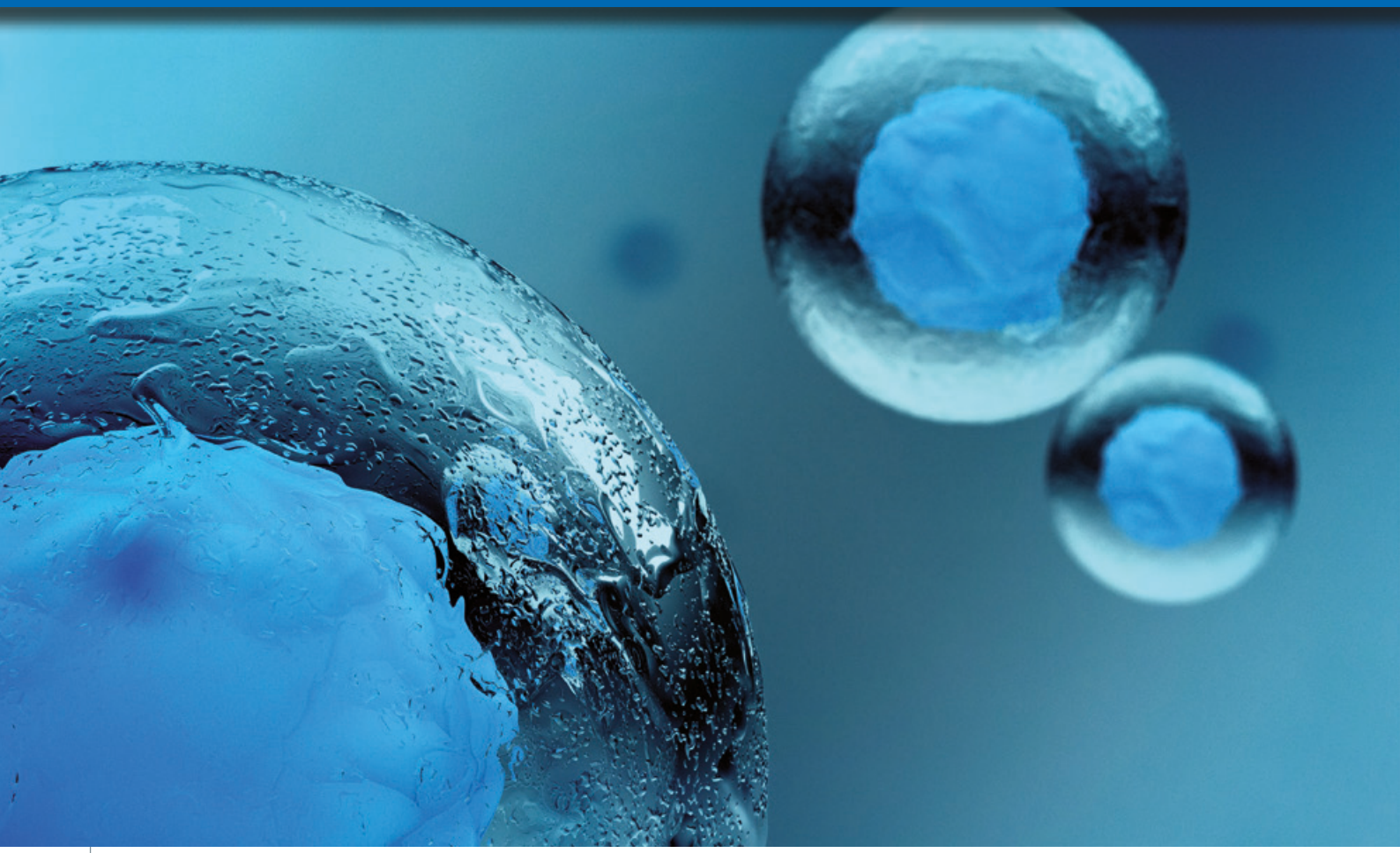

timsTOF Pro and PASEF for High Sensitivity Proteomics and Phosphoproteomics

Introduction

In data dependent acquisition experiments only around 20% of the eluting peptide features are targeted by current mass spectrometers due to limitations in sequencing speed, sensitivity and resolution. We recently described the parallel accumulation serial fragmentation (PASEF) method that has shown promise to increase the sequencing speed and sensitivity of MS/MS scans on a TIMS-QTOF instrument[1]. We show the instrument performance on low sample amounts, offering unprecedented possibilities to investigate samples at high sensitivity and high-throughput. Sensitivity experiments are shown on 12-100 ng of HeLa digest and enrichments of phosphopeptides from < 200 µg of starting material.

