

Application of a Data Dependent™ Neutral-Loss Experiment on the Finnigan LTQ™ for the Identification of Phosphorylated Peptides

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Abstracted from posters presented at ASMS 2003 and IMSC 2003, with co-authors: Amy Zumwalt, Jae Schwartz, Julian Phillips, Iain Mylchreest, Eric Hemenway, and Melissa Chen

Goal:

What we set out to demonstrate ...

- The functionality and applicability of the Data Dependent™ neutral loss algorithm for the detection of phosphorylated peptides
- The use of TurboSEQUENT™ in BioWorks for the identification of phosphopeptides from MS³ data
- The ultra-high sensitivity of the new Finnigan LTQ™ linear ion trap mass spectrometer to produce high-quality MS³ data

Background: Why look for phosphorylation sites?

Phosphorylation

- Recognized as one of the most important post-translational modifications of proteins
- Associated with many proteins that have a regulatory function in cells

The Phosphoproteins Challenge

Low-level abundance

- Generally found in low quantities within cells
- Complexity of analysis by ESI (+) MS, due to the higher acidity of phosphopeptides

Limited fragmentation

- Exhibit a prominent neutral loss of a phosphate group (98 Da)

Identification and site determination

- Identification of the phosphoprotein and determination of the exact site of phosphorylation is often limited by inadequate peptide fragmentation and diagnostic sequence ion information

The Solution

Data Dependent Neutral Loss Experiment

- Selectively trigger MS³ scans on only the MS/MS fragment ions for which a prominent neutral loss ion is detected

standard on the Finnigan LTQ!

Finnigan LTQ's Ultra-high Sensitivity LC/MSⁿ

- Required for low abundance phosphoproteins
- To generate high quality MS/MS, and MSⁿ data

Further Confirmation by MS³

- To identify the phosphopeptides and phosphorylation sites

Experimental Conditions

Samples

- Alpha and Beta Casein standards were reduced, alkylated and buffer exchanged, and subsequently enzymatically digested

Finnigan Surveyor LC Conditions

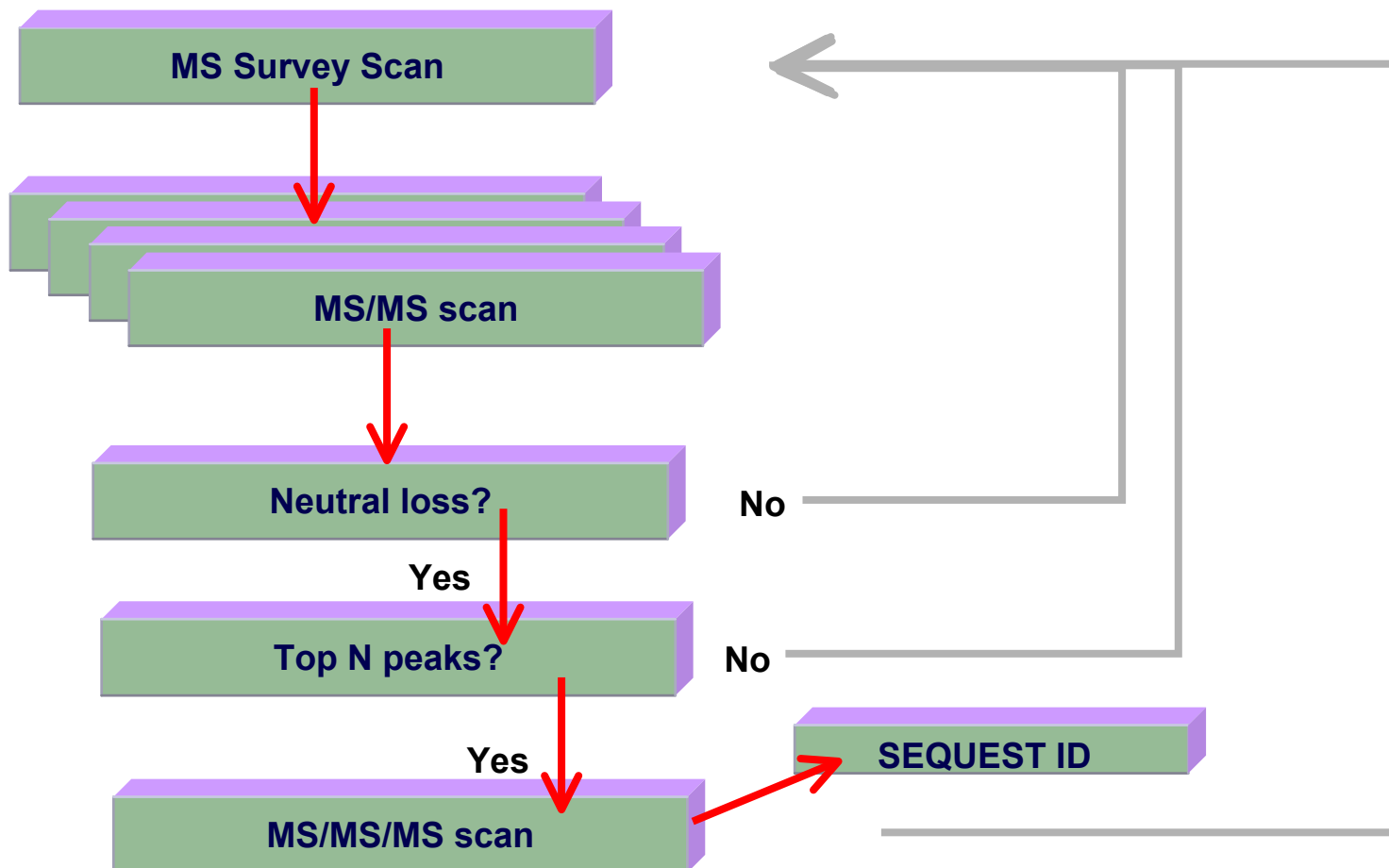
- Column: C18 Column (2.1x100mm, 3 μ m)
- Flow rate: 400 μ L/min
- Gradient: 2-60% acetonitrile with 0.1% formic acid in 30min, 60-80% in 3 min, at 80% for 5 min

