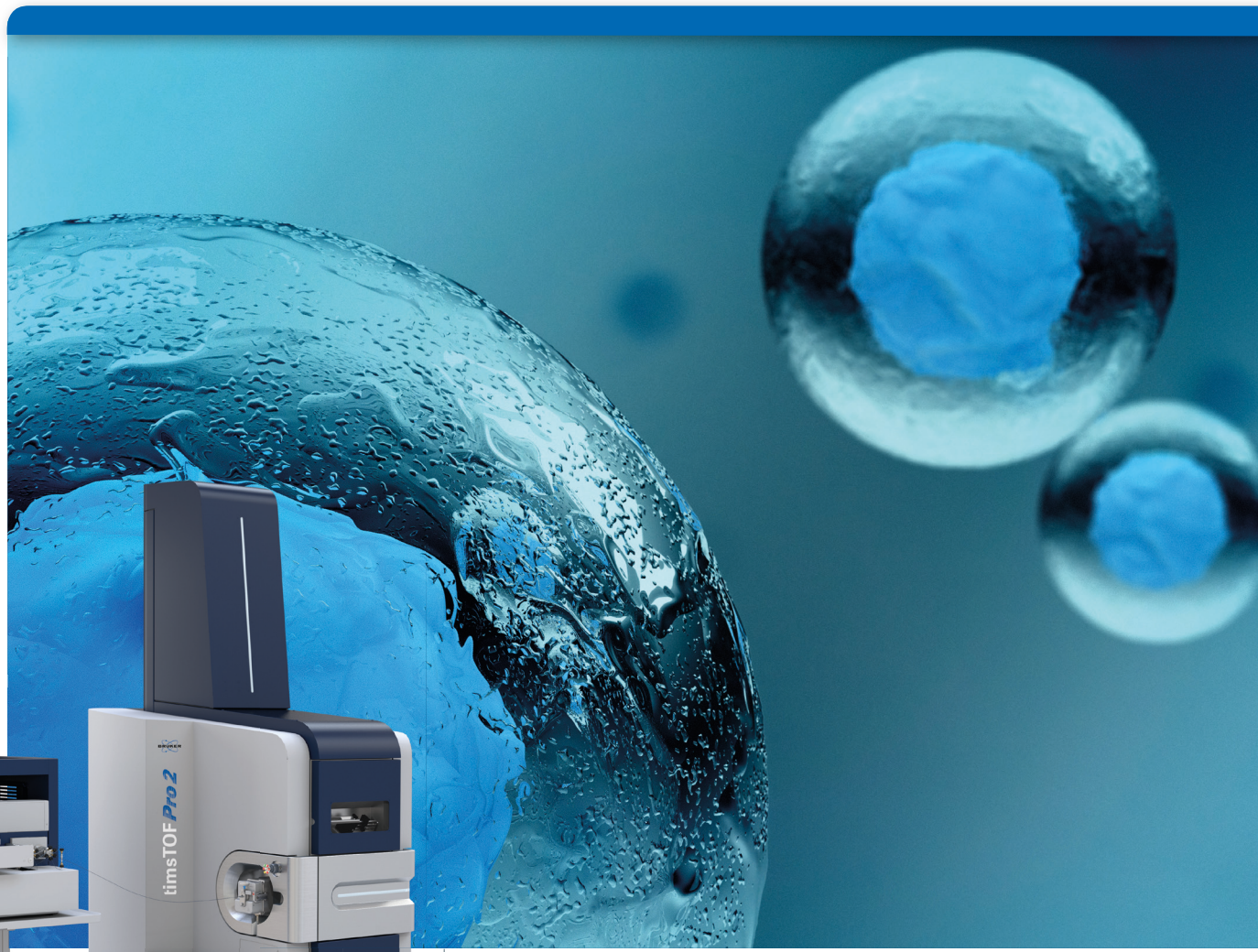
BRUKER



timsTOF Pro 2

- The new standard for high speed, high sensitivity 4D-Multiomics

TIMS-QTOF MS

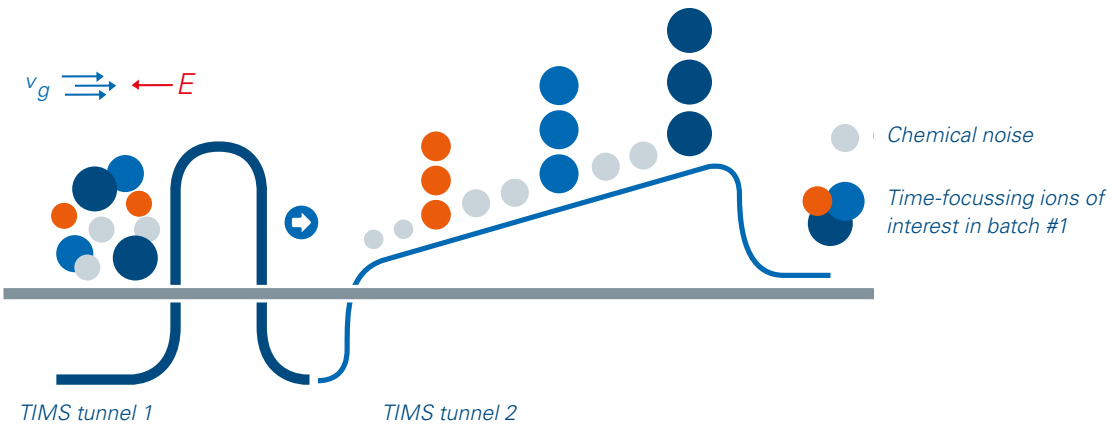
Innovation with Integrity

timsTOF Pro 2

The standard for high speed, high sensitivity, 4D-Multiomics

Introducing the timsTOF Pro 2 mass spectrometer with our newest generation dual-TIMS analyzer offers three times higher ion capacity. Simplified ion optics maximize ion transfer and sensitivity to set a new standard in 4D-Multiomics. Uncompromised depth of coverage with short gradients, and CCS-enabled precision makes the timsTOF Pro 2 indispensable for translational multi-Omics applications.

Dual-TIMS and CCS-enabled analysis



Trapped ion mobility spectrometry (TIMS) resolves sample complexity through an added dimension of separation in the gas phase 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), which allows for higher fidelity separation of noise from signal, which enables an increase in sensitivity with speed (> 100 Hz TIMS duty cycle)

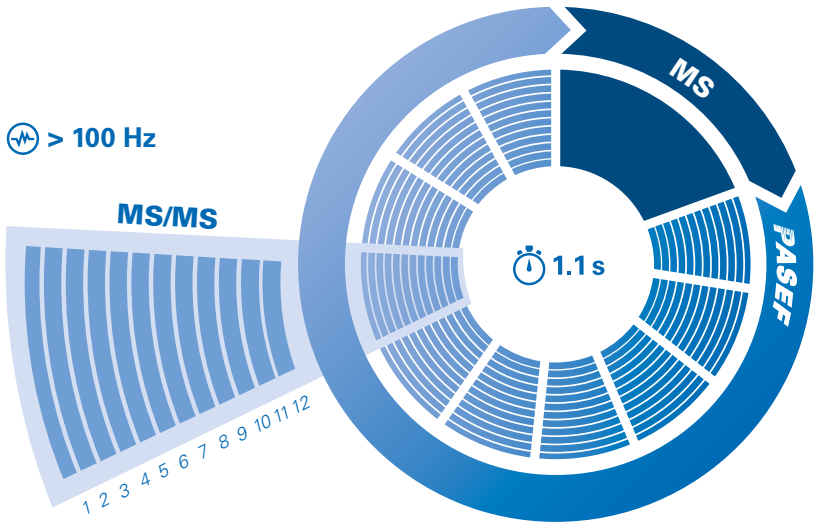
Dual TIMS achieves a near 100% duty cycle by accumulating ions in TIMS tunnel 1, while ions in TIMS tunnel 2 are released sequentially (> 100 Hz). This process of parallel accumulation serial fragmentation (PASEF®) enables collisional cross section (CCS) analysis with high speed.

PASEF digs deeper into complex samples

