Evaluation of TIMS MS and MS/MS data for targeted proteomics

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Markus Lubeck[1], Heiner Koch[1], Scarlet Koch[1], Oliver Raether and Pierre-Olivier Schmit[2]
[1]Bruker Daltonik GmbH, Bremen, Germany
[2]BrukerFrance S.A, Wissembourg, France

Introduction

Targeted proteomics approaches are used to perform targeted biomarker candidate discovery or to validate candidate biomarkers. Within complex matrices quantitative measurements of a large number of proteins within a short time requires speed, selectivity and sensitivity. Current approaches are preferential for either selectivity and sensitivity(MRM/PRM), or the analysis of a larger library with lower selectivity and sensitivity (DIA). Bruker recently introduced Parallel Accumulation Serial Fragmentation (PASEF[1]) acquisition strategy for untargeted discovery. PASEF allows for acquisition of data dependent MS/MS spectra at very high speed (> 100 Hz) after a Trapped Ion Mobility Spectrometry (TIMS) separation and concentration step, thus ensuring a combination of maximum specificity and enhanced sensitivity. Here, in this proof-of concept study, we evaluate TIMS-UHR-Q-TOF for targeted proteomics through the use of existing PASEF spectra and present the very first dataset acquired in tims-broadbandCID mode (tims-bbCID, Figure 1).

Methods

Hela cells, yeast, BSA, UPS1 and UPS 2 (Sigma) digests were separated by HPLC (nanoElute, Bruker Daltonics) on a 250 mm pulled emitter columns (IonOpticks, Australia) with 90 min LC-Gradients and analyzed on a high-resolution timsTOF Pro instrument (Bruker Daltonics) operated in PASEF or tims-bbCID. Post-processing analysis was performed with a new version of Data Analysis™ and a prototype version of Skyline™ .

References

(1) Meier et al.; Journal of Proteomics Research 2015

Results

Combining LC, TIMS and MS separation allows for the dramatic increase in the instrument's peak capacity[(2)], and thereby the propensity to track peptide abundance changes within complex mixtures.

tims-PASEF

The dynamic range, accuracy and specificity of PASEF have already been illustrated in untargeted label-free approaches (see WP753 and WP446); more than 5200 proteins, covering 4 orders of magnitude in concentration are reproducibly identified and quantified (CV < 10%) from triplicate analysis of 200 ng Hela cell digest injections.

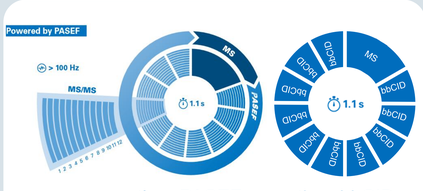


Fig. **1 tims-PASEF and tims-bbCID acquisition methods**: A PASEF cycle (total: 1.1s) consists of 1 TIMS MS scan (100 ms) and 10 PASEF MS/MS scans (each 100 ms, during which an average of 12 precursors are selected for MS/MS). A tims-bbCID consists of 1 TIMS ms Scan followed by 10 tims-bbCID (fragmentation without precursor selection) scans.

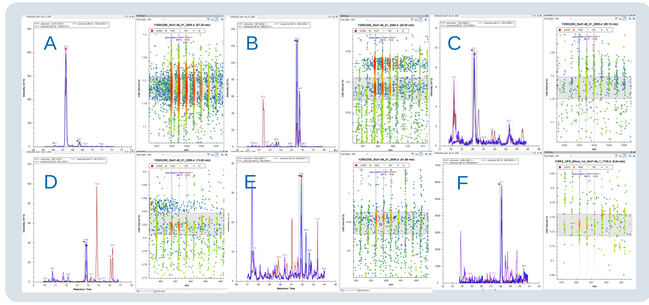


Fig. **2 tims-PASEF sensitivity test with Skyline™**: 100ng of a yeast cell digest have been spiked with and UPS2 digest. The chromatographic traces of peptides representative from the various concentration levels of UPS2 proteins are displayed together with the corresponding heatmap. **A:** 200fmol, Hemoglobin; **B:** 20fmol, Cytochrome b5; **C:** 2fmol, Retinol binding protein4; **D:** 200 amol, Antithrombin-3; **E:** 200 amol, Glutathione S-transferase; **F:** 20 amol, Gelsolin.