Finnigan LTQ Conditions

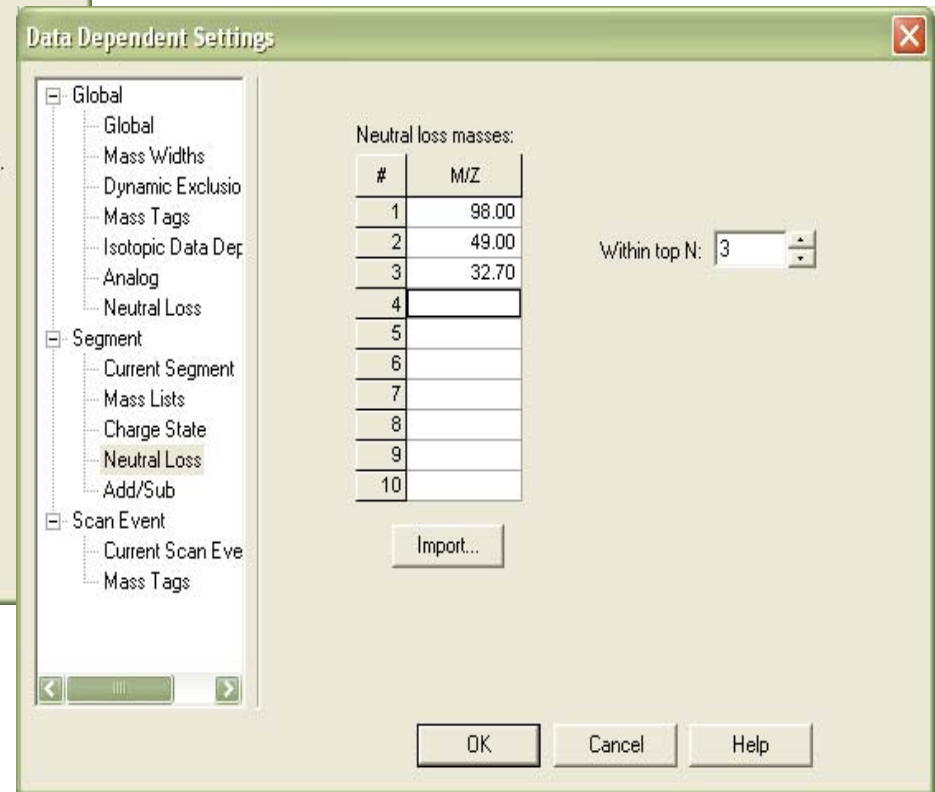
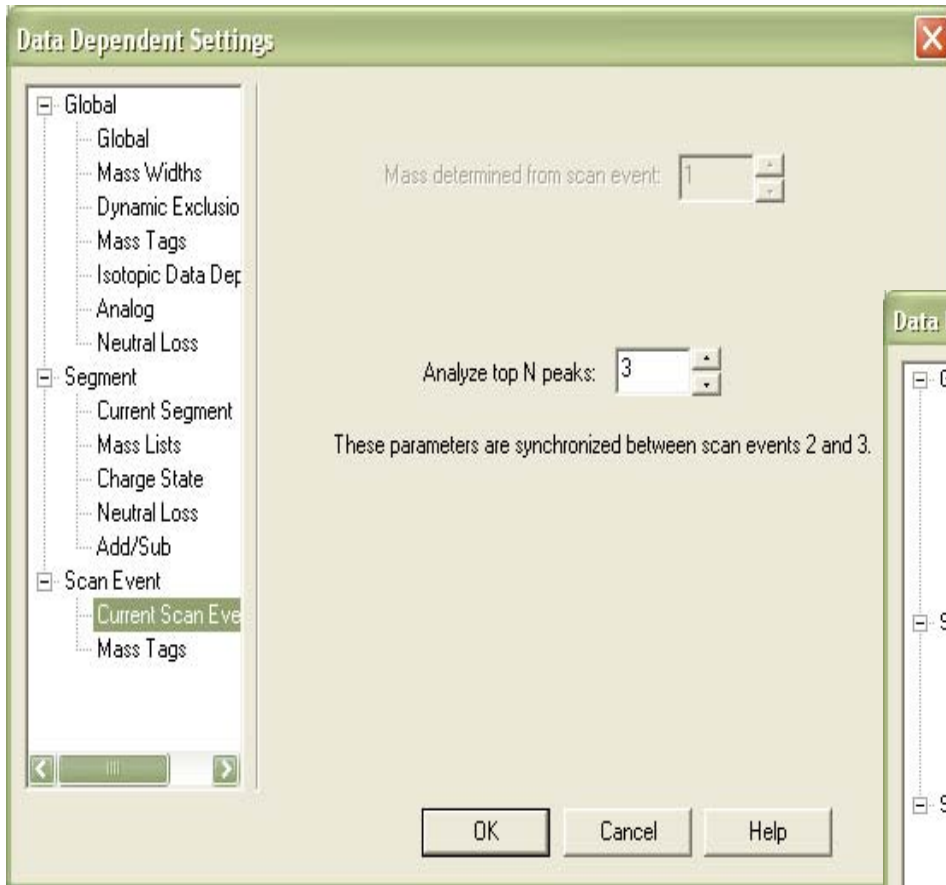
- NanoSpray ionization



