# Smart Note

## Accelerate your translational workflows with effortless selectivity enhancements

**I need a seamless experimental workflow that overcomes sample complexity to maximize detection, characterization, and quantitation of biological compounds for putative biomarker determination. What do you recommend?**

Biological mass spectrometry is the preferred experimental method to analyze complex samples to determine their individual compositions and to measure the differential expression of molecules between samples collected from different states and/or conditions. Combining liquid chromatography with state-of-the-art mass spectrometers (LC-MS) has expanded the breadth and depth of sample coverage, increased confidence in characterization, and improved global quantitation. However, current research demands even greater profiling capabilities to allow expanded performance with fewer sample preparation steps and higher throughput, in the face of ever-increasing sample complexity. To maximize productivity, any workflow change should complement, rather than disrupt, existing experimental workflows.


ThermoFisher SCIENTIFIC

The Thermo Scientific™ FAIMS Pro™ interface enhances the analytical capabilities of existing LC-MS methods with increased sample coverage, confidence in characterization, and quantitative performance for complex biological samples. LC-FAIMS-$MS^n$ analyses offer the combined separating power and selectivity of LC, differential (or non-linear) ion mobility, and Thermo Scientific™ Orbitrap™ mass analyzer-based high-resolution, accurate-mass (HRAM) $MS^n$ analysis, resulting in greater breadth and depth of untargeted profiling and targeted quantitation, while requiring only minor modifications to existing LC-MS methods. In only milliseconds, differential ion mobility spatially disperses precursor ions prior to their entry into the mass spectrometer, fitting perfectly into the existing acquisition cycle times for untargeted data acquisition [e.g., data-dependent acquisition (DDA) or data-independent acquisition (DIA)], or targeted data acquisition [e.g., timed $MS^2$ ($tMS^2$)], or selected reaction monitoring (SRM).

The FAIMS Pro interface provides ultimate flexibility for biological mass spectrometry experiments and can be coupled to any next-generation Thermo Scientific™ LC-MS instruments without tools or breaking vacuum on the mass spectrometer. Subsequent setup, calibration, and optimization can be performed in minutes, making the conversion easy and straightforward. The FAIMS Pro interface is ideal for low-flow chromatography or direct infusion methods and is compatible with multiple nanospray ionization sources.

**The Thermo Scientific next-generation mass spectrometers that work with the FAIMS Pro interface include:**

- Thermo Scientific™ Orbitrap™ Tribrid™ mass spectrometers
- Thermo Scientific™ Orbitrap Exploris™ mass spectrometers for LC-MS
- Thermo Scientific™ TSQ Quantis™ triple quadrupole mass spectrometer
- Thermo Scientific™ TSQ Altis™ triple quadrupole mass spectrometer

## How does differential ion mobility separate ions?

High-Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS) is an atmospheric gas-phase separation technique. A FAIMS device separates ions based on differences between the various ions' mobilities in strong and weak electric fields. The FAIMS Pro interface comprises a cylindrical set of electrodes (inner and outer), with an entrance orifice that allows the newly formed ions to enter the interface and then exit to the mass spectrometer (Figure 1). The inner electrode blocks line-of-sight of neutrals from entering the mass spectrometer, thus improving workflow robustness. The cylindrical electrodes focus ions through the electrode assembly with the aid of nitrogen carrier gas, significantly improving ion transmission compared to parallel, planar electrodes.

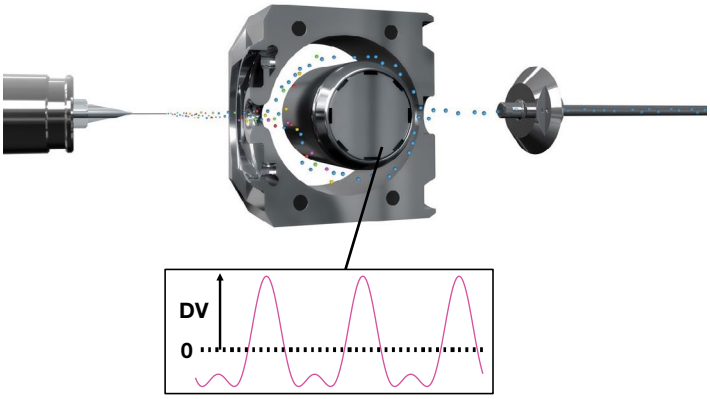


**Figure 1. The FAIMS Pro interface and its spatial relationship to the electrospray ionization tip (left) and entrance to the mass spectrometer.** Precursor ions are formed and introduced into the FAIMS device and then directed around the inner electrode. Precursors with a stable trajectory are transmitted through the interface and into the mass spectrometer for analysis. The inset shows the high-field asymmetric waveform that is applied to the inner electrode, while the outer electrode is held at ground.

A high voltage—the dispersion voltage (DV)—is applied to the inner electrode for a short time, followed by a low voltage (50% of high voltage) of opposite polarity that is applied for twice the length of time. The Figure 1 inset shows the voltage profile of these strong and weak electric fields. The waveform completes tens of thousands of cycles during the precursor ions' short residence times in the FAIMS Pro interface. Differential ion mobility results in spatial filtering where only those ions with stable trajectories move through the FAIMS Pro interface into the mass spectrometer. All other ions are quenched on the electrodes.

Figure 2 provides an example of differential ion mobility that uses a simplistic model of two ions with different three-dimensional volumes. The ions have two different mobilities under the high and low fields, and their combined mobilities determine if the ion has a stable trajectory through the FAIMS Pro interface to the mass spectrometer. Here, the trajectory of the orange ion falls off the median line following each high-low cycle. After a series of cycles, the orange ion quenches into an electrode. Conversely, the blue ion has a combined mobility that results in a stable trajectory through the interface to the mass spectrometer and is thus detected. The overall difference in an ion's mobility in the high and low fields creates spatial selectivity.

