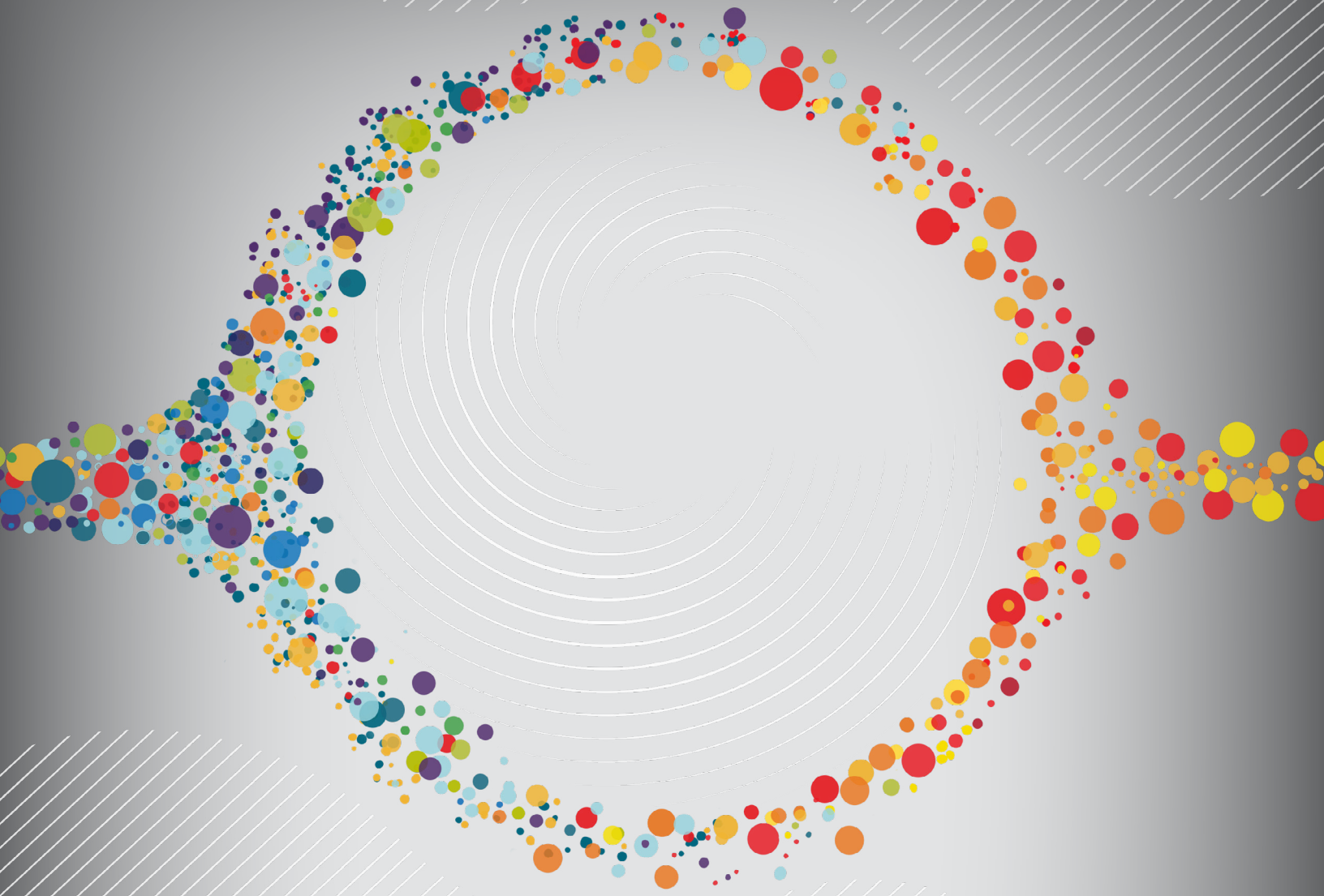
ThermoFisher SCIENTIFIC



Mass spectrometry

Enhanced selectivity, ultimate flexibility

FAIMS Pro Duo interface

thermo scientific

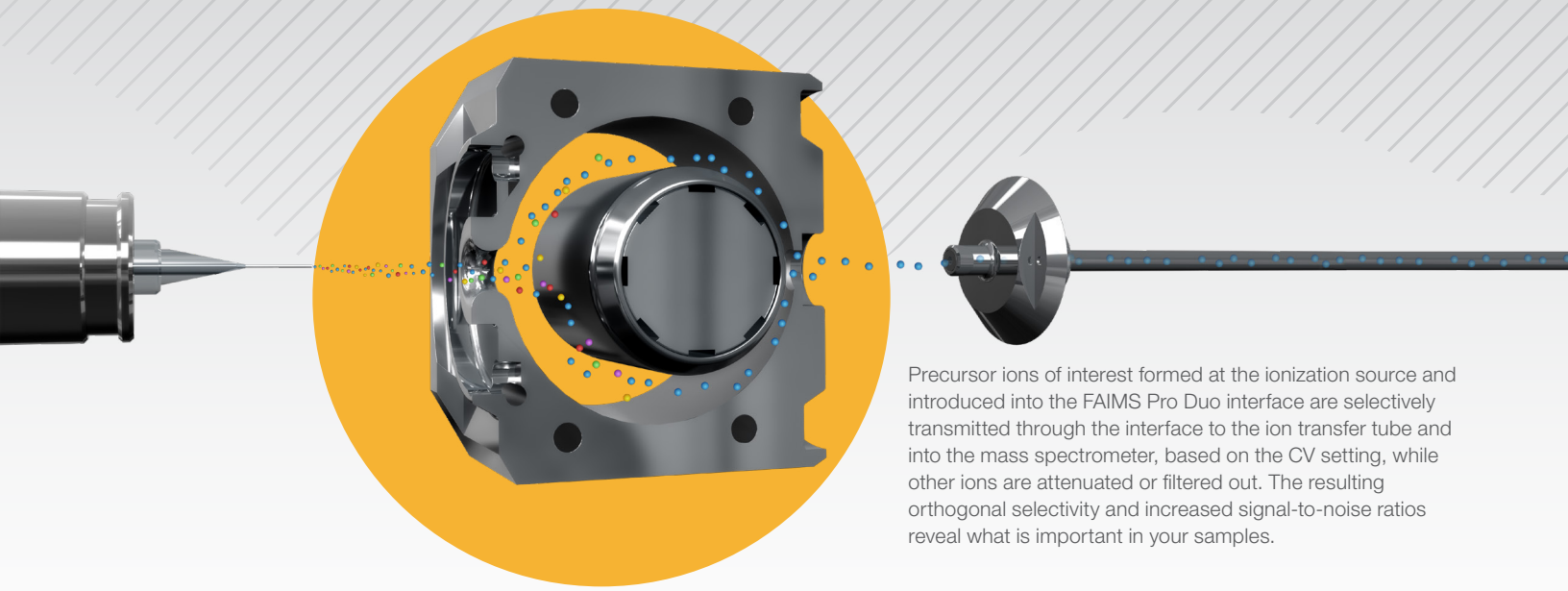
Enhanced selectivity

accelerates your analytical workflow

The growing complexities of qualitative and quantitative analyses in both research and routine applications demand more selective and sensitive analytical techniques, reduced sample preparation, and confident data processing for a wider range of compound types. With industry-leading technical depth in mass spectrometry innovation, Thermo Fisher Scientific enables broader and deeper qualitative and quantitative analyses for small- and large-molecule applications than ever before.

The Thermo Scientific™ FAIMS Pro Duo interface extends differential ion mobility capability to multiple Thermo Scientific™ next-generation mass spectrometers, overcoming sample complexity and matrix interferences regardless of chromatographic flow rate or sample loading amount. Whether it's the discovery of novel analytes and putative biomarker panels, or a need for enhanced quantitative performance, the orthogonal selectivity offered by the FAIMS Pro Duo interface delivers increased productivity and data quality for every user.

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



Precursor ions of interest formed at the ionization source and introduced into the FAIMS Pro Duo interface are selectively transmitted through the interface to the ion transfer tube and into the mass spectrometer, based on the CV setting, while other ions are attenuated or filtered out. The resulting orthogonal selectivity and increased signal-to-noise ratios reveal what is important in your samples.

Flexible to fit your work

