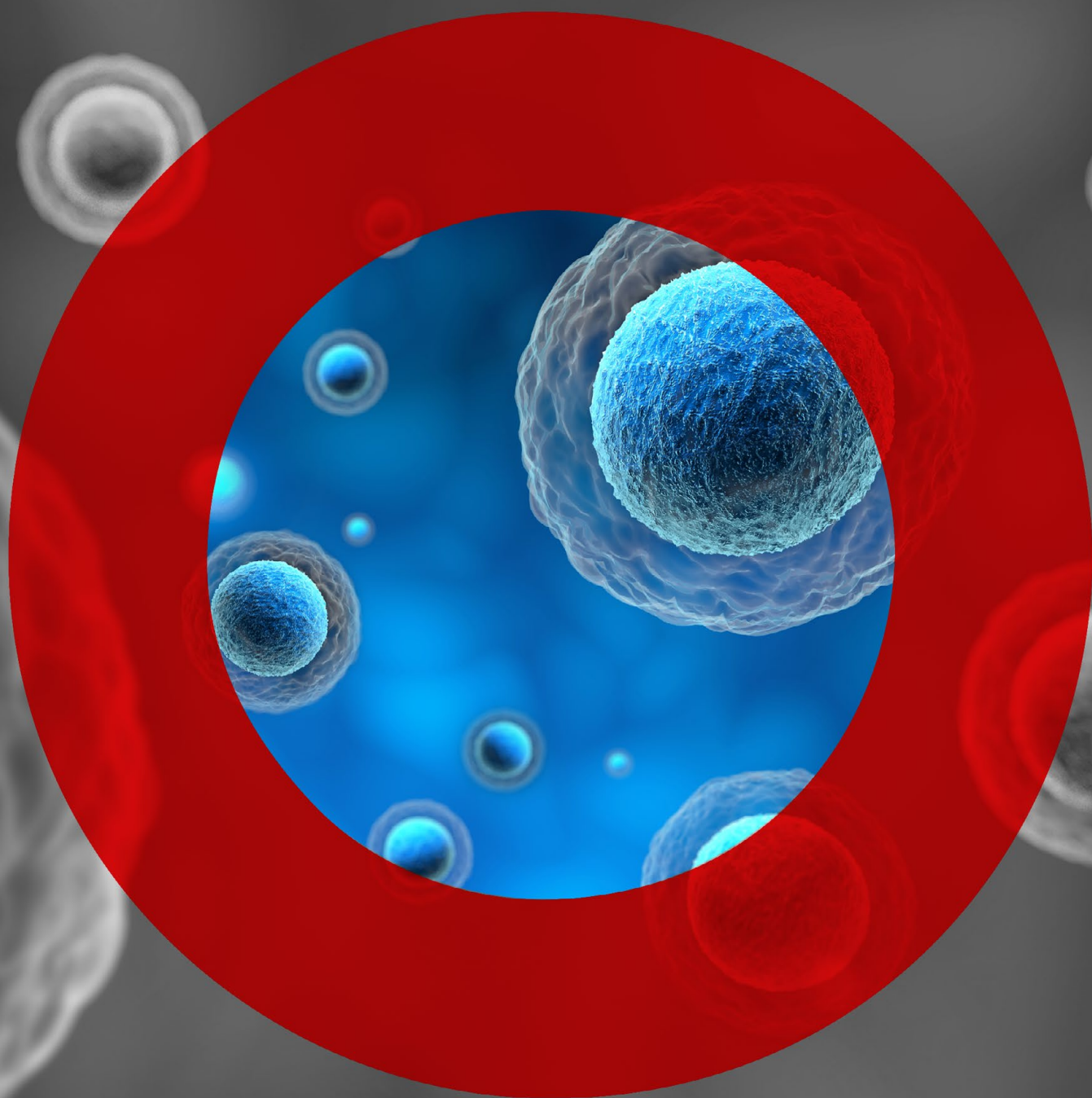


thermoscientific



Reveal what matters

with effortless selectivity

Thermo Scientific FAIMS Pro interface

ThermoFisher
SCIENTIFIC

Effortless selectivity

accelerates your proteomics workflow

The growing complexities of qualitative and quantitative analyses in proteomics demand faster sample preparation, selective and sensitive analytical techniques, and efficient software solutions. With industry-leading technical depth in mass spectrometry innovation, Thermo Fisher Scientific enables broader and deeper analysis into the proteome than ever before.

The Thermo Scientific™ FAIMS Pro™ interface is a next-generation, differential ion mobility device that seamlessly works with multiple Thermo Scientific™ next-generation mass spectrometers to enhance selectivity and enable identification and quantitation of more proteins while reducing time-consuming sample preparation. This results in increased productivity and data quality for every proteomics user—from discovery of disease biomarkers to identification of new therapeutic targets.

The FAIMS Pro interface seamlessly connects with Thermo Scientific™ LC and MS systems such as the Thermo Scientific™ EASY-nLC™ 1200 system and the Orbitrap Exploris™ 480 mass spectrometer, to provide selective identification of more proteins and unique peptides, increasing coverage without extra work.



Flexible to fit your work

Effortlessly fits into existing workflows, maximizing sample profiling across wide dynamic loading amounts, gradient lengths, and with low-flow chromatography, to increase the productivity of proteomics experiments.

Easy to install, use, and maintain

Tool-free, one-way assembly requiring no fine-tuning nor breaking of vacuum, and predefined workflows for data acquisition and processing, ensure ultimate usability and high data quality for all users.

Increases depth of analysis without extra work

Minimizes the time, expense, and variability of offline LC fractionation by carrying out online gas phase fractionation prior to ion introduction into the mass spectrometer.

Conserves sample

Increases selectivity and sensitivity to maximize proteome coverage while conserving sample.

