Exploring the proteomics capabilities of a new Trapped Ion Mobility Q-TOF designed for enhanced metabolomics performances

Benoit Fatou[1], Helena Alamil[1], Manuel Chapelle[1], Andreas Schmidt[2], Verena Tellstroem[2], Sabine Jourdain[1], Jocelyn Dupuy[1], Silke Neustifter[2] and Pierre-Olivier Schmit[1] [1]Bruker Datonique S.A., Wissembourg, France;[2] Bruker daltonik GmbH and Co KG, Bremen, Germany

Introduction

The growing adoption of multi-omics approaches in translational research highlights the need for LC–MS systems capable of delivering high performance across diverse analyte classes. While previous Trapped Ion Mobility Q-TOF instruments (timsTOF) have shown excellent proteomics performance, the new timsMetabo is optimized for enhanced transmission of low m/z analytes. Its synergistic integration of the TIMS-MX cartridge, the Athena Ion Processor (AIP), and the Mobility Range Extension (MoRE) scan mode enables high-throughput analyses over a broad m/z and 1/K_0 range. In this study, we assessed whether these innovations preserve robust proteomics capabilities despite off-axis optimization, ultimately evaluating the suitability of timsMetabo as a high-performance benchtop platform for multi-omics applications.

Methods

Commercial cell line protein digest (k562, Promega) and neat plasma samples were injected on a 25 cm x 75 µm column (Ion Opticks) and separated with using 22-, 44-, and 60-min (from 5% to 35% ACN) gradients at 250 nl/min using using a nanoElute2 nano-HPLC system. The nanoElute system was coupled to a timsMetabo system operated in both dda- and dia-PASEF modes. LC-MS/MS Peptide Reference mix (V7491, Promega) was spiked into the samples for prm-PASEF measurements. The dia-PASEF method was built with 24x25Th windows covering a 400 – 1000 m/z range and distributed over 8 PASEF ramps. The PASEF method was scheduled to perform 5 PASEF ramps per cycle with 300-Hz acquisition speed enabled. Data were analyzed using DIA-NN 2.0.1 in library-free mode, with the SwissProt Homo sapiens database for HeLa samples (release 02-2025, 20,417 entries) and the Mus musculus database for tissue lysates (release 01-2025, 17,229 entries). The analysis was performed without using the match-between-runs (MBR) functionality. The prm-PASEF data were analyzed using Skyline-daily v26.1. (Fig.1)

Results

For all experiments, the sample load on column was set to 100 ng. As preliminary experiments have shown that a 30% duty cycle setting was a sweet spot for prm-PASEF, we decided to use a similar setting for prm-PASEF in order to facilitate methods sensitivity comparison. While keeping the injected amount stable, we tested the influence of the gradient length using the reduced duty cycle dia-PASEF acquisition method .

Up to 69k precursors and 7.2k protein groups were identified using a 22-min gradient, which compares positively to previous results obtained from a 200ng load using100% duty cycle method with an identical gradient. The dynamic range of the k562 cell proteome covers up to 5 orders of magnitude in terms of quantified proteins. Increasing the gradient time to 60-min yielded to 103k precursors and 8.2k protein groups identified. A PASEF acquisition (DDA) demonstrated a number of 28k peptides and 4.7k protein groups using a 22-min gradient, and 62k peptides and 6.5k protein groups identified using a 60-min gradient time, highlighting the system potential for studies requiring a maximized selectivity and enabling DeNovo studies, like discovery Immunopeptidomics or Metaproteomics, or cross-linking experiments. Switching to a 100 ng neat plasma proteome injection, with no specific dia-PASEF method optimization we obtained an average of 412 protein groups and 5050 precursors. The dynamic range of the neat plasma proteome span from up to 5 orders of magnitude, which is expected from this type of sample (Fig.2).

To investigate the targeted proteomics performances of the timsMetabo, we have used a prm-PASEF method targeting isotopolog peptides from Promega (see Methods for more details) in the neat plasma background. Doing so, we demonstrated a consistent sensitivity, highlighting a Limit Of Quantitation (LOQ) down to 25 amol (on column) with a R^2 > 0.99. Despite the complex background (100 ng neat plasma) and short gradient (22 min), the spectral quality was still good (Fig.3).

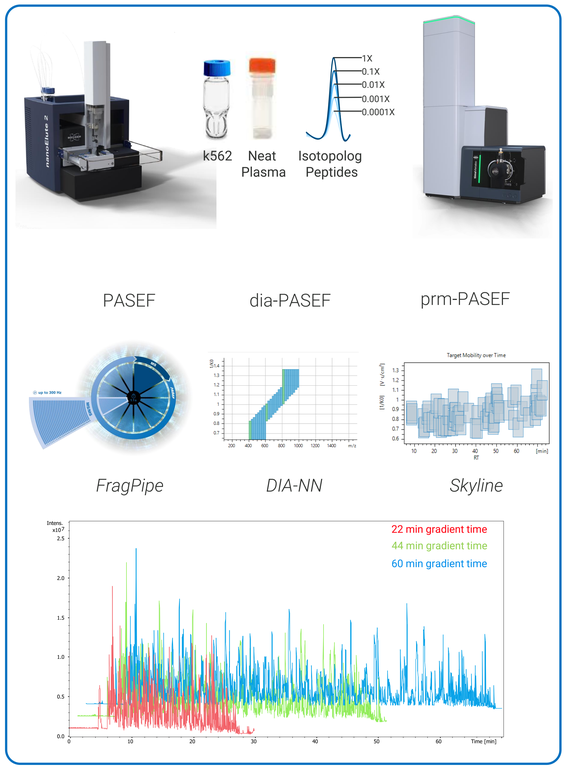

Fig.1: Experimental workflow.
Plasma samples were spiked with the isotopolog mixture to evaluate the LOQ in prm-PASEF and dia-PASEF