Together with Prof. Dr. Matthias Mann, Bruker scientists worked together to address the shortcomings of proteomics mass spectrometry by inventing the PASEF (Parallel Accumulations Serial Fragmentation) scan function based on dual-TIMS technology. Peptide ions are separated using trapped ion mobility spectrometry, eluted (~ 100 ms) and detected in the quadrupole time of flight (QTOF), generating the TIMS MS heat map.

PASEF then uses the same TIMS separation to serially fragment the ions using MS/MS: the quadrupole isolates a certain ion species during its elution for MS/MS and then immediately shifts to the next precursor. Parent and fragment spectra are aligned by mobility values.

PASEF® technology can achieve a sequencing speed of > 100 Hz and the MS/MS spectra quality of the low abundant peptides can be enriched by selecting them several times, resulting in higher confidence peptide spectrum matching (PSM).



PASEF®: the perfect fit for complex samples: *The timsTOF Pro powered by PASEF® offers a sequencing speed of > 100 Hz without losing sensitivity or resolution. This is achieved by synchronizing the quadrupole isolation mass window with the elution time of the specific peptide packages from the TIMS funnel.*

Prof. Dr. Matthias Mann, Director, Department of Proteomics and Signal Transduction, Max-Planck-Institute of Biochemistry, Martinsried, Germany

"We now know that the peptide mixtures are still extremely complex when analyzing them in two dimensions (retention time and m/z). Adding one more dimension should in principle get us a long way ahead. In addition to the additional dimension of separation, the timsTOF Pro 2 gives us extremely high speed and sensitivity to get deeper into the proteome and using less sample material."

