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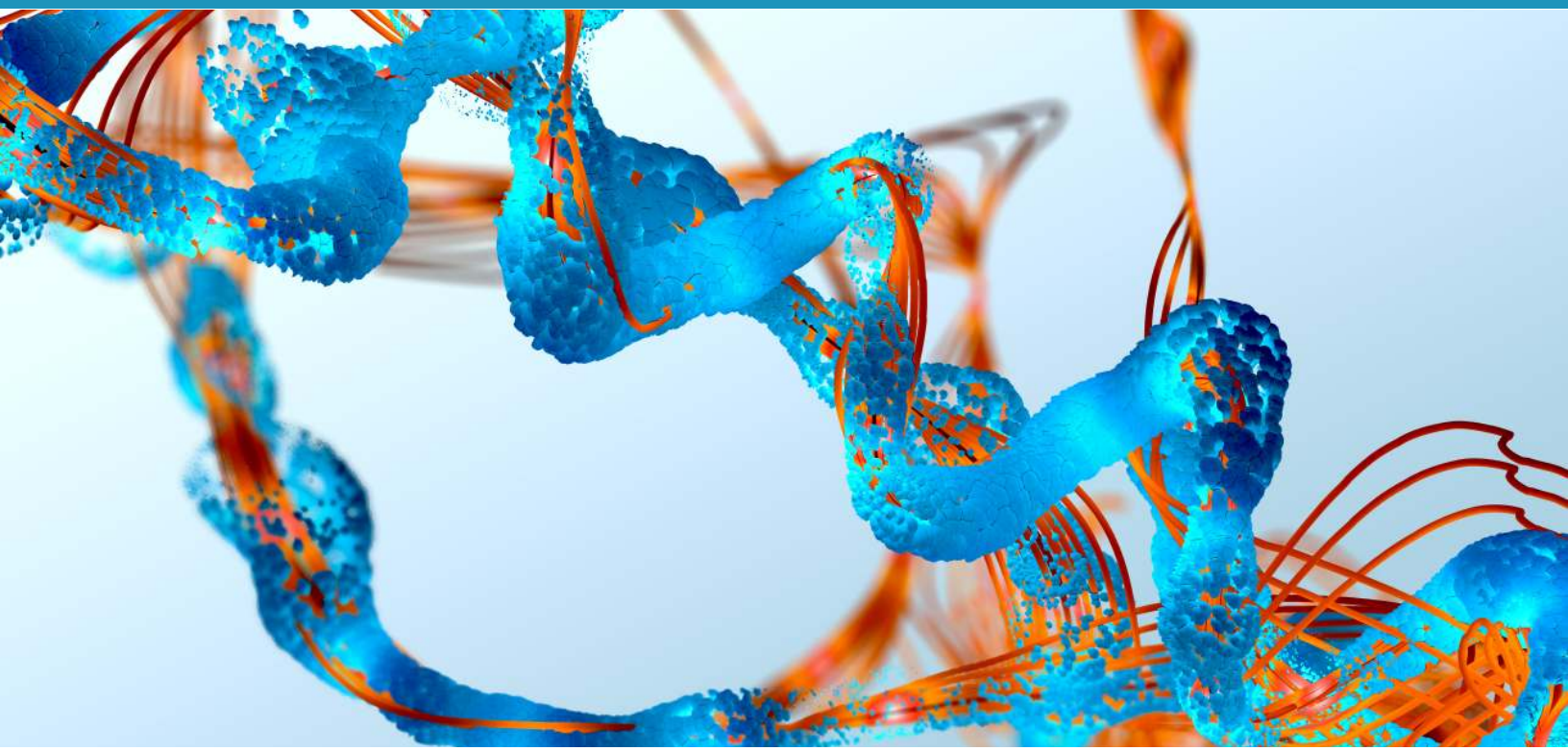

NEW PEAKS PTM: an accurate and sensitive workflow for finding confident PTMs

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Abstract:

IUnderstanding cellular signaling pathways and protein regulation requires accurate identification and localization of post-translational modifications (PTMs). The standard research approach to PTM analysis is to use liquid-chromatography mass spectrometry techniques combined with bioinformatics tools. Here we present our latest improvements with PEAKS 11 PTM tool and demonstrate the accuracy and sensitivity it provides for PTM analysis.

Introduction:

Post-translational modifications (PTMs) play a critical role in various biological process in vivo. More than 70% of proteins contain PTMs and now more than 600 types of PTMs are recorded in UniProt database [1]. Mass spectrometry (MS)-based proteomics requires bioinformatics tool to detect mass shifts between modified and unmodified peptides, and to determine the type of PTM and modification site(s).

Software	Modified PSMs (mode 1)	Modified PSMs (mode 2)
SW1	32557	N/A
SW2	43245	43603
PEAKS 11	44951	45851

Table 1. Number of modified PSMs from different software platforms.

Here we introduce new PEAKS PTM search, which could help users identify confident PTMs and find potential signature ions. With the help of versatile quality-related filters and Deep Learning-based rescoring, new PEAKS PTM search is highly accurate and sensitive in PTM characterization.

Methods and Results:

A published dataset was used [2] to demonstrate the accuracy and sensitivity of PTM characterization. The data were processed in PEAKS 11 using the workflow named PEAKS PTM search. All search parameters were aligned with the original publication. The result (SW1) and a re-analysis result (SW2) from another publication [3] were also used for benchmarking.

The original paper (SW1) reported 32557 modified PSMs and the latest paper (SW2) reported 43063 PSMs. PEAKS 11 identified 44951 and 45851 PSMs w/o pre-configurated signature ions (Table. 1).

