Reliable and Deep Proteome Coverage by Gas-Phase Fractionation of Peptides with a FAIMS Pro Interface on a Modified Quadrupole Orbitrap

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ABSTRACT

Purpose: Demonstrate the benefit of Thermo Scientific™ FAIMS Pro™ interface for proteomic data-dependent experiments and establish an optimized method setup with the new Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer.

Methods: A commercially available tryptic HeLa digest was separated over different gradient lengths and subsequently analyzed in data-dependent acquisition mode on a Orbitrap Exploris 480 equipped with a FAIMS Pro interface.

Results: The use of peptide gas-phase fractionation with the novel Orbitrap Exploris 480 and optimized method setup increases protein identification rate by up to 15% and can be achieved with both nano and capillary flow conditions.

INTRODUCTION

While great advances have been achieved in the performance of mass spectrometers, deep proteome coverage is still impaired for highly complex samples with high dynamic concentration ranges. To address this challenge a multitude of offline fractionation techniques are employed. However, these are time-consuming and mostly consume higher sample amounts.

The use of high-field asymmetric ion mobility spectrometry (FAIMS) with an Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer has been shown to lead to comparable results regarding protein and peptide identifications in complex proteomic samples, or even exceed them[1]. For the first time, a FAIMS Pro interface has been used for offline gas-fractionation with a new hybrid quadrupole-Orbitrap mass spectrometer, providing higher sensitivity for low-abundant species and reducing chemical background. As a consequence, the mass spectrometer is exposed to fewer ions which could potentially lead to less instrument downtime. For future experiments, by reducing the co-isolation of reporter ions for MS^2, quantitative experiments could be enhanced on the Orbitrap Exploris 480 in combination with a FAIMS Pro interface[2].

MATERIALS AND METHODS

Sample preparation and LC-MS methodology

As a standard sample the commercially available tryptic digest of HeLa (Thermo Scientific™ Pierce™ HeLa Protein Digest Standard) which was reconstituted in 2 % acetonitrile in 0.1 % formic acid to a concentration of 500 ng/µL.

Three different gradients lengths were used as shown in Table 1. All separations with nanoflow were performed using the Thermo Scientific™ EASY-nLC™ 1200 HPLC system on a Thermo Scientific™ Acclaim™ PepMap™ C18, 75 µm 50 cm, 2 µm, 100 Å analytical column using 0.1 % FA in water as solvent A and 0.1 % FA in 80 % acetonitrile (Fisher Chemicals) as solvent B. For separations with capillary flow, Acclaim PepMap C18, 150 µm x 15 cm, 2 µm, 100 Å analytical column was used.

Mass spectrometric analysis was achieved by use of the novel Orbitrap Exploris 480 mass spectrometer. It was operated in data-dependent acquisition mode. The summary of the MS parameters is shown in Table 2.

The FAIMS Pro interface is compatible to the new source design of the Orbitrap Exploris 480 and was simply mounted directly in front of the ion inlet. Needle position and spray voltage were optimized with the dispersion voltage (DV) set to 'off'. For all experiments the standard FAIMS parameters were applied and compensation voltages (CV) were varied during the acquisition.

Table 1. Nano and capillary flow LC gradients on an EASY nLC 1200

solvent B [%]	60 min	90 min	nano-flow [nL/min]	30 min	capillary flow [nL/min]
8	00:00	00:00	300	00:00	1000
8	02:00	03:00	300	01:00	1000
31	62:00	93:00	300	31:00	1000
50	70:00	108:00	300	36:00	1000
90	75:00	113:00	300	38:00	1000
90	80:00	121:00	300	43:00	1000
8	82:00	122:00	300	44:00	1000
8	88:00	130:00	300	46:00	1000

Data analysis

Raw data files were processed using Thermo Scientific™ Proteome Discoverer™ 2.4 software. The peptides/proteins were identified using the SEQUEST® HT search engine after offline mass recalibration of mass spectra and with the following modifications: carbamidomethylation as fixed, methionine oxidation, aspartic and glutamic acid deamidation and N-terminal acetylation as dynamic. False-discovery rate (FDR) was set at 1 % for proteins and peptides. Less than high-confident proteins were filtered out.

Table 2. MS parameters of DDA experiments with FAIMS on the novel Orbitrap Exploris™ 480 for different gradient lengths and sample loads.

