Precursor Mono-Isotopic Mass And Charge Determination with Almost 100% Accuracy

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Overview

Purpose: To design an algorithm to determine the correct charge state and mono-isotopic mass of the precursor ion from high-resolution MS data.

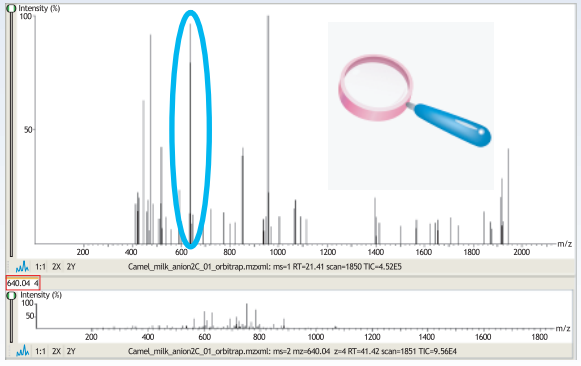
Methods: Generate all the potential isotopic envelope candidates in the given m/z range, compare their theoretical isotopic distributions with the observed distribution, and pick up the most similar one.

Results: Greatly increased the accuracy of the mono-isotopic mass and charge state of the precursor ion.

Introduction

The mono-isotopic precursor ion mass is crucial for most existing software to identify a peptide from its MS/MS spectrum. High-resolution MS/MS instruments promise to significantly enhance proteomics analysis by providing smaller mass error tolerance for both the precursor and the fragment ions. Thermo Fisher LTQ-FT and LTQ-Orbitrap instruments are among the most popular high-resolution instruments. However, very often the precursor mass reported by these instruments is offset by one or more isotopes from the correct value. This would cause the software analysis to fail unless, contrary to the nature of high-resolution experiments, a bigger mass error tolerance is used. Here we propose an algorithm to automatically determine the correct charge state and mono-isotopic mass of the precursor ion from high-resolution MS data.

Figure 2. An example: MS and its MS/MS



Methods

1.Candidate Generation.
2. Candidate Evaluation.

Figure 1 Algorithm for predicting precursor m/z value and charge state

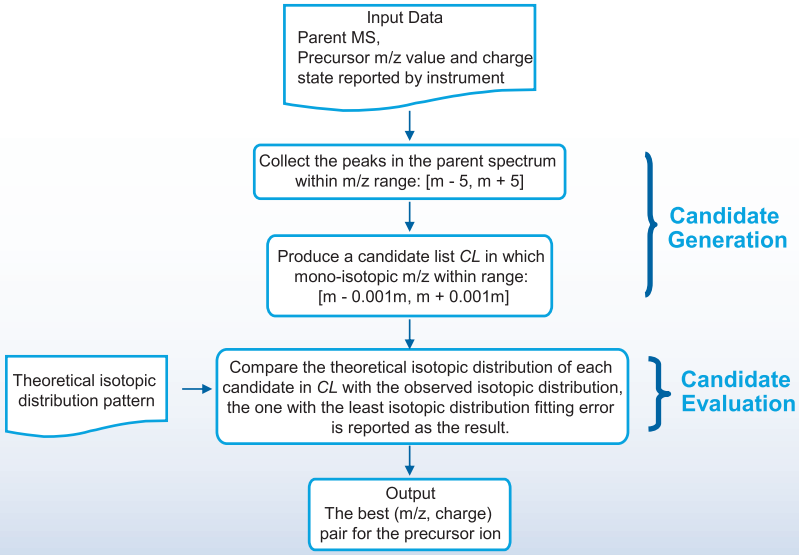
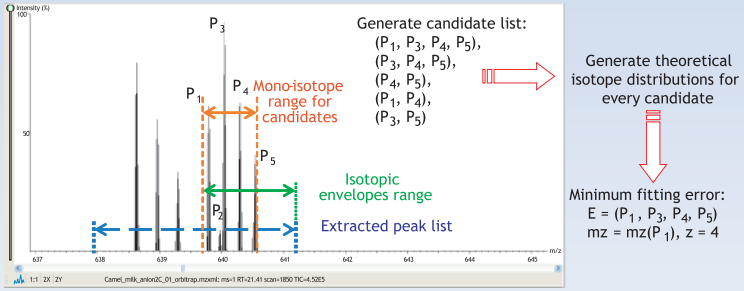


Figure 3 An example: steps to determine the mono-isotopic mass and charge state



Results

The algorithm was implemented in Java and tested with two standard protein mixtures. The standard mixtures were reduced and alkylated by iodoacetamide, then digested by trypsin overnight. The peptide mixture (2 µl injected) were separated via Surveyor™ LC equipped with MicroAS™ autosampler (Thermo Fisher Scientific) using a reversed phase analytical column (75 µm inner diameter, 10cm length, 3m particle size, both Nanoseparations, NL), at a flow rate of 250nl/min. A gradient of 5-30% acetonitrile in 90 minutes was used.

Both data sets were obtained from Thermo LTQ-Orbitrap XL. One contains 6317 MS spectra and 711 MS/MS spectra. The mono-isotopic m/z of 100 spectra were manually annotated by a human expert (the annotator). The annotation showed that 68 out of the 100 precursor m/z reported by the instrument were not mono-isotopic. The other dataset contains 32 MS spectra and 96 MS/MS spectra. All 96 precursor masses reported by the instrument were not mono-isotopic. All the charge states of both datasets are from 1 to 5.

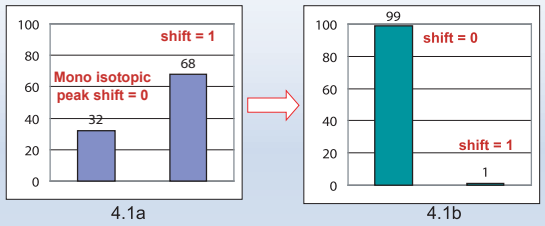


Figure 4.1
Experiment on data set A: precursor mono-isotopic peak correction: before (a) vs. after (b)

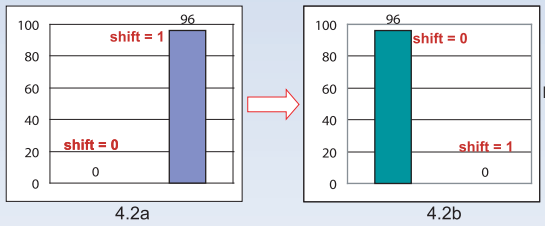


Figure 4.2
Experiment on data set B: before (a) vs. after (b)

Conclusion

The experiment results showed that our mono-isotopic mass and charge determination algorithm has almost 100% accuracy on our testing data sets.

Reference

B. Ma, K. Zhang, C. Hendrie, C. Liang, M. Li, A. Doherty-Kirby, G. Lajoie. PEAKS: Powerful Software for Peptide De Novo Sequencing by MS/MS Rapid Communications in Mass Spectrometry, 17(20):2337-2342. 2003. Early version appeared in 50th ASMS Conference 2002.

Screenshots courtesy of PEAKS Studio 5.1 (proteomic mass spectrometry software).

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