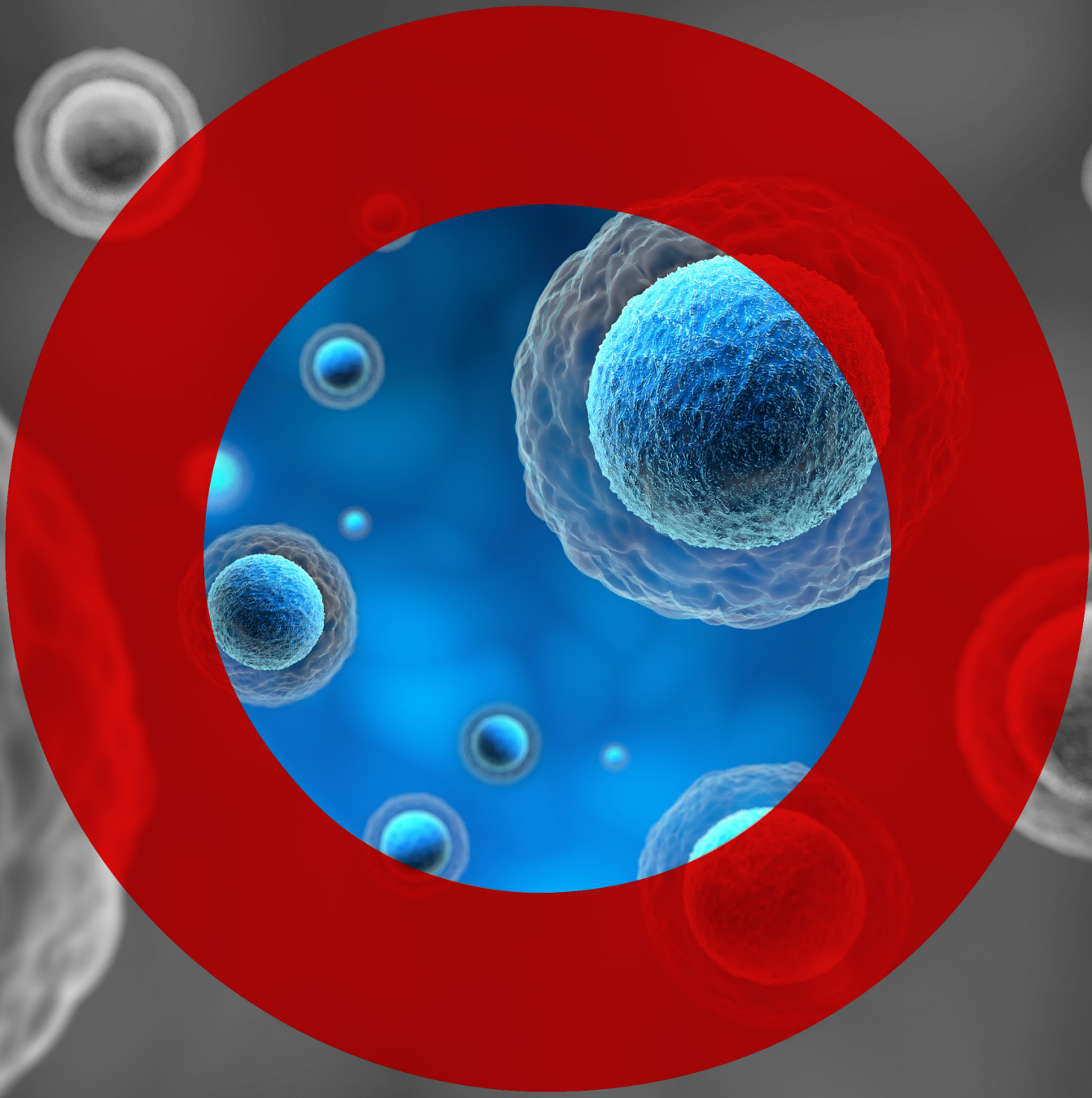
proteomics

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 both Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ and Orbitrap Fusion Tribrid 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 Fusion Lumos Tribrid 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

