The Latest Evolution of Ion Trap Technology: 

Velos Pro and Orbitrap Elite

Jae Schwartz

Indianapolis Users Meeting
September 25, 2011
New Instruments

Velos Pro
Quadrupole Linear Ion Trap

Orbitrap Elite
Quadrupole Linear Ion Trap + Orbitrap
Hybrid Instrument
## The Velos Pro - Motivation

<table>
<thead>
<tr>
<th>Feature</th>
<th>Application Benefit</th>
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| **1** Faster Scanning                  | • UHPLC Cycle Times  
• More points across a chromatographic peak gives better quantitative performance  
• More MS\(^n\) Scans During a Run, Or Shorter Run  
• More Information/Unit Time |
| **2** Enhance the Robustness of Ion Optics | • Improved Robustness to Reduce Instrument Down Time with High sensitivity S-Lens technology |
| **3** Competitive Quantitation on Ion Traps | • Single digit RSDs,  
• Up to 6 orders of linear dynamic range  
• First Ion Trap Designed for Quantitation. |
| **4** Improve Dissociation No Low mass cut off/TMT | • Increases flexibility for structural elucidation (both proteomics and small molecule applications)  
• Allows Highly Sensitive TMT Based Protein Quantitation |
Ion Trap Relationship Between Scan Rate and Peak Width

Scan Rate (amu/sec) vs. FWHM (amu)

- Scan Rate Range 1
- Scan Rate Range 2
- Scan Rate Range 3

* At ~1 mtorr
New Mass Analysis Scan Rate: “Rapid” Scan Rate

- **Ultra Zoom**: 27 Da/sec
- **Zoom**: 2.2 KDa/sec
- **Enhanced**: 10 KDa/sec
- **Normal**: 33 KDa/sec
- **Rapid**: 66 KDa/sec

- **3,500 M/ΔM FWHM=.52**
- **5,400 M/ΔM FWHM=.34**
- **7,000 M/ΔM FWHM=.26**
- **10,100 M/ΔM FWHM=.18**
- **36,440 M/ΔM FWHM=.05**
More Real Scan Speed Improvement:

Normal Scan Rate

Rapid Scan Rate

Up to 10% More Scans with Rapid
Velos Pro Speed: Full Ion Tree in 1 second!

RT: 6.19 - 6.82

8 MS\textsuperscript{n} Spectra in 1 second!
LTQ Velos - Generation I Ion Optics

• Dual ID Variable Spaced Stacked Lenses:
  • Larger ID to capture entire expansion from transfer tube
  • Smaller ID to focus ion beam through conductance limiting aperture
  • Increasing spacing = increasing field penetration to focus ion beam
    • Small number of electrodes (18 total in one assembly)

• Stainless Steel Electrodes and Supports for Ruggedness and Easy Cleaning

• Alternate Phase RF on Lenses (650KHz, 300Vpp max)

• Relatively Large Exit Aperture versus Skimmer

• **5-10x Transmission Improvement!**
“S-LENS” in Velos increased Sensitivity by 10
Bent Q0 Optics Designed to Catch Neutrals
Can Improve Robustness Further
To prevent material from building up on the rods, the quadrupole has been rotated 45°. This opens up a new line of sight for the neutral beam.

A beam blocker is now able to capture the neutral beam outside the body of the ion optics!

This allows, for the first time, neutrals and ions to be separated in a linear optics design.
The majority of material strikes the beam blocker outside of the ion path.
Using the new rotated Q0, the Velos Pro is 2x as robust as the LTQ Velos.
Ion Traps for Quantitation

Early Ion Traps had a narrow dynamic range due to inherent design limitations.

- Automatic Gain Control
- >10X More Ions
- 10 Hz Fast Scanning
- Isolation during loading
- High Dynamic Range Detection
Injection (Isolation) Waveforms

With new injection=isolation waveforms, the HPC acts just like a very good single quadrupole!