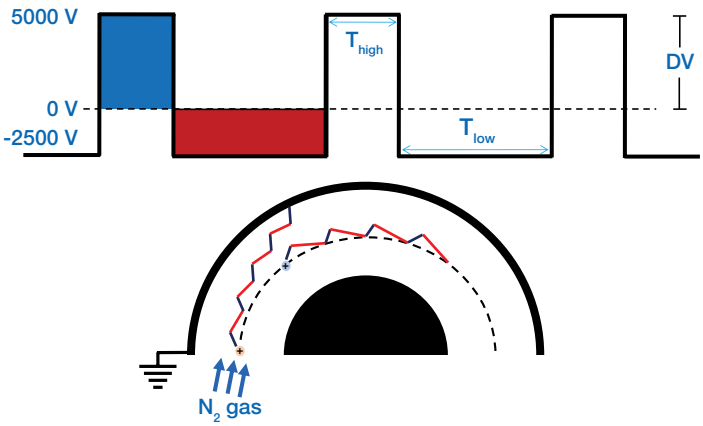


**Figure 2. FAIMS Pro Interface: differential ion mobility.** The dashed line represents the uniform ion trajectory that maintains an equal distance between the two electrodes as it is carried by the nitrogen gas towards the exit orifice and into the mass spectrometer. The solid lines represent the ion trajectories under the high (blue) and low (red) field voltage potentials that are applied to the center electrode.

To compensate for ion displacement during repeated asymmetrical waveform cycles, a compensation voltage (CV) is applied to the inner electrode. The CV value dictates which ion groups pass through the electrodes into the mass spectrometer. CV values can be empirically determined and applied to a user's existing instrument methods to improve signal-to-noise for the analytes of interest. Figure 3 shows that a narrow range of CV settings provides maximum ion transmission. Initial research for bottom-up shotgun proteomics experiments has shown that at CV settings of -40 V or lower, the selectivity of the FAIMS Pro interface reduces singly charged precursor ions that are measured in the mass spectrometer. Multiply charged peptides on the other hand are preferentially transmitted to the mass spectrometer for detection and characterization (Figure 4.) As a result, over 15% more proteins and peptides can be confidently sequenced, compared to similar experiments performed without the FAIMS Pro interface. (Hebert, 2018)

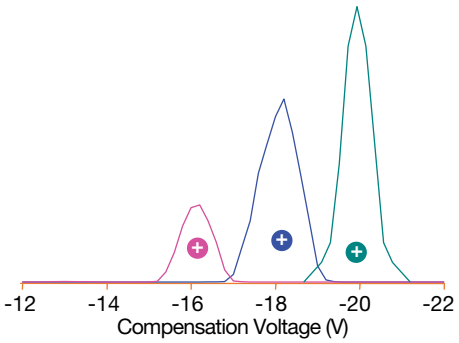


**Figure 3. CV values dictate which ion groups are transmitted through the FAIMS Pro interface to the mass spectrometer for detection.** A small CV setting range provides maximum ion transmission.

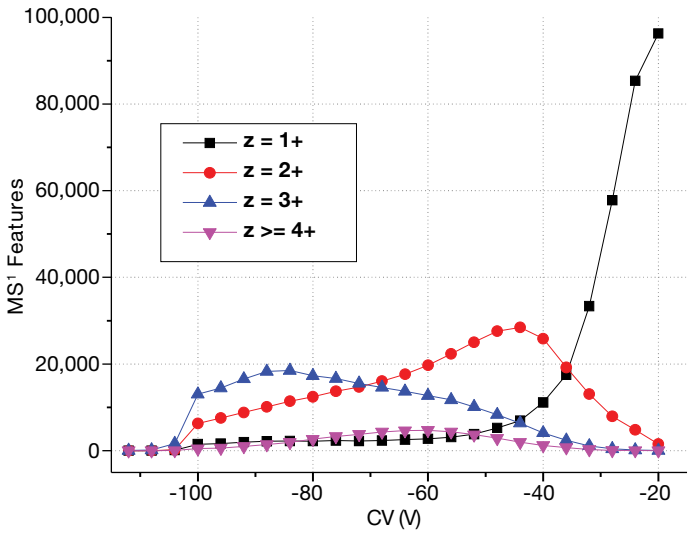


**Figure 4. HRAM MS measurement of precursor features in a HeLa tryptic digest as a function of compensation voltage.** The experiment was performed using replicate injections.

## How does differential ion mobility increase experimental selectivity for analysis of complex samples?

The FAIMS Pro interface is placed between the LC column (at the electrospray ionization tip) and the mass spectrometer. Each component performs a separation that contributes to the overall peak capacity of the system. Chromatographic separation results from hydrophobic interactions between molecules, solvents, and packing material. In reverse-phase LC, molecules elute in order of more hydrophilic to more hydrophobic as a function of organic mobile phase composition (gradient). LC column chemistries, particle sizes, dimensions, and gradient lengths govern the chromatographic separation.

The mass spectrometer separates compounds based on mass-to-charge (*m/z*) ratios. Selectivity can be performed using the quadrupole mass filter and the mass detector. Thermo Scientific mass spectrometer quadrupoles have the highest transmission efficiency at the narrowest isolation ranges, enabling selective ion accumulation and mass detection. In addition, the resolving power of the mass spectrometer can further differentiate co-eluting isobaric molecules. Orbitrap mass analyzers typically operate at resolving powers between 60,000 and 240,000 (at *m/z* 200)—significantly higher than Q-TOF mass spectrometers—while maintaining acquisition speeds that are on the LC timescale.

