Comprehensive Evaluation of Bottom-up Proteomics using an Orbitrap Fusion Lumos Tribrid Mass Spectrometer with FAIMS Pro Interface

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ABSTRACT

Purpose: Evaluate the performance of novel Thermo Scientific™ FAIMS Pro™ Interface in bottom-up proteomics.

Methods: A FAIMS Pro Interface was coupled to a Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer. Different compensation voltages of the FAIMS Pro interface were set to separate precursor ions in the gas phase.

Results: Gas phase ion separation bythe FAIMS Pro interface prior to mass spectrometric analysis substantially increased the coverage of shotgun proteomics. With FAIMS, proteins and peptides identified in bottom-up proteomics increased by 10-20%.

INTRODUCTION

State-of-the-art proteomics studies rely on utilization of high-performance mass spectrometers. Current data-dependent acquisition (DDA) methods can only identify about half of the proteins in the human proteome. To increase proteome coverage, we coupled a novel FAIMS Pro (high-field asymmetric waveform ion mobility spectrometry) interface[1] to an Orbitrap Fusion Lumos Tribrid mass spectrometer. The peptide ions are separated in the gas phase by the FAIMS Pro interface, leading to increased peak capacity and DDA performance. In this study, we evaluated the performance of the FAIMS Pro interface for various proteomics applications, including single-shot proteomics, phosphoproteomics, reporter ion quantification, and chemical crosslinking.

MATERIALS AND METHODS

Sample Preparation

Trypsin digest of human 293T cell was prepared by the common digestion workflow[2]. Phosphopeptides from 500 µg trypsin digest of 293T cell were prepared using Thermo Scientific™ High-Select™ Fe-NTA Phosphopeptide Enrichment Kit according to the included instructions.

A plasma sample was kindly donated by a healthy donor in China. High abundant proteins were depleted using Thermo Scientific™ Pierce™ Top 12 Abundant Protein Depletion Spin Columns or Thermo Scientific™ High Select™ Top14 Abundant Protein Depletion Mini Spin Columns according to manual. After tryptic digestion, peptides were desalted using Thermo Scientific™ SOLA™ SPE Plates (SOLA HRP).

Crosslink sample was prepared using bovine serum albumin (BSA) in HEPES buffer (pH 8.0), BSA was reacted with DSS in a molar ratio of 1:50 (protein: cross-linker) for 1 h at room temperature. Reaction was quenched with 1 M NH_4HCO_3.

Test Method

Liquid chromatography was performed on a Thermo Scientific™ Easy-nLC™ 1200 system. A FAIMS Pro Interface was connected to an Orbitrap Fusion Lumos Tribrid mass spectrometer. The compensation voltage (CV) of the FAIMS Pro interface was set either to a fixed CV or combinations of different CVs. The common DDA workflow was conducted employing the default template of Orbitrap Fusion Lumos Tune Software 3.1.2412.24. Briefly, each of the three CVs used was set to run DDA mode for 1 s cycle to build a big cycle of 3 s. We used Orbitrap full MS scan and ion trap HCD MS/MS scan for DDA. We used “top speed” of 3 s cycle time without FAIMS Pro interface.

Data Analysis

Raw data files were analyzed by Thermo Scientific™ Proteome Discoverer™ software (2.3.0.523) to identify peptides and proteins. False-discovery rate of PSM, peptide and protein was set to 1% for whole proteome, phosphoproteome and TMT-labeled sample. Crosslinked sample was analyzed by XlinkX2.0 node in Proteome Discoverer software.

RESULTS

Principle and hardware of FAIMS Pro interface

In its early years, bottom-up proteomics experiments were limited by the mass spectrometer scan speed. The development of faster scan speeds in the last decade[3-6], enabled researchers to explore the proteome more deeply. However, the complex nature and high dynamic range of the proteome impose additional limits on bottom-up proteomics. Even with the significantly higher scan speeds available with modern instruments, proteome coverage has been constrained by masking of proteins present at low levels by high-abundant proteins having the same or similar mass-to-charge ratios.

FAIMS Pro interface provides an additional dimension of selectivity through gas-phase ion mobility-based separation[1]. With the combination of dispersion voltage (DV) and compensation voltage (CV), only a fraction of ion are selected to pass through FAIMS Pro interface (Figure 1). The hardware and software of the FAIMS Pro interface have been redesigned to not only deliver the highest sensitivity for low flow (< 20 µL/min) applications, but also to make the interface simple to attach and use (Figure 2).

Figure 1. Ion path with use of FAIMS Pro interface. Only a fraction of ions from ESI source could pass the FAIMS device by helping of nitrogen carrier gas flow.