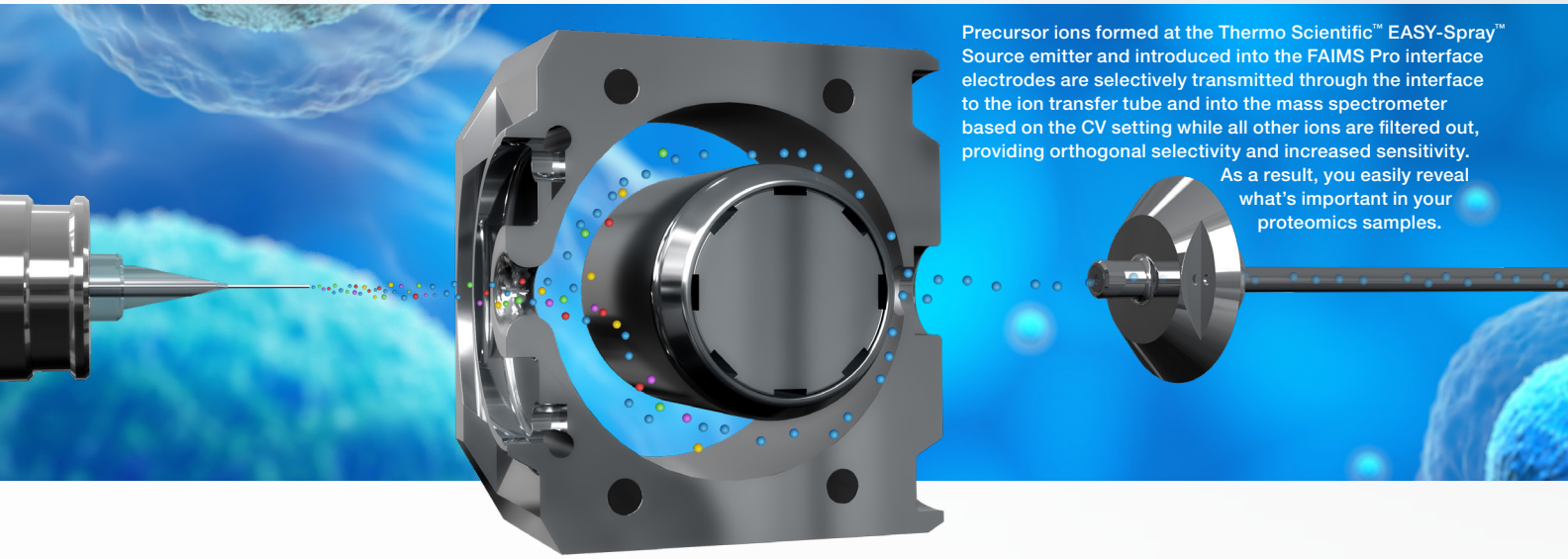
Tool-free, one-way assembly requiring no fine-tuning nor breaking of vacuum, and predefined workflows for data acquisition and processing, ensure ultimate usability and high data quality for all users.

Increases coverage without extra work

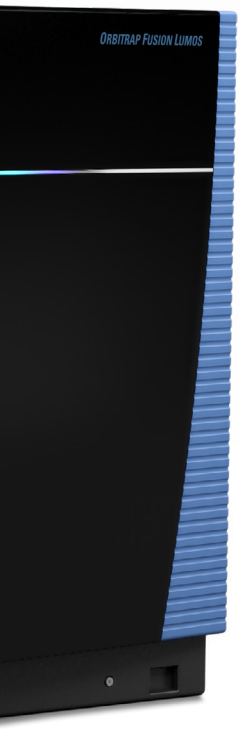
The FAIMS Pro interface minimizes the time, expense, and variability of offline LC fractionation by carrying out online gas phase fractionation prior to ion introduction into the mass spectrometer.

Conserves sample

Increased selectivity and sensitivity maximize proteome coverage while conserving sample.



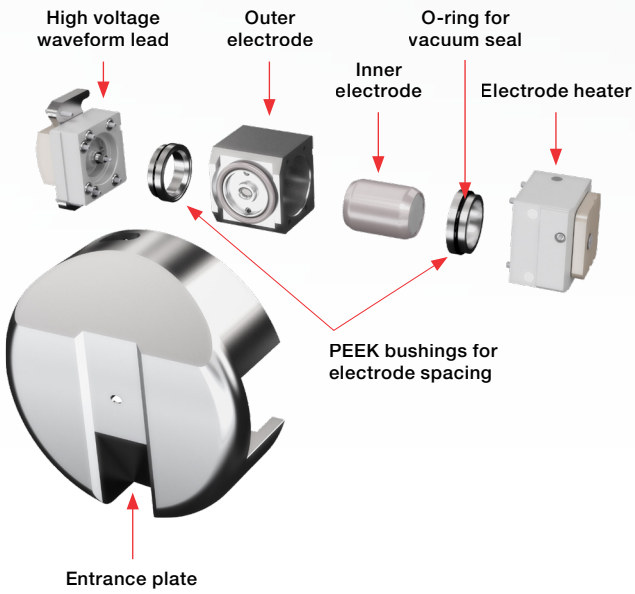
Precursor ions formed at the Thermo Scientific™ EASY-Spray™ Source emitter and introduced into the FAIMS Pro interface electrodes are selectively transmitted through the interface to the ion transfer tube and into the mass spectrometer based on the CV setting while all other ions are filtered out, providing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



