Exploring linear sequence determinants of inferred Collisional Cross-Sections of unmodified and phosphorylated peptides in an Orbitrap Mass Analyzer

Ulises H. Guzman[1], Konstantin Aizikov[2], Kyle Fort[2], Martin Rykaer[1], Jeppe Madsen[1], Ana Martinez Del Val[1], Alexander Makarov[2], Jesper V. Olsen[1].

[1] The Novo Nordisk Foundation Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark.
[2] Thermo Fisher Scientific (Bremen) GmbH, Bremen, Germany.

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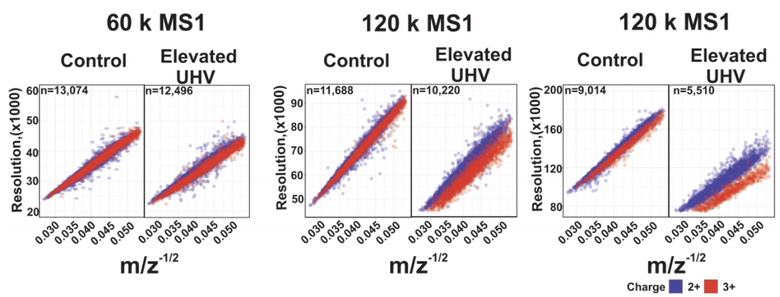
1 HIGHLIGHTS

- High-Throughput CCS values inference from common proteomic workflows.
- Comparable results to previous published CCS values
- Does not require a dedicated ion mobility cell
- Require only minor hardware modifications to an Orbitrap Exploris 480
- Capable to discriminate between unmodified and phosphorylated peptides In the CCS vs m/z space

2 INTRODUCTION

Ion collisional cross-sections can add an extra dimension to proteomics workflows. 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 FTIR and Orbitrap analyzers. Herein, we extend this strategy to peptide ions in complex proteomics samples trough introducing a minor modification to an Orbitrap Exploris 480 mass spectrometer. Briefly, this strategy takes advantage of the observed decrease in full-scan resolution of Individual peptide ions at high UHV pressures and high MS1 resolution enabling the inference of peptide CCS values without dedicated ion mobility cell.

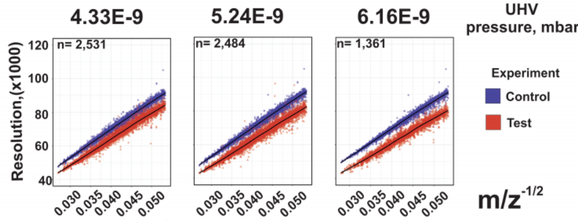
3 WORKFLOW OPTIMIZATION. MS1 RESOLUTION



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 480TM 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.

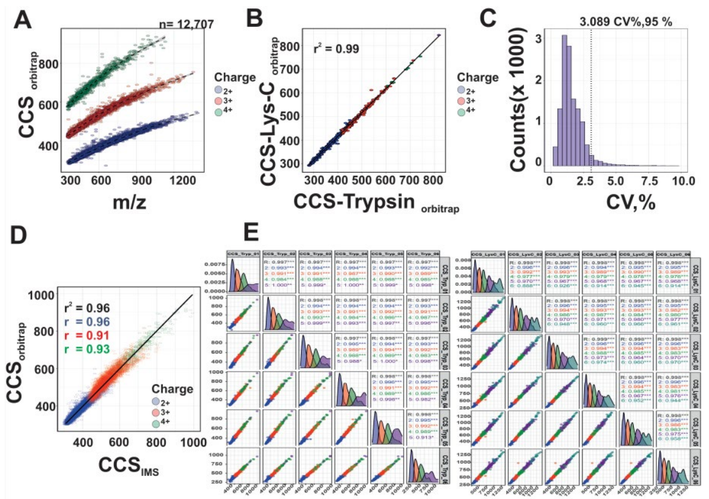
4 EFFECT OF BACKGROUND PRESSURE ON MS1 RESOLUTION



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.

