TIMS-QTOF MS

timsTOF Pro 2

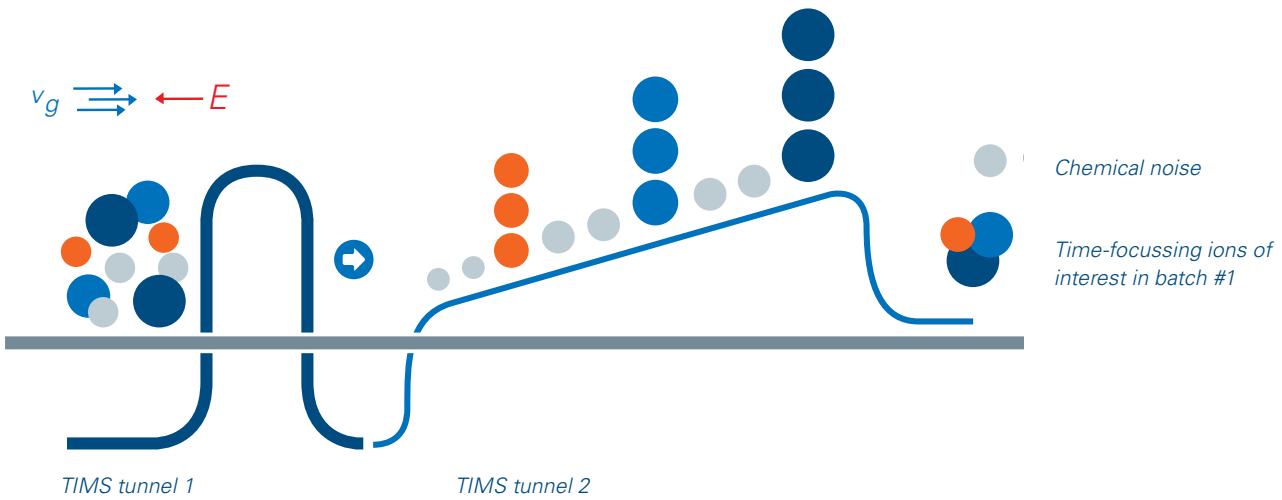
The new standard for high speed, high sensitivity 4D-Multiomics

Innovation with Integrity

timsTOF Pro 2

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

The timsTOF Pro 2 mass spectrometer comes with our innovative dual-TIMS analyzer that 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 multiomics 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. This enables an increase in sensitivity and unlocks unprecedented MS/MS coverage.

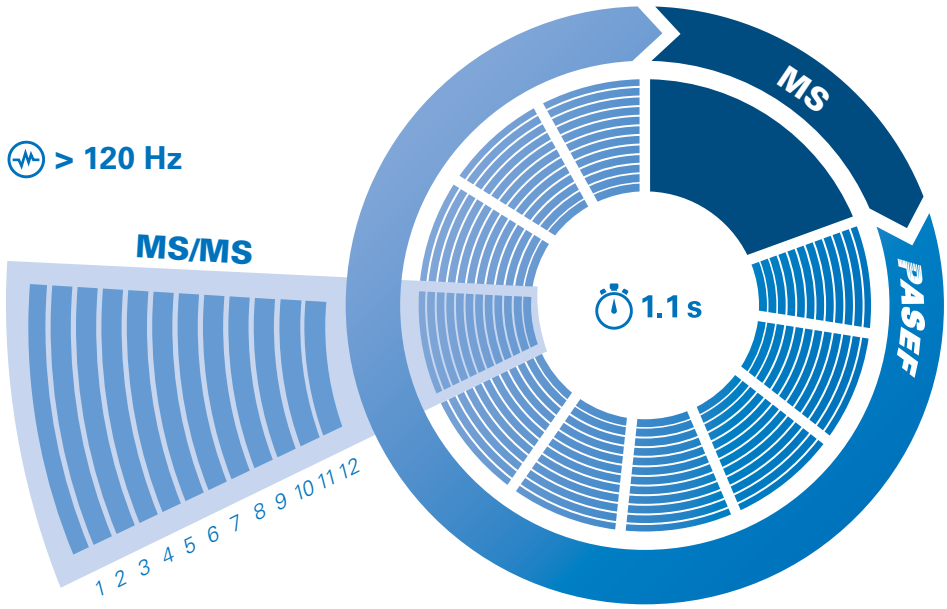
Dual TIMS achieves a near 100% duty cycle by accumulating ions in TIMS tunnel 1, while ions in TIMS tunnel 2 are released sequentially (> 120 Hz). This process of parallel accumulation serial fragmentation (PASEF®) enables collisional cross section (CCS) analysis with high speed.

