# Performance of a timsTOF Pro mass spectrometer in shotgun proteomics of a tryptic HeLa digest mixture

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## Introduction

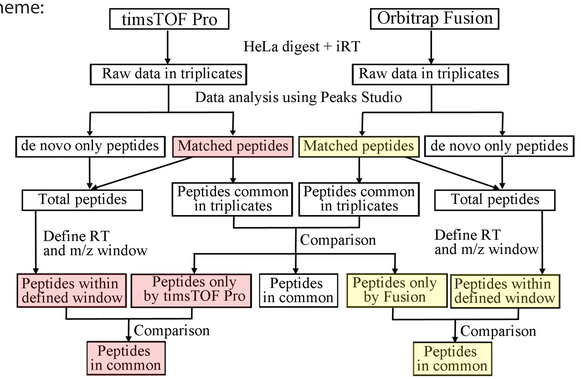
Trapped ion mobility spectrometry coupled with quadrupole time-of-flight mass spectrometry (timsTOF Pro) offers a unique dimension of characterization and separation in complex samples.[1] The timsTOF Pro instrument also enables the "Parallel Accumulation Serial Fragmentation" (PASEF) method that allows continuous ion accumulation, sorting and eluting, therefore reaching a duty cycle of nearly 100%.[1] Notably, the timsTOF Pro powered by PASEF offers sequencing speed of more than100 Hz.[1] In this work, we tested the effects of PASEF in shotgun proteomic analysis of a HeLa digest sample using the timsTOF Pro instrument. Proteomic data from the same sample were also collected using a Thermo Orbitrap Fusion mass spectrometer. The results from these two instruments were compared.

[1] https://www.bruker.com

## Methods

A tryptic peptide mixture derived from HeLa cells (Pierce) mixed iRT (Biognosys) (100 ng or 200 ng) was loaded onto a C18 column (25 cm X 75 µm, 1.6 µm, IonOpticks, Australia) using a nanoElute UHPLC that was coupled to the timsTOF Pro mass spectrometer (Bruker), or a self packed column (5 cm x 150 µm, 1.9 µm) (abbreviaed as selfpack hereafter) using a NanoLC coupled to the Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific). Chromatographic separation was carried out using a linear gradient of 2-37% of acetonitrile with 0.1% formic acid (FA) in water with 0.1% FA at a flow rate of 400 nL/min over 90 min (Ionopticks) or at a flow rate of 800 nL/min over 75 min (Selfpack). The acquired data were analyzed for peptide and protein identifications using the Peaks Studio 8.5 (Bioinformatics Solutions).

Data analysis scheme:




Data highlighted were listed in Tables 1 and 2.

## Results

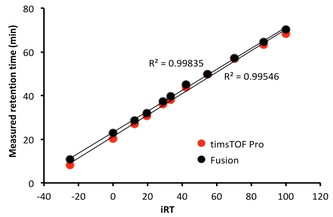



Figure 1. Correlation between measured retention time (min) and iRT hydrophobicity using the timsTOF Pro and Fusion systems. Data were collected using selfpack columns over a 75 min gradient. (0-71 min, 4-25% B; 71-73.5 min, 25-90%B; 73.5-75 min, 90-2%B). Data were expressed as mean ± SD (n=3). Raw data were processed by Peaks Studio 8.5.

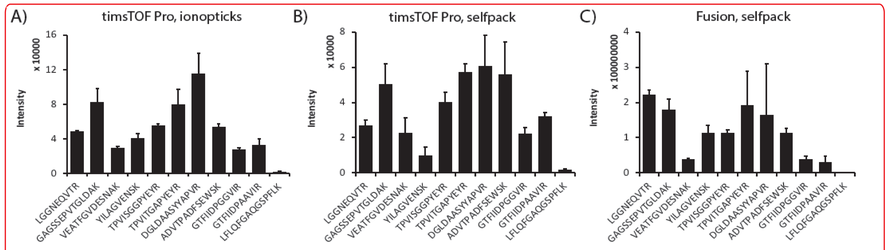



Figure 2. The intensity of iRT peptides determined by A) timsTOF Pro using a commercial column from Ionopticks, B) timsTOF Pro using a self packed column, and C) Fusion using a self packed column. One hundred nanograms of HeLa-iRT sample were used for all experiments. Data were expressed as mean ± SD (n=3). Raw data were processed by Peaks Studio 8.5. Detailed column information was in Methods section.

Table 1. Comparison between timsTOF Pro and Fusion using selfpack columns.

| | HeLa + iRT (ng) | Matched peptide [2] | Peptide within defined RT & m/z window [3] | Peptide only found by timsTOF Pro or Fusion [4] | Peptide in common [4] | Percentage [5] |
|---|---|---|---|---|---|---|
| timsTOF Pro | 100 | 21377 ±363 | 2129 ± 67 | 8407 | 155 ± 11 | 1.8 ± 0.1 |
| | 200 | 23166 ± 629 | 2512 ± 37 | 8678 | 195 ± 10 | 2.2 ± 0.1 |
| Fusion | 100 | 14434 ± 418 | 250 ± 33 | 3202 | 5 ± 2 | 0.2 ± 0.1 |
| | 200 | 17975 ± 345 | 228 ± 31 | 5451 | 7 ± 1 | 0.1 ± 0.0 |

Table 2. Comparison between a commercial column from Ionopticks and a selfpack column using the timsTOF Pro.

| | HeLa + iRT (ng) | Matched peptide [2] | Peptide within defined RT & m/z window [3] | Peptide only found by Ionopticks or Selfpack [4] | Peptide in common [4] | Percentage [5] |
|---|---|---|---|---|---|---|
| Ionopticks | 100 | 25849 ± 211 | 2275 ± 68 | 8407 | 144 ± 15 | 1.9 ± 0.2 |
| | 200 | 31776 ±684 | 2957 ± 72 | 11746 | 230 ± 6 | 2.0 ± 0.1 |
| Selfpack | 100 | 21377 ± 363 | 2129 ± 67 | 4137 | 128 ± 55 | 1.5 ± 0.7 |
| | 200 | 23166 ± 629 | 2512 ± 37 | 4515 | 110 ± 10 | 2.4 ± 0.2 |

[2] Matched peptide list was obtained from Peaks Studio 8.5.

[3] Peptide search within the defined RT and m/z windows was achieved using a custom program written in R. The defined RT and m/z windows were 1.2 s and 0.015, respectively.

[4] Peptides that were uniquely found in each scheme was searched against the matched peptide lists using a custom program written in R. Peptides in common were found by the same program.

[5] Percentage = 100*Peptide in common/peptide only found by timsTOF or Fusion (Table 1) or peptide only found by Ionopticks or Selfpack (Table 2).

## Summary

- The timsTOF Pro identified 30-50% more peptide sequences compared to Fusion.
- Both timsTOF Pro and Fusion exhibited good LC performance.
- The fast scanning rate of the timsTOF Pro may be the major contributor for its superb performance in peptide identification.
- Ion mobility of the timsTOF Pro may contribute to its superb performance in peptide identification, however, it's unlikely to be the major contributor.

## Conclusion

The PASEF method utilized in the timsTOF Pro represents a robust approach for shotgun proteomics.

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