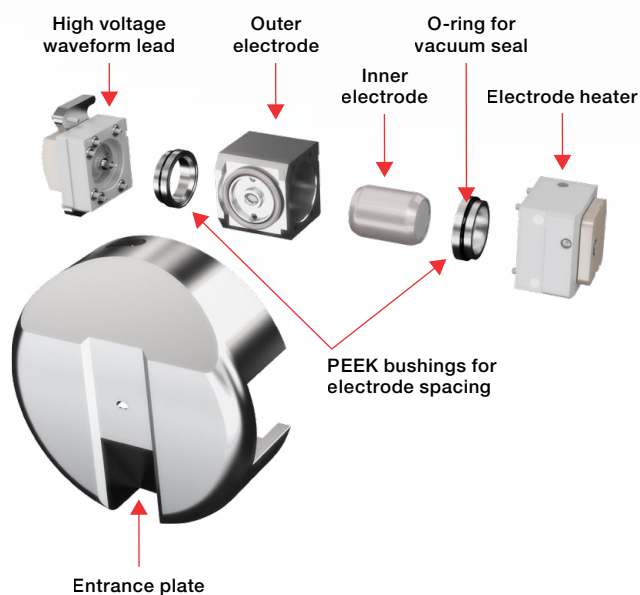


Precursor ions formed at the Thermo Scientific™ EASY-Spray™ Source emitter and introduced into the FAIMS Pro interface electrodes are selectively transmitted through the interface to the ion transfer tube and into the mass spectrometer based on the compensation voltage (CV) setting, while all other ions are filtered out, providing orthogonal selectivity and increased sensitivity. As a result, you easily reveal what's important in your samples.

State-of-the-art interface enhances performance and usability

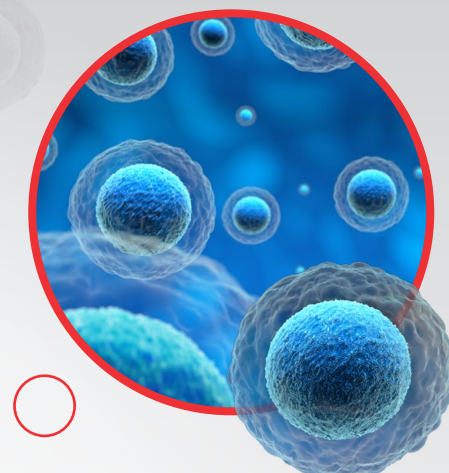
With advanced hardware, the FAIMS Pro interface not only enhances experimental performance and data quality, a unique design also makes it easy to set up, use, and maintain.

- Optimized cylindrical geometry substantially increases ion transmission to the mass spectrometer with short residence times, allowing multiple compensation voltage (CV) settings per data acquisition method
- Ideally operates with typical nanoflow (100 to 1000 nL/min) chromatography separations or direct infusion experiments
- Assembly and disassembly are fast, tool-free, and do not require breaking vacuum
- Assembly, including mounting to the instrument, takes less than two minutes and is one way with perfect alignment and no fine-tuning



Orthogonal selectivity adds efficiency to proteomics workflows

The FAIMS Pro interface integrates directly into existing workflows to enhance selectivity for proteome profiling, multiplexed protein quantitation, and top down protein characterization. Differential ion mobility reduces the complexity of precursor ions accumulated and analyzed per CV setting, increasing proteome coverage, decreasing interference, and improving quantitative confidence. In most cases, the FAIMS Pro interface provides the same coverage using less sample and in less time.



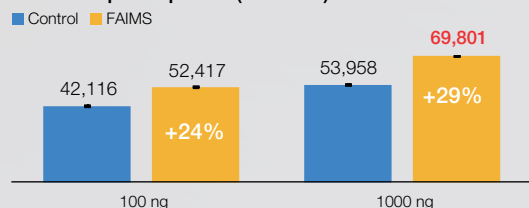
Enhanced selectivity, unmatched performance

The FAIMS Pro interface provides orthogonal precursor ion selectivity based on differential gas phase mobility. The CV setting determines which groups of ions are transmitted to the mass spectrometer for detection. A wide range of possible CV settings (-120 to 120 V) increases

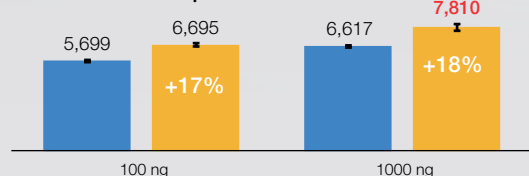
instrument performance for proteomics experiments. Multiple CV settings may be repetitively sampled to increase the number and type of precursors detected and sequenced, resulting in greater ID rates using the same experimental parameters. Each CV setting uses

the same Data Dependent Acquisition (DDA) method to acquire high-resolution accurate-mass (HRAM) MS and a series of MS/MS spectra, maximizing proteome coverage. The increased selectivity and sensitivity enable researchers to increase proteome coverage, or to maximize efficiency and conserve sample.

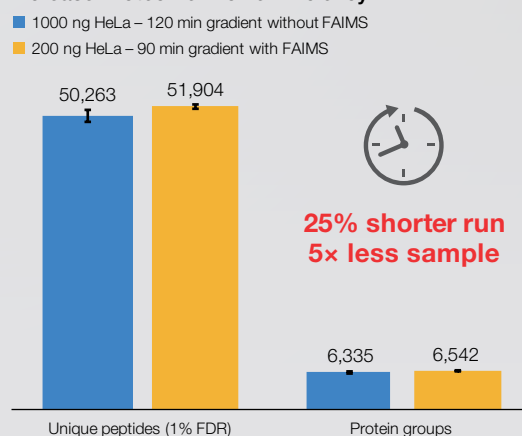
More Unique Peptides (1% FDR)



More Protein Groups



Increase Proteome Profile Efficiency



Proteome analysis using the FAIMS Pro interface increases proteome coverage for both low and high sample loading amounts. Comparative analysis of the number of proteins and peptides confidently identified in an analysis of a tryptic digest of HeLa cell lysate under identical chromatographic conditions. While the proteome coverage measured using the standard DDA method at low and high sample loading amounts is impressive, simply incorporating the FAIMS Pro interface substantially increased protein and peptide coverage with almost 8,000 proteins confidently measured in the 140-minute method.

Incorporation of the FAIMS Pro interface into an existing DDA experimental workflow increases proteome profiling efficiency. Compared to using a standard DDA method, the FAIMS Pro interface method provided similar proteome coverage while using substantially less sample and shorter chromatographic gradients, addressing the two primary concerns for translational proteomics.



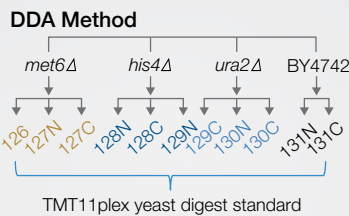
“We have been extremely impressed with the performance of the Thermo Scientific FAIMS device for shotgun proteomics. FAIMS enables considerably more sample depth per unit time and could eliminate the need for prefractionation of peptides for most applications.”

Joshua J. Coon Thomas and Margaret Pyle Chair at the Morgridge Institute for Research; Professor, Biomolecular Chemistry and Chemistry, University of Wisconsin; Director, NIH National Center for Quantitative Biology of Complex Systems.
For more information about Coon's work with FAIMS see, A. S. Hebert et al. *AnalChem* 2018, 90(15), pp. 9529–9537.