PASEF unlocks deep investigation of complex samples with high speed

Together with Prof. Dr. Matthias Mann, Bruker scientists worked 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 ions. 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 > 120 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.



PASEF®: The perfect fit for complex samples: The timsTOF Pro 2 powered by PASEF offers a sequencing speed of > 120 Hz without losing sensitivity or resolution. This is achieved by synchronizing the quadrupole isolation mass window with the elution time of the specific ion packages from the TIMS tunnel.

Prof. Dr. Matthias Mann

Director, Department of Proteomics and Signal Transduction, Max-Planck-Institute of Biochemistry, Martinsried, Germany

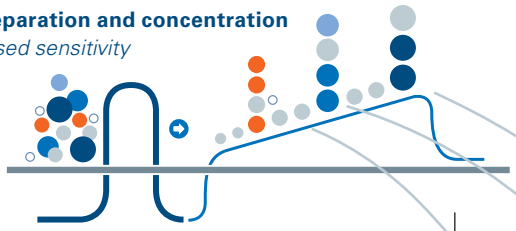
"We now know that the peptide mixtures are still extremely complex when analyzing them in two dimensions (retention time and *m/z*). Adding one more dimension should in principle get us a long way ahead. In addition to the additional dimension of separation, the timsTOF Pro 2 gives us extremely high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

dia-PASEF adding confidence to your identifications

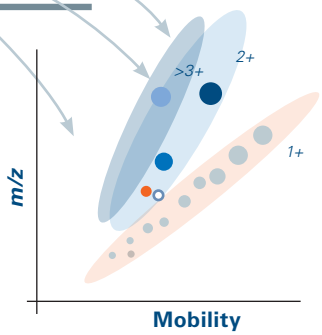
Boosting data-independent-analysis with the speed of PASEF and unmatched specificity of TIMS-derived Collisional Cross Sections (CCS)

dia-PASEF
Powered by 4D-Multiomics

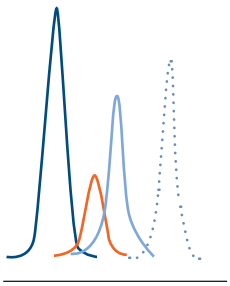
1 **TIMS separation and concentration**
- *Increased sensitivity*



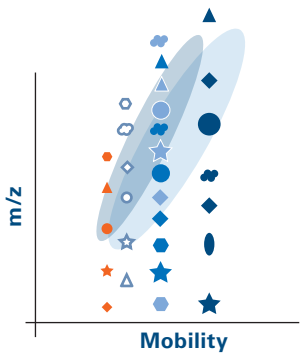
2 ***Separation by rt, m/z and mobility***
Increased selectivity reveal sample complexity signal clean-up



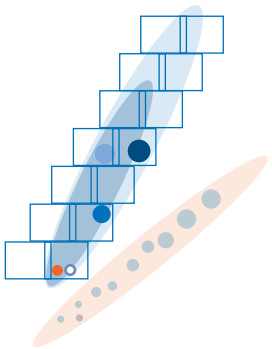
3 ***Increased CCS-coded analytes***
- ID Confidence
- Increased Quant accuracy
- MOMA ID & Quant



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



5 **MS/MS based, CCS-enabled**
Quantitation accuracy
- Ultimate selectivity
- Results confidence



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

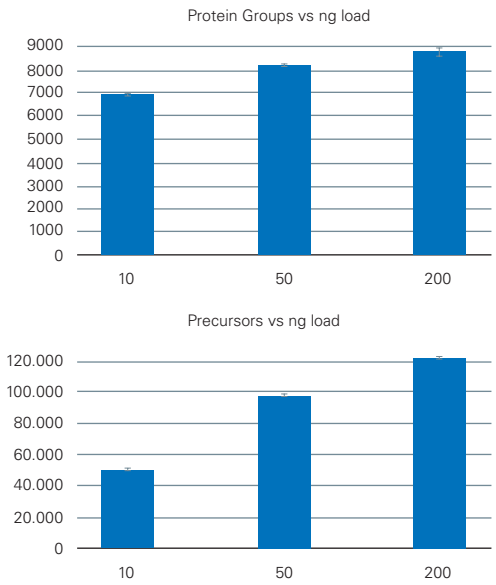
Chemical noise *Ion 1 (high CCS)* *Ion 2 (intermediate CCS)* *Ion 3 (intermediate CCS)*
Isobaric Ion 4 (MOMA) *Isobaric Ion 5 (MOMA)*

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.

