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Evaluation of diagonal-PASEF for Data Independent Acquisition in Proteomics

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Introduction

Data independent acquisition with and without additional ion mobility separation or fractionation became the most widely used method for proteomics due to its high level of sensitivity, reproducibility, and data completeness. To increase sensitivity and sample throughput, a lower number of broader isolation windows needs to be used, which leads to reduced selectivity. The combination with dispersive ion mobility separations like Trapped Ion Mobility (TIMS) prior to quadrupole isolation increases selectivity, however the quadrupole has to switch isolation position in multiple steps to follow the mobility separation. A more efficient way to sample the mass-mobility range is to scan the quadrupole in a way to continuously follow the ion cloud eluting from the TIMS (diagonal-PASEF). This type of acquisition scheme should show advantages for low sample amounts or very short gradients where high sampling speed while maintaining selectivity is especially important.

The DIA processing software needs to be adapted for diagonal-PASEF. Spectronaut 19 (Biognosys) is able to process these type of data.

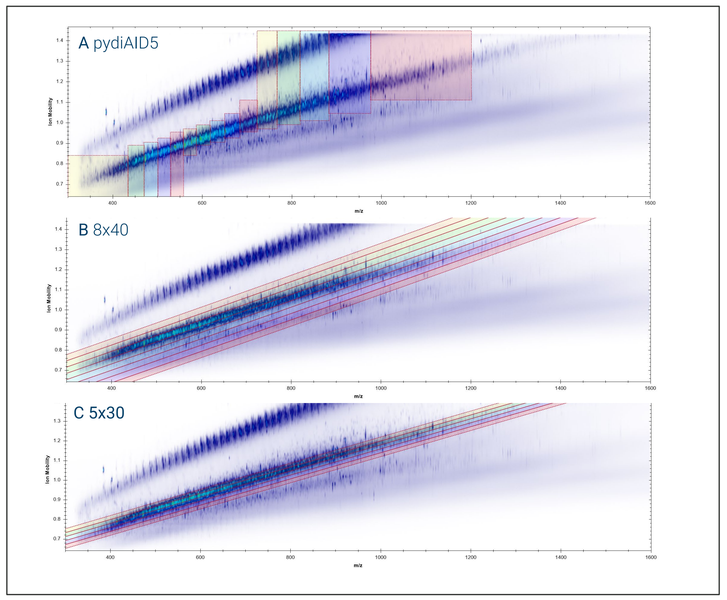

Fig. 1 diaPASEF and diagonalPASEF acquisition schemes
A: classic dia-PASEF isolation with the quadrupole switching isolation position and width twice during a TIMS scan, isolation widths are normalized to precursor density (pydiAID[1]). B: diagonal-PASEF acquisition using scanning quadrupole isolation covering a broad (320 Th) precursor range and C selectively focussing on region of highest precursor density with 150 Th total width.

Methods

Tryptic digest from human cell line K562 (Promega) was diluted with 0.1 % formic acid with 0.015% n-Dodecyl-β-D-Maltosid (DDM) added to prevent peptide losses due to absorption to hydrophobic surfaces.

Single HeLa cells were separated and digested using the CellenOne system (Cellenion).

Samples were separated on a nanoElute 2 (Bruker) using linear gradients from 5 % to 35 % acetonitrile in 0.1% formic acid at 250 nl/min with gradient lengths as indicated. Columns of 75 µm ID with integrated nanospray emitter (5 or 25 cm length, depending on gradient, Aurora, IonOpticks) were used in combination with a CaptiveSpray Ultra 2 source.

Data were acquired on a timsTOF Ultra 2 mass spectrometer, or a timsTOF HT in case of high sample amounts. Data were processed with Spectronaut 19 (capable of processing diagonal-PASEF data) in directDIA+ mode against a uniprot human .fasta file (20432 entries) or a library derived from a 10-20x higher concentrated sample.

