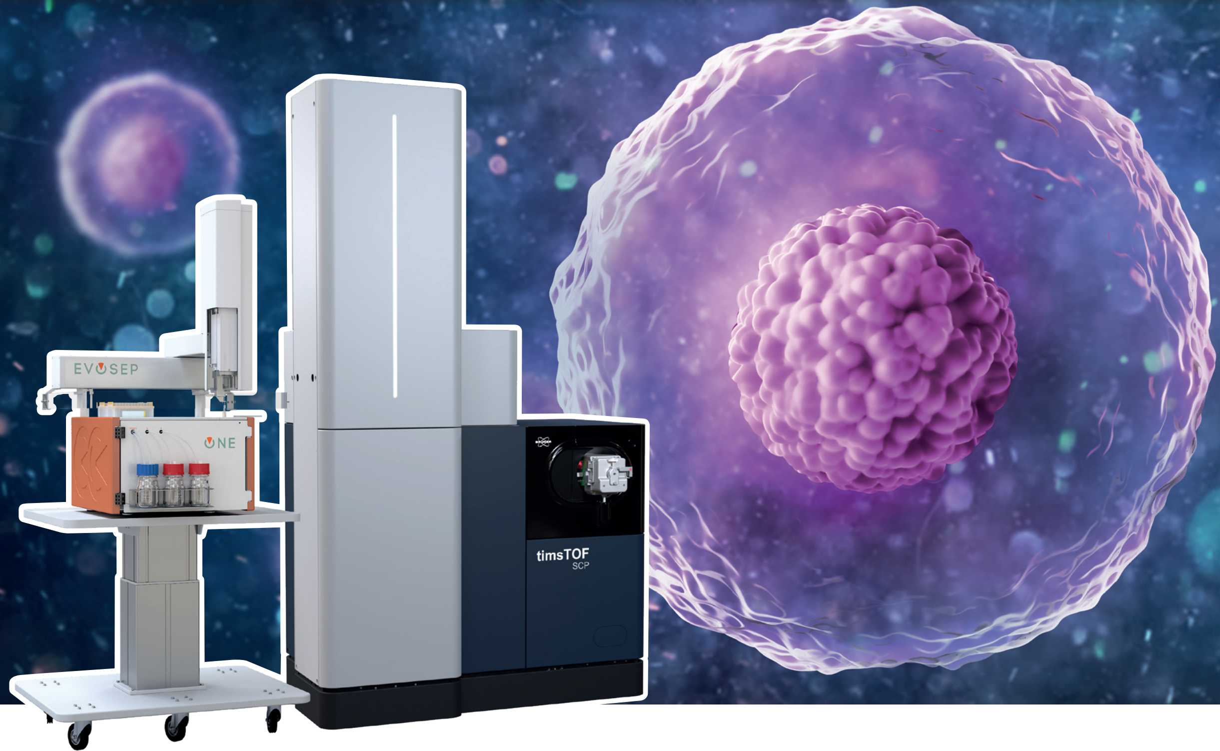
BRUKER



Proteomics at the single cell level with Evosep Whisper and the timsTOF SCP

Single cell proteomics is a rapidly developing field with the potential to make important contributions to the understanding of cellular heterogeneity.

Abstract

Recent enhancements in trapped ion mobility spectrometry (TIMS) coupled to fast and sensitive mass spectrometry, established in the timsTOF SCP, paired with fast and robust liquid chromatography, enabled by the Evosep One, open new possibilities for proteome analyses at the single cell level. HeLa peptide digests, ranging from picogram to low nanogram amounts, were analyzed with Evosep's Whisper 40 samples per day method and detected on a timsTOF SCP mass spectrometer. The Evosep One – timsTOF SCP combination gives market leading sensitivity and the data presented here shows the reproducible identification of ~1250 proteins from 250 pg of protein, the typical amount in a single cell. This will allow scientists to investigate cellular heterogeneity and measure phenotypic responses to drugs and other external stimuli on the single cell level in an unbiased way, without the need for antibodies and affinity reagents.

Keywords: Single cell proteomics, dia-PASEF, Evosep, Whisper, timsTOF SCP

EVOSEP

Authors: Christoph Krisp[1], Nagarjuna Nagaraj[1], Renata Blatnik[1], Heiner Koch[1], Markus Lubeck[1], Gary Kruppa[1], Dorte Bekker-Jensen[2], Nicolai Bache[2], Ole Bjeld Hørning[2];
[1] Bruker Daltonics GmbH & Co. KG, Bremen, Germany, [2] Evosep Biosystems, Odense, Denmark.