Unprecedented proteomic depth

Maximize peptide and protein sequence coverage

Unprecedented proteome coverage in 35 min gradient times



K562 digest, 35 min gradient (≈ 30 sample per day) dia-PASEF analysis of a K562 digest. Data processing was performed using TIMS DIA-NN.

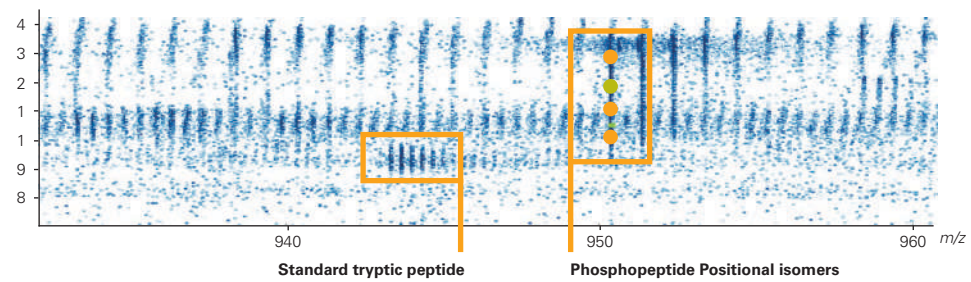
The robust stainless steel stacked ring ion guide (SRIG) configuration and optimized standard dia-PASEF® methods in timsTOF Pro 2 provides an unprecedented depth of proteome coverage in single shot proteomics. Nearly 9000 protein groups identified from 200 ng of K562 lysate in 35 minute gradients (~30 sample per day) using a PepSep 25 cm column with nanoElute on a timsTOF Pro 2 and a TIMScore™ powered spectral library.

timsTOF Pro 2 provides in-depth proteome coverage for everyday cell line proteome quantification experiments directly by database searching and matching between 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 challenging proteomics and PTM applications

Sensitivity is not the sole benefit brought by 4D-Proteomics™ when it comes to analyze challenging PTM-containing samples. PASEF allows to detect, characterize and quantify co-eluted, isobaric, ion mobility-separated position isomers (MOMA), delivering an information which is not accessible to standard appraoches, at no cost for sensitivity or speed. The extensive use of the CCS information by the TIMScore™ algorithm gives further confidence for the allocation of the modification site, even for very low intensity MS/MS spectra.

Zoom on a 26.6-29 min elution time window of a phosphopeptide enriched K562 measurement (200 ng sample load on column, 70 min gradient, dda-PASEF run with PaSER™). The analysis yielded 47,800 peptide identifications . The snapshot displays the mass overlap of four positional isomers from the ISSSSFSR phosphopeptide. Three of these isomers are overlapping, but separated in the ion mobility dimension, allowing for their identification and characterization. Combining MS/MS information with TIMScore™ further yields localization information for the isomers.



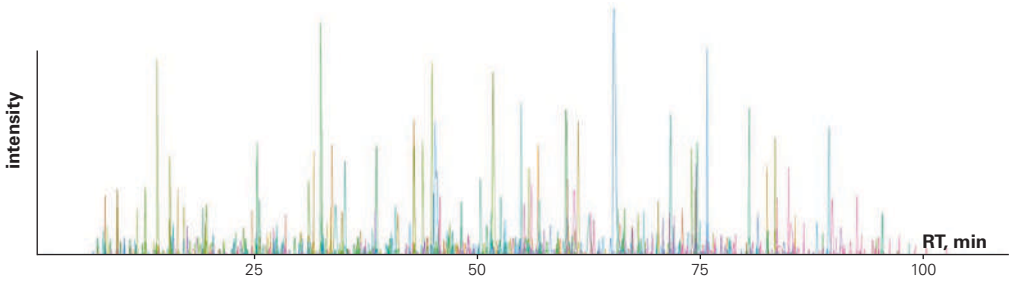
	Sequence	RT	*m/z*	1/K0	
Co-eluted	R.ISSSS(79.9666331)FSR.V	26.8	950.402	1.30	TIMS-separated
	R.ISSS(79.9666331)SFSR.V	26.9	950.401	1.12	
	R.ISS(79.9666331)SSFSR.V	27.0	950.398	1.02	
	R.IS(79.9666331)SSSFSR.V	28.5	950.402	1.29	

