# High Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS) expands the comprehensiveness and precision of multiplex proteomics

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## Overview

**AIM:** Improved coverage and quantification in proteomic analysis using differential ion mobility.
**METHODS:** A novel high field asymmetric waveform ion mobility (FAIMS) interface was coupled to an Orbitrap Fusion Tribrid (Thermo Fisher Scientific). Isobaric labeling of peptides was used to profile dynamic changes in protein abundance upon heat shock.
**RESULTS:** LC-FAIMS-MS2 provided 2.5-fold higher number of quantifiable peptides compared to the SPS-MS3 strategy for TMT labeling with comparable fold changes.

## Introduction

Isobaric labeling of peptides provides a convenient approach to enhance the throughput of quantitative proteomic measurements, and can be achieved using different reagents including Tandem Mass Tag (TMT) labeling. However, the fragmentation of co-eluting isobaric ions can lead to chimeric MS/MS spectra and distorted reporter ion ratios. Synchronous precursor selection (SPS)-based MS3 method can alleviate this problem, though this approach can result in a reduced number of quantifiable proteins compared to traditional MS2 method. Here, we compared the analytical merits of SPS-MS3 to that of LC-MS/MS that combines a new high field asymmetric waveform ion mobility spectrometry (FAIMS) interface. LC-MS/MS experiments performed using FAIMS enhanced peak capacity and sensitivity while reducing peptide co-fragmentation thus extending the coverage of multiplex proteomic measurements[1].

## Method

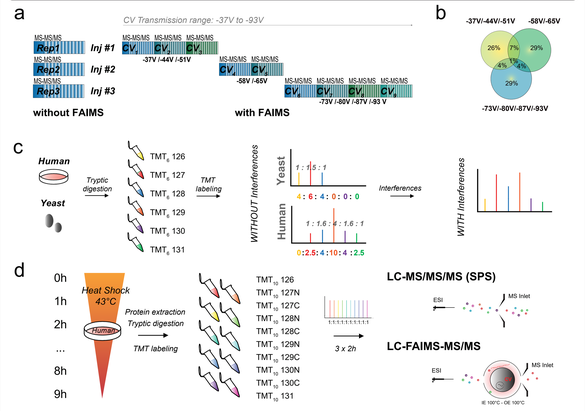
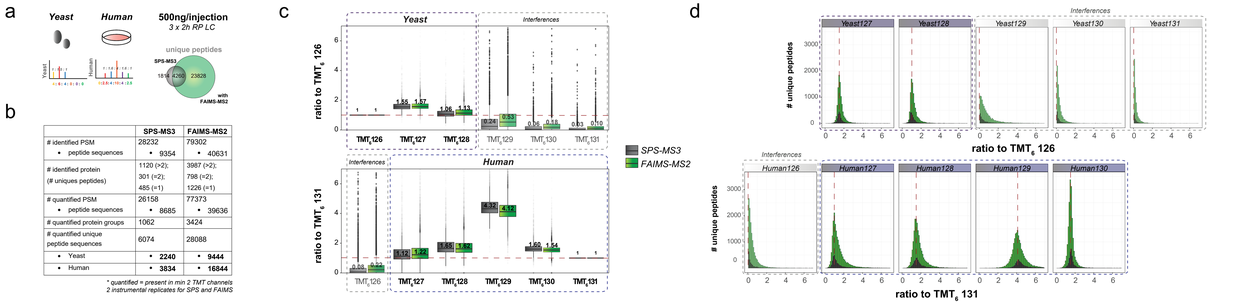



***Fig.1**: Experimental workflow. (a) All experiments were performed using LC-MS/MS on a Orbitrap tribrid Fusion mass spectrometer with a two hours LC gradient, 500ng/injection with 3s cycles. For SPS-MS3, 10 notches were selected[2]. Without FAIMS we did 3 replicates, with FAIMS we combined CVs together to cover in 3 injections the whole transmission range. (b) Optimised FAIMS method shows little overlap between the different injections and comparable number of identification. (c) Yeast and HEK293 cell extracts were reduced, alkylated and digested with trypsin prior to labeling with TMT 6-plex. (d) HEK293 cells were incubated at 43°C, and collected at 1h intervals up to 9 h prior to digestion and TMT 10-plex labeling.*

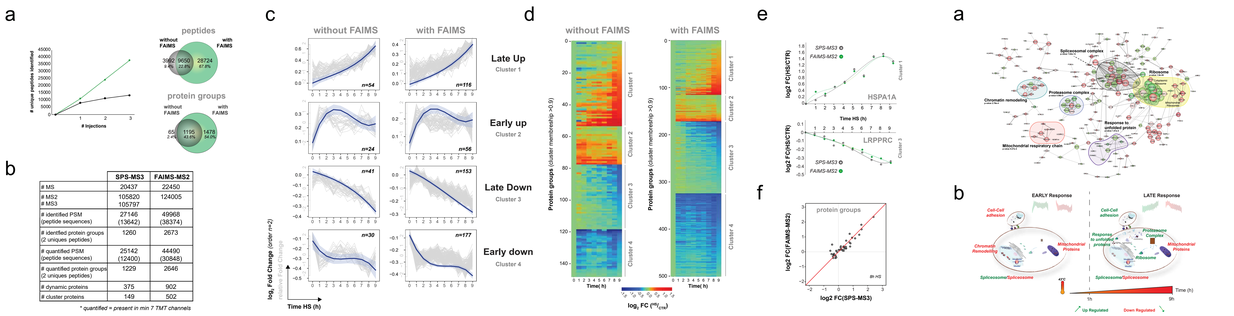
## Results




| | SPS-MS3 | FAIMS-MS2 |
|---|---|---|
| # identified PSM | 28232 | 79302 |
| • peptide sequences | • 9354 | • 40631 |
| # identified protein (# uniques peptides) | 1120 (>2); 301 (=2); 485 (=1) | 3987 (>2); 798 (=2); 1226 (=1) |
| # quantified PSM | 26158 | 77373 |
| • peptide sequences | • 8685 | • 39636 |
| # quantified protein groups | 1062 | 3424 |
| # quantified unique peptide sequences | 6074 | 28088 |
| • Yeast | • 2240 | • 9444 |
| • Human | • 3834 | • 16844 |