PASEF selectivity and sensitivity for targeted proteomics has been assayed with an UPS2 mixture spiked in a yeast digest. UPS2 proteins could be detected/quantified over 4 orders of magnitude in dynamic range, down to a 20 amol on column (Figure 2). TIMS MS therefore enables sensitive quantitation, but further LOQs will require the extra selectivity brought by MS/MS

tims-bbCID

bbCID is the most simple DIA acquisition mode as the complete mass range is selected for every MS/MS. It allows shorter cycle time than multiple windows DIA but yields more complex MS/MS spectra. tims-bbCID allows to reduce the MS/MS complexity with prior TIMS separation allowing to reduce the noise level of extracted chromatographic traces (Figure 3) A 1/10 ratio of BSA spiked in 100ng of a yeast digest background could be measured accurately (Figure 4).

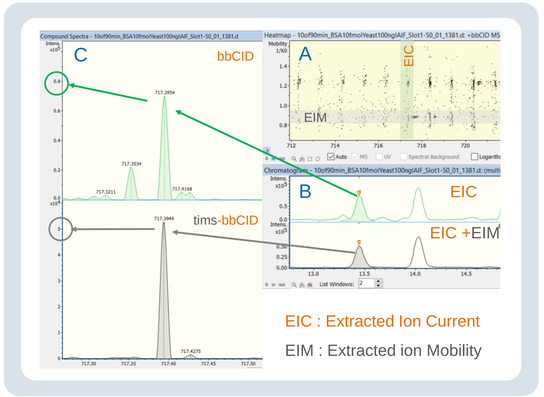


Fig. **3 tims-bbCID data extraction for increased specificity**: tests have been performed with 10 fmol of BSA spiked in 100 ng of a yeast digest **A :** extract of the tims-bbCID heat map (m/z vs ion mobility) acquired @ 10Hz; B :EIC of the corresponding $[y6]^+$ ion of the CCTESLVNR peptide with and without ion mobility filtering. **C:** zoom on the corresponding peak in the bbCID spectrum. **The 60% difference** in the resulting MS/MS intensities is highlighted.
This illustrates the benefit of the ion mobility filtering in terms of selectivity and quant accuracy.

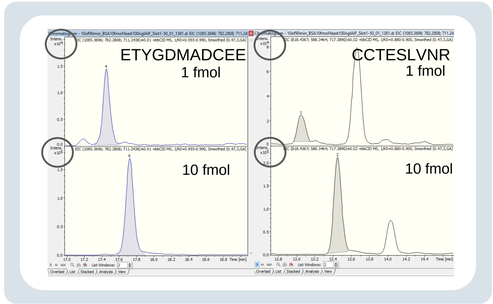
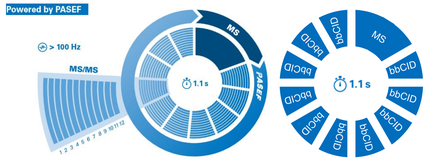


Fig. 4: ratio accuracy obtained from a 10 Hz tims-bbCID acquisition. BSA has been spiked @ 1 and 10 fmol levels in 100ng of a yeast digest background. The three most intense fragment ions have been used for data extraction. Calculated area ratios are 9,9 for the first peptide and 13,1 for the second.