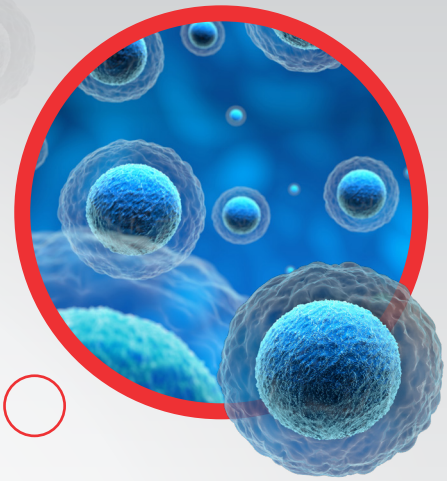
State-of-the-art interface enhances performance and usability

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 is one way with perfect alignment and no fine-tuning, and mounting to the instrument takes less than two minutes



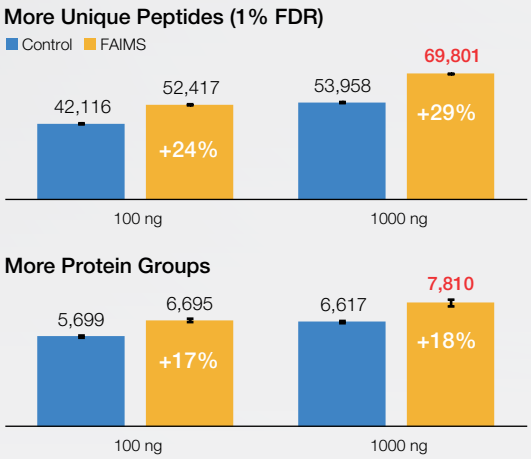
Orthogonal selectivity
adds efficiency to proteomics workflows



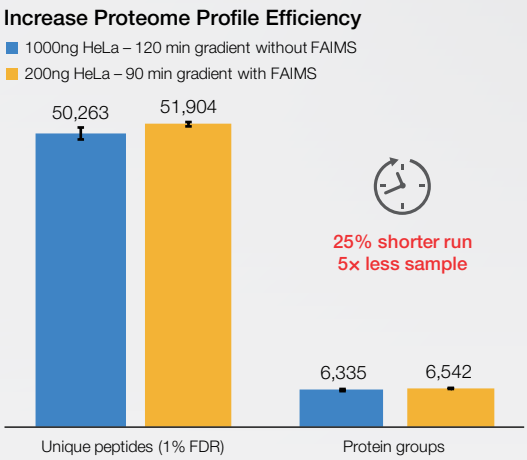
The FAIMS Pro interface integrates directly into existing workflows to enhance selectivity for proteome profiling, multiplexed protein quantitation, and top down protein characterization. Differential ion mobility reduces the complexity of precursor ions accumulated and analyzed per CV setting, increasing proteome coverage per experiment and 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 DDA method to acquire high-resolution accurate-mass (HRAM) MS and a series of HRAM MS/MS spectra, maximizing proteome coverage. The increased selectivity and sensitivity enables researchers to increase proteome coverage, or to maximize efficiency and conserve sample.



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."