Unprecedented proteomic depth

Maximize peptide and protein sequence coverage

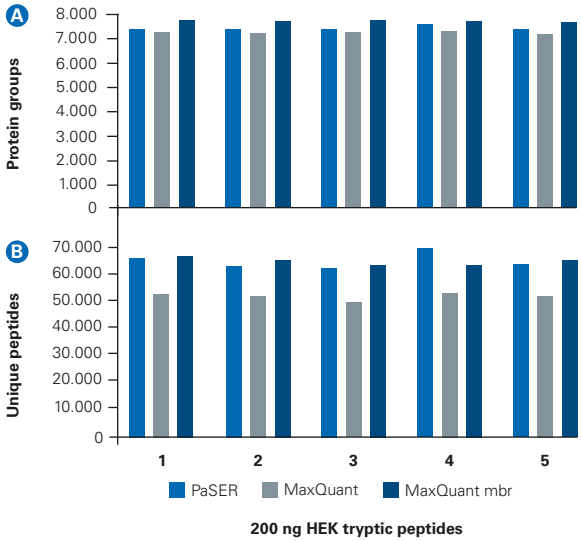
The new robust stainless steel stacked ring ion guide (SRIG) configuration and new optimized standard dda-PASEF methods in timsTOF Pro 2 provides an unprecedented depth of proteome coverage in single shot proteomics. From inhouse HEK tryptic digests of 200 ng, in 60 minute gradients using an Aurora-25 cm column, more than 7000 protein groups and 60,000 peptides were identified.

timsTOF Pro 2 provides in-depth proteome coverage for everyday cell line proteome quantification experiments directly by database searching and matching between the runs without the need for a spectral library. Different database search strategies resulted in very comparable results. PaSER, enabling real time protein identifications, and MaxQuant resulted in similar ID numbers at both protein and peptide level.

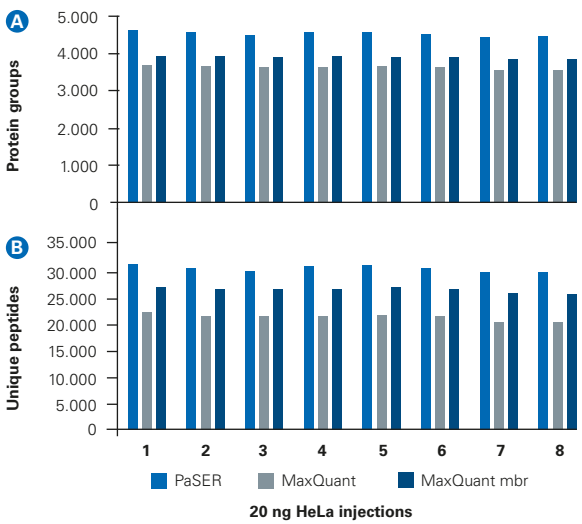
High sensitivity for tackling the most difficult proteomics and PTM challenges

Proteome quantification using low sample amounts is crucial for a growing number of biological applications such as specialized cells, rare cell populations, or fine needle aspiration tumor biopsies. Proteome quantification of such low sample amounts using a sensitive mass spectrometer is crucial. From 20 ng of HeLa (Pierce) digest measured on an Aurora-25 cm column in 30 minute gradients, the PaSER realtime search engine identifies more than 4200 protein groups and close to 30,000 peptides.

Unprecedented proteome coverage in 60 min gradient times



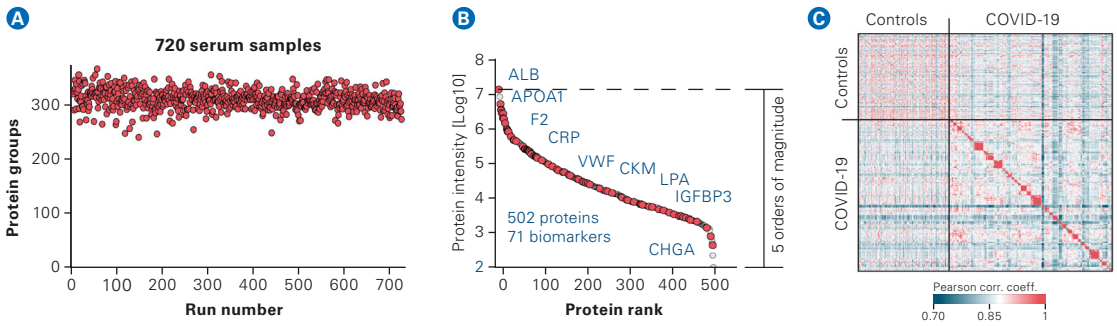
Reproducible identification of more than 6000 protein groups Ⓐ and about 60,000 peptides, in 60 minutes Ⓑ from 200 ng HEK digests. PaSER and MaxQuant deliver comparable results.



Ⓐ Protein group identifications (~4000) in 30 min gradients from 20 ng HeLa proteolytic digest and PaSER, MaxQuant and MaxQuant with match between runs (mbr), respectively. Ⓑ Peptide identifications (~25,000) on 30 min gradients from 20 ng HeLa proteolytic digest and PaSER, MaxQuant and MaxQuant with match between runs, respectively.

