Improved proteome coverage combined with reproducible quantitation on the timsTOF platform using a narrow-window dia-PASEF method

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Introduction

In the realm of proteomics, accurate and precise protein quantitation is the key to unravel the complex secrets of biological processes. In recent years there has been a shift towards analyzing complex proteomics samples in shorter time to keep up with the increasing demand of sample throughput. dia-PASEF is an advanced variant of Data-Independent Acquisition (DIA), capitalizing on the additional dimension of separation by TIMS. We optimized a dia-PASEF acquisition mode by using narrow isolation windows of 5Da combined with focused m/z and ion mobility window placements to evaluate their performance on complex proteomics samples.

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.

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-level quantitation activated).

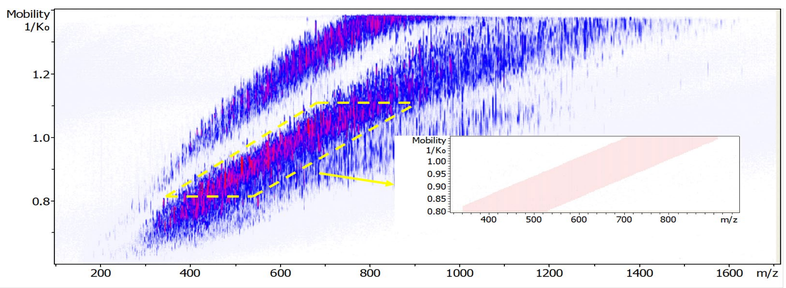
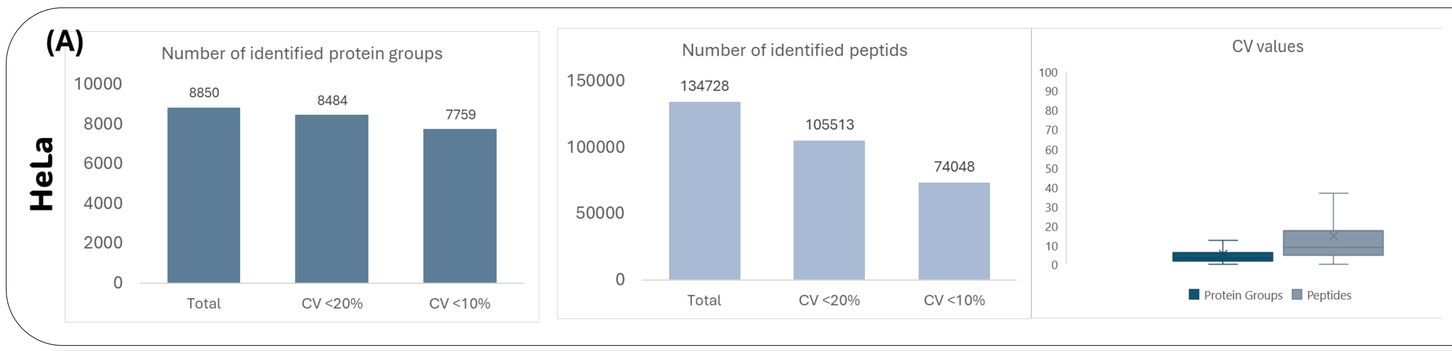
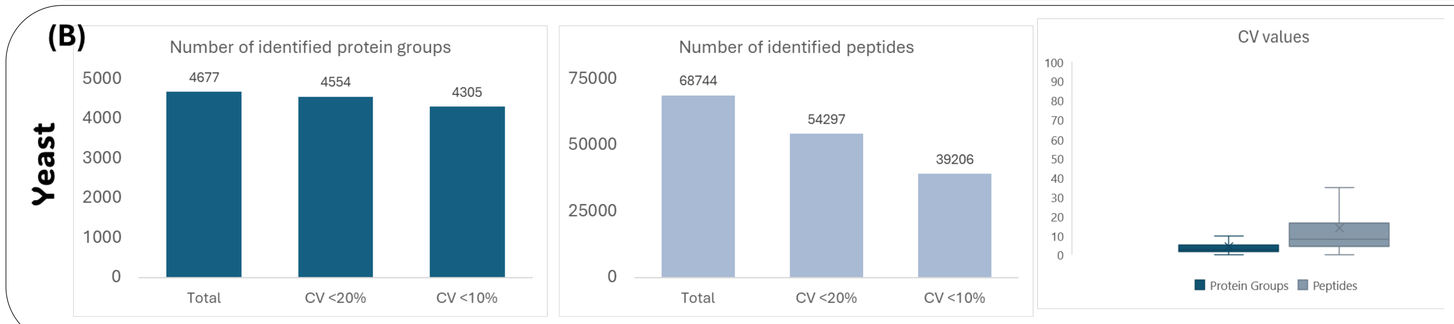


Fig. 1: Overview narrow-window dia-PASEF method

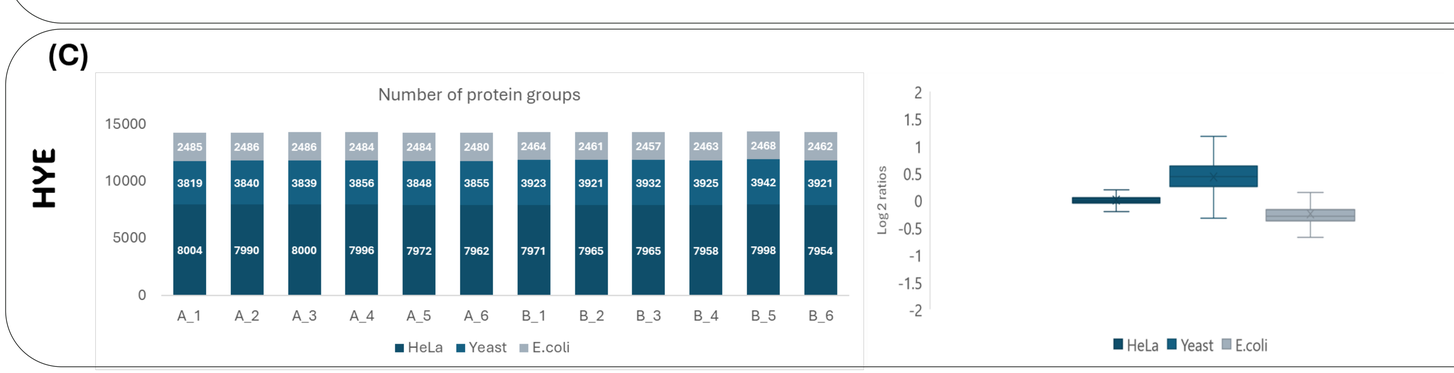
Results



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. When applying the optimized method to a human cell line digest, we were able to identify 8850 protein groups and 134,728 peptides from triplicate injections with a 15-minute active gradient. 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.



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. We found the relative quantitation of human proteins to be centered at around 1 and measured ratios for yeast and E.coli were close to the expected ratios.

Figure 2: Improved proteome coverage from the optimized method for different proteomics samples.

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

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