Inference of Collisional Cross-Sections of peptides in an Orbitrap Mass Analyzer

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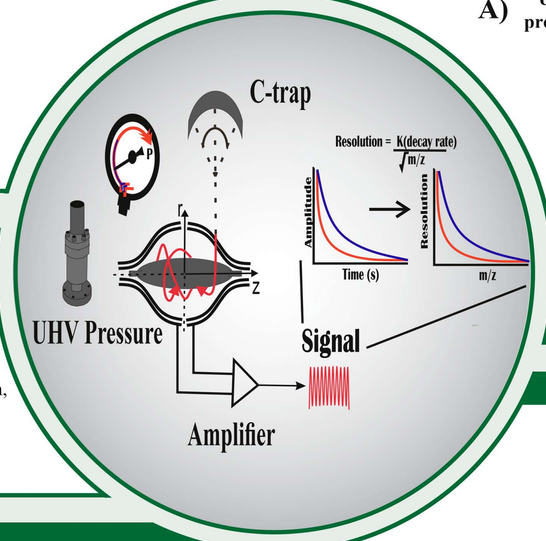
1 HIGHLIGHTS

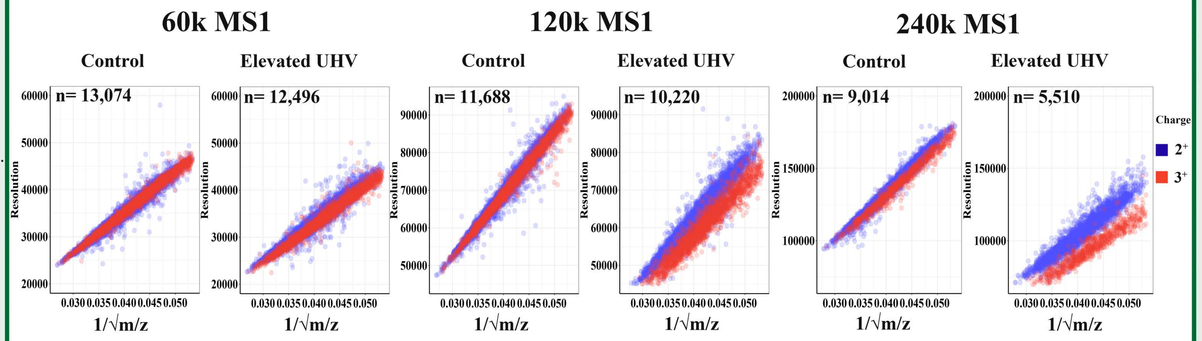
- >Inferred CCS values from common proteomic workflows
- >Requires only minor hardware modifications to an Orbitrap Exploris 480 ™
- >Comparable results to previous published CCS values
- >Does not require a dedicated ion mobility cell

2 INTRODUCTION

Ion collisional cross section (CCS) can add an extra dimension to proteomic workflows; however, these workflows often require the integration of an ion mobility cell within a mass spectrometer. Recently, a novel method for determining ion CCS, which does not require a dedicated ion mobility cell, was described[1,2], whereby the CCS values were based on ion decay rates in the time-domain transient signal measured in the FTICR and the Orbitrap™ analyzers. Herein, we extend this strategy to peptide ions in complex proteomic samples through introducing a minor modification to an Orbitrap Exploris 480 ™ mass spectrometer.



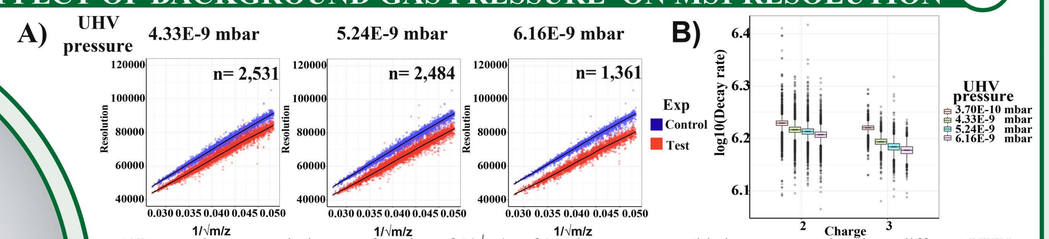
3 WORKFLOW OPTIMIZATION



Reported mass resolution for charge state 2+ and 3+ of tryptic peptides as a function of 1/√m/z for different resolution settings acquired at 3.70E-10 (Control UHV) or 4.33E-9 (Elevated UHV) mbar, n = median of detected peptides.