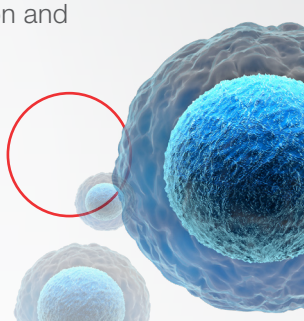
Joshua J. Coon Thomas and Margaret Pyle Chair at the Morgridge Institute for Research; Professor, Biomolecular Chemistry and Chemistry, University of Wisconsin; Director, NIH National Center for Quantitative Biology of Complex Systems.
For more information about Coon's work with FAIMS see, A. S. Hebert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

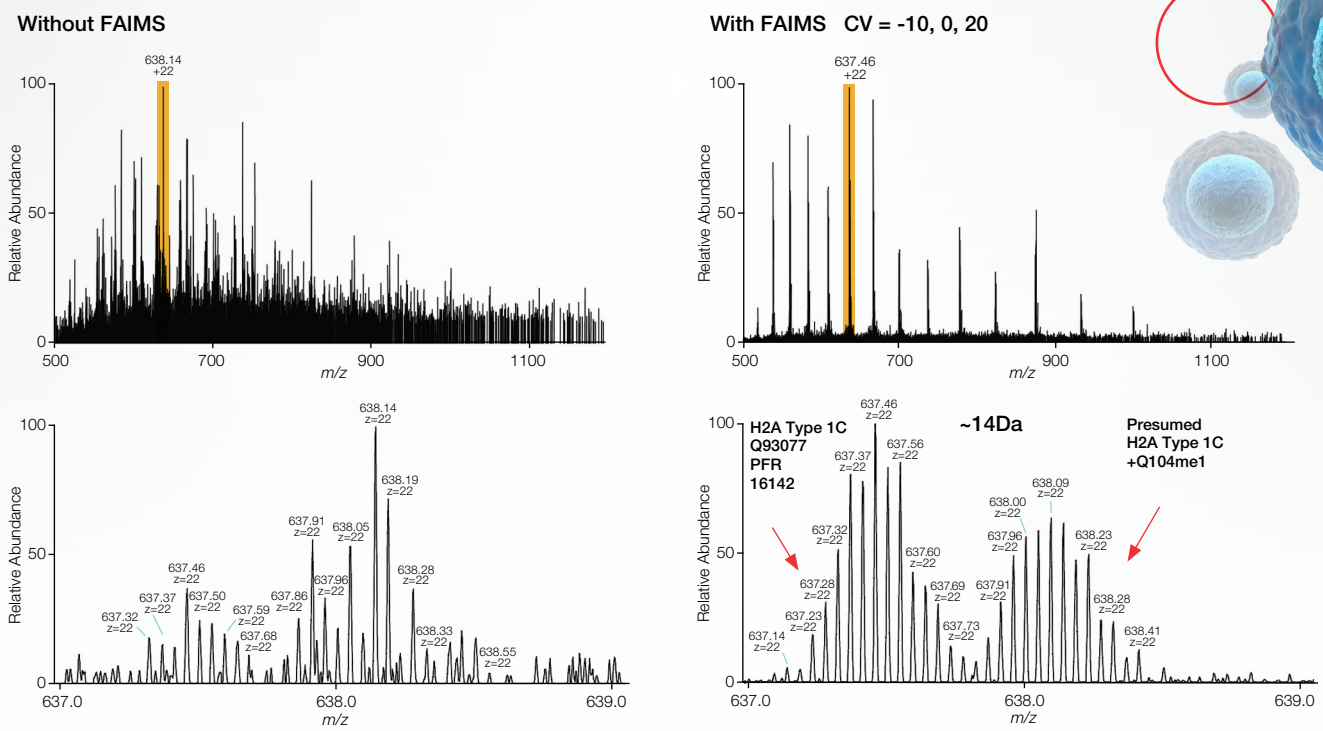
"Using the FAIMS Pro interface on the Orbitrap Fusion Lumos significantly enhances the detection and deep characterization of proteoforms in either the direct infusion or LC-MS/MS modes of operation. Our data has generated a lot of excitement about using FAIMS, in part due to robustness of the ion source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

Neil Kelleher Professor, Northwestern University

Selectivity expands confidence in intact proteoform analyses

Proteoform analysis focuses on detecting and characterizing cSNPs, mutations, and PTMs on a protein molecule. Samples such as cell lysates are complex mixtures with large numbers of proteins that are present at wide expressed copy numbers, making MS detection of individual proteoforms difficult without time-consuming sample preparation. Adding the FAIMS Pro interface to the Orbitrap Fusion Lumos Tribrid mass spectrometer enhances intact protein analysis by reducing interferences and enhancing sensitivity, enabling more accurate proteoform detection and characterization.





