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Improved proteome coverage combined with reproducible quantitation on the timsTOF platform

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Introduction

In proteomics studies, accurate and precise relative protein quantitation, together with high protein sequence coverage, is the key to unravel the complex secrets of biological processes. Scientists in the pharmaceutical and biopharmaceutical industry, as well as in the life sciences are committed to advancing knowledge, often relying on using cutting-edge technologies to achieve this goal. Data-independent acquisition (DIA) offers the advantage of comprehensive proteome coverage and reliable quantitation of proteins, while also reducing missing information, leading to more robust results. dia-PASEF is an advanced variant of DIA, capitalizing on the additional dimension of separation unlocked on the timsTOF platform by trapped ion mobility separation (TIMS). Due to the two-dimensional mass and mobility space in combination with varying TIMS scan/accumulation times, dia-PASEF enables method creation with tailored window schemes and duty cycles. When introduced in 2020 [1], the standard dia-PASEF method consisted of constant 25 Da m/z window widths covering the mass range of interest. Already with the introduction of py_diAID [2] variable window schemes became frequently used, showing a benefit of smaller isolation windows in the very dense ion cloud region. Recently a new flavor of dia-PASEF has been introduced, called thin-PASEF [3], which applies very small isolation windows of 10 Da focusing exclusively on regions of high ion density. The application of thin-PASEF resulted in the identification of nearly 11,000 protein groups from human cell line digests within a 100-minute active gradient. While identification of 11,000 proteins is impressive, the field is tending towards shorter gradients as high throughput is crucial in proteomics. Thus, we further optimized the approach 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. 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).

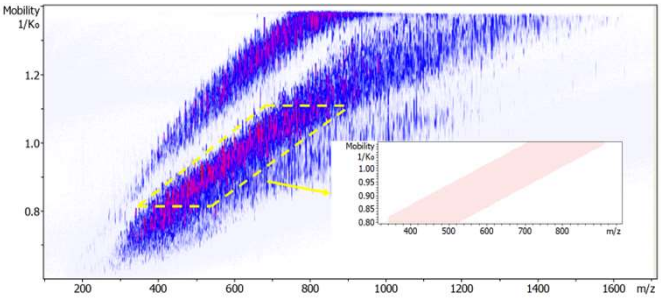
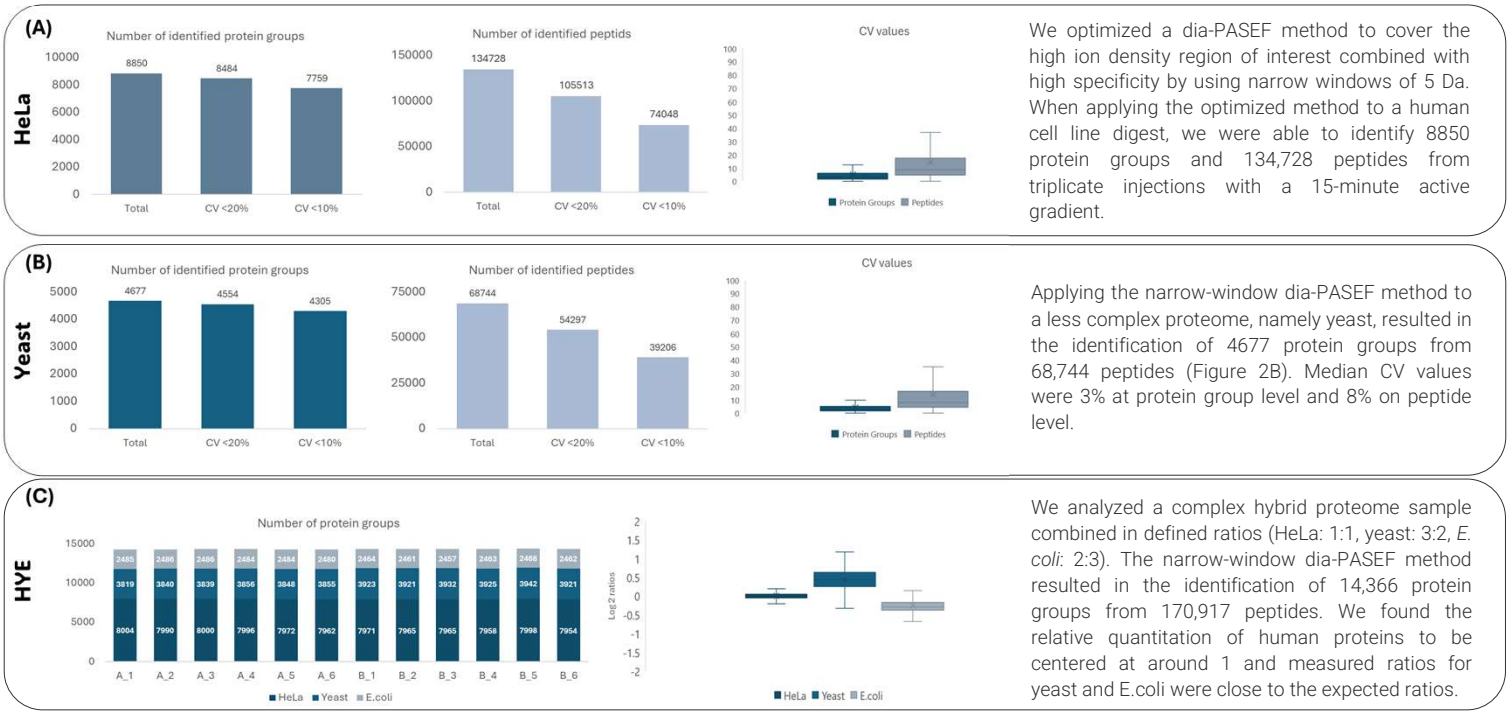


Figure 1: Overview narrow-window dia-PASEF method.

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.

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. 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.

[1] Meier et al., Nat Methods, (2020), 17(12): 1229-1236
[2] Skowronek et al., Mol Cell Proteomics, (2022), 21(9): 100279
[3] Konno et al. (2024). bioXriv. https://doi.org/10.1101/2024.04.26.591246

- 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.

nanoElute 2 + timsTOF HT

Conflict of interest: SK-S, DV, AS, ML, PS, TM, and SK are employees of Bruker Daltonics.