Automated phosphorylation site analysis using Data Dependent MS³

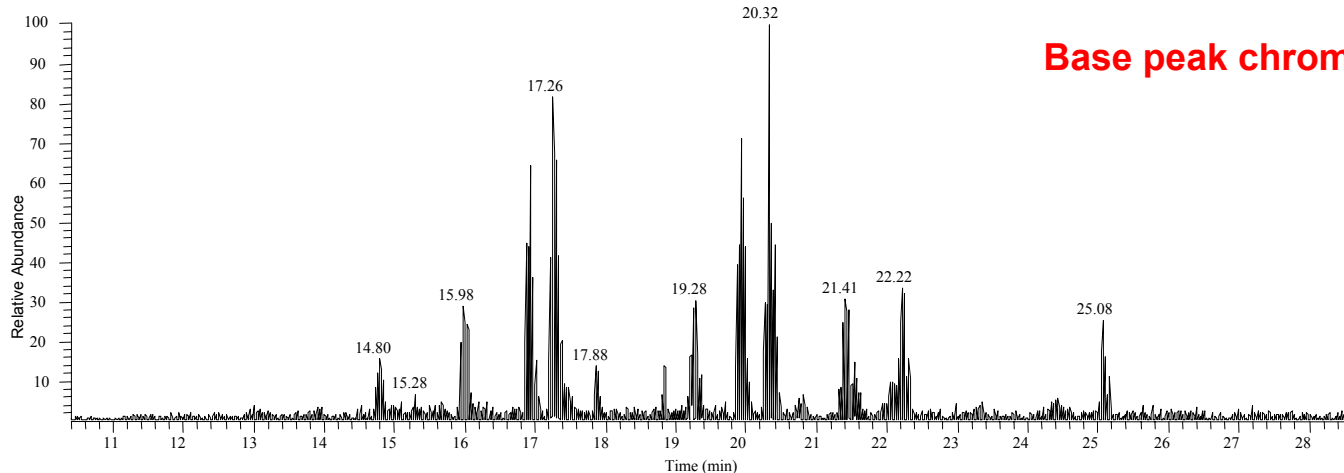


Data Dependent acquisition parameters for phosphorylated peptides

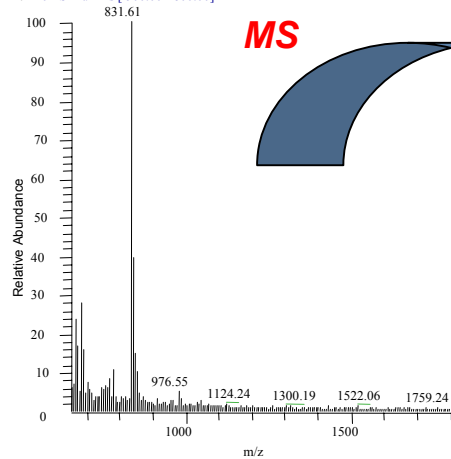


Casein Digest 10 fmol on column

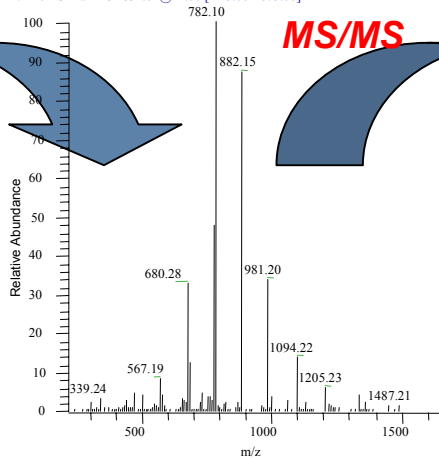
RT: 10.39 - 28.68



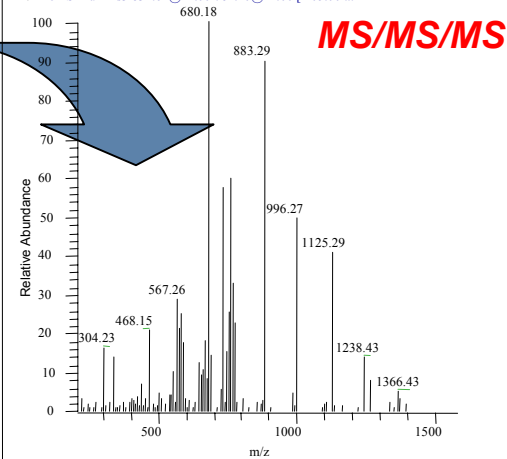
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T: + c ESI Full ms [500.00-1800.00]



alphabeta10fmole1_2msms # 3149 RT: 16.75
T: + c ESI Full ms2 831.61@22.00 [215.00-1675.00]

