Versatility of timsTOF platforms for 4-D high throughput and sensitive proteomics studies

Diego Assis[1], Sebastian Vaca[2], Elizabeth Gordon[1], Paul Speir[1], Christopher Adams[2], Jonathan Krieger[3], Tharan Srikumar[3], Matthew Willetts[2],

[1]Bruker Scientific LLC, Billerica, MA, USA

[2]Bruker Scientific LLC, San Jose, CA, USA

[3]Bruker Ltd, Milton, ON, Canada

Introduction

Significant improvements in proteomics have been achieved in recent years regarding identifications, sensitivity and throughput. These include advances in software, hardware, a combination of chromatography, columns, and mass spectrometers. PASEF acquisition modes (dia-PASEF[1], dda-PASEF[2] and prm-PASEF[3]) for example are now well established in the proteomics field. Moreover, the proteomics community also has proposed and tested additional acquisition modes such as slice-PASEF[4] and synchro-PASEF[5]. Here, we show some recent advances of this technology implementing the use of ion mobility prediction combined with different timsTOF instrumentation, column setups and chromatography; improving proteomic depth using dia-PASEF mode. This extends to injecting samples in the order of micrograms or the level of single cell.

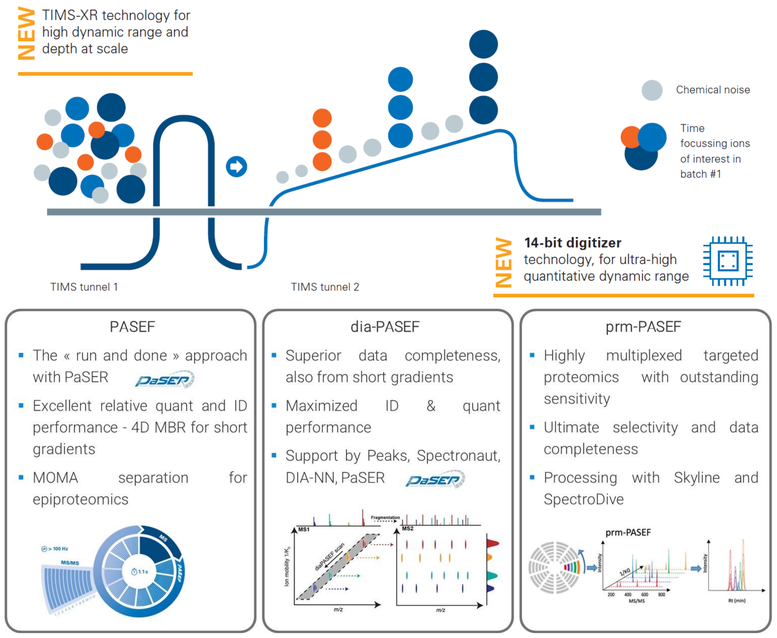


Figure 1: timsTOF HT mass spectrometer is equipped with the 4th generation high capacity TIMS-XR analyzer and advanced digitizer technology (above). All acquisition modes are demonstrated in the panels (below).

Methods

Sample: K562 tryptic digest (Promega) 60 pg to 1 ug

LC-MS setup: EVOSEP One

- timsTOF Pro or HT: 30SPD, Bruker/PepSep 15cm
- timsTOF SCP: Whisper 40 SPD, IonOpticks 15 cm

MS method: dia-PASEF (windows are shown in the figure 2)

Data analysis: PaSER (Bruker) and DIA-NN 1.8 with and without spectral libraries built with TIMScore where measured CCS values are referenced against the predicted CCS values.

[1]Meier et. al., Nat Methods, 2020 (10.1038/s41592-020-00998-0); [2]Meier et. al., J. Proteome Research, 2015 (10.1021/acs.jproteome.5b00932); [3]Lesur et. al., Analytical Chemistry, 2021 (doi.org/10.1021/acs.analchem.0c03180); [4]Szyrwiel et. al., BioRxiv, 2022 (doi.org/10.1101/2022.10.31.51454); [5]Skowronek et. al., BioRxiv, 2022 (doi.org/10.1101/2022.11.01.514654).

