

# Demonstrating a Novel Decision Tree Strategy for Proteomics Experiments on a New Dual-Cell Linear Ion Trap

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## Overview

**Purpose:** Evaluation of a rules-based method for selection of optimum fragmentation on a new dual-cell (dual-pressure) linear ion trap that allows for higher duty cycle and more efficient charge state determination.

**Methods:** A simple peptide mixture was analyzed with a novel dual-pressure linear ion trap by LC/MS/MS using CID or ETD.

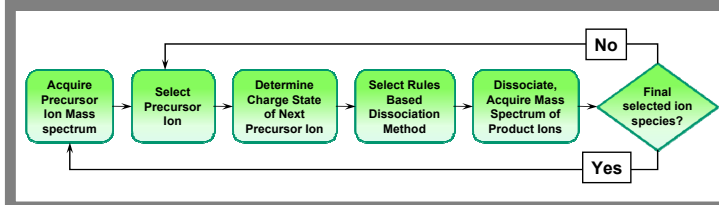
**Results:** Approximately 20% more unique precursors with assigned charge states of up to 7+ are fragmented and produce high quality spectra using a rules-based algorithm for MS/MS.

## Introduction

Protein identification by mass spectral methodologies typically involves proteolytic digestion followed by detection of the resulting peptides by Data Dependent™ LC-MS/MS. Maximizing coverage for the purpose of sequencing a protein including its site-specific post-translational modifications requires maximizing the number of peptides detected in a single experiment and the application of multiple activation methods, i.e. collision-induced dissociation (CID) or electron transfer dissociation (ETD). Here,  $\mu$ LC-MS/MS data for a mixture of peptides including a proteolytic digestion of a horse heart myoglobin, were acquired using data-dependent CID and ETD MS/MS as well as a rules-based, data-dependent MS/MS method in which the fragmentation method is automatically selected according to the algorithm. The rules based algorithm determines the optimal fragmentation method based on the charge state and mass-to-charge of the precursor ion.

The decision tree algorithm, discussed in US patent application number 2008/0048109, is depicted in the flow chart in Figure 1 for a data-dependent top N experiment.

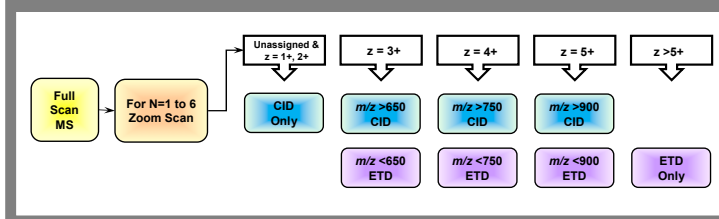
FIGURE 1. Flow chart depiction of the decision tree algorithm for a data-dependent top N experiment



## Methods

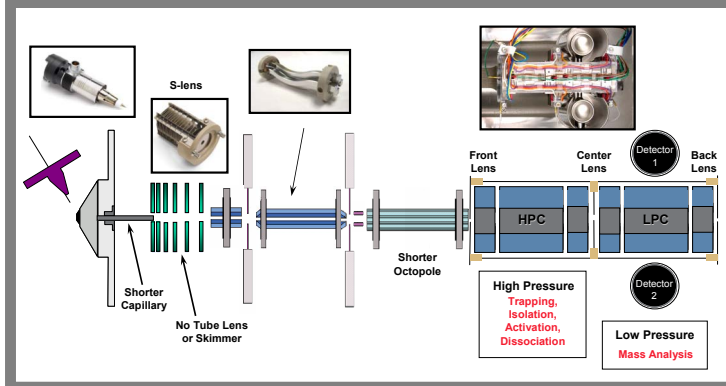
Separation of peptides was achieved using a MicroTech Vydac® 3- $\mu$ m particle size, 0.150 mm x 100 mm column. The HPLC pump was operated at 100  $\mu$ L/min with a precolumn split ratio of approximately 1:50 giving a flow rate at the column of slightly less than 2  $\mu$ L/min. An autosampler equipped with a 2  $\mu$ L sample loop was used for injection. Data-dependent LC tandem mass spectral data were acquired using a Thermo Scientific LTQ Velos dual-pressure linear ion trap (shown in Figure 3, discussed in the following paragraph) which was equipped with electron transfer dissociation (ETD) and a nanospray ionization source operated in positive ion mode. Charge states for the decision tree logic were obtained using a zoom scan "triple play" experiment (experimental design shown in Figure 2). Rules for the decision tree are also shown in Figure 2. Results from the decision tree experiment were compared with a data-dependent top 6 CID/ETD switching experiment.