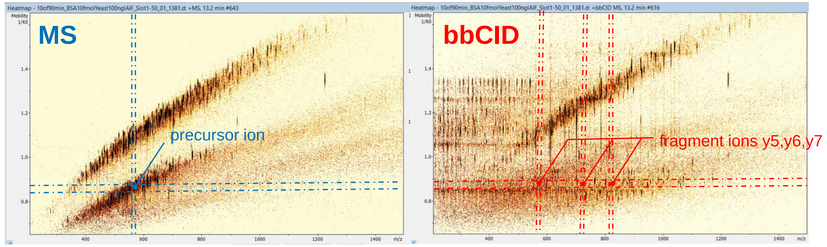
Conclusions

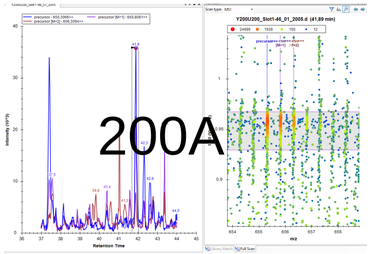
- Extremely high selectivity with the combination of tims, LC and MS separation
- Straightforward, accurate and sensitive quantitation from tims-PASEF with Skyline
- tims-bbCID for increased specificity and compatible with Ultra-sharp peaks
- Increased specificity expected from tims-multiple window DIA
- Proof of concept reveals an extremely high potential of tims-UHR-Q-TOF for fast, sensitive and accurate targeted proteomics