Data at the two different UHV pressure conditions were collected in quadruplicates. For each single replicate, 100 ng of HeLa tryptic digest was separated using 21 min gradients via nanoflow liquid chromatography coupled online to a research grade Orbitrap Exploris 480™ mass spectrometer. The experiments were run at two different UHV pressure conditions (4.33E-9 and 3.70E-10 mbar for control) in quadriplicates. The experiments were conducted with MS1 resolution settings of 240k, 120k, and 60k, while the MS2 was kept at 15k. The data analysis was performed using a custom software suite built on top of MaxQuant. The results show that 240k resolution setting provides more discriminatory power in terms of CCS; however 120k, resolution provides the best compromise between identification rates and CCS discriminatory power.

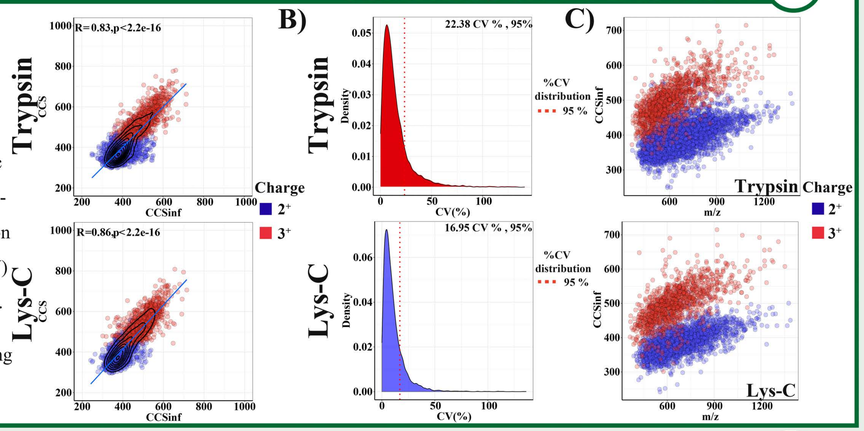
4 EFFECT OF BACKGROUND GAS PRESSURE ON MS1 RESOLUTION



A)Reported mass resolution as a function of 1/√m/z of 3+ charge state peptide ions measured at three different UHV pressure settings (labeled "Test") as compared to a UHV pressure of 3.70E-10 (Control).Data was collected in triplicates and n = median of detected 3+ ions; B) The decay rates in log 10 scale for 2+ and 3+ ions at the three different UHV pressure conditions.

The results show that as pressure in the UHV region increases, the ion transient decay also increases, allowing for better resolution in the CCS domain; however, with this increase in pressure and transient decay, the quality of the individual spectra decreases and may reduce the rate of identification.Therefore,a balance between UHV and identification rate must be achieved.

5 COMPARISON TO IMS DATA



Comparison of published CCS values[3] for tryptic (top panels) and Lys-C Hela peptides (bottom panels) vs. the inferred CCS (CCSinf) value determined by the decay constant is shown (A).

The experimental results show a linear correlation between the two data sets.

Panel B shows the coefficient of variation (%CV) of the calculated CCSinf values for tryptic and Lys-C peptide ions (top and bottom, respectively).

Panel C shows the CCSinf as a function of ion m/z, showing that the distribution of all detected peptide ions separate by charge state, likely owning to an increase of amino acid length, and thus subsequent increase in CCS, with an increase in observed peptide charge state.

6 CONCLUSIONS & REFERENCES

>The optimization of the experimental protocol across the entire LC-MS process demonstrated the feasibility of FTMS instruments to provide comparable with IMS results on the complete mammalian proteome scale, which has a promise of adding an additional analytical dimension to the technique.

[1] Yang, F.; Voelkel, J. E.; Dearden, D. V. (2012). Collision Cross Sectional Areas from Analysis of Fourier Transform Ion Cyclotron Resonance Line Width: A New Method for Characterizing Molecular Structure. Analytical chemistry, 84, 4851−4857.
[2] Sanders, J. D., Grinfeld, D., Aizikov, K., Makarov, A., Holden, D. D., & Brodbelt, J. S. (2018). Determination of collision cross-sections of protein ions in an orbitrap mass analyzer.Analytical chemistry, 90(9), 5896-5902.
[3] Meier, F., Köhler, N. D., Brunner, A. D., Wanka, J. M. H., Voytik, E., Strauss, M. T., ... & Mann, M. (2021). Deep learning the collisional cross sections of the peptide universe from a million experimental values. Nature communications, 12(1), 1-12

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