High-throughput targeted proteomics at unprecedented sensitivity

Targeted mass spectrometry is a powerful technique used in proteomics experiments to verify for example biomarker candidates in large sample cohorts. However, the approach is limited by a necessary compromise between the number of measurable targets in a single acquisition versus the duration of the liquid chromatography and the overall sensitivity that is needed.

prm-PASEF® benefits from TIMS as a 4th dimension of separation for unmatched selectivity, the time-focusing of ions in the TIMS cartridge to increase sensitivity, and the high speed facilitated by PASEF to increase the number of precursor targets. In a single acquisition several hundred precursors can be targeted without compromising the unique selectivity and high sensitivity of the timsTOF Pro 2 instrument.

Combining sensitivity, speed, and selectivity, the prm-PASEF acquisition method delivered high reproducibility and accurate quantitation for either a high number of targets or for application with short chromatography gradients (i.e. <5 minutes).



Easily set up prm-PASEF® methods within timsControl software.

Targeted protoemics analysis in a colorectal cancer plasma study. 1565 precursors (Heavy/Light) from 565 proteins were targeted in a prm-PASEF® experiment, using Biognosys's PQ500 kit.

Prof. Dr. Gunnar Dittmar

Group Leader Proteomics of Cellular Signalling, Department of Infection and Immunity, Luxembourg Institute of Health, Luxembourg

"In 2019, my laboratory started a collaboration with Bruker to develop the prm-PASEF® method on the timsTOF 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, Department 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"

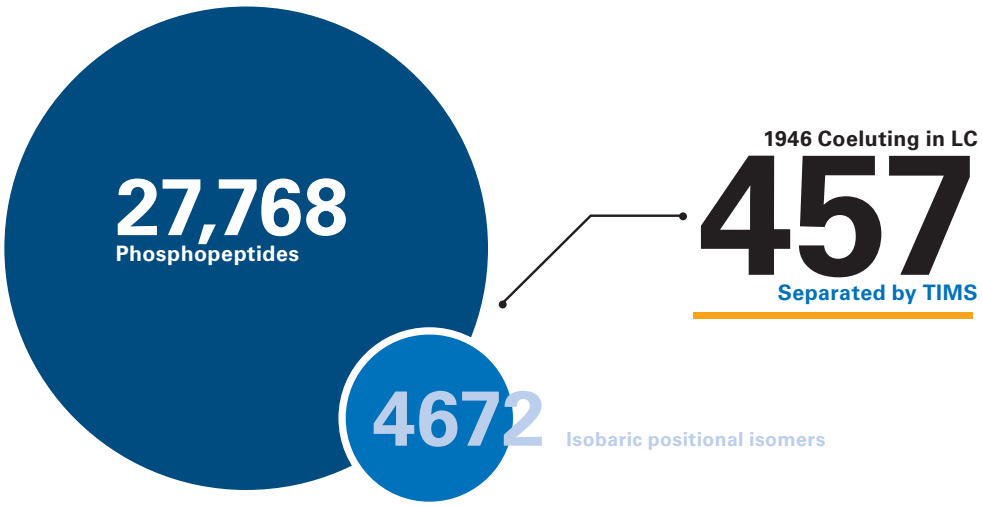
High sensitivity PTM analysis and isomer separation

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 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. Dr. Stefan Tenzer.

