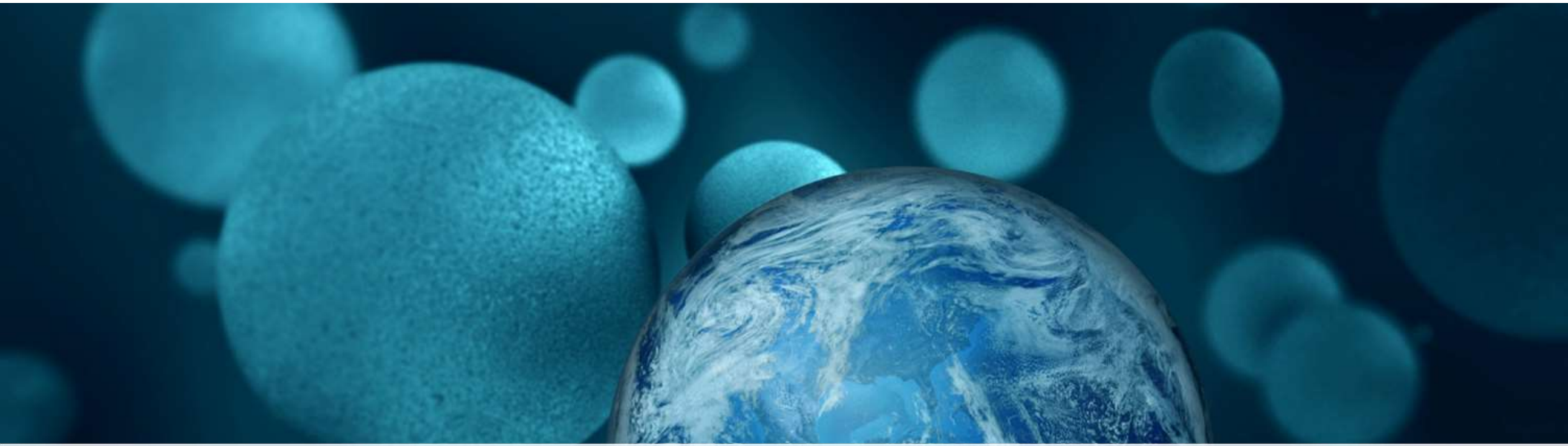

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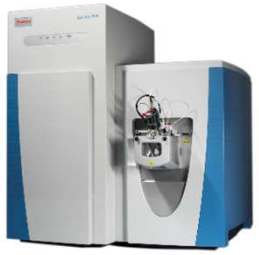
Q Exactive UHMR Introduction

Luka Milivojevic
Technical Sales Manager LSMS

Sofia, October 25th 2018

Exactive Series Portfolio

Small molecules
- Screening



Exactive Plus

Octapole – D30

Small molecules
– Qual/Quan

Q Exactive Focus

Quadrupole – D30

Small molecules / Proteomics – Qual/Quan/ID

Q Exactive

Quadrupole – D30

Small molecules / Proteomics – Qual/Quan/ID

Q Exactive Plus

Segmented Quadrupole – D30

Proteomics - Native

Q Exactive UHMR

Segmented Quadrupole – D30

Proteomics – ID/Quan

Q Exactive HF

Segmented Quadrupole – D20

Proteomics – ID/Quan

Q Exactive HF-X

Ion Funnel -Segmented Quadrupole – D20

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Exactive EMR compared to Q Exactive UHMR

Increased Performance

Very Large molecules

- Orbitrap analyzer (D30)
- Mass Range EMR m/z 350 - 20,000
- Mass Accuracy: <1ppm (internal)
- Mass Resolution >140,000
- Scan Speed up to 12 Hz
- Octapole transfer for MS

D30 Orbitrap

2013

Exactive Plus EMR

New

2018

Q Exactive UHMR

- Orbitrap analyzer (D30)
- Mass Range m/z 350 – 80,000
- Mass Accuracy <1ppm (internal) and <3ppm (external) for CsI cluster ions under defined conditions
- Max. Mass Resolution 200,000 at m/z 400
- Scan speed up to 12Hz at resolution of 12,500 @ m/z 400
- Quadrupole selection up to m/z 25,000 (SIM, MS/MS & pseudo-MS^3)
- Full Scan, AIF, SIM and ddHCD (TopN)

- High resolution, High sensitivity, Ultra-High Mass Range
- Characterize large proteins & protein assemblies
- Ribonucleoprotein, membrane protein, protein assembly
- Characterize proteins in intact form, high res native MS

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Thermo Scientific™ Q Exactive™ UHMR Hybrid Quadrupole-Orbitrap™ MS

Performance Characteristics

Max resolution	200,000 at m/z 400
Mass range	m/z 350 to 80,000
Scan rate	12 Hz at resolution setting of 12,500 @ m/z 400
Mass Accuracy*	Internal: < 1 ppm RMS External: < 3 ppm RMS
Quadrupole Selection	Up to m/z 25,000 (SIM, MS/MS, pseudo-MS^3)
Dissociation	Source CID, In-source trapping, HCD
Analyzer	Orbitrap
Scan Functions	FS: Full Scan AIF: All Ion Fragmentation SIM: Selected Ion Monitoring ddHCD: data dependent HCD (Top N)

*For CsI cluster ions under defined conditions

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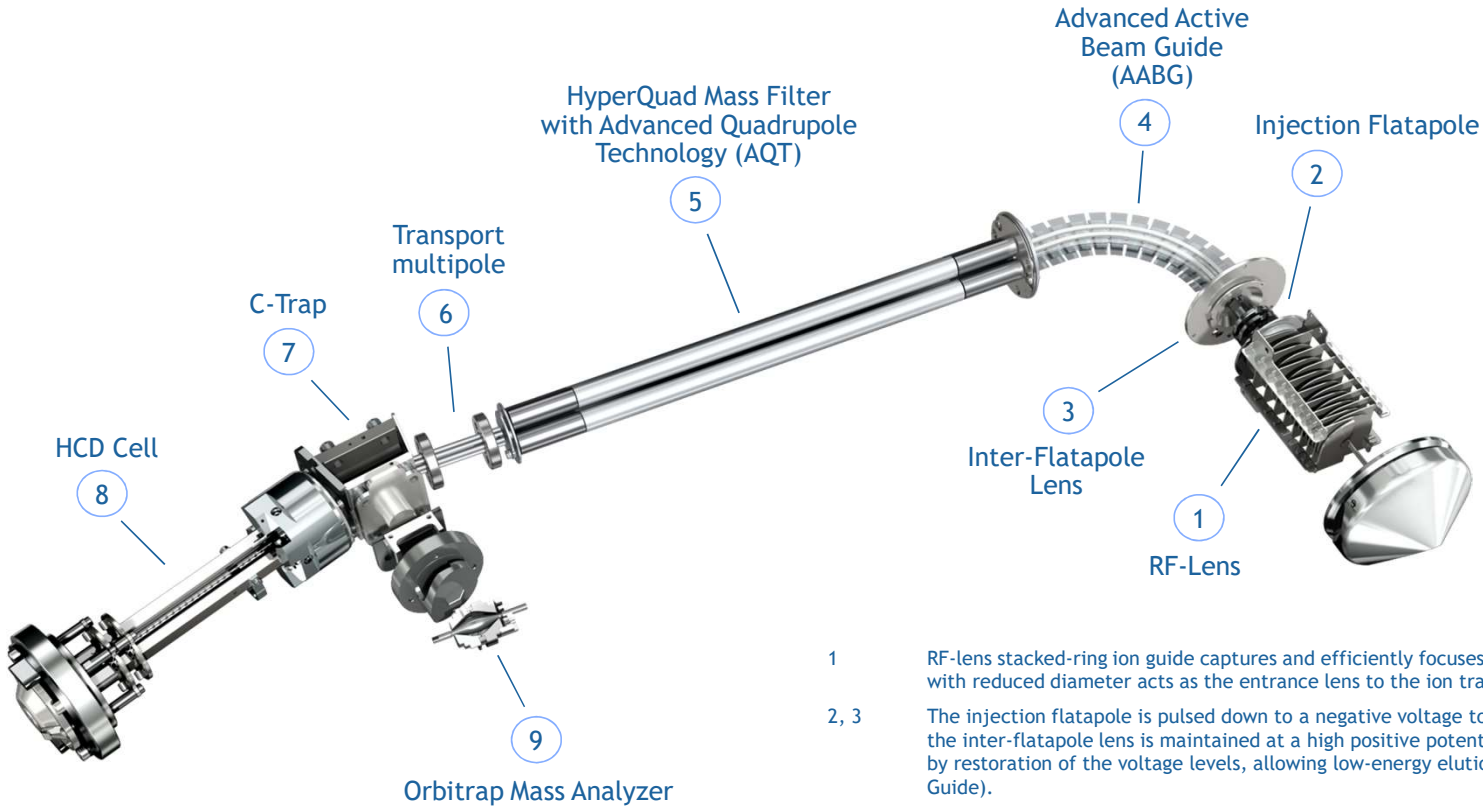
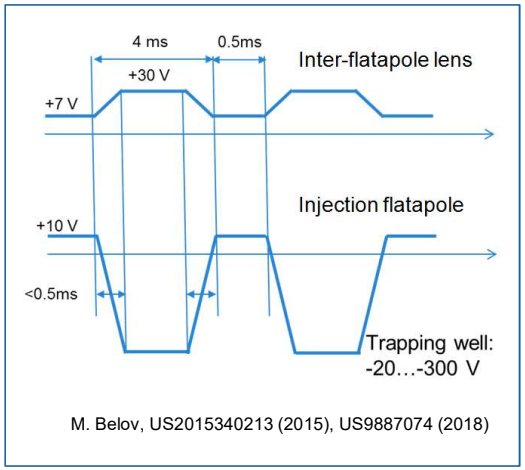
Schematic of the Q Exactive UHMR Mass Spectrometer



In-source trapping



M. Belov, US2015340213 (2015), US9887074 (2018)

1	RF-lens stacked-ring ion guide captures and efficiently focuses the ions into a tight beam. The RF-lens exit aperture with reduced diameter acts as the entrance lens to the ion trapping region.
2, 3	The injection flatapole is pulsed down to a negative voltage to improve desolvation of large protein complexes, while the inter-flatapole lens is maintained at a high positive potential to prevent ions from eluting out. Trapping is followed by restoration of the voltage levels, allowing low-energy elution of ions into the bent flatapole (Advanced Active Beam Guide).
4	The bent flatapole guides and focuses ions using an axial DC field and a focusing RF field, enhancing sensitivity.
2, 4–8	The RF frequencies of all ion routing multipoles—the injection and bent flatapoles, quadrupole, transport multipole, C-Trap and HCD cell—are reduced to improve ion transmission.
9	High mass ions are efficiently injected into the Orbitrap mass analyzer by adjusting the slew rate of the high-voltage pulse that captures ions in the analyzer.

