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Improved proteome coverage combined with reproducible quantitation on the timsTOF platform

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Introduction

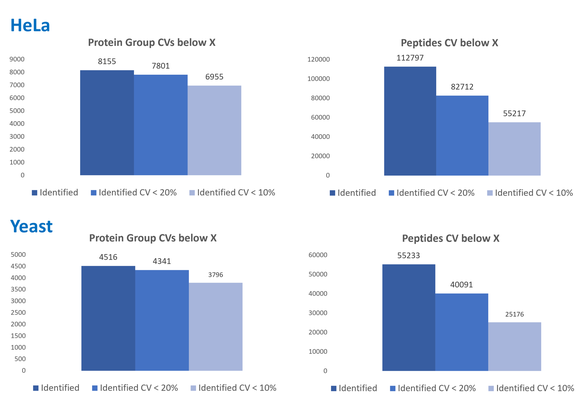
In the realm of proteomics, accurate and precise relative protein quantitation is the key to unravel the complex secrets of biological processes. In recent years there has been a shift towards analyzing complex proteomics samples in shorter time to keep up with the increasing demand of sample throughput. Nowadays Data-Independent Acquisition (DIA) is widely used as it typically outperforms Data-Dependent Acquisition (DDA) for protein identification and quantitation. dia-PASEF is an advanced variant of DIA, capitalizing on the additional dimension of separation by trapped ion mobility separation (TIMS). We applied more advanced (py_diAID, diagonal PASEF) window schemes to evaluate their performance on complex proteomics samples analyzed from different amounts in short gradient times.

Methods

Tryptic digests of a human cell lysate (in-house digest), *Saccharomyces cerevisiae* (Promega) and *E. coli* (Waters) were used to evaluate the performance of sophisticated DIA methods (py_diAID and diagonal PASEF) for short gradients. Tryptic digests were either loaded directly on column or combined in defined ratios according to Guzman et al. (2024).
Digests were loaded on a 15cm C18 column (75µm, 1.6µm, Aurora, IonOpticks) using a nanoElute 2 nano HPLC (Bruker) coupled to a timsTOF instrument (Bruker) via a CaptiveSpray source (Bruker) using a 15-min ACN gradient.
Data were processed in Spectronaut (v19, Biognosys) using library-free mode (directDIA+™). For direct database identifications from dia-PASEF runs, we used human, *E. coli*, and yeast Uniprot fasta files. False discovery rate (FDR) was controlled at 1% for peptide and protein group level.

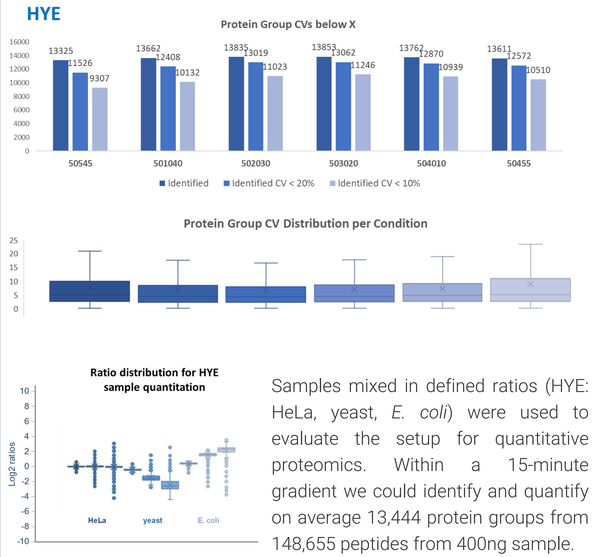
Results

High proteome coverage from 15-minute gradients



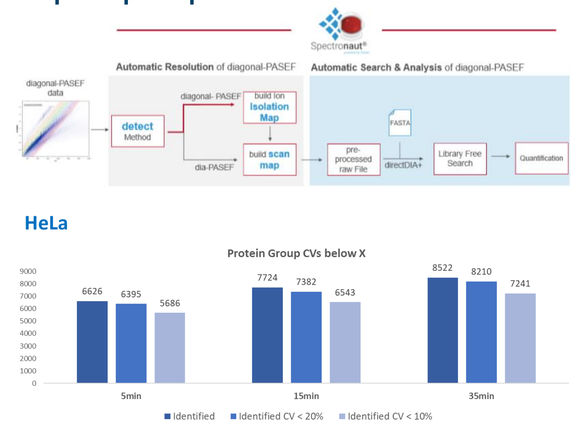
We investigated the performance of the timsTOF HT mass spectrometer for full proteome analysis using short gradients of 15 minutes. Using a variable window-based dia-PASEF method enables measurement of nearly the complete yeast proteome in 15 minutes by identifying on average 4516 protein groups from 55,233 peptides using library-free data processing. From a human protein digest sample, representing a higher complexity proteome, 112,797 stripped peptide sequences from 8155 protein groups have been identified.

Accurate and reproducible label-free quantification from complex proteome mixtures



Samples mixed in defined ratios (HYE: HeLa, yeast, *E. coli*) were used to evaluate the setup for quantitative proteomics. Within a 15-minute gradient we could identify and quantify on average 13,444 protein groups from 148,655 peptides from 400ng sample.
Investigation of the quantitative performance showed low median coefficient of variation for the replicate runs at around 5% on protein group level. The measured ratios obtained for human, yeast and *E. coli* were close to the expected ratios with low levels of standard deviation.

diagonal-PASEF synchronizes the TIMS ramp with the quadrupole speed



diagonal-PASEF is an additional DIA acquisition scheme, which allows to scan the quadrupole synchronously with the TIMS ramp during the mobility scan to optimally cover the ion population in the ion-mobility-mass to charge plane. Main advantage is a better utility of the available cycle time due to better sampling of the relevant part of the ion cloud. Data processing of diagonal-PASEF is enabled in Spectronaut v19 (Biognosys). Initial results show high identification rates across different gradients combined with 95% of the protein groups quantified with CV values below 20%.

Summary

We investigated the performance of the timsTOF HT mass spectrometer for full proteome analysis using short gradients of 15 minutes. Working with optimal placement of the dia-PASEF windows is important to ensure that all theoretical precursors are covered even within these very short gradients and without compromising coverage of the chromatographic peak. We used py_diAID, which automatically adjusts the isolation window width to the precursor density, and optimally positions the isolation design in the mass to ion mobility space.

Results show nearly full coverage of the yeast proteome with more than 4500 protein groups identified using a 15-minute gradient. For the complex hybrid proteome sample on average 13,444 protein groups were identified and reproducibly and accurately quantified applying library-free data processing (directDIA+, Spectronaut).
The novel scan mode diagonal-PASEF enables easy method setup combined with reduced cycle times leading to better peak coverage. Initial tests showed 6626 protein groups identified using a very short gradient of 5 minutes up to 8522 protein groups using a 35-minute gradient already highlighting the future potential of diagonal PASEF.

Conclusion

- dia-PASEF on the timsTOF HT enables high proteome coverage and accurate quantitation in short gradients of 15 minutes.
- Nearly full yeast proteome coverage can be achieved with on average 4516 protein groups identified.
- The novel scan mode diagonal-PASEF allows scanning the quadrupole synchronously with the TIMS ramp. Data processing is fully supported in Spectronaut.

dia-PASEF and diagonal-PASEF on the timsTOF HT

COI disclosure: SKS, DV, AS, LL, POS, and TM are current employees of Bruker Daltonics

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