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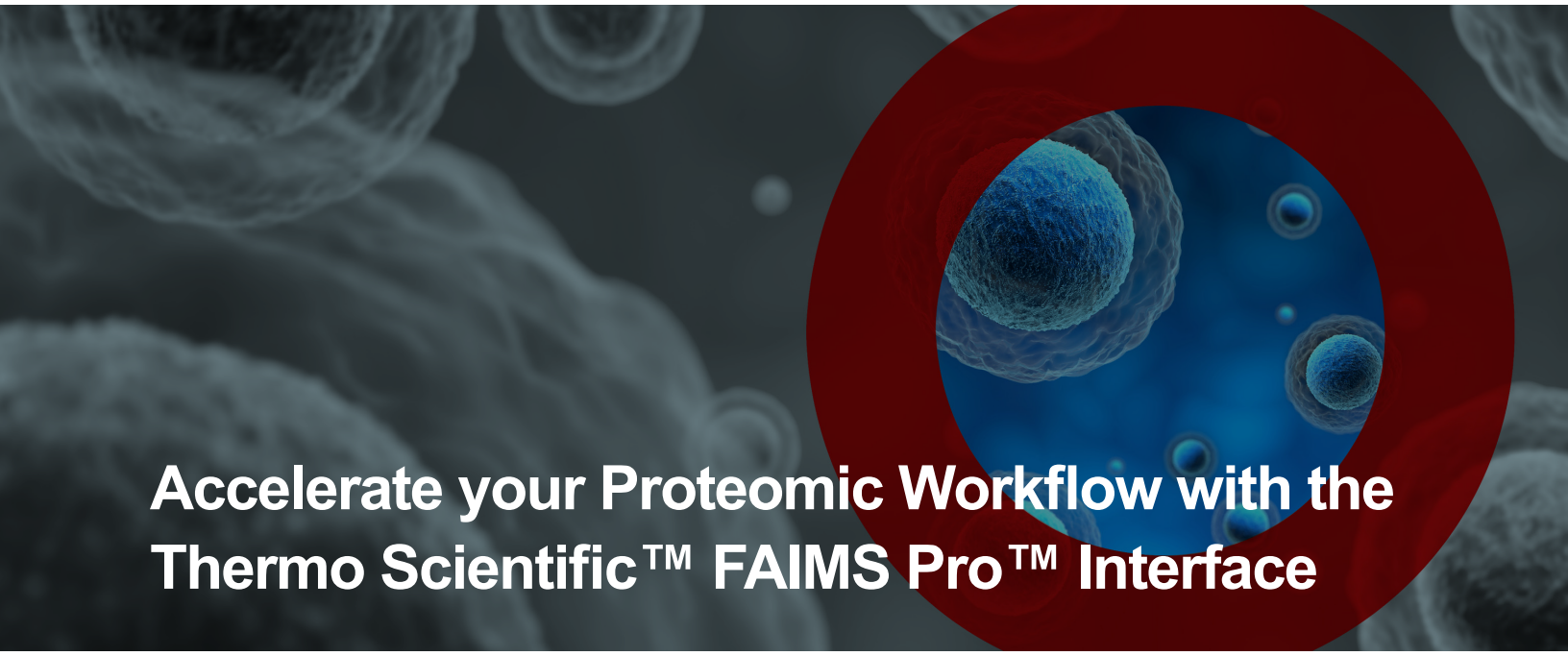

Accelerate your Proteomic Workflow with the Thermo Scientific™ FAIMS Pro™ Interface

Proteomics is the systematic and simultaneous study of numerous and diverse proteins. Its emergence was catalyzed by the realization that complexity is principally generated by protein variation, rather than by large numbers of genes[1]. The genome is thought to be largely static. However, the proteome demonstrates substantial plasticity that is a function of splicing, post-translational modification (PTM), protein complexation, and both the spatial and temporal regulation of proteins[2]. Given that the proteome reflects the dynamic state of cells, tissues, and therefore the organism, proteomics is crucial to our understanding of both health and disease.