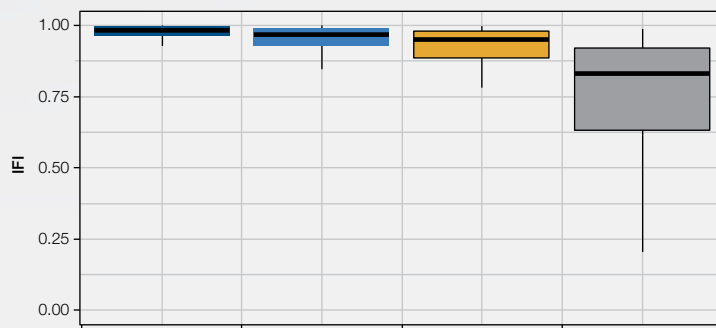
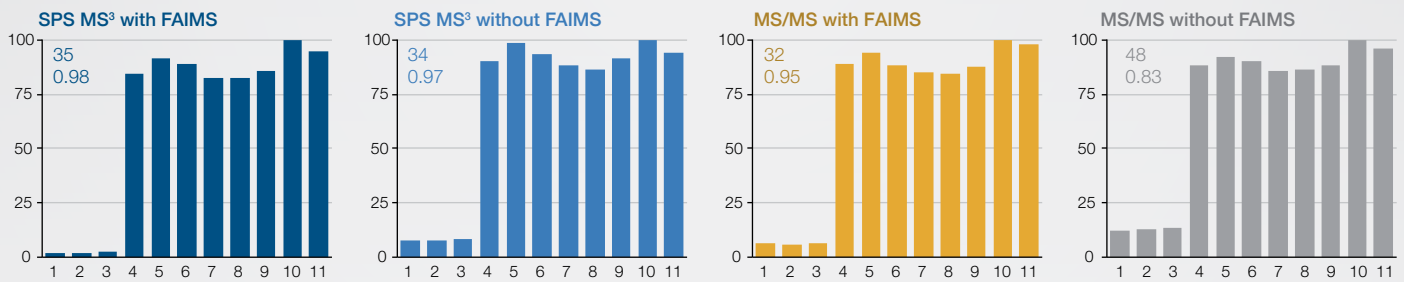
Increased confidence in multiplexed protein quantification

To address the large scope of translational proteomics experiments, multiplexed proteome analysis using Thermo Scientific™ Tandem Mass Tags™ (TMT™) isobaric tags is used to simultaneously compare protein abundances across time, perturbations, tissues, etc. The challenge is to not only detect more peptides, but to maintain relative quantitative accuracy across the different samples. Using isobaric tags, the reporter ions used for relative quantitation are generated at the MS/MS or MS³ stage. However, quantitative accuracy can be compromised by sample complexity due to co-isolation of isobaric peptides resulting in distortion of reporter ion ratios. Incorporating the FAIMS Pro interface in the workflow increases precursor selectivity, resulting in greater confidence in peptide and protein quantification.

Higher-confidence quantification



Met6 Normalized ProteinSumSN



Observed in Met6 KO:
Median IFI:

Method	Observed in Met6 KO	Median IFI
SPS MS ³ with FAIMS	35	0.98
SPS MS ³ without FAIMS	34	0.97
MS/MS with FAIMS	32	0.95
MS/MS without FAIMS	48	0.83

Comparative TMT analysis evaluating the knock out MET6 protein ratios across four proteomes and 11 replicates. At top is the breakdown of the four-proteome mix. Each proteome was mixed 1:1 after preparation and labeling. Samples were analyzed using four DDA methods to evaluate selectivity and relative quantitation accuracy.

Relative quantification of TMT reporter ions of proteins regulated by the MET6 gene is for each acquisition method. Only the response for the MET6 knock out samples should have measured reporter ion ratios close to zero.

The interference free index plot depicts the isobaric interferences for the targeted peptides. The greater the index, the better the quantitation shown in the middle chart. The greatest selectivity and quantitative accuracy was obtained for the SPS MS³ FAIMS Pro interface method. Use of the FAIMS Pro interface for MS/MS analysis also produced a substantial increase in quantitative accuracy. Data courtesy of D. Schweppe and S. Gygi.



"FAIMS is appearing with perfect timeliness. We desperately need robust and reliable separation approaches that allow access to previously undetected low abundance populations of precursor peptides. FAIMS can do that with ease. And we need techniques to remove interference in isobaric tagging experiments. FAIMS does that, too."

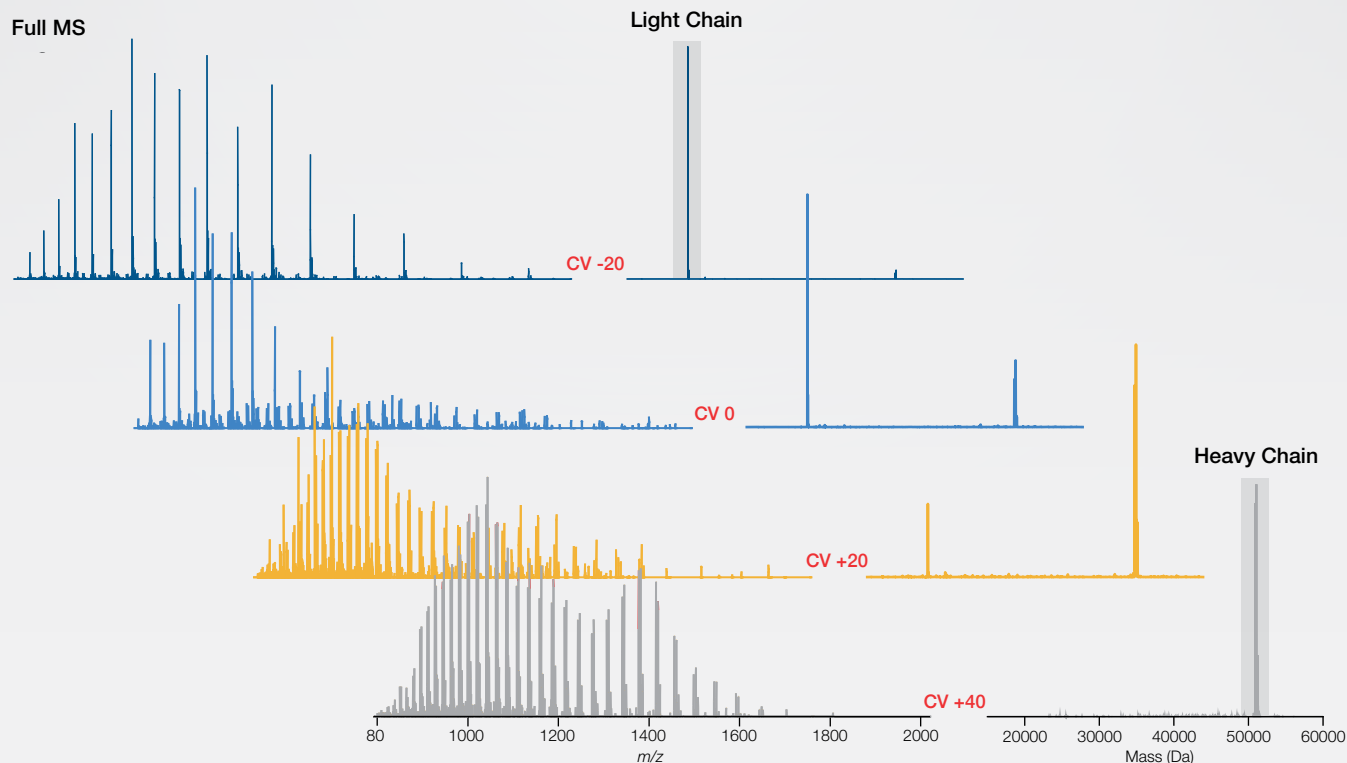
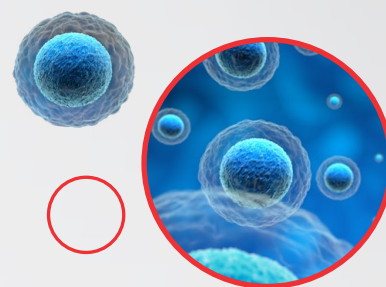
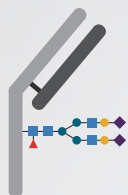
Steven Gygi Professor of Cell Biology, Harvard Medical School