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Not Just UHMR

Native MS must retain the structure and optimally the function of a protein or protein complex while it is measured

Solution: In-source-trapping of ions in the injection flatapole gently desolvates non-covalent protein complexes and efficiently removes detergent micelles for the analysis of intact proteins and protein complexes

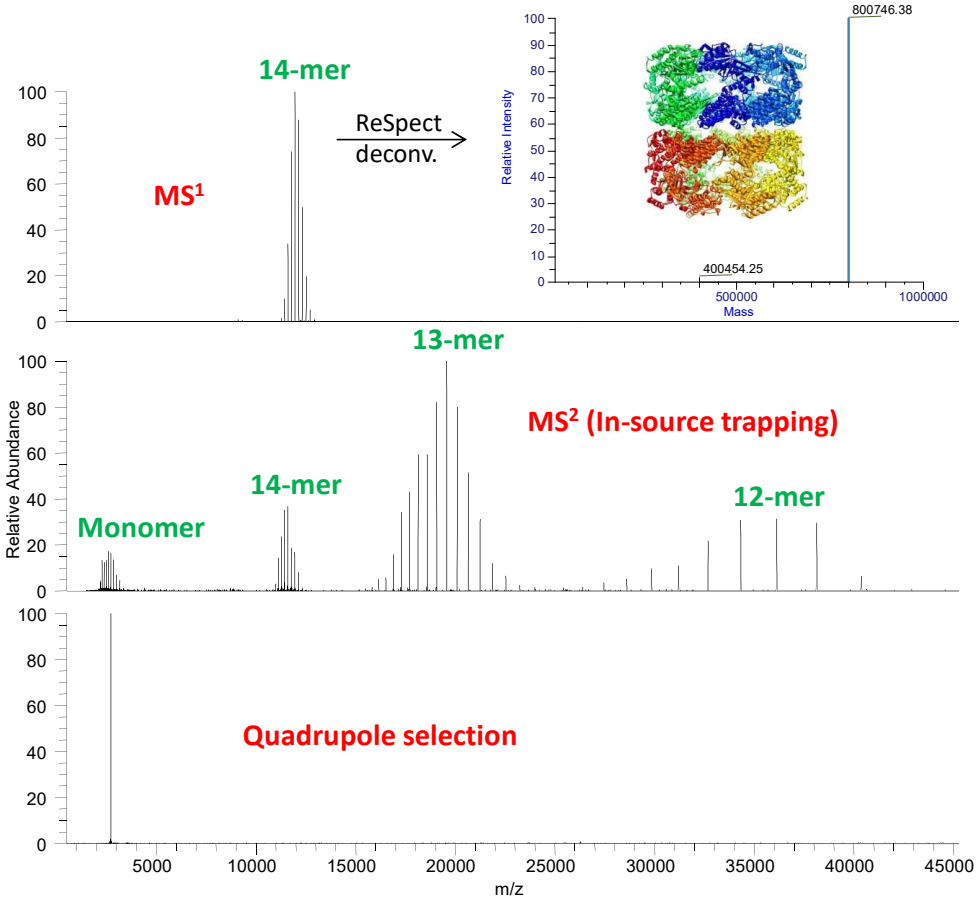
Top-Down sequencing of native protein complexes has been limited by poor fragmentation into subunits

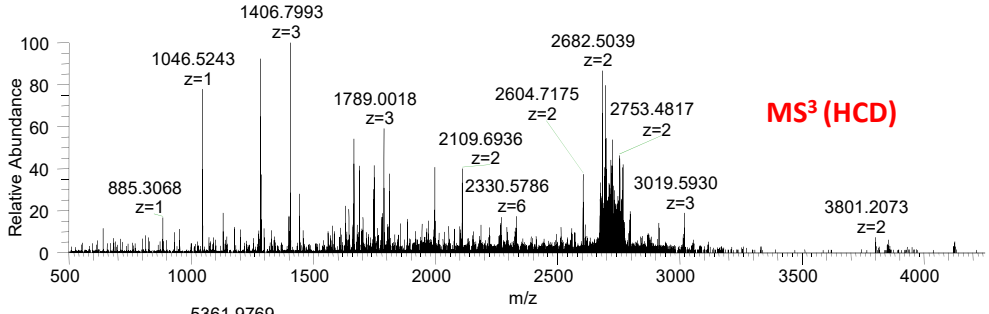
Solution: Ultra-high mass quadrupole selection up to 25k m/z and higher fragmentation efficiency in the injection flatapole and the HCD cell allow native top-down analysis. A protein complex can be fragmented the front end of the instrument and the subunits fragmented downstream in HCD cell for high resolution accurate mass measuring

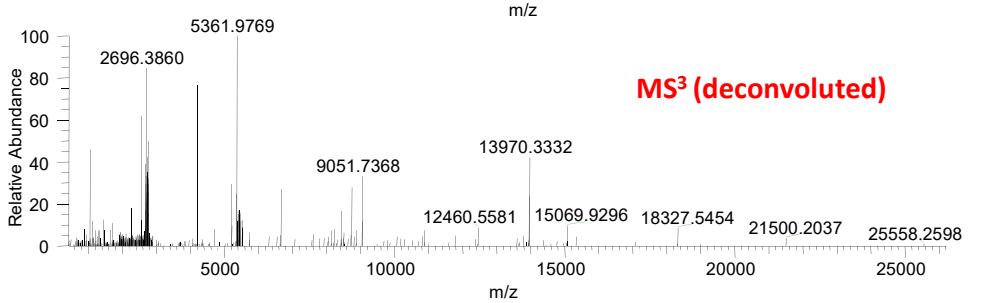
Native MS has suffered from low transmission efficiency at high m/z, which has limited obtainable sensitivity and resolution

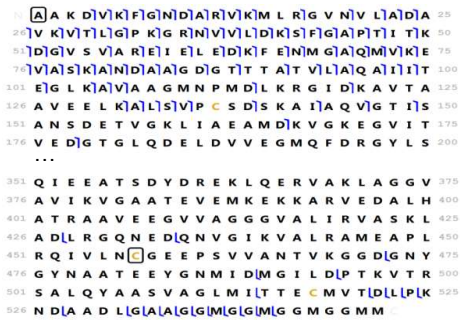
Solution: Reduction of the frequencies of the RF voltages applied to injection and bent flatapoles, quadrupole, transfer multipole, C-Trap and HCD cell together with adjustment of the voltage ramp rate on the central Orbitrap electrode, significantly improve transmission of high mass ions with no known limit.

Native Top-down Pseudo-MS^3 Analysis of the GroEL Complex









21% Residue Cleavages

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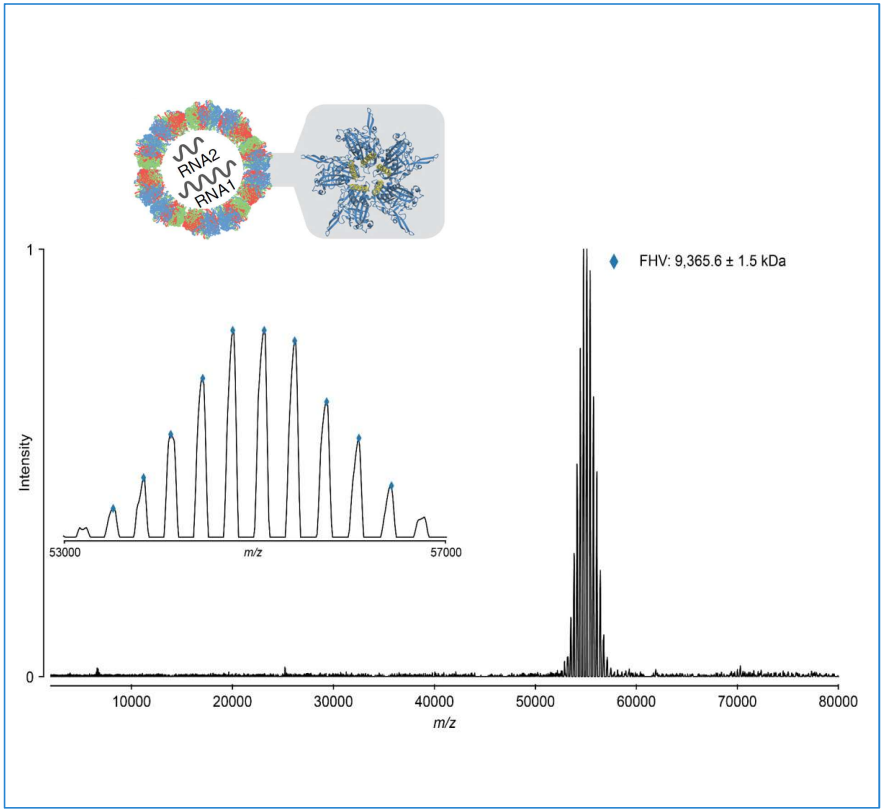
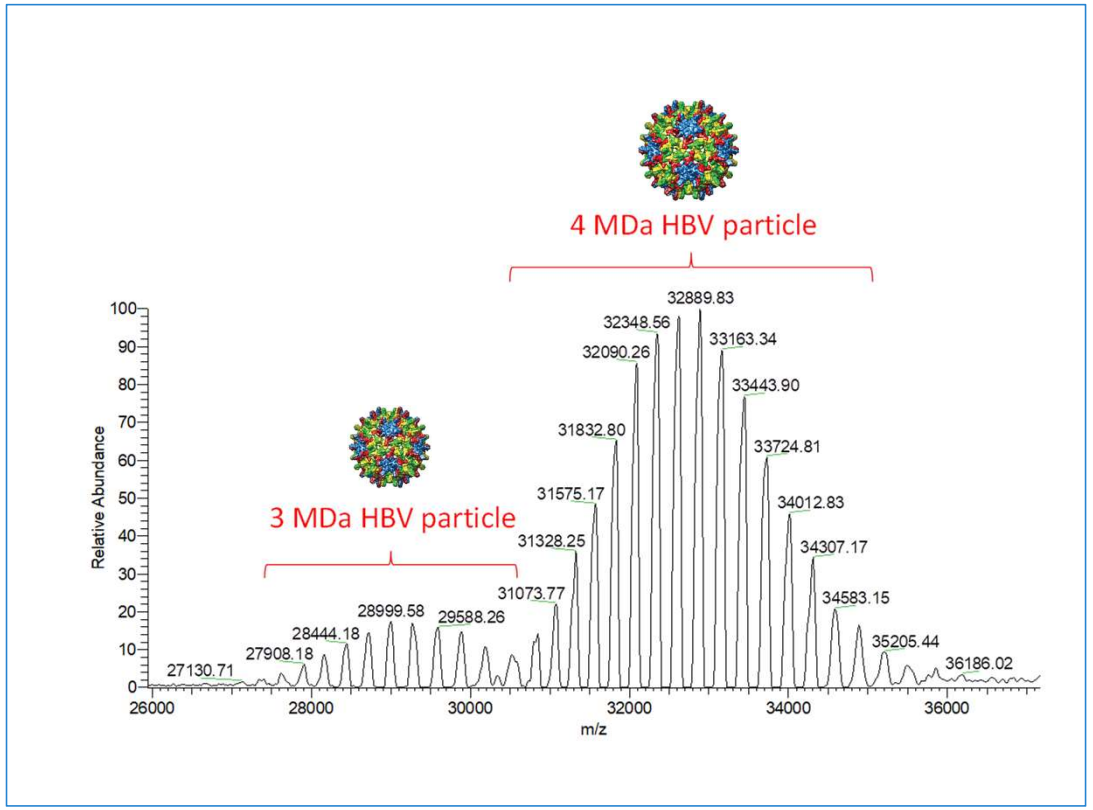
9.3MDa Flock House Virus (left) and 3MDa and 4MDa Hepatitis B Virus Capsids (right)