High-throughput plasma proteomics applied to COVID-19 research

Very recently we applied this powerful combination of a timsTOF Pro 2 and the Evosep One to investigate proteome alterations in one of the largest serum proteome studies on COVID-19 infected patients which is available as a preprint[1]. Serum samples from 31 patients in up to 54 days of hospitalization resulting in 458 analyzed samples. Samples from PCR negative controls from 262 patients with COVID-19 like symptoms served as control. Data were acquired using the Evosep 60 samples per day (SPD) method with PASEF and processed with a matching library in MaxQuant[1]. From this set of 720 patient samples, 310 ±18 proteins were quantified including more than 70 potential biomarkers covering five orders of magnitude in protein abundance (Figure below). Remarkably no maintenance or cleaning was necessary during this study which is crucial for obtaining homogenously high precision protein quantity values resulting in very low CVs and the ability to tease out even minor changes in the protein level. This study led to the description of highly detailed longitudinal trajectories of regulated serum proteins and potential novel biomarkers. The ability to measure similar and larger datasets using this pipeline would provide precious insights about the question in hand.



Plasma proteome analysis of COVID-19 patients and control individuals. Ⓐ number of proteins quantified Ⓑ dynamic range of proteins quantified with a few known biomarkers highlighted Ⓒ clustering heatmap of correlation across all samples.

[1] www.medrxiv.org/content/10.1101/2021.02.22.21252236v1

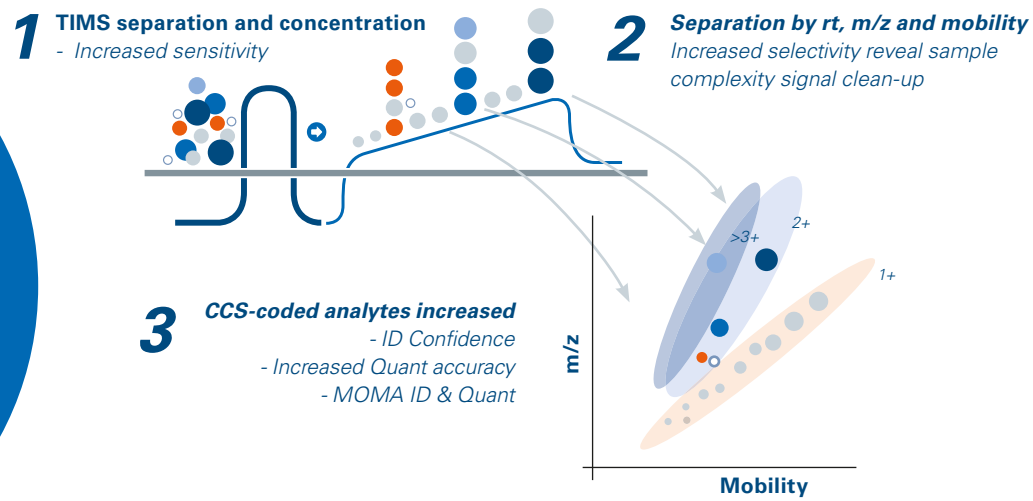
Roman Fischer, Ph.D., Associate Professor in Clinical Proteomics, Target Discovery Institute, University of Oxford, Oxford, United Kingdom

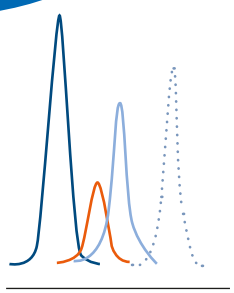
"Since we started to work with the timsTOF Pro in February 2019, we have run more than 25000 LCMS samples, of which about 5000 have been non-depleted plasma digests. We had virtually zero downtime so far."

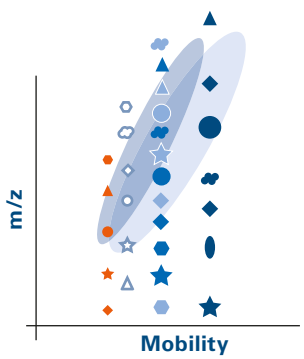
dia-PASEF adding confidence to your identifications

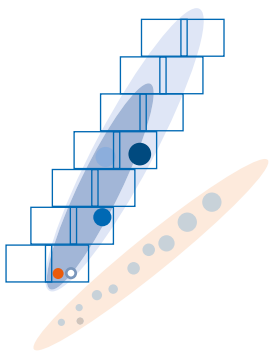
Boosting data-independent-analysis with the speed of PASEF and unmatched specificity of TIMS-derived Collisional Cross Sections (CCS)

dia-PASEF
Powered by 4D-Multiomics









6 ***Accuracy - Selectivity - Sensitivity Robustness - Confidence - Speed***

5 **MS:MS based, CCS-aware**
- *Quantitation accuracy*
- *Ultimate selectivity*
- *Results confidence*

4 ***Bi-dimensional dia-PASEF windows***
- *Improved ion usage: sensitivity*
- *Shortened cycle time: throughput*
- *1+ removal: spectral quality*