Results

High sensitivity/single cells

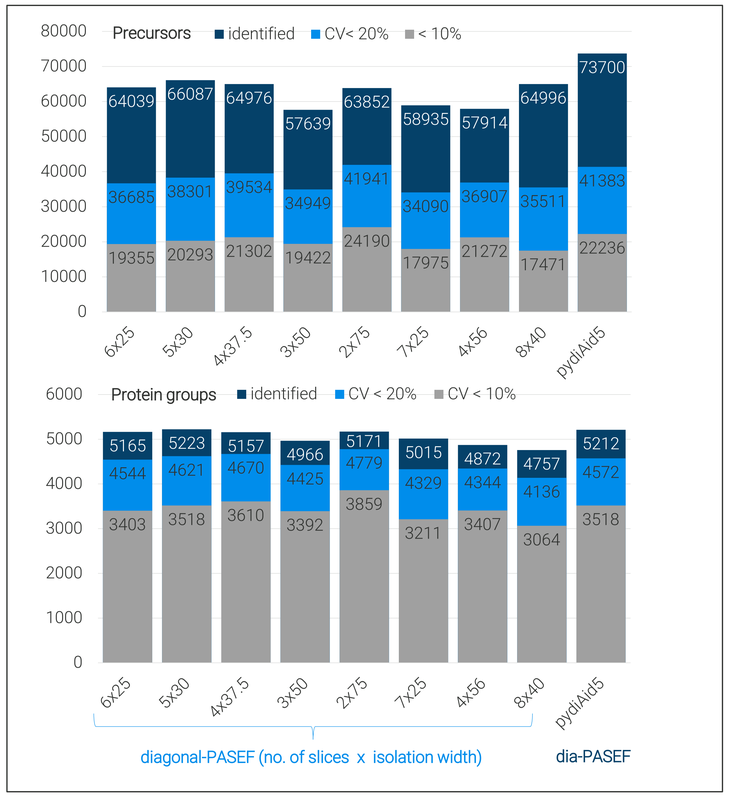

Fig. 2: Identifications from 0.25 ng K562 digest, acquired on a timsTOF Ultra 2, either with dia-PASEF or different diagonal-PASEF window schemes, using a 22 min gradient and processed with Spectronaut 19, The individual libraries were derived from 8 ng samples acquired with the corresponding method.

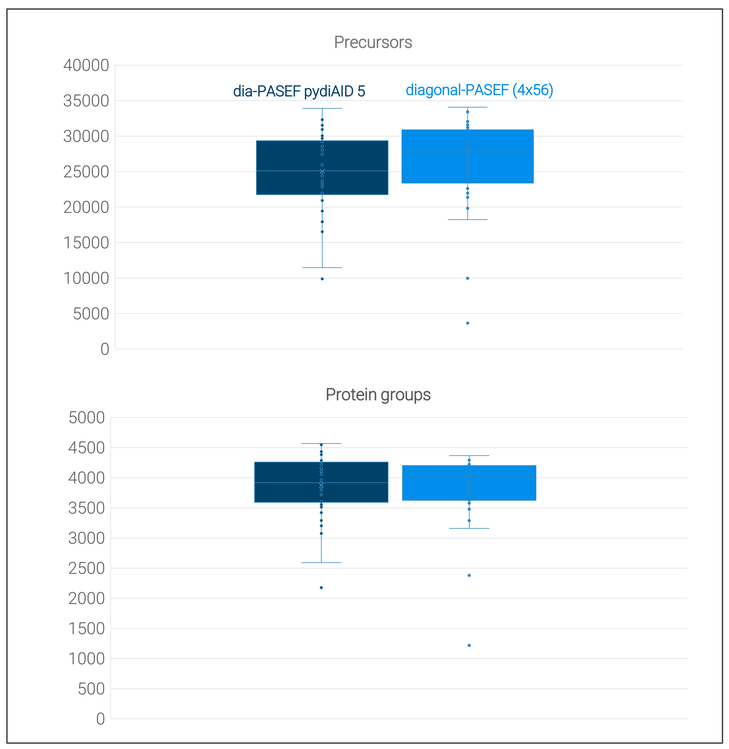

Fig. 3: Identifications from digests of single HeLa cells, acquired either with dia-PASEF (pydiAID5) or diagonal-PASEF (4x56 Th) on a timsTOF Ultra 2, using an Evosep One system running a 40 SPD whisper method and processed with Spectronaut 19 (matched with a 10 cell sample, acquired under same conditions).