Native MS analysis under charge reducing conditions

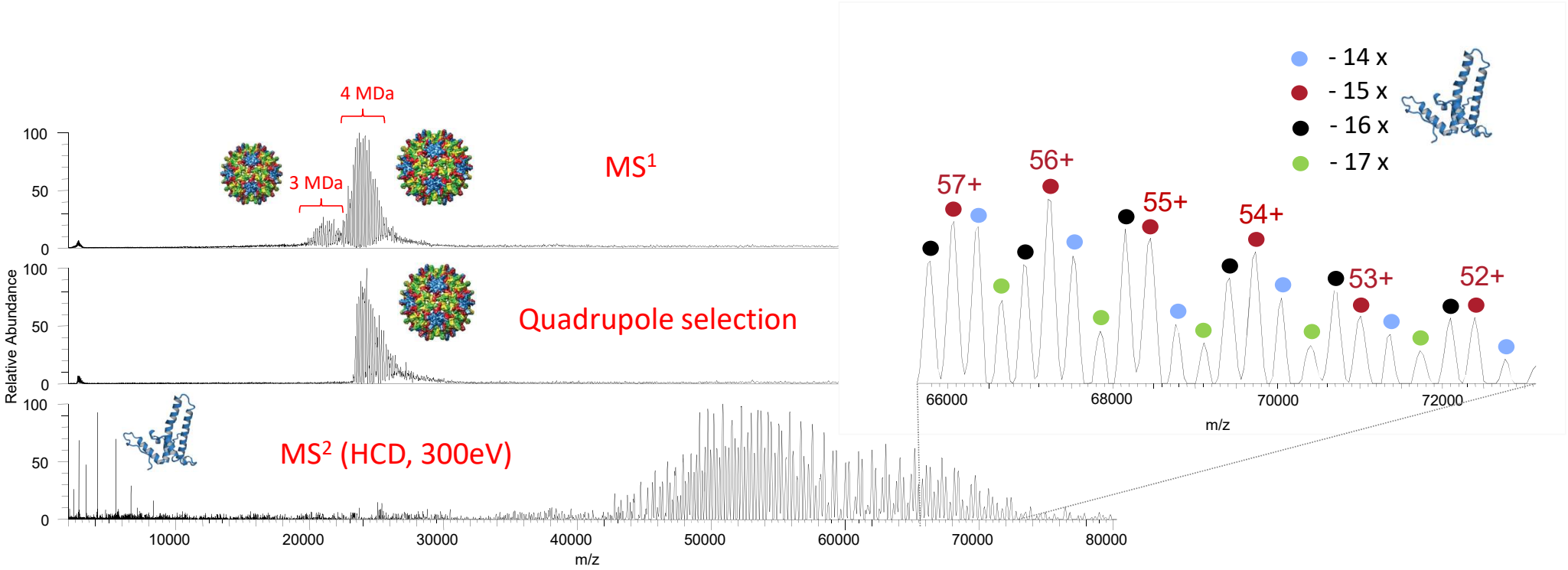




ASMS 2018, MOF am 09:50, Tobias Woerner, Utrecht University

Nat Methods. 2017 Mar;14(3):283-286. doi: 10.1038/nmeth.4147

Native MS and MS/MS Analysis of Hepatitis B Virus Particles



Nat Methods. 2017 Mar;14(3):283-286. doi: 10.1038/nmeth.4147

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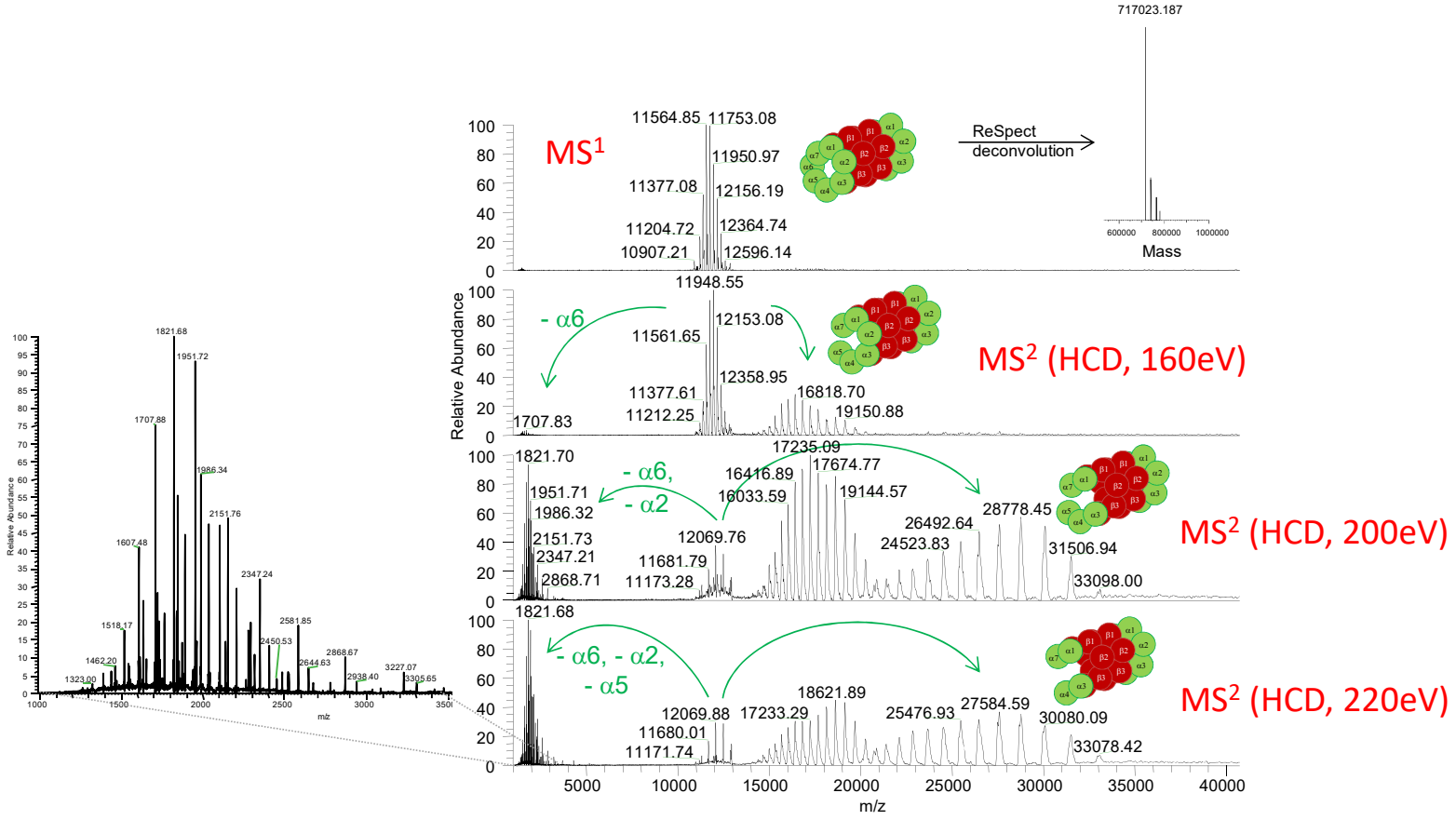
Native MS and MS/MS Analysis of the Rabbit 20S Proteasome complex



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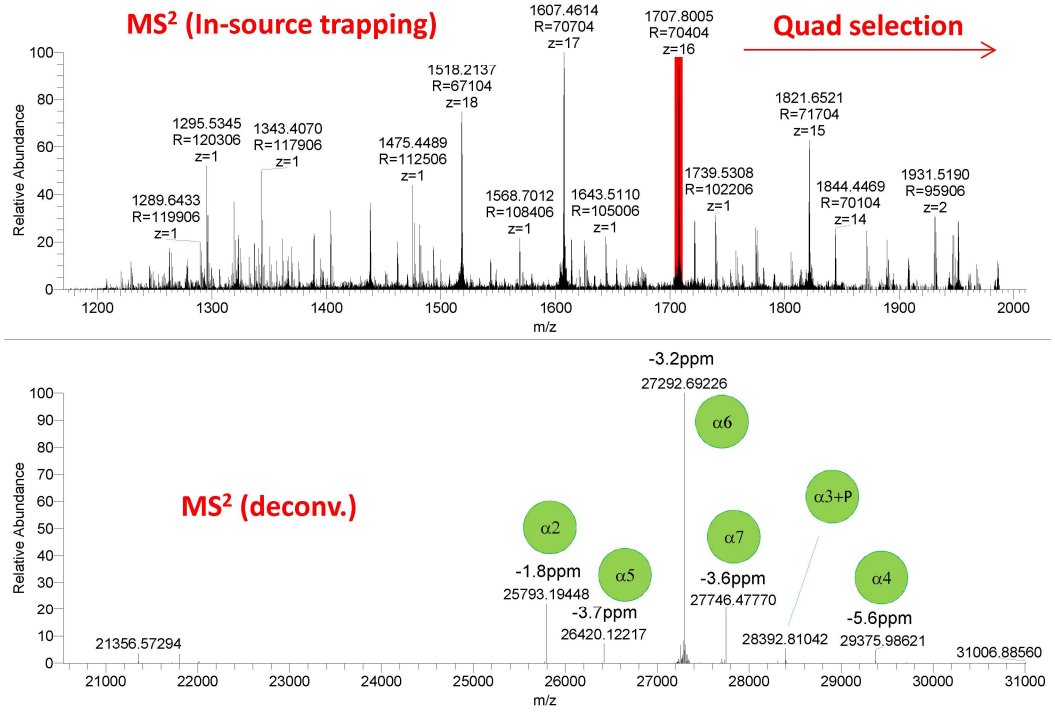
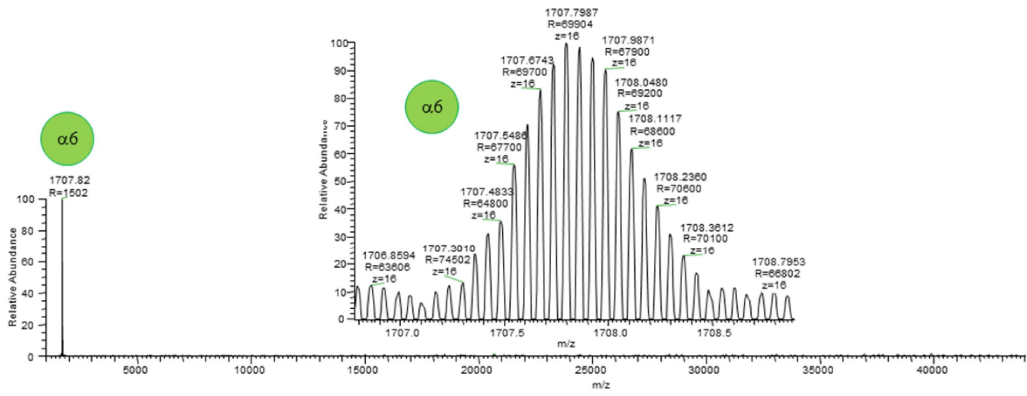
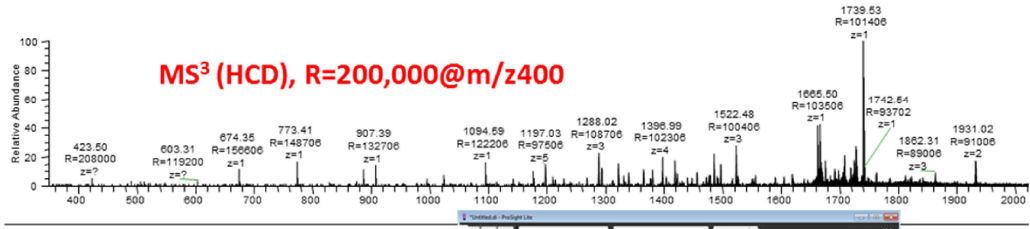
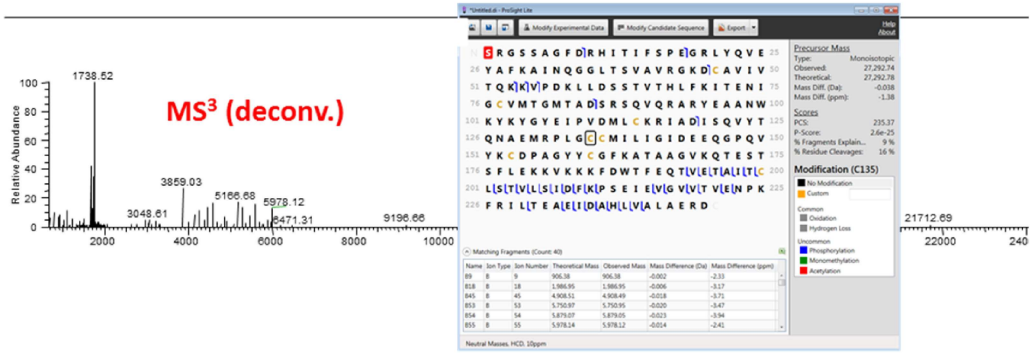
ID: Rabbit 20S Proteasome α-6 Subunit with Top-down Pseudo-MS3 Analysis









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Unique Value to Scientists

Features

- Unprecedented resolution and orders of magnitude enhanced sensitivity at high m/z
- In-source trapping capability that enables improved transmission and controllable desolvation and fragmentation.
- High mass quadrupole selection and higher HCD fragmentation efficiency for native top-down analysis