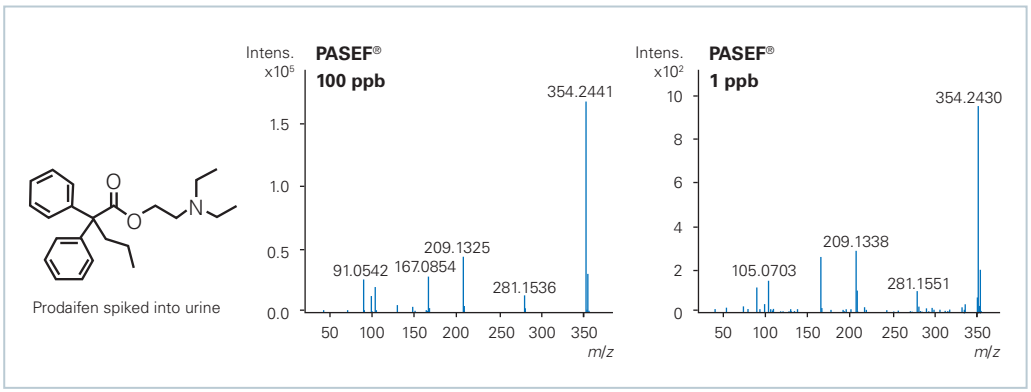


Phosphopeptide identifications and separation of positional phosphopeptide isomers by TIMS and PASEF®. Results are provided courtesy of Prof. Dr. Stefan Tenzer.

Expanding the Horizons with 4D-Metabolomics

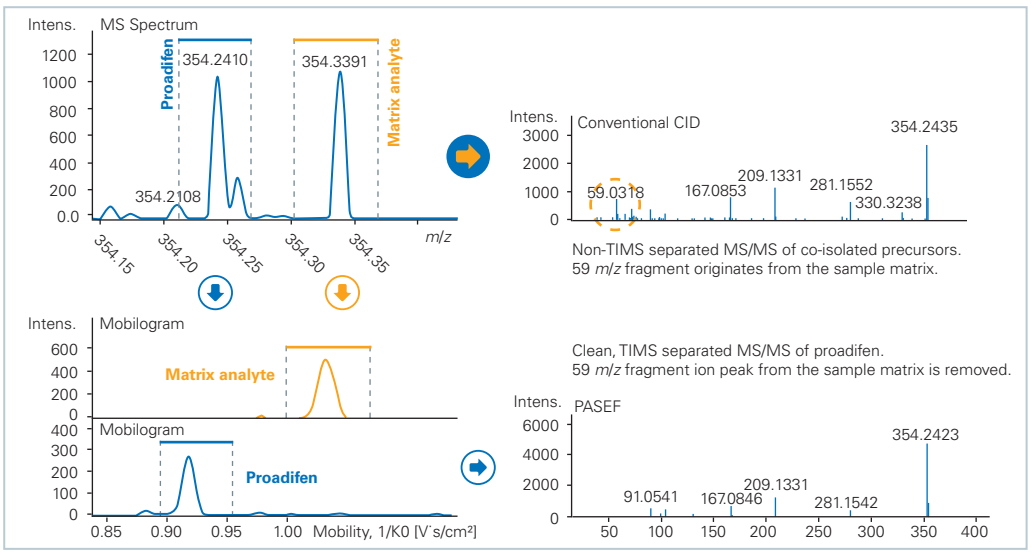
Reproducible MS/MS spectra even at low concentrations

Non-targeted metabolomics benefits from high MS/MS coverage as a key component of compound annotation. Our optimized 4D-Metabolomics™ methods provide both quantitative MS profiling data and MS/MS fragmentation spectra in the same sample analysis, achieving majority feature coverage even at low analyte concentrations. PASEF provides mobility selection prior to fragmentation resulting in clean and unambiguous fragment spectra. Highly accurate CCS measurements are intrinsic to the workflow, providing an orthogonal criterion for confident compound annotation that augments the accurate mass, isotopic pattern fit, retention time, and MS/MS routinely generated.



4D-Metabolomics™ analysis of a forensic toxicology analyte mixture spiked into urine highlights the reproducibility of MS/MS spectra provided by PASEF® at both high and low analyte concentrations.

