TIMS-QTOF MS

timsTOF HT

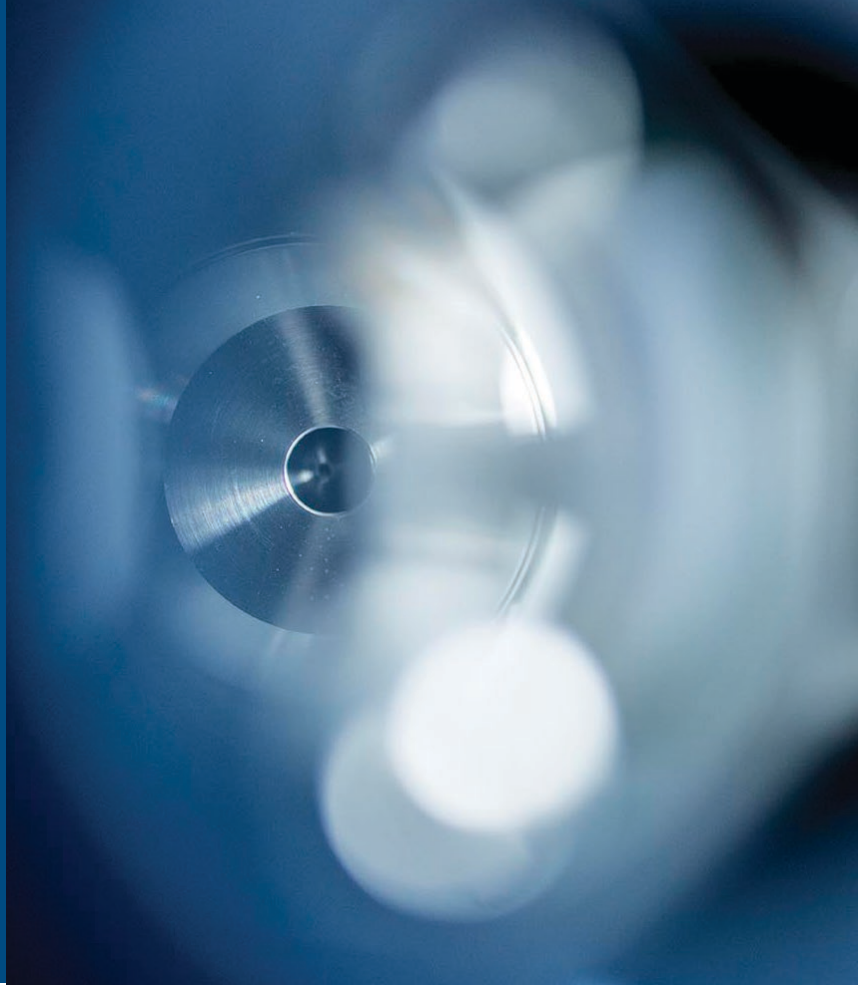
Expanding the capabilities of high-throughput, 4D-Proteomics™

Innovation with Integrity

TIMS benefits

Expanding the capabilities of high-throughput, 4D-Proteomics™

Introducing the timsTOF HT mass spectrometer with 4th generation high capacity TIMS-XR analyzer and advanced digitizer technology (ADT). The timsTOF HT offers higher dynamic range for unmatched analytical depth and quantitation in high throughput proteomics experiments. The timsTOF HT is fully CCS-enabled and supports all PASEF© acquisition modes including PASEF, dia-PASEF© and prm-PASEF© achieving flexibility in 4D-Proteomics™. Simply uncompromised proteomic depth at scale!