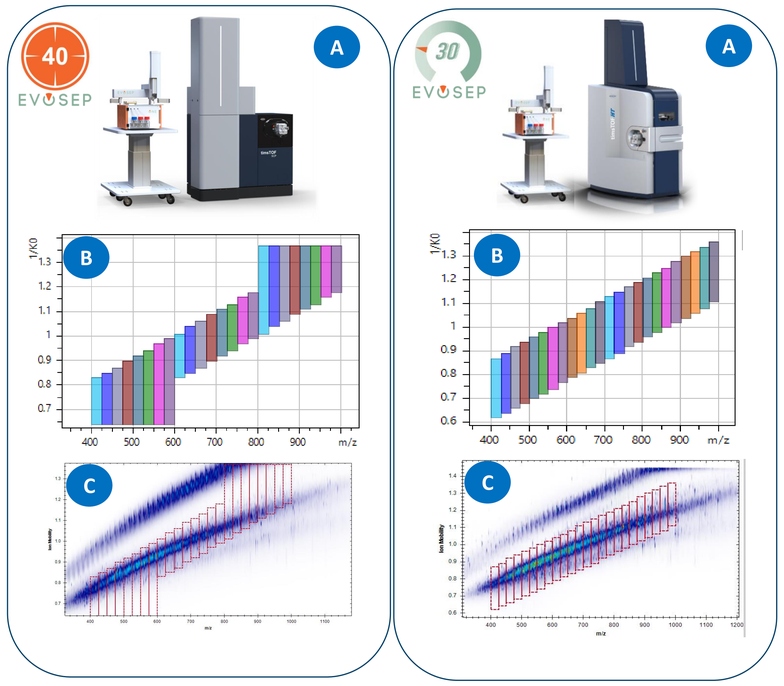
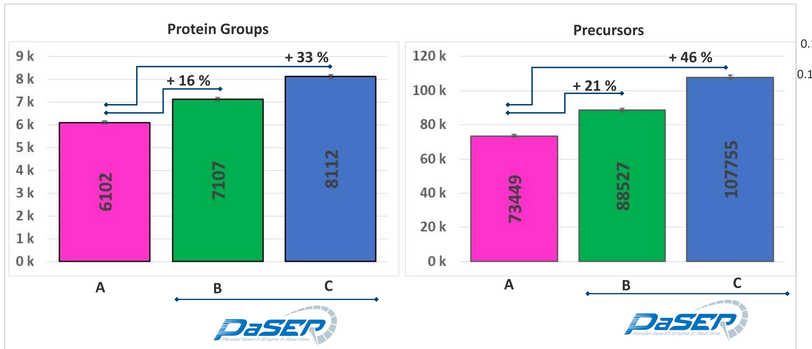


Figure 2: A) LCMS setup: EVOSEP with timsTOF SCP (left) and timsTOF HT (right). B) dia-PASEF windows scheme where same color coded on schemes indicates windows which are collected in the same mobility cycle. Mobility heat maps from Spectronaut (below) showing peptides distributions across dia-PASEF windows (16 ng on left and 200 ng on right) .

Results

Proteomics experiments have shown remarkable developments in throughput and proteomics depth in a short period of time. This has been the result of combining most recent advances in mass spectrometry, columns, chromatography and software as demonstrated in figure 3. Here we have attempted to illustrate one example of those achievements. Results previously acquired on a timsTOF Pro2 one year ago were researched against a library created using TIMScore (PaSER) which utilizes CCS values in a database search algorithm to boost the number of peptide spectrum matches (Bruker App Note LCMS 192). Later this year, similar experiment was performed combining a timsTOF HT and a column with 1.5 um particle size. This new setup shows an increase of 1.3 and 1.5 times in the number of proteins groups and peptides , respectively.



A) 2021 – EVOSEP 30SPD, column 15 cm x 150um – 1.9 um, timsTOF Pro 2, MSFragger DDA Library
B) 2021 – EVOSEP 30SPD, column 15 cm x 150um – 1.9 um, timsTOF Pro 2, Human TIMScore Library (PaSER)
C) 2022 – EVOSEP 30SPD, column 15 cm x 150um – 1.5 um, timsTOF Pro HT, Human TIMScore Library (PaSER)

Different gradients show the same gain in number of identifications across experiment A, B and C – data not shown. 200 ng of K562 on column.