High sample load/high throughput

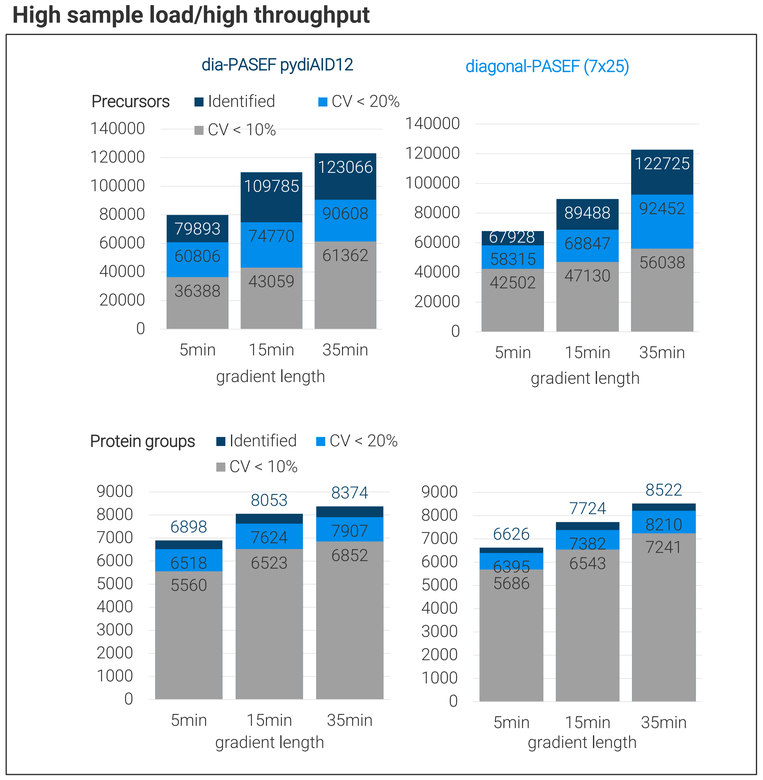

Fig. 5: Identifications from 400 ng HeLa digest, acquired on a timsTOF HT either with dia-PASEF (12 pydiAID isolation windows) or diagonal-PASEF (7 slices, 25 Th) using 5, 15 or 35 min nanoLC gradients. For the 5 min gradients a 5 cm column was used, and a 25 cm column for the 15 min and 35 min gradients. DirectDia+ processing (Spectronaut 19) against human Sprot (20432 entries).

Summary

For low sample amounts of 0.25 ng cell digest, searched against a library derived from an 8 ng sample, widely different diagonal-PASEF acquisition schemes resulted in a similar number of identified protein groups (Figure 2). When an overall acquisition stripe of 150 Th is divided in different window schemes from 2x75 Th to 6x25 Th slices, only around 5 % variation in identified protein groups were observed. Broader isolation schemes of 4 x 56 Th or 8 x 40 Th windows, covering a wider precursor range of the m/z mobility heatmap, showed no dramatic improvement. In this study the classic dia-PASEF method using 5 pydiAID windows slightly outperformed diagonal-PASEF in terms of precursors identified from 0.25ng of cell digest, whereas for real single cell samples diagonal-PASEF showed on average somewhat better IDs on precursor as well as on protein level (Figure 3).

For high sample loads and very short gradients, improved CVs would be expected for diagonal-PASEF due to a faster acquisition cycle while maintaining high selectivity. When analyzing high loads of 400 ng HeLa digest (Figure 4), no obvious benefit could be observed for IDs or for reproducibility, independent of the gradient length used. On precursor level, diagonal-PASEF resulted in fewer IDs for the two faster gradients.

More in-depth investigations are required to understand diagonal-PASEF in order to further optimize acquisition and data processing.

- Acquisition and processing of diagonal-PASEF timsTOF data enabled with latest control and processing software
- Robust Identification rate and quantitative reproducibility over a wide range of different isolation window schemes.
- diagonal-PASEF results in general not (yet) superior to classic dia-PASEF.

timsTOF

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

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