NEW TIMS-XR technology for high dynamic range and depth at scale

Chemical noise

Time focussing ions of interest in batch #1

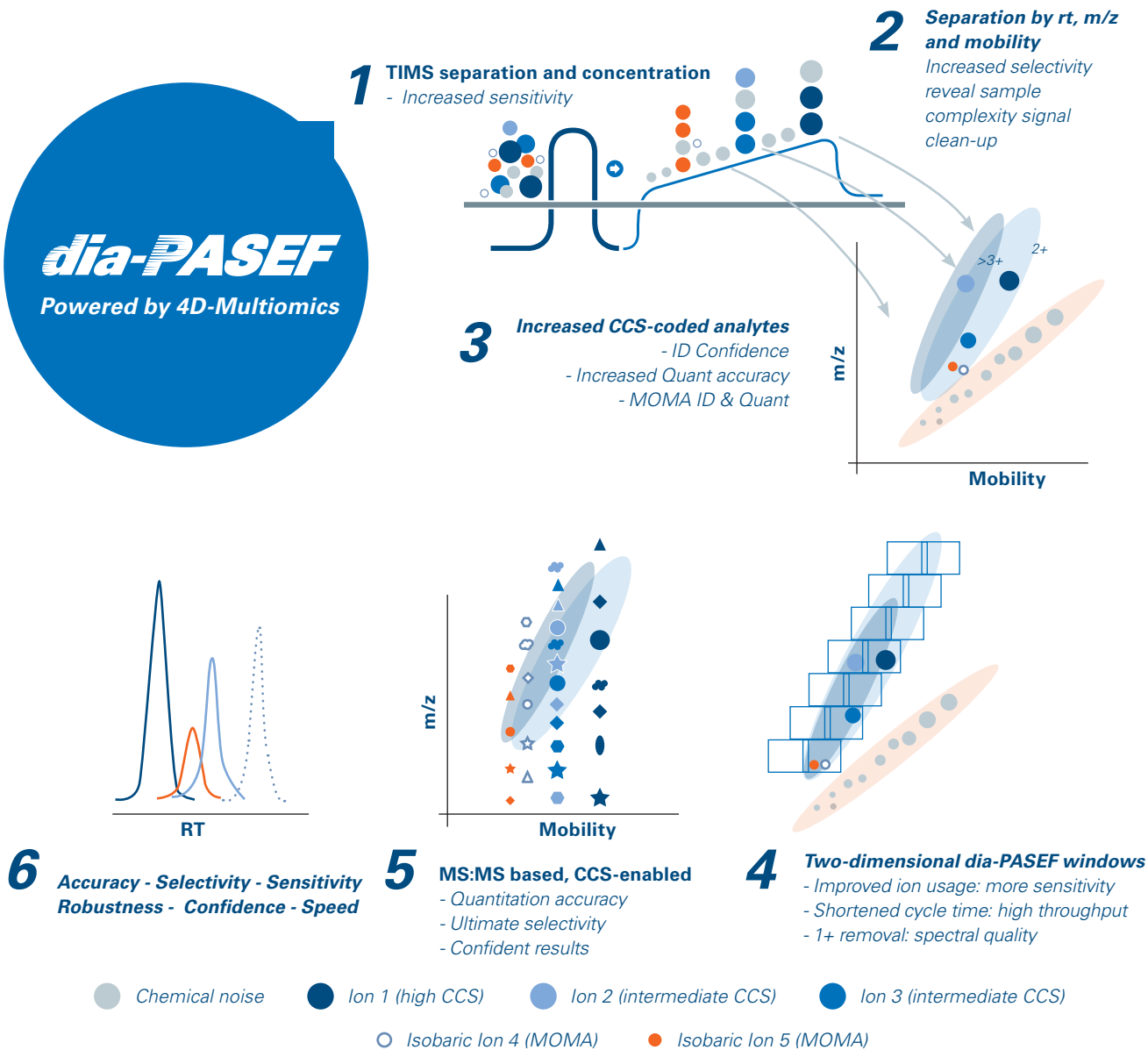
NEW **14-bit digitizer** technology, for ultra-high quantitative dynamic range

Dual-TIMS and CCS-enabled analysis

Trapped ion mobility spectrometry (TIMS) resolves sample complexity through an added dimension of gas phase separation on top of LC-MS. TIMS accumulates and concentrates ions (time-focusing effect) of a given mass-to-charge and mobility (based on cross sectional attributes). This allows for higher fidelity separation of noise from signal, which enables an increase in sensitivity with speed (> 100 Hz TIMS duty cycle). The 4th generation dual TIMS-XRTIMS achieves a near 100% duty cycle by accumulating ions in TIMS funnel 1, while ions in TIMS tunnel 2 are released sequentially (> 150 Hz). This process of parallel accumulation serial fragmentation (PASEF®) enables high speed collisional cross section (CCS) analysis.