Introduction

In recent years, with ongoing improvements in mass spectrometry platforms and proteomics sample preparation for single cell, the analysis of single cell proteomes has become an achievable goal. However, the predominant aim of single cell analysis is to decipher the cellular heterogeneity in samples, especially in tumor tissue, requiring dozens to hundreds of individual single cells to be analyzed in a short timeframe. This requires not only highly sensitive MS systems, but also systems capable of high throughput. With the introduction of trapped ion mobility spectrometry (TIMS), a new level of sensitivity and speed has been reached when coupling this technology with fast scanning, high resolution time of flight (TOF) analyzers (1). The latest enhancements in ion transfer with a larger transfer capillary for up to 5-fold higher ion transfer, additional higher-pressure ion transfer optics for more effective ion collection and two orthogonal deflections to maintain robustness, introduced with the timsTOF SCP have pushed the limits of detection even lower (2). The speed of the instrument is more than sufficient to keep up with the fast and robust chromatography, achieved with the Evosep One system's short gradients (3). The recently introduced Whisper methods designed for ultra-sensitive analyses, operating at the low flow rate of 100 nL/min (4) are perfectly suited for the sensitivity level reached with the timsTOF SCP. The combination of fast and robust chromatography of the Evosep One system coupled with rapid and sensitive mass spectrometry delivered by the timsTOF SCP meets the demands of single cell proteomics for both speed and sensitivity.

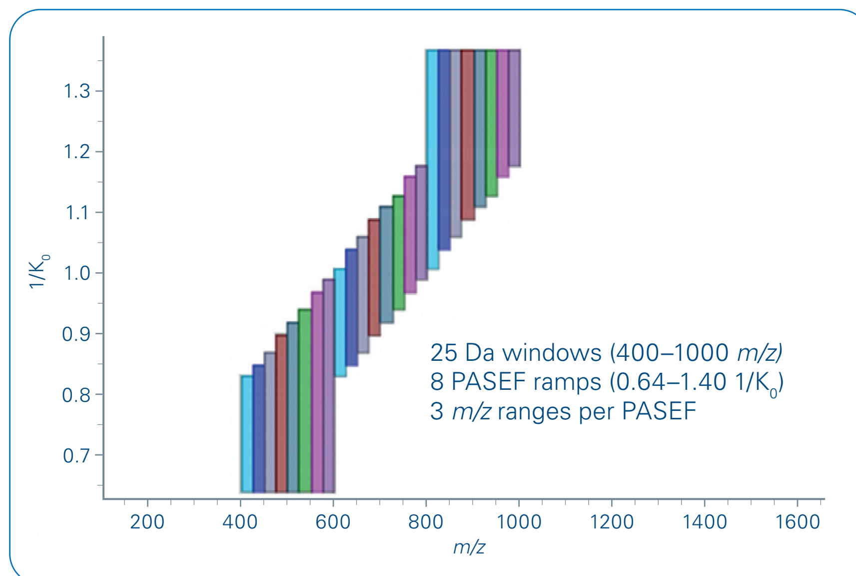


Figure 1: dia-PASEF window placement scheme

Untargeted proteome-wide data-dependent quantification has been the most widely used strategy in proteomics. More recently, data-independent acquisition (DIA) methods are becoming the routine strategy for quantification of samples for various purposes (e.g. clinical research proteomics discovery, companion diagnostics and personalized medicine research).

Data Independent Acquisition - Parallel Accumulation and SErial Fragmentation (dia-PASEF®) on the timsTOF platforms have the advantage of separating ions based on space and time therefore increasing sensitivity and efficient precursor scheduling for fragmentation, providing collisional cross-section (CCS) values and separation of isomeric species that are mobility offset but mass aligned (MOMA) (5).

Here we show the performance of the powerful combination of low flow Whisper methods on the Evosep One and the dia-PASEF acquisition strategy on the timsTOF SCP platform. In a concentration range down to the single cell level this combination delivers fast and in-depth proteome quantification with >1000 protein groups from 250 pg of peptide samples.