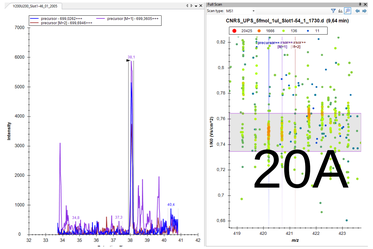
timsTOF Pro

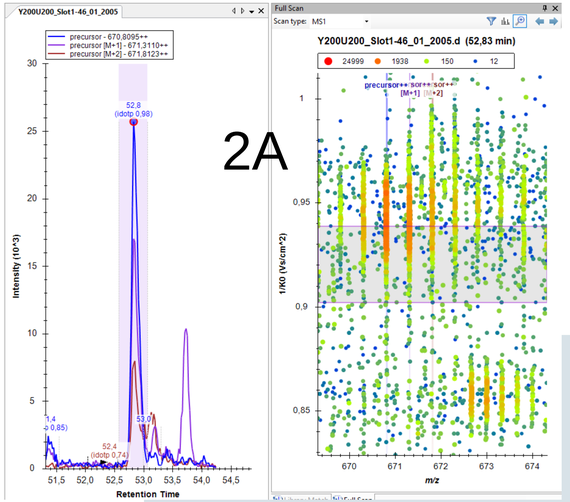


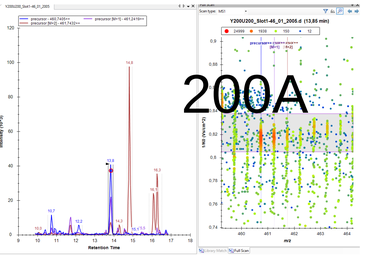
tims-PASEF tims-bbCID

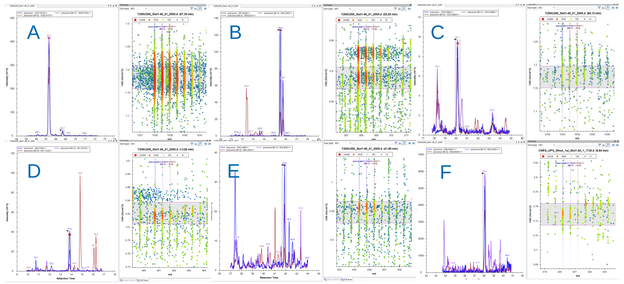






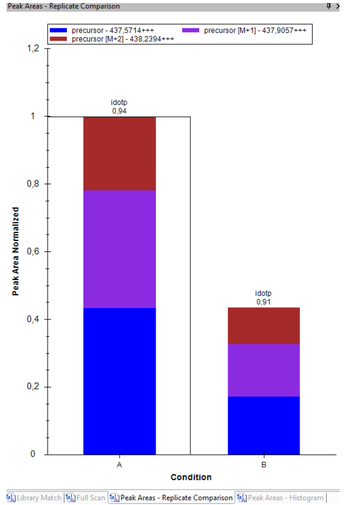






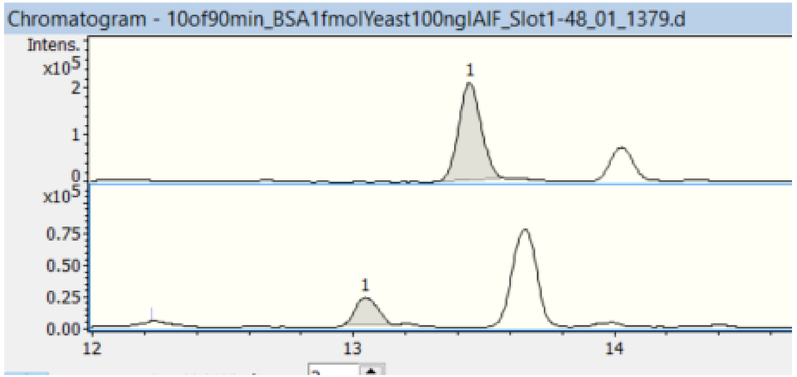
20F 200A

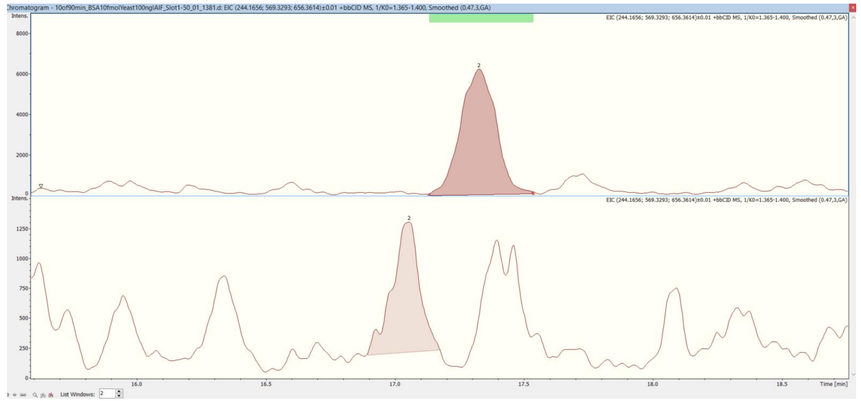
2F 200A 20A



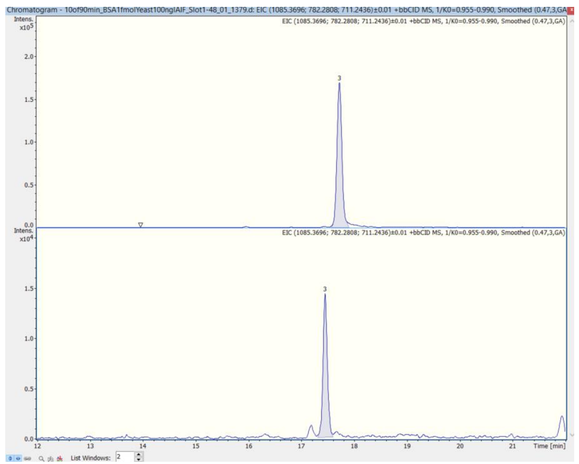
Human catalase 1/2