Differential ion mobility provides separation that is orthogonal to chromatographic and mass spectral separations. Ion mobility spectrometry generally uses electric fields and neutral gases to differentiate ions based on charge state and three-dimensional gas-phase volume. For similarly charged ions, larger ions undergo more ion-neutral collisions, reducing their mobility relative to more compact ions. In differential ion mobility devices, ions are exposed to high and low field strengths, and their combined mobility determines which ion maintains a stable trajectory through the FAIMS Pro interface into the mass spectrometer. The degree of orthogonality for the ion mobility device further differentiates the overall selectivity of the analysis as measured in the mass spectrometer.

Shotgun proteomics experiments on complex tryptic digests result in peptide co-elution. More abundant peptides can cause ion suppression during ion accumulation in the mass spectrometer, limiting its dynamic range for the detection of low-abundance peptides. The FAIMS Pro interface increases the breadth and depth of peptide detection and subsequent sequencing via gas-phase fractionation of ions prior to entering the mass spectrometer for DDA detection. Figure 5 shows full-scan HRAM MS detection without (Figure 5A) and with (Figure 5B and 5C) the orthogonal selectivity provided by differential ion mobility. Though there were ions in common measured at each CV setting for experiments performed without the FAIMS Pro interface, the greatest number of peptides were measured using the FAIMS Pro interface. In addition, no singly charged ions were measured in full-scan mode when the FAIMS Pro Interface was used.

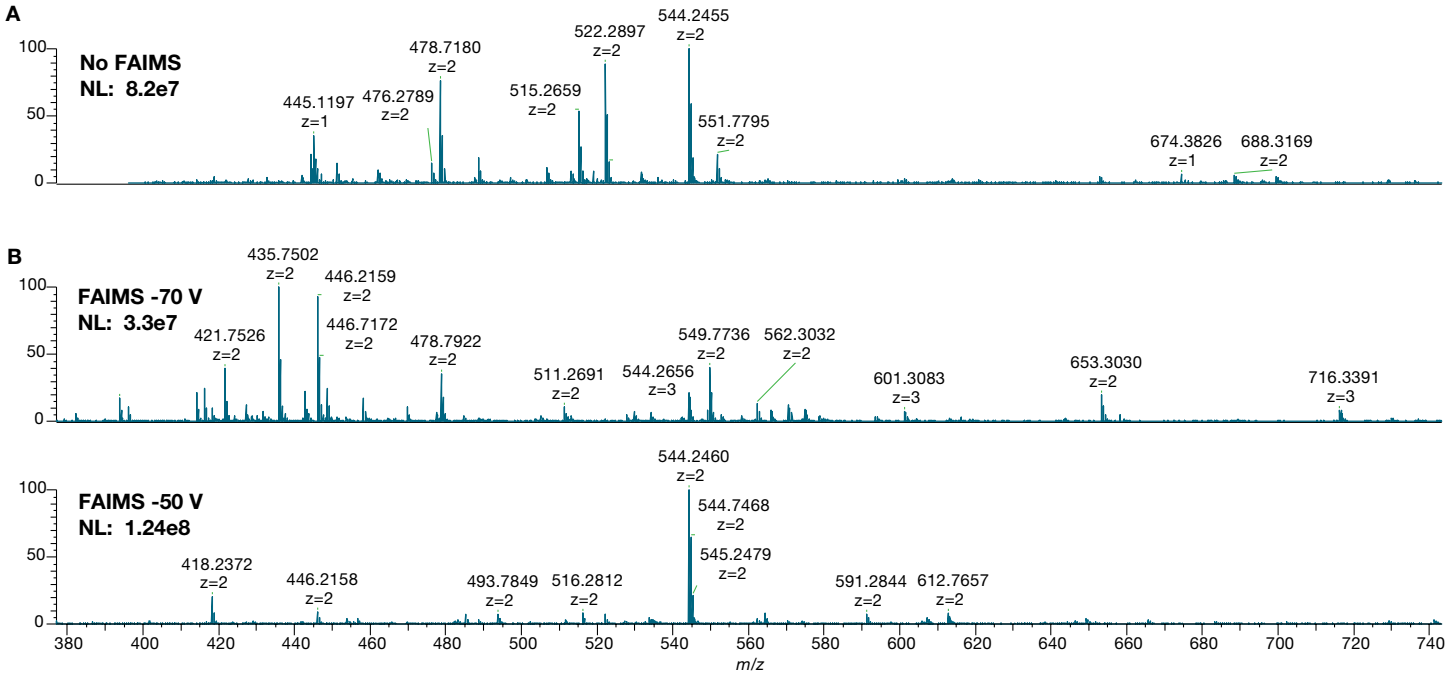


**Figure 5. Comparative HRAM MS spectra acquired with (A) and without (B) the FAIMS Pro interface from the analysis of 200 ng of a tryptic digest of HeLa cell lysate.** All experimental parameters were the same for both experiments, except for the experiment performed with the FAIMS Pro interface. Here the DDA method was repeatedly stepped between two instrument methods: DDA with a CV setting of -70 V and DDA with a CV setting of -50 V. Total cycle time was the same for both methods.

