Optimizing dia-PASEF isolation window schemes for proteomics measurements on a timsTOF ultra instrument

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Introduction

Data-Independent Acquisition (DIA) is widely used for proteomics as it usually outperforms Data-Dependent Acquisition (DDA) for protein identification and quantitation, due to its higher ion usage and reproducibility, resulting from a fixed scheme of rather broad isolation windows. This advantage can be further increased by combining it with trapped ion mobility separation (TIMS), as the additional separation dimension reduces complexity and the sequential elution of condensed ion packages from the TIMS device allows for even more efficient ion usage (dia-PASEF). The two-dimensional mass and mobility space enables method creation with extensively different window schemes.

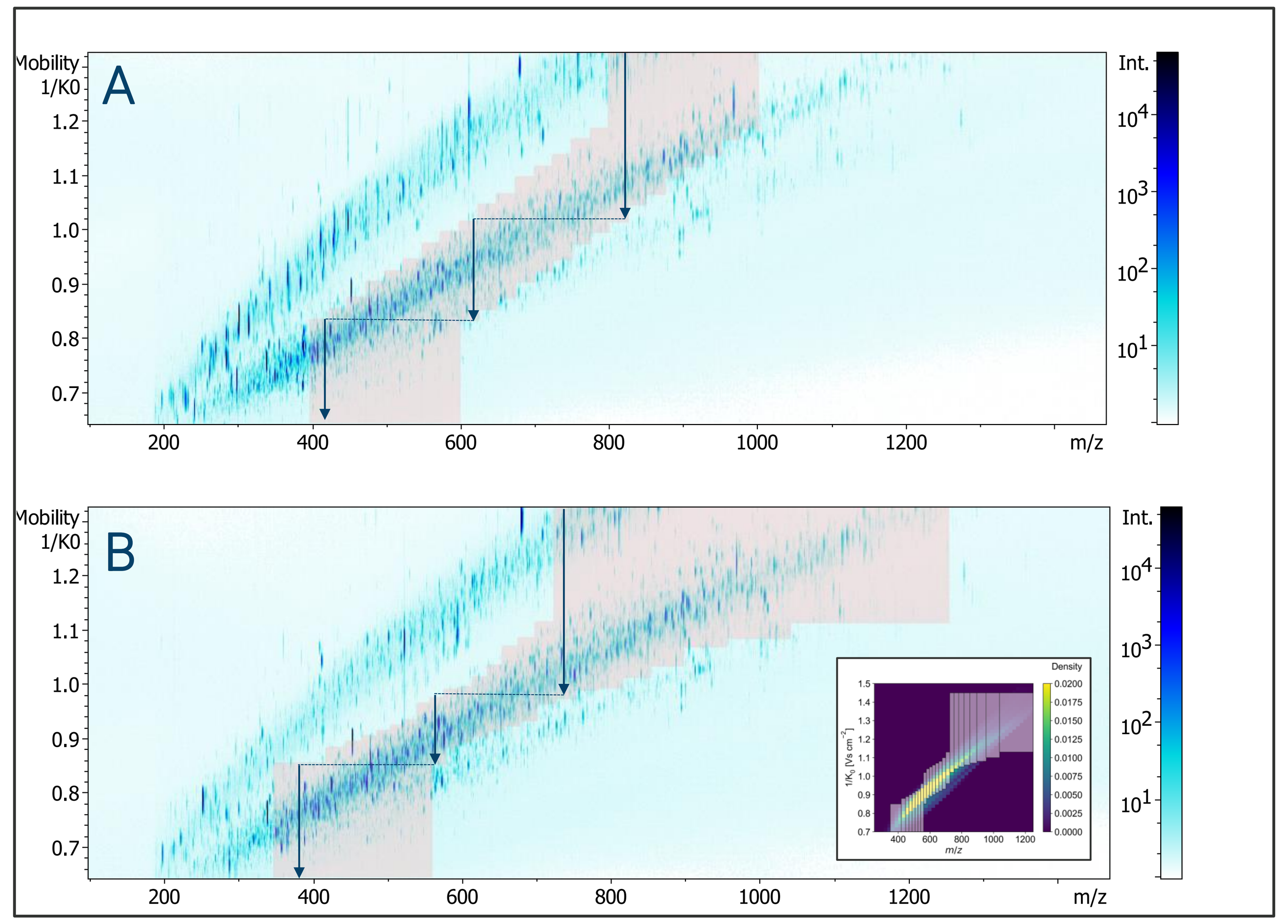

Fig. 1: dia-PASEF window schemes (3x8). During an ion mobility scan the quadrupole switches its isolation position so that 3 isolation windows are covered within a single TIMS scan, thus only 8 TIMS scans are required to cover all 24 windows.
A: Scheme with a fixed 25 m/z isolation width for all windows.
B: Individual isolation widths normalized to precursor density of a spectral library, optimized by the py_diAID software tool[1].

Methods

Tryptic digest from human cell line K562 (Promega) was diluted with 0.1 % formic acid to a final concentration of 100 ng/µl. To achieve a concentration representing digests of single cells the stock solution was further diluted in 4 steps to a final concentration of 0.250 ng/µl. To prevent peptide losses due to absorption to hydrophobic surfaces, 0.015% n-Dodecyl-β-D-Maltosid (DDM) was added to the dilution buffer.

Peptide samples were separated on a NanoElute (Bruker) using 22minute linear gradients from 5 % to 35 % acetonitrile in 0.1% formic acid at 250 nl/min. A 25 cm, 75 µm ID column with integrated nanospray emitter (Aurora Ultimate, IonOpticks) was used in combination with a CaptiveSpray ultra source.

Data were acquired on a timsTOF-ultra mass spectrometer. Dia-PASEF window schemes were initially calculated using the py_diAID[1] software tool and further fine-tuned manually (Fig. 1 B).

Data were processed with dia-NN 1.8.1 using a library with 560,000 precursors derived from a deeply fractionated K562 sample. All measurements were performed in triplicates; cross-run normalization was switched off.

