Segmented Ion Fractionation using High Field Asymmetric Waveform Ion Mobility Spectrometry expands the depth and comprehensiveness of proteomics analyses

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OVERVIEW

AIM: Improvement of dynamic range for large scale proteome analyses
METHODS: A novel high field asymmetric waveform ion mobility (FAIMS) interface was coupled to a Q Exactive™ BioPharma. MS1 scans were acquired in 100 *m/z* segments.
RESULTS: Segmented Ion Fractionation in combination of FAIMS provides unparalleled identification and quantification capabilities for proteomic analyses.

INTRODUCTION

In spite of recent advances in MS sensitivity and resolution, the instrument performance has been mitigated by the overwhelming sample complexity that limits the comprehensiveness of proteomics analyses [1-2]. These limitations have profound impact not only on the sensitivity and depth of proteomics analyses but also on the capability to quantify trace level proteins in complex biological extracts [3]. Here, we present a novel gas phase ion fractionation approach based on segmented m/z ranges [4] that leverages the separation capability of a new high-field asymmetric waveform ion mobility spectrometry (FAIMS) interface to enrich multiply-charged ions of low abundance contained within different segments of precursor mass range. Segmented ion fractionation provides up to 3-fold improvement in peptide identification compared to traditional LC-MS/MS experiments.

Fig.1**: Schematic overview of the FAIMS-QE biopharma interface. (a) Electrode assembly with 1.5mm gap between outer and inner electrode. (b) Side view of EASY spray ionsource, FAIMS electrodes and transfer tube to the MS. (c) FAIMS research interface coupled to the Q Exactive Biopharma (Thermo Fisher Scientific) via a custom mounting flange. CVs were changed via an external in-house script before every acquision.* ***The FAIMS enabled QE interface is an experimental research system exclusively developed within the context of the Genomic Applications Partnership Program (GAPP) of Genome Canada

METHOD

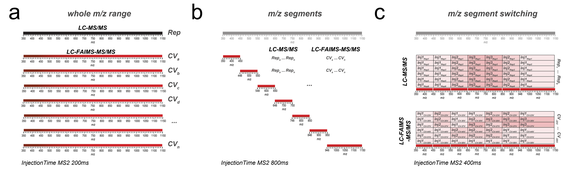
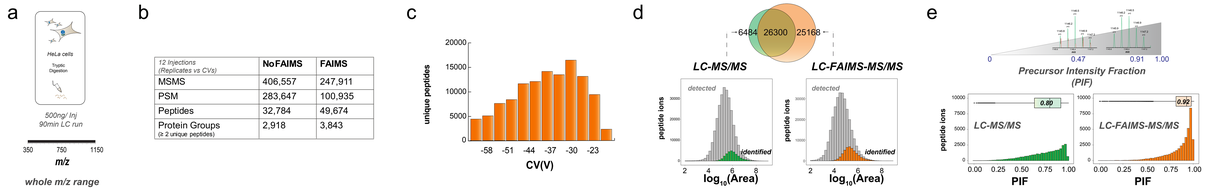


***Fig.2**: Workflow overview. HeLa digest (500ng) was separated on a 50 cm long C18 EASY spray column with a 90min gradient a) Transmitting the whole m/z range from 350-1150 m/z, (b) m/z range divided in 7 segments: 6 x 100 m/z wide and one 200 m/z wide. (c) Segments were combined in a single LC-MS run. Without FAIMS 3 segments were combined per run. For FAIMS, 1 to 3 segments were combined in each run.*

RESULTS



12 Injections (Replicates vs CVs)	NoFAIMS	FAIMS
MSMS	406,557	247,911
PSM	283,647	100,935
Peptides	32,784	49,674
Protein Groups (≥ 2 unique peptides)	2,918	3,843

***Fig.3**: HeLa proteome analysis by LC-MS/MS on m/z range 350-1150. (a) HeLa Protein Digests (500ng/inj.) were analyzed with and without FAIMS. (b) Summary of analyses for t HeLa digest. (c) Distribution of unique peptide identification for each CV. (d) Venn diagram of identified peptides with and without FAIMS and intensity distribution for detected (grey) and identified peptides with (orange) and without (green) FAIMS. (e) Precursor intensity fraction (PIF) available in Maxquant [5]. A PIF value approaching 1 indicates that most of the ion current comes from the precursor of interest and contains a low level of contaminating ions. Distribution of PIFs with (orange) and without (green) FAIMS.*

