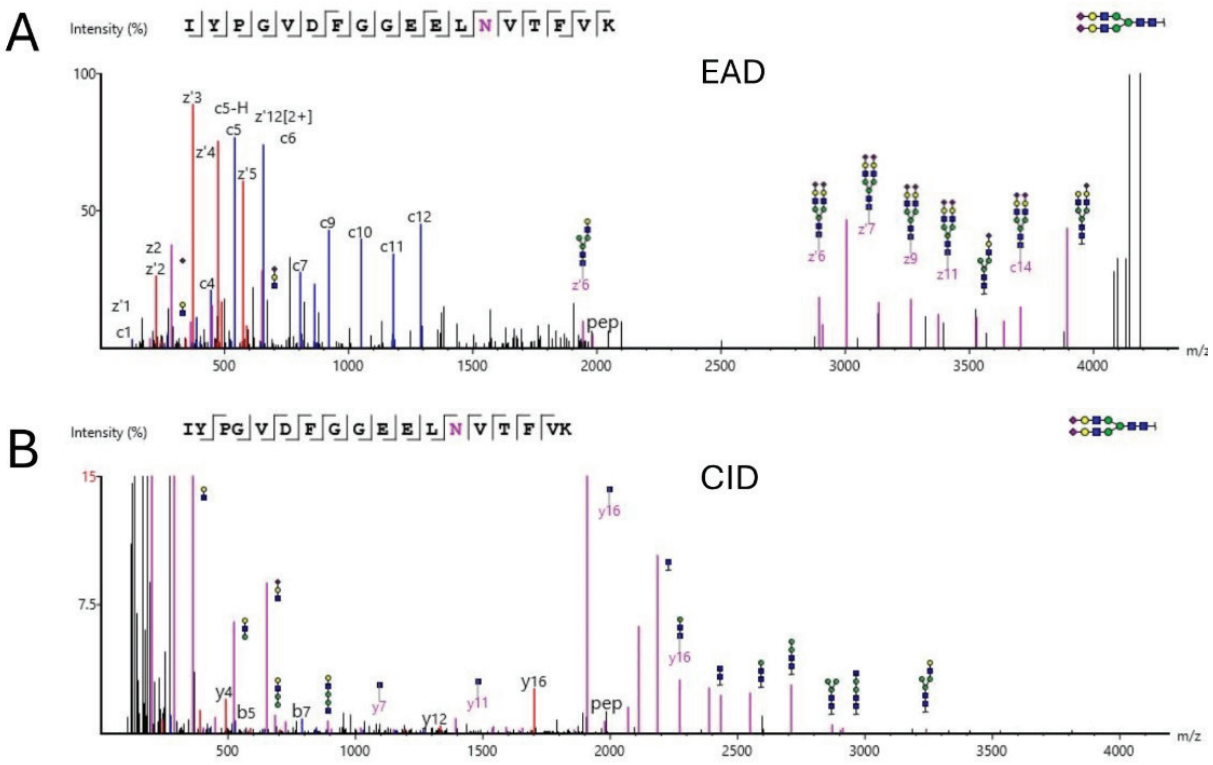

Figure 4. Comparison of EAD versus CID MS/MS spectra of a glycopeptide from Kallikrein B. (A) EAD spectrum, with the peptide backbone fully sequenced with c- and z-ion fragments, while several peptide-glycan fragments are evidence for the proposed glycan structure. (B) CID spectrum of the same glycopeptide, which, while showing some evidence of the glycan, lacks many peptide backbone fragments. Spectra were processed and annotated using PEAKS GlycanFinder software. Figure from [4].

Identification and reporter ion quantification of released glycans

In PEAKS GlycanFinder 2.5, Glycans are reported at the compositional and structure level. In cases where multiple glycan structures cannot be differentiated by the MS2 fragments, a mirror plot will be available showing the top two structures with their MS2 spectra. All glycan candidate structures can be viewed.

PEAKS GlycanFinder 2.5 identified 34 released glycans in the sample using the dataset from PXD038501.

In the reporter ion result, a heatmap is provided to easily view the glycan quantification trends across different samples and groups.

In the reporter ion glycan table result, glycans are by default listed from highest to lowest quality. A high quality score indicates that a glycan is more quantifiable. The quality score is calculated based on the glycan identification score, the noise around the reporter ions, and the mass error of reporter ions.