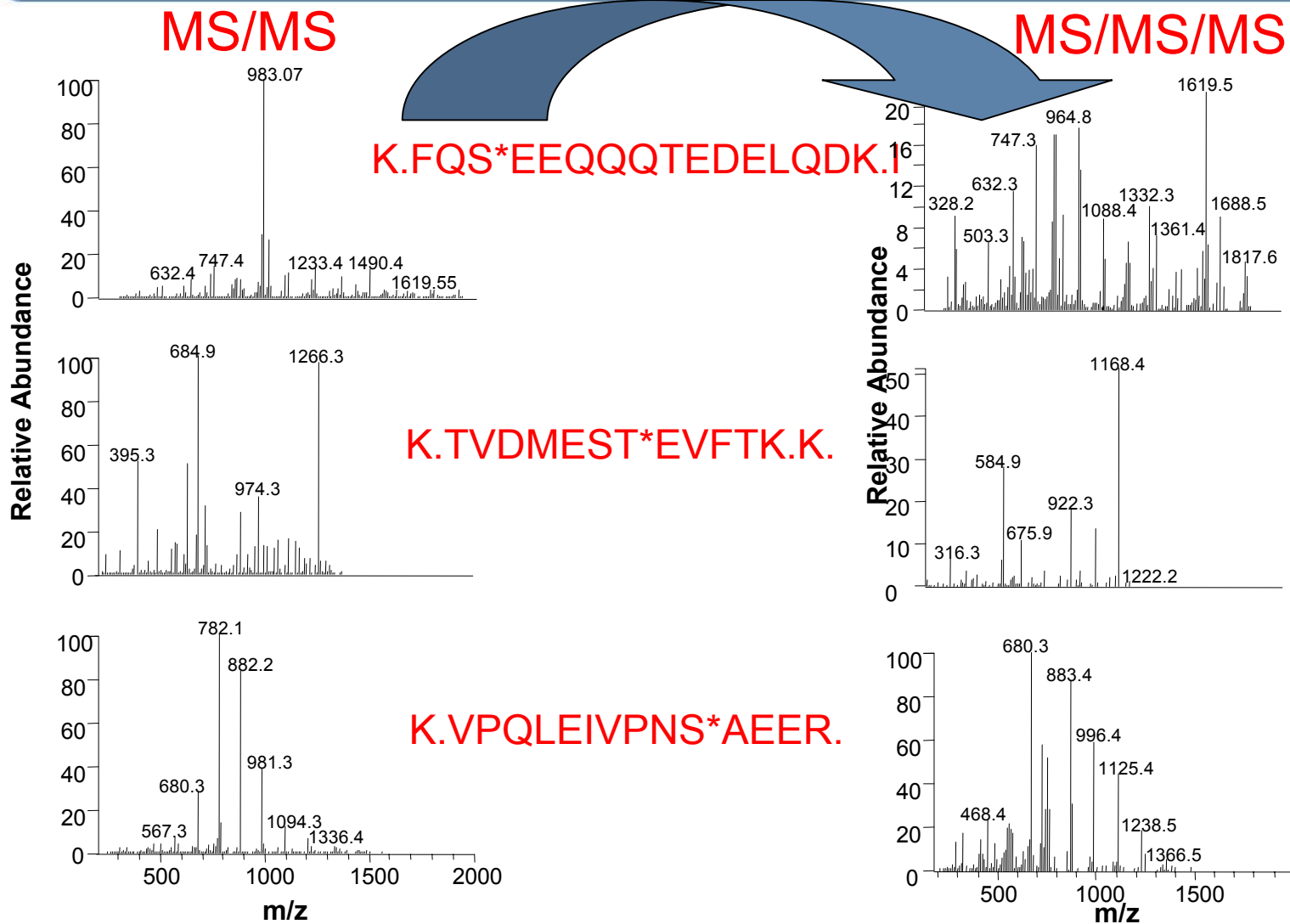


alphabeta10fmole1_2msms # 3150 RT: 16.76
T: + c ESI Full ms3 831.61@22.00 782.10@22.00 [205.00 ...]