PASEF improves MS/MS spectra for better library matching results

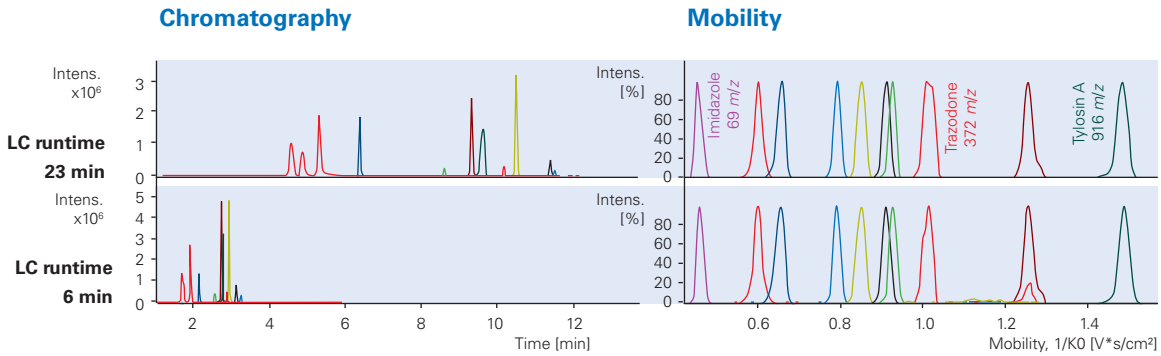


When measuring Proadifen in a complex biofluid, its MS/MS spectrum can become compromised by the coelution, co-isolation and simultaneous fragmentation of similar mass matrix analytes.

PASEF® is able to mobility select and fragment each compound separately, resulting in clean fragment spectra. Removal of matrix-derived MS/MS fragments provides better library matching for Proadifen in MetaboScape® and hence higher confidence in results.

Increase throughput and maintain confidence

Increasing demand for higher sample throughput in many metabolomics labs requires optimizing chromatographic methods to speed up analysis time without compromising confidence in results. TIMS mobility values are characteristic for each metabolite and remain unaffected by choice of speed or prior sample separation, offering measurement orthogonality regardless of the upstream sample separation approach.



A mixture of reference standards, ranging from Imidazole at 69 *m/z* to Tylosin A at 916 *m/z*, separated by a conventional 23 minute and a runtime-optimized six-minute reversed-phase UHPLC gradient. Extracted ion chromatograms for the two different LC methods highlight the expected condensation in retention time, however the mobility remains unaffected when changing chromatographic runtimes. Hence, CCS values can be used as an important identification criterion during method optimization and routine sample analysis.

MetaboScape: CCS values substantiate annotations

Because of this methodological independence, experimentally acquired CCS values are being generated and shared at a rapid rate, including in the Unified CCS Compendium (https://doi.org/10.1039/C8SC04396E) which houses >3800 values from traceable reference standards. TIMS data provides highly accurate matching values, accelerating the process of automated metabolome annotation using MetaboScape®. Furthermore, with CCSPredict Pro, any metabolite library containing the common InChI identifier for chemical substances can be readily made into a searchable CCS library featuring highly accurate predicted values, greatly expanding the utility of the 4th dimension in metabolomics research.

CCSPredict Pro

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 Bruker timsTOF Pro's combination of high scanning speed and the ability to generate CCS values is a unique combination, which can significantly improve our capabilities in high-throughput metabolomics"

Enable higher throughput with 4D-Lipidomics

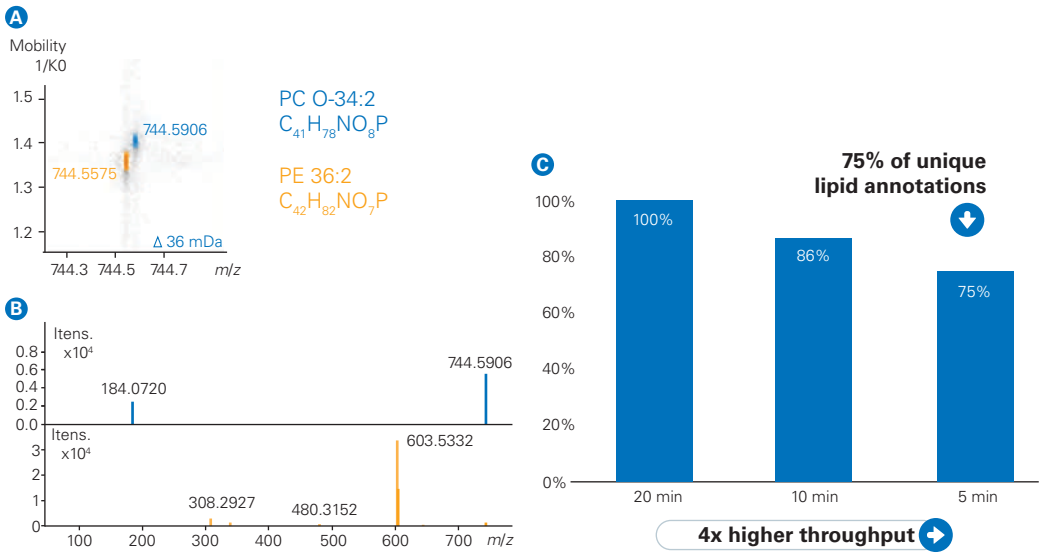
Analogous to proteomics samples, lipid extracts have a high sample complexity caused by the structural diversity of lipids. High quality MS/MS spectra are integral to obtaining confident lipid annotations. PASEF unlocks CCS-enabled workflows which can be used to additionally boost lipid annotation confidence.

