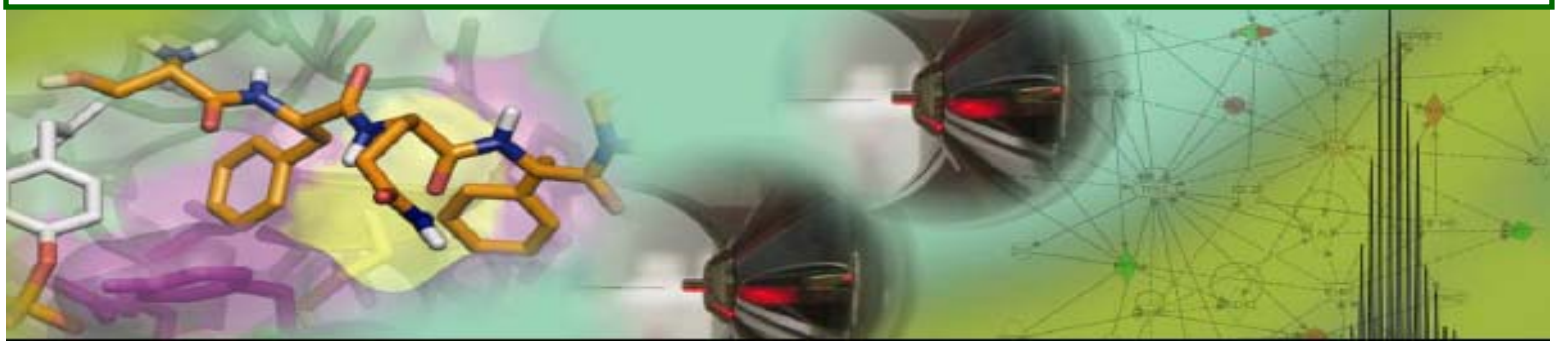
MS & Proteomics Resource

Yale School of Medicine
Keck Biotechnology Resource Laboratory



Application Note 6: Using MD-Score and PhosphoRS in YPED to aid in localization and verification of phosphosite(s)

Phosphosites

Due to the growing number of phosphopeptide identification assays, we have added tools to the Yale Protein Expression Database (YPED), which enable researchers to filter their LC-MS results based on phosphoprotein peptide identification data. The identified phosphopeptide (usually identified in a Mascot database search) can then be further queried to view the probability of a site that is actually phosphorylated using either MD-score (1) and/or PhosphoRS (2) scoring algorithms. Both of these algorithms enable us to automate phosphopeptide localization on large phosphopeptide datasets and have high confidence that the sites are assigned correctly based on strong experimental and statistical evidence.

In YPED, you can view the peptides identified by clicking on the “view” link under the peptides column in the bottom table. The underlined amino acid Ser(S), Thr(T), or Tyr(Y) are the phosphosite(s) that Mascot has determined is the most likely site of modification. The site can be manually validated by reading the MS/MS fragmentation pattern. However, sometimes the fragmentation pattern is insufficient to determine the site of modification. Hence, computer algorithms use other criteria for locating the site. This includes looking for the neutral loss of -98Da for pS and pT. If in YPED you click on the “View PhosphoProteins” link, (above the bottom table), you will see the phosphoproteins identified, and a count of the number of phosphopeptide matches (called Phospho ct, and then if it is S, T or Y under the Phospho (ST) ct and Phospho (Y) ct columns). If you click on the “view” link under the phosphopeptide column in the table, you will see the identified phosphopeptides. Again, the underlined amino acids are the sites identified using the Mascot algorithm. There are also now new columns called MD-Score, and PhosphoRS with a PhosphoRS score for each site identified.

MD-Score

The Mascot Delta Score (MD-Score) simply reflects the difference of Mascot ion scores between the highest and second highest ion scores for candidate phosphorylation sites on an identical peptide sequence in a database search. This comes into play when there is more than 1 possible phosphorylation site in a peptide. The goal is to determine if there is any evidence in the MS/MS spectra to assign the most probable site. The accuracy of assignment depends on the sequence separation of the 2 sites, if there are any ions between the sites, and if the signal to noise ratio for the assigned fragment peaks are sufficient to confirm the sites. In Mascot, a MD-Score of 10 means that one site has a 91% probability of being correct and other only 9% (for 2 possible sites). Hence, for confident

scores, 10 or greater is required. Scores at less than 10 mean that there is not significant evidence to assign the site.

PhosphoRS

In PhosphoRS, the algorithm also uses the peptide sequence with the MS/MS spectra in order to localize the phosphorylation to 1 site, with a probability assigned to each site. It then calculates individual probability values for each putatively phosphorylated site based on estimating the probability that the observed match between theoretical and acquired fragment ions is a random event. Hence, probability scores of > 0.99 means that you can confidently assign that site from the MS/MS spectra. For lower probability scores, applying a phosphoRS site probability cutoff of 0.75 to your data should still lead to an acceptable False Localization Rate (FLR) for publication.

(1) Savitski MM, Lemeer S, Boesche M, Lang M, Mathieson T, Bantscheff M, Kuster B. (2011) Confident phosphorylation site localization using the Mascot Delta Score. Mol Cell Proteomics. 2011 Feb;10(2):M110.003830.

(2) Taus T, Köcher T, Pichler P, Paschke C, Schmidt A, Henrich C, Mechtler K. (2011) Universal and confident phosphorylation site localization using phosphoRS. J Proteome Res. Dec 2;10(12):5354-62.