Benefits

- Analyze intact MegaDalton assemblies and resolve small differences in masses that reveal key ligands, modifications and interactions
- Gain detailed structural insights for deeper understanding of biological processes
- Quickly verify sample quality prior to analysis by cryo-electron microscopy (cryo-EM), and determine sample composition and homogeneity to assure successful cryo-EM analysis

Product Compatibility

Front-end

- Thermo Scientific™ Vanquish™ (F & H) UHPLC
- Thermo Scientific™ UltiMate™ 3000 HPLC
- Thermo Scientific™ UltiMate™ 3000 RSLC
- Thermo Scientific™ Easy-nLC™ systems

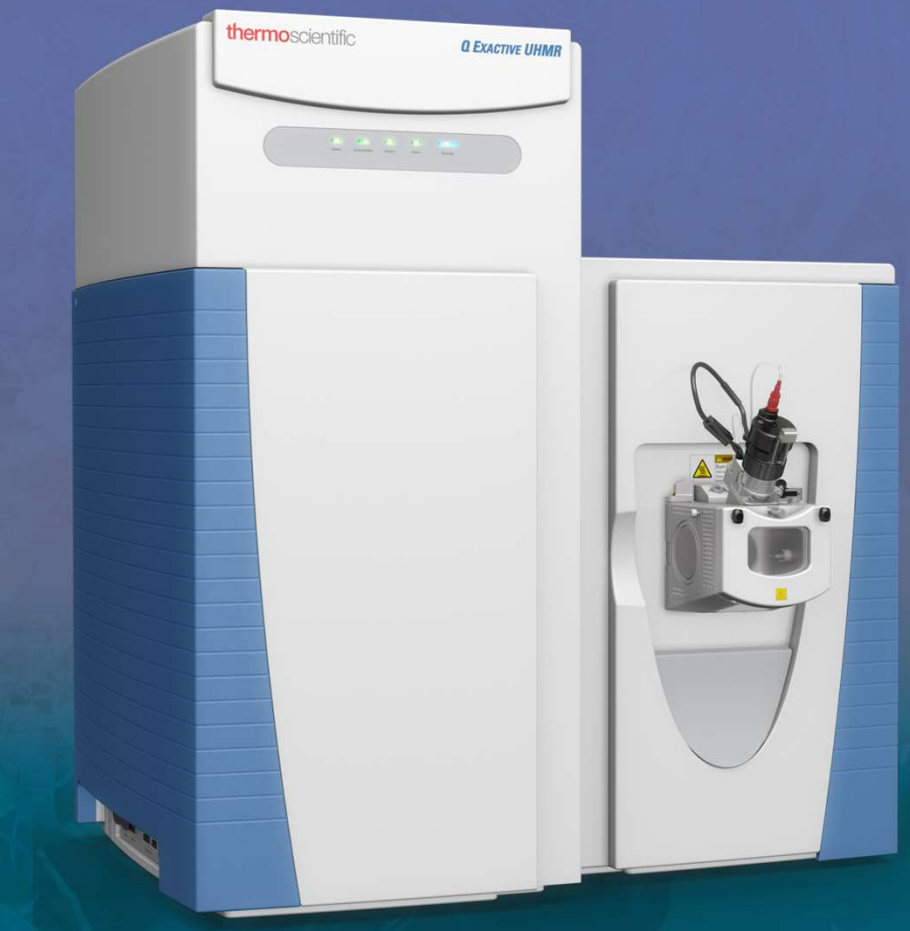
Sources

- Thermo Scientific™ H-ESI II™ ion source
- Thermo Scientific™ Nanospray Flex™ ion source
- Triversa NanoMate, Advion
- ZipChip™, 908devices

Software

- Thermo Scientific™ Xcalibur™
- Thermo Scientific™ BioPharma Finder™
- Thermo Scientific™ Respect™ Deconvolution
- Thermo Scientific™ ProSightPC™

New Member to the Thermo Scientific™ Exactive™ Family



Q Exactive UHMR

Unmatched Native MS and Native Top-Down Performance

- Innovate in Structural Biology & BioPharma research
- Accelerate native protein structure analysis
- Study protein interactions for deeper understanding of biological processes
- Achieve accurate characterization of non-covalent protein complexes
- Determine ligand biomolecular interactions under native conditions

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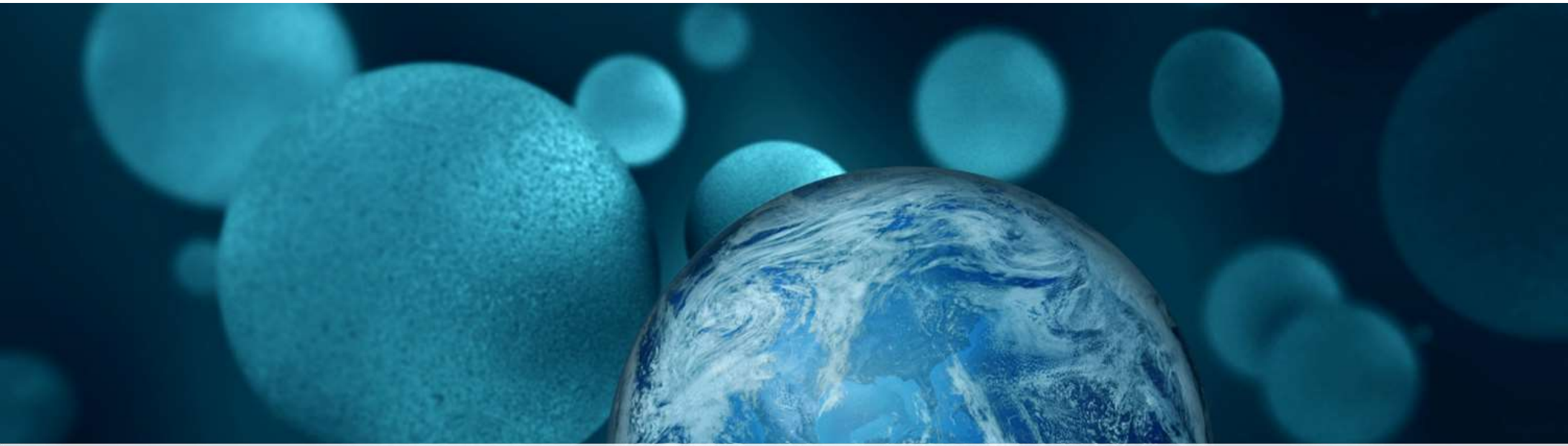
Questions?

thermofisher.com/QExactiveUHMR | www.planetorbitrap.com



ThermoFisher SCIENTIFIC

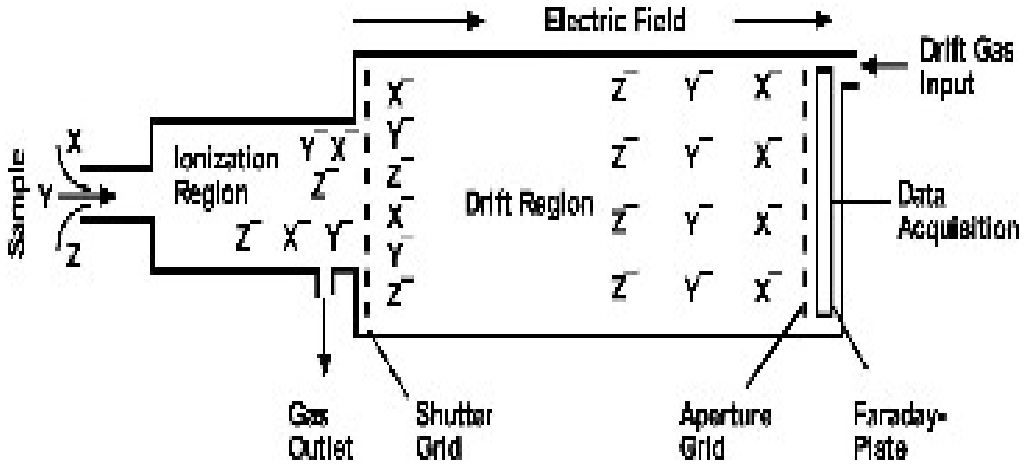
FAIMS Pro Introduction

Luka Milivojevic
Technical Sales Manager LSMS

Sofia, October 25th 2018

Ion Mobility?

Ion Mobility Spectrometry (IMS) – Analytical technique used to separate and identify ionized molecules in the gas phase based on their mobility in a carrier buffer gas.

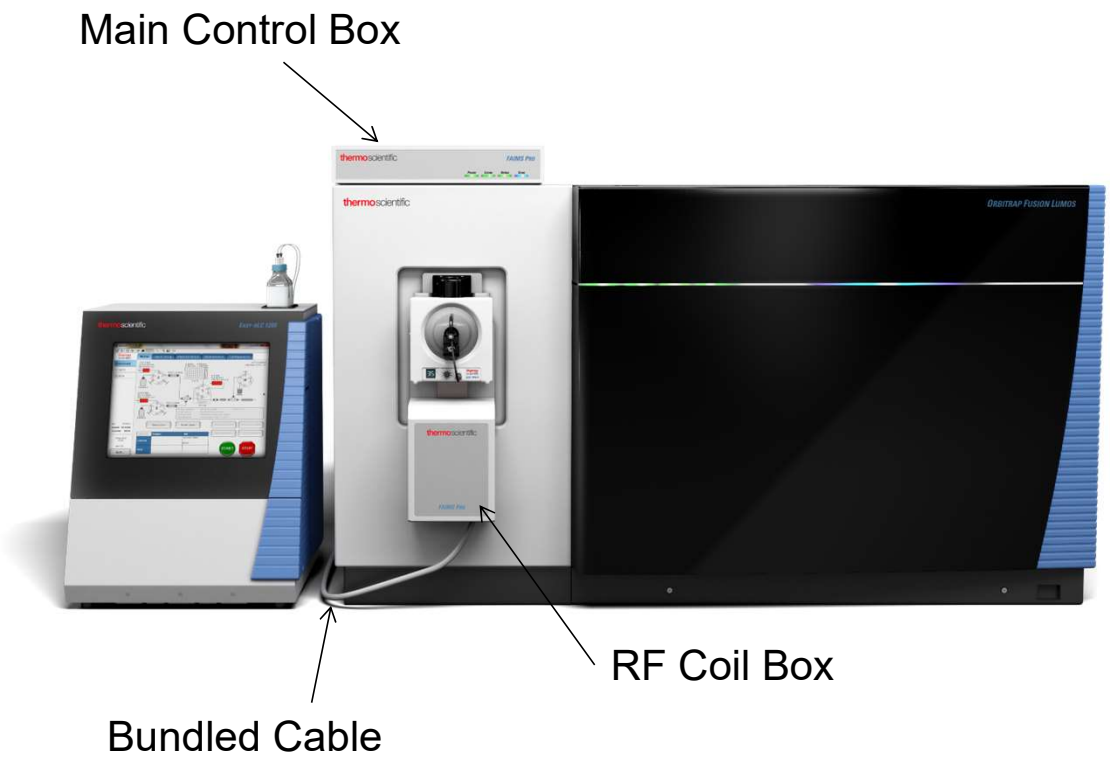


Conventional ion mobility spectrometer

FAIMS– **F**ield **A**symmetric waveform **I**on **M**obility **S**pectrometry

New FAIMS Pro Design

Improved Ion Mobility-Based Separation Option for Proteomics



Thermo Scientific™ FAIMS Pro™ Details

- FAIMS Pro hardware comprises the main control box (MCB), the RF coil box and electrode assembly mounted to a collar flange, and a bundled cable connecting the two.
- Hardware and software ease-of-use makes it simple to attach and use in <2 minutes
- Method templates help a customer to hit the ground running for DDA proteomics experiments
- The result is improved peptide and protein IDs for nanoflow applications