Traditional technologies for proteome analysis

Mass spectrometry (MS) has established itself as the preferred method for direct protein identification and measurement. This has been driven by significant improvements in the sensitivity and resolution of MS instrumentation[3]. The two main MS-based approaches to identifying and characterizing proteins are the "top-down" workflow that analyses intact proteins, and "bottom-up" which analyses peptides derived by proteolytic digestion. In "top-down" proteomic workflows proteins are introduced into the gas phase by electrospray ionization (ESI) and are subsequently fragmented in the mass spectrometer. The top-down workflow is more amenable to protein variant identification[4]. A mainstay of the MS analysis is the "bottom-up" approach, which begins with enzymatic digestion, and the resultant peptides are then analyzed by ESI[5]. This approach is largely preferred for comprehensive protein identification; first the mass of the intact peptides is determined, followed by fragmentation in the gas phase to provide sequence information/information about the protein sequence. The conversion of proteins to peptides increases sample complexity, resulting in > 25,000 peptides, even for comparatively small proteomes such as those found in yeast[6,7]. The identification and quantification of poorly represented peptides can be overwhelmed by confounding background ions[8]. Greater sample complexity also increases the likelihood that multiple precursors are concomitantly fragmented, resulting in chimeric mass spectra[9]. These limitations not only affect the dynamic range of peptide identification and the depth of proteome analyses but can also limit quantitative measurements[8,10]. Additionally, peptide loss during sample preparation and digestion limits the ability of top-down MS workflows to identify all proteoforms such as splice variants[11]. Given the limitations of more traditional workflows and the growing complexities of qualitative and quantitative analyses, new approaches were

required to provide more selective and sensitive analysis. Technological advances have led to the development of an improved high field asymmetric waveform ion mobility spectrometry (FAIMS) interface that can be used in conjunction with mass spectrometers[8]. FAIMS delivers workflow improvements including, ease of operation, high ion transmission, robustness, and increased confidence for identification and quantitation.

FAIMS Pro interface

FAIMS is an ion mobility technique that separates gas-phase ions based on their behavior at low and high electric fields (Figure 1). Ions of differing mobilities are transmitted sequentially by scanning the compensation (CV) voltage. FAIMS pre-fractionation of ions enables the removal of interfering isobaric species, and singly charged ions. FAIMS modules are easily interfaced with Thermo Scientific™ mass spectrometers and provide additional selectivity that is often necessary in challenging liquid chromatography-mass spectrometry (LC-/MS/MS) experiments. FAIMS separation is used as a means of fractionation to improve detection of peptides in complex samples. FAIMS improves signal-to-noise ratio, detection limits, and throughput[10].

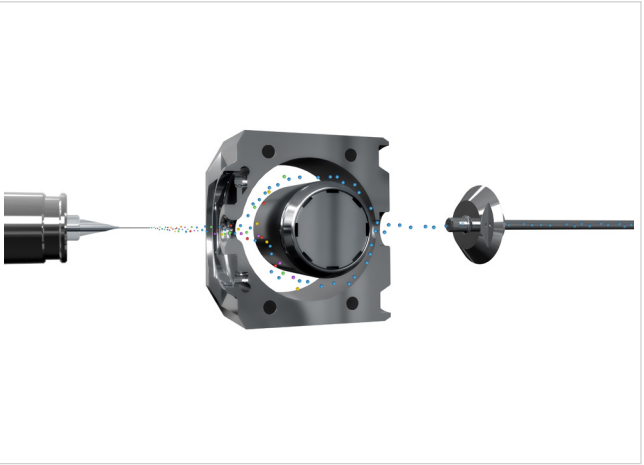

Figure 1. High field asymmetric ion mobility spectrometry (FAIMS) spatially separates ions between strong and weak electric fields. Ions (multi-colored) transverse the interface between the cylindrical electrodes based upon differences in the ion mobility. Only ions with a stable flight path (blue) enter the mass spectrometer. Therefore FAIMS increases selectivity and enables gas-phase fractionation.

General benefits

FAIMS Pro technology is uniquely designed to improve nano, capillary, and microflow applications. This reduces sample consumption whilst still achieving higher data quality. The FAIMS Pro design includes optimized cylindrical geometry that substantially increases ion transmission to the MS with short residence times, allowing multiple CV settings per acquisition. FAIMS Pro workflows enable gas-phase fractionation within a LC/MS analysis, which is faster and consumes less sample than offline chromatography-based separation methods, reducing or omitted sample pre-fractionation preparation. FAIMS technology employs electrical fields to achieve (post-ionization) separation, therefore reducing solvent use in comparison with traditional methodologies, this complies with the American Chemical Society's principles[12] for more environmentally friendly "green" bioanalytical techniques[13].

No consumable parts mean lower long-term cost and maintenance. Assembly and disassembly are fast, tool-free and, do not require the vacuum to be broken. The interface is easy to use and install, meaning that the system is accessible to all users. The menu-driven software incorporates pre-configured method templates with recommend parameters, resulting in faster workflows that retain the ability to be customizable.