Easily incorporated into existing targeted and untargeted workflows, maximizes sample profiling across wide dynamic loading amounts and chromatographic flow rates to increase experimental productivity.

Easy to install, use, and maintain

One-way assembly and installation without breaking instrument vacuum, simple calibration, and online Compensation Voltage (CV) optimization routines ensure ultimate usability and high-quality data for users of all skill levels.

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.

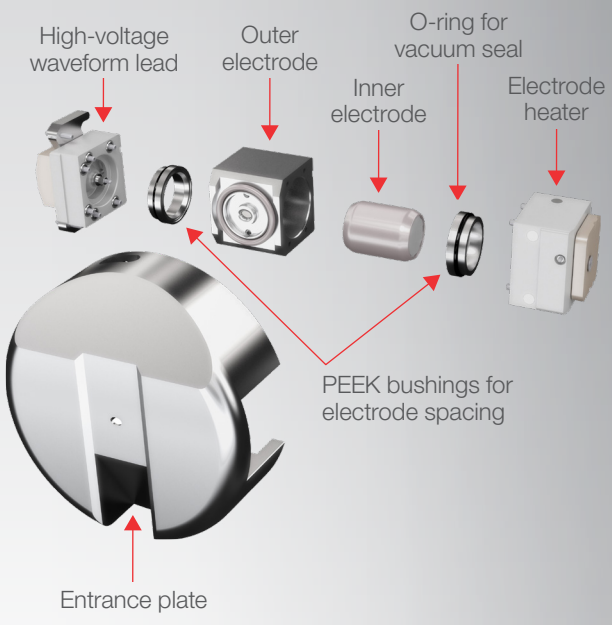
Conserves sample

Increases selectivity and sensitivity to maximize signal-to-noise ratios and sample coverage while conserving sample.

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

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

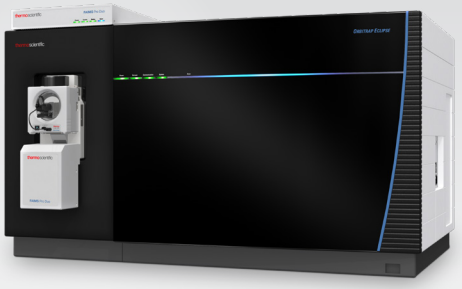
- Optimized cylindrical geometry substantially increases ion transmission to the mass spectrometer with short residence times, allowing multiple CV settings per data acquisition method
- Operates ideally across a wide range of chromatographic flow rates (100 nL to 1 mL/min) or direct infusion
- Assembly and disassembly are fast 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
- Does not require standards for calibration



Orthogonal selectivity

adds efficiency to proteomics workflows

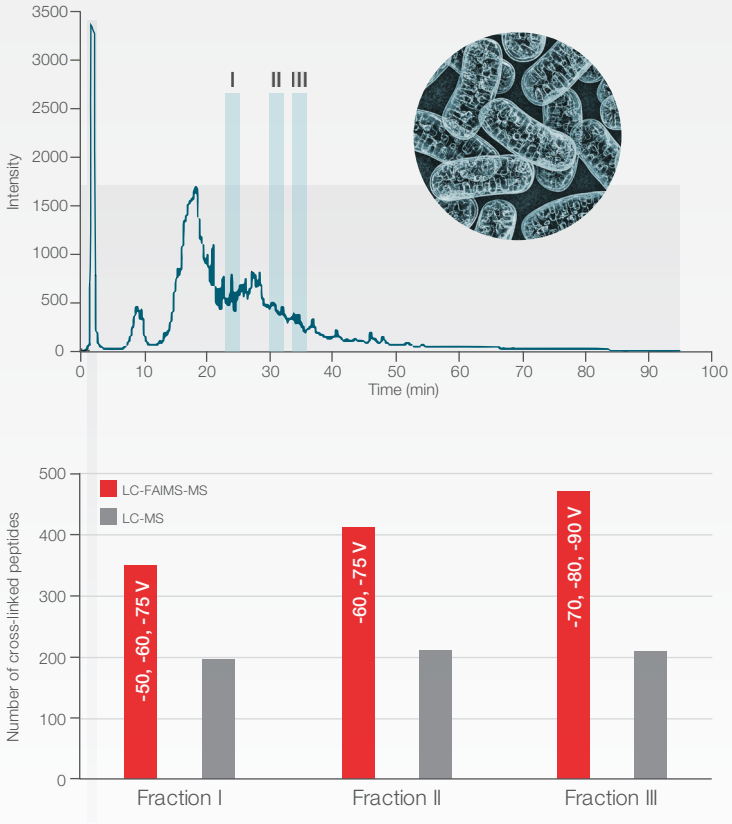
The FAIMS Pro Duo interface integrates directly into existing workflows to enhance selectivity for a wide range of nanoflow proteomics applications, including post-translational modification (PTM) characterization, single-cell proteomics, structural biology, and targeted quantification. Gas-phase fractionation through differential ion mobility can increase proteome coverage and improve qualitative and quantitative data confidence. In many cases, the FAIMS Pro Duo interface provides the same coverage as time-consuming offline preparation.