Velos symmetric rod stretch rf field: E=0 on axis

Isolation profiles for Velos instruments during loading. Even better isolation after loading.
Isolation while loading is key

Select only this ion while loading into the trap!
Isolation while loading is key

Effect of Injection Waveforms: MS/MS of m/z 735 Lactoperoxidase Digest

- **Waveform OFF**
  - Low precursor intensity
  - Produces poor quality MS/MS

- **Waveform ON**
  - High precursor intensity
  - Produces high quality MS/MS

- Injection waveforms allow selective ion accumulation
- Injection waveforms prevent signal variation due to matrix based space charge
Faster Scan Rates – Higher Detected Ion Currents

- **Rapid**: 15 usec/amu
- **Normal**: 30 usec/amu
- **Enhanced**: 100 usec/amu
- **Zoom**: 455 usec/amu
- **Ultra Zoom**: 3700 usec/amu

- **3,500 M/ΔM FWHM=.52**
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Continuous Dynode Electron Multiplier (CDEM) Versus Discrete Dynode Electron Multiplier (DDEM)

**Advantages:**
- Larger Surface Area
- Each multiplication stage can be optimized
- Larger linear Dynamic Range
- Longer life times

**Advantages:**
- Smaller
- Cost Effective
Linear Dynamic Range - 195/196 Intensity Ratio

Comparison of High End of Linear Range

~20 μA

~160 μA

Peak Ratio

Current (μA)
New Higher Dynamic Range Detection System

• Discreet Dynode Electron Multipliers (ETP-SGE)
  • Packaged as a Direct Replacement for Previous Continuous channel (Channeltron) Electron Multipliers
  • Extended dynamic range
  • Slower aging and longer lifetimes

• 24 bit High Dynamic Range Electrometer/Pre Amplifier Circuit
  • Novel 160 uA electrometer board designed to handle high peak currents found in fast scanning micropackets. (now 24bit)
  • Packaged to replace current LTQ Velos Electrometer
Alprazolam 6 Order Quantification on Velos Pro

\[ Y = 6852.68 \times X \quad R^2 = 0.9918 \quad W: \frac{1}{X} \]

<table>
<thead>
<tr>
<th>Pg On Column</th>
<th>RSD%</th>
</tr>
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<tbody>
<tr>
<td>0.01</td>
<td>14.58</td>
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<tr>
<td>0.02</td>
<td>10.76</td>
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<tr>
<td>0.04</td>
<td>6.63</td>
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<tr>
<td>0.1</td>
<td>4.25</td>
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<tr>
<td>0.2</td>
<td>3.21</td>
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<tr>
<td>0.4</td>
<td>3.70</td>
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<tr>
<td>1</td>
<td>3.59</td>
</tr>
<tr>
<td>2</td>
<td>3.57</td>
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<tr>
<td>4</td>
<td>3.47</td>
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<tr>
<td>10</td>
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<td>20</td>
<td>1.98</td>
</tr>
<tr>
<td>40</td>
<td>4.03</td>
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<td>100</td>
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<tr>
<td>200</td>
<td>2.03</td>
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<td>400</td>
<td>3.40</td>
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<tr>
<td>1000</td>
<td>3.85</td>
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<tr>
<td>2000</td>
<td>1.66</td>
</tr>
<tr>
<td>4000</td>
<td>1.27</td>
</tr>
<tr>
<td>10000</td>
<td>2.16</td>
</tr>
</tbody>
</table>
Dynamic Range Summary: 12 Compounds + 1 IS in plasma (5 min run)

Norcodeine 286.30 CID 4
Codeine-d3 303.37 CID 5
Hydrocodone 300.37 CID >5

Methamphetamine 150.24 CID 5
Methoxymethamphetamine 180.26 CID >5
Lidocaine 235.34 CID >5
Sulfamethazine 279.09 CID 4

Methythioamphetamine 165.22 CID IS 4.2%
Bupropion 240.11 CID >5

Propranolol 260.16 CID >5
Alprazolam 309.09 PQD >5
Testosterone 289.43 CID 5
Terfenadine 472.32 CID 6
# Dissociation Diversity