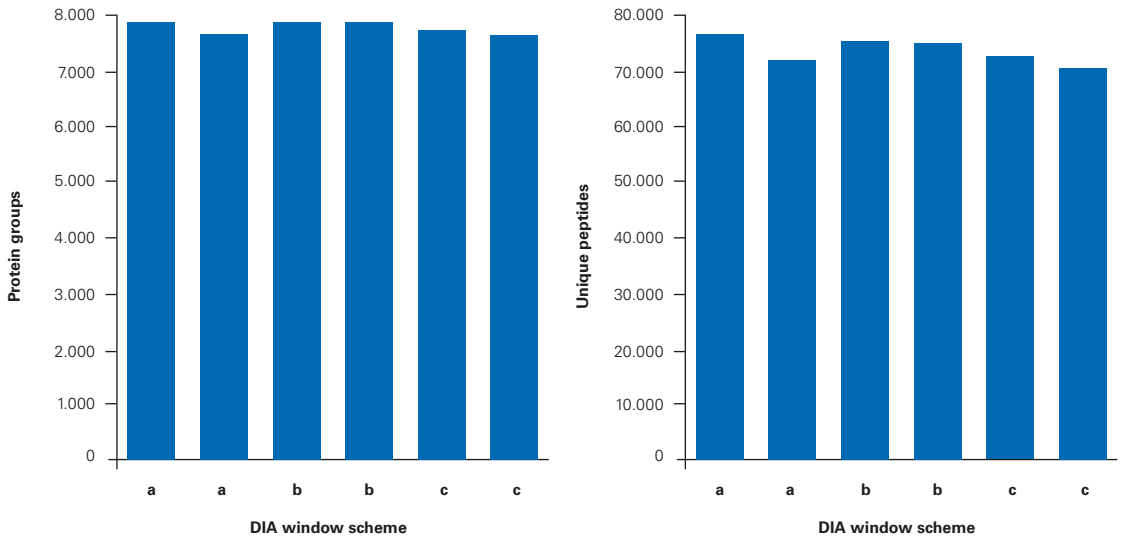
Chemical noise *Ion 1 (high CCS)* *Ion 2 (intermediate CCS)* *Ion 3 (intermediate CCS)*
Isobaric Ion 4 (MOMA) *Isobaric Ion 5 (MOMA)*

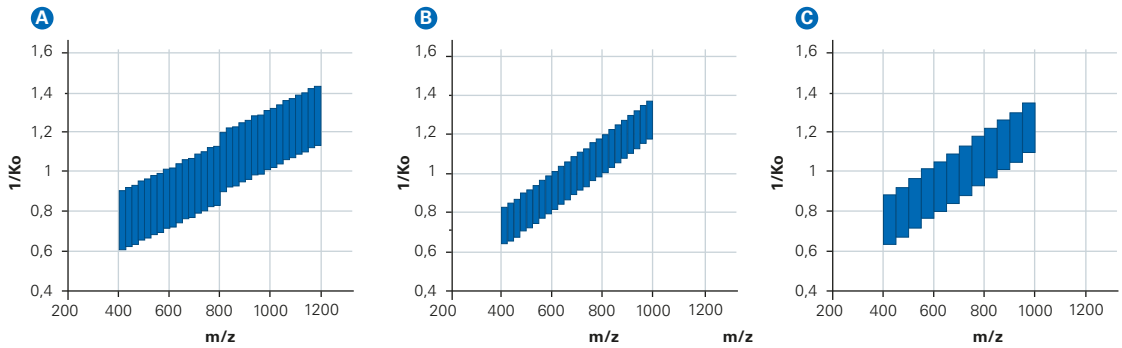
Data-independent acquisition (dia)-PASEF is both more sensitive and selective than traditional DIA approaches as it applies the PASEF principle to combine the advantages of DIA with the inherent ion efficiency of PASEF. Over the entire liquid chromatography-mass spectrometry (LC-MS)/MS dia-PASEF run, 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.

dia-PASEF

Unmatched data completeness and analytical depth for high-throughput quantitative proteomics

Data independent acquisition (DIA) using standard dia-PASEF methods and in-house generated libraries provide reproducible identifications in multiple runs. Three different dia-PASEF window schemes allow for the quantification of close to 8000 protein groups and more than 70,000 unique peptides in 60 minute gradients from 200 ng using Aurora-25 cm columns, while demonstrating quantitative precision.





Protein group and peptide identification rates for duplicate injections using three different dia-PASEF window settings Ⓐ Ⓑ Ⓒ.

