Rapid and Comprehensive Proteome Analysis Using LC-FAIMS-MS/MS

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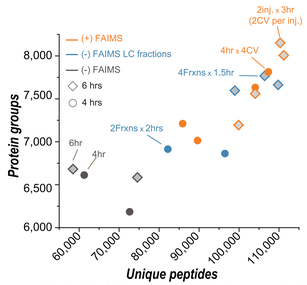
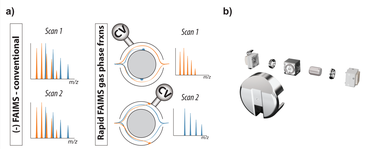
Conclusion

1) Single shot experiments with FAIMS produce >8,000 protein and >100,000 peptide identifications.
2) 1,000 more proteins quantified with addition of FAIMS.
3) FAIMS gas phase fractionation works similarly or better than LC fractionation.

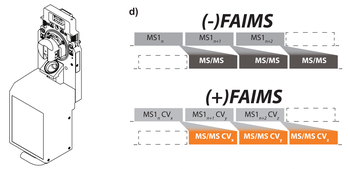


Comparing proteomic depth with and without FAIMS. Comparison of proteomic depth achieved with FAIMS (red), without FAIMS (dark grey), or from LC fractions without FAIMS (blue), in 4 hours (◆) or 6 hours (●) of instrument time. The top point consists of 2x3 hour runs, each with two CV internal stepping (-45V|-75V and -60V|-90V). The top protein result for each method type is annotated.

Introduction



FAIMS sources transmit ions through inner and outer electrodes based on their difference in mobility when in a high or low electric field is generated from an asymmetric waveform. Since this field causes the ion to disperse, the maximum peak amplitude of the asymmetric waveform is referred to as the dispersion voltage (DV). Ions with a large enough difference in mobility between the high and low field migrate towards the electrodes, while ions with no or limited difference in mobility are transmitted. **The trajectory of an ion may be altered by the addition of a DC voltage. Termed as the compensation voltage (CV), the selection of an appropriate DC level will compensate for the drift of a specific ion or group of ions, allowing them to pass through the device.** By changing the CV (**a**), alternate groups of ions will pass. Thus, the CV provides a handle by which one can control which population of ions are traversing the FAIMS device. The mobility, and thus the appropriate CV to use cannot be easily predicted and must be determined empirically. (**b**) Schematic of device used in these experiments.



FAIMS holds the promise of delivering true gas-phase fractionation for proteomics analyses by stepping through multiple CVs. Previous implementation of FAIMS instruments have typically imparted reduced sensitivity to mass spectrometry experiments because of low ion transmission. Planar electrodes suffer from diffusion causing a portion of the ion population to crash into the electrodes. The gas dynamics of cylindrical electrodes cause the vast majority of ions to collide with the center electrode upon entrance into the device. Recently a new FAIMS device with improved gas dynamics has been described. In addition to reducing losses due to collisions with the inner electrode the flow rate of the carrier gas has also been increased. Part of this redesign resulted in the gap between the inner and outer electrode being reduced, allowing for an increased electric field strength from existing drive electronics. The result is a reduced ion transit time (~20 ms). This reduction in ion transit time combined with ultrafast MS/MS sampling (60 Hz) makes internal CV stepping (intra-analysis CV switching) practical, allowing for the simultaneous analysis of multiple gas-phase fractions during a single analysis. Here we characterize how peptide ions behave in the improved FAIMS device interfaced with nanoLC and a fast-scanning Orbitrap hybrid mass spectrometer. With these data we recommend strategies for analysis of tryptic peptides and benchmark our results against fractionated and unfractionated analyses without FAIMS. (**c**) FAIMS source interfaces between nanoLC emitter and Orbitrap Fusion Lumos mass spectrometer. (**d**) Data collection scheme for standard analyses and analyses with internal CV stepping.

All results are from 60 minute analyses of human cell line K562 derived tryptic peptides, analyzed on an Orbitrap Fusion Lumos except where noted.

Characterization of CV space for proteomics

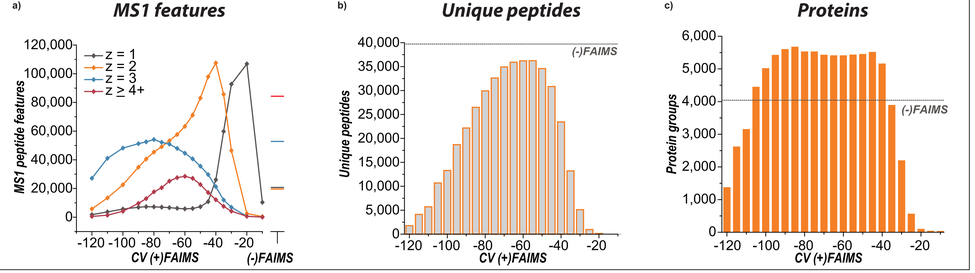
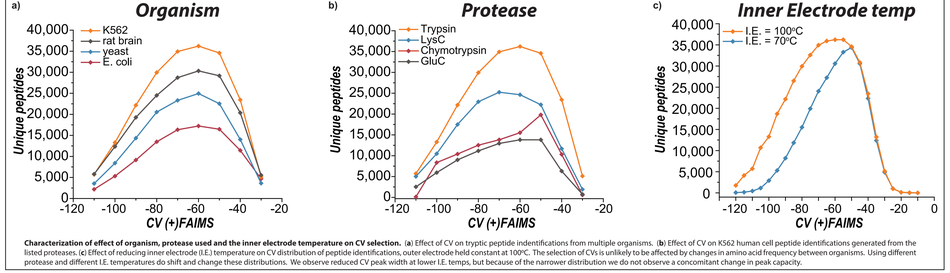