The FAIMS Pro Duo interface integrated with the Thermo Scientific™ Orbitrap Eclipse™ Tribrid™ mass spectrometer.

Enhanced selectivity, expanded sample coverage

The FAIMS Pro Duo interface provides orthogonal precursor ion selectivity based on differential gas-phase mobility. The CV setting determines which group of ions are transmitted to the mass spectrometer for detection. To expand sample coverage in one experiment, multiple CV settings can be used and repetitively sampled to increase the number and type of precursors detected and sequenced. The CV settings can be optimized to enhance transmission for subclasses of peptides and proteins, such as cross-linked peptides which generally have higher precursor charge states and molecular weights than co-eluting unmodified tryptic peptides. Compared to experiments performed without FAIMS separation, stepping CV values results in gas-phase fractionation that increases detection of desired peptide types. By simply changing CV values, FAIMS selectivity can provide results that are similar to offline physical methods such as strong cation exchange (SCX) or size-exclusion chromatography (SEC).



Mouse-heart mitochondrial interactome fractions were evaluated for cross-linked peptide identification to demonstrate the benefit of the FAIMS Pro Duo interface. The three SCX fractions were collected at the shaded retention times and analyzed using LC-XL-MS (standard method) and LC-FAIMS-XL-MS. Additionally, three replicate injections were performed using different combinations of CV values. The bar graph shows an approximately twofold increase in the number of cross-linked peptides identified when FAIMS selectivity was applied. Interestingly, later SCX fractions required higher CVs as the charge-state distribution increased.*

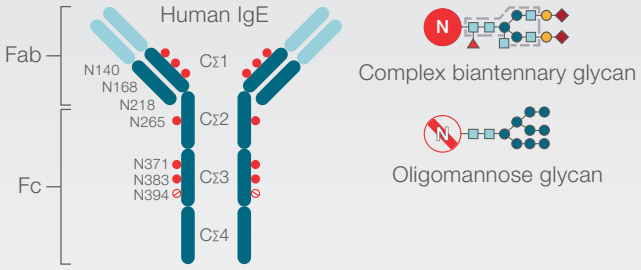
* Schnirch, L et al. *Anal. Chem.* 2020, 92(15), 10495.

Selectivity expands confidence

for characterization of glycoproteins

Glycosylation is an abundant protein PTM that plays fundamental roles in various biological processes. A specific challenge in profiling protein glycosylation using LC-MS^n is identifying all glycosylation sites and determining the microheterogeneity at each site. The resulting digested sample can be extremely complex, containing modified and unmodified peptides. Additionally, the same peptide can be modified with different glycans that have minimal chromatographic separation and are present over wide dynamic ranges. Acquiring informative product ion spectra for lower abundance glycopeptides can also be difficult. The FAIMS Pro Duo interface enhances N- and O-linked glycoprofiling by modifying gas-phase fractionation using different CV settings to improve signal-to-noise ratios for glycopeptides, resulting in greater coverage and confidence.**

Increased glycoprotein characterization, simplified



Representation of Human immunogobulin epsilon heavy chain (IgE P01854) with predicted N-glycosylation sites in each constant domain. Identifying each glycosylation site and profiling peptide glycoforms is challenging. The predominant glycoforms for six N-glycosylation sites are complex biantennary, whereas the N394 site is expected to be comprised predominantly of oligomannose glycans.

Glycans per instrument condition

Comprehensive N-linked glycosylation profiling of the IgE N394 site was evaluated using LC-MS and LC-FAIMS-MS. External CV stepping (one CV setting per analysis) and internal CV stepping (two CV settings per analysis) experiments were performed to determine optimal parameters to improve signal-to-noise ratios for enhanced detection of glycoforms and quality of resulting HCD and EThcD product ion spectra. The bar graph shows that changing the CV settings provided greater glycoform coverage and relative abundance compared to experiments performed without FAIMS selectivity.†

	Without FAIMS	With FAIMS				
		-30 CV	-40 CV	-50 CV	-60 CV	-45/-60 CV
N140	A2S1G1F	Nonglycos	A2S2F	A2S2FB	A2G0F	A2G0F
N168	A2S1G1B	Nonglycos	A2S2B	A2S1G1	A2G1	A2G1
N218	A2S2F	Nonglycos	A2S2F	A2S2F	A2S1G1F	A2S2F
N265	A2G2F	Nonglycos	A2S2	A2S2	A2S1G1FB	A2S2
N371	A2S1G1FB	A2S1G1	A2S2FB	A2S1G1FB	A2S1G0FB	A2S1G1FB
N383	A2G2F	A2G2F	A2S2FB	A2G2F	A2G2F	A2G2F
N394	M5	M5	GnF	M7	M5	M5

This table lists the most abundant IgE glycoform identified per site without and with the FAIMS Pro Duo interface operated at different CV values. A CV setting of -30 V enabled detection and characterization of the unmodified peptide, while the more negative CV settings enhanced glycosylated peptide abundance. This study demonstrated that FAIMS selectivity provided detection and characterization of all seven IgE N-linked glycosites in both non-glycosylated and glycosylated states.