Figure 6 shows the expanded mass range around the *m/z* 544 precursor ion detected in each experiment. The measured *m/z* value for the most abundant ion in the narrow mass range was used to generate the extracted ion chromatograms (XICs), which showed co-elution. The narrow mass range spectra show that the same peptide is detected using a CV value of -50 V in the FAIMS and non-FAIMS experiment. However, the ion intensity of the peptide measured using the FAIMS Pro interface is about two times greater. Acquiring HRAM MS data using a FAIMS Pro interface CV setting of -70 V prevents NYYEQWGK peptide transmission, and instead preferentially transmits two different peptides that were sequenced using $MS^2$ DDA. The ion intensity for the IVLDSDAAEYGGHQR peptide is 6.8e6 and with a S/N of approximately 400, demonstrating a substantial increase in the depth of detection compared to the shotgun proteomics experiment carried out without the FAIMS Pro interface. The data was acquired at a resolving power of 86,000 at *m/z* 544, enabling baseline resolution between the two peptides. Evaluation of overall peptide detection for the digested HeLa sample demonstrated that—in a single experiment—only a 7% overlap of commonly detected and sequenced peptides for CV settings of -50 and -70 V. Adding the FAIMS Pro interface to the shotgun proteomics workflow increased protein and peptide detection by about 30% without the need to modify the LC gradient.

The comparative full-scan $MS^2$ spectra for the co-eluting, isobaric peptides IVLDSDAAEYGGHQR and NYYEQWGK shown in Figure 7 further demonstrate the orthogonal selectivity obtainable using the FAIMS Pro interface. Without use of the FAIMS Pro interface, the DDA scan would have co-isolated both precursors, causing the product ion ($MS^2$) spectrum to include the product ions associated with both peptides. Instead, neither product ion spectra are chimeric for increased confidence in automated sequencing.

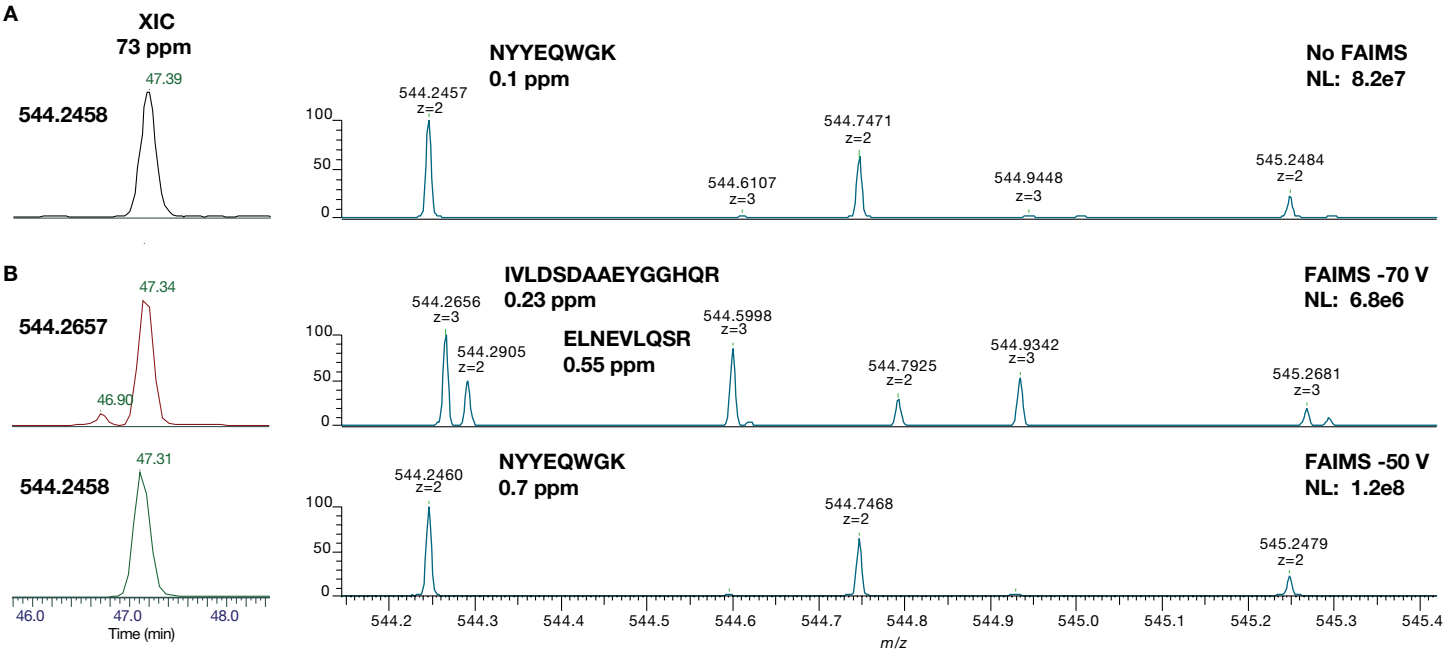


**Figure 6. Orthogonal selectivity: LC-FAIMS-MS analysis of the HeLa cell lysate sample.** The XIC of the narrow mass range around *m/z* 544 is shown for a standard DDA method without the FAIMS Pro interface (A) and with the FAIMS Pro interface stepped between two CV settings (B). For each peptide, the XIC reveals co-elution of the peptides, both with and without FAIMS. The peptide sequence was determined using DDA $MS^2$ processing capabilities of Thermo Scientific™ Proteome Discoverer™ software version 2.4.