Selectivity expands confidence

for intact protein characterization

Protein and proteoform analysis require comprehensive and accurate molecular and sub-structural characterization to enable identification and location of cSNPs, mutations, PTMs, and sequence clippings. The mass spectral method must be able to differentiate the various proteoforms to increase the confidence in automated data processing, while maintaining efficiency to address sample throughput. Proteoforms significantly increase mass spectral complexity, requiring additional workflow selectivity to increase profiling capabilities. Adding the FAIMS Pro interface enhances intact protein characterization by reducing interferences and enhancing sensitivity, enabling more accurate proteoform detection and characterization.

Comprehensive protein and proteoform characterization



Rapid characterization of the reduced and alkylated NIST mAb standard through direct infusion analysis using the Thermo Scientific™ Orbitrap™ Eclipse™ Tribrid™ mass spectrometer. Introduction of the FAIMS Pro interface prior to HRAM MSⁿ analysis facilitated gas-phase separation based on the CV setting. Using discreet CV settings selectively transmitted either the light or heavy chain and corresponding proteoforms into the mass spectrometer for comprehensive analysis.



“Using the FAIMS Pro interface on the Orbitrap Eclipse Tribrid MS significantly enhances the detection and deep characterization of proteoforms in either the direct infusion or LC-MS/MS modes of operation. Our data has generated a lot of excitement about using FAIMS, in part due to robustness of the ion source over time.”

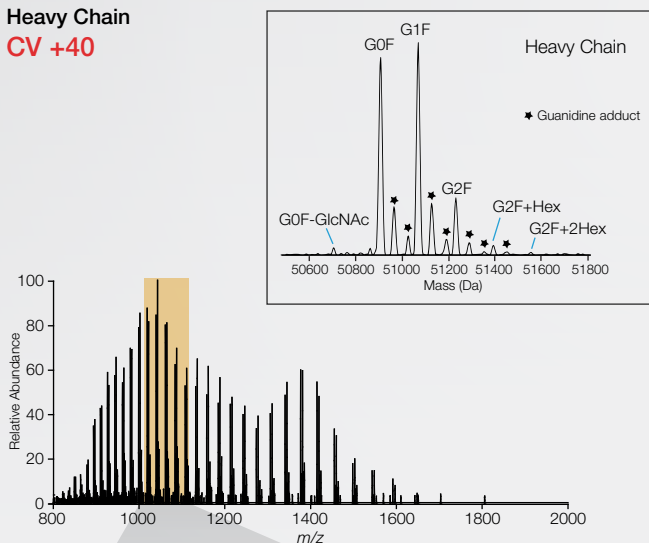
Neil Kelleher Professor, Northwestern University

Thermo Scientific™ EASY-nLC™ 1200 system and the Thermo Scientific™ Orbitrap™ Eclipse™ Tribrid™ mass spectrometer. Ready-to-use method templates for the FAIMS Pro Interface simplify method setup.

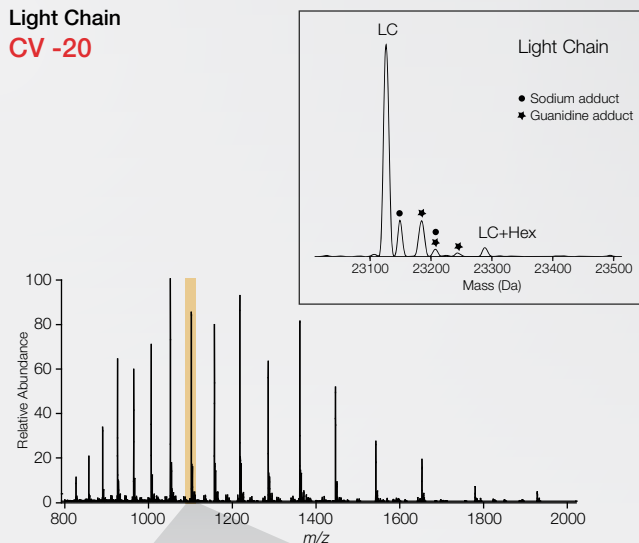


Enhanced top-down characterization

Heavy Chain CV +40



Light Chain CV -20



Heavy Chain (HC)