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Long Title</th>
<th>Pros/Cons</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>CID</td>
<td>Collision Induced Dissociation</td>
<td><strong>Universal, no tuning required, high efficiency</strong>&lt;br&gt;<strong>Low mass cutoff</strong></td>
<td>Resonance excitation of precursor at low q; low energy collisions with He</td>
</tr>
<tr>
<td>PQD</td>
<td>Pulsed “q” Dissociation</td>
<td><strong>Selective, no low mass cutoff, low noise</strong>&lt;br&gt;<strong>Tuning required, low efficiency</strong></td>
<td>Short resonant excitation of precursor at high q, rapid shift to very low q, high energy collisions with He</td>
</tr>
<tr>
<td>HCD</td>
<td>Higher Energy Collision Induced Dissociation</td>
<td><strong>High efficiency, no low mass cutoff</strong>&lt;br&gt;<strong>Tuning required</strong></td>
<td>Acceleration of ion packet through N2 cell, high energy collisions with N2</td>
</tr>
<tr>
<td>ETD</td>
<td>Electron Transfer Dissociation</td>
<td><strong>Highly selective, no low mass cutoff, very low noise</strong>&lt;br&gt;<strong>Doesn’t work on singly charged species</strong></td>
<td>Transfer of electron from radical destabilizes molecule</td>
</tr>
</tbody>
</table>
Use Existing High Pressure Multipole Collision Cell

**TRAP-HCD**
1. Isolate parent ions in the HPC
2. Pass the ions back to the Q00 (100mTorr) at high energy.
3. Send fragments back to the Dual pressure trap for analysis.
Replace Square Quadrupole with Octopole for Q00

Advantages:
- Higher Ion Capacity
- Lower Mass Discrimination
TRAP-HCD – “No” Low Mass Cut Off, Faster than CID

CID (10 ms)

PQD (0.1 ms)

HCD (3 ms)
Metabolite Quantification

**CID Dextromethorphan**

HCD gives a complex spectrum with multiple fragments

**HCD Dextromethorphan**

CID gives a simpler spectrum with a single, dominant fragment.

Can select fragmentation mode to maximize sensitivity
Fast and Sensitive TMT Quantitation

- Prior to Velos Pro only PQD was available for TMT analysis on our ion trap instrument.

- Velos Pro Trap-HCD:
  - higher quality fragmentation spectra than PQD for more confident identifications
  - More intense reporter ions
    - Quantify lower abundance proteins.
    - Improves precision
    - Improves accuracy

Optimized CE HCD

Optimized CE PQD
Protein Quantitation – TMT labelled Peptides
Low Mass Ion Intensity Signal with TRAP-HCD

1. TMT intensities dramatically increased
2. Better ion statistics = better quantitation.
3. Potentially, increased peptide ID’s! Pro
More IDs with Velos Pro

1 ug C. elegans loaded, 90 min gradient

Avg CID NN: 1212
Avg CID RR: 1227
Avg HCD NN: 1372
Avg HCD RR: 1462

CID

HCD

5.4%
8.1%
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• More Information/Unit Time                                                                                                                                   |
| **2 Enhance the Robustness of Ion Optics** | • Generation II Ion Optics with Neutral Beam Blocking Technology  
• Improved Robustness to Reduce Instrument Down Time with High sensitivity S-Lens technology                                                                                     |
| **3 Competitive Quantitation on Ion Traps** | • New EM and electrometer detection system matched to handle the ultra-high peak currents from the VELOS trap.  
• Single digit RSDs,  
• Up to 6 orders of linear dynamic range  
• First Ion Trap Designed for Quantitation.                                                                                                                  |
| **4 Improve Dissociation No Low mass cut off/TMT** | • Trap-HCD fragmentation (HCD,CID, PQD,ETD)  
• Increases flexibility for structural elucidation (both proteomics and small molecule applications)  
• Allows Highly Sensitive TMT Based Protein Quantitation                                                                                                    |
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<td><strong>1</strong> Incorporate All Features of Velos Pro</td>
<td>• See all above</td>
</tr>
<tr>
<td><strong>2</strong> Faster Scanning</td>
<td>• More points across a chromatographic peak for quantitative performance</td>
</tr>
<tr>
<td></td>
<td>• More MS and MS(^n) Scans During a Run, Or Shorter Run</td>
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<tr>
<td></td>
<td>• More Information/Unit Time</td>
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<td><strong>3</strong> Higher Resolution</td>
<td>• Isobaric Interference Separation</td>
</tr>
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<td></td>
<td>• Enhances Mass Accuracy (Fewer ions needed, eliminate interferences)</td>
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<tr>
<td></td>
<td>• Intact Protein Analysis</td>
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**Diagram:** Diagram of the Orbitrap Elite with signal processing highlighted.
Principles of the Orbitrap – Alexander Makarov Inventor