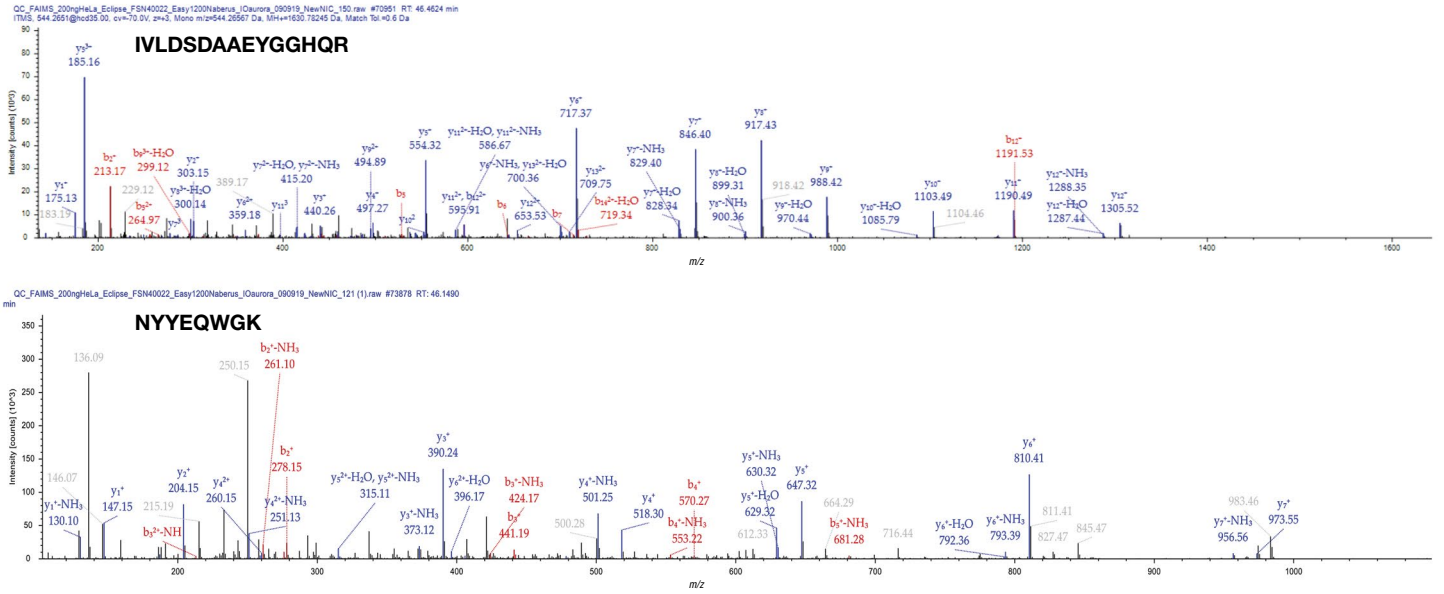


**Figure 7. Comparative full-scan MS² spectra of the isobaric peptides IVLDSDAAEYGGHQR and NYYEQWGK**

## What is the difference between differential ion mobility provided by the FAIMS Pro interface and other commercially available ion mobility devices?

Differential ion mobility spatially disperses ions as they migrate through the FAIMS Pro interface. Ions with unstable trajectories migrate off the optimal flight path and are either quenched or have reduced signals relative to ions that have optimal flight trajectories. On the other hand, linear ion mobility devices separate ions based on temporal or field dispersive mechanisms by using opposing electric fields and neutral gas flow. In this case, ions are separated based on mobility differences as they move through a drift tube in a linear path from the ion source to the mass analyzer.

Figure 8 shows a schematic of a drift tube ion mobility (DTIMS) device in which an electric field drives ions into a counter flow of neutral gas. (Madhu, 2018) The size and charge of the ion determine its mobility within the drift tube. Smaller ions have greater mobility than larger ions of the same charge state, and thus arrive at the detector first, resulting in temporal dispersion. Since the field, temperature, and neutral gas density are constant, a single mobility is measured, enabling determination of the collisional cross section (CCS) area, which is measured in Å$^2$. A commercially available set of compounds can be used to calibrate the DTIMS device and assign a CCS to each compound as a diagnostic value. Thus, the differences in measured CCS values help determine the effectiveness of the ion mobility device's strength in classifying molecules based on resolution (an individual ion's peak width) and resolving power (ion-ion separation).

However, due to the gas pressure and temperature limitations of DTIMS devices, resolution is generally governed by the length of the drift tube. (Dodds, 2017)

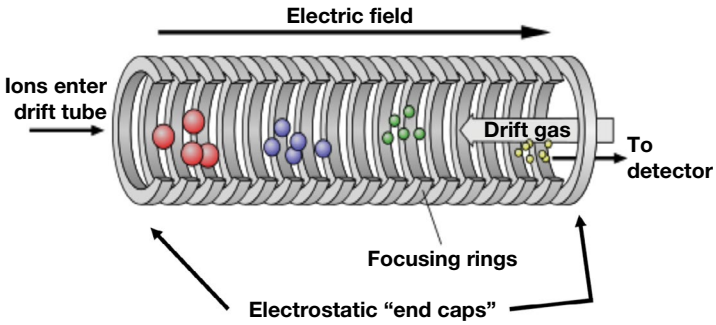


**Figure 8. Drift tube ion mobility (DTIMS) device schematic.** An electric field drives ions into a counter flow of neutral gas. The size and charge of the ion determine its mobility within the drift tube. Smaller ions have greater mobility than larger ions of the same charge state, and thus arrive at the detector first, resulting in temporal dispersion.

Trapped ion mobility spectrometry (TIMS) was recently introduced to commercially available Q-TOF instruments to overcome the limitations associated with DTIMS. While both DTIMS and TIMS are linear, a TIMS device reverses the electrostatic field and neutral gas flow directions (Figure 9.) Ions are first accumulated in the front half of the TIMS tunnel based on the desired charge density, and then released into the TIMS analyzer where an electrostatic field is applied. Ions migrate into the electrostatic field against the moving buffer gas and reach an axial equilibrium where the ion drift velocity is equal to the opposing gas velocity (Figure 9 inset). Next, the ions are sequentially eluted for mass analysis by gradually reducing the electric field strength. The rate at which the electric field is dropped dictates the resolution.

