PASEF for sensitive shotgun proteomics: toward single cell analysis

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Romano Hebeler[1], Matt Willetts[2], Heiner Koch[1], Markus Lubeck[1], Scarlet Koch[1]

[1]Bruker Daltonik GmbH, Bremen, Germany

[2]Bruker Daltonics, Billerica, USA

Introduction

The "Parallel Accumulation Serial Fragmentation" (PASEF) method utilizing trapped ion mobility spectrometry time of flight mass spectrometer (timsTOF Pro) described previously (Meier et al, JPR 2015) enables faster acquisition of fragment ion spectra from isolated precursor ions while improving sensitivity without sacrificing spectral quality. Here, we illustrate the performance obtained with a fully automated instrument, which increases sequencing speed by a factor of 10 simultaneously improving sensitivity. We show how the combined increase of the sensitivity and the sequencing speed can be used to obtain deep proteome coverage using very low sample amounts, toward the characterization of the proteome of a single cell.

Methods

The performance for shotgun proteomics has been evaluated by using tryptic digests of human cancer cell lysates (Pierce HeLa Protein Digest Standard) and *E. coli* cell lysates (Bruker in-house). Chromatographic separations were performed on a nanoElute (Bruker Daltonics) nano-flow LC using a 25 cm, 75 µm ID Odyssey C18 nano column with integrated emitter (IonOpticks) using a non-linear gradient of 2-36% buffer B (100% ACN and 0.1% FA) at a flow rate of 400 nl/min. Data were acquired using the PASEF method on a timsTOF Pro mass spectrometer (Bruker Daltonics).

Results

PASEF as sensitivity enhancer

A standard 1.1 s PASEF acquisition cycle was used to measure sample amounts from 3.1 ng up to 100 ng on column. The HeLa cell digest was separated using a 60 min gradient in technical duplicate. The corresponding TIC and BPC traces reflect the increased sample amount loaded onto the nanoLC separation column (Figure 1). Even with the lowest amount on column (3.1 ng) more than 1,650 protein families from 7,000 peptide sequences can be identified (Figure 2).

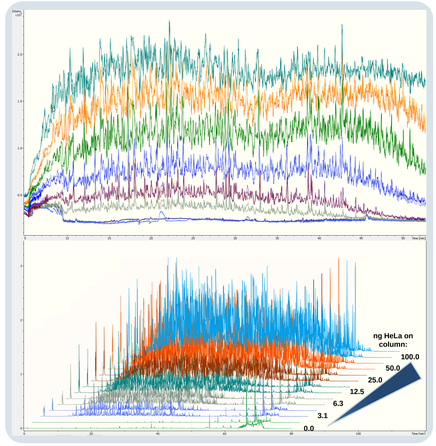

Fig. 1: Total ion chromatogram (TIC) and base peak chromatogram (BPC) of 3.1 to 100 ng HeLa injection.

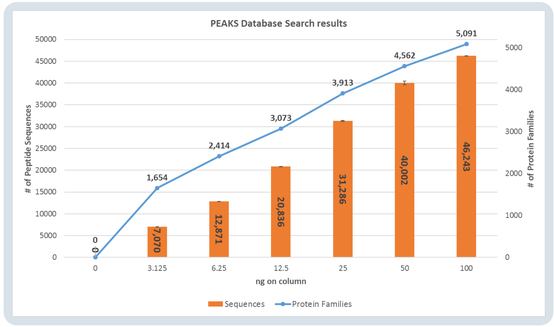

Fig. 2: Number of peptide and protein identifications using low sample amounts of HeLa digest (3.1 ng – 100 ng) and a gradient lengths of 60 min. Data analysis was performed using PEAKS studio (Version 8.5). For database search settings refer to Table 1.

In another experiment, four different admixed proteomes of HeLa and *E. coli* at various ratios were analyzed in quadruplicate. The Mascot database search results show very good reproducibility and sensitivity (Figure 3).

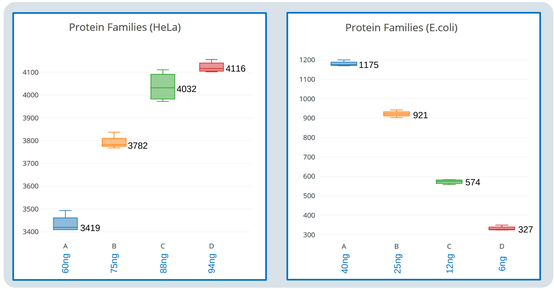

Fig. 3: Box plot analyses of the identified protein families of mixed proteomes (n = 4) where sample A is 60/40ng, B is 75/25ng, C is 88/12ng, D is 94/6ng of HeLa and *E. coli*, respectively.

PASEF as selectivity enhancer

Trapped ion mobility separation (TIMS) enables the separation of co-eluting peptides that only differ slightly in mass (Figure 4). The so called space focusing makes it possible to distinguish a low abundant peptide in the presence of a high abundant peptide. This is nicely demonstrated in sample D (Figure 3), where 6 ng of an *E. coli* digest was analyzed in the presence of 94 ng HeLa.

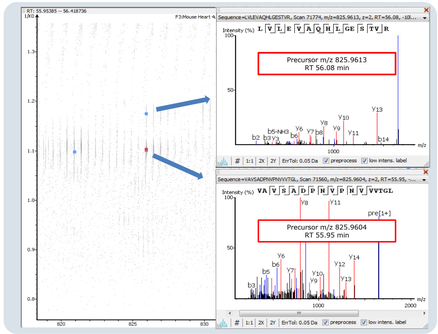

Fig. 4: Co-eluting peptides that cannot be distinguished by mass spectrometry alone but by their mobility term using TIMS.

Search Engine	PEAKS 8.5 / *Mascot 2.6.1*
Parent Mass Error Tolerance	20.0 ppm / *50 ppm*
Fragment Mass Error Tolerance	0.05 Da
Precursor Mass Search Type	monoisotopic
Enzyme	Trypsin
Max Missed Cleavages	2 / *1*
Fixed Modifications	Carbamidomethylation
Variable Modifications	Oxidation (M), Acetylation (N-term) / *Deamidated (NQ)*
Taxon	Human / Human or E. coli
FDR	1%

Tab. 1: Database search parameters.

Summary

Applying PASEF on the timsTOF Pro mass spectrometer provides deep proteome coverage without the need of large sample amounts. Due to the additional dimension of separation provided by TIMS a deeper insight into the proteome is provided by separating co-eluting peptides not only by mass-to-charge but also by size-to-charge resulting in a higher confidence in identification.

References

(1) Meier et al.; JPR 2015,

Conclusions

- PASEF provides deep proteome coverage even with low sample amounts
- PASEF provides high reproducibility
- TIMS separation makes low abundant peptides visible in the presence of high abundant ones
- TIMS separation delivers separated high quality MS/MS spectra from low-abundant co-eluted isoforms

timsTOF Pro