HiLight-PTM: an online application to aid matching peptide pairs with isotopically labelled PTMs

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Abstract

**Motivation:** Database searching of isotopically labelled PTMs can be problematic and we frequently find that only one, or neither in a heavy/light pair are assigned. In such cases, having a pair of MS/MS spectra that differ due to an isotopic label can assist in identifying the relevant m/z values that support the correct peptide annotation or can be used for de novo sequencing.

**Results:** We have developed an online application that identifies matching peaks and peaks differing by the appropriate mass shift (difference between heavy and light PTM) between two MS/MS spectra. Furthermore, the application predicts, from the exact-match peaks, the mass of their complementary ions and highlights these as high confidence matches between the two spectra. The result is a tool to visually compare two spectra, and downloadable peak lists that can be used to support de novo sequencing.

**Availability and implementation:** HiLight-PTM is released using shinyapps.io by RStudio, and can be accessed from any internet browser at https://harrywhitwell.shinyapps.io/hilight-ptm/

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**Supplementary information:** Supplementary data are available at Bioinformatics online.

1 Introduction

The use of stable isotopes to heavy label proteins (e.g. SILAC) is a common proteomic approach (Chen et al., 2015), allowing the simultaneous analysis of multiple samples whilst reducing experimental variation. In such an experiment, proteins may be analyzed by tandem mass spectrometry (MS/MS) and peptide fragmentation spectra searched against a database of in silico fragmentation peak lists for proteins of interest. It is common that many of the peptides’ fragmentation spectra will go unassigned, for example due to mixed spectra arising from co-fragmentation, fragment ions having too low an intensity, isotopically labelled amino acids having ambiguous masses or the presence of post-translational modifications (Hart-Smith et al., 2016). In such cases, having a pair of MS/MS spectra, that differ due to an isotopic label or post translational modification, can assist in identifying the relevant m/z values that support the correct peptide annotation.

Consider the histone H3 peptide ‘EIADSFK_{me}TDLR’ where the lysine is monomethylated and MS/MS spectra exist for both the light (CH₃) and heavy (13CD₃) methylated peptide. The y and b ion series for both spectra will match prior to the methylated lysine (i.e. y-ions 1-4 and b-ions 1-6). After the methyl lysine, the fragmentation series will differ by 4 Da (i.e. the difference between heavy and light methylation) (Fig. 1A). In studies using heavy labelled PTMs [e.g. methylation (Ong et al., 2004; Zee et al., 2010), acetylation (Everts et al., 2013)], the difference in mass between the heavy and light modification is known in advance. The m/z’s that match exactly between the two spectra can be used to predict what m/z’s should be present if that modification was there, and the supporting y and b ion can be identified.

We have developed an online web application to compare two MS/MS spectra. The application graphically presents the spectra showing m/z’s that match exactly or are separated by the specified mass shift, with added confidence if they are calculated from their complementary ion series.

The user is able to adjust the graph in order to output high-quality figures as well as download the matched peak lists that may be used for de novo sequencing.

2 Description

The application, available online at https://harrywhitwell.shinyapps.io/hilight-ptm/, was created in R (version 3.5.0) as a shiny (1.1.0) app that uses packages ggplot2 (3.0.0) and gridExtra (2.3) to generate and output plots. It takes one or two comma separated value (csv) files as input, each containing a peak list from a centroided MS/MS scan with m/z and intensity values in the first and second columns respectively.

The peak list is filtered by binning the m/z values and returning the most intense values in each bin (Cox et al., 2011). The size of the bin (parameter: Bin width) and the number of peaks in each bin...
3 Evaluation

Within our group, we use this program as a rapid visual aid for comparing two MS/MS spectra in the identification of protein methylation. Following culture with heavy-labelled methionine, methyl groups are indeed present as a mixture of CH₃ and ¹³CD₃, thus co-eluting peptides are separated by a 4 Da shift. Often, we find that whilst MS/MS spectra are acquired for both heavy and light peptides, only 1 or neither may be assigned by database searching (Hart-Smith et al., 2016). For these MS/MS pairs, we wish to visually ascertain if they are indeed the same methylated peptide or not. For example, Figure 1C and D shows the MS/MS of two doubly-charged peptides with a mass shift of 2 m/z (4 Da). Database searching only identified the heavy-labelled peptide (733 m/z) (Fig. 1B), despite MS/MS being present for both (Fig. 1C). Initial comparison of the MS/MS spectra is not conclusive—as the spectra for 731 m/z has more abundance ions and more peaks around 1000 m/z compared to the spectra for 733 m/z. We then visualize the peaks that are the same between the two spectra, mass-shifted and predicted from the exact-matched ions (Fig. 1D).

We can see that exact-matched and mass-shift matches are present throughout the spectra and the relative intensities between them are similar. Furthermore, the higher-confidence matches—those predicted from the exact-mass matches—correspond to the fragment masses that contain the monomethyl-lysine in the annotated spectra. For example, the highlighted peak at m/z 964 or 968 in the light or heavy spectra respectively (Fig. 1D) is annotated as the y7 fragment (‘DFK{me}TDLR’) (Fig. 1B).

This visualization allows for easy identification of matched peaks and the identification of the mass-shifted ion series providing additional confidence to the annotated spectrum. Similarly, identifying the mass-shifted peaks in this manner provides information for de novo sequencing.

The peak list and optional inputs used to generate these figures are provided in Supplementary Material.

4 Conclusion

We have developed a simple to use tool for comparing two fragment ion peak lists for peptides with isotopically labelled PTMs. The application could also be used to evaluate heavy/light SILAC pairs, assess peptide assignment from database searching and identify de novo sequencing. The software is open source and free to use from https://harrywhitwell.shinyapps.io/hilight-ptm/

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Conflict of Interest: none declared.

References