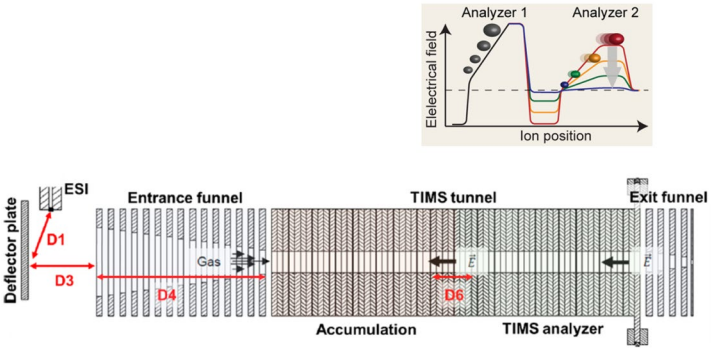


**Figure 9. TIMS device schematic with graph of ion position versus the electric field**

Both DTIMS and TIMS have commonalities with the FAIMS Pro interface. All of these IMS devices are positioned between the LC and the mass spectrometer to provide orthogonal separation that enhances MS performance for the detection and characterization of complex samples. In addition, they can provide selectivity based on charge state, which is particularly advantageous for proteomics experiments where peptides and proteins are multiply charged, while solvent and matrix ions are singly charged and thus reduced prior to detection. Each of these devices can also be operated for broad or targeted transmission, although DTIMS and TIMS operate with about a tenfold increase in resolution compared to the FAIMS Pro interface. For linear ion mobility devices, the resolution is inversely proportional to overall data acquisition speed, and is therefore maintained between 40 and 100 as defined by the following equation: (Dodds, 2017)

Equation 1: $R_P = x / \Delta x$

Here, x is the CCS value measured at the apex and Δx is the difference in CCS values measured at full width half maximum (FWHM). The CCS values represent the technique-specific mobility dispersion dimension, where DTIMS is based on drift time and TIMS on dispersion voltage. While most commercially available LC-IMS-Q-TOF MS instruments can operate at higher resolving powers, overall performance is reduced for complex sample analysis because fewer $MS^2$ spectra can be acquired. In other words, there is a tradeoff between sample coverage and resolution. Standard operational TIMS resolution has been reported to be about 40 to 100 despite maximum resolution capabilities approaching 200.

Linear ion mobility devices perform separation at much faster time scales and are therefore better suited to TOF detection. To maintain fast data acquisition speeds, most commercial IMS Q-TOF MS instruments do not perform post-IMS ion accumulation prior to detection. Rather, TIMS accumulate ions prior to entrance into the ion mobility separation device. Since the effectiveness of IMS is limited by a user-defined charge density (about one to three million charges), performance can be limited by intra-scan dynamic range in both full-scan MS and more importantly, DDA $MS^2$ (or DIA). By performing ion accumulation prior to separation, dissociation (for DDA), and detection, the initial charge density per molecule becomes critical for spectral quality. Data-dependent acquisition schemes within commercially available TIMS Q-TOF mass spectrometers redundantly acquire $MS^2$ spectra for precursors below a pre-set intensity threshold to improve spectra quality. This redundant product ion spectral acquisition reduces sample coverage. Recent publications on bottom-up proteomics limit sample loading amounts to 200 ng on column. (Meier F. B., 2015) (Meier F. B., 2018)

Similarly, the space charging effects in the ion mobility device can limit performance for experiments using higher chromatographic flow rates. Published studies comparing lipid detection and identification at low and high flow rates show 3- to 3.5-fold improvement for low flow separation and requires loading 100× more sample to achieve the same identification rates using high flow rates. (Vasilopoulou, 2020) The need to utilize low flow rates reduces sample analysis throughput that is critical for clinical and translational research applications.

In sum, ion accumulation post-FAIMS Pro interface separation for all scan types and spectra increases the dynamic range of detection for mass spectrometer systems with Orbitrap and/or ion trap mass analyzers. Single-cell proteomics applications using FAIMS demonstrated increased proteome coverage improvements as compared to single-cell analysis without FAIMS. (Schoof, 2019) In addition, the FAIMS Pro interface has not exhibited negative effects of high charge densities associated with higher sample loading amounts (up to 4 µg of plasma digest) and the spatial filtering helps maintain optimal charge densities in the Orbitrap and ion trap mass analyzers in both MS and $MS^n$ operation.

## Do I need CCS measurements to differentiate compounds?

No. While linear ion mobility devices facilitate CCS determination per compound, it alone does not uniquely differentiate one compound from another. FAIMS and IMS devices were introduced into LC-MS workflows to enhance sample coverage by providing selectivity that is orthogonal to chromatographic and mass spectrometric selectivity. The combination of all three of these empirical measurements contributes to the workflow's ability to differentiate between closely related compounds. As reported by Meier and coworkers (Meier F. B., 2018) a set of peptides sequences from digested HeLa cell lysate analysis using a timsTOF™ Pro mass spectrometer system was compared (Table 1). The table lists two sets of peptides, one isomeric and the other isobaric, along with the experimentally determined precursor *m/z* and CCS values. Although the tryptic digest was analyzed using LC-MS and data-dependent $MS^2$, limited information was provided in the reported table to enable peptide differentiation.