Hyper-logarithmic potential distribution in the Orbitrap: “ideal Kingdon trap”

\[ (r, \phi) = \frac{1}{2} \left\{ r^2 - \frac{r^2}{2} + \frac{\ln(2/2)}{\omega} \right\} \]

- Characteristic frequencies:
  - Frequency of rotation \( \omega_\phi \)
  - Frequency of radial oscillations \( \omega_r \)
  - Frequency of axial oscillations \( \omega_z \)

\[ \omega_\phi = \frac{\omega}{\sqrt{2}} \sqrt{\left( \frac{r}{\omega} \right)^2 - 1} \]

\[ \omega = \omega \sqrt{\left( \frac{r}{\omega} \right)^2 - 2} \]

\[ \omega = \sqrt{\frac{1}{\ln(2/2)}} \]

Only this frequency does not depend on energy, angle, etc. and is used for mass analysis.
Smaller size- 1.8x frequency at the same voltage
New miniature lenses for focusing onto Orbitrap entrance
Higher tolerance requirements
Lower capacitance and new preamplifier transistors bring increased sensitivity.
Space charge shift: ca. 70% rel. to the standard trap at the same target
2x Resolution Or 2x Scan Speed!
Detection process in the Orbitrap analyzer

All ions are ejected from the C-trap at the same moment

Coherent nature of ion packets allows for a new advanced signal processing procedure

Another 2x Resolution Or 2x Scan Speed!

Narrow peaks in Enhanced FT

\[ F(\omega_m) = \sum_{n=0}^{N-1} f(t_n) \exp\left(-\frac{inm}{N}\right) \]

+ zero filling
+ apodization

...
OT Elite: 240,00 @1 Hz @ m/z 400!
OT Velos: 60,000 @ 1Hz @ m/z 400

LC/MS at 250,000 Resolution

A+1 zoom-in

Buspirone 0.2 ppm

RT: 3.73 - 6.21

Base Peak m/z = 386.25307 - 386.25693 F: ms

M.S. Bile_FTfullMS_Buspir one2_110414232108

387.2528
R=164404
C_{21}H_{32}N_{4}\text{^{15}}\text{NO}_2

387.2582
R=252704
C_{20}\text{^{13}}\text{CH}_{32}N_{5}\text{O}_2

388.2615
R=220004

A+1

A+2

OT Elite: 240,00 @1 Hz @ m/z 400!
OT Velos: 60,000 @ 1Hz @ m/z 400

386.2550
R=252701

387.2582
R=252701

388.2615
R=252701

0.2 ppm
Resolution vs. Competition

- Outperforming High Res QTofs by a factor >5
Combination of Compact Orbitrap Analyzer and Enhanced FT

FTICR Magnet Equivalent

<table>
<thead>
<tr>
<th>m/z</th>
<th>$R_{FWHM}$ in 0.76s</th>
</tr>
</thead>
<tbody>
<tr>
<td>195</td>
<td>345,000</td>
</tr>
<tr>
<td>393</td>
<td>248,000</td>
</tr>
<tr>
<td>524</td>
<td>215,000</td>
</tr>
<tr>
<td>1222</td>
<td>140,000</td>
</tr>
<tr>
<td>1822</td>
<td>106,000</td>
</tr>
</tbody>
</table>

0.76 sec acquisition (std for $R=60,000$)
Hamming apodization, $T=5e5$
Large Molecule Ultra-High Resolution Example

47+ charge state of yeast enolase showing resolution of > 100,000 and baseline resolution
Analysis of intact yeast enolase (46.64 kDa) on the Orbitrap Elite hybrid MS

47+ charge state of yeast enolase showing resolution of > 100,000 FWHM at m/z 1,000