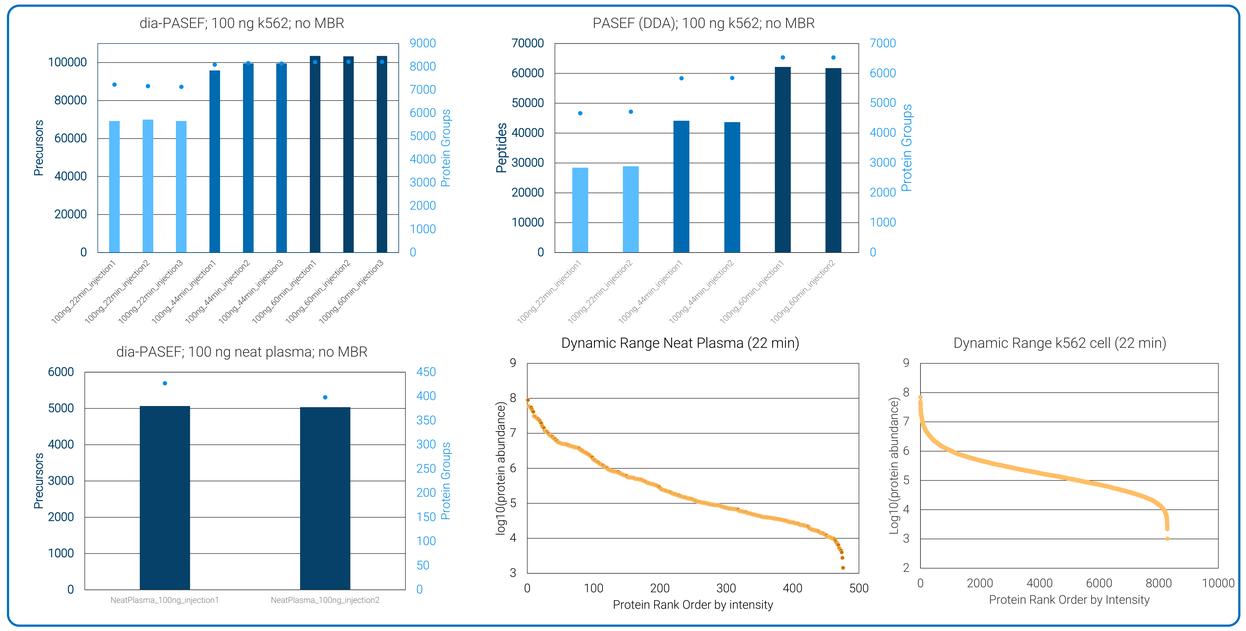

Fig. 2: Proteomics performances from cell and neat plasma samples.
The dia-PASEF data were searched against DIA-NN v2.0 and the PASEF data were searched with FragPipe v22.0. The light blue dots indicates the number of protein groups and the dark blue bars the number of precursors. The orange dots indicates the averaged protein abundance.

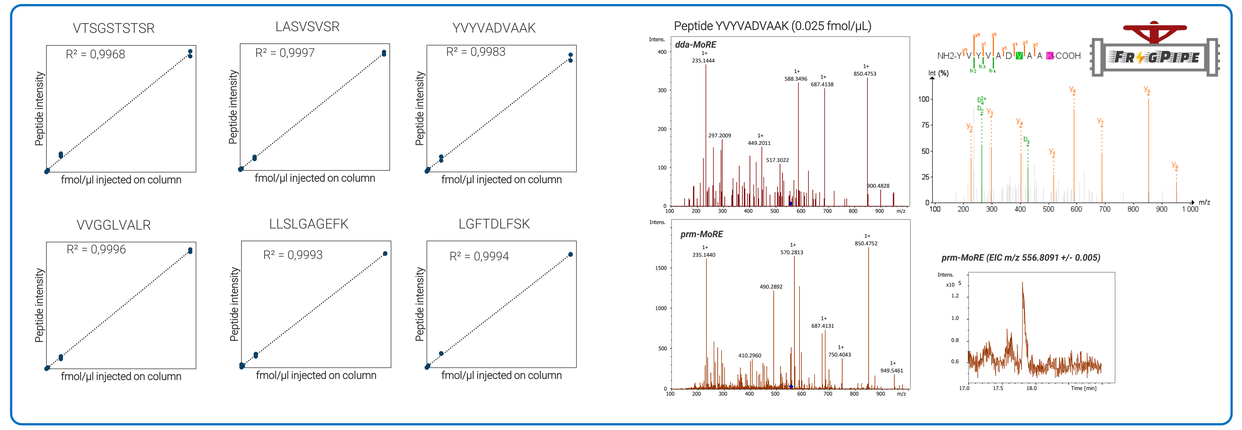

Fig.3: LOQ and spectral quality @ LOQ
Left hand : Peptide quantification at the MS2 level extracted from Skyline-daily using a linear regression fit with no normalization enabled. Right hand : MS/MS spectra of the YVYVADVAAK peptide @ LOQ for the PASEF and prm-PASEF acquisitions.

Conclusions

- While being designed to offer ultimate performances for metabolomics and lipidomics applications, the timsMetabo preserves the proteomics capacities of its predecessor, in terms of depth, throughput, dynamic range and sensitivity where it can be compared to a timsTOF SCP for performance in discovery proteomics operations.
- The targeted proteomics insights obtained from neat plasma also highlight the system suitability for targeted proteomics.
- this study complements the previous ones that had assayed the system sensitivity and quantitative accuracy

timsMetabo

© 2026 Bruker All authors are employees of Bruker and may hold financial interests in the company.

For Research Use Only. Not for use in diagnostic procedures.

Innovation with Integrity