LC-MS analysis of H2A Type 1C in a human whole nuclear extract comparing a standard DDA method to a method that adds the FAIMS Pro interface. In this experiment, sample preparation steps were significantly reduced for the DDA analysis, resulting in greater chemical background. The FAIMS Pro interface effectively suppressed this background, providing easier detection of modified proteoforms. The highlighted region covering the 22+ charge state showed two baseline-resolved isotopic envelopes that were analyzed using the FAIMS Pro interface. The mass difference between the two isotopic envelopes of ca. 14 Da indicates methylation. Data courtesy of R. Gerbasi and N. Kelleher.

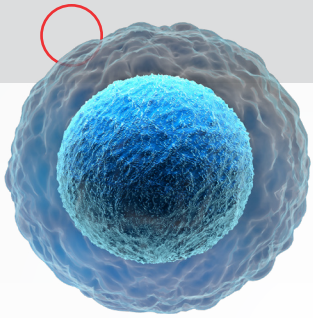
Top down sequencing analysis of the MS/MS data acquired during chromatographic separation identified Histone H2A Type 1C and its modified form. The sequence was determined by matching HRAM MS and MS/MS spectra against the human database in Thermo Scientific™ ProSightPC™ software. Sequence coverage maps show that the FAIMS Pro Interface method nearly doubled (37 versus 71) the number of fragments found, many of which covered the middle of the protein sequence, substantially increasing the certainty of the identification.

Increased confidence
in multiplexed protein quantification

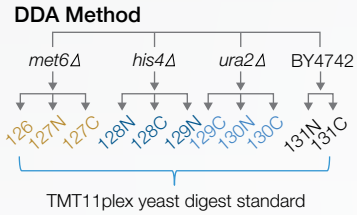
To address the large scope of translational proteomics experiments, multiplexed proteome analysis using TMT isobaric tags is used to simultaneously compare protein abundances across time, perturbations, tissues, etc. The reporter ions used for relative quantitation are generated in the MS/MS stage, but 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.

"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."

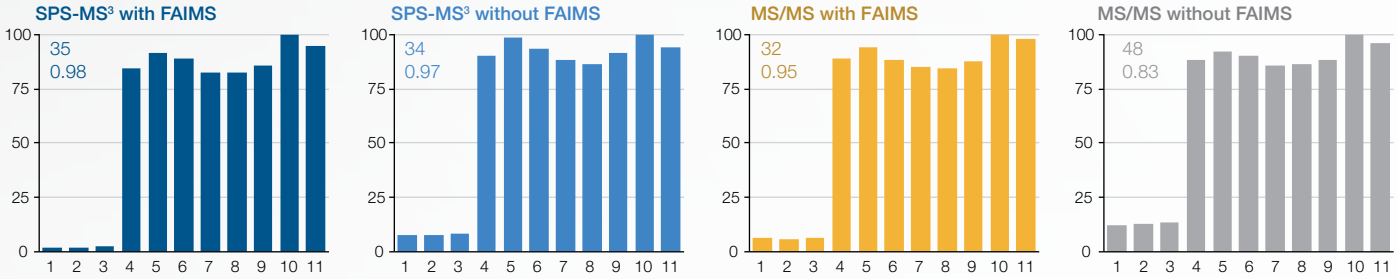
Steven Gygi Professor of Cell Biology, Harvard Medical School

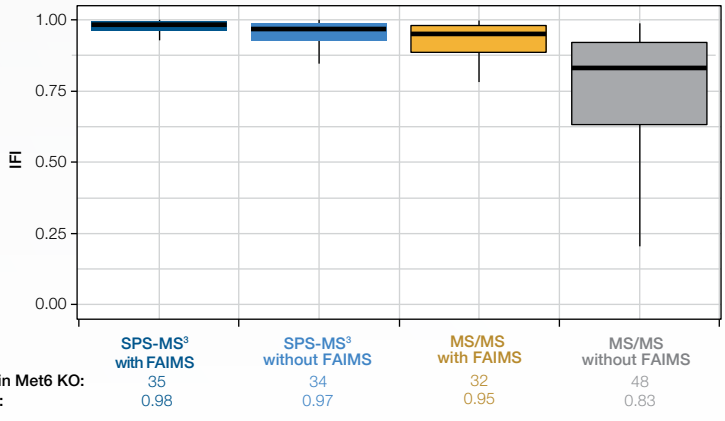


Higher-confidence quantification



Met6 Normalized ProteinSumSN





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.