Methods

Human cervical cancer cell digests (HeLa, Pierce, Cat. 88328) were loaded on Evotips (EV2001) according to Evosep sample loading instructions (6) for reproducibility assessment of 5 ng on tip and for dilution series of 0.125, 0.25, 0.5, 0.75 1, 1.5, 2, 5, 10, and 20 ng on tip. Peptides were separated on a 15 cm performance column (15 cm x 75 μm, 1.9 μm particle size, EV1112) with the Whisper 40 samples per day method on the Evosep One coupled to a timsTOF SCP mass spectrometer via a CaptiveSpray ionization source.

Eluting peptides were analyzed with a dia-PASEF method with high sensitivity mode enabled. For dia-PASEF acquisition, a window placement scheme consisting of 8 TIMS ramps with 3 mass ranges per ramp spanning 400 – 1000 *m/z* and a mobility range of 0.64 – 1.40 $1/K_0$ with a cycle time of 0.9 seconds, including one MS1 frame, was utilized (Figure 1).

All dia-PASEF data were processed in DIA-NN v1.8 (7) in library-free mode without match between runs using the human reviewed protein sequence database including isoforms (Uniprot, downloaded November 2021). Variable modifications were excluded, except carbamidomethylation and methionine loss on the protein N-term. The number of identified protein groups and precursor as stated in the statistics .tsv file was used for comparison.