Keywords: PASEF, TIMS, shotgun proteomics, phosphoproteomics, high sensitivity

Authors: Heiner Koch[1], Markus Lubeck[1], Scarlet Beck[1], Michael Krause[1], Florian Meier[3], Andreas-David Brunner[3], Oliver Raether[1], Gary Kruppa[2], Matthias Mann[3]
[1]Bruker Daltonik GmbH, Fahrenheitstraße 4, 28359 Bremen, Germany
[2]Bruker Daltonics Inc., 40 Manning Road, Manning Park, Billerica, MA 01821, USA
[3]Max Planck Institute of Biochemistry, Am Klopferspitz 18, 82152 Martinsried

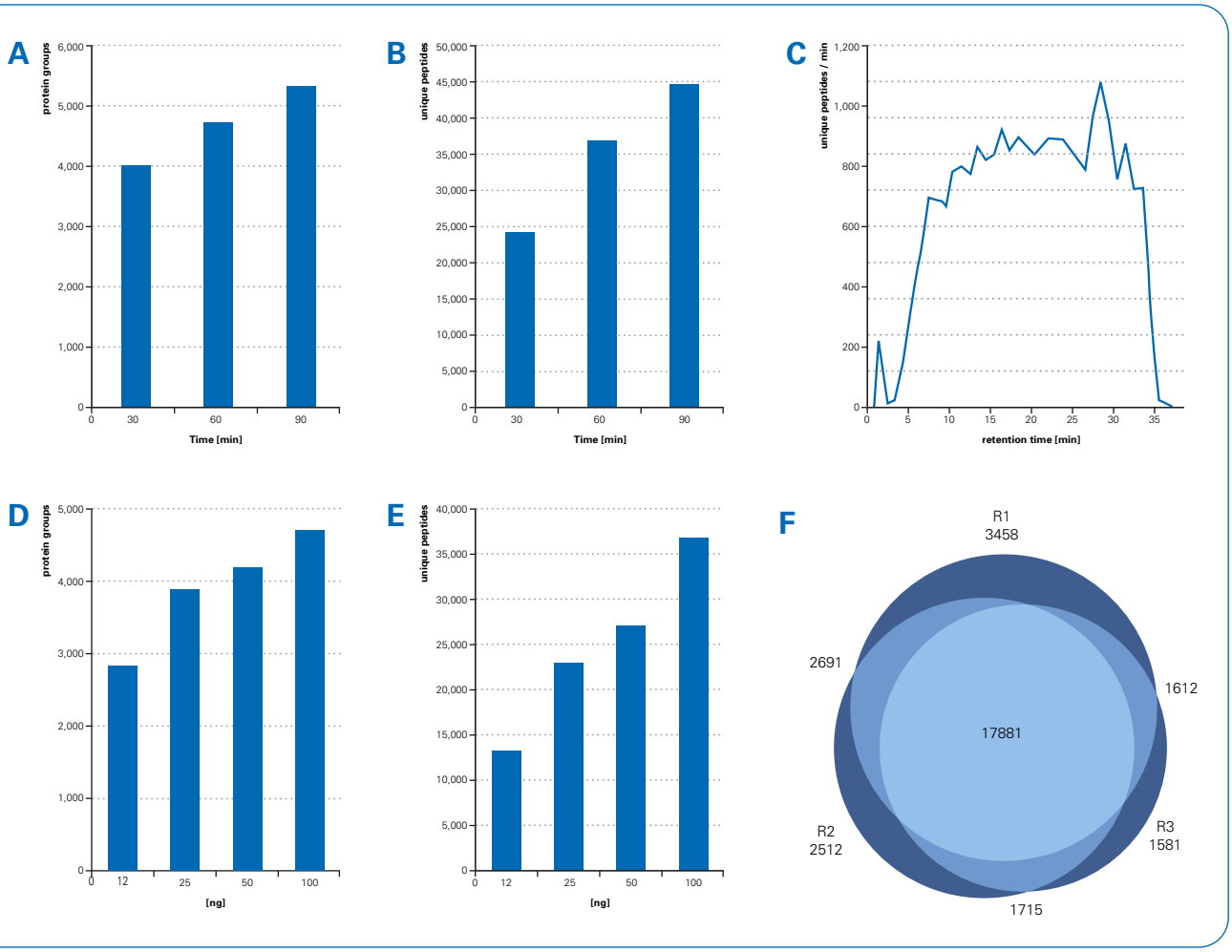

Fig. 1: Single shot proteomics at high depth and sensitivity *A) Identified protein groups on nanoLC gradients from 30-90 min length with 4000-5300 protein groups identified from 100 ng crude digest. B) Identified unique peptides on nanoLC gradients from 30-90 min length with 25,000-45,000 peptides identified from 100 ng crude digest. C) Number of identified unique peptides/min with high identification rates. D) Numbers of identified proteins of 60 min gradients on sample amounts from 12-100 ng. E) Numbers of identified unique peptides of 60 min gradients on sample amounts from 12-100 ng. F) Venn diagram of three technical replicates of 25 ng without match between runs on MS1 level, illustrating high reproducibility of peptide identifications.*

Methods

Low peptide sample amounts (12-100 ng) of a HeLa protein digest and IMAC phosphopeptide enrichments from 200 µg proteolytic digests were HPLC separated (nanoElute, Bruker Daltonics) on 250 mm pulled emitter columns (IonOpticks, Australia) and analyzed on a high resolution timsTOF Pro instrument (Bruker Daltonics) on different LC gradients from 30-90 min.

Postprocessing analysis was performed in Mascot 2.5.1 (MatrixScience). Peptides and phosphopeptides were filtered to <1 % PSM FDR.

Results

- High-throughput DDA measurements with high depth at 30-90 min gradient length resulting in identifications of 25,000-45,000 unique peptide and 4000 to 5300 protein groups (Fig. 1A, B).
- Extremely fast acquisition speed leading to high identification rates/min (Fig. 1C).
- Very sensitive measurements possible at only 12 ng sample amount (3000 proteins groups, ~13,000 peptides, Fig. 1D, E).
- Reproducible peptide identification from low sample amounts (Fig. 1F).
- Phosphopeptide analysis from low sample amounts resulting in phosphopeptide identifications of more than 14,000 unique peptides in 90 min LC gradient time (Fig. 2)