Precise intact protein characterization
IgG Analysis

![IgG Analysis Graph](image-url)
LTQ Orbitrap Velos: Raw Spectrum zoom-in

 Isoforms

Orbi Velos @ 7500 Res
Orbitrap Elite: Raw Spectrum zoom-in

Glycoforms – Δm = 162 Da

Orbitrap Elite @15000 Res
Orbitrap Elite vs. LTQ OT Velos: Cycle times for TOP10 HCD Method

LTQ OT Velos (MS@60k, HCD@15k) - 3.76 s

Orbitrap Elite (MS@60k, HCD@15k) - 1.51 s

Calmix, MS: 1e6 ions, IT~2 ms; MS2: 2e4 ions, IT~1 ms with pAGC
Number of peptide spectrum matches for different LC gradients

Sample: E.coli, HCD top 15 method
TMT quantification: Speed and Sensitivity of MS2

<table>
<thead>
<tr>
<th>Sample load</th>
<th>Orbitrap Elite</th>
<th>LTQ Orbitrap Velos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># proteins</td>
<td># unique peptides</td>
</tr>
<tr>
<td>20 ng</td>
<td>703</td>
<td>4032</td>
</tr>
<tr>
<td>80 ng</td>
<td>873</td>
<td>5078</td>
</tr>
<tr>
<td>200 ng</td>
<td>933</td>
<td>5696</td>
</tr>
</tbody>
</table>

E.coli digest

*Identification data shown for 1%FDR, mean of 2 runs*

1x FTMS + 15 x ddHCD

+30%
LC-MS analysis of histone standard H4

RT: 0.00 - 59.99

Relative Abundance

Time (min)

NL: 1.19E8
TIC MS
Histone_II-S_1

NL: 5.36E5
m/z = 808.19999-809.00281
MS
Histone_II-S_1
Extended top-down capabilities

Sequence coverage

Orbitrap Elite using ETD: 81%
LTQ Orbitrap XL using ETD: 43%

Increased coverage due to more data points and improved S/N

Histone H4 HCD (top trace) and ETD (bottom trace) spectra acquired on the Orbitrap Elite; identification and annotation using ProSight.
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• Faster - Now scanning at 66kDa/sec (Fastest Trap on Market)  
• More Robust - Generation II Ion Optics with Neutral Beam Blocker  
• Quantitative Ion Trap - New EM and Detection System  
• Trap-HCD fragmentation (HCD, CID, PQD, ETD) |
| **2 Faster Scanning**                        | • High-Field Orbitrap Analyzer/Advanced Data Processing/EFT  
• 4x Speed at Same Resolution  
• 2x Speed and 2x Resolution  
• More MS or MS\(^N\) Scans During a Run, Or Shorter Run  
• More Information/Unit Time  
• More points across a chromatographic peak - better quantitative performance |
| **3 Higher Resolution**                      | • High-Field Orbitrap Analyzer/Advanced Data Processing/EFT  
• 4x Resolution at Same Speed – 240,000 Resolution  
• 2x Resolution and 2x Speed  
• Isobaric separation  
• Enhances Mass Accuracy (Fewer ions needed, eliminate interferences)  
• Intact Protein Analysis/PTM Analysis/TMT Quantitation |
Pushing “Ion Trap” Technologies Forward….

**Velos Pro**
- Less Down Time
- Novel Detection System – Allows high performance Quantitation
- Faster Scanning - Rapid scan Rate
- HCD Dissociation on an Ion Trap – TSQ Like Fragmentation, TMT Analysis
- Ideal Front End for Hybrid

**Orbitrap Elite**
- Increased Peptide/Protein ID Using Bottom Up Approach
- Enhanced Protein Quantitation Capabilities
- Extended Top Down Capabilities
- Complementary Fragmentation Technologies (CID,HCD,ETD)
- Bottom up (Protein ID)
- PTMs (especially those requiring MS\textsuperscript{n} such as glycomics/glycoproteomics )
- Comprehensive Quan (SILAC, label free, targeted peptide quan with HRAM)
Acknowledgements

• San Jose Velos Pro R&D Team
• Bremen Orbitrap Elite R&D Team
• San Jose Proteomics Marketing Team