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Improved MS/MS Quality and Higher ID Rates Through Charge Tailored Collision Energies in dda-PASEF

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Introduction

Collision energy (CE) is a key parameter in data dependent acquisition (DDA) workflows, directly influencing peptide fragmentation efficiency and MS/MS spectral quality. Default CE settings are typically optimized in a global manner and therefore do not account for the combined dependencies on precursor m/z, ion mobility, and charge state. This can lead to suboptimal fragmentation and reduced identification performance. Here, we systematically developed and evaluated charge dependent CE optimization strategies for dda-PASEF on a timsTOF platform, aiming to improve MS/MS quality and peptide identification rates.

Methods

CE database generation: 26 human digest files acquired with distinct CE settings across m/z 100–1750 and ion mobility 0.65–1.35 Vs/cm² (dda_PASEF, timsUltra AIP, timsControl 7.0, Bruker Daltonics).

Database search & metrics extraction: Searches performed with SAGE (1); peptide- and spectrum-level metrics extracted, complemented by custom Python scripts.

MS/MS quality evaluation: Fragmentation rate, SAGE hyperscore, matched fragment intensities, summed b- and y-ion intensities.

CE optimization & implementation: Optimal CE values derived using boxplot statistics, 2D binning, and model fitting to generate continuous CE functions; implemented via the timsControl Python API and evaluated against the default ion-mobility-based CE method

Results

Based on these optimized CE values, three charge dependent CE optimization strategies were developed:

a) an m/z based approach,
b) an ion mobility based approach ($1/K_0$), and
c) a combined m/z and ion mobility based approach.

(1) Lazear, M. Sage: An Open-Source Tool for Fast Proteomics Searching and Quantification at Scale, Journal of Proteome Research. 22(11), 2023.

Conflict of Interest Disclosure: all authors are currently employed by Bruker.

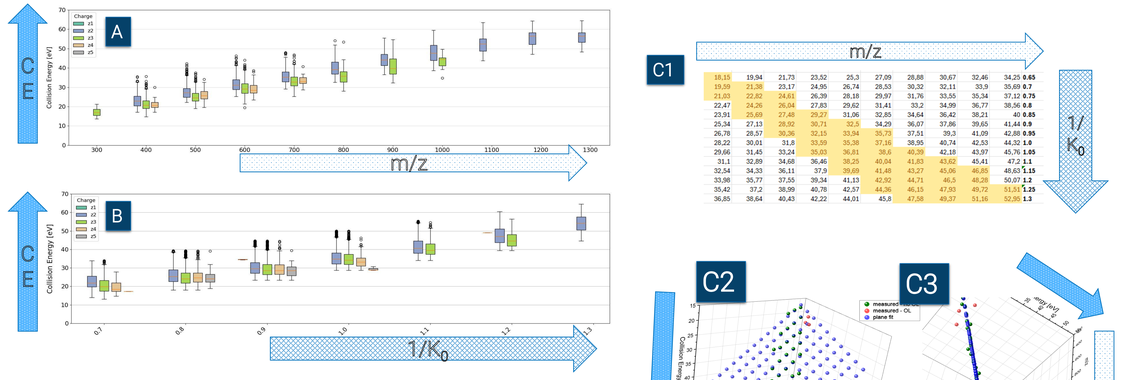


Fig. 1 Optimized Collision energies for 3 charge dependent approaches.
A: m/z approach; B: ion mobility approach
C: combined m/z ion mobility based approach. C1 shows the derived and extrapolated CE functions. C2 and C3 display 3D CE landscapes with determined values (green), outliers (red), and fitted functions (blue).

All three strategies were implemented using the timsControl Python API, which allows real time modification of DDA acquisition schemes. This enables dynamic precursor scheduling as well as precursor-specific collision energy assignment during data acquisition. Consequently, collision energies could be explicitly defined as a function of precursor charge state and either m/z, ion mobility ($1/K_0$), or a combined dependence on both parameters (Fig 1).

Application of the optimized CE strategies resulted in comparable numbers of peptide identifications (Fig 2) across all three approaches for a human cell line digest, while consistently outperforming the default CE method. Overall, peptide identifications increased by approximately 11 % across all charge states relative to the default setting.

In addition to higher identification rates, multiple MS/MS quality metrics showed systematic improvements. These included:

- increased fragmentation rate,
- higher SAGE hyperscores,
- increased MS2 intensity,
- enhanced summed intensities of b- and y-type fragment ions,
- and higher matched intensity (%).