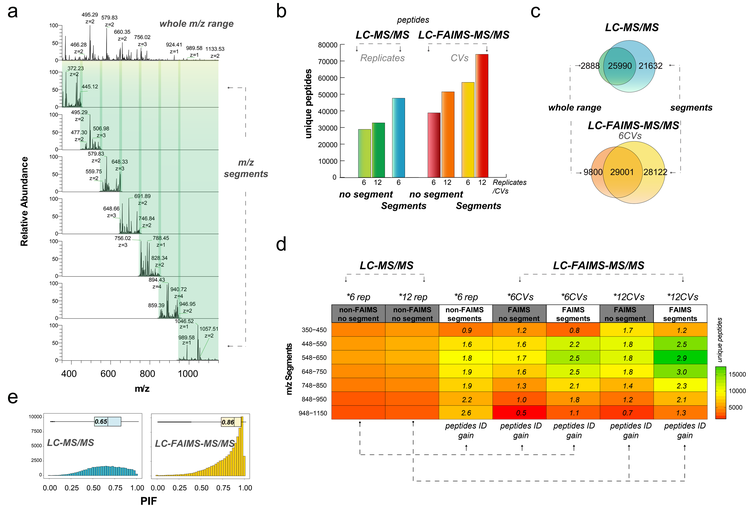


***Fig.4**: Segmented Ion Fractionation. (a) Comparison of MS1 pectra for full m/z and segmented m/z acquisition. (b) Number of unique peptides for segmented and non-segmented conventional LC-MS/MS and for LC-FAIMS-MS/MS analyses. For FAIMS we used either 6CVs or 12CVs with 7V and 3.5V steps respectively. (c) Venn diagram comparing the number of identified peptides for segmented and non-segmented analyses with and without FAIMS. (d) Heatmap for the number of identified peptides per segment for all experiments from Figs.2 a and b. Numbers show the gains in peptide identification. (e) PIF distribution for segmented MS1 analysis.*

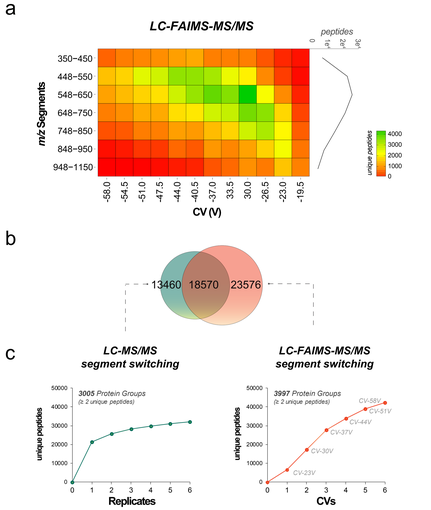


***Fig.5**: Impact of combined m/z segments on peptide identification. (a) Heatmap from segmented m/z acquisition with FAIMS. Segments were combined according to Fig.2c. Peptide distribution for the 7 segments are shown on the right. (b) Venn diagram comparing identified peptides for segment switching with and without FAIMS. (c) Cummulative increase in peptide identification for additional replicates or CVs.*

RESULTS

- FAIMS enables a 50% gain in peptide identification compared to conventional LC-MS/MS analyses ***(Figs.3b-c).*** The combination of differential ion mobility and *m/z* based gas phase fractionation increases the dynamic range of the C-trap and significantly reduces the contribution of interfering isobaric precursor ions (higher PIF values) ***(Fig.3e)***, and leads to improved peptide detection and identification ***(Fig.3d)*** .
- The use of 100 m/z segment for LC-MS/MS analyses enhances the detection of low abundance ions ***(Fig.4a)*** and leads to ~40% increase in peptide identification ***(Figs.4b-c)***.
- An additional 50% gain in peptide identification is achieved using FAIMS and segmented LC-MS/MS acquisition ***(Figs.4b-c).*** Dense ion population (*m/z* 550-650 and *m/z* 650-750) showed up to 3-fold gain in peptide identification ***(Fig.4d).*** PIF values are significantly higher for FAIMS (median: 0.86) compared to non-FAIMS (median: 0.65) experiments ***(Fig.4e)***.
- Segments of different m/z ranges can be combined within the same LC-MS/MS run according to the density of peptide ion population in order to maximize instrument use ***(Figs.2c, 5a)***.
- The combination of *m/z* segments in LC-FAIMS-MS/MS provides a 40% gain in peptide identification compared to segmented LC-MS/MS experiments ***(Figs.5b-c)***.

CONCLUSION

- The use of *m/z* segments enhances peptide detection by up to 40% compared to that of a standard full scan.
- The combined use of *m/z* segments and FAIMS in LC-MS/MS experiments provides up to 3-fold improvement of peptide identification compared to conventional LC-MS/MS experiments.

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