Analysis of post translational modifications with AB SCIEX 5600 TripleTOF System

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Introduction

Finding post-translational modifications (PTMs) is still a challenging task in mass spectrometry-based proteomics. Association of Biomolecular Resource Facilities (ABRF) sPRG 2010 study evaluated the performance of available PTM analysis methods against a comprehensive PTM standard. The study showed the need for improving the sensitivity and accuracy of PTM analysis. In addition, to achieve better protein coverage and explain more input spectra, the peptide score in database search is designed to represent the overall matching quality of the peptide, not for a specific modified residue. A high-score peptide may still have PTMs poorly assigned at specific locations, if key fragment ions are missing. In this abstract, a successful procedure using an AB SCIEX 5600 TripleTOF system with PEAKS PTM software was demonstrated.

Methods

1. A list of highly confident proteins is identified by database search. Each peptide from the highly confident proteins is "modified" in-silico by trying all possible modifications in Unimod database. These theoretically modified peptides are compared with the spectra to identify modified peptides.

2. High mass accuracy of precursor and fragmentation ions was used to resolve the ambiguity of modifications with very close mass differences.

3. For modified peptide identification with high score, the intensities of two consecutive peaks spanning the modified residue are used to measure modification confidence.

Results

Two samples from ABRF sPRG2011 were used for LC-MS and MS/MS by AB SCIEX 5600 TripleTOF with CID fragmentation. One contains only a lyophilized mixture of 70 synthetic modified peptides, and the second contains the same mixture combined with a tryptic digest of the six proteins from which the synthetic peptides were derived. The data was analyzed with PEAKS 6 with 20 ppm of precursor mass error and 0.1 Da fragment mass error. The suspected modifications were static Carbamidomethylation on C and variable Oxidation on M and Deamidation on NQ. The result was reported with 1% of FDR at peptide-spectrum match level.

61 and 63 synthetic modified peptides were identified respectively in sample one and two. The recovery of spiked modifications was shown in Figure 1. 40 and 42 modification sites were localized (the intensities of two consecutive peaks spanning the modified residue are great than 5%) respectively in sample one and two. 67% of modifications was localized as shown in Figure 2.
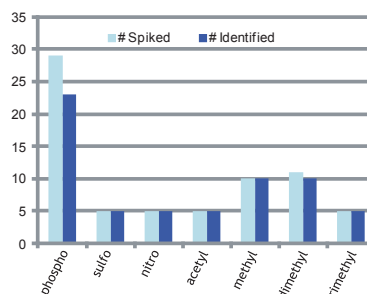
To achieve better protein coverage and explain more input spectra, the peptide score in database search engines is designed to represent the overall matching quality of the peptide, not for a specific modified residue. A high-score peptide may still have PTMs poorly assigned at specific locations. The fragment peaks closely related to the modification site provide direct evidence of PTM location. For modified peptide identification with high score, the intensities of two consecutive peaks spanning the modified residue are used to measure modification confidence.

In order to evaluate ability of PEAKS 6 to identify modified peptides in a complex peptide mixture, the data set from ABRF iPRG study 2012 was tested. The performance was shown in Figure 3. One of the spiked-in peptides with variety of modifications was shown in Figure 4.

Figure 1. Recovery of the identification for the spiked peptides
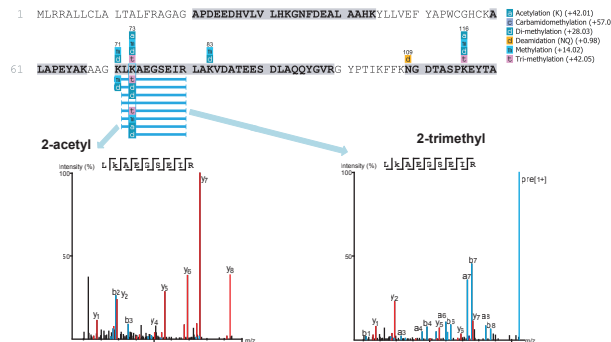
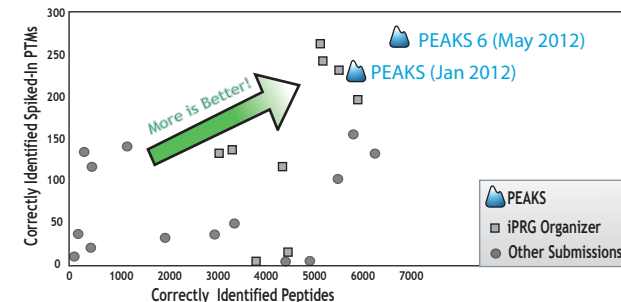Figure 2. Identification of 2-acetyl (a) vs 2-trimethyl (b) peptides

Figure 3. PEAKS software reported several PSMs with the same mutation, indicating a highly confident mutation site.
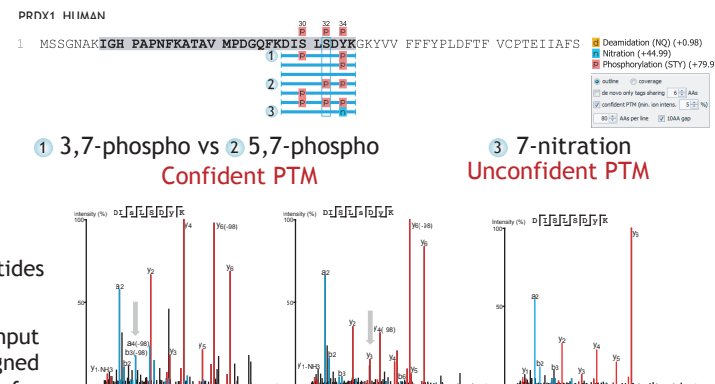

Figure 4. The peptide-spectrum annotation reports strong evidence peaks for the mutation at site.

Conclusions

Excellent performance of PTM analysis with 5600 TripleTOF using PEAKS software.

References

X. Han, L. He, L. Xin, B. Shan and B. Ma. PeaksPTM: Mass Spectrometry Based Identification of Peptides with Unspecified Modifications. Journal of Proteomics Research, 2011, 10(7): 2930-2936.