dia-PASEF adding confidence to your identifications

Boosting data-independent-analysis with the speed of PASEF and unmatched specificity of TIMS-derived Collisional Cross Sections (CCS)

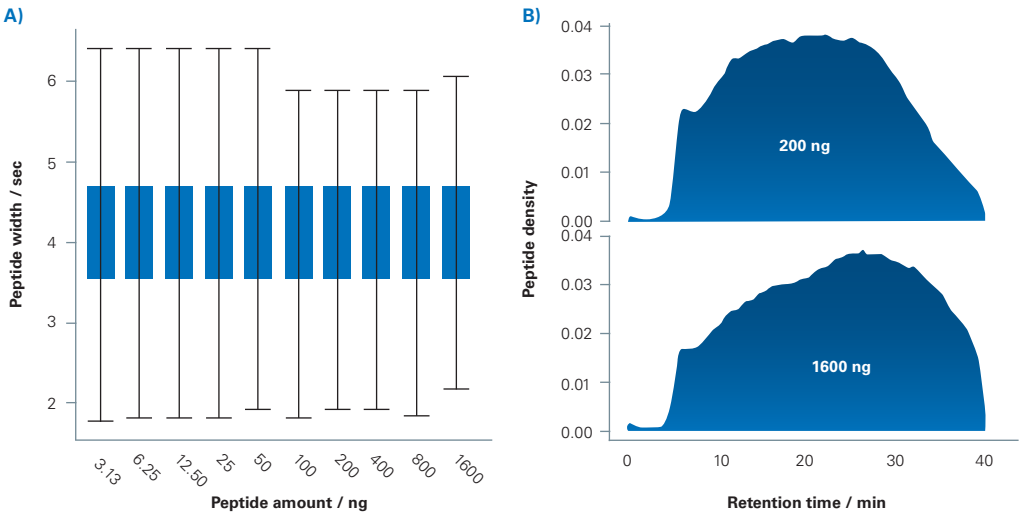


Data-independent acquisition dia-PASEF is both more sensitive and selective than traditional DIA approaches as it applies the PASEF principle to combine the advantages of DIA with the inherent ion efficiency of PASEF. Over the entire liquid chromatography-mass spectrometry (LC-TIMS-MS)/MS dia-PASEF run, a perfect data cuboid is created containing m/z, ion mobility (CCS), retention time and intensity. TIMS separation increases selectivity, excludes singly charged precursors from fragmentation and cleans up the sample by concentrating signals from noise. Making use of the correlation of molecular weight and CCS coded information from the dual-TIMS funnel, dia-PASEF enables highly confident identification.

Higher sample capacity for nano LC-MS/MS

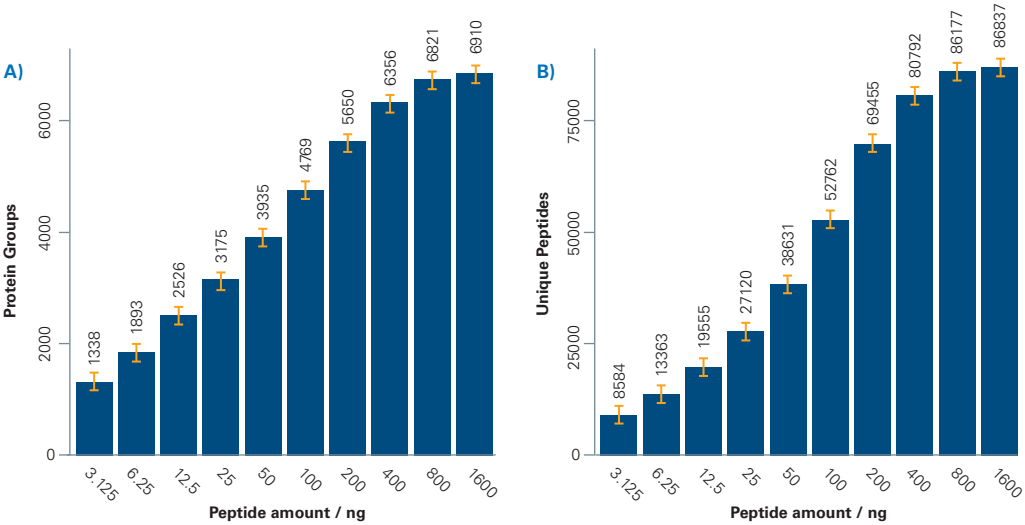
Maximize peptide detection capabilities for high sample loads