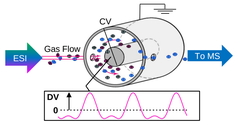
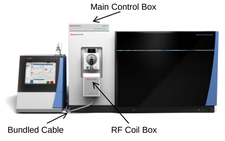
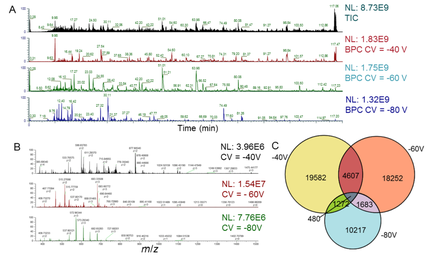


Figure 2. Overview of FAIMS Pro interface. FAIMS Pro hardware comprises the main control box, the RF coil box, and a bundled cable connecting the two.



To evaluate the possibilities of bottom-up proteomics studies utilizing this novel interface, we first used trypsin digest of human cell line (Thermo Scientific™ Pierce™ HeLa Protein Digest Standard) to assess the effect of the FAIMS Pro interface in common bottom-up proteomics experiment. With FAIMS Pro on, we found the base peak chromatogram and full MS scan in different CVs are almost completely different at the same timepoint (Figure 3A-B). When we analyzed the whole raw file, we found that the peptide and protein identifications are dependent on the CV we used. More importantly, the number of overlapping peptide precursors in different CVs are relatively small (Figure 3C) comparing to each of the CV, which shows the promising possibility of using FAIMS Pro interface as a gas-phase fractionation device.

Figure 3. The FAIMS Pro interface simplifies the complex proteome for mass spectrometry-based bottom-up proteomics. A. Total ion chromatogram and base peak chromatogram of different CVs in a 120 min gradient. B. Full MS scan in different CVs at ~50 min. B. Venn diagram of peptide identifications in different CVs.

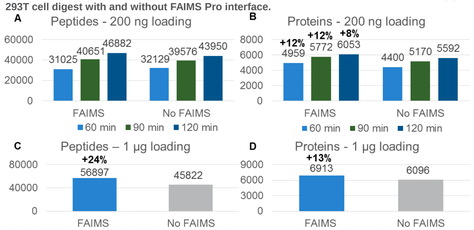


Evaluation of performance using FAIMS Pro interface for single-shot bottom-up proteomics and phosphoproteomics

Inspired by the new possibilities from FAIMS Pro device, we designed a set of comprehensive evaluations to explore proteome coverage of mass spectrometry-based proteomics.

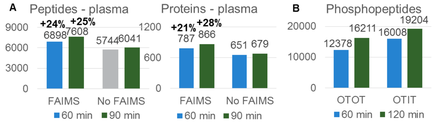
First, we evaluated the performance of single-shot proteomics using human cell digest (Figure 4). Using 200 ng HeLa Protein Digest Standard, we observed a substantial gain in protein identification for all three LC gradient lengths tested (Figure 4A-B). Notably, the Orbitrap Fusion Lumos mass spectrometer could identify ~5,000 proteins per hour with the FAIMS Pro interface, representing a gain of 12% relative to results obtained without FAIMS. As the ultra-high-field Orbitrap and commonly used nano-flow LC columns could hold far more peptides than 200 ng, we also tested the peak performance of single shot bottom-up proteomics with the FAIMS Pro interface. Using 1 µg 293T cell trypsin digest, with 24% more peptides and 13% more protein identification than without FAIMS Pro interface (Figure 4C-D), we identified ~7,000 proteins at 1% FDR in only 2h in a single human cell line.

Figure 4. The FAIMS Pro interface elevates the performance of bottom-up proteomics in common human cell lines. A. Peptides identified in 200 ng HeLa Protein Digest Standard with and without FAIMS Pro interface in different LC gradient lengths. B. Proteins identified in 200 ng HeLa digest with and without FAIMS Pro interface. C. Peptides identified in 1 µg 293T cell digest in 2 h LC gradient with and without FAIMS Pro interface. D. Proteins identified in 1 µg 293T cell digest with and without FAIMS Pro interface.



As proteomes with higher dynamic range (e.g., body fluids such as plasma and urine; post-translational modification enriched proteomes) often suffer more from ion suppression effects and other related drawbacks from co-eluted ions than cell lines, we then investigated whether the FAIMS Pro interface could also help in these cases (Figure 5). We found even more exciting results from top-12 abundant protein depleted human plasma digest, as more than 700 proteins and ~7,000 peptides were identified from plasma in only one hour (Figure 5A). Identification of post-translational modification also benefits from the FAIMS Pro interface (Figure 5B). Data-dependent acquisition using Orbitrap-ion trap (OT-IT) mode outperformed Orbitrap-Orbitrap (OT-OT) mode because of the higher MS/MS scan speeds in ion trap analyzer. We also note the even more interesting potential of better separation and identification of peptide isoforms having different sites of chemical modifications from view of ion mobility using FAIMS Pro interface, which is especially beneficial for research of complex post-translational modifications and their crosstalks, such as histone codes and phosphorylation signal transductions[7-8].