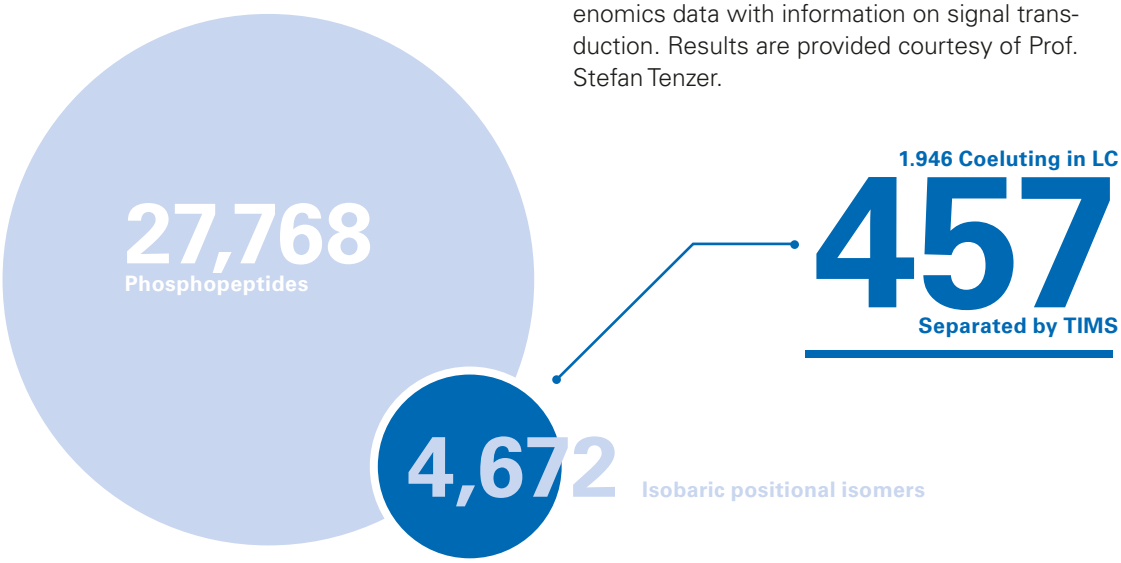
High sensitivity phosphoproteomics and isomer separation

CCS-enabled quantification of proximal phosphorylation sites

At the point of chromatographic co-elution, quantification of p-peptide isomers is not possible in traditional proteomics approaches without CCS information due to the isobaric nature and signal overlay. PASEF analyses from standard 150 µg TiO2-based enrichment workflows identifies 27,768 phosphopeptides, as shown below and reveals the benefits of ion mobility separation with Mobility Offset Mass Aligned (MOMA). From 1946 identified co-eluting isomers, 20% could be fully separated by TIMS, enabling a better understanding of proximal protein phosphorylation sites.

Analyze cell signaling where sample amounts are limited

The high sensitivity, sequencing speed and reproducibility of dia-PASEF on the timsTOF Pro 2 even enables quantitative phosphoproteomic analyses of limited sample amounts. Label-free quantification of phosphoproteomes is feasible from as little as 25 µg of total protein obtained from mouse brain samples. dia-PASEF analysis of enriched phosphopeptides using a 30 SPD (samples per day) Evosep method resulted in the identification of up to 4473 unique phosphopeptides across three enrichment replicates. These results hold further promise for the application in needle biopsies, complementing cancer proteogenomics data with information on signal transduction. Results are provided courtesy of Prof. Stefan Tenzer.



Phosphopeptide identifications and separation of positional phosphopeptide isomers by TIMS and PASEF. Results are provided courtesy of Prof. Stefan Tenzer.

Prof. Dr. Stefan Tenzer, Institute for Immunology, University Medical Center of the Johannes-Gutenberg University, Mainz, Germany

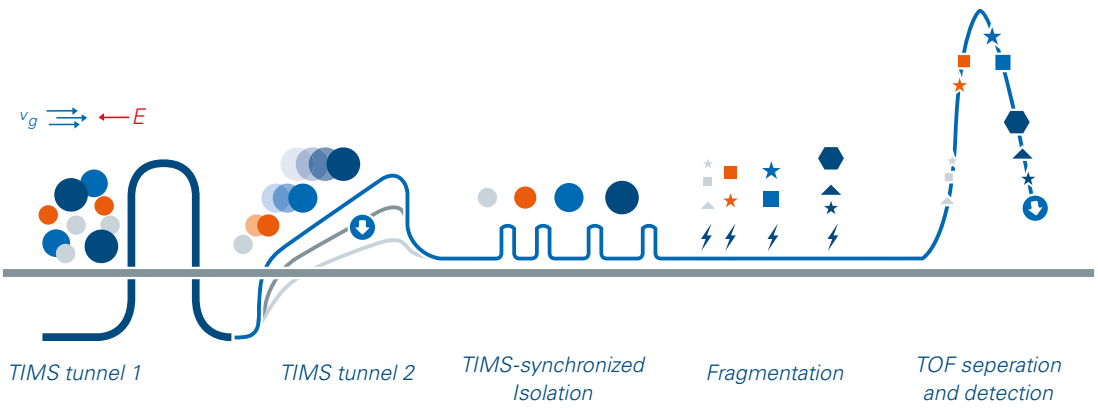
"Besides its high sensitivity, a unique aspect of the timsTOF Pro instrument is its capability to resolve positional phosphorylation isomers in the gas phase, thus providing more detailed insight into signaling pathways."

High-throughput targeted proteomics at unprecedented sensitivity

In comparison with standard selected and parallel reaction monitoring (SRM and PRM), prm-PASEF increases the number of peptides that can be targeted in a single acquisition method, without compromising the selectivity or the sensitivity.