** Izaham, ARA et al. *J. Proteome*. Res. 2021, 20(1), 599.

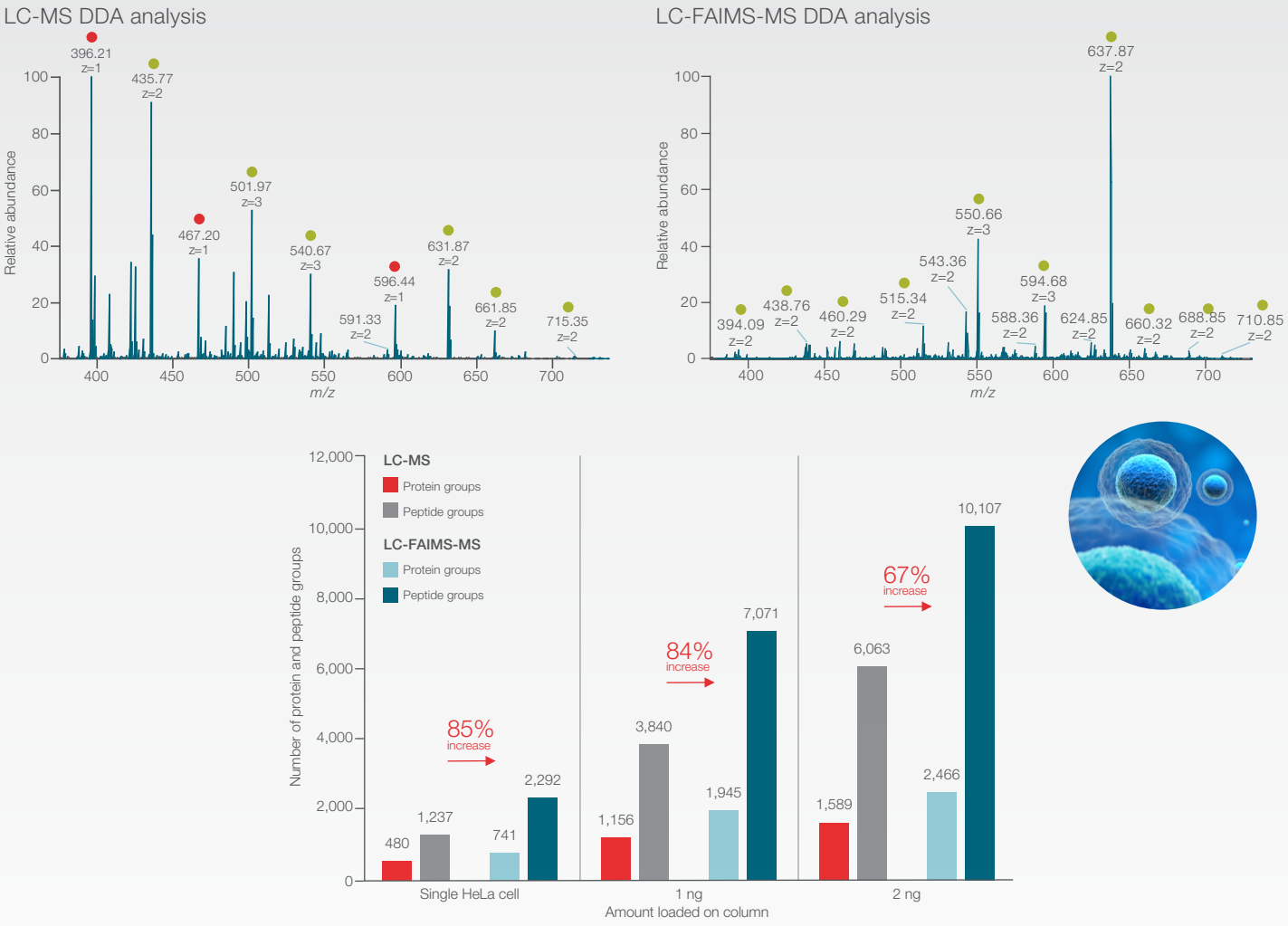
† Makaju, A et al. ASMS 2020 and Thermo Fisher Scientific poster PO65801EN-0422S.

Increased confidence

for low-level protein coverage

Recent research has focused on developing LC-MS methods capable of performing routine proteome profiling of very low protein loading amounts, including single-cell lysates. While extraction, sample preparation, and chromatography have been optimized to introduce peptides into the mass spectrometer, orthogonal selectivity is needed to enhance accumulation of multiply charged ions relative to the more abundant singly charged background solvent and matrix ions. The FAIMS Pro Duo interface performs gas-phase fractionation, enabling preferred accumulation of multiply charged ions to maximize the efficiency of data-dependent acquisition (DDA) routines and increase proteome coverage. Short-ion residence time in the FAIMS Pro Duo interface electrode assembly enables use of multiple CV settings in a single run to increase proteome coverage. The improved signal-to-noise ratios provide higher match scores that reduce false discovery rates (FDR) and that enable researchers to maintain high proteome coverage when using shorter chromatographic gradients and improve quantitative accuracy when using Thermo Scientific™ Tandem Mass Tag (TMT™) multiplexing.

Preferentially transmits peptides, not solvent ions, for improved sample coverage



Comparative performance for proteome coverage at low loading amounts of digested HeLa cell lysate analyzed with an Orbitrap Exploris 480 mass spectrometer. The full-scan spectra acquired using LC-MS and LC-FAIMS-MS demonstrate the benefits of orthogonal selectivity in attenuating singly charged ion transmission through the FAIMS Pro Duo interface, resulting in increased accumulation of multiply charged precursors that are attributed to peptides. The bar chart shows the increased proteome coverage obtained from differential ion mobility. The percent increase in protein and peptide groups identified is even greater at lower loading amounts.

