Assessment of a narrow-window dia-PASEF method for high-throughput proteomics

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Introduction

In proteomics, accurate protein quantitation and high sequence coverage are crucial for understanding biological processes. Scientists in pharmaceuticals and life sciences use advanced technologies like Data-independent acquisition (DIA) for comprehensive proteome coverage and reliable quantitation. dia-PASEF, an advanced DIA variant, leverages trapped ion mobility separation (TIMS) on the timsTOF platform for enhanced separation. Initially, dia-PASEF used 25 Da m/z windows, but with py_diAID, variable window schemes became common. Recently, thin-PASEF, with 10 Da windows, identified nearly 11,000 protein groups in 100 minutes. To meet high-throughput demands, we further optimized a narrow-window dia-PASEF method for shorter gradients of 15-minutes, resulting in a narrow-window dia-PASEF method with 5 Da windows.

Methods

Tryptic in-house digests of a human cell lysate, yeast, and *E. coli* were used to evaluate the proteomics performance of the timsTOF HT for short gradients. Samples (800ng) were directly loaded onto a 25cm x 75µm Aurora Ultimate column (IonOpticks) and separated using a 15-min gradient. Eluting peptides were measured using an optimized dia-PASEF window scheme with a significantly smaller m/z range (350 to 900 m/z) combined with a focused mobility range from 0.8 to 1.1 1/K0 (Figure 1). 110 MS/MS windows with 5 Da window width were covered in 44 TIMS frames with 10 MS1 frames in between to ensure good peak coverage. Accumulation and ramp time were set to 30ms.

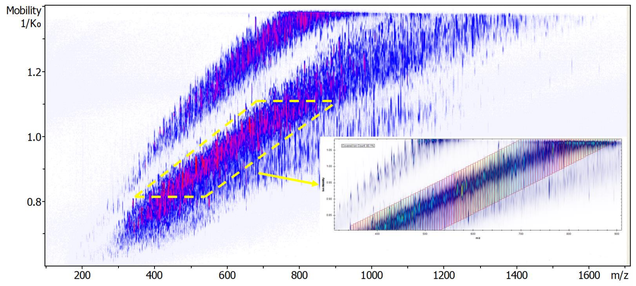

Fig. 1 Overview narrow-window dia-PASEF method.

To optimize deep proteome coverage a method has been generated with focus on the most intense ion region regarding m/z and mobility pane.

Data were processed in Spectronaut (v19, Biognosys). Data files were converted via HTRMS converter (deselecting "Sum multiple MS1 scans per Cycle") and then processed using the directDIA+ workflow (MS1 Quant activated).

Results

We optimized a dia-PASEF method to cover the high ion density region of interest combined with high specificity by using narrow windows of 5 Da, resulting in reduced complexity of MS2 spectra. The multiplexing capabilities of dia-PASEF allowing measurements of more than one window per frame combined with the ability to use very short accumulation and ramp times of 30 ms in the TIMS cartridge resulted in an overall cycle time of 1.76 s on MSMS level and 0.45 s on MS level.

When applying the optimized narrow-window dia-PASEF method to a human cell line digest, we were able to identify 8,850 protein groups and 134,728 peptides from triplicate injections with a 15-minute active gradient (Figure 2A) using library-free directDIA+ in Spectronaut (v.19, Biognosys). Impressively, 88% of the protein groups were quantified with a coefficient of variation (CV) below 10%.

Applying the narrow-window dia-PASEF method to a less complex proteome, namely yeast, resulted in the identification of 4677 protein groups from 68,744 peptides (Figure 2B). Median CV values were 3% at protein group level and 8% on peptide level.

In a next step we analyzed a complex hybrid proteome sample combined in defined ratios (HeLa: 1:1, yeast: 3:2, E. coli: 2:3). The narrow-window dia-PASEF method resulted in the identification of 14,366 protein groups from 170,917 peptides from six injections per sample within a 15-minute gradient (Figure 2C). The chosen experimental design enabled the evaluation of the quantitative accuracy of the narrow-window dia-PASEF method in complex proteomics mixtures with pre-determined theoretical ratios. Background human proteins were spiked in equal amounts resulting in a theoretical ratio of 1:1. We found the relative quantitation of the corresponding human proteins to be centered at the expected ratio across the complete dynamic range (Figure 3B, median log2 ratio of –0.01, measured ratio = 0.99). The measured ratios obtained for yeast (1.4) and E. coli (0.8) were close to the expected ratios with low levels of standard deviation making the developed narrow-window dia-PASEF method perfectly suited for investigation of proteome-wide changes in protein abundance in different biological contexts.

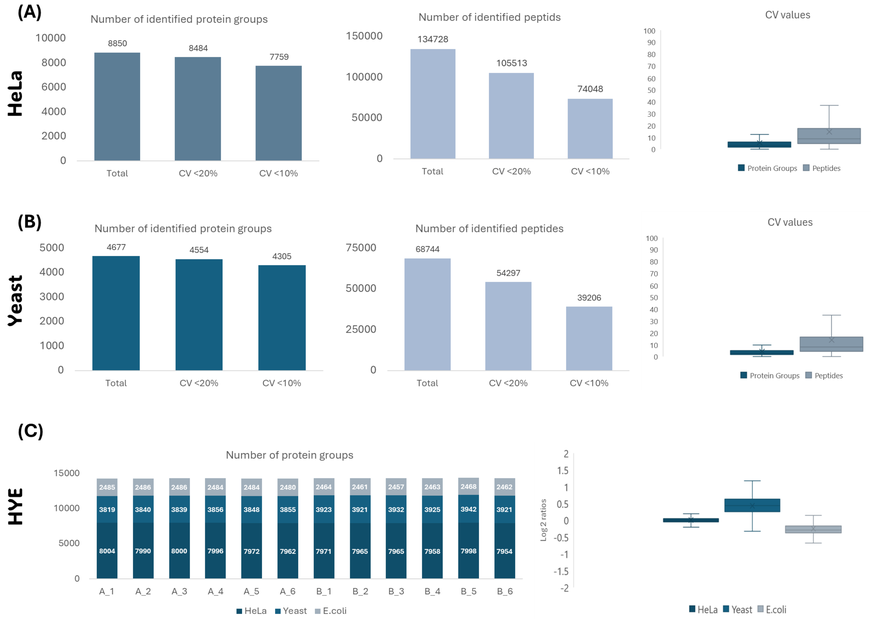

Fig. 2 Reproducible and in-depth peptide and protein identification from (A) human and (B) yeast digest as well as in-depth protein identification and quantitation from a (C) hybrid proteome sample (HeLa, Yeast, Ecoli).

Conclusion

- The two-dimensional mass and mobility space of dia-PASEF enables generation of various methods tailored to sample complexities and throughput demands.
- The presented method using 5 Da isolation windows over a condensed ion mobility and m/z region resulted in high proteome coverage and accurate quantitation in short gradients of 15 minutes.
- Analysis of complex mixed proteomes resulted in identification of 14,366 protein groups using library-free data processing.

timsTOF HT

 Conflict of Interest Disclosure: S.K.-S., J.K., S.S., and M.L.. are currently employed by Bruker.