Mobility Offset Mass Aligned (MOMA) data of isobaric lipids

PASEF is able to fragment >10x more precursors by using mobility separation. 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.

High throughput with confident annotation

MS/MS spectra are acquired for on average 70% 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.



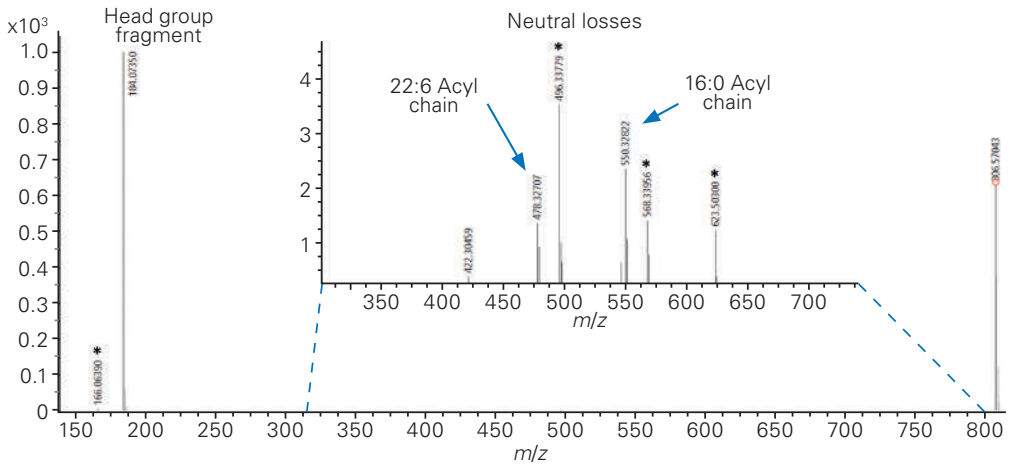
(A) Heatmap showing the mobility separation of two isobaric phospholipids co-eluting from reverse phase LC.

(B) Clean PASEF® MS/MS spectra after mobility separation.

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

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 of PC 16:0_22:6 based on head group and acyl chain fragments.

Rule-Based Annotation

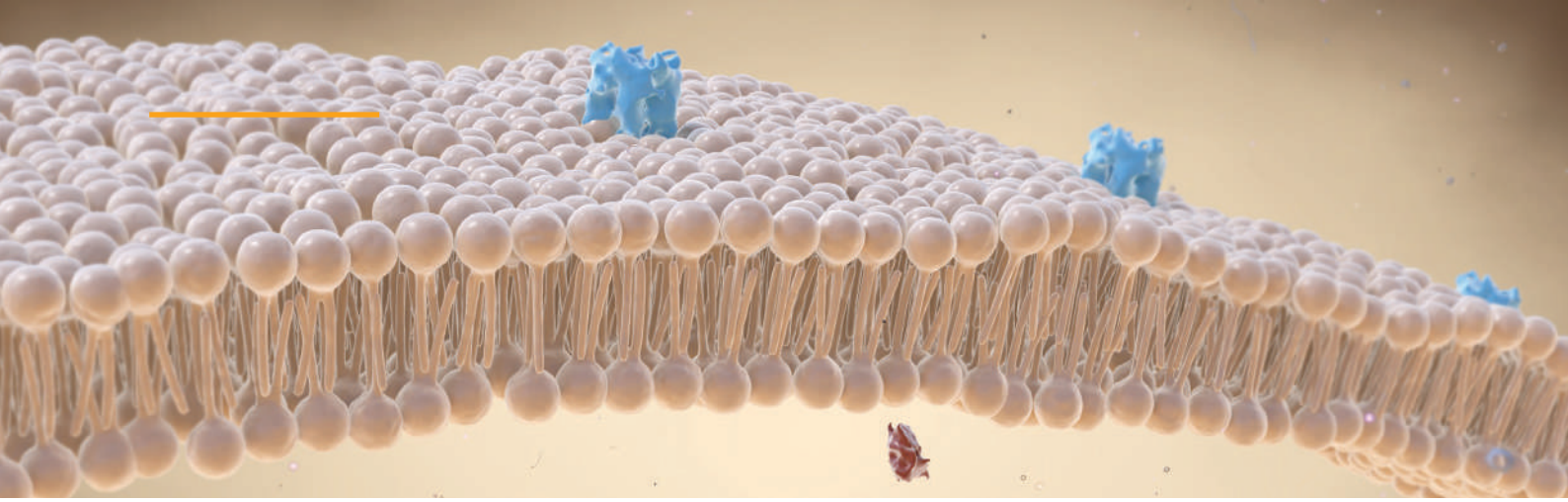
Besides the typical database- or spectral library-based annotation, MetaboScape features a library-free annotation tool that utilizes published fragmentation rules. Depending on the fragments and neutral losses, MetaboScape is able to annotate on a species or molecular species level.

