Evaluation of a timsTOF PRO UHR-Q-TOF bottom-up proteomics platform for Proteoform Profiling and Top-Down approaches

ASMS 2018, TP 754

Jim Kapron[1], Kristina Marx[2], Pierre-Olivier Schmit[3] and Gary Kruppa[4]

[1]Bruker Daltonics, Billerica, USA,
[2]Bruker Daltonik GmbH, Bremen, Germany
[3]Bruker France S.A, Wissembourg, France.

Introduction

Hardware and software developed for bottom up approaches has evolved at a very rapid pace in recent years, making it possible to detect, quantify and characterize more peptides from complex mixtures than ever before. As the bottom up performance has been increasing, the complementarity of proteoform profiling and Top-Down approaches is also becoming clearer, as they provide information on the distribution of protein isoforms and degradation products which cannot be distinguished after digestion.
Ultra-high resolution Q-TOFs analyze highly complex protein mixtures without compromise in dynamic range, mass resolution and accuracy. This poster examines if these these characteristics are changed by the introduction of trapped ion mobility spectrometry (TIMS). At the same time, we have tested the first version of the MASH[1] suite Pro software to automate proteoform identification.

Methods

Undigested protein mixtures of E. coli (Bruker Daltonics),Yeast (Promega) and human cerebrospinal fluid (patient samples), are separated on a 150X2.1 mm Aeris Widepore C4 column (Phenomenex), or on a 50 cm X 100 µm monolithic ProSwift column (ThermoFisher) using 45 and 180 minutes methods, respectively. HPLC's are coupled to a timsTOF Pro UHR-Q-Tof (Bruker) operating in TIMS-off LC-MS or auto LC-MS/MS or to an Impact II UHR-QTOF. Bruker data was automatically processed using the Proteoform Profiling 1.0 routine (Bruker Daltonics) for comparison between impact II and timsTOF Pro. Identifications were performed using Byonic (ProteinMetrics) and MASH (UW Madison - Ge lab) software.

References

(1) https://gecrb.wiscweb.wisc.edu/group-software-mash-suite-pro/

Results

timsTOF Pro/Impact II comparison

As the new timsTOF Pro platform exchanges the standard funnel of the impact II for a new dual TIMS cartridge, the first question that was to be answered was the reproducibility of results formerly obtained on the impact II platform. Results from a 15min separation of an E. coli lysate processed with the Proteoform Profiling 1.0 workflow were compared. The number of proteoforms detected in these conditions is similar on both platforms – slightly higher than 800 – Isotopic resolution could be obtained for 30KDa proteins in complex mixtures. LC-MS and the mass accuracy of deconvoluted proteins was well within 5ppm, while the isotopic ratio accuracy was preserved. The intra-spectral dynamic range for intact proteins was well over 3.5 orders of magnitude (Figure1)

Evaluation of MASH for proteoform identification

The proteoform profiling workflow is a label-free approach to point out regulated proteoforms in a set of samples with limited pre-fractionation, in order to maintain high sample preparation reproducibility over large cohorts of samples. Consequently, the MS/MS identification is preferentially performed after the statistical analysis, in order to fragment the regulated compounds in priority (Top-Down MS/MS speed in the range of 4 – 0.5 Hz does not allow the comprehensive fragmentation of all eluted proteoforms on the fly). In the past we used Byonic™ to perform an automated identification of proteoforms as gene products. A complete characterization was then finalized in BioTools™. MASH allows to combine both steps in one, and the results obtained on an E. coli test analysis point to superior ID performances for MASH (using TopPic as a search engine), while setting the cutoff values at a similar 1% false discovery rate at spectrum level (Table 1).

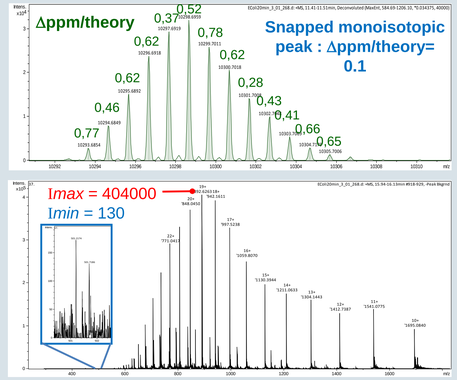

Fig. 1: **timsTOF Pro compatibility with Proteoform Profiling.** Both upper and lower spectra were extracted from a 20 min analysis of an undigested E. coli lysate after automated processing. Upper spectrum: Zoom on the deconvoluted peak of the RS 19 protein (P0A7U3) at 11.5 min retention time, illustrating the resolution and mass accuracy on each peak. Lower Spectrum: Extract @ 16 min, illustrating > 3.5 orders of magnitude in dynamic range. Instrument is far from saturation.

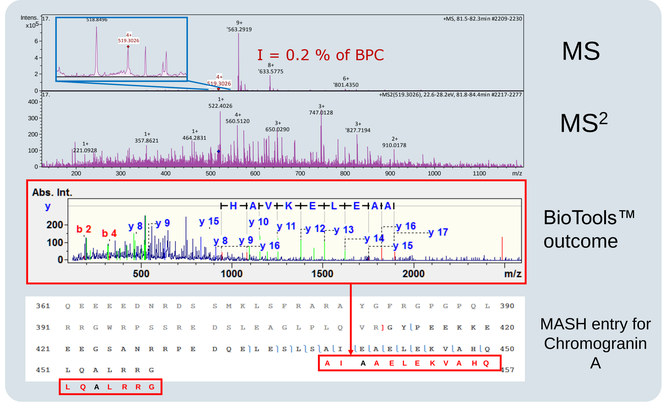

Fig. 2**: identification of low-abundance proteoforms**: Here shown from a CSF extract. The parent ion is of extremely low intensity and the Mascot identification followed by a BioTools™ characterization allows to characterize a mutated (E3A) C-terminal fragment of Chromogranin A. MASH identified 14 proteoforms of Chromogranin A and only the displayed one contains the C-terminal moiety. The lighter proteoform was not identified by MASH but was detected and quantified by Proteoform Profiling 1.0.

	Byonic™ Search	MASH Search
E.Coli Standard : proteins	54 @ 1% FDR	111 @ 1% FDR
E.Coli : Number of identified proteoforms	226	248
E.Coli : Number of characterized proteoforms	Manual With BioTools	248

Table 1**. Illustration of the benefits Brought by the use of MASH for automated searches** Results have been obtained from the 60 min LC-MS/MS separation of an E. coli cell Lysate. Search parameters and Database (SwissProt E. coli) are similar.

Using MASH to reprocess data from a clinical study on cerebrospinal fluid allowed to increase the number of identified proteoforms, however some identifications obtained from Low abundance parent ions could not be reproduced, suggesting a limitation in the feature detection step for low abundance-0,5% of base peak - compounds (Figure2).

Conclusions

- Proteoform Profiling capabilites were maintained between Impact II™ to the timsTOF Pro™ platform.
- MASH offers new capabilities for a higher level of proteoform identification and automation.
- Some algorithm tuning is required to to take into account very low abundance compounds detected by the standard MASH program.
- Proteoform Profiling 1.0 application is fully compatible with the last generation timsTOF Pro platform, identifying and quantifying low level proteoforms.

Proteoform Profiling

For research use only. Not for use in diagnostic procedures.