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26  Q V T L R E S G P A L V K P T Q T L T L T C T F S 25
26  G F S L S T A G M S V G W I R Q P P G K A L E W L 50
51  A D I W W D D K K H Y N P S L K D R L T I S K D T 75
76  S K N Q V V L K V T N M D P A D T A T Y C A R D 100
101 M I F N F Y F D V W G Q G T V T V S I S A S T K I G 125
126 P S V F P L A P S S K S T S G G T A A L I G C L V K 150
151 D Y F P E P V T V S W N S G A L T S G V H T F P A 175
176 V L Q S S G L Y S L S S V V T P S S V L G T Q T 200
201 Y I C N V N H K P S N T K V D K R V E P K S C D K 225
226 T H T C P P C P A P E L L G G P S V F L F P P K P 250
251 K D T L M I S R T P E V T C V V D V S H E D P E 275
276 V K F N W Y V D G V E V H N A K T K P R E E Q Y N 300
301 S T Y R V V S V L T V L H Q D W L N G L R L E Y K C K 325
326 V S L K A L P A P I E K T I I S K A L K G L Q P R E P Q 350
351 V Y T L P P L S R E L E M T K N Q V S L T C L V K G F 375
376 Y P S D I A V E L W E S N G L Q P E L N N Y K T T P P V 400
401 L L D S D G S F L L Y S K L T L V D K S R L W L Q L G N I V 425
426 F L S C S I V M H E A L L H N H Y T L Q K S L S L S P G C
  
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Light Chain (LC)

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26  N D I Q M T Q S P S T L S A S V G I D R V T I T C S A 25
26  S S R V G Y M H W Y Q Q K P G K A P K L L I Y D I T 50
51  S K L A S G V P S R F S G S G S G T E F T L T I S 75
76  S L L Q P D I F A T Y Y C F Q G S G L Y P F T F G L G I 100
101 T K V E I K R T V A A P S V F I F P I S D E Q L K 125
126 S G T A S V V C L L N N F Y P R E A K V Q W K V D 150
151 N A L Q S G L N S I Q E L S V T E Q D I S K D S T Y S L S 175
176 S T L T L S K A D Y L E K H K V Y A C E V T H Q G L I 200
201 S L S P V T K S F N R G E C
  
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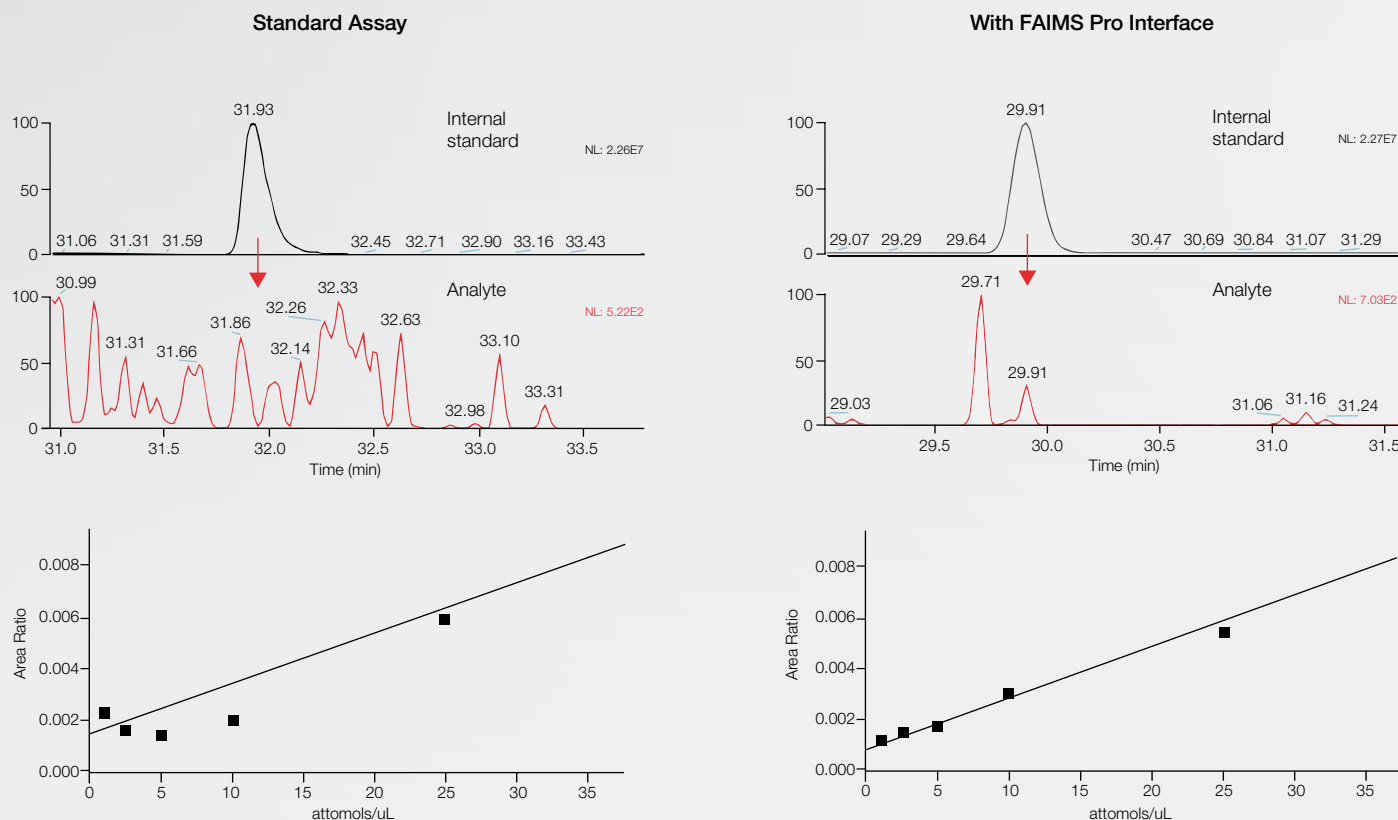
Comprehensive light and heavy chain characterization results at different CV settings using a HRAM MSⁿ Orbitrap Eclipse Tribrid mass spectrometer method. The inset shows the deconvoluted MS spectrum with annotated glycoforms (heavy chain) and adducts (light chain). The increased selectivity provided by the FAIMS Pro interface enabled use of both wide and narrow quadrupole mass filters for subsequent top-down characterization using HCD, ETD, EThcD, and UVPD to generate complementary fragmentation. The sequence coverage map shows 45 and 65% coverage for the heavy and light chain, respectively. The red brackets represent c- and z- ions, blue brackets b- and y- ions, and green brackets a- and x- ions.