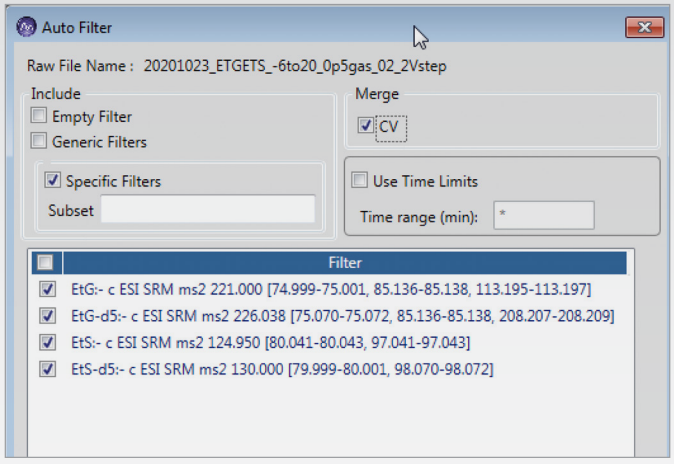
Everyday usability

ensures performance

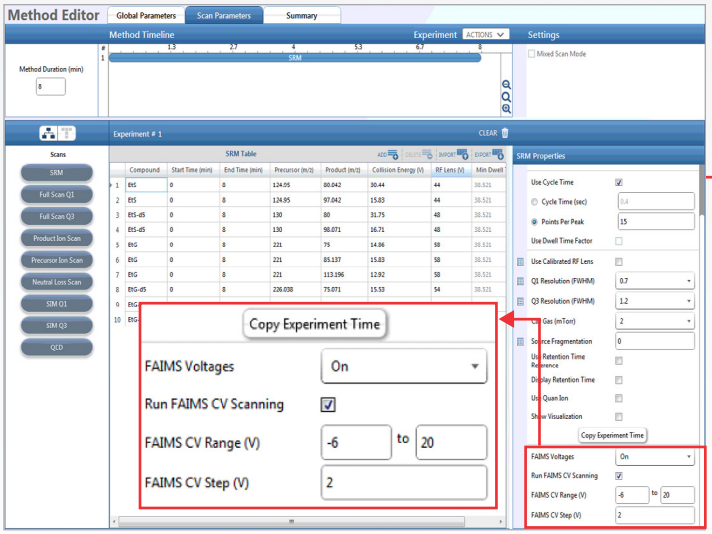
Intuitive tuning and CV optimization for one compound or many using direct infusion or online chromatographic separation simplify setup and use of the FAIMS Pro Duo interface with Thermo Scientific next-generation mass spectrometers. Simplified optimization routines enhance experimental performance regardless of user experience or availability of purified standards. Seamless integration is designed to support maximum productivity for your most demanding science.

CV optimization routines for Orbitrap and triple quadrupole mass spectrometers

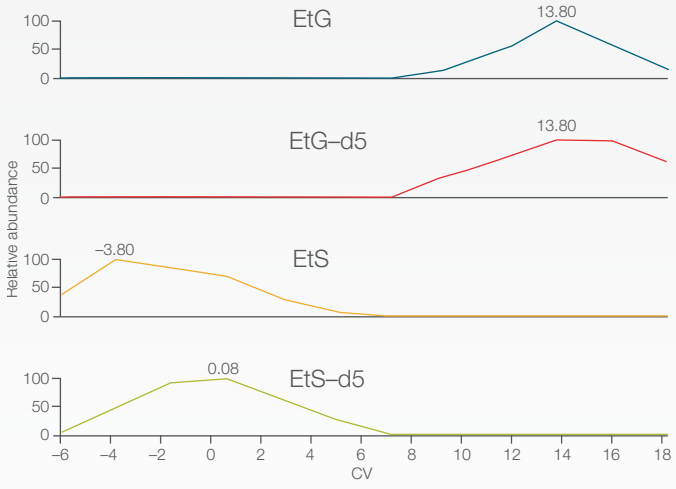
Screen capture displaying the data acquisition method used to perform online CV optimization for Thermo Scientific™ Orbitrap™-based mass spectrometers. CV optimization can be performed for a wide range of sample components. The number of CV steps repeatedly cycled through depends on the chromatographic peak width up to 20 CV settings.



Thermo Scientific™ FreeStyle™ software is used to process the CV optimization data from both instrument types. For each compound, the RAW data contains measured intensities at each CV setting. In FreeStyle software, checked options automate data extraction based on scan filters used to acquire the data. This is an example of SRM optimization on a TSQ Altis mass spectrometer.



CV optimization of selected reaction monitoring (SRM) methods for Thermo Scientific™ TSQ Altis™ and Quantis™ mass spectrometers. The inset shows the parameter settings that define the range of CV voltages and the step size throughout the direct infusion or LC gradient.



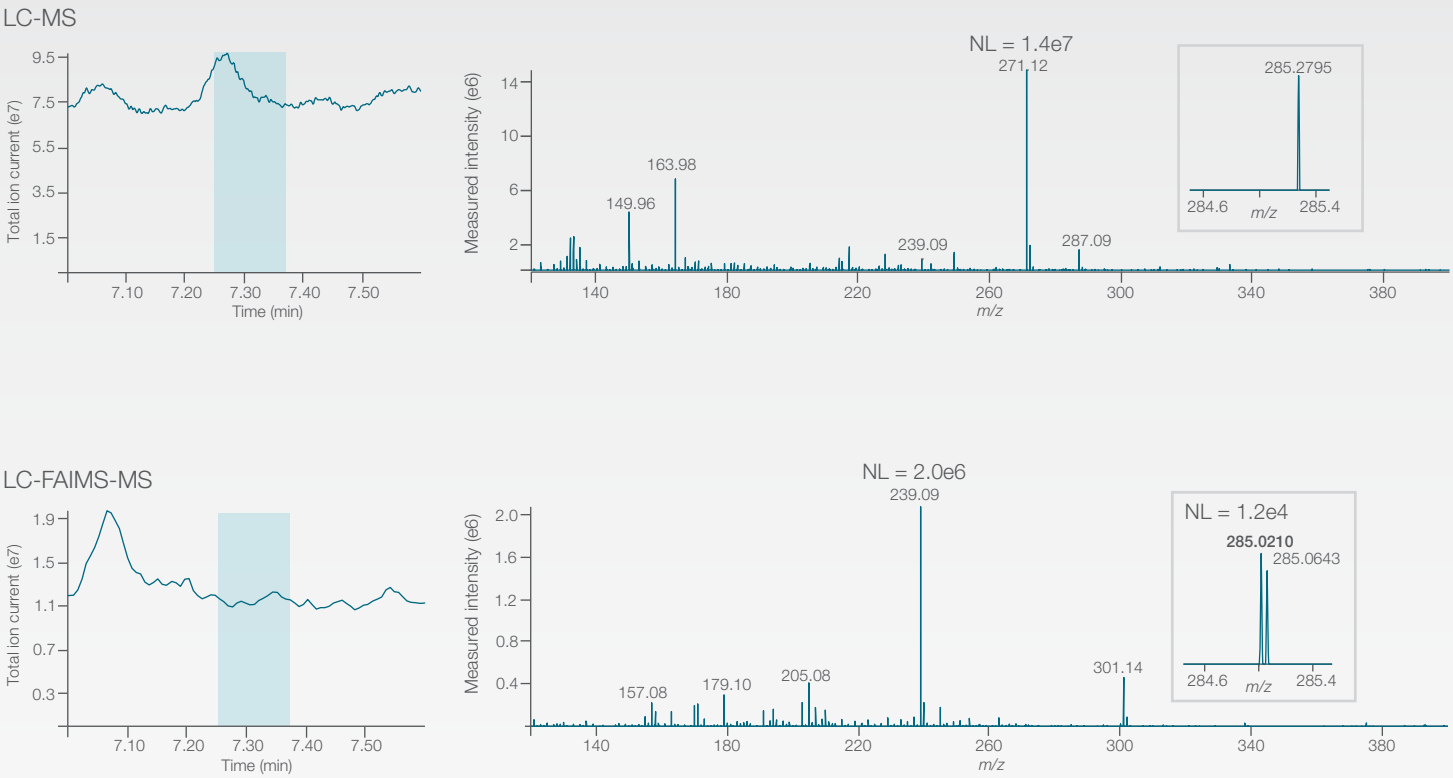
Automating separation enables straightforward determination of optimal CV settings per compound or groups of compounds.

