Overview

For theoretical modeling of the approach, the Home sapiens proteins were digested with Trypsin and GluC. The fragment peptides were then separated by HPLC and the LC retention time prediction accuracy and +/- 0.5 pH units isoelectric point accuracy provides enough specificity in order for the peptides with MW>1000 Da to be identified with high confidence. Indeed, at least >91% of the peptides with MW >1500 are unique.

Methods

• For experimental verification, the Home sapiens proteins were digested with Trypsin and GluC. The fragment peptides were then separated by HPLC and the LC retention time prediction accuracy and +/- 0.5 pH units isoelectric point accuracy provides enough specificity in order for the peptides with MW>1000 Da to be identified with high confidence. Indeed, at least >91% of the peptides with MW >1500 are unique.

Results

• Peptide frequency

Peptide MW (mass bin of 25)

<table>
<thead>
<tr>
<th>Peptide MW</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td></td>
</tr>
<tr>
<td>1250</td>
<td></td>
</tr>
<tr>
<td>1500</td>
<td></td>
</tr>
<tr>
<td>1750</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td></td>
</tr>
</tbody>
</table>

Conclusion

A novel shotgun proteomics method is proposed and was successfully implemented. The method modeling and preliminary results imply that the present method can be a standard alternative to traditional MS/MS-based shotgun proteomics methods.

The “in-solution fragmentation” approach is anticipated to provide high throughput and increased throughput. Furthermore it can be further extended and used in novel ways: In combination with accurate MS/MS information the “in-solution fragmentation” approach can provide more highly specific peptides and be used as a powerful de novo sequencing approach.

The present method uses full MS spectra (rather than MS/MS) for peptide identification, making it possible to use/explore a more powerful database mode for their identification. An appropriate set of proteomes might be necessary for optimized results (i.e., AspN as first digestion instead of LysC).

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References


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