Enhanced targeted peptide quantification using selected reaction monitoring

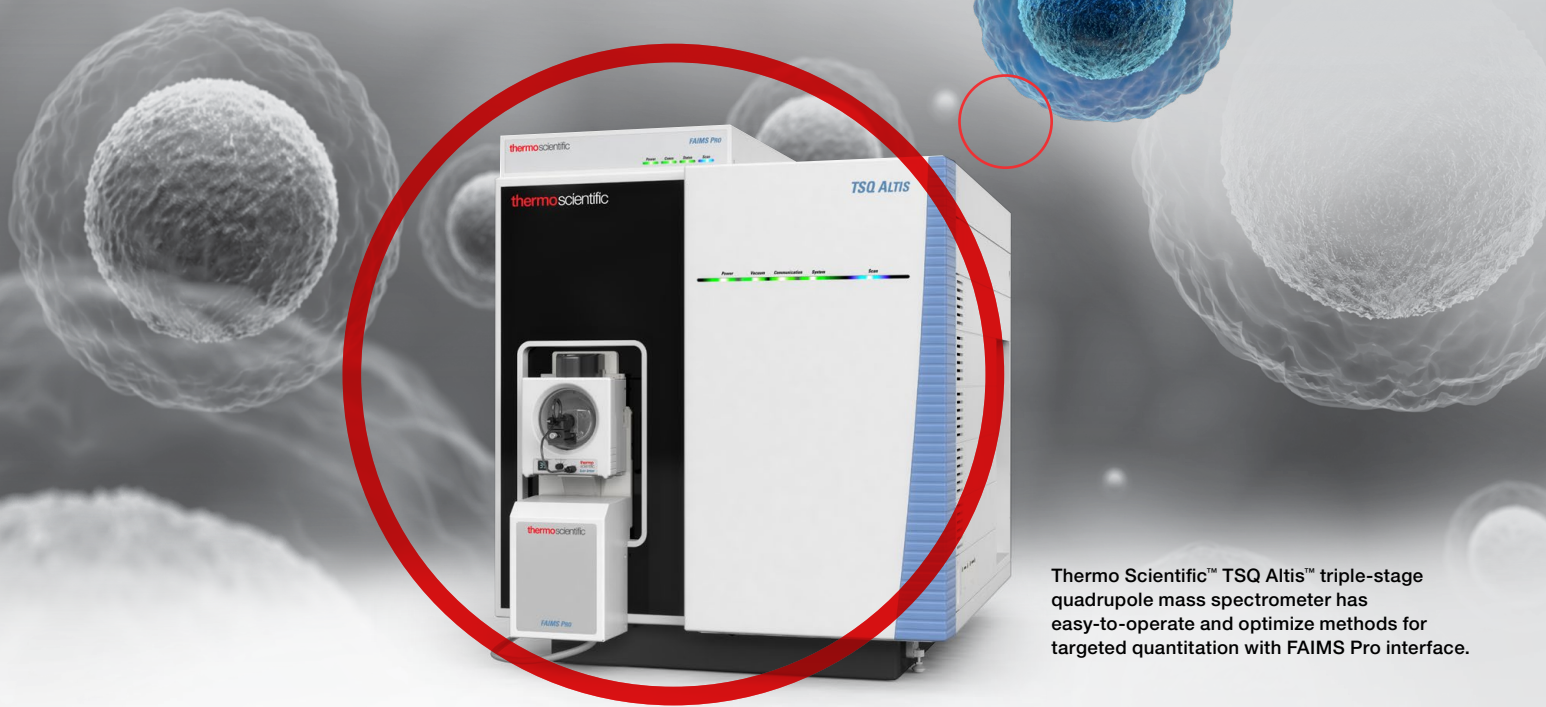


Routine biomarker verification and validation studies require robust, precise, and accurate quantification across wide dynamic ranges and in the presence of complex biological matrices. Successful quantification at the LOQ and LLOQ is critical to establish values for early detection opportunities, but automated data processing becomes challenging due to matrix interference co-adding to the measured selected reaction monitoring (SRM) signal. When automated data processing becomes compromised, manual peak integration is required, resulting in reduced efficiency and increased chance of introducing bias. Incorporating the FAIMS Pro interface into existing nLC-SRM methods enhances selectivity, extends quantitative dynamic range, and permits confident automated data processing.