Figure 3: Comparison of the number of identifications from dia-PASEF mode.

In addition, here we demonstrate using 30 SPD (44 minutes) on EVOSEP but loading 1 ug on column, the number of identifications increases to 8467 protein groups and 116k precursors. Moreover, in experiments where a deep depth is required, 1 ug injection in gradients of 60 min using a nanoElute has identified over 10k protein groups. Additionally, demonstrating versatility and sensitivity provided by timsTOF platforms, only 60 picograms on timsTOF SCP mass spectrometer combined with the new method for Evosep systems (Whisper 40 SPD) and Ion Opticks 15 cm, about 1204 protein groups and 6379 precursors were identified (figure 4).

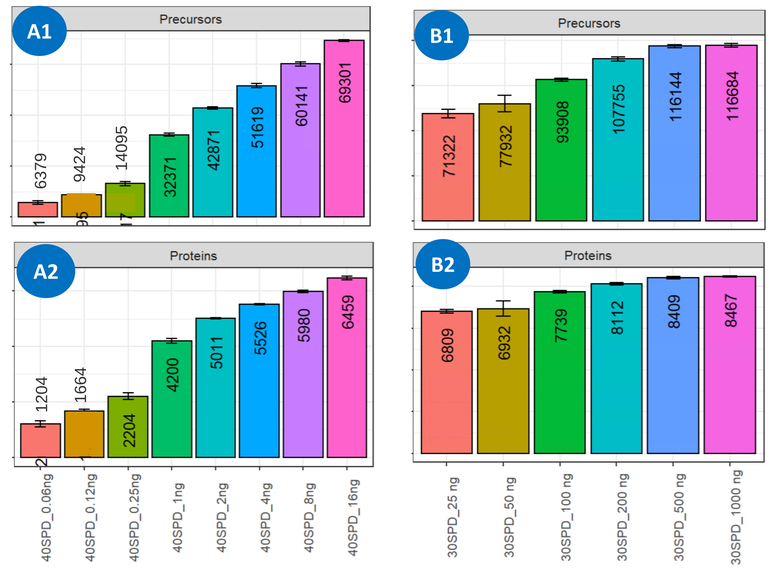


Figure 4: Number of identifications from a dilution series. A1 and A2 shows the number of protein groups and precursors on a timsTOF SCP while B1 and B2 on a timsTOF HT.

Since proteomics quantification is as important as identification, the same experiments mentioned above resulted in tight coefficient of variation (< 20%, data not shown) and intensity in the expected range according to the dilution series, demonstrated in the figure 5 as ratios.

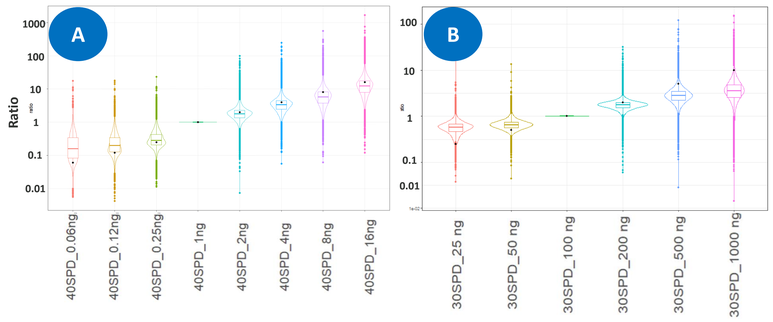


Figure 5: Intensity Box plots illustrating intensity response (in ratio) according to different amounts on the column (dilution series) on timsTOF SCP (A) and timsTOF HT (B).

Conclusion

Results show the versatility of timsTOF platforms enabling high-throughput and high sensitivity experiments. Additionally, we have demonstrated how a combination of mass spectrometer, chromatography, column and software can impact and improve performance providing deep proteomics analysis.

timsTOF