Two-dimensional difference plots demonstrated (Fig 3 Top) consistent quality gains across the explored m/z and ion mobility space. Manual inspection of representative MS/MS spectra with delta CE 4.6 eV further confirmed these findings (Fig 3 Spectra), revealing higher signal-to-noise ratios and more clearly resolved fragment ion series when applying the optimized CE settings.

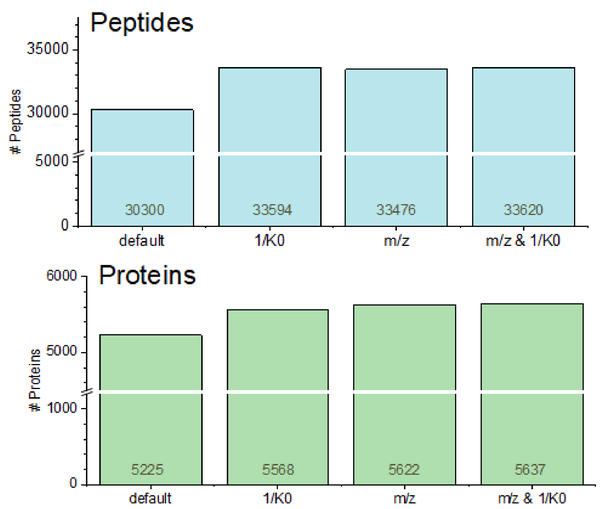
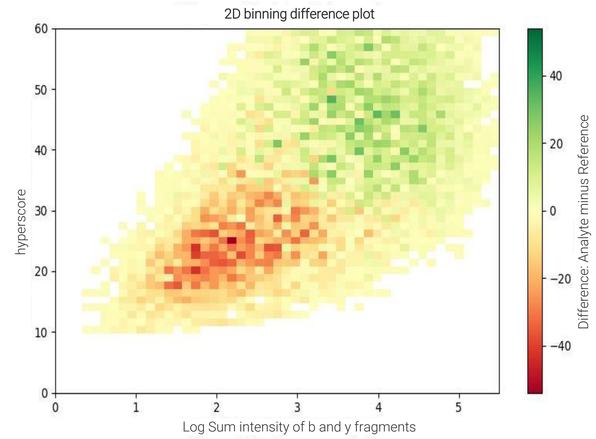
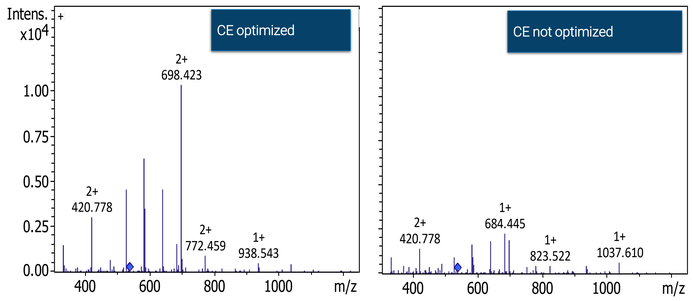


Fig. 2 Peptide and protein IDs
Peptide IDs were 11 % higher for all 3 CE optimization approaches compared to the results from data acquired with default method.





Parameter	CE not optimized	CE optimized
Fragmentation Rate [%]	71	88
Sum Intensities b and y fragments	2799	23221
Matched intensity [%]	17	42
Number of matched peaks	24	30
SAGE hyperscore	63.1	77.2
MS2 intensity	16125	54794
Collision Energy [eV]	33.70	27.81
RT [min]	23.1	23.1

Fig. 3 Comparison of fragment spectrum quality
2D binning difference plot (50 × 50 bins) shows higher summed b/y-ion intensities and hyperscores with optimized CEs (green vs red; ion-mobility approach, charge 2). Representative MS/MS spectra illustrate improved fragmentation with optimized CE.

Summary & Conclusion

- Three charge dependent approaches for CE optimization for dda-PASEF have been investigated.
- All strategies demonstrated a clear positive impact of incorporating precursor charge state into CE selection for DDA data acquisition.
- This led to both an increased number of identified peptides and a systematic improvement in MS/MS spectral quality, which will have a pronounced impact in driving new biology in immunopeptidomics identification

timsUltra AIP

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