Improved S/N and LOQs

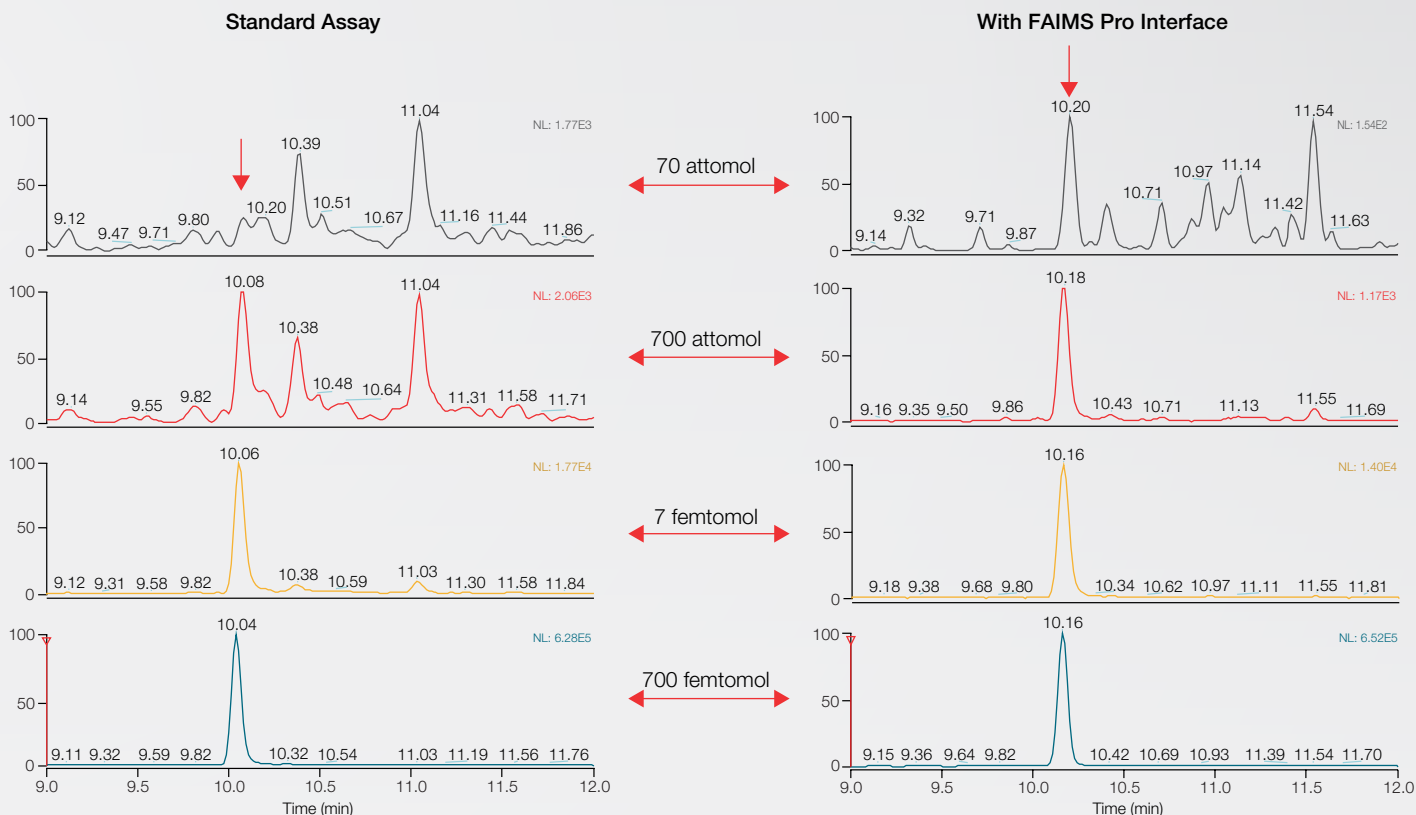


The peak is selectively cleared from background noise in the FAIMS method and confirmed by SRM and retention time (RT) match with an internal standard. Using the FAIMS Pro interface (right panels) provides quantifiable signal at low concentration, which extends calibration curve linearity towards lower limits of detection (with FAIMS Pro interface: Slope = $2.02E-4$; $R^2 = 0.9981$, without FAIMS Pro interface: Slope = $1.94E-4$; $R^2 = 0.9975$).



Thermo Scientific™ TSQ Altis™ triple-stage quadrupole mass spectrometer has easy-to-operate and optimize methods for targeted quantitation with FAIMS Pro interface.

Extended quantitative accuracy

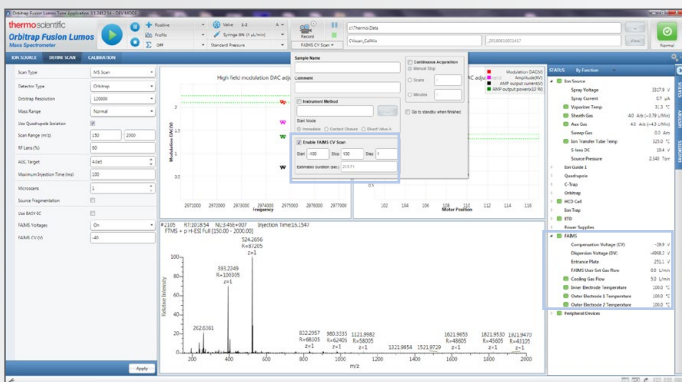


Quantification of Angiotensin I in digested plasma. Using FAIMS Pro interface with optimized CV value as part of the SRM table provides selective noise reduction allowing a quantifiable signal (right panels) even at concentration levels where analyte signal is impacted by noise interference in a standard run without using the FAIMS Pro interface (left panels).

Everyday usability assures performance

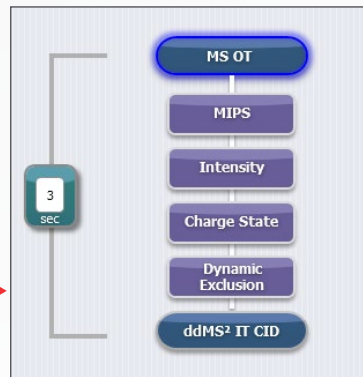
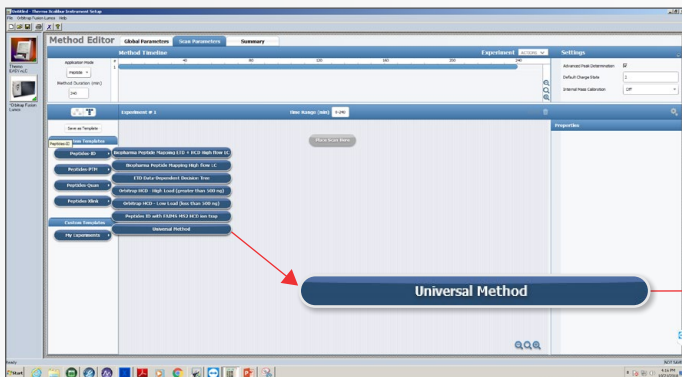
With automated tuning and optimization, data-acquisition method templates, and streamlined data processing, setup and use of the FAIMS Pro interface with Thermo Scientific next-generation mass spectrometers are simplified, enhancing experimental performance and productivity regardless of user expertise. Seamless integration is designed to support maximum productivity for your most demanding science.

Instrument tuning and optimization



The instrument control software enables rapid CV evaluation and optimization. Thermo Scientific™ Orbitrap™ Tribrid™ Instrument Control software (version 3.1 or later), Thermo Scientific™ TSQ Instrument Control software (version 3.1 or later) and Thermo Scientific™ Orbitrap™ Exploris™ 480 Instrument Control software (version 1.0 or later) automatically recognize the FAIMS Pro interface assembly. In addition, the CV Scan Tool in the Tune application of the Instrument Control software can be used for infusion-based CV optimization for either total ion current (TIC), selected ion monitoring (SIM), or parallel reaction monitoring (PRM)-based detection. The resulting optimization files are automatically saved for further interrogation in Thermo Scientific™ Freestyle™ software.

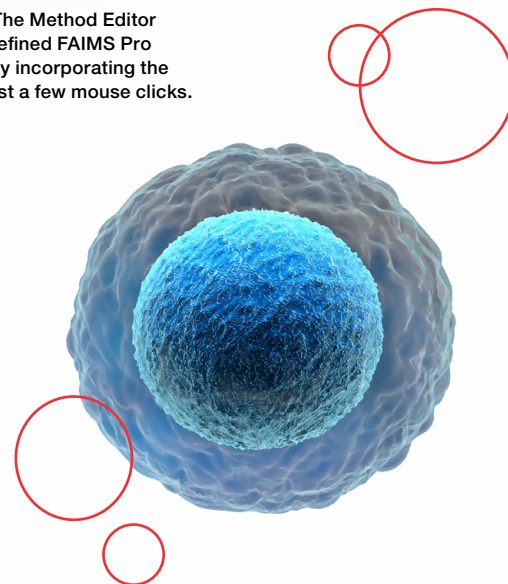
Xcalibur software data acquisition methods



Predefined instrument method templates provide comprehensive, high-confidence data acquisition. The Method Editor application within the Instrument Control software provides flexibility to incorporate a variety of user-defined FAIMS Pro interface CV settings. The instrument method templates simplify complex data acquisition schemes by incorporating the optimized parameters needed to acquire high-quality MS and MS/MS data into a process requiring just a few mouse clicks.