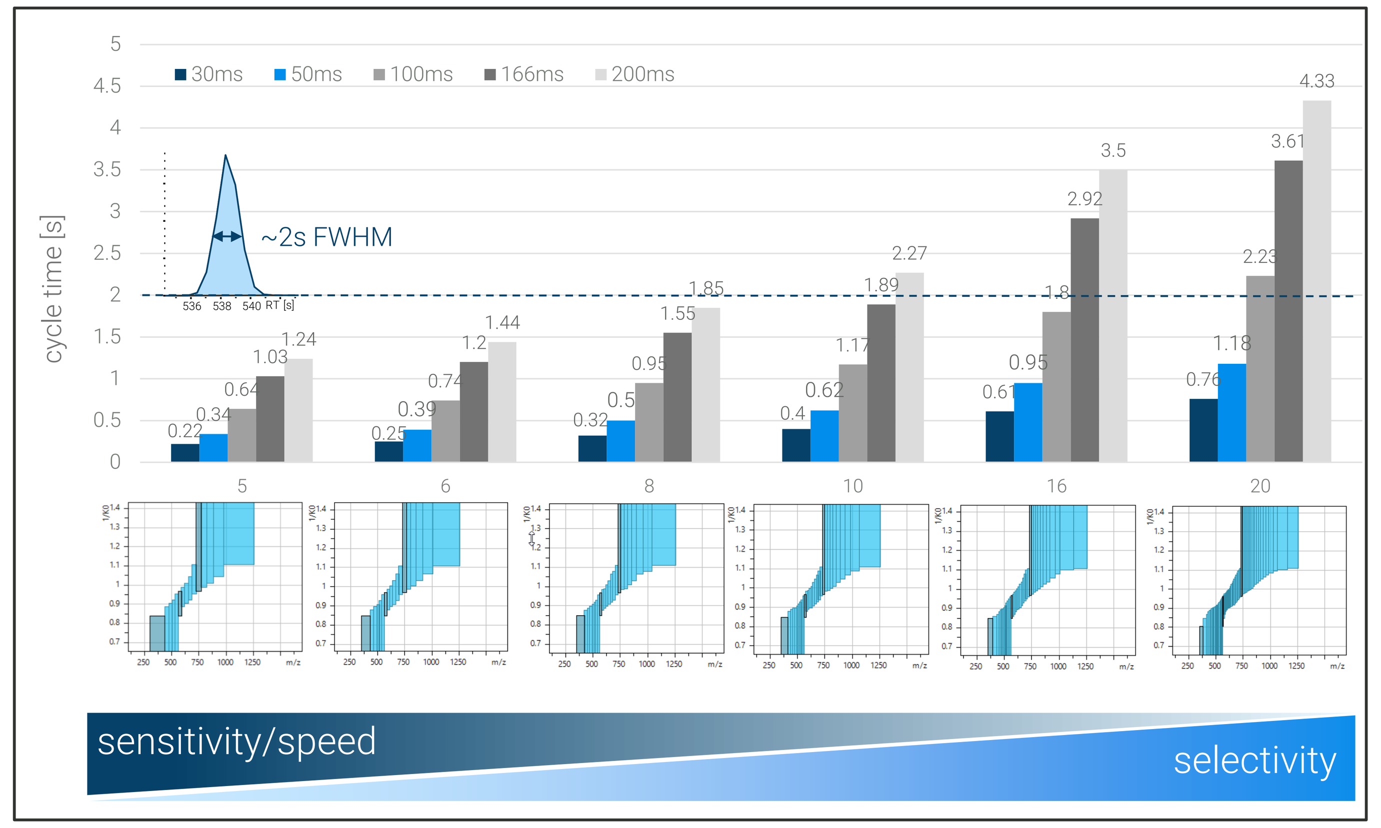

Fig. 2: dia-PASEF cycle times for different window schemes in combination with varying TIMS scan/accumulation times. All schemes cover the same m/z-mobility range. A higher number of narrower windows will increase selectivity, but also result in a loss of overall sensitivity, as individual ions will be fragmented less frequently. For the detection of very low abundant ion species longer accumulation of ions prior to detection would be preferred over averaging a multiplicity of sparse signals. However, if the cycle time exceeds a certain threshold, quantitation will be impaired by undersampling chromatographic peaks, thus acquisition methods with a cycle time greater than 2s (~FWHM) were not considered.