The nanoElute series provides robust and reliable gradient separation, resulting in narrow peak widths and excellent peak capacities. Using a PepSep Twenty-five series column (150 µm ID, 1.5 µm particle size), average peak widths less than five seconds were achieved for K562 tryptic digest (Promega) sample loads up to 1600 ng. A total runtime of 41 min was adequate for efficient peptide separation, resulting in homogenous peptide elution across the gradient even at high peptide loads.



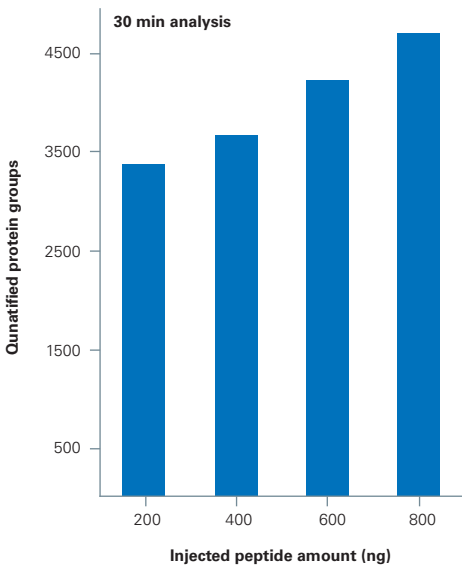
A) Narrow average peak widths for 3.13 ng to 1600 ng K562 tryptic digest. Boxes and whiskers correspond to 50% and 1.5 times the interquartile data range, respectively (Outliers not shown).
B) Homogenous elution of peptides as indicated by kernel density estimate plots for 200 ng and 1600 ng K562 tryptic digest.

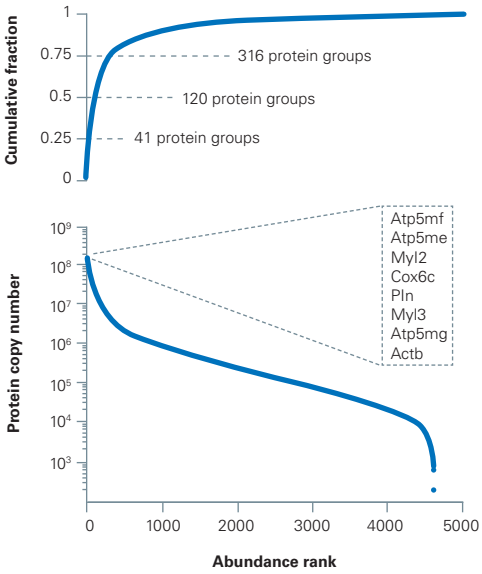
Excellent separation is the prerequisite for successful mass spectrometric detection. With improved ion storage capacity, the timsTOF HT provides the means to increase the dynamic range for the detection of eluting peptides. This leads more than 6900 protein group identifications and 86,000 unique peptide identifications within a 41 min gradient length for 1600 ng K562 tryptic digest by data dependent acquisition parallel accumulation serial fragmentation PASEF (DDA).



A) The TIMS XR allows for increased ion capacity for sample loads beyond 200 ng. Reproducible identification of ~ 6900 protein group identifications and more than 86,000 unique peptide identifications
B) using the PaSER real-time search engine with TIMScore for dda-PASEF of up to 1600 ng K562 tryptic digest. Error bars indicate standard deviation for 5 replicates with mean displayed above histogram.

Enabling rapid tissue proteome analysis with dia-PASEF





Rapid cardiac tissue proteomics. The new timsTOF HT featuring the well-known dia-PASEF technique enables robust and accurate quantitation of proteomes in tissues at only 30 min LC-MS gradient time. Different amounts of a murine heart proteome digest were analyzed in triplicates with short liquid chromatography gradients and dia-PASEF.