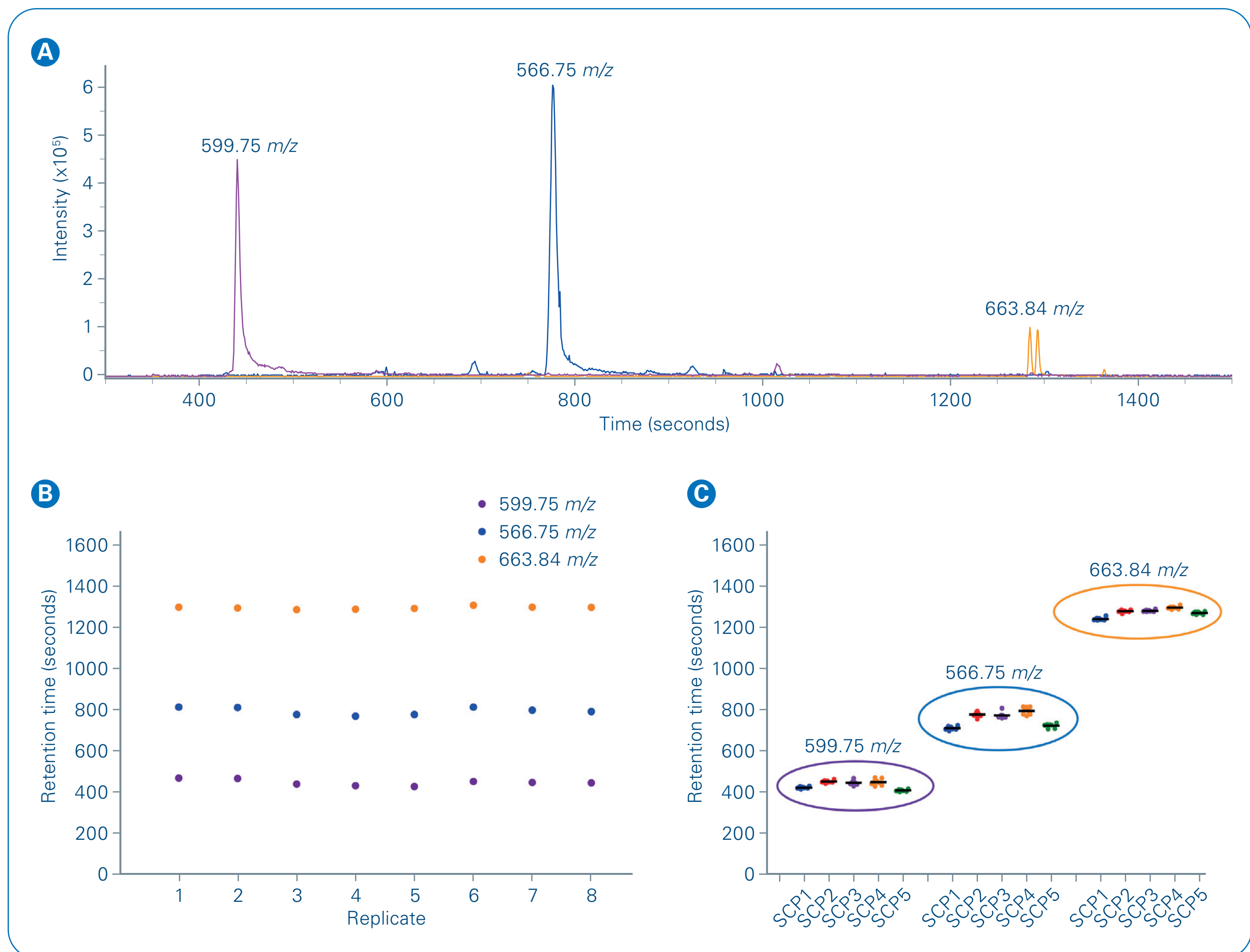


Figure 2: Chromatographic separation assessment. (A) Overlayed EICs of m/z values 599.75 +/- 0.02 Da, 566.75 +/- 0.02 Da, 663.84 +/- 0.02 Da representing a typical elution profile from a 5 ng peptide load of a HeLa digest, (B) retention times of the three peptides across 8 replicates of a 5 ng peptide load of a HeLa digest, (C) retention times of the three peptides across 8 replicates run on 5 timsTOF SCP instruments of a 5 ng peptide load of a HeLa digest.

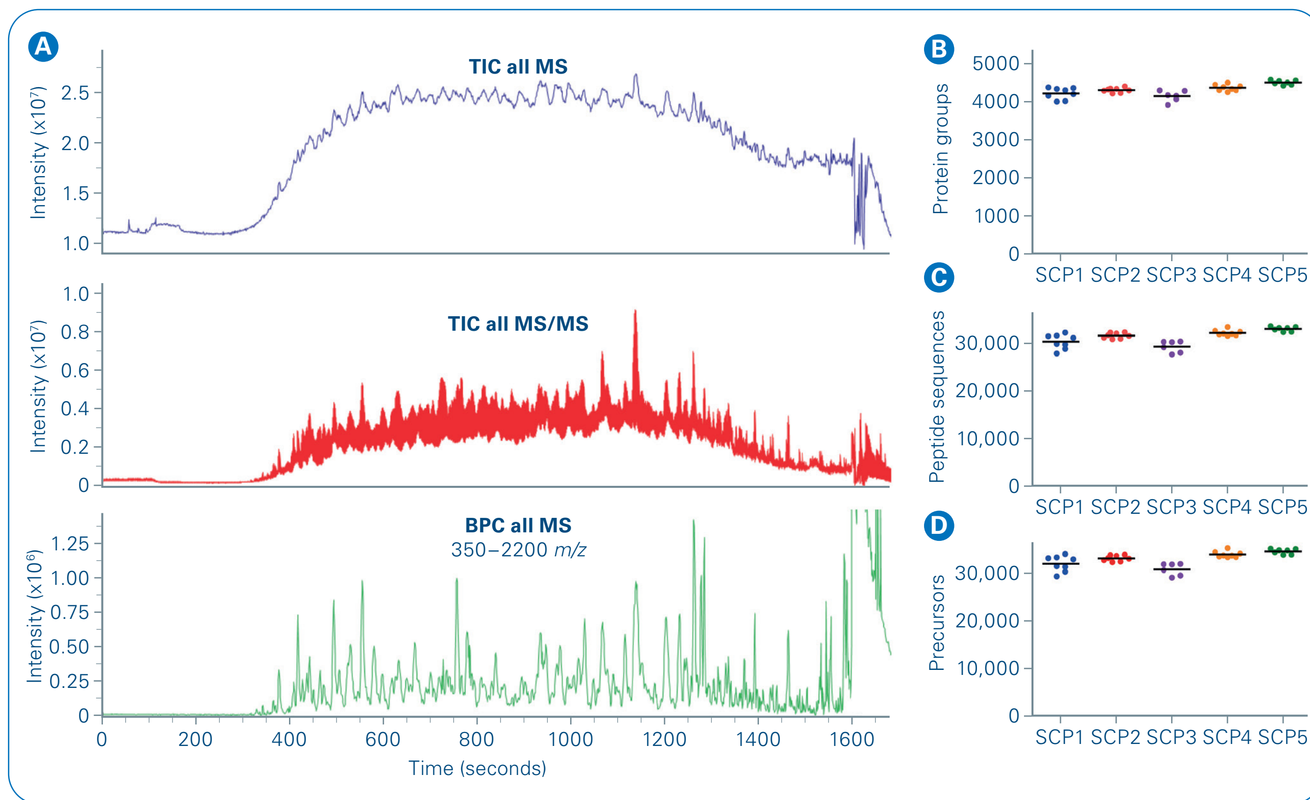


Figure 3: Identification rate reproducibility at the protein and peptide level. (A) representation of a typical TIC all MS, all MS/MS and BPC of 5 ng peptide load of HeLa digest analysed in dia-PASEF, (B) reported protein group, (C) stripped peptide sequences and (D) precursors of 5 ng peptide load of HeLa digest on Evotips processed with DIA-NN v1.8 in library-free mode.