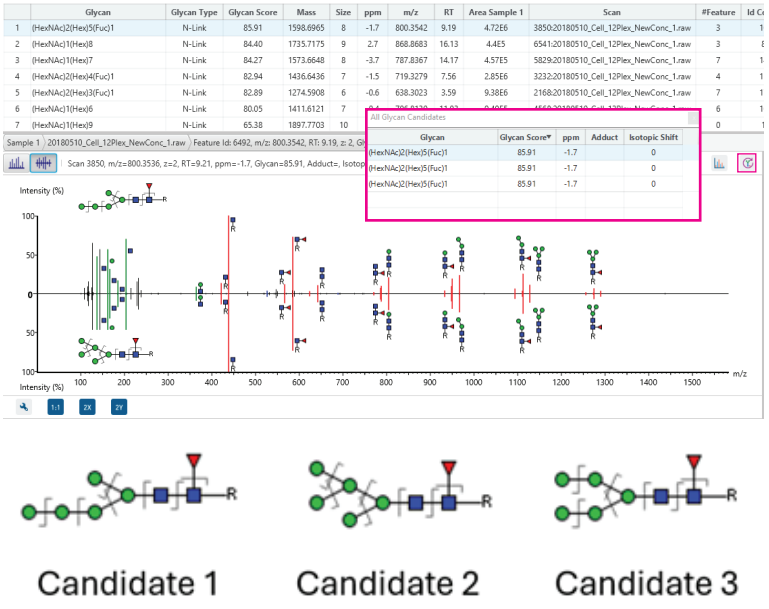

Figure 5. An example released glycan spectrum. In this spectrum, three glycan structure candidates are possible based on the fragmentation evidence. A mirror plot is shown with the annotation for each structure shown. Any structure candidate can be selected and displayed in the spectrum view.

The seven glycans with the highest quality scores quantified in PEAKS GlycanFinder 2.5 are displayed in Figure 6. A similar trend as seen in the publication was observed. The overall glycan level decreased 48 h after the treatment with Atovaquone compared to the control but returned to levels near the control group after 72 h. The explanation provided in the publication was that the N-glycan expression may have decreased due to Atovaquone causing mitochondrial electron transport inhibition but returned to normal after the cells adapted to the new conditions and compensation processes began.

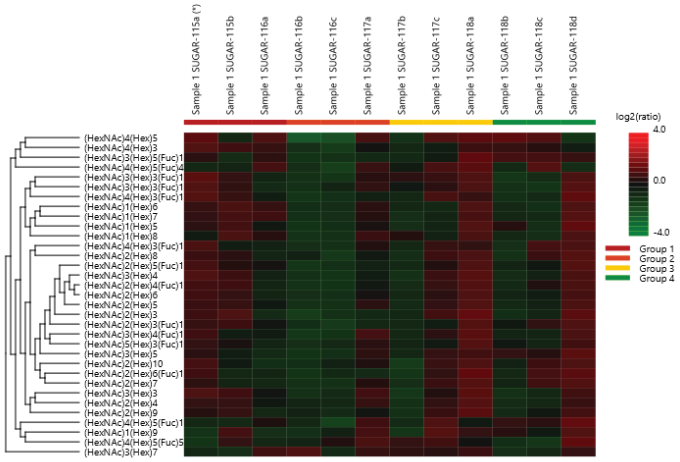

Figure 6. The heat map showing the reporter ion quantification results as log2(ratio) of the sample over the average of the experiment in PEAKS GlycanFinder 2.5 using dataset PXD038501.

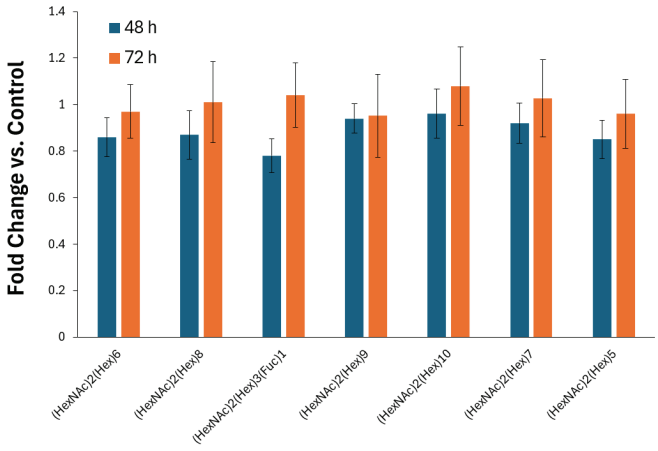

Figure 7. The top seven glycans from dataset PXD038501 with the highest quality scores quantified in PEAKS GlycanFinder 2.5.

Conclusions

PEAKS GlycanFinder 2.5 provides a comprehensive solution for qualitative and quantitative analysis of both glycopeptides and released glycans. It offers accurate and sensitive glycans identifications at the composition and structure levels. In this note we showed that PEAKS GlycanFinder 2.5 identified an increased number of glycopeptides at more collision energies compared to PEAKS GlycanFinder 2.0. In addition, we demonstrated the new released glycan analysis workflow, which allows for automatic quantitative analysis and annotation of glycomic data with scoring and quality information. Finally, PEAKS GlycanFinder 2.5 allows the researchers to gain deeper insights into the glycans' structures, site profiling, and expression levels.

References:

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[4] Remco van Soest, R.1, Pribil, P.2, Chen, Z.1, and Jurcic, K.3 Glycopeptide identification using the ZenoTOF 7600 system with EAD fragmentation and PEAKS GlycanFinder software. 1SCIEX USA; 2SCIEX Canada; 3Bioinformatics Solutions Inc. Technical Note: June 11, 2024

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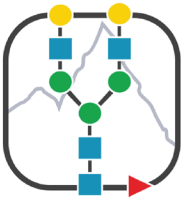

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