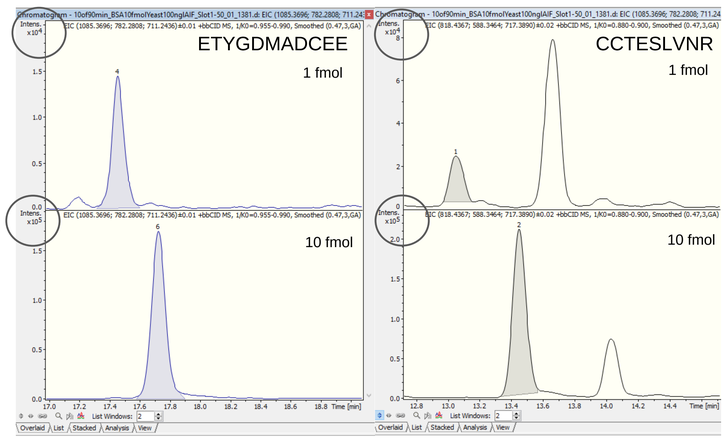
bbCID 1-10 fmol BSA

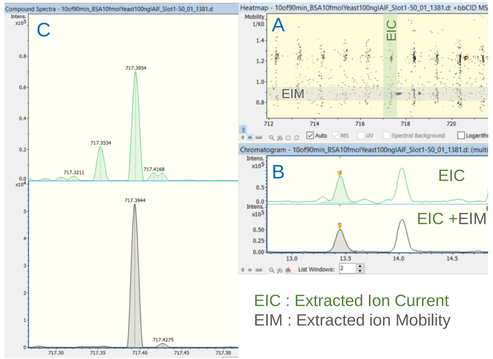




ddspdlpk

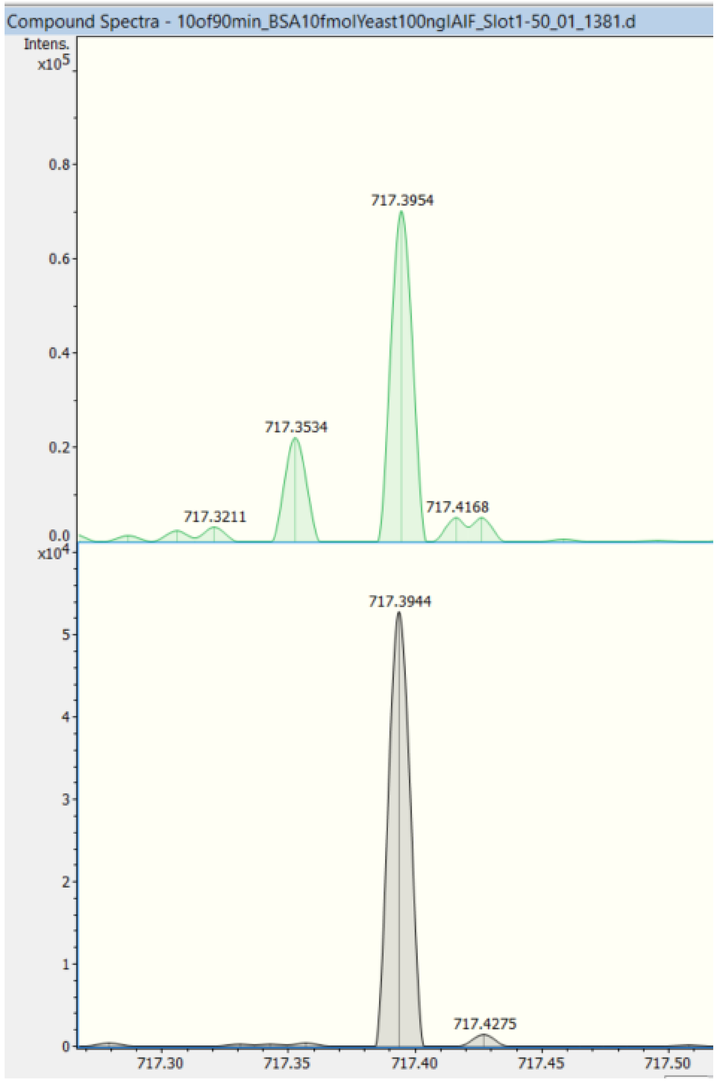






EIC : Extracted Ion Current

EIM : Extracted ion Mobility


Compound Spectra - 10of90min_BSA10fmolYeast100ngIAIF_Slot1-50_01_1381.d
Intens.
x10^5
0.8
0.6
0.4
0.2
0.0
717.3954
717.3534
717.3211
717.4168
x10^4
5
4
3
2
1
0
717.3944
717.4275
717.30
717.35
717.40
717.45
717.50