Results and discussion

For quality control assessment, 5 ng of tryptic peptides from a commercially available HeLa cell digest were loaded on Evotips and analyzed by dia-PASEF to assess chromatographic and quantitative reproducibility. Three peptides eluting throughout the gradient were picked to assess chromatographic reproducibility by extracted ion chromatograms (EIC) for *m/z* values 599.75 +/- 0.02 Da, 566.75 +/- 0.02 Da, and 663.84 +/- 0.02 Da (Figure 2A). The full width at half maximum (FWHM) varied between 3.5 and 6.0 s demonstrating excellent chromatographic separation, ideally matched to the cycle time of the applied dia-PASEF method. The retention time for these three peptides varied in average by 30 s (Figure 2B) within 8 replicates. on the Evosep One and timsTOF SCP system and across five timsTOF SCP instruments with the same Evosep One by 50 s (Figure 2C).

The identification rate reproducibility on the protein and peptide level was assessed across multiple timsTOF SCP instruments. A typical total ion current (TIC) of all MS, MS/MS as well as base peak chromatogram (BPC), generated from 5 ng peptide load of HeLa digest on Evotip, are shown in Figure 3A. The raw files were processed with DIA-NN v1.8 in library free mode. The reported ID rates in the "stats.tsv" for precursor and protein group IDs as well as unique stripped sequences from the main .tsv report were compared between 5 different timsTOF SCP instruments (Figure 3B - D). On average 4305 +/- 152 protein groups, 31,324 +/- 1543 stripped peptide sequences and 32,950 +/- 1597 precursors were identified per instrument with an intra-instrument CV of 1 – 5 % at protein, peptide, and precursor identification level.

For a quantitative analysis, a dilution series from 20 ng down to 125 pg peptide loads of a HeLa digest on Evotips was prepared (6 replicates) and processed with DIA-NN v 1.8 in library-free mode. From 125 pg, this yielded 2870 precursors and 2850 peptides corresponding to 690 protein groups. When increasing the load to 20 ng, 45,700 precursors and 42,800 peptides were identified corresponding

to 5400 protein groups (Figure 4A– C). The mean CV of the quantified protein groups for all concentrations was less than 10%, where the lowest peptide loads resulted in the highest mean CV, and the highest peptide loads resulted in the lowest mean CV (Figure 4D). For visualization of the quantification accuracy assessment the protein high mobility group box 1 (HMGB1) was used because it was quantified in each sample of each concentration throughout the entire concentration range loaded onto the Evotips. The mean protein group area (sum of the area under the curve of all precursors quantified from HMGB1) per concentration was correlated with the concentration range. The Pearson correlation (PC) score was 0.999, demonstrating a linear concentration response (Figure 4E). This was also performed for all protein groups with quantitative values (n=6) in at least three concentrations (3991 protein groups) (Figure 4F). The mean PC score was 0.94 with 3539 protein groups showing a correlation greater than 0.9 demonstrating excellent concentration responses at the whole proteome level.