At the highest sample load, we identified in total 4700 protein groups spanning more than five orders of magnitude in relative abundance. Raw data were processed with DIA-NN 1.8 and UniProt fasta in library-free mode and protein copy numbers were estimated with the "proteomic ruler" approach.

Professor Dr. Florian Meier-Rosar

Independent Research Group Functional Proteomics, Jena University Hospital, Jena, Germany

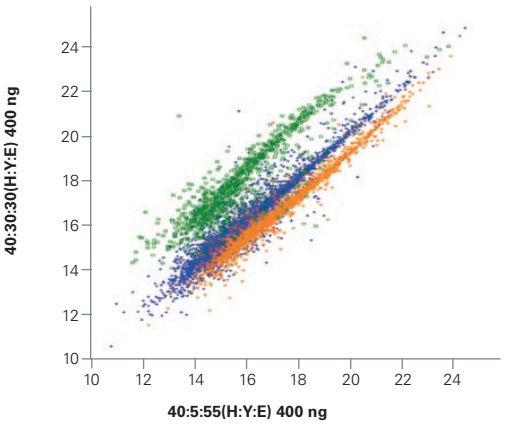
"Our ongoing collaboration with Bruker to stream-line tissue analysis is a key area in clinical proteomics, yet extremely challenging as tissue sections and biopsies comprise very heterogenous cell populations. The dia-PASEF acquisition mode on the timsTOF HT instrument quantifies proteins across a large dynamic range even in notoriously difficult samples, such as cardiac tissue, without sacrificing throughput or sensitivity."

Achieve the most accurate quantification results

Identify and quantify more than 10.000 proteins in your LC-MS run

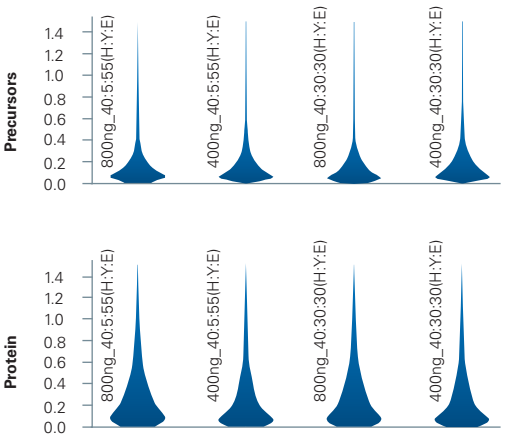
The new timsTOF HT featuring a 14-bit digitizer for ultimate quantitative performance was tested for quantitative accuracy and linearity with proteolytic digest from three different species (Human, Yeast, E. coli) in two different mixing ratios. dia-PASEF and MS2 based quantification results show accurate ratio determination across more than five orders of magnitude in dynamic range.

Proteolytic digests of HeLa, Yeast and E. coli were mixed in two different ratios and each experiment mixture was applied to the timsTOF HT mass spectrometer for dia-PASEF analysis on 60 min LC-MS gradients. **A)** Data analysis using TIMS DIA-NN on the PaSER platform shows protein intensity distributions similar to the expected values from sample mixing, highlighting very accurate quantification across the abundance range. **B)** CV analysis of peptide precursor intensities and protein intensities showing low CV values at 12% and 5%, respectively.