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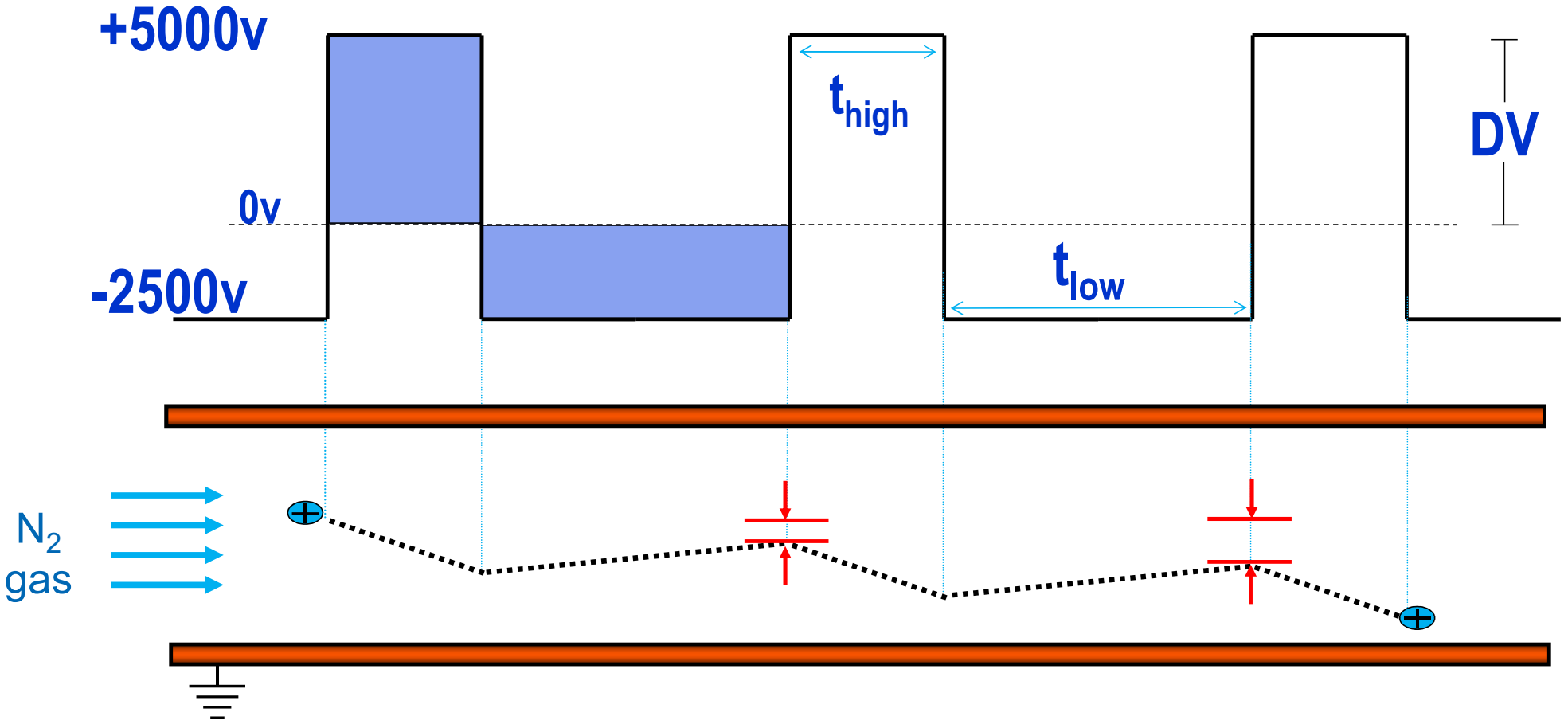
How Does FAIMS Technology Work?

Ion displacement using a dispersion voltage (DV)



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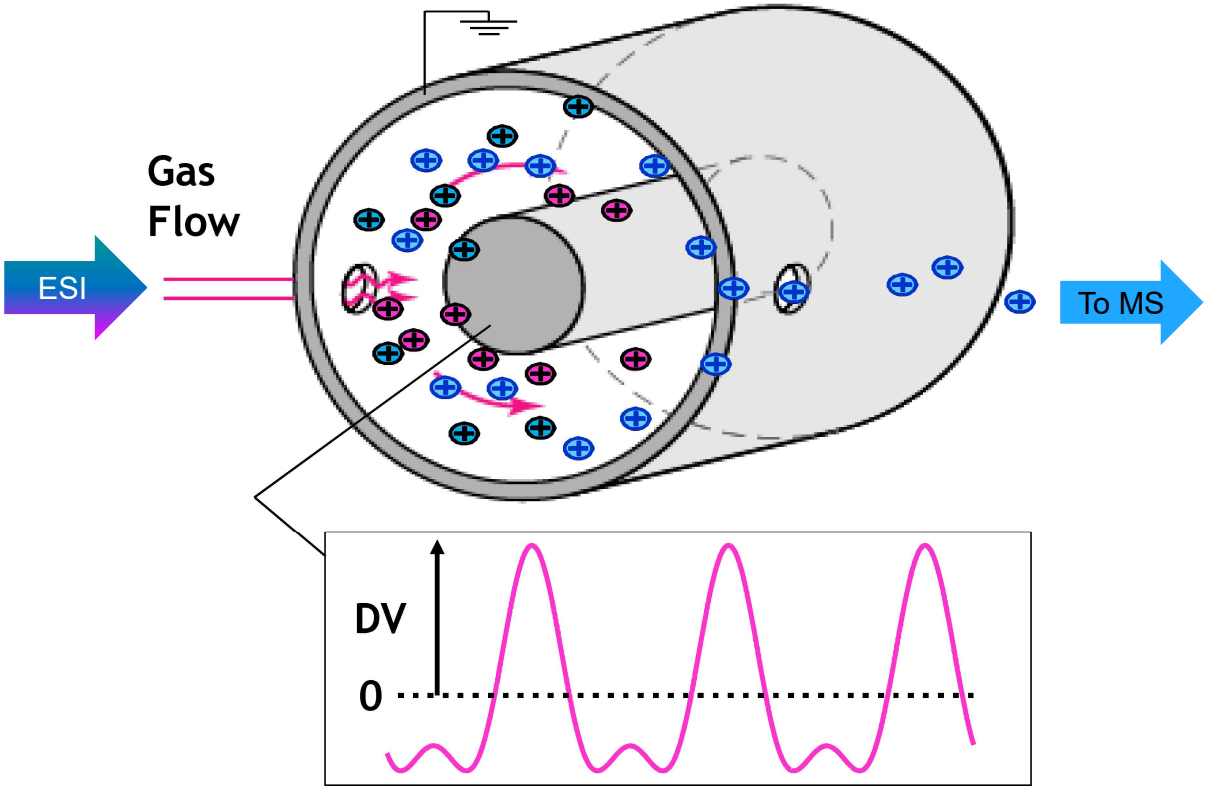
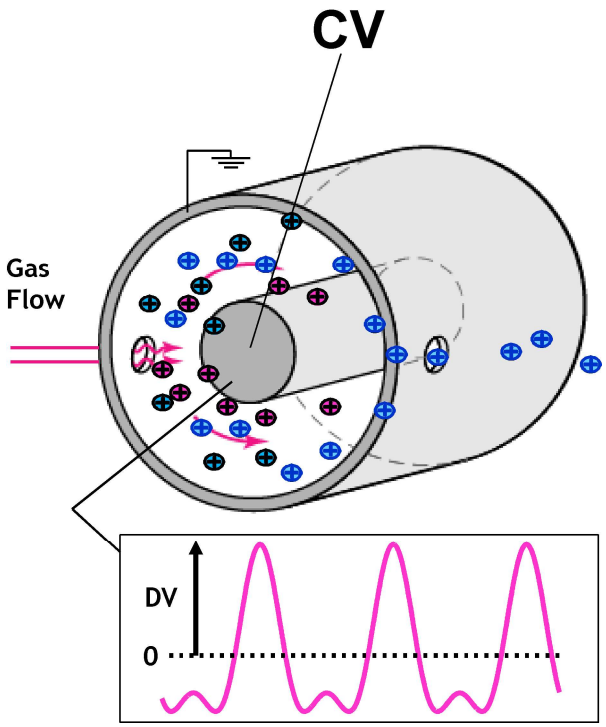
Why is FAIMS Pro Technology Unique?



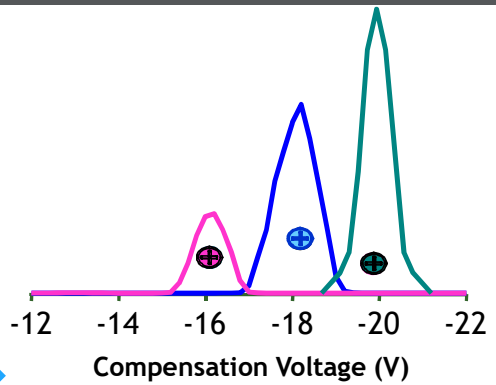
Cylindrical Electrodes

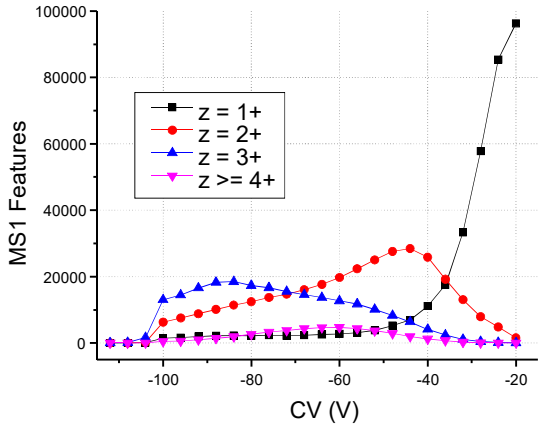
- Cylindrical Electrodes help focus ions through the electrode assembly
- Nitrogen carrier gas moves the ions through from front to back
- The result is better ion transmission into the MS compared to parallel, planar electrodes
- The inner electrode blocks “line of sight”, but the gas and fields direct ions to the MS inlet

FAIMS Compensation Voltage Selects Ions of Interest



To MS

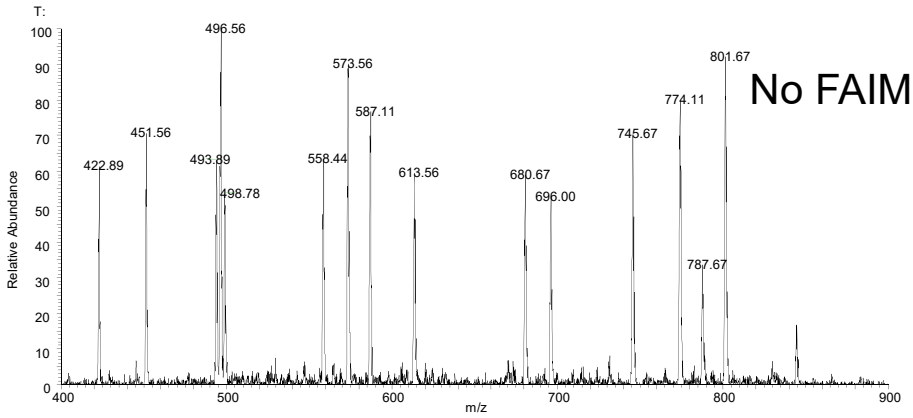




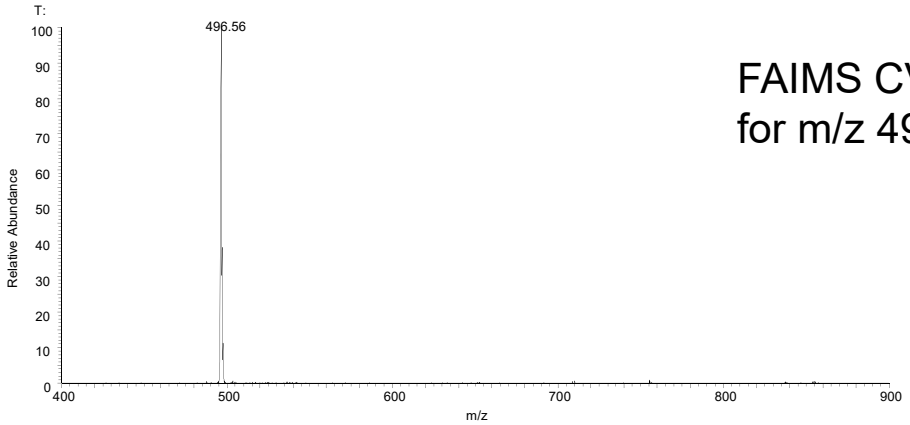
Compensation Voltage Provides Selectivity