Results

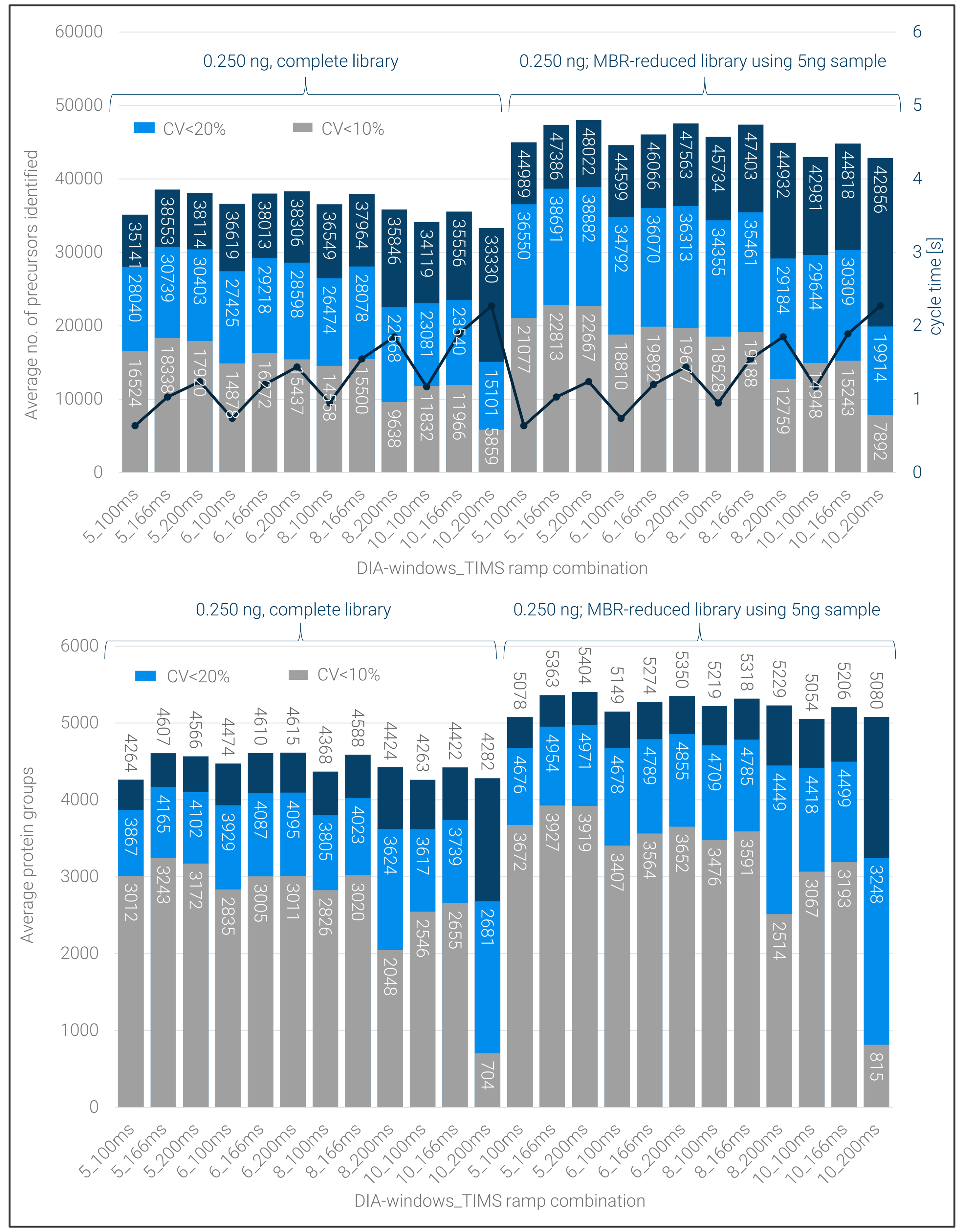

Fig. 3: dia-NN results from 0.250 ng K562 digest separated using a 22min gradient and a selection of the dia-PASEF window schemes and TIMS ramp time combinations described in Figure 2.
The same data were processed either solely with a library of 560,000 precursors or using an initial search with the same library together with data from a 5ng sample, resulting in a reduced library for the final 2nd -pass search (Match Between Runs, MBR).

[1]Skowronek et.al., Mol Cell Proteomics (2022) 21(9) 100279 https://doi.org/10.1016/j.mcpro.2022.100279