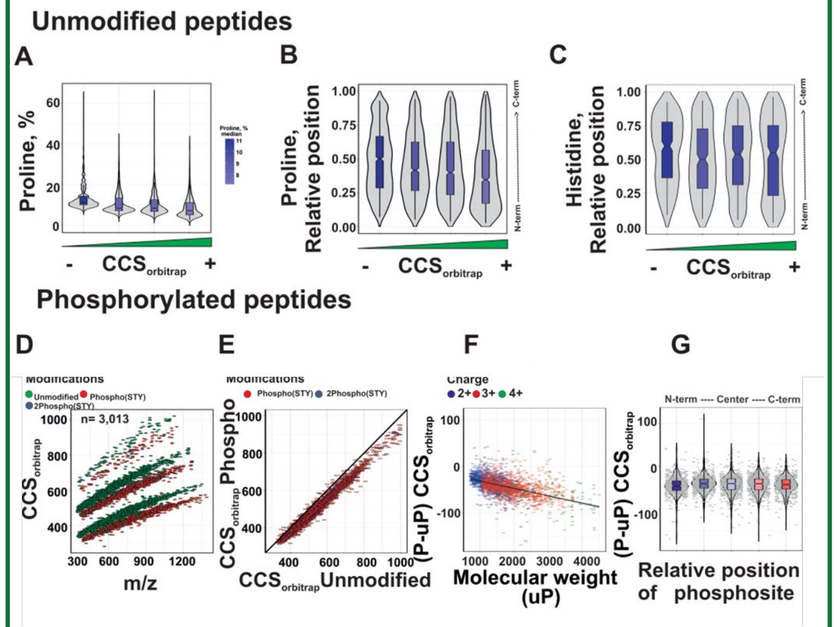
The results show that as pressure in the UHV region increases, the ion transient decay also increases, allowing for better resolution in the CCS domain; however, with this increase in pressure and transient decay, the quality of the individual spectra decreases and may reduce the rate of identification.Therefore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

5 REPRODUCIBILITY AND COMPARISON TO IMS DATA



Panel A shows the distribution by charge of 12,707 unique data points including modified sequences (Methionine oxidation and Nterm-acetylation) and charge state in the CCS vs m/z space. The distribution of the detected peptide ions separates by charge state, likely owning to an increase of flexibility and amino acid variability of longer peptides, commonly observed in multiple charge state. CCS values between tryptic and Lys-C shared peptides are shown in (B) (n=840). The coefficient of varaiation distribution (%CV) of the calculated CCS ($CCS_{orbitrap}$) is presented in (C). The linear aligment between peptide CCS values which share sequence but originating from different sources and a relative low CV% point out the feasibility of comparing CCS values across different data sets. Comparison between plublished CCS values[3] for tryptic and Lys-C peptides vs CCS orbitrap inferred by the decay constant is shown in (D). Both datasets show a linear correlation, with a coefficient of determination (r2) of 0.96 and pearson correlation of about 0.9 per charge state. Finally, Panel E shows a correlation matrix of seis independent tryptic and Lys-C peptides LC-MS runs (left and right, respectively). The pearson correlation between all replicates is nearly 1 for all the cases.

6 EXPLORING THE LINEAR SEQUENCE DETERMINATS OF CCS



The relationship between the proline abundance and its relative position with CCS values is shown in Panel A and B. Briefly, peptides with a large proportion of prolines and internal prolines have small CCS values, likely owing to increased solvation capacity of positively charge sites, provided by the proton high affinity of the proline backbone amide. Panel C shows the effect of histidine position on CCS values on triply charged peptides. The histidine-containing peptides with a histidine nearby the C-terminus(K,R) have a compact conformation, which illustrates the dependency of CCS with the peptide charge solvation capacity. CCS values of phosphopeptides and their corresponding unmodified version as function of m/z is shown in (D). The phosphorylated peptides follows a similar trend to their unmodified version, but their CCS are reduced, which agrees with IMS reports[4]. Panel E shows the phosphorylation compaction effect on CCS. Delta CCS values (P-uP; phosphorylated and unphosphorylated peptide, respectively) as function of the uP molecular weight (MW) is shown in (F). The negative trend between MW and the phosphorylation compression of CCS points out that CCS of large and multi-charge peptides are more strongly affected by phosphorylation. The relationship between phosphorylation sites and CCS is shown in (G). Phosphosites nearby terminal regions of a peptide appear to increase the compression effect of phosphorylation

7 CONCLUSIONS AND REFERENCES

The Collisional Cross-Section infearence on a orbitrap analyzer demostrate to provide reproducible and comparable data to IMS in proteome scale without the need of a dedicated ion mobility cell. Likewise, the geerated CCS data allows to explore gas-pase structural determinants of CCS in the m/z vs CCS space, which has promise to add an additional dimensión of FTMS instruments.

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