* quantified = present in min 2 TMT channels
2 instrumental replicates for SPS and FAIMS

***Fig.2**: Precision of TMT quantification (a) To determine the extent of co-fragmentation using FAIMS, we labeled separate aliquots of yeast and HEK293 tryptic digests with TMT reagents, and mixed those aliquots to obtain final TMT ratios of 1:1.5:1:0:0:0 for yeast and 0:1:1.6:4:1.6:1 for human extracts. Human peptides were not labeled with TMT-126, whereas channels TMT-129 to TMT-131 were not used for yeast peptides. This labeling scheme facilitated the identification of co-fragmentation arising from interfering peptides of each species. 70% of all unique sequences in SPS-MS3 were also quantified in FAIMS-MS2. (b) Summary table comparing MS analysis between SPS-MS3 (middle column) and FAIMS-MS2 (right column) for identified and quantified features. (c) Distortion of TMT ion ratios and extent of ion contamination for the two-proteome model analysed with SPS-MS3 (grey) and FAIMS-MS2 (green). Box plot and (d) frequency distribution of TMT reporter ion ratios normalized using TMT-126 and TMT-131 for for unique yeast and human peptide sequences, respectively.*




| | SPS-MS3 | FAIMS-MS2 |
|---|---|---|
| # MS | 20437 | 22450 |
| # MS2 | 105820 | 124005 |
| # MS3 | 105797 | |
| # identified PSM (peptide sequences) | 27146 (13642) | 49968 (38374) |
| # identified protein groups (2 uniques peptides) | 1260 | 2673 |
| # quantified PSM (peptide sequences) | 25142 (12400) | 44490 (30848) |
| # quantified protein groups (2 uniques peptides) | 1229 | 2646 |
| # dynamic proteins | 375 | 902 |
| # cluster proteins | 149 | 502 |

* quantified = present in min 7 TMT channels

***Fig.3**: FAIMS improves TMT quantification of the human proteome. HEK293 cells were exposed to a 43˚C heat stress for up to 9 h in 1h increments. (a) Cumulative number of unique peptides identified as a function of repeat injections for SPS-MS3 (black) or CV stepping program with FAIMS-MS2 (green). Overlap in peptide and protein identifications between the two methods are depicted in Venn diagrams to the right of the curves.(b) Summary table comparing MS analysis parameters between SPS-MS3 (middle column) and FAIMS-MS2 (right column). (c) Dynamic clusters for heat shock regulated proteins without FAIMS (left) and with FAIMS (right). The grey lines show the relative fold changes of the individual proteins with high membership (≥0.9), the blue lines the average fold changes of all the proteins in the corresponding cluster. (d) Corresponding heat map for all proteins in the clusters from (c). (e) Representative dynamic profile of HSPA1A (assigned to a late up regulation) and LRPPRC (assigned to a late up downregulation) for SPS-MS3 (grey) and FAIMS-MS2 analysis (green), highlighting virtually identical profiles/quantifications for both acquisition methods. (f) Scatterplot representations for the common dynamic (c) proteins at time point 8h.*

## Results

- The New FAIMS interface provides significant advantages in terms of instrument speed compared to the old generation FAIMS, allowing in three injections to cover the CV transmission range with optimized CV distribution ***(Fig.1a** and **Fig.1b)**.
- Using a known two-proteome model of Yeast - Human peptides ***(Fig.1b),*** FAIMS MS2 improves peak capacity[3] and reduces the occurence of co-fragmentation of isobaric precursors with precision comparable to the SPS MS3 approach ***(Fig.2)**.
- The dynamic changes of HEK293 cells exposed to hyperthermia was analyzed with total 1.5ug of peptides. FAIMS increases the number of quantifiable TMT-labeled peptides by 3-fold and proteins by 2-fold compare to the SPS MS3-based strategy for LC-MS/MS analyses ***(Fig.3b)**.
- Proteins showing changes in abundance upon heat shock were separated in 4 groups: early up- or down-regulation and late up- or down-regulation and showed similar trends for FAIMS and SPS ***(Fig.3c-d)**.
- Hyperthermia affects several key cellular processes that impact protein homeostasis, such as responses to unfolded proteins ***(Fig.4)**.

## Conclusion

- A compact FAIMS interface combined with an Orbitrap tribrid mass spectrometer improves accuracy and coverage in multiplex quantification.
- TMT-based MS2 quantitation made possible with FAIMS enabled a 2-3 fold gain in identification with high TMT ratio accuracy compared to SPS-MS3 quantitation.




***Fig.4**: Heat stress affects several key cellular processes that impact protein homeostasis. (a) Interaction network for upregulated proteins (green) and down regulated proteins (red) based on the clustering shown in Figure 3c. Proteins that belong to enriched GO-terms are outlined by coloured shapes. (b) Cellular processes affected in early and late response to heat shock.*

## Acknowledgments

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## References

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