Automated data processing

Complex FAIMS Pro interface data are easily processed for high-level data interrogation. Thermo Scientific™ Proteome Discoverer™ software (version 2.3 or greater) manages data acquired at different CV settings and then merges the results into a concise, actionable report. In addition to detected and quantified proteins and peptides, processed results can also include biological functions, groupings, and pathway evaluations.

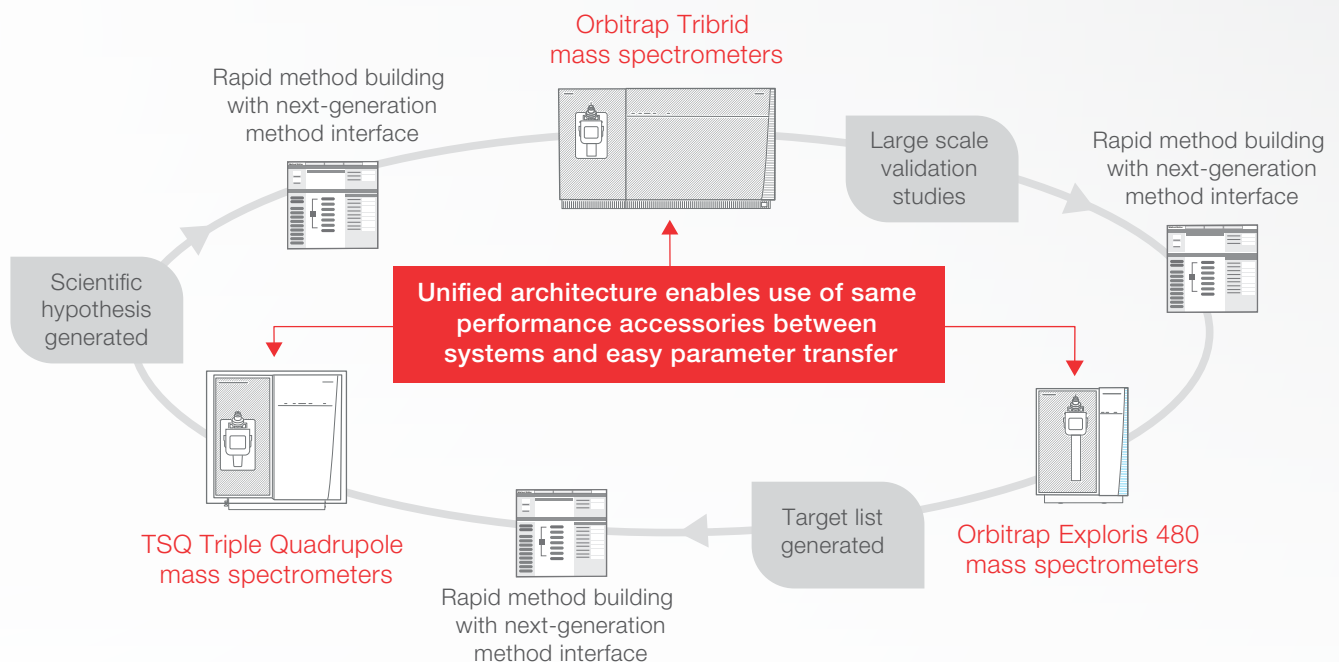


Integrated intelligence — a foundation for compatibility between systems

The FAIMS Pro interface is compatible with a range of next-generation Thermo Scientific mass spectrometers, all of which deliver ease without sacrificing high performance. Combining the FAIMS Pro interface with the intelligent acquisition strategies that are built into the instrument control software enables parameter portability across multiple platforms for a true sample to knowledge workflow solution. Common capabilities include One-Click method set-up, Application mode with best-practice default parameters, and a common instrument method interface on the Orbitrap Tribid, Orbitrap Exploris 480, and TSQ Triple Quadrupole mass spectrometers.

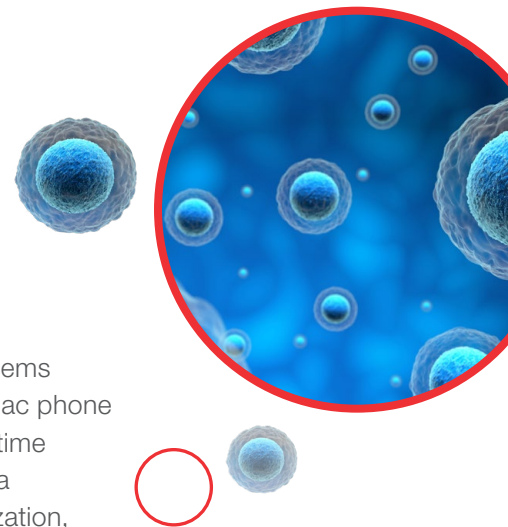
The FAIMS Pro interface is specifically compatible with:

- Thermo Scientific™ Orbitrap™ Exploris™ 480 mass spectrometer
- Thermo Scientific™ Orbitrap™ Eclipse™ Tribid™ mass spectrometer
- Thermo Scientific™ Orbitrap™ Fusion™ Lumos™ Tribid™ mass spectrometer
- Thermo Scientific™ Orbitrap™ Fusion™ Tribid™ mass spectrometer
- Thermo Scientific™ TSQ Altis™ mass spectrometer
- Thermo Scientific™ TSQ Quantis™ mass spectrometer
- Thermo Scientific™ EASY-Spray™ Source
- Thermo Scientific™ Nanospray Flex™ ion source
- Thermo Scientific™ VeriSpray™ PaperSpray ion source



“FAIMS provides up to 50% enhancement of identified peptides compared to LC-MS/MS analyses performed without FAIMS, and can extend the limit of detection by almost an order of magnitude. More importantly, the reduction in chimeric MS/MS spectra using FAIMS also improves the precision and the number of quantifiable peptides when using isobaric labeling of peptides.”

Pierre Thibault Principal Investigator, Proteomics and Bioanalytical Mass Spectrometry Research Unit, IRIC; Professor, Department of Chemistry, Faculty of Arts and Science, Université de Montréal. For more information about Thibault's work with FAIMS see Molecular & Cellular Proteomics S. Pfammatter, et al. 2018, 17(10), pp. 2015–2067.



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