- The Compensation Voltage (CV) is applied to the inner electrode to compensate for ion displacement through the electrodes
- The CV dictates which ions pass through the electrodes into the MS
- The CV can be empirically determined and applied during methods for improved signal-to-noise of analytes of interest
- This has benefits for tryptic peptides in complex samples, as 1+ ions are separated from multiply charged ions

FAIMS Pro Technology Improves Selectivity



No FAIMS



FAIMS CV Optimized for m/z 496

Peptide Separation without LC

- Gas phase separation provides an additional level of selectivity for peptides
- Selective transmission of ions improves MS1 signal to noise
- Minimal loss of transmission
- The result is reduction in isolation interference, even without chromatographic separation

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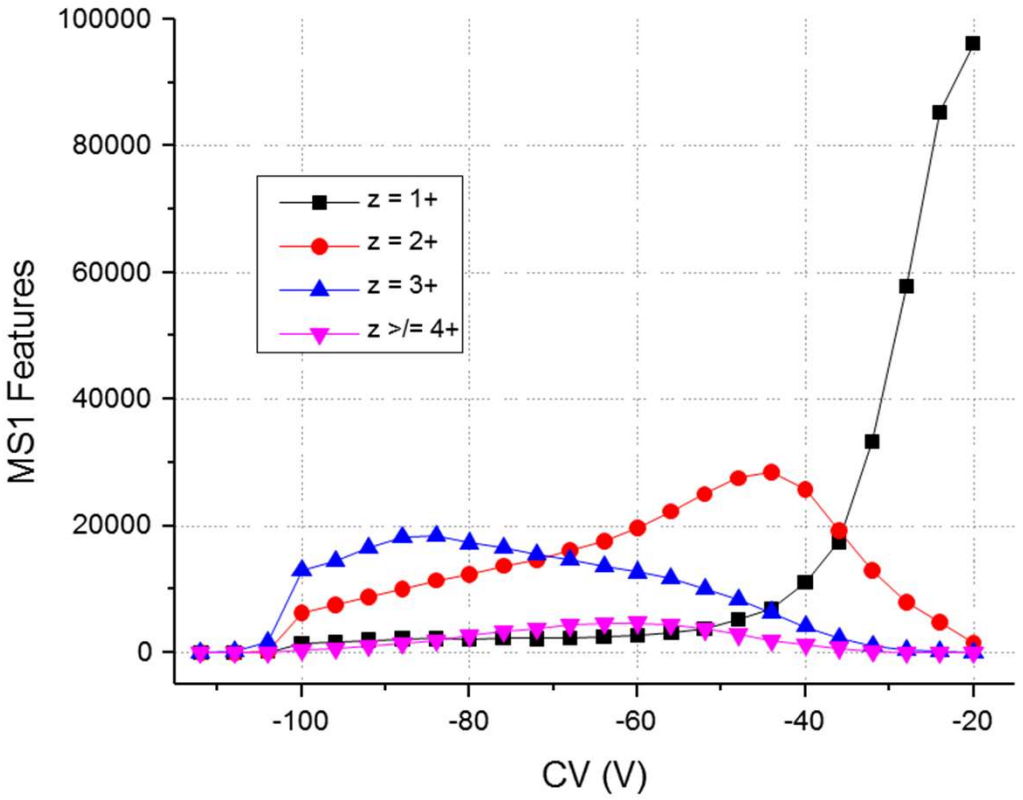
Implementation of FAIMS Pro Technology is Simple on the Orbitrap Fusion Lumos

Data Dependent Experiment: OTMS>ITMS^2 or OTMS^2



Method Templates Make it Easy

Full OTMS scan at CV1

DDA MS2 acquired at CV1

Full OTMS scan at CV2

DDA MS2 acquired at CV2, etc

- **CVs are pre-selected based on tryptic peptide transmission**
- **Gas-phase separation at different FAIMS CVs sends different populations of ions to the MS, increasing number of precursors available for MS/MS**
- **The result is improved peptide and protein IDs in one DDA run compared to not using FAIMS Pro technology**

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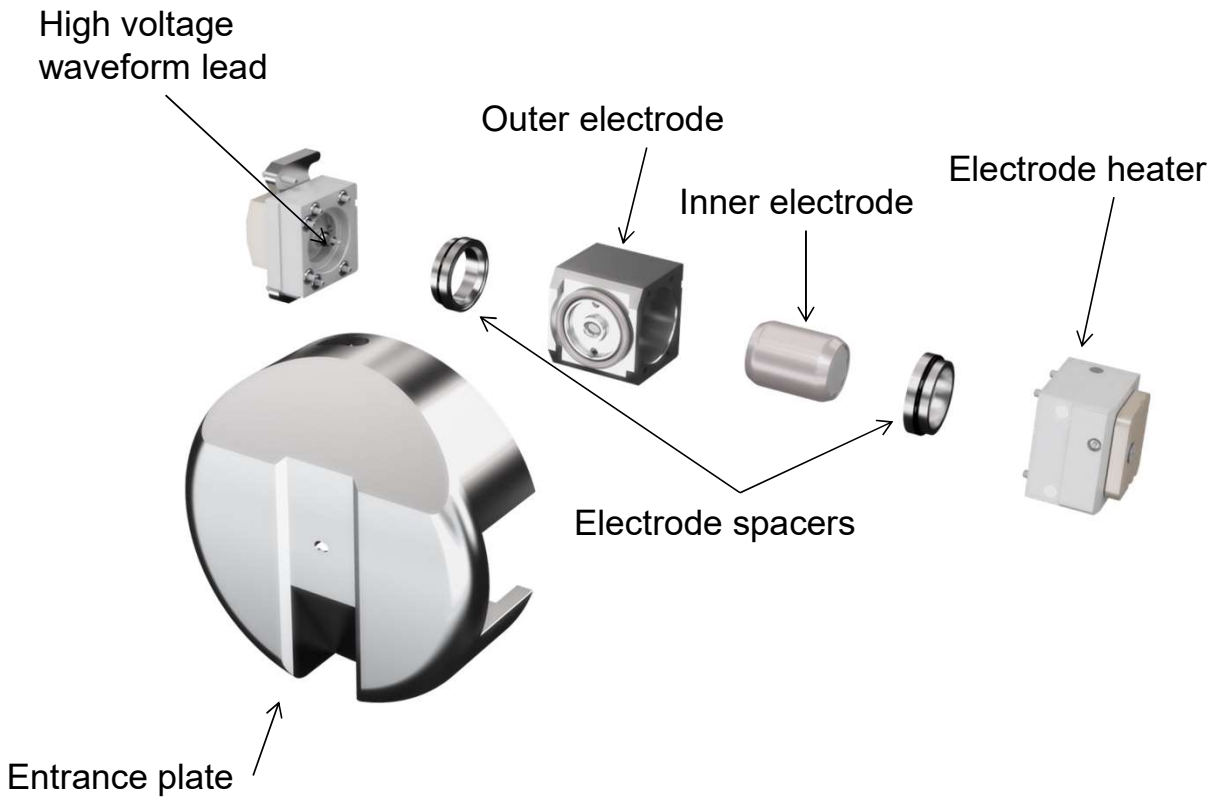
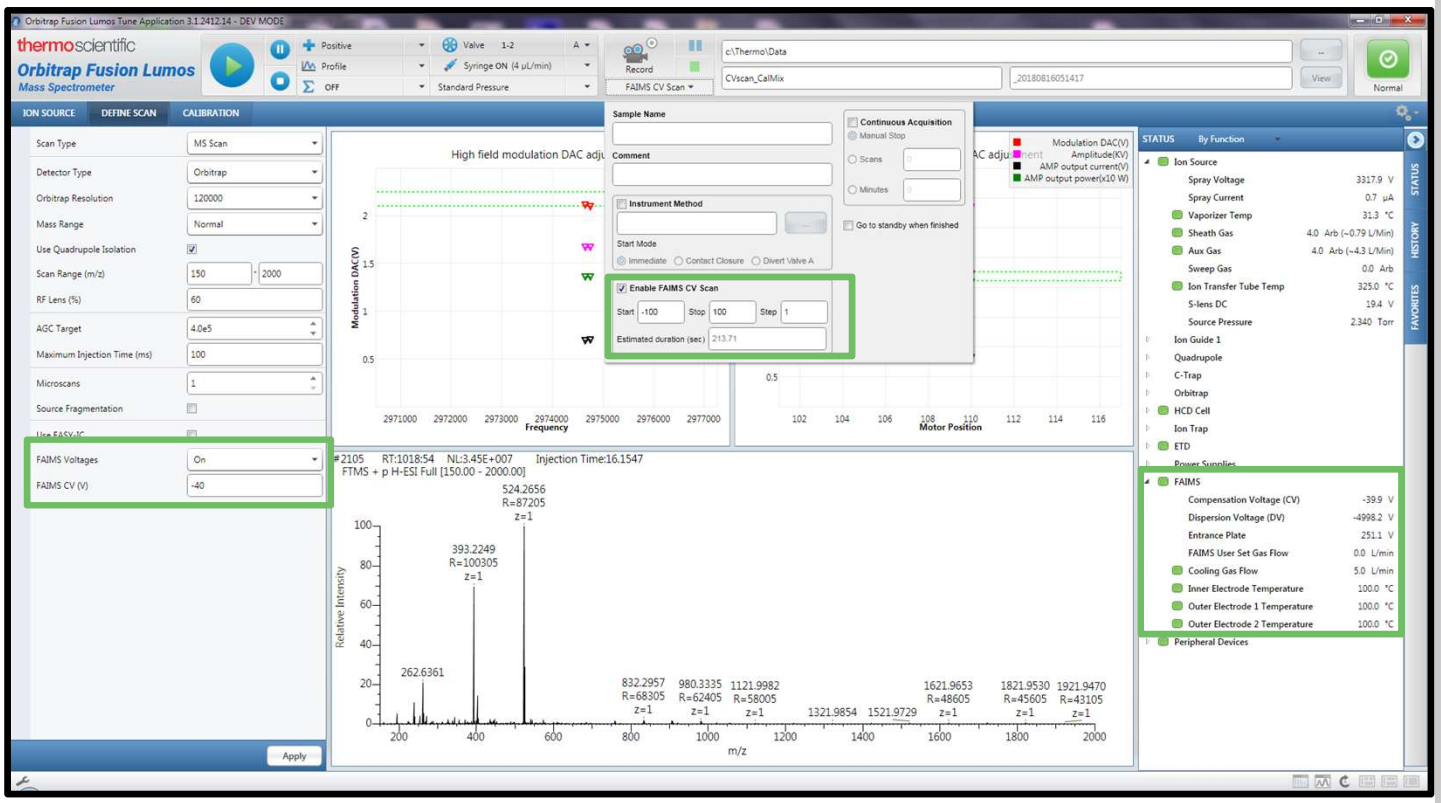
FAIMS Pro Hardware is Tool-Free and Easy to Maintain



User-Friendly Implementation

- One-way, tool free assembly and disassembly
- No venting required for maintenance
- Inner electrode blocks line-of-sight, which improves MS robustness
- Maintenance is simple and electrodes can be cleaned by sonication, at needed frequency (≥ 1 week)
- The only parts requiring cleaning are stainless steel

FAIMS Pro Hardware is Easy To Use



User-Friendly Implementation

- Minimal set-up required by user
- No Instrument Configuration necessary, FAIMS Pro interface is recognized by the software when mounted and powered up
- FAIMS support is in Tune for CV optimization
- Optimization files are automatically saved for further interrogation

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Example Application of the FAIMS Pro Interface