Improved targeted quantification

using full-scan HRAM MS methods

Confident targeted quantification across a wide dynamic range must be robust, accurate, and precise while maintaining throughput. Full-scan high-resolution, accurate-mass (HRAM) MS detection can simplify method development for small-molecule screening and quantification by using a single instrument setting to simultaneously acquire data on all precursor ions with *m/z* values within the user-defined range. While the resolution and mass accuracy achieved using Thermo Scientific™ Orbitrap™ mass analyzers is sufficient to separate ions associated with compounds of interest from co-eluting background ions, intense background ions transmitted through the quadrupole mass filter can limit the intra-scan dynamic range for very low-level targets. Incorporating the FAIMS Pro Duo interface into existing full-scan HRAM MS methods introduces orthogonal selectivity that can extend the lower limit of detection (LOD) and quantification (LOQ) by eliminating unwanted matrix ions before they enter the mass spectrometer. Multiple CV settings can be scheduled to enhance screening and quantification on multiple compounds.

Orthogonal selectivity lowers limits of detection



Sulfachlorpyridazine spiked into an acetonitrile extract of meat-muscle matrix at 0.5 ppb, vortexed, and analyzed with and without the FAIMS Pro Duo interface using optimized CV values for enhanced performance. The total ion current (TIC) plots at left show the benefits of FAIMS selectivity in suppressing transmission of unwanted background ions through the interface by almost sevenfold during elution (marked in grey) compared to the standard method. The comparative full-scan mass spectra shows that the highly abundant background ions are greatly attenuated by FAIMS selectivity and that the *m/z* 239 ion, which was one of the lower abundance ions measured without FAIMS selectivity, becomes the most abundant ion measured. The inset shows the narrow mass range for the monoisotopic ion of sulfachlorpyridazine (*m/z* 285.0210) that was only detected with LC-FAIMS-MS. The selectivity of the Orbitrap mass analyzer measurement was sufficient to perform post-acquisition data extraction with mass measurement accuracy below 1 ppm.

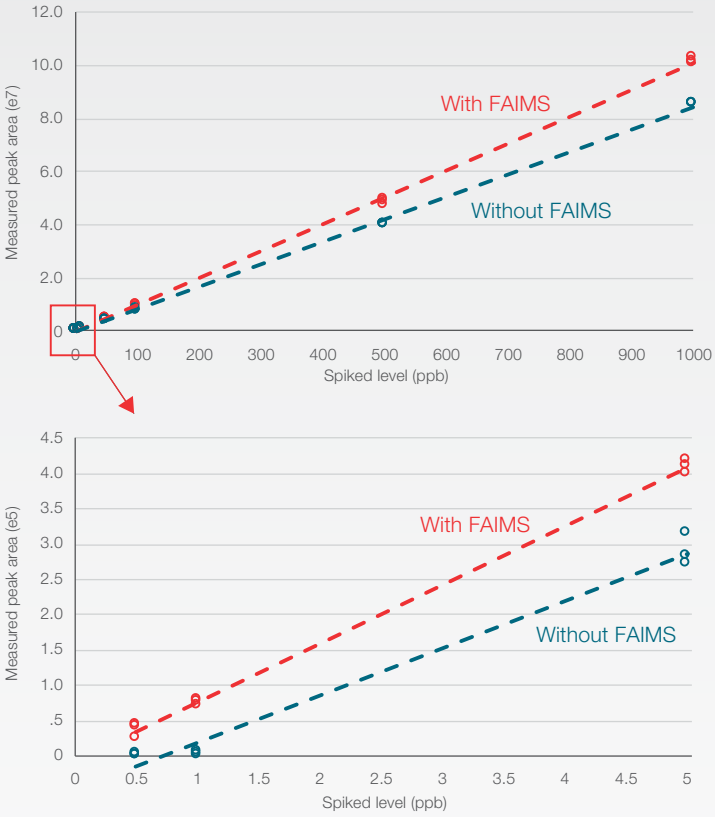
The FAIMS Pro Duo interface integrated with the Thermo Scientific™ Vanquish™ Core HPLC system with the Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer.