Benefits of combining FAIMS Pro interface with Orbitrap™ technology

The use of FAIMS Pro interface with Thermo Scientific™ Orbitrap™ MS technology improves signal-to-noise ratio by limiting chemical noise/ interferences from entering the MS by transmitting more homogeneous population of ions to the MS. The combination of a FAIMS Pro interface with high resolution, accurate mass instrumentation also increases the limits of detection and quantitation. The Orbitrap analyzer enables more unique peptide identifications per unit time, shortening the time-to-results and adding depth at any level of chromatographic throughput. Combining this with the FAIMS Pro interface boosts protein and peptide identification efficiency further. Strong orthogonality between FAIMS and Orbitrap MS makes their combination a powerful approach for the separation of isomers and isobars[14].

The increase in selectivity and sensitivity that a FAIMS Pro interface enables assists a variety of application areas as detailed below, including site-identification of post-translational modifications, higher peptide IDs, improved separation for top-down analysis and quantitation of isobaric tags.

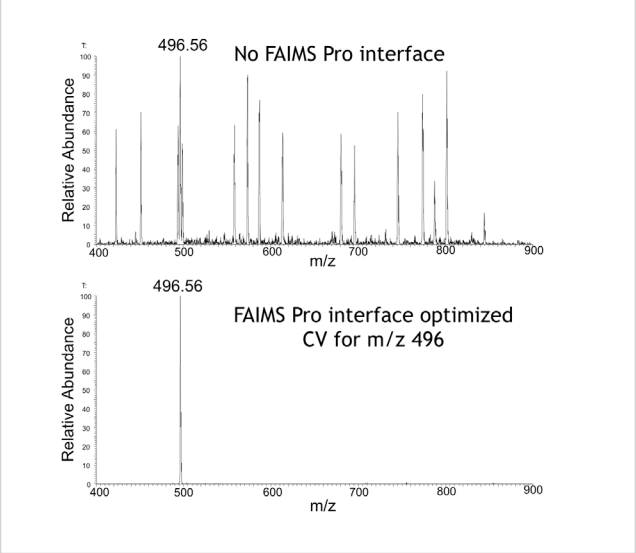

Figure 2. Gas phase separation provides an additional level of selectivity for precursors. The result is a reduction in isolation interference, even without chromatographic separation. Here the compensation voltage (CV) was optimized for transmission of precursor m/z 496, selectively enriching for this target. Data collected on a Orbitrap Eclipse Tribrid MS.

FAIMS has shown great utility in identifying protein post-translational modifications, such as phosphorylation. The incorporation of FAIMS into a phosphoproteomics discovery workflow for a fibroblast growth factor receptor signaling study, conducted in breast cancer carcinoma cells, revealed an additional 685 high-confidence phosphorylations, of which 20% were novel[15].

In a single-shot analyses of human cell line tryptic peptides, the addition of a FAIMS device doubled the number of precursors presented to the mass spectrometer, improved peptide identification by up to 2-fold, and increased protein identifications up to 55%[16].

FAIMS technology has been employed for monoclonal antibody analysis because its gas-phase fractionation excludes the liquid separation step making the process more rapid, robust, and less expensive. Melani *et al.* have used the FAIMS Pro™ interface coupled to a Thermo Scientific™ Orbitrap Eclipse™ Tribrid™ mass spectrometer (FAIMS-MS^n) in a proteomic analysis of reduced light and heavy chain antibody moieties[17].

Isobaric labeling methods, such as Tandem Mass Tagging™ (TMT™) (Proteome Sciences plc, London, United Kingdom), enable multiplexed up to 16 samples in a single LC/MS analysis. However co-isolated ion interference can suppress ratio quantification and thereby mask true differences in protein abundance. Methods to ameliorate this bias can result in protein loss and limit peptide identifications. FAIMS, however, has been shown to robustly improve the quantitation in high-resolution MS and synchronous precursor selection MS^3 methods[18].

In summary, FAIMS Pro interface enables orthogonal gas-phase separation while increasing ion selectivity and sensitivity, enabling users to reveal what matters effortlessly. Designed to enhance performance and usability, it easy to set up, use, and maintain with pre-built data-acquisition method templates, and streamlined data processing. Thus, the FAIMS Pro interface is a next-generation, differential ion mobility device that seamlessly works with a Thermo Scientific Orbitrap mass spectrometers to help proteomic researchers' answer challenging biological questions.

Thermo Scientific™ EASY-nLC™ 1200 system and FAIMS Pro interface coupled to the Orbitrap Eclipse Tribrid MS.

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