BOTTOM UP PROTEOMICS

Data-dependent experiments with complex peptide mixtures

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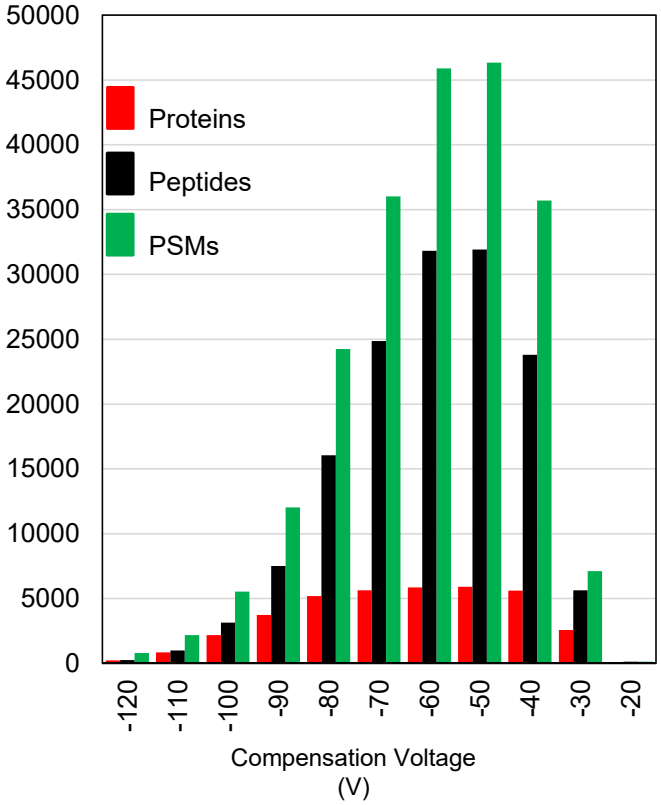
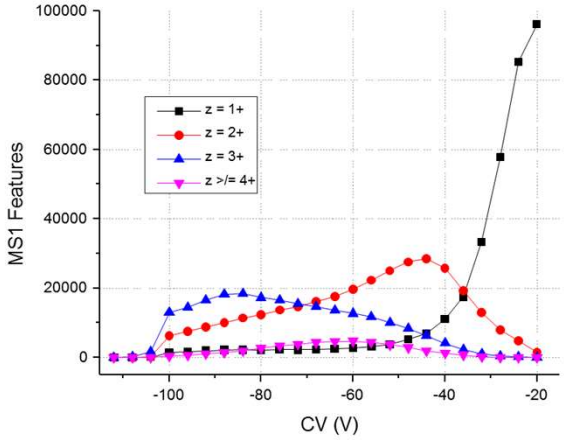
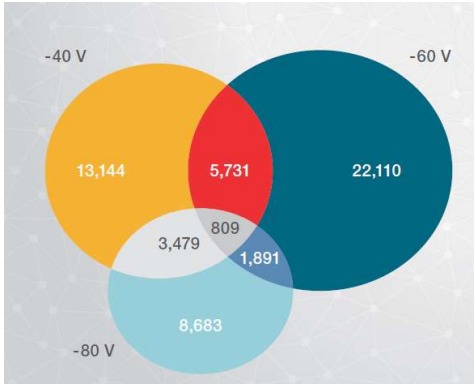
FAIMS Pro CV Fractionation of HeLa Lysate Digest





Peptides IDed in 1 run



Unique Sequence Coverage

- LC-MS analysis of HeLa lysate digest
- Individual runs at single CVs show a distribution of identifications
- Singly charge ions are passed at different CVs than multiply charged peptide ions
- When select CVs are combined in 1 LC-MS/MS run, orthogonal selection results in increased peptide IDs

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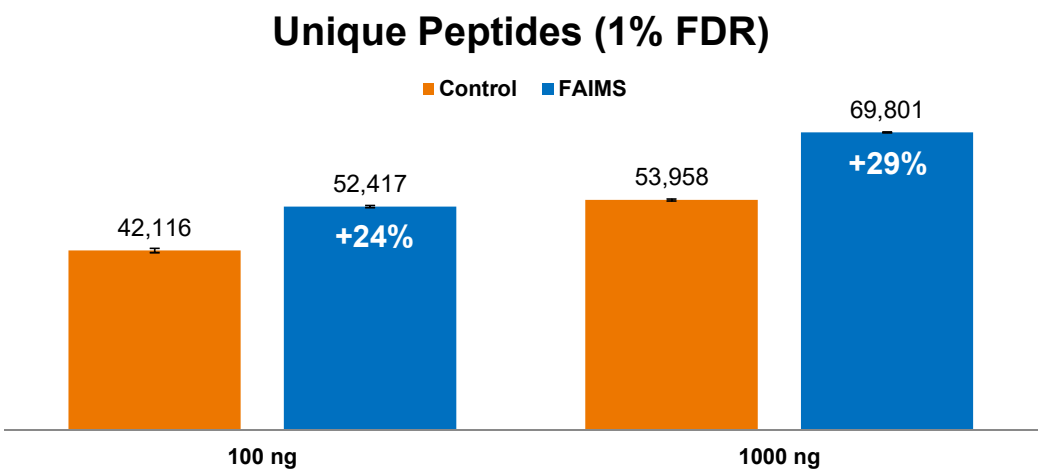
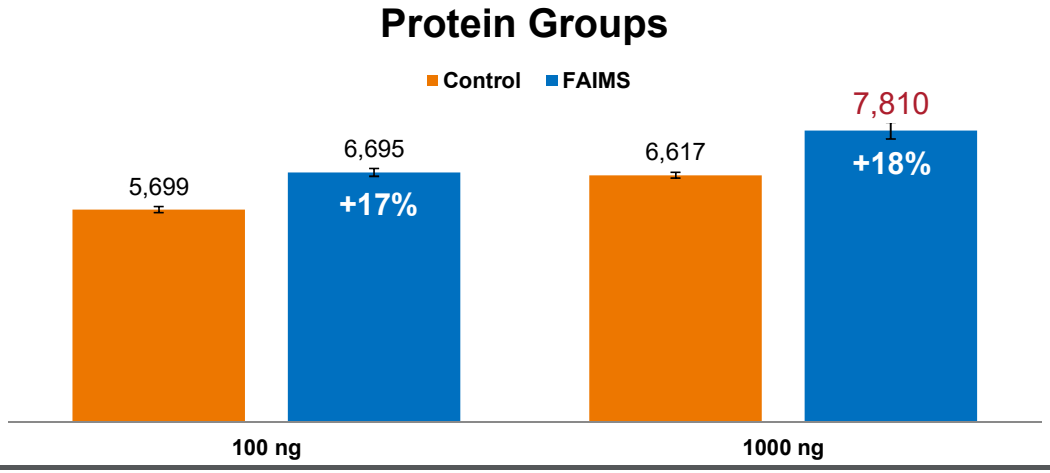
FAIMS Pro Technology Improves Peptide and Protein Coverage





Improved Peptide Identifications

- HeLa lysate digest analysis with 140 min gradient, detected with OTIT and 3 FAIMS CVs in one run
- Peptide improvements exceeded 20% for both 100 ng and 1000 ng loads compared to no FAIMS
- Protein ID improvements with FAIMS Pro technology were nearly as high, approaching 8000 protein group IDs at the 1000 ng load
- Data were searched against the Uniprot human database using Sequest in Proteome Discoverer 2.3 SW with 1% FDR using Percolator
- Simply using the FAIMS Pro interface with the Orbitrap Fusion Lumos MS can improve peptide and protein IDs with little effort

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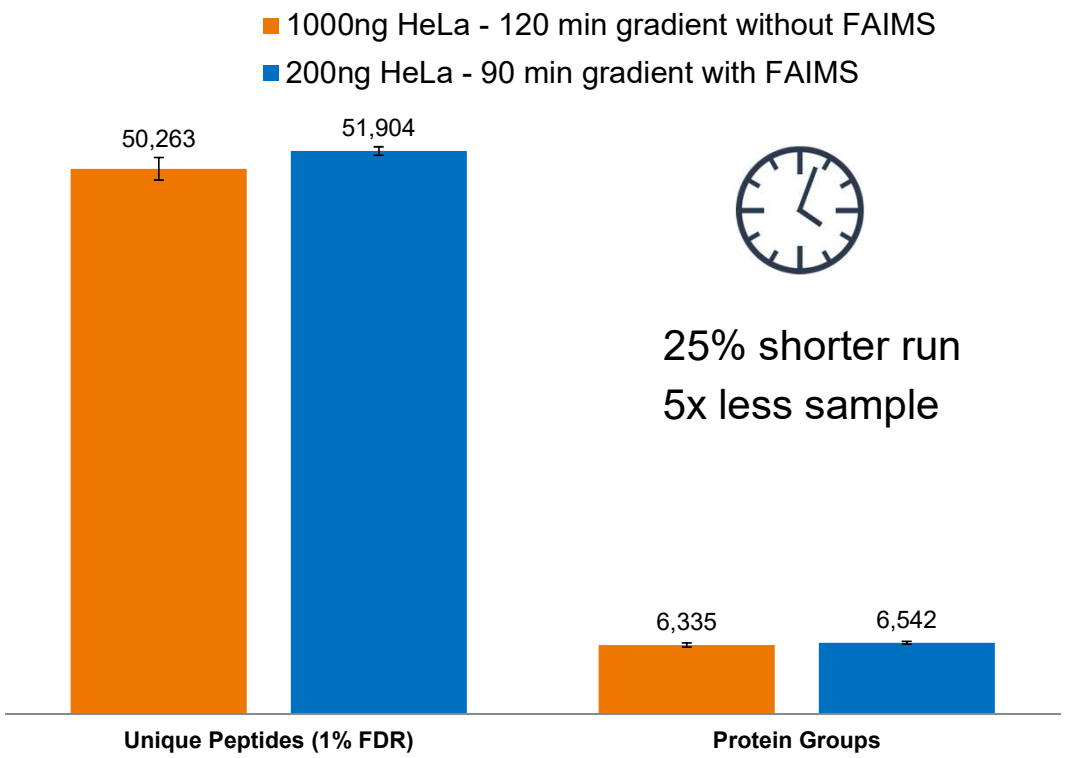
FAIMS provides same IDs with 5x less sample and shorter gradient



Increased Sample Throughput

- FAIMS can save sample throughput time by providing similar IDs in shorter chromatographic runs
- Loading only 200 ng and analyzing on a 90 minute gradient with FAIMS achieves >1000 more peptide IDs and >200 protein IDs than a 1000 ng load and a longer gradient without FAIMS
- Over time, this could lead to savings in time and samples