FIGURE 2. Decision tree methodology implemented for data-dependent top 6 triple play experiment



Mass spectral data were acquired on the new LTQ Velos™ dual-pressure linear ion trap depicted in the block diagram in Figure 3. Here, we introduce a dual pressure configuration which separates ion manipulation processes such that each can be carried out at its optimum pressure. Trapping, isolation, activation and dissociation occur in the high-pressure cell while mass analysis occurs in the low-pressure cell. Mass analysis occurring in the low-pressure cell (LPC) affords higher resolution and faster scan speed for all scan modes. This enables ready charge state assignment using zoom scan (2200 amu/sec) providing the ability to apply rules-based fragmentation methods.

FIGURE 3. Schematic diagram of the LTQ Velos dual-pressure linear ion trap featuring a stacked-ring ion guide (S-lens), a curved multipole and the dual-cell configuration.



## Results

The peptide mixture used for infusion and chromatographic separations is shown in Table 1. Peptides were chosen on the bases of mass-to-charge ratio, theoretical charge state distribution in common aqueous liquid chromatographic solvents and amenability to reversed phase chromatography. Examples of higher charge states acquired in zoom scans for ACTH 6+ and Amphoterin 7+ is shown in Figure 4.

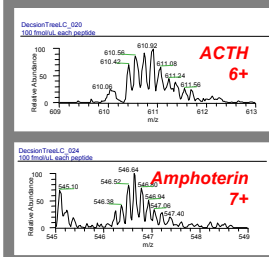
Unique precursors of all charge states are compared for the switching and decision tree experiments to determine the average cycle time (the time for completion of one scan event cycle), 13 scans in both cases and the number of unique precursor ions.

Application of the rules-based decision tree method (Figure 2) compared to the switching CID/ETD LC/MSMS experiments resulted in 20% more precursor ions on average selected for fragmentation under all concentrations examined. Results are presented in Table 2. The cycle time for the decision tree experiment was 2.83s and 3.35 s for the switching experiment. (data based on 100 fmol/ $\mu$ L experiment).

TABLE 1. Mixture of peptides used for decision tree and CID/ETD switching experiments

Peptide	[M+H] <sup>+</sup>
ACE2 Inhibitor	3034.29
ACTH (7-38) Human	3656.92
Amphoterin (150-183)	3804.22
Glucagon (1-29) Bovine, Porcine, Human	3481.62
Growth Hormone Releasing Factor (1-29)	3357.81
[4-Ser] Human Angiotensin II	970.51
[4-pSer] Human Angiotensin II	1050.48
Myoglobin Equus caballus Tryptic Peptides	Various

FIGURE 4. Zoom scans for ACTH 6+ & amphoterin 7+ from 500 fmol/ $\mu$ L and 100 fmol/ $\mu$ L decision tree runs



In addition to this 15.5% decrease in overall cycle time for the 13 scan event sequence, we note that a decision tree experiment yields an average of 20% more unique precursor ions selected for MS/MS analysis.

Conventionally, addition of zoom scans to the acquisition method is considered to consume valuable time that is better used for fragmentation. These results, however, suggest that the addition of a zoom scan not only provides correct charge state information, but also an improvement in duty cycle as well as the optimal fragmentation method for the analysis of simple mixtures.

TABLE 2. Number of unique precursors in decision tree and switching CID/ETD chromatography experiments

Peptide Concentration	Number of Unique Precursors Switching CID/ETD	Number of Unique Precursors Decision Tree
500 fmol/ $\mu$ L	1442	1857
100 fmol/ $\mu$ L	1203	1478
50 fmol/ $\mu$ L	1060	1356
10 fmol/ $\mu$ L	1042	1295
1 fmol/ $\mu$ L	1002	1232

FIGURE 5. Consecutive zoom scans and MS2 spectra for precursor ions with z>1 for t<sub>R</sub>~11.3 min. from the 100 fmol/ $\mu$ L for the decision tree LC/MSMS experiment.

