timsTOF Pro powered by PASEF and the Evosep One for high speed and sensitive shotgun proteomics

The “Parallel Accumulation Serial Fragmentation” (PASEF) method for trapped ion mobility spectrometry (TIMS) quadrupole time of flight (QTOF) instruments described previously\(^1\) enables faster acquisition of fragment ion spectra from isolated precursor ions, without sacrificing spectral quality.

**Introduction**

During an ion mobility scan the quadrupole switches its isolation position several times, isolating a certain ion species only for the period of time it actually elutes from the mobility separation device as a focused package (Figure 1). With further hardware and software improvements, PASEF has been fully implemented on a new QTOF based instrument, the timsTOF Pro, which was used to produce the results presented here.

Precursor selection in two dimensions occurs in multiple steps:
- coarse detection of intensities in the mz/mobility pane
- extract MS spectra around intensities found
- refine and correct isolation position
- filter for precursors detected more than once
- filter by 2D dynamic exclusion list

When the final list is compiled, individual precursors have to be distributed among the following PASEF fragmentation scans in the most efficient way. The optimal order of measurements would correspond to the shortest itinerary.

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in a classical ‘travelling salesman problem’ which cannot be calculated on an LC timescale. A non-perfect but fast (~ 1 ms per full cycle) approach was implemented which also takes into account that low intensity precursors have to be scheduled multiple times to obtain meaningful fragment spectra (Figure 2).

**Methods**

Helacells (CIL Biotech, Mons, Belgium) were digested using the protocol from Wang et al. Chromatographic separations were performed on a nanoE-lute (Bruker Daltonics) using a 25 cm, 75 µm ID Odyssey C18 nano column with integrated emitter (IonOpticks, Australia) at 400 nL/min and 50°C with direct loading. High speed separations were performed on an Evosep One (Evosep Biosystems). Data were searched against SwissProt database using Mascot 2.5.1 or Byonic 2.12.0. Results were normalized to < 1% FDR on PSMs (Mascot) or 1% protein FDR (Byonic).

*Figure 1: Principle of PASEF acquisition. Peptide ions are trapped for 100 ms and then separated by trapped ion mobility (~ 100 ms) generating the MS m/z/mobility heat map (right). For acquisition of MS/MS spectra (left) the same TIMS separation is used with the quadrupole isolating a certain ion species only during its mobility elution time and immediately shifting to next precursor.*

*Figure 2: A representative MS1 TIMS heat map from a 90 min HeLa run at 60.7 minutes, 50 different precursors were automatically scheduled for the following 10 PASEF MS/MS scans. Low abundant precursors were scheduled for multiple PASEF scans, resulting in an equivalent of 118 separate MS/MS events without TIMS PASEF in a single topN cycle (1.1 s) and without compromising sensitivity. Each of the ten colored lines connects precursor ions for one particular PASEF MS/MS scan. Note that the most abundant precursors have been sequenced in previous scan cycles and were dynamically excluded from re-sequencing.*
Figure 3: Distribution of the number of precursor ions fragmented per PASEF MS/MS scan during a 90 min LC autoMS/MS gradient (200 ng HeLa digest). On average 12.6 precursors are addressed during a 100 ms PASEF scan, resulting in > 100 Hz acquisition speed. However, due to the 100 ms accumulation time, the signal intensity per fragment spectrum is still comparable to a 10 Hz acquisition.

Figure 4: Distribution of intensity based repetitive measurements of the same precursor: In a 90 min gradient of 200 ng HeLa digest, precursors were measured 2.3 times on average with the set target values.
Figure 5: Peptide identification results from different search engines for 200 ng HeLa digest separated by 90 min gradients (average numbers from triplicate runs).

Figure 6: Peptide identification results (Mascot/Percolator) for 200 ng HeLa digest separated by LC gradients of 30, 45, 60 and 90 minutes, using the nanoElute (Bruker Daltonics).
**Results**

PASEF on the timsTOF Pro instrument allows 10 times higher acquisition speed without losing sensitivity.

Fast acquisition speed for targeting more precursors

The ability to fragment more unique precursor ions per unit time enables an in-depth analysis from limited sample amounts: > 5000 protein groups can be identified from 200 ng HeLa digest using a single 90 min gradient.

Fast acquisition speed for reduced analysis time

The acquisition speed of > 100 MS/MS/s enables reduction of analysis time per sample. Even with 30 min gradients more than 4000 protein groups can be identified.

**Acknowledgment**

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**Conclusions**

PASEF allows for 10 times higher acquisition speed during data dependent autoMS/MS and 10 times higher sensitivity, enabling

- in-depth proteome analysis even from low sample amounts
- fast acquisition with short analysis time for high sample throughput
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www.bruker.com/timstofpro

References


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