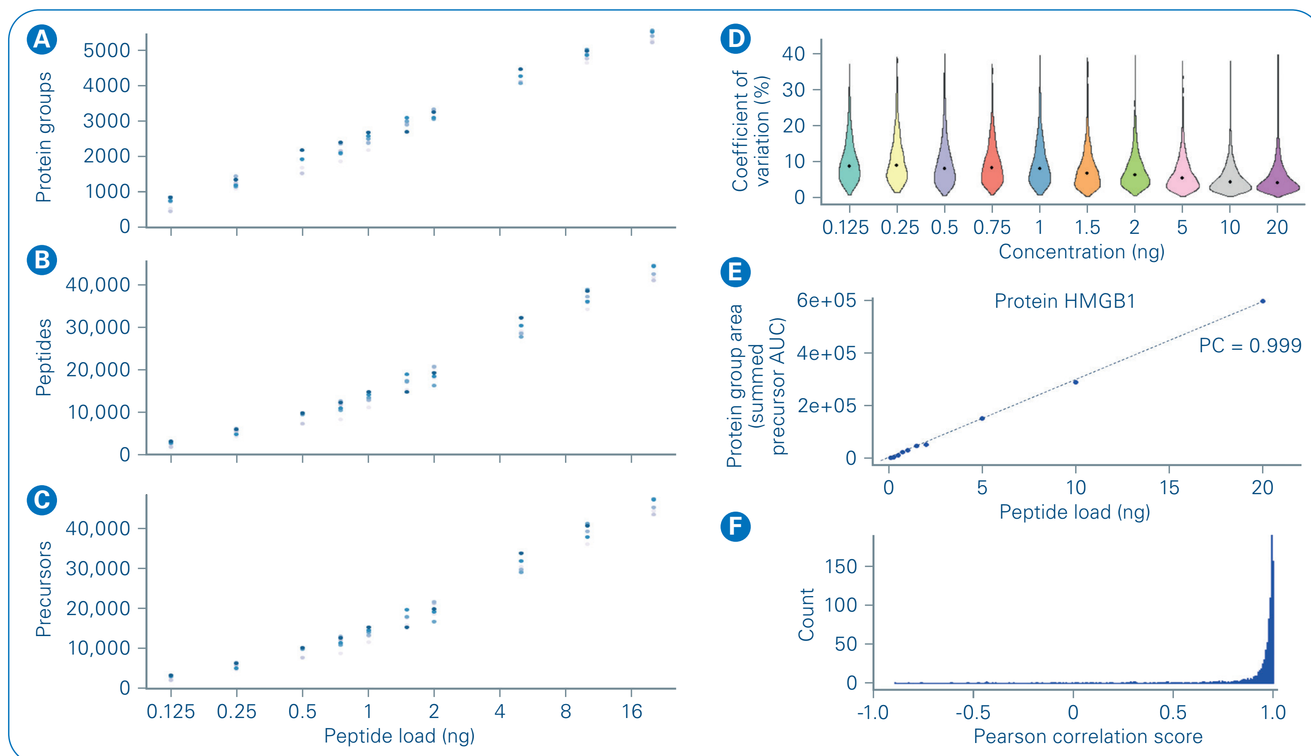


Figure 4: Quantitative assessment of HeLa digest dilution series. (A) reported protein group, (B) stripped peptide sequences and (C) precursors of a HeLa digest dilution series with peptide loads of 0.125, 0.25, 0.5, 0.75, 1, 1.5, 2, 5, 10 and 20 ng processed with DIA-NN v1.8 in library-free mode, (D) CV of quantification within individual concentration ranges (n=6), (E) correlation of peptide load on Evotip with protein group area (summed precursor area under the curve), (F) Histogram of Pearson correlation scores of protein groups with quantitative values (quantified in all replicates) in at least 3 concentrations with the peptide amount loaded onto an Evotip.

Conclusion

- The low flow Evosep Whisper methods (100 nL/min in the core part of the gradient) enables high sensitivity with good chromatographic reproducibility and robustness with short gradients and low overhead time between gradients
- The novel ion optics of the timsTOF SCP instrument, optimized for high-sensitivity helps in efficient transmission of generated ions, adding another layer of sensitivity enhancement
- High instrument to instrument identification rate reproducibility demonstrated with 5 ng peptides of a HeLa digest loaded onto Evotips
- dia-PASEF provides a further sensitivity gain with a mean of 5600 peptides from 1250 protein groups identified at 250 pg and a linear concentration response in quantification from 125 pg up to 20 ng

Learn More

You are looking for further Information?
Check out the link or scan the QR code.

www.bruker.com/timstof-scp

References

[1] Meier, F et al. (2015), J. Proteome Res.

[2] Application Note (2021), Bruker Daltonics, LCMS-185, 1888581

[3] Bache, N et al. (2018), Mol Cell Proteomics

[4] Brunner, A et al. (2020), bioRxiv 2020.12.22.423933

[5] Meier, Fet al. (2018), Mol Cell Proteomics

[6] Application Note (2020), Evosep, PR-001A-Sample loading protocol

[7] Demichev, V et al. (2020), Nat Methods

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

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

Bruker Daltonics is continually improving its products and reserves the right
to change specifications without notice. © Bruker Daltonics 02-2022, LCMS-193, 1894933