Targeted mass spectrometry (MS) is a powerful technique that is used in proteomics experiments – to verify biomarker candidates in large sample cohorts, for example. This technique is limited by a necessary compromise between the number of targets measured in a single run, the duration of the liquid chromatography separation stage and the overall sensitivity.

prm-PASEF increases the number of peptides that can be targeted in a single acquisition by benefiting from the 4th dimension of separation using Bruker's timsTOF Pro to improve selectivity and sensitivity, and adding the speed of PASEF to increase the number of precursor targets.



Prof. Dr. Gunnar Dittmar, Group Leader Proteomics of Cellular Signalling, Department of Infection and Immunity, Luxembourg Institute of Health, Luxembourg

"About a year ago, my laboratory started a collaboration with Bruker to develop the prm-PASEF method on the tims-TOF Pro. During the development of the prm-PASEF method, we saw that the dual trapped ion mobility device could store ions and release them as very sharp, intense peaks coupled to the high-resolution TOF is a wonderful way to increase the signal and gain in intensity. Moreover, we also have been positively impressed by the instrument's reliability; it's fantastic!"

Jarrod A. Marto, Ph. D. Associate Professor, Dept. of Pathology, Brigham and Women's Hospital and Harvard Medical School, Dana-Farber Cancer Institute, Boston, USA

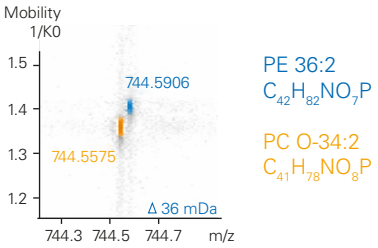
"We've been working closely with Bruker to build prm-PASEF from the ground-up. Throughout our collaboration, we've been impressed by the world-class combination of speed, sensitivity, and robustness provided by prm-PASEF. We are excited to take this performance to the next level on the timsTOF Pro 2"

Enable higher throughput with 4D-Lipidomics

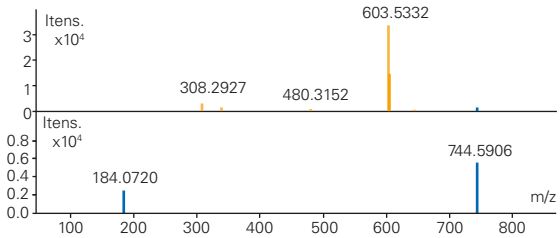
Analogous to proteomics samples, lipid extracts have a high sample complexity caused by the structural diversity of lipids. High quality MS/MS spectra are integral to obtaining confident lipid annotations. PASEF unlocks CCS-enabled workflows which can be used to additionally boost lipid annotation confidence.

Mobility Offset Mass Aligned (MOMA) data of isobaric lipids

PASEF is able to fragment > 10x more precursors by using mobility separation to select more than 10 precursors in a timeframe of just 100 ms. This removes overlapping contaminants and resolves isobaric as well as isomeric lipids. The resulting MS/MS spectra show unique fragments for each lipid class resulting in annotations with increased confidence.



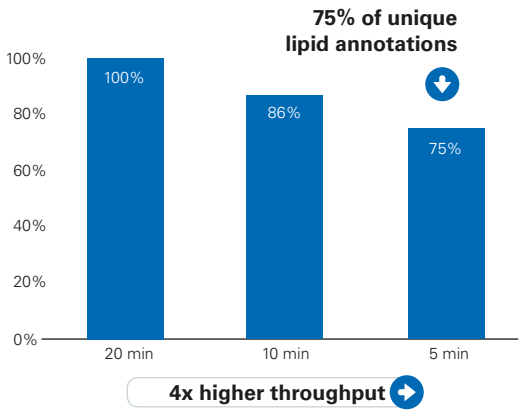
Heatmap showing the mobility separation of two isobaric phospholipids co-eluting from reverse phase



Clean PASEF MS/MS spectra after mobility separation

High throughput with confident annotation

MS/MS spectra are acquired for on average 65% of precursors in a single injection with no need to waste precious sample on multiple runs. This allows for throughput to be increased more than 4 fold In combination with fast LC gradients, enabling high throughput lipid profiling. Accurate and precise ^{TIMS}CCS values are used as additional qualifiers to increase the confidence via automatic CCS prediction for annotations from the library-free rule-based approach.



Unique lipid annotations from NIST SRM 1950 (ESI-(+) mode) for different gradient run times

Zheng-Jiang Zhu, Ph.D. Principal Investigator, Director of Metabolomics Research Center, Interdisciplinary Research Center on Biology and Chemistry (IRCBC), Shanghai Institute of Organic Chemistry (SIOC), Chinese Academy of Sciences (CAS)