Automated data processing

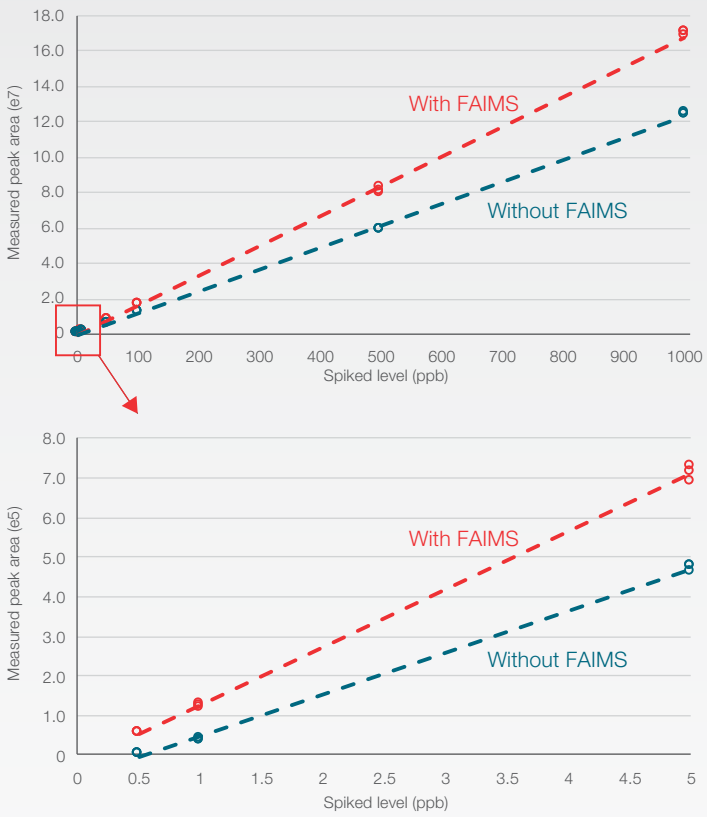
Complex, targeted FAIMS Pro Duo interface LC-MS data are easily processed for high-level data interrogation. Thermo Scientific™ Tracefinder™ software manages data acquired at different CV settings and then merges the results into a concise, actionable report. In addition to detected and quantified compounds, processed results can also include dose-response, linearity, and LOD/LOQ.

Extends orthogonal selectivity over wide dynamic ranges

Sulfachlorpyridazine



Sulfamerazine



Veterinary drugs were spiked into an acetonitrile extract of meat-muscle matrix across a wide dynamic range (0.1 to 1000 ppb) to compare detection and quantification performance for a standard method versus a method with the FAIMS Pro Duo interface. Introduction of FAIMS selectivity demonstrated linear performance for both sulfachlorpyridazine and sulfamerazine, with a linear regression ≥0.995 without internal standards. All levels met the 20% reproducibility requirement. Performance was similar to the standard method, except the FAIMS method lowered the LOQ to 0.5 ppb (bottom graphs covering the lowest three spiking levels).

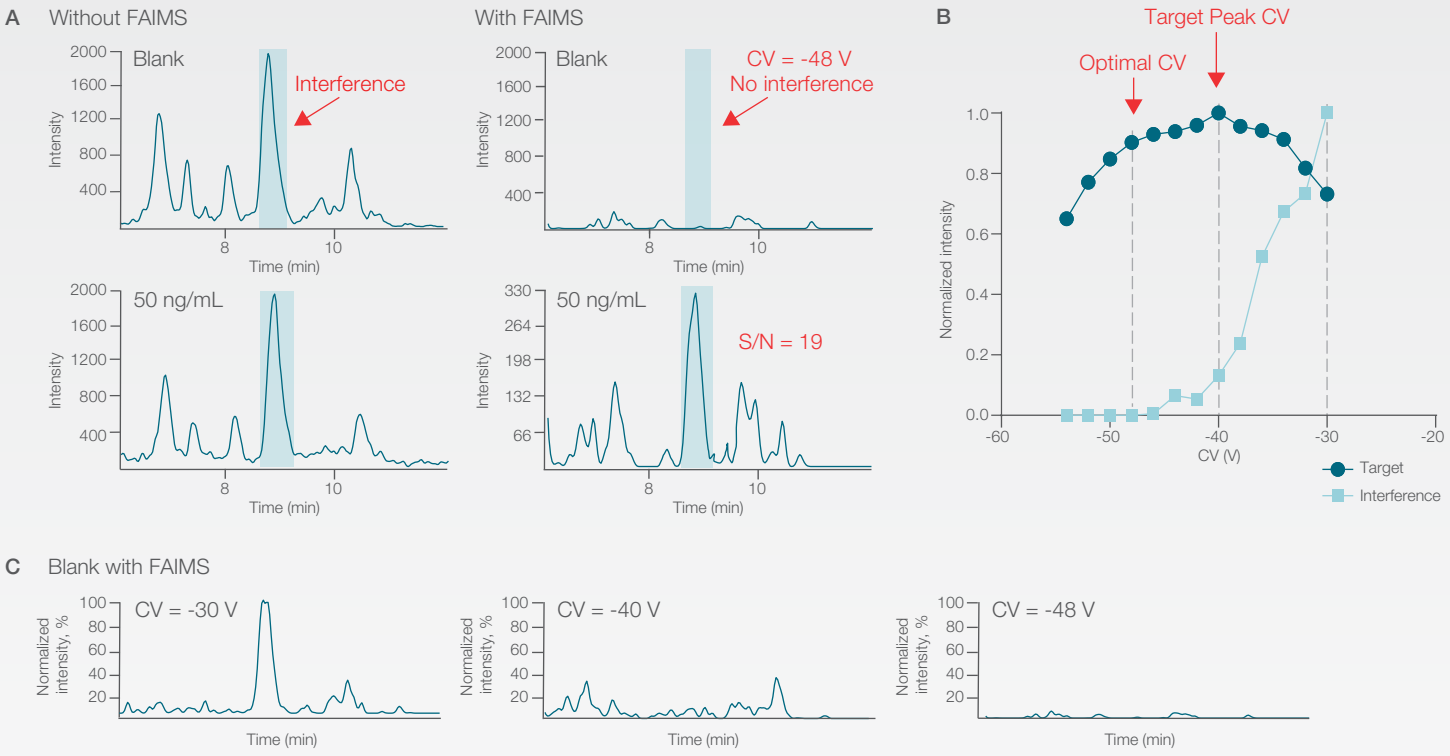
Suppress chemical noise

for improved SRM quantification

Targeted biotherapeutic quantification in biological matrices relies on enzymatic digestion to improve detection. Quantifying surrogate peptides covering unique protein sequence regions can improve the detection performance to enable reliable Pharmacokinetics (PK) studies. Protein digestion, however, increases complexity by converting every protein into tens to hundreds of peptides, resulting in an increased probability of isobaric interference with SRM transitions, failure to detect a true blank, and lower LOQ. Researchers often develop antibody-based extraction to improve selectivity, which is costly and time-consuming. The FAIMS Pro Duo interface can be used to suppress chemical noise for one or more peptides, independently, improving LOQ using the optimal CV value to improve workflow efficiency.