PEAKS 11 also offers a comprehensive viewer including MS2 mirror plot and show RT shifts between modified and unmodified peptides to help users review their results (Fig. 2). FDR control (here 1% peptide-level FDR was used) and ion matches indicate the confidence PTM identification. Furthermore, A-score and support ions with predefined intensities (not shown here) help to localize the confident PTM site.

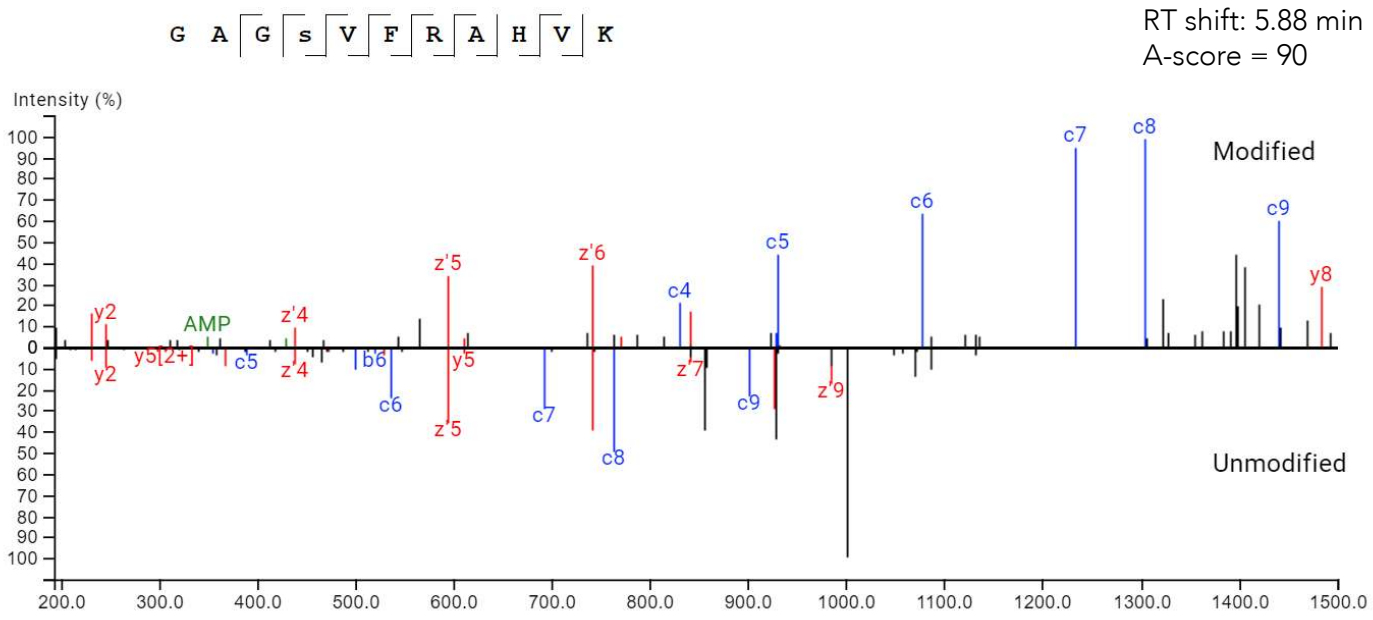

Fig 1. Mirror plot of modified and unmodified peptide

The search with pre-configured signature ions finds 3% more PSMs since it uses signature ion-based rescoring to boost sensitivity. On the other hand, if the signature ions are unknown, PEAKS PTM will also try to find potential signature ions to facilitate further spectra interpretation. For example, the search without signature ions reported X potential signature ions based on their frequency in modified and unmodified PSMs (Fig. 3).

Modification	Signature Ion Name	Mass	#PSM with modification	#PSM with modification with signature ion	#PSM without modification	#PSM without modification with signature ion	Specificity (Expected/Potential)
ADP-Ribose	ADP_136	136.0700	12981	5593	13397	1003	Potential
	ADP_159	159.0900		1726		935	
	ADP_232	232.0900		2913		197	
	ADP_250	250.0900		2813		47	
	ADP_259	259.1900		891		182	
	ADP_348	348.0700		681		37	
	ADP_428	428.0300		844		32	
Acetylation (Protein N-term)	Ace_136	136.0700	589	179	25789	6417	Potential
Acetylation (K)	Ace_136	136.0700	456	199	25922	6397	Potential

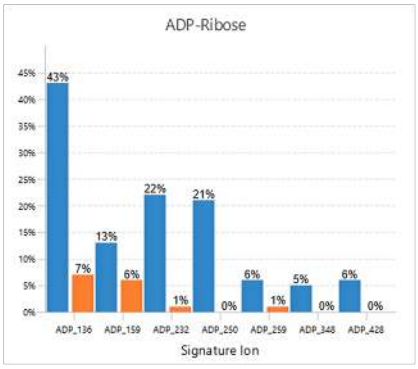

Fig 2. Potential signature ions reported by PEAKS PTM search.

Conclusion:

New PEAKS 11 PTM search improves the accuracy and sensitivity of PTM identification.

References:

1. Long et al., (2023) Nonenzymatic Posttranslational Modifications and Peptide Cleavages Observed in Peptide Epimers. JASMS. DOI: 10.1021/jasms.3c00092
2. Buch-Larson et al., (2020) Mapping Physiological ADP-Ribosylation Using Activated Ion Electron Transfer Dissociation. Cell Reports. DOI: 10.1016/j.celrep.2020.108176
3. Polasky et al., (2023) MSFragger-Labile: A Flexible Method to Improve Labile PTM Analysis in Proteomics. Mol Cell Proteomics. DOI: 10.1016/j.mcpro.2023.100538

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