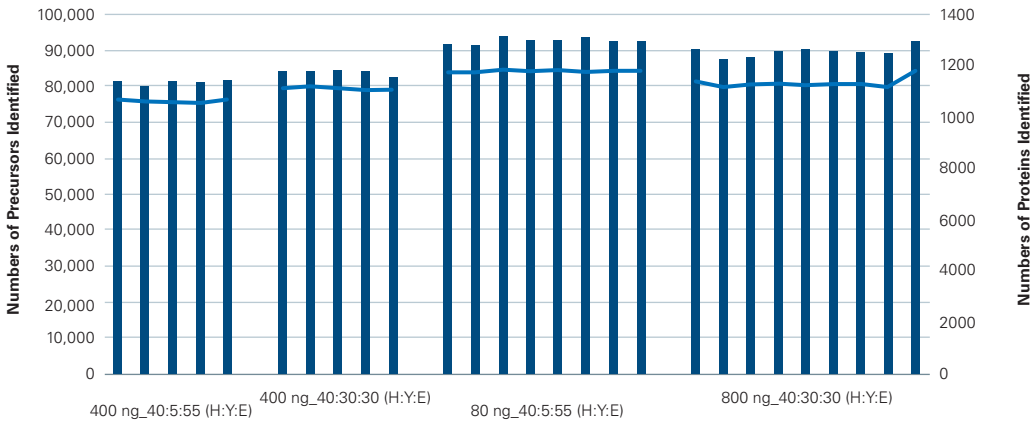


Human, Yeast, E. coli were combined in 2 ratios (40:30:30 or 40:5:55 HYE) and either 400ng or 800ng was loaded onto the timsTOF HT

- Yeast
- Human
- E. coli



Data processed with TIMS DIA-NN shows expected protein intensity distributions as well as low CV values at protein and precursor level



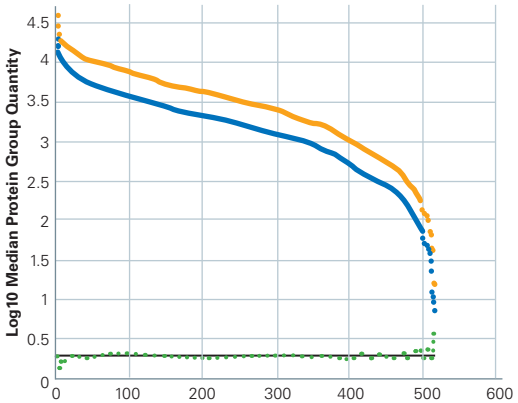
Proteins and precursors are reliably identified across several replicates showing robustness of the instrument

Targeted plasma proteomics with dia-PASEF

Reliable and easy to setup targeted quantitation of more than 500 plasma proteins

Ranked protein group view for different amounts of SIS PQ500 peptides spiked in non-depleted human plasma background. Measured ratios (green dots) correspond well with the theoretical ratios (black line) over the complete dynamic range.

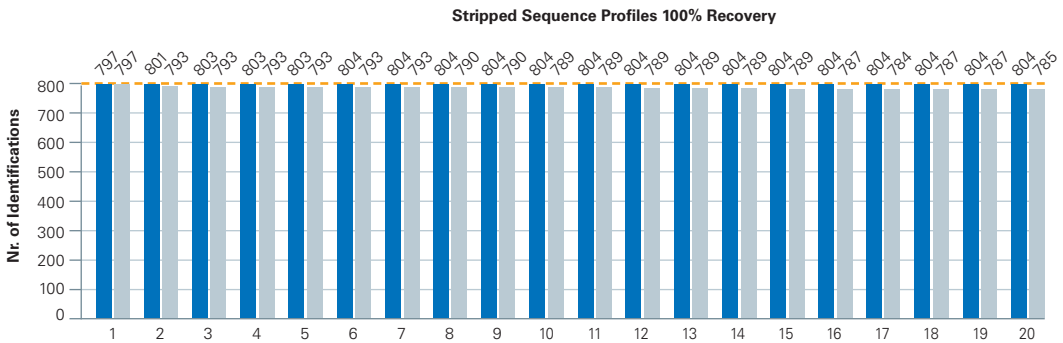
- Average 0.1IE
- Average 0.2IE
- Measured Ratio
- Theoretical Ratio