> "The primary factor limiting the sensitivity of targeted protein quantification is the high interference from numerous peptides in highly complicated digested samples, rather than MS or SRM signal intensity. The FAIMS Pro Duo interface provides orthogonal selectivity that effectively separates the target from chemical noise with high reproducibility and robustness and can be integrated into an existing workflow to dramatically improve quantitative sensitivity without antibody enrichment."
>
> **Jun Qu**, Professor, SUNY Buffalo

Selectively removes chemical noise to improve LOQ



(A) High-throughput targeted biotherapeutic quantitative analysis in mouse plasma using a trapping µLC-FAIMS-MS/MS workflow. The targeted protein analysis was based on surrogate peptide measurements that used a high-flow sample loading step with a 25 µL/min chromatographic flow rate to balance loading capacity, sample throughput, and sensitivity. FAIMS selectivity removed chemical noise, enabling determination of a true blank and a 50 ng/mL LOQ. Without orthogonal selectivity, a loading amount of 1600 ng/mL was required to overcome the chemical noise for the targeted peptide. (B) Evaluation of the optimal CV setting to best attenuate chemical noise while preferentially transmitting the targeted peptide through the FAIMS Pro Duo interface. (C) Evaluation of the chemical noise suppression at the three marked CV settings using the FAIMS Pro Duo interface.‡

‡ Data provided courtesy of Prof. Jun Qu, The Dept. of Pharmaceutical Sciences, Uni. Of Buffalo, State Uni. Of New York.
Background information on µTrapping LC-MS/MS methods can be found in *Anal. Chem. 2020*, 92, 15152-15161.

Integrated intelligence

a foundation for compatibility between systems

The FAIMS Pro Duo interface is compatible with a range of Thermo Scientific next-generation mass spectrometers, all of which deliver ease of use without sacrificing high performance. Combining the FAIMS Pro Duo interface with the intelligent acquisition strategies that are built into the instrument control software offers parameter portability across multiple platforms for a true sample-to-knowledge workflow solution. Common capabilities include One-Click method setup, Application mode with best-practice default parameters, and a common instrument method interface on the Thermo Scientific™ Orbitrap™ Tribrid™ mass spectrometer and Thermo Scientific™ Orbitrap Exploris™ mass spectrometer platforms, as well as the TSQ Altis and TSQ Quantis triple quadrupole mass spectrometers.

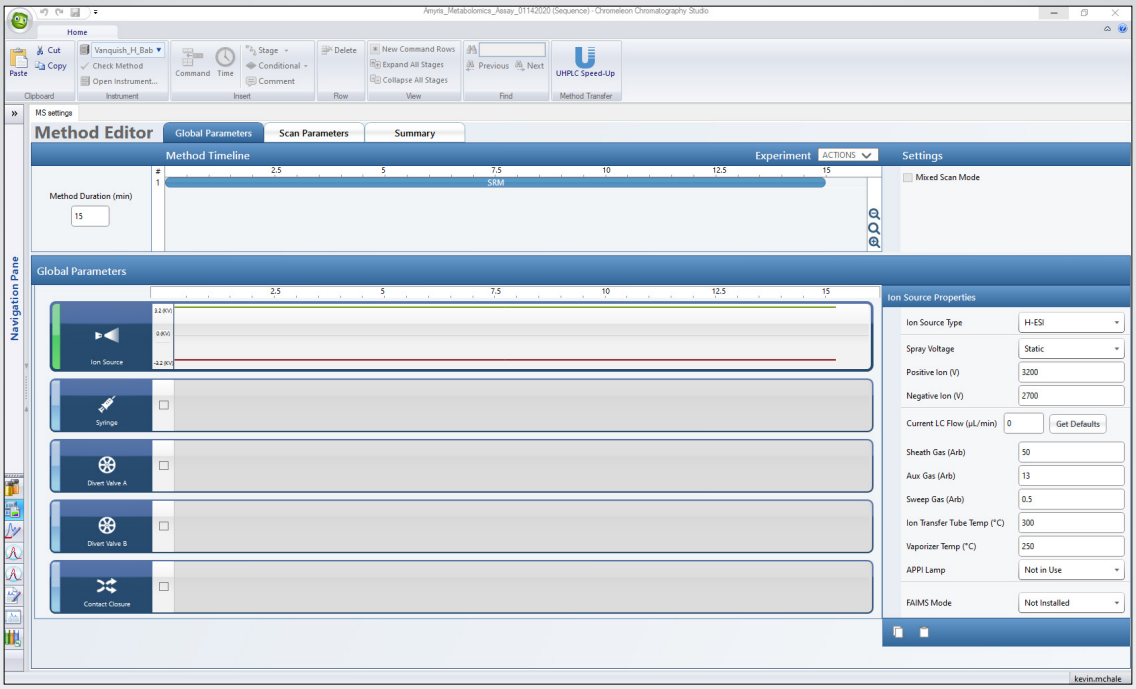
The FAIMS Pro Duo interface is compatible with:

- Thermo Scientific™ Orbitrap Exploris™ mass spectrometers
- Thermo Scientific™ Orbitrap™ Tribrid™ mass spectrometers
- 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
- Thermo Scientific™ OptaMax™ NG ion source

The Thermo Scientific Vanquish Core HPLC system with the Orbitrap Exploris 240 mass spectrometer.

The Thermo Scientific™ Vanquish™ Horizon UHPLC system with the TSQ Altis mass spectrometer.

The Thermo Scientific EASY-nLC 1200 with the Orbitrap Eclipse Tribrid mass spectrometer.



The Method Editor that is consistent across the Thermo Scientific next-generation mass spectrometers.

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