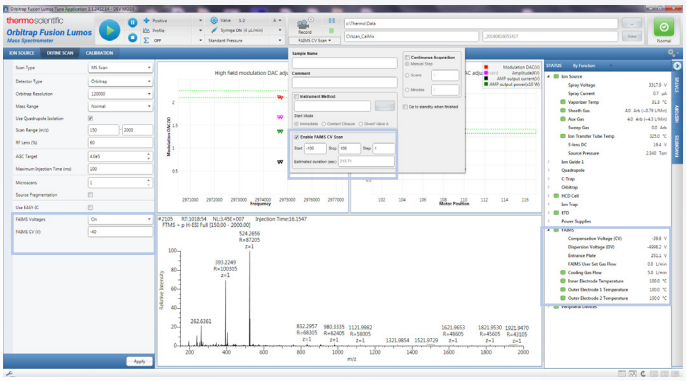
Relative quantification of TMT reporter ions of proteins regulated by the MET6 gene is for each acquisition method. Only the response for the MET6 knock out samples should have measured reporter ion ratios close to zero.

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.

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 the Orbitrap Fusion or Fusion Lumos Tribrid 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

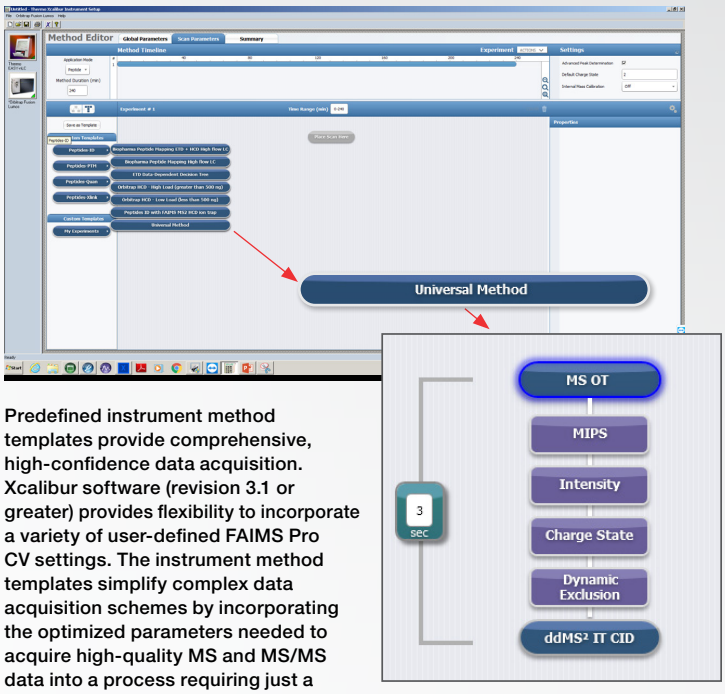


The instrument control software enables rapid CV evaluation and optimization. Thermo Scientific™ Xcalibur™ Tune (revision 4.2 or greater) automatically recognizes the FAIMS Pro interface assembly. In addition, the CV Scan Tool can be used for infusion-based CV optimization for either total ion current (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.

Automated data processing

Complex FAIMS Pro interface data are easily processed for high-level data interrogation. Thermo Scientific™ Proteome Discoverer™ software (revision 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.

Xcalibur software data acquisition methods



Predefined instrument method templates provide comprehensive, high-confidence data acquisition. Xcalibur software (revision 3.1 or greater) provides flexibility to incorporate a variety of user-defined FAIMS Pro 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.

Thermo Scientific™ UltiMate 3000™ HPLC system and the Orbitrap Fusion Lumos MS with the FAIMS Pro Interface.

"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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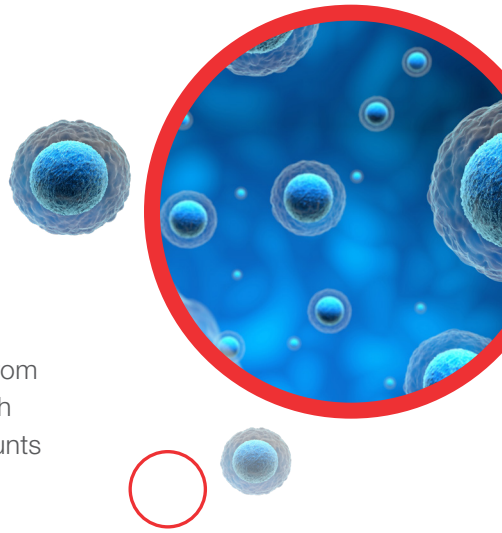

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