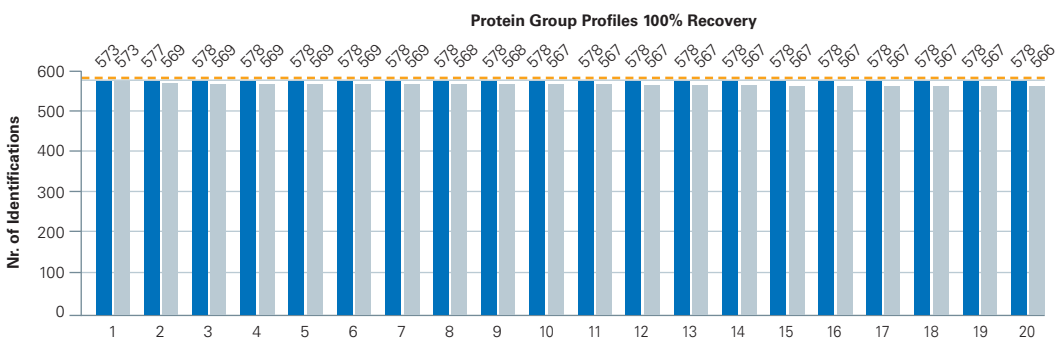


New reference kit developments allow for absolute quantification of more than 500 proteins in plasma samples. There is strong demand for easy-to-use methods that do not compromise sensitivity and specificity known from classical targeted approaches like SRM and MRM. The new timsTOF HT featuring the well-known dia-PASEF technique enables robust and accurate quantitation of all 804 peptides and 578 proteins from Biognosys' PQ500TM kit using a 30 min gradient. Analyzing samples consisting of two different concentrations of PQ500 spiked into a non-depleted human plasma background revealed accurate quantitation over the complete dynamic range. Measured ratios (1:1.98 (± 0.15)) correlate extremely well with the theoretical ratio (1:2).

Robust identification of all 804 SIS PQ500 peptides and 578 proteins in 20 different non-depleted human plasma samples. Data processing was done using Spectronaut software (Biognosys).

- Cumulative Sparse Profiles
- Cumulative Full Profiles
- Library/Experiment Size

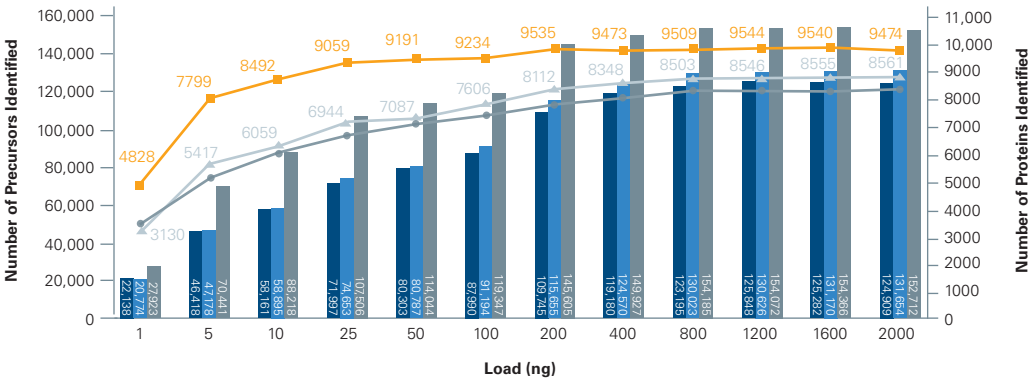




Unprecedented depth in cell lines with dia-PASEF, TIMS DIA-NN and PaSER

Getting the most out of your samples with the timsTOF HT and PaSER

For even higher proteome depth, extending the gradient improves identifications even further. A tryptic digest of human cell line K562 (Promega) was analyzed using a nanoElute system at 250 nL/min with 60 min separation time on an Aurora column (25 cm, 75 µm ID, IonOpticks). timsTOF HT operated in dia-PASEF mode using 100 ms TIMS separations. Using PaSER, data was processed with TIMS DIA-NN using a TIMScore library generated from K562 and MOLT-4 cell lines containing more than 500,000 peptide sequences.



1 ng to 2000 ng of K562 material was loaded onto timsTOF HT run in dia-PASEF mode and searched with TIMS-DIA NN against a library made with and without TIMScore and Match Between Runs (MBR)

- no TIMScore
- TIMScore noMBR
- TIMScore_MBR
- no TIMScore
- TIMScore
- TIMScore_MBR

Professor Dr. Markus Ralser

Director of the Institute of Biochemistry, Group Leader Biochemistry and Systembiology of Metabolism, Charité, Berlin

"Our ongoing collaboration with Bruker to tailor DIA-NN to a streamlined processing tool for dia-PASEF data with a CCS focus has been rewarding. It simplifies and accelerates identifying and quantifying thousands of proteins in very short gradients. We are pleased that within our close collaboration with Bruker, the vendor-integrated version of DIA-NN called TIMS DIA-NN now becomes part of the PaSER bioinformatics platform."

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