Figure 1. Characterization of FAIMS CV settings. All results are from 60 minute analyses of human cell line K562 tryptic peptides analyzed on an Orbitrap Fusion Lumos, except where noted. Distributions of (**a**) precursor charge states, (**b**) unique peptide identifications, and (**c**) protein groups across CV settings from -10V to -120V.

The vast majority of multiply charged features lie in the CV range -60V to -120V, while the charge state +1 features are almost entirely constrained to CVs between -10V and -45V. Analyses without FAIMS always yield more peptide identifications than any analysis with a single CV. Yet, there is a broad range of CVs (-40V to -105V) that can produce more protein identifications (≤ 40%) than the same analysis without FAIMS. This reduced ratio of peptides per protein is also observed when analyzing a subset of LC fractions. As more fractions (gas phase or LC) are analyzed the peptide per protein ratio increases.

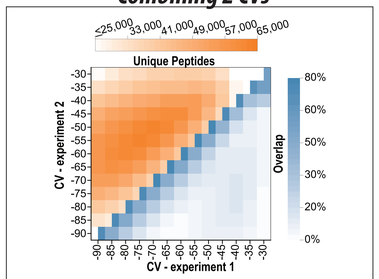
Organism, protease, and inner electrode temp



Characterization of effect of organism, protease used and the inner electrode temperature on CV selection. (**a**) Effect of CV on tryptic peptide indentifications from multiple organisms. (**b**) Effect of CV on K562 human cell peptide identifications generated from the listed proteases. (**c**) Effect of reducing inner electrode (I.E.) temperature on CV distribution of peptide identifications, outer electrode held constant at 100ºC. The selection of CVs is unlikely to be affected by changes in amino acid frequency between organisms. Using different protease and different I.E. temperatures do shift and change these distributions. We observe reduced CV peak width at lower I.E. temps, but because of the narrower distribution we do not observe a concomitant change in peak capacity.

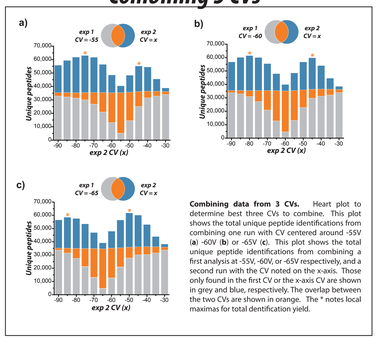
Which CVs to use and how many?

Combining 2 CVs



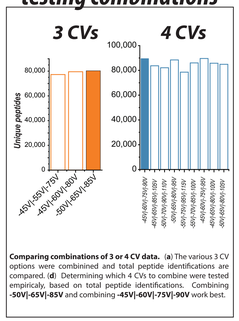
Combining data from 2 CVs. (**a**) Heat map of unique peptides combined from two CVs (orange), and the percent overlap of those identifications (blue). From these data we conclude that combining data from **-50V|70V**, or **-55V|-75V** provides the greatest proteomic depth.

Combining 3 CVs



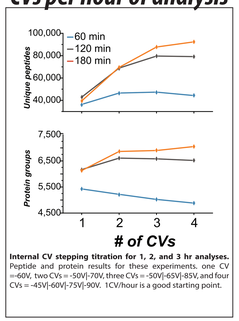
Combining data from 3 CVs. Heart plot to determine best three CVs to combine. This plot shows the total unique peptide identifications from combining one run with CV centered around -55V (**a**) -60V (**b**) or -65V (**c**). This plot shows the total unique peptide identifications from combining a first analysis at -55V, -60V, or -65V respectively, and a second run with the CV noted on the x-axis. Those only found in the first CV or the x-axis CV are shown in grey and blue, respectively. The overlap between the two CVs are shown in orange. The * notes local maximas for total identification yield.

testing combinations



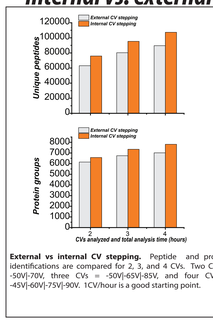
Comparing combinations of 3 or 4 CV data. (**a**) The various 3 CV options were combined and total peptide identifications are compared. (**d**) Determining which 4 CVs to combine were tested empirically, based on total peptide identifications. Combining **-50V|-65V|-85V** and combining **-45V|-60V|-75V|-90V** work best.