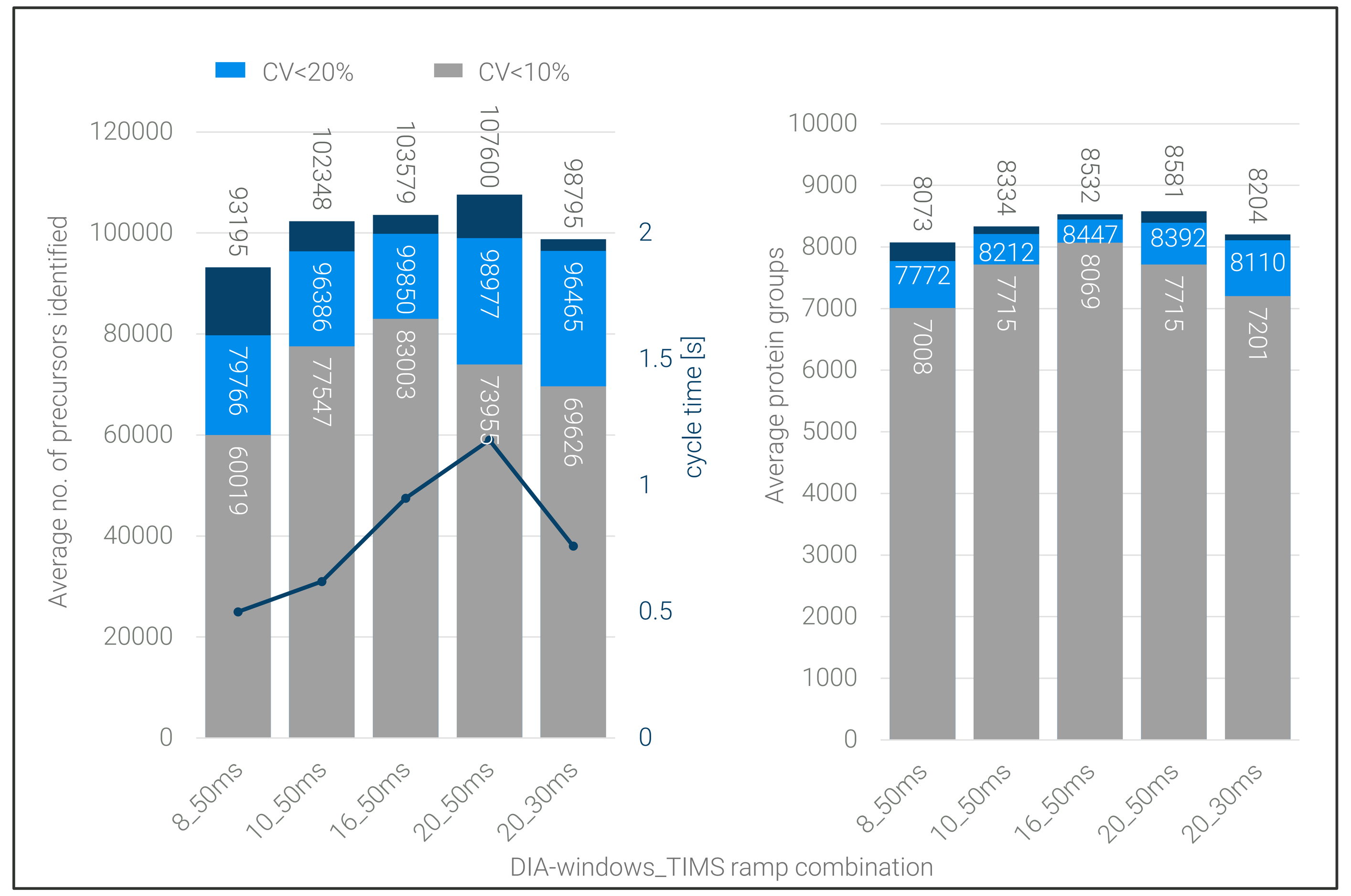

Fig. 4: dia-NN results from 100ng K562 digest separated with a 22min gradient using window scheme and TIMS ramp combinations from Figure 2 with a higher selectivity (higher number of windows and shorter TIMS ramp times).

Summary

Dia-PASEF results are remarkably robust towards changes in acquisition schemes (isolation widths and TIMS ramp times), if the cycle time allows for at least 4-5 points across the chromatographic peak. For high sensitivity a slight benefit of longer TIMS ramps with fewer, broader windows can be observed.

In general variations are low (10-15 %) and allow for easy adaption to individual chromatographic separations.

Conclusion

- Highest sensitivity and depth: 5400 protein groups can be identified and quantified with >47,000 precursors from 0.250 ng cell digest; 8500 protein groups with >105,000 precursors from 100 ng.
- TIMS enables efficient ion usage and selectivity due to pre-separation of precursor ions into condensed ion packages, that are fragmented consecutively.
- A broad precursor range can be covered, while maintaining high acquisition speed without extensive method optimization.

timsTOF-ultra