Parameter	30 min	60 min	90 min
Global parameter			
Spray voltage [kV]		2.3	
Expected peak width [s]		15	
FAIMS mode		Standard resolution	
FAIMS user gas flow [L/min]		0	
FAIMS mode		Standard resolution	
MS1 properties			
AGC target [%]	300	300	300
Mass range [*m/z*]	350-1200	350-1200	350-1200
Resolution	60k	60k	60k
MS2 properties			
TopN	20	20	15
Intensity threshold	5e4	5e3	5e3
MIPS	Peptide	Peptide	Peptide
Charge states	2-6	2-6	2-6
Isolation width [*m/z*]	1.4	1.4	1.6
Max IT [ms]	22	22	45
AGC target [%]	75	75	75
First mass [*m/z*]	120	120	120
Resolution	15k	15k	15k
NCE [%]	29	29	29
Dynamic exclusion [s]	20	20	30

RESULTS

Improving protein identification with FAIMS under nano-flow conditions

The FAIMS interface is compatible with the TNG source configuration and was used for proteomic experiments with the new hybrid quadrupole Orbitrap mass spectrometer for the first time. Figure 1 shows the results of proteomic DDA experiments with a 60 min gradient under nano-flow conditions that the FAIMS interface was ideally designed for. In single CV runs, peptide ID rates are decreased compared to the baseline without FAIMS. In contrast, the identified peptides in CV runs lead to significantly increased protein groups, up to 15 % compared to the baseline. However, when using CV switching peptide IDs are completely restored with highly increased number of identified protein compared to the baseline. In other words, even if the same number of peptides can be identified in both runs, the switching between two appropriate CVs leads to an inherently different subset of peptide ions that reach the mass analyzer.

Similarly, Figure 2 illustrates the behavior of peptide ions with different properties at varying compensation voltages. While higher masses seem to be passing the FAIMS electrode space best at -40 V, a compensation voltage of -65 V favors the smallest masses of the precursor mass range. This is in accordance with lower *m/z* in MS^2 spectra at -65 V and higher *m/z* at -40 V (B) which also lead to most peptide-spectrum-matches (PSM) at these voltages (C). Without FAIMS, ions with *m/z* 350 are predominantly selected for fragmentation while only a very small percentage thereof leads to PSM. As a consequence, the most intense ions are characterized by very low *m/z* while not leading to actual peptide identification.

Figure 1. Results of LC-MS runs with different single CVs and CV switching within a 60 min gradient and 500 ng sample load (HeLa). Single CV runs lead to less peptide but increased protein IDs compared to without FAIMS. CV switching enhances peptide ID rates.

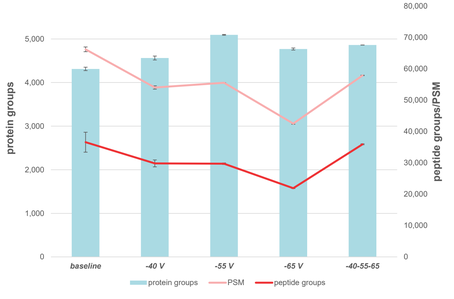
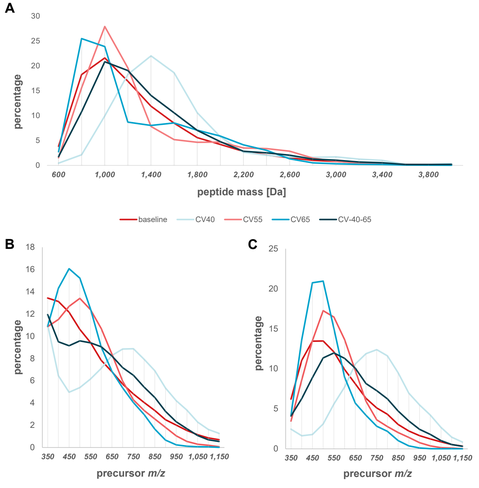


Figure 2. Distribution of peptide ions with different properties at varying FAIMS voltages. A) Density plot of original precursor mass distribution at different CV and without FAIMS. B) Density plot of precursor *m/z* in all acquired MS^2 spectra. C) Density plot of precursor *m/z* of all peptide-spectrum matches.