For each peptide set, the measured CCS values are the only apparent diagnostic values provided in the table of supplementary data for the Q-TOF mass analysis. The measured *m/z* value is isomeric (identical) or reported with minimal mass difference (4.4 mmu), requiring mass resolution of almost 200,000, which is easily obtained using Orbitrap mass analyzers but not with Q-TOF instruments. The reported CCS values have differences of about 2.5 $Å^2$, which appears significant. However, previous studies had found that the average precision of CCS apex determination is 0.25%, (Barsch, 2020), which could result in direct overlap of measured CCS values for both sets of peptides, limiting the effectiveness of CCS values in differentiating between peptides. In addition, the data was acquired with a time-dispersive resolution of 40–50, which for the average peptide-pair CCS values shown in Table 1, would result in a FWHM CCS distribution of 7.3 and 9.4 $Å^2$, respectively. Low resolution coupled with the reported precision decreases overall selectivity for unambiguous peptide sequencing based on measured CCS and precursor *m/z* values.

The measured retention times associated with each peptide shown in Table 1 were not reported in the supplemental data provided by Meier *et al.* All peptide fractions and the total sample were analyzed using a two-hour chromatographic gradient and should have a retention time associated with all peptides that indicate when MS and $MS^2$ spectra were acquired for peptide sequencing. To assign the retention times (listed in Table 1) at which tandem mass spectra were acquired to confirm peptide sequence 1, data were evaluated from similar LC-MS experiments performed on an Orbitrap Eclipse Tribrid mass spectrometer equipped with the FAIMS Pro interface. Clearly the peptides within each group show large differences in measured retention times. To further evaluate relative retention times, the reported peptide sequences were evaluated using SSRCalc per Krokhin *et al.* based on estimated hydrophobicity factors. Lower values are associated with more hydrophilic peptide sequences, while higher values are associated with more hydrophobic peptide sequences. Thus, chromatographic separation and the combination of precursor and product ion spectral analysis provides the diagnostic metrics needed to confidently differentiate peptides.

Experimentally measured CCS and precursor *m/z* values can be associated with a public CCS compendium repository or machine-learning based predictions. (Zhou, 2017) (Dodds, 2017) However, the reported accuracy of measured CCS values to compendium values is within ±1% to 2% for predicted CCS values based on modeling. (Barsch, 2020) This poor accuracy introduces a substantial

**Table 1. Identified and sequenced peptides from a tryptic digest of HeLa cell lysate.** Forty-eight peptide-fraction samples were created using high-pH reversed-phase separation, and each fraction was sequentially analyzed using the same two-hour gradient. The reported peptide sequence, with corresponding charge state, *m/z* value, and CCS value, is taken from the Supplemental Data Table 1 in the work by Meier *et al.* The retention times listed in Table 1 were obtained from similar analysis of HeLa cell digests using a Thermo Scientific™ Orbitrap Eclipse™ Tribrid™ mass spectrometer. To evaluate the relative retention times, the hydrophobicity factor was calculated using SSRCalc. (Krokhin, 2017)

| Peptide classification | Peptide sequence | Charge | *m/z* | CCS ($Å^2$) | Eclipse retention time (min) | SSRCalc value |
|---|---|---|---|---|---|---|
| Isomeric | GSNLDAPEPYR | 2 | 609.7911 | 362.4 | 42.54 | 20.86 |
| | HVATEYQENK | 2 | 609.7911 | 364.1 | 7.86 | 6.16 |
| Isobaric | LPVDLAEELGHR | 3 | 450.2455 | 473.3 | 79.60 | 32.30 |
| | VPLHKPTDWQK | 3 | 450.2506 | 470.8 | 28.52 | 15.94 |

range for consideration when trying to identify a single peptide sequence. Using the supplementary data provided Meier, for a peptide measured in the 2+ charge state with a CCS value of 400 $Å^2$, there are 5,587 peptides assuming a ±1% tolerance or 23,580 peptides assuming a ±2% tolerance. While the measured *m/z* values stratify the possibilities to two peptides, the combination of measured values still requires retention time and product ion spectra to confidently identify peptides. Of the 23,580 peptides that would be considered for a peptide having a CCS value of 400 $Å^2$, 6,814 isomeric pairs were listed. For a peptide mass difference of 5 mmu, which would be difficult for a Q-TOF mass spectrometer to resolve, 20,589 isobaric peptides were reported.

The bottom line is that high-confidence unknown compound characterization requires all experimental information, including retention time, CCS/FAIMS CV, HRAM MS and $MS^2$, and even higher order $MS^n$ spectra.

**Does the higher resolving power of linear ion mobility devices compared to the FAIMS Pro interface substantially enhance compound separation to provide greater sample coverage?**

No. Both FAIMS and IMS devices are incorporated into the overall LC-$MS^n$ sample analysis workflow. Therefore, the devices' contribution to selectivity is multidimensional. High peak capacity is achieved by the combined resolving power of each component and the difference in their separation mechanisms (orthogonality). Thus, peak capacity can be defined using the following equation from Kurulugama, *et al.* (Kurulugama)

Equation 2: Peak capacity = UHPLC resolving power × IM resolving power × MS resolving power × fraction orthogonality

While the resolution of linear ion mobility devices is 10- to 40-fold higher than that reported for cylindrical FAIMS devices, FAIMS provides greater orthogonality than linear ion mobility selectivity. In addition, Orbitrap mass analyzers provide 2 to 8 times greater mass resolution. As shown in Figure 5, when used together in a workflow, similar, or in many cases, greater peak capacity can be obtained. Comparing the effects of TIMS resolution on peptide detection using the timsTOF Pro mass spectrometer, reported 32,000 peptides identified using a TIMS trapping time of 100 ms (estimated to have a $R_p$ of 40–50). (Meier F. B., 2018) However, increasing the trapping time by twofold (doubling the resolution) resulted in a 23% decrease in peptide detection in 200 ng of digested HeLa cell lysate. Therefore, increasing the IM resolving power actually hurt peptide detection performance. A similar study performed on the Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer with and without the FAIMS Pro interface resulted in 42,548 peptides identified without FAIMS and 51,904 peptides with FAIMS—an increase of 22%, which is in line with recent publications. (Hebert, 2018)