Casein Digest

MS/MS and MS3 spectra of three phosphorylated peptides



TurboSEQUEST™ search on MS³ spectra for peptide at m/z 831.6 using BioWorks

TurboSEQUEST Search Parameters

Basic | Advanced | Modifications | Charge State (ZSA) | SEQUEST Queue

Tolerance & Limits for Dta Generation

Precursor Mass: 1.40

Group Scan: 1

Minimum Group Count: 1

Minimum Ion Count: 20

Use Charge State: Auto

Subsequence:

MSn Level: MS3

Tolerance & Limits for Dta Search

Peptide: 1.40

Fragment Ions: 1.40

Number results scored: 500

Ion Series Calculated in Dta Search

A Ions B Ions Y Ions

Output Options for Dta Search

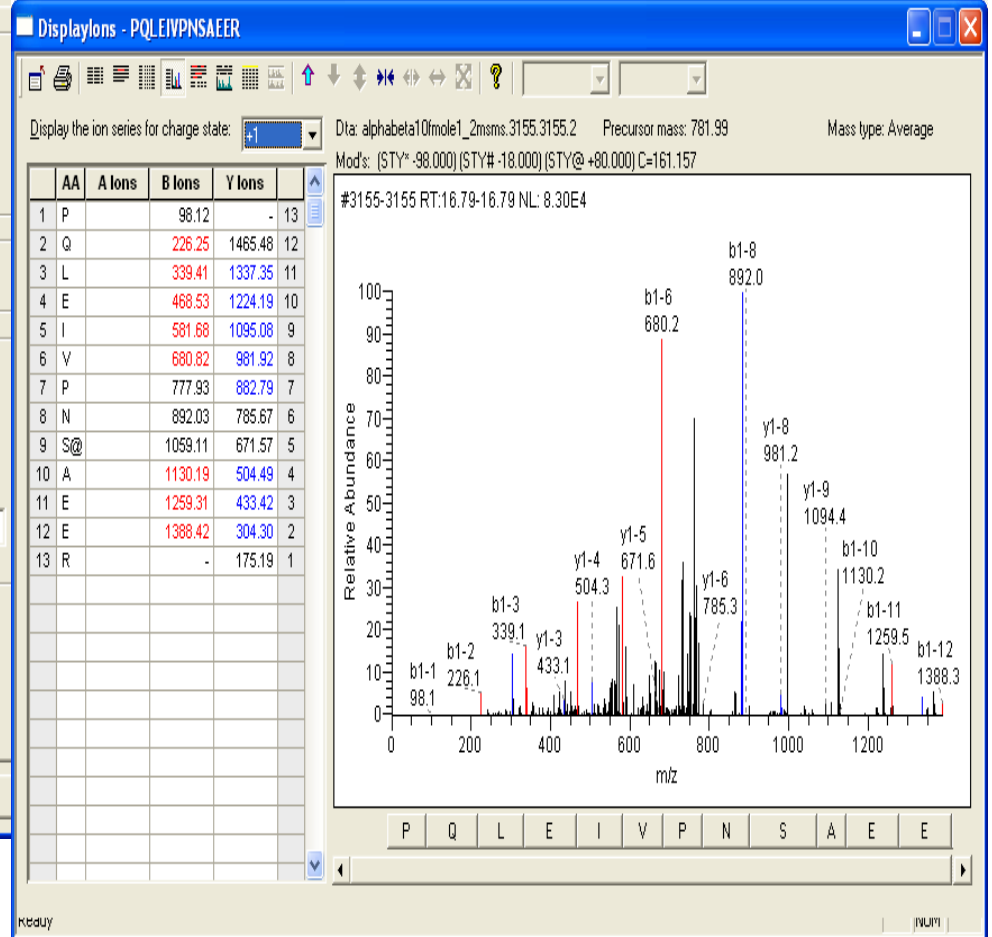
Number Output Lines: 10

Number Protein Lines: 5

Show Fragment Ions

Report Duplicate Reference 40

Start Search | Close | Save | Defaults | Help



Conclusions

- The Finnigan LTQ linear ion trap mass spectrometer shows unparalleled sensitivity for the analysis of a digest of casein, which is evident by the detection of peptides at levels as low as 10 fmol digest on-column
- The automated Data Dependent neutral loss scanning algorithm outlined in this poster is of great utility in identifying sites of phosphorylation on peptides whose fragmentation using only MS/MS would not normally be sufficient for identification.
- The MS³ data was searched using TurboSEQUENT within BioWorks and was successful in identifying the exact site of phosphorylation on the peptides.

Acknowledgements

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- Jae Schwartz
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- Amy Zumwalt