CVs per hour of analysis



Internal CV stepping titration for 1, 2, and 3 hr analyses. Peptide and protein results for these experiments. one CV =-60V, two CVs = -50V|-70V, three CVs = -50V|-65V|-85V, and four CVs = -45V|-60V|-75V|-90V. 1CV/hour is a good starting point.

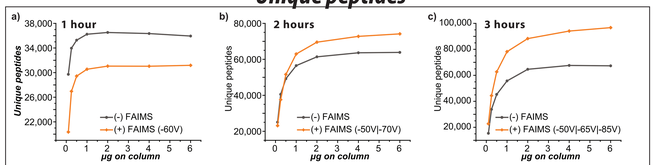
internal vs. external



External vs internal CV stepping. Peptide and protein identifications are compared for 2, 3, and 4 CVs. Two CVs = -50V|-70V, three CVs = -50V|-65V|-85V, and four CVs = -45V|-60V|-75V|-90V. 1CV/hour is a good starting point.

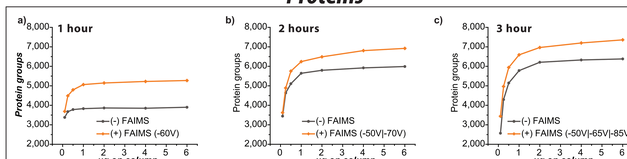
Loading Experiments

Unique peptides



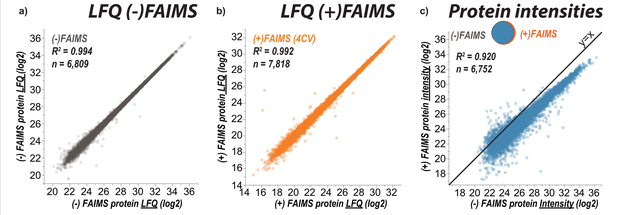
Loading-response comparison with and without FAIMS for multiple analyses durations. All data is from tryptic peptides derived from K562 human cells. FAIMS experiments use internal CV stepping with the noted CVs. Loading amounts tested are 0.1, 0.25, 0.5, 1, 2, 4, and 6 µg on column. Unique peptide comparison for 1, 2, and 3 hour analyses. (**a**,**b**,**c**). Internal CV stepping was performed for FAIMS enabled runs using the number of CVs and voltages noted.

Proteins

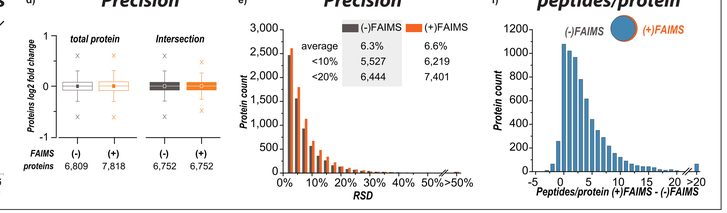


Loading-response comparison with and without FAIMS for multiple analyses durations. All data is from tryptic peptides derived from K562 human cells. FAIMS experiments use internal CV stepping with the noted CVs. Loading amounts tested are 0.1, 0.25, 0.5, 1, 2, 4, and 6 µg on column. Protein comparison for 1, 2, and 3 hour analyses. (**a**,**b**,**c**). Internal CV stepping was performed for FAIMS enabled runs using the number of CVs and voltages noted.

Quantitation



Comparison of injection replicate LFQ with and without FAIMS. All data is from 4 hour analyses of 6 µg K562 tryptic peptides injected back-to-back. Data points have 70% transparency to show data density. For the FAIMS analyses 4 CVs (-45V|-60V|-75V|-90V) were rastered across during the analysis (internal CV stepping). MS/MS were collected for 0.6 sec at each CV for a total cycle time of 2.4 sec. Correlations of log2 LFQ protein intensity values for injection replicates without FAIMS (**a**), and with FAIMS (**b**). (**c**) Correlation of averge log2 total protein intensity values for proteins quantified in both modes. (**d**) Box plots of protein LFQ fold change measurements between injection replicates. Box = inner 50%, whiskers = 5% and 95%, x's = 1% and 99%. (**e**) Histogram of RSD values for protein measurements between injection replicates in both modes. (**f**) Histogram of difference in peptides per protein identified with FAIMS compared to without. The total cycle time impacts quantitation, the FAIMS cycle time for these analyses is 0.6 seconds per CV translating to a total cycle time of 2.4 seconds between MS survey scans with the same CV. As expected the cycle time has an inverse correlation with quantitative reproducibility. Regardless, the protein measurement reproducibility is similar with and without FAIMS, and FAIMS-enabled quantitation produces nearly 1,200 more protein measurements with <20% RSD. FAIMS enabled experiments access quantitative values from more peptides per protein (3.7 more unique peptides per protein on average), balancing out any negative effects from the 2.4 second cycle time. Nearly twice as many isotope patterns are observed in the FAIMS runs and fewer protein measurements rely on the matching between runs function, which points to higher quality data overall.



References and Acknowledgments

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LC-MS/MS methods:

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