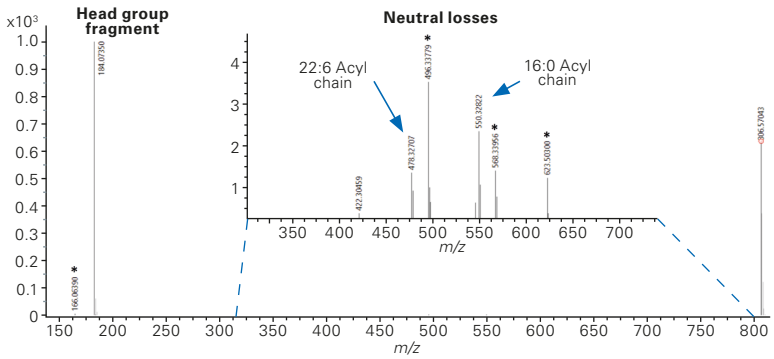
"The timsTOF Pro is a very versatile platform; you can do all applications on one platform. The instrument design exceeds anything else on the market"

Integrated annotation tools designed for lipidomics beginners and experts alike

An important step in lipid profiling is the validation of annotations to ensure proper reporting of results. To simplify this task, several tools are streamlined in MetaboScape.

Rule Based Annotation

Besides the typical database- or spectral-library based annotation, MetaboScape® features a library-free annotation tool that utilizes published fragmentation rules. Depending on the fragments and neutral losses, MetaboScape is able to annotate on a species or molecular species level.



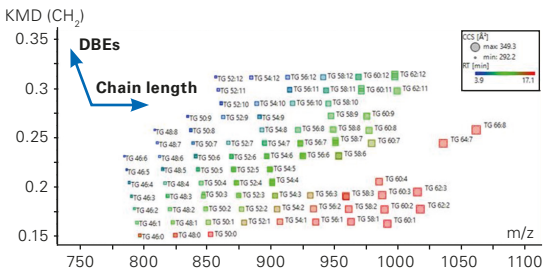
Rule-based annotation of PC 22:6_16:0 based on head group and acyl chain fragments

To unite with the lipidomics community as it continues to develop and grow, MetaboScape uses the latest recommendations on shorthand nomenclature and hierarchy and is regularly updated with novel lipidomics tools to simplify profiling workflows.

Kendrick Mass Defect analyses

Different Kendrick Mass Defects can be calculated across multiple lipid classes and displayed in multidimensional plots to screen for outliers, identify non-annotated species and remove false positives.

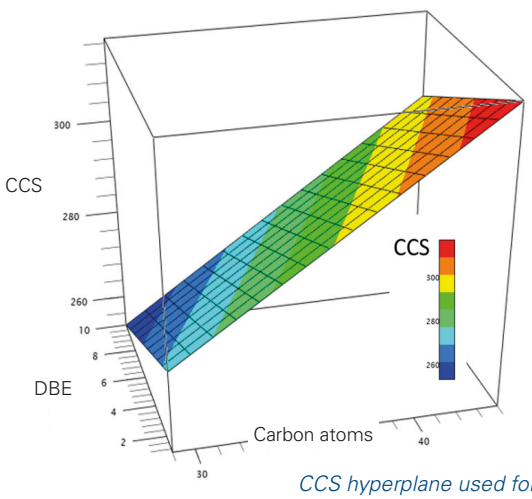
An automatic outlier detection simplifies the deep analysis of homologous series of lipids.



Kendrick Mass Defect plot showing homologous Triacylglycerides of a fish oil extract

CCS-enabled tools for confident lipid annotation

MetaboScape is a fully CCS-enabled solution with multiple tools utilizing CCS values to improve annotation quality. Besides MS/MS spectral libraries with integrated CCS values, e.g. the LipidBlast library for ½ million lipids, a new tool for the automatic prediction of CCS values based on CCS hyperplanes is implemented in MetaboScape.

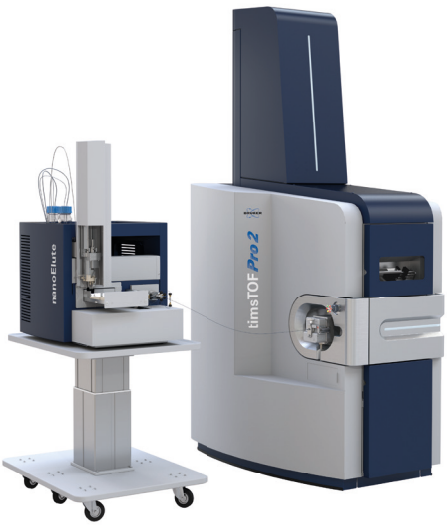


CCS hyperplane used for the automatic prediction of CCS values

timsTOF Pro 2

Prof. Janne Lehtiö, Science for Life Laboratory, Department of Oncology-Pathology, Karolinska Institute, Sweden

"We have been impressed with the performance of the timsTOF Pro. In particular the speed and sensitivity of the instrument enable us to see more immunopeptides from limited amounts of starting material, which we expect to be particularly valuable for neoantigen discovery and the development of personalized therapies for cancer treatment."



PaSER
Parallel Search Engine in Real-time

timsTOF Pro 2 and PaSER (Parallel Search Engine in Real-time) is a combined hardware and software solution enabling fully integrated GPU based real-time database searches and results-based sample queue management. PaSER delivers results with uncompromising speed, including PTM searches. This uncompromised search speed of PaSER allows you to have results on hand, seconds after the acquisition ends, Run & Done!

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