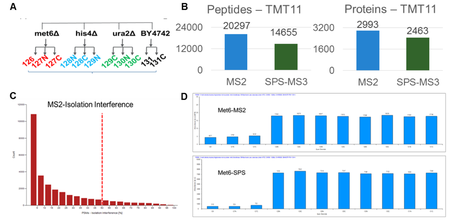
Figure 5. The FAIMS Pro interface boosts the performance of bottom-up proteomics in proteomes with higher dynamic range. A. Peptides identified from trypsin digest of top-12 abundant protein depleted human plasma with and without FAIMS Pro interface in different LC gradient lengths. B. Phosphopeptides enriched and identified from 100 µg trypsin digest of 293T cell in different scan modes and LC gradient lengths.



Evaluation of possibilities using FAIMS Pro interface for reporter ion-based quantitative proteomics and chemical crosslinking based structural biology research

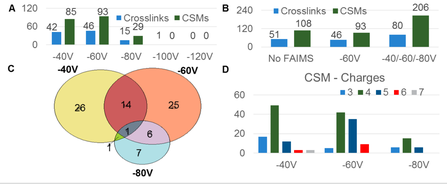
Informed by the increase in protein identifications usingthe FAIMS Pro interface, we decided to extend our study to assess if protein quantification also benefits from this gas-phase separation of co-eluted ions. Reporter ion-based quantitative proteomics often suffers from isolation interference[9], especially when the peptides are not heavily and carefully fractionated before LC-MS/MS. Using Thermo Scientific™ Pierce™ TMT11plex Yeast Digest Standard, we found substantially reduced isolation interference of precursors with FAIMS Pro (Figure 6). More than 90% of precursor ions have isolation interference less than 50% (Figure 6C). We also note that the FAIMS Pro interface combined with synchronous precursor selection (SPS)-MS^3 provides higher than ever precision of reporter ion-based protein quantification (Figure 6D).

Figure 6. The FAIMS Pro interface greatly reduced isolation interference and set new standard for reporter ion-based protein quantification. A. Composition of quantification channels in TMT11plex Yeast Digest Standard. B. Peptides and proteins identified from 1 µg TMT11plex Yeast Digest Standard in different scan modes in 180 min LC gradient. C. Distribution of precursor isolation interference in MS^2-based TMT quantification. D. Quantification Ratio of Met6 in MS^2 and SPS-MS^3 workflow.



With the help of modern Tribrid mass spectrometers and novel MS-cleavable chemical crosslinker, advanced data acquisition modes and dedicated searching algorithm[5], chemical crosslinking mass spectrometry is becoming a powerful method for structural biology. Crosslinked peptides are bigger in size and have more charges in reversed-phase chromatography, but are much less abundants (<5%) than peptides without crosslink. Researchers havee utilized the nature of crosslinked peptides, employing size exclusion chromatography (SEC) or strong cation exchange (SCX) chromatography to enrich and increase identification of crosslinkings. As ion mobility separation by FAIMS Pro interface shows good separation of different peptide charge states[1], we evaluated if the FAIMS Pro interface could help in identification of chemically crosslinked peptides (Figure 7). We found that the FAIMS Pro interface almost doubles the crosslinks we found from DSS-crosslinked bovine serum albumin (BSA) without enrichment. This additional information enabled by the FAIMS Pro interface can boost the confidence of structure alignment, as well as provide more details of interfaces in protein-protein interactions and interactome studies.

Figure 7. The FAIMS Pro interface unveils substantially more structure details in chemical crosslinking-based structural biology research. A. Identified crosslinks and crosslink-spectrum matches (CSMs) in different CVs. B. Identified cross-links and CSMs with and without FAIMS Pro interface. C. Venn diagram of crosslinks in different CVs. D. Distribution of CSMs of different charges in different CV of -40V, -60V and -80V.



CONCLUSIONS

Though there are many commercial mass spectrometers that could utilize gas-phase ion mobility to simplify ion population, the FAIMS Pro interface is singularly well-suited for bottom-up proteomics. Through our evaluation, Orbitrap Fusion Lumos Tribrid mass spectrometer with FAIMS Pro interface offers the unprecedented sensitivity and selectivity to dive deeper into the ultra-complex proteomes:

- Super high proteome coverage, especially for proteomes with high dynamic range.
- Substantially higher precision of reporter ion-based quantification.
- Much more confidence and structural details in chemical crosslinking-based structural biology.

With these technical advances, researchers will be able to find new possibilities from their bottom-up proteomic studies. More confident identifications and more accurate quantifications will finally translate into novel biological findings.

Promisingly, we note that there are further possibilities to be assessed, such as comprehensive PTM analysis and targeted proteomics. FAIMS Pro interface could also be utilized in data-independent acquisition (DIA) mode, together with spectral library and retention time library based methods, e.g. high precision column-specific chromatographic library[10], high resolution and high mass precision MS1 based peptide identification and quantification[11-12]. These possibilities may assist in attaining a key goal of bottom-up proteomics: whole proteome coverage in a single-shot LC-MS experiment.

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ACKNOWLEDGEMENTS

We thank our colleagues from Thermo Fisher Scientific China and all over the world, as well as our customers in China for insightful discussions.

TRADEMARKS/LICENSING

PO65481-EN0419S

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