Discovery of Unanticipated Modifications using Protein Prospector

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Introduction

• In LC-MSMS analyses large numbers of spectra are not identified by standard database searching strategies.
• Several of these spectra correspond to peptides with unanticipated modifications.
  • This is especially true when some proteins are present at very high abundance relative to others.
• It has been proposed that there are 8-12 modified versions (mainly chemical/artifactual) of each unmodified tryptic peptide.1
• If you want to fully characterize your sample you need to be able to explain as much of your data as possible.
  • What is the best strategy to try to identify these peptides?

• Strategies that have been proposed:
  • Identify ‘good looking’ spectra and search only these for more modification types.2
  • Compare unidentified spectra to those identified to find families.3
  • Perform database searching allowing for unexpected modifications, searching only against those peptides4 or proteins5,6 identified in a standard database search.
  • With very high mass accuracy data de novo interpretation.7
In this poster we present the performance of unexpected modification searching using Protein Prospector:

- How is the search/analysis performed?
- What does it find?
- How does it compare to other software?
- What are the advantages of using Protein Prospector for doing this type of search?
Heterogeneity of Cysteine Modifications

- Cysteines pick up many different modifications.
- Peptides containing two cysteines can be very heterogeneous.
- Single peptide identified in 11 different forms:

  1. GFC(dehydro)TLC(dehydro)FIEYR (internal disulfide)
  2. GFC(carboxymethyl)TLC(carboxymethyl)FIEYR
  3. GFC(propionamide)TLC(carboxymethyl)FIEYR
  4. GFC(carboxymethyl)TLC(trioxidation)FIEYR
  5. GFC(trioxidation)TLC(carboxymethyl)FIEYR
  6. GFC(trioxidation)TLC(sulfide+carboxymethyl)FIEYR
  7. GFC(oxidation+carboxymethyl)TLC(carboxymethyl)FIEYR
  8. GFC(trioxidation)TLC(Cys->Dha)FIEYR
  9. GFC(propionamide)TLC(sulfide+carboxymethyl)FIEYR
  10. GFC(Cys->Dha)TLC(carboxymethyl)FIEYR
  11. GFC(carboxymethyl+DTT)TLC(carboxymethyl)FIEYR
Can manually type in sequence (with modifications) to see how it matches e.g. to assess evidence for correct site assignment.

Change the number of peaks used for fragment assignment – useful for PTM analysis.

View raw data (available for ABI and Thermo instruments).
Tryptophan Modification

• Tryptophan is also very prone to modification:
e.g.

LLDNWDSVTSTFSK
LLDNW(oxidation)DSVTSTFSK
LLDNW(dioxidation)DSVTSTFSK
LLDNW(Trp->Kynurenin)DSVTSTFSK
LLDNW(-71)DSVTSTFSK
LLDNW(+61)DSVTSTFSK
Identification of Cross-linked Peptides

• Data from a study of protein binding interfaces using cross-linking.

<table>
<thead>
<tr>
<th>ID</th>
<th>Score</th>
<th>Q-value</th>
<th>Type</th>
<th>Spectrum</th>
<th>Assignments</th>
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<tbody>
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<td>11.12</td>
<td>8.6</td>
<td>7.7e-4</td>
<td>3.39</td>
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<tr>
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<td>869.4754</td>
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<tr>
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<td>16.1</td>
<td>1.6e-4</td>
<td>3.12</td>
</tr>
</tbody>
</table>

Clicking here will allow you to re-search this one spectrum with different search parameters; e.g. allow for a modification of (817 x 3 =) 2450 Da to try to identify the other peptide.

MS-Bridge Assignment of Cross-linked Peptides (from different proteins)

1. 180 (L)MFDNLTDRL
2. 247 258 (K)KIITNLTEGR
Comparison of Protein Prospector Mass Modification Searching with Alternative Software

• InsPecT is freely available software designed for finding modified peptides in iontrap MSMS data.9
  • It works by finding sequence tags and has no bias towards particular modifications.

• We compared mass modification searching in Protein Prospector to InsPecT results of the same dataset.

Software Results Comparison

• The dataset: One LCMSMS analysis of a gel-purified modified protein.
• The sample was initially searched without looking for modifications and 17 database entries were subsequently used for the modification searches using Protein Prospector and InsPecT.
• Number of spectra acquired: 521.
• Number of unmodified spectra identified (PP E-value <0.01): 73

• After modification searching:
  • Number of assignments Prospector and InsPecT agree completely upon: 116
  • Number agree on modification but not site: 27
  • Number agree on peptide but not modification: 37

• Both search engines returned 163 matches with E-value/P-value <0.3, of which the majority of spectra uniquely identified by one or other were due to slightly different search parameters.

• Conclusion: the two different searching and scoring strategies produce essentially the same assignments, with neither being significantly more sensitive/reliable than the other.
Where Protein Prospector is better

• Protein Prospector allows the user to search for a combination of specified and unexpected modifications: e.g. search allowing for methionine oxidation and one unknown modification:
  • InsPecT only allows searching for either specified or unknown
  • Hence, Protein Prospector is able to more reliably identify multiply modified spectra and more consistently assign the correct site where a common modification is present.
• In Protein Prospector you can specify to allow modification to only certain amino acids*:
  • Reduction in ‘search space’ can give more confident and reliable answers whilst still identifying unexpected modifications.
• Protein Prospector user-friendly interface allows easier assessment and verification of results.

*not utilized in this software comparison

Conclusions

• Protein Prospector is able to identify many MSMS spectra to peptides with modifications:
  • It does find many biological modifications as well as the chemical modifications highlighted in this poster; e.g. ubiquitination, tyrosine nitration...
  • In some cases the ‘modification’ is an unexpected cleavage site.
  • Modification can be large (a cross-linked peptide).
  • Site assignment is much less reliable than peptide and modification assignment.
• Certain residues account for a large percentage of all the modifications, most notably cysteines and tryptophans.
• Protein Prospector allow easy assessment and manual verification of assignments.

• Performance of software is at least as good as InsPecT (most other alternative software is not freely available).

• A new version of Protein Prospector (including mass modification searching) will be available to the public in the next month or two!
References


Acknowledgements

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