For LC-MS applications, overall peak capacity is most important. Combining the selectivity of LC, differential ion mobility, and mass spectrometry increases breadth and depth of sample characterization. Routine IMS resolution is generally between 40 and 100 when used on chromatographic time scales, which is insufficient to baseline resolve co-eluting isomers. UHPLC separation significantly reduces the probability of co-eluting isomers, and the combination of FAIMS and Orbitrap-based high resolution mass analysis distinguishes between co-eluting isobaric compounds.

**Are there any other differences between the FAIMS Pro interface and commercially available mass spectrometers with ion mobility spectrometry?**

The FAIMS Pro interface is mounted to Thermo Scientific mass spectrometers as an interface and can be added on or taken off without breaking instrument vacuum. In addition, the FAIMS Pro interface can be mounted to many Thermo Scientific mass spectrometers. These include the Thermo Scientific TSQ Altis and Quantis triple quadrupole mass spectrometers and the Thermo Scientific™ Orbitrap Exploris™ and Orbitrap Tribrid™ product series. Commercially available IM-Q-TOF mass spectrometers on the other hand are integrated into the instruments' ion flight path and can only be turned on or off.

For simplified operation, FAIMS Pro interface calibration is performed without calibrants and takes less than two minutes. System performance per set of CV values for similar sample types across instruments and FAIMS Pro interfaces enable robust method transfer. Conversely commercially available IMS devices require calibration with a set of calibrant compounds to ensure reproducible performance on the same instrument, as well as to align performance with other instruments. IM-Q-TOF MS systems must be routinely calibrated with study-specific standards to match global CCS library values. The CV settings used on the FAIMS Pro interface, however, are consistent between instruments without internal standards or frequent calibration routines.

thermo scientific

## Conclusion

The FAIMS Pro interface is a differential ion mobility device that seamlessly works with a broad range of Thermo Scientific next-generation mass spectrometers for identification and/or quantitation across a broad range of compounds. Differential ion mobility provides orthogonal selectivity to that of LC and MS, expanding breadth and depth of compound detection and quantitation. Introducing orthogonal selectivity at the ion source maximizes MS instrument capabilities for discovery and targeted analytical workflows.

### For more information

- Single-Cell Proteomics
- Thermo Scientific FAIMS Pro interface
- Thermo Scientific Orbitrap Tribrid mass spectrometers
- Thermo Scientific Orbitrap Exploris mass spectrometers for LC-MS
- Thermo Scientific TSQ Quantis triple quadrupole mass spectrometer
- Thermo Scientific TSQ Altis triple quadrupole mass spectrometer
- Thermo Scientific Proteome Discoverer software

### References


Barsch, A. M.-H.-k. (2020). 4D-Lipidomics investigation of in C. elegans daf-2 mutants related to aging and longevity. *ASMS 2020 Reboot,* (p. TP 352).

Dodds, J. N. (2017). Correlating resolving power, resolution, and collision cross section: unifying cross platform assessment of separation efficiency in ion mobility spectrometry. *Anal. Chem., 89*(22), 12176–12184.

Hebert, A. S. (2018). Comprehensive single-shot proteomics with FAIMS on a hybrid Orbitrap mass spectrometer. Anal. Chem., 90(15), 9529–9537.

Krokhin, O. V. (2017). Peptide retention time prediction in hydrophilic interaction liquid chromatography: data collection methods and features of additive and sequence-specific models. *Anal. Chem., 89*(10), 5526-5533.

Kurulugama, R. I. (n.d.). Agilent Products. Retrieved from https://www.agilent.com/en/product/liquid-chromatography-mass-spectrometry-lc-ms/lc-ms-instruments/quadrupole-time-of-flight-lc-ms/6560-ion-mobility-lc-q-tof#literature: https://www.agilent.com/cs/library/technicaloverviews/public/5991-3244EN.pdf

Madhu. (2018, December 16). Retrieved from DifferenceBetween.com: https://www.differencebetween.com/difference-between-ionic-mobility-and-ionic-velocity/

Meier, F. B. (2015). Parallel accumulation-serial fragmentation (PASEF): multiplying sequencing speed and sensitivity by synchronized scans in a trapped ion mobility device. *J. Proteome Res., 14,* 5378–5387.

Meier, F. B. (2018). Online parallel accumulation-serial fragmentation (PASEF) with a novel trapped ion mobility mass spectrometer. *Mol. Cell. Proteomics, 17,* 2534–2545.

Schoof, E. M. (2019). A quantitative single-cell proteomics approach to characterize an acute myeloid leukemia hierarchy. *BioRxiv Preprint.* doi: https://doi.org/10.1101/745679

Vasilopoulou, C. G. (2020). Trapped ion mobility spectrometry and PASEF enable in-depth lipidomics from minimal sample. *Nat. Commun., 11,* 331–342.

Zhou, Z. T.-J. (2017). LipidCCS: Prediction of collision cross-section values for lipids with high precision to support ion mobility-mass spectrometry-based lipidomics. *Anal. Chem., 89*(17), 9559–9566.


Find out more at **thermofisher.com**

ThermoFisher SCIENTIFIC