SINGLE SHOT FAIMS-DIA OPTIMIZATION FOR DEEP COVERAGE OF THE PROTEOME

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BIOGNOSYS
NEXT GENERATION PROTEOMICS

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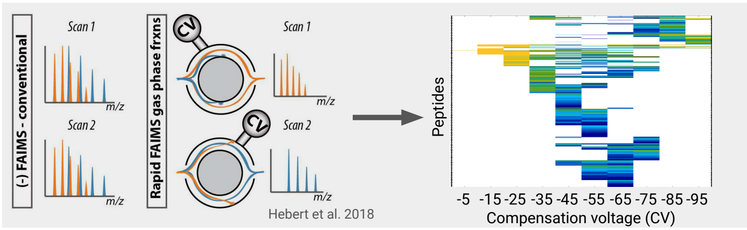
INTRODUCTION

To investigate health and disease states of biological tissues, label-free discovery proteomics is a powerful method. It enables identification and quantification of thousands of proteins in large sample cohorts. Despite this progress, there is still a large fraction of the proteome routinely not sampled. Therefore, increasing proteome coverage remains highly relevant.

We present an investigation of the suitability and benefit of the newly released ion mobility device: FAIMS Pro with the orbitrap platform for deep single shot data independent acquisition (DIA). We specifically focus on acquisition lengths that enable most comprehensive proteome coverage and benchmark the improvements on body fluids and human tissues.



Hebert et al. 2018

CONCLUSIONS

- **The FAIMS Pro device efficiently filters out a set of peptides entering the mass spectrometer**
- **Analysis of the different FAIMS spaces (CVs) enabled the selection of combinations of orthogonal CV spaces resulting in the highest protein identifications**
- **The final optimized methods result in the highest reported identified proteins in the human cell lines HeLa to date in single shots**
- **From a acquisition length of 1h and longer, more modified peptides were identified with FAIMS-DIA than in standard DIA**

RESULTS

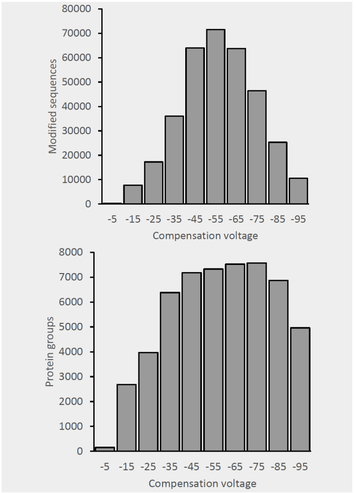


Figure 1: Characterization of FAIMS CVs in DIA
To develop optimized DIA methods for various gradient lengths, single compensation voltages (CV) were recorded spanning -5 to -95 CV.. An EASY-nLC 1200 was connected to a Thermo Scientific Orbitrap Exploris 480 equipped with a FAIMS Pro device. Precursors, proteins and charge states were calculated per CV. Importantly, the distribution of peptide and protein groups differ significantly.

Figure 2: Assessment of Productive CV Combinations
Based on the single CV FAIMS-DIA, all possible combinations were generated for 2,3,4 and 5 CVs combined. This was performed on precursor and protein level. Two CV combinations on protein level were visualized for illustration. Then for each CV multiplexing level, the combination with the highest protein level was selected

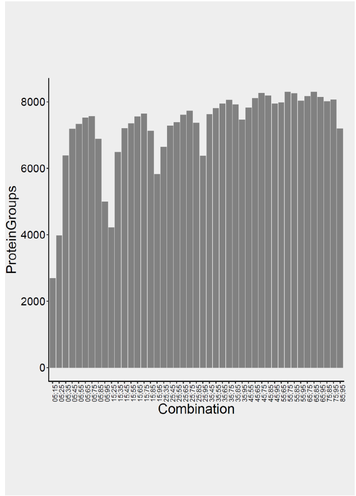
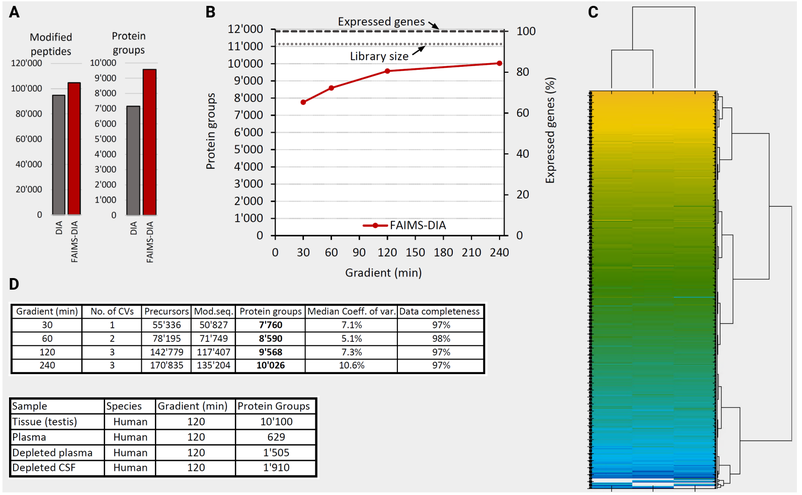


Figure 3: Benchmark of Gradient Lengths with Optimized FAIMS-DIA Methods
For benchmarking of the performance of FAIMS-DIA, an EASY-nLC 1200 was connected to a Thermo Scientific Orbitrap Exploris 480 equipped with a FAIMS Pro device. Then optimized methods were generated for non-linear gradients of 30min, 1h, 2h and 4h. Additionally, a Pierce HeLa digest, human tissue, plasma, depleted plasma and depleted CSF were used as benchmark samples.

The different gradient lengths were acquired in consecutive triplicate injections. The data were analyzed using Spectronaut 14 with a library generated from off line HPRP fractionation and FAIMS-DDA. (**A**) Comparison standard DIA to FAIMS-DIA at 2h gradient with HeLa. (**B**) The average protein group identifications per gradient length were calculated. (**C**) A heat map on protein level of the 2h HeLa acquisitions. (**D**) Tables listing the results of the FAIMS-DIA benchmark.



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Gradient (min)	No. of CVs	Precursors	Mod.seq.	Protein groups	Median Coeff. of var.	Data completeness
30	1	55'336	50'827	**7'760**	7.1%	97%
60	2	78'195	71'749	**8'590**	5.1%	98%
120	3	142'779	117'407	**9'568**	7.3%	97%
240	3	170'835	135'204	**10'026**	10.6%	97%

Sample	Species	Gradient (min)	Protein Groups
Tissue (testis)	Human	120	10'100
Plasma	Human	120	629
Depleted plasma	Human	120	1'505
Depleted CSF	Human	120	1'910