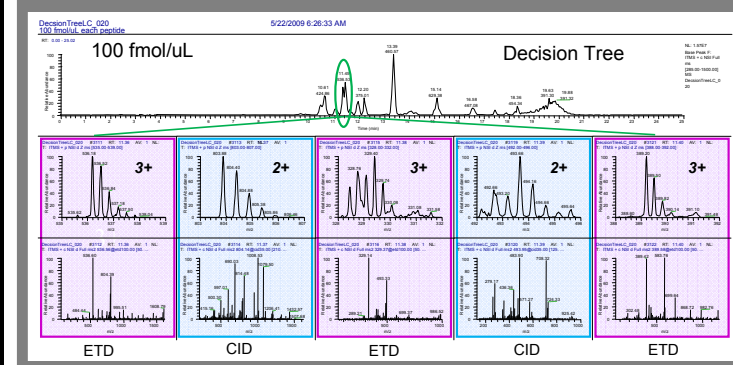
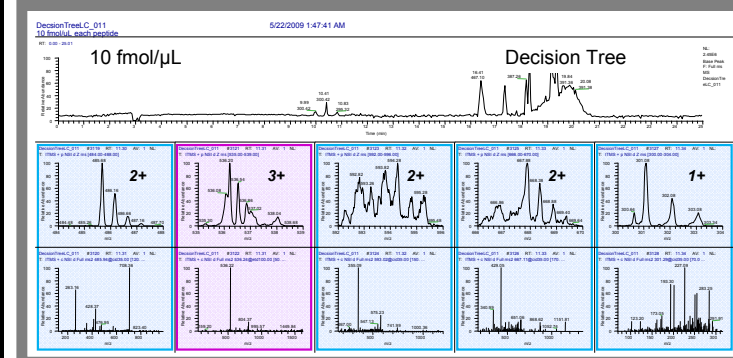


FIGURE 6. Consecutive zoom scans and MS2 spectra for precursor ions with z>1 for t<sub>R</sub>~11.3 min. from the 10 fmol/ $\mu$ L for the decision tree LC/MSMS experiment.

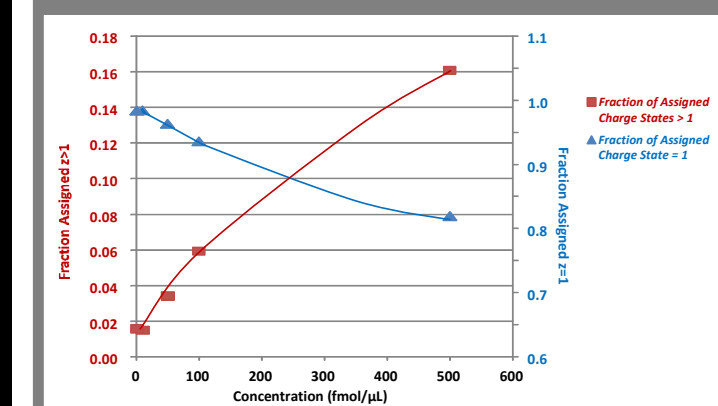


It is noteworthy that the decision tree logic is implemented to avoid selection of singly charged ions or ions with unassigned charge for any other fragmentation method but CID. This selection rule eliminates the acquisition of many low quality MS/MS spectra thus optimizing the information content of every LC/MS experiment.

This is demonstrated in Figure 5 which shows 5 of 6 consecutive zoom scans and MS2 spectra for all precursor ions for retention time ~11.3 min from the 100 fmol/ $\mu$ L decision tree LC/MSMS experiment (singly charged ion spectra are excluded).

We examined a range of concentrations of the mixture to evaluate the ability of the rules-based method to assign charge states at lower concentrations. Charge states for the 500 fmol/ $\mu$ L (not shown) and 100 fmol/ $\mu$ L (Figure 5) samples are always correctly assigned with the exception of charge states > +7 (amphoterin and ACTH have species up to +10) due to the high resolution and spectral quality of the zoom scans in the LTQ Velos instrument. When the concentration of sample is decreased to 10 fmol/ $\mu$ L (Figure 6) the percentage of charge state assignment labeled as >1 (red trace in Figure 7) decreases at lower concentration while assignments of +1 increases (blue traces). This is expected since the relative intensity of peptide ions (z>1) to background ions (z=1) in the full scan decreases with decreasing concentration.

FIGURE 7. Fraction of charge states assigned as +1 (blue trace) and assigned as >1 (red trace) vs concentration for the decision tree experiments.



## Conclusions

We have compared decision tree methodology for charge and mass-to-charge based selection of fragmentation method to a standard CID/ETD switching experiment and find the following:

- The >10,000 resolving power of the zoom scan mode of the LTQ Velos dual-pressure linear ion trap makes possible the assignment of charge states up to 7+ on the fly during an LC run.
- The decision tree experiment affords a 15.5% increase in duty cycle and a 20% increase in the number of unique precursor ions selected for fragmentation over the switching experiment for all concentrations studied.
- The application of rules-based method combines the advantages of a more efficient duty cycle with the acquisition of high quality MS/MS spectra.

## References

- US Patent Application 2008/0048109