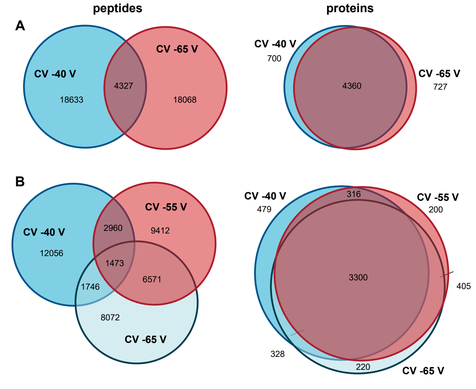


Gas-phase fractionation: reducing sample complexity of HeLa digests with FAIMS Pro™

Sample complexity, and resulting dynamic concentration ranges, are still presenting a challenge in proteomics workflows. This is often overcome by offline fractionation techniques or sample depletion which is time-consuming and prone to sample loss. The ability of peptide ions to pass through the FAIMS electrode space – with maximized transmission at a certain compensation voltage – is dependent on their mass and charge, predominantly. Therefore, the applied CV in an LC-MS run acts as a filter for a certain subset of peptide precursors and can be interpreted as a fractionation step in the gas-phase.

Accordingly, the data from two FAIMS experiments shown in Figure 3 with switching of two (A) and three CV values (B), respectively, over a 90 min gradient, show a clear separation of peptide species among the chosen voltages. Peptides identified with both CV: -40 V and -65 V, only amount to 10 % of the total number of identifications. When switching between three CV, -40 V, -55 V, and –65 V, the overlap between all three is less than 4 %. These findings demonstrate that peptide identification can be significantly enhanced with CV switching while not increasing instrument and sample preparation time. An LC-MS run applying two CV is also feasible for shorter gradient lengths, as shown in Figure 2 for a 60 min gradient, while switching between three voltages gives more benefit in longer gradients.

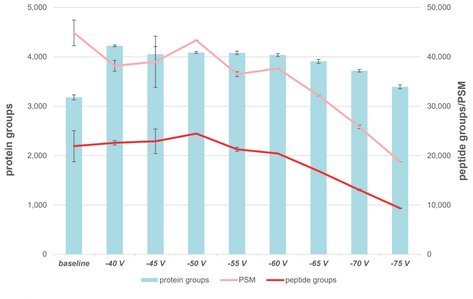
Figure 3. Overlap of peptide and protein populations from 90 min gradients and 1000 ng sample load at different compensation voltages. A) Overlap of peptide and proteins in a single LC-MS run with alternating switches between two CV of -40 V and -65 V. B) Overlap of peptide and proteins in a single LC-MS run with alternating switches between three CV of -40 V, -55 V, and -65 V.



Application of FAIMS Pro™ under capillary flow conditions with short gradients

Originally, FAIMS Pro™ has been used predominantly for nanoflow applications not exceeding 300 nL/min. The data shown in Figure 4 demonstrate that FAIMS can be used in combination with capillary flow and very short gradients. The number of protein IDs can be increased by 25 % even with single CV settings. In fact, at each of the applied CV settings the protein IDs exceeded those of the baseline. Additionally, within a CV range of -40 to -55 V, peptide identification is not decreased compared to the baseline. The distribution of numbers of proteins and peptides is similar as with longer gradients (see Figure 1) with the exception that highest protein ID rates could be achieved at a compensation voltage of -40 V. However, it does not appear reasonable to use CV switching for such short gradients so that no optimal combinations of CV were established.

Figure 4. Results of LC-MS runs with different single CV within a 30 min gradient and capillary flow, and 1000 ng sample load (HeLa). Applying different compensation voltages after a 30 min separation increases protein identification without compromising peptide IDs.



CONCLUSIONS

- FAIMS Pro interface was successfully used on a hybrid quadrupole Orbitrap mass spectrometer for the first time and method parameters, gradient lengths and sample load were optimized for this instrumental setup.
- Protein identification rates can be increased by as much as 15 % even in single compensation voltage (CV) experiments. CV switching within a single LC-MS run enhances peptide identification rates due to the fractionation of precursor ions based on their physicochemical properties.
- Separation of peptide and protein groups by applying different compensation voltages could be demonstrated and optimized CV combinations for longer and shorter gradients were determined.
- FAIMS Pro technology applied to Orbitrap Exploris 480 is also giving increased protein and peptide identifications under capillary flow conditions with short 30 min gradients.

REFERENCES

1. A. Hebert *et al.*, Anal Chem **2018**, *90*, pp 9529−9537.
2. Pfammatter *et al.*, Mol Cell Proteomics, **2018** ;*17*(10):2051-2067.

TRADEMARKS/LICENSING

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