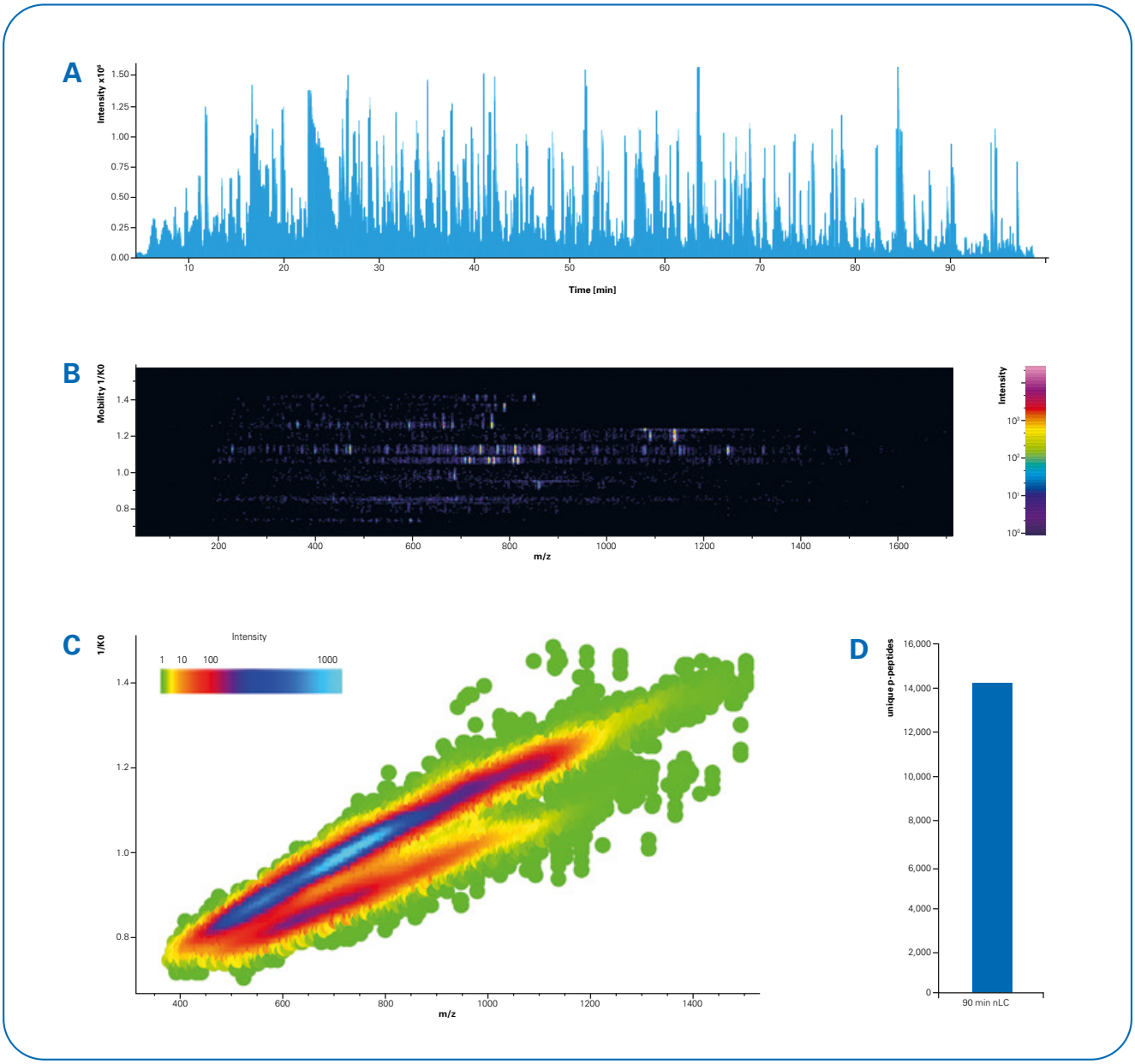

Fig. 2: Deep phosphoproteomics on 90 min LC gradients *A) Base peak intensity of a 90 min LC gradient from IMAC phosphopeptide enrichments and 200 µg starting material. B) PASEF MS/MS heat map of selected precursors. C) Density heat map of identified phosphopeptide features eluting from the nLC over the 90 min gradient in the 1/K0 to m/z space. The 1/K0 separation of peptides has several further applications including potential p-peptide isomer separation. Only multiply charged precursors are shown, singly charged species are excluded for MS/MS precursor selection. D) Number of unique p-peptides identified in a 90 min gradient at 1% PSM FDR.*

Outlook

PASEF on a timsTOF Pro instrument offers the possibility to investigate samples with limited amounts (e.g. phosphopeptides or clinical samples) to an unprecedented depth.

Conclusion

- High sensitivity; PASEF offers the possibility for deep single shot proteomics in ng amounts
- Sensitive PTM analysis to investigate signal transduction pathways
- High-throughput; PASEF enables deep proteomics analysis on short gradients

Learn More

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Check out the Link or scan the QR Code.

www.bruker.com/timstofpro

References

[1] Meier F., Beck S., Grassl N., Lubeck M., Park M., Raether O., and Mann M., Parallel Accumulation - Serial Fragmentation (PASEF): Multiplying Sequencing Speed and Sensitivity by Synchronized Scans in a Trapped Ion Mobility Device, J. Proteome Res., 2015

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Bruker Daltonics GmbH & Co. KG

Bremen · Germany
Phone +49 (0)421-2205-0

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660

ms.sales.bdal@bruker.com - www.bruker.com