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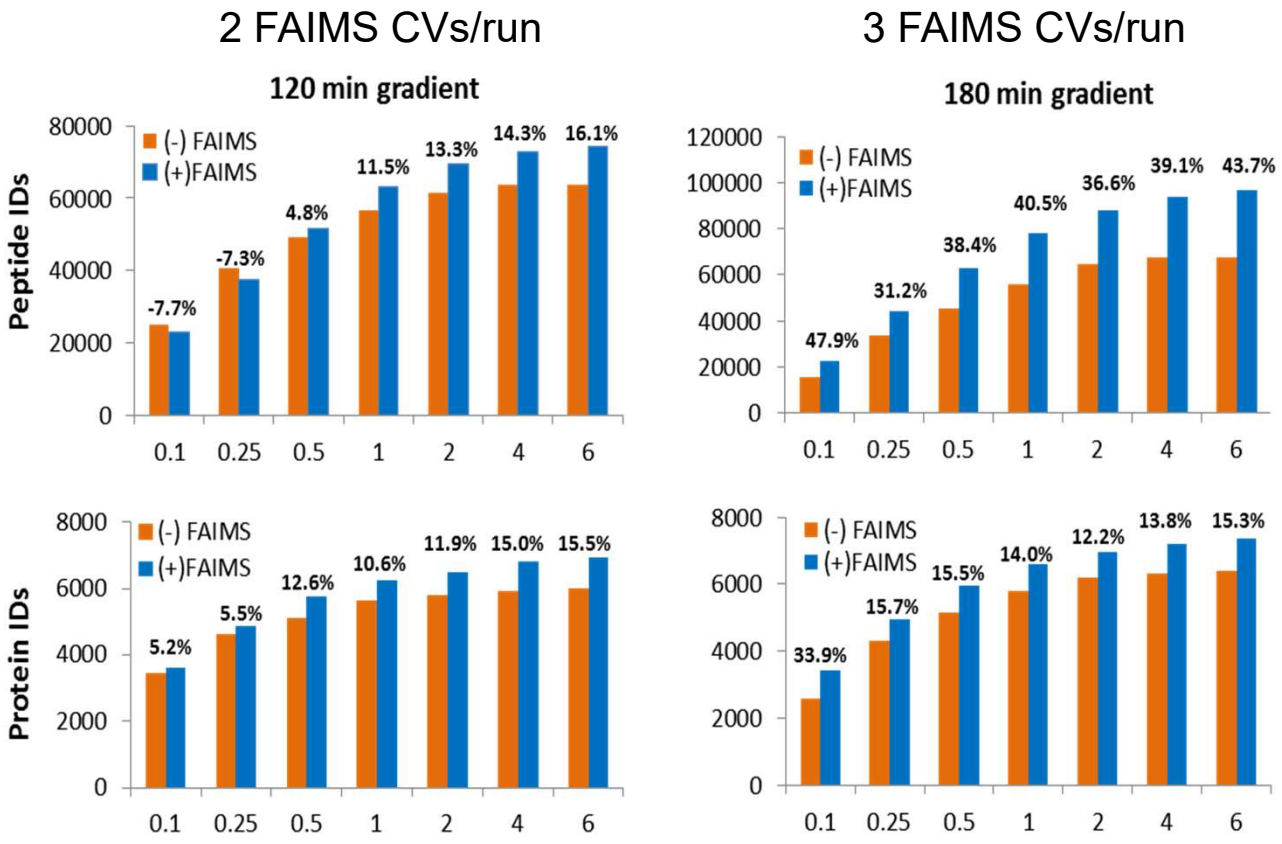
Peptide Improvements with FAIMS Pro Interface at Different Sample Load Amounts



Hebert *et al,* Anal Chem, 2018 Aug 7; 90(15):9529-9537

Improved Peptide Identifications

- Sample load and gradient conditions were evaluated with 2 and 3 FAIMS CVs per run
- Improvements were best observed for low sample loads at longer gradients, with 3 FAIMS CVs
- Protein ID improvements with the 180 min gradient were consistent with Peptide ID improvements, but more obvious at lower loading amounts
- Data were searched with OMMSA and filtered to 1% FDR with the COMPASS software suite
- Proteins grouped based on parsimony with 1% FDR
- FAIMS Pro technology can improve proteome coverage

Example Application of the FAIMS Pro Interface

BOTTOM UP PROTEOMICS

Data-dependent experiments with complex peptide mixtures compared to off-line fractionation

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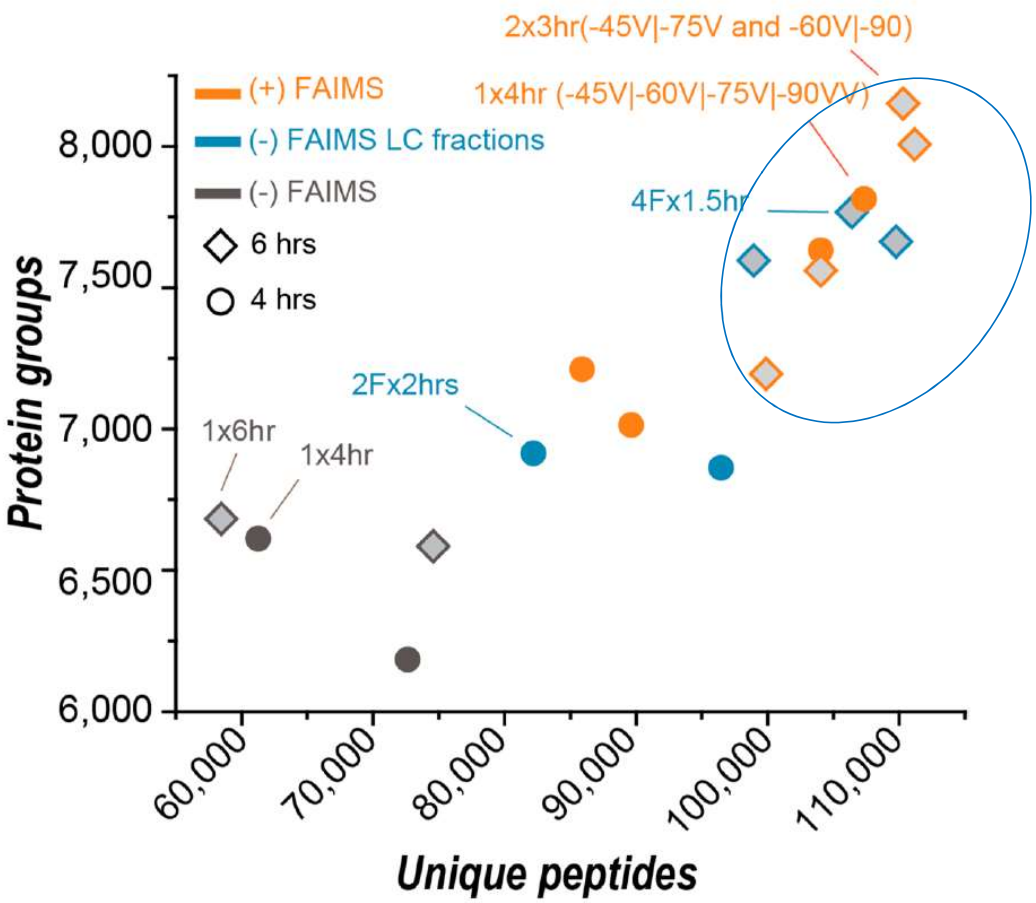
FAIMS Pro Separation Mimics Off-Line Fractionation



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Improved Throughput

- Each FAIMS CV is similar to an off-line fraction
- Combining multiple CVs in one LC-MS/MS run improves peptide and protein IDs
- Two 3 hour runs with the FAIMS Pro technology can exceed 4 off-line fractions with 1.5 hour LC-MS/MS run times
- The result is improved throughput and proteome coverage, without extra sample handling

Example Application of the FAIMS Pro Interface

BOTTOM UP PROTEOMICS

Improved Quantitative Accuracy in TMT experiments

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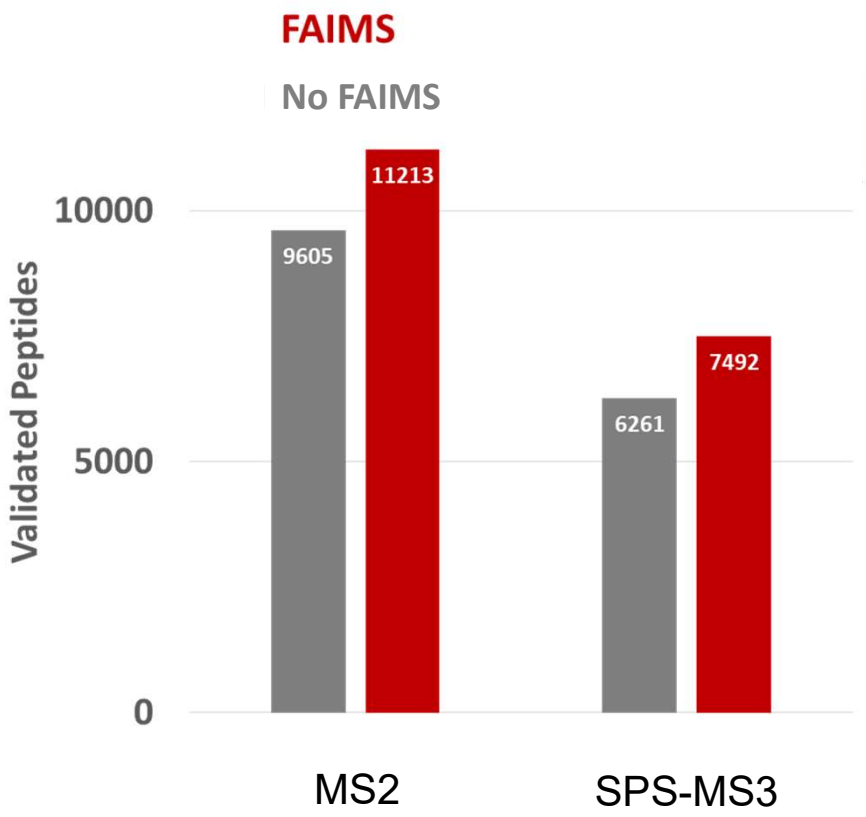
FAIMS Pro Technology Increases Identifications with TMT



Slide courtesy of Devin Schweppe and Steve Gygi
Harvard Medical School, Boston, MA

Improved Data Quality

- The selectivity of the FAIMS Pro Interface provides additional separation of peptides in the gas phase prior to MS detection
- This results in improved peptide identifications for both MS2 and SPS-MS3 methods
- Higher quantitative accuracy with reduced interference (2-8 fold) and increased peptide IDs (17-20%) over the same retention time (120 min gradient)

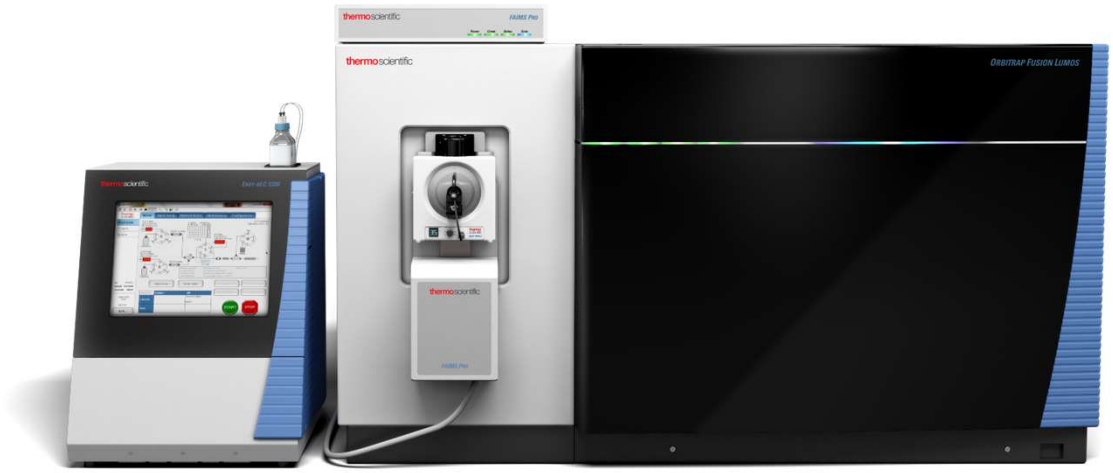
Benefits of FAIMS Pro Technology for Proteomics Applications

- Improved peptide and protein identifications through CV Fractionation
- Improved intact protein detection for top-down experiments
- CV Fractionation very similar to off-line fractionation, but without extra sample handling
- Smaller sample loads in shorter times result in the same DDA results
- Ease-of-use
  - Method templates for DDA work with FAIMS
  - Automated CV optimization tool in Tune
  - Processing software in FreeStyle to observe spectra and extracted ion chromatograms at different CVs
  - Simple installation
  - Easy maintenance

FAIMS Pro Option on the Orbitrap Fusion Lumos MS: Specifications at a Glance

Ion Source Compatibility	HESI for Tune only; TNG nanoflow ion sources
MS Compatibility	Orbitrap Fusion; Orbitrap Fusion Lumos
FAIMS resolution	2-10
Sample flowrate compatibility	100 nL/min – 25 uL/min
CV switching time	25 msec on Lumos; 40 msec on Fusion
N2 gas requirements	~ 18 L/min, 99.9% pure
Applications	Nanoflow bottom-up proteomics, TMT workflows, top down proteomics
Software Version	3.1
Shipping Schedule	September 2018

Questions?



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