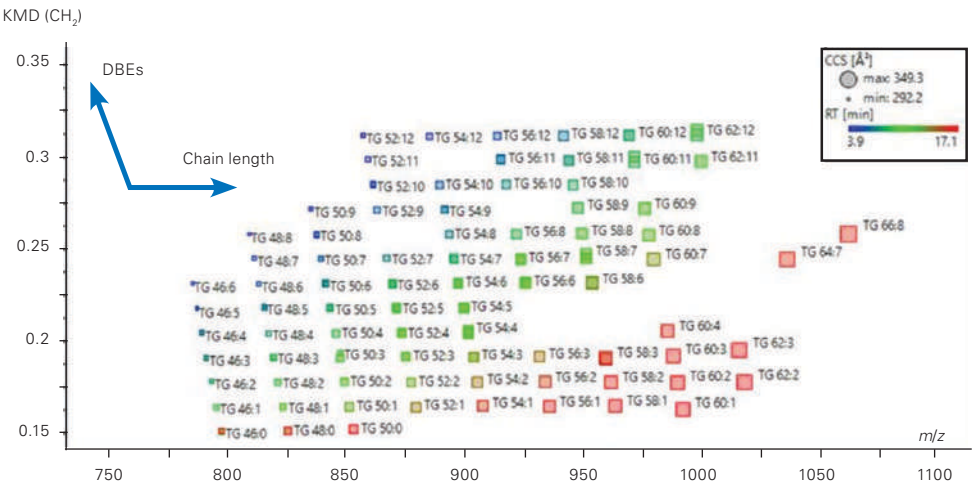
To unite with the lipidomics community as it continues to develop and grow, MetaboScape uses the latest recommendations on shorthand nomenclature and hierarchy and is regularly updated with novel lipidomics tools to simplify profiling workflows.

Dr. Michael Witting

Co-Head Metabolomics and Proteomics Core,
Helmholtz Zentrum Munich, Neuherberg, Germany

"PASEF® already out of the box increases the precursor coverage of lipids with an associated MS2 spectra to 70%. These MS2 spectra are essential for the correct identification of lipids. On top, the use of CCS adds additional confidence in annotation by adding an additional layer of information to RT, MS and MS/MS."

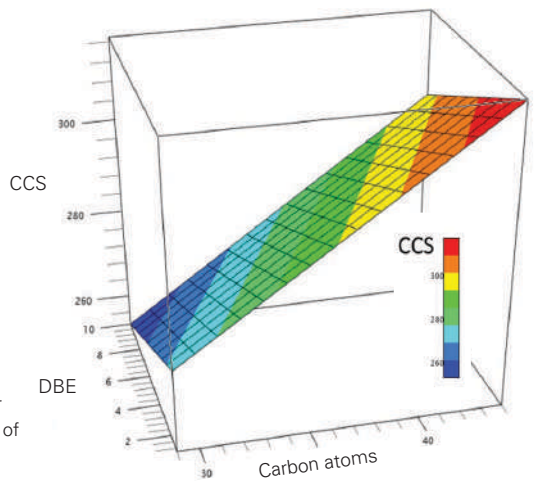




Kendrick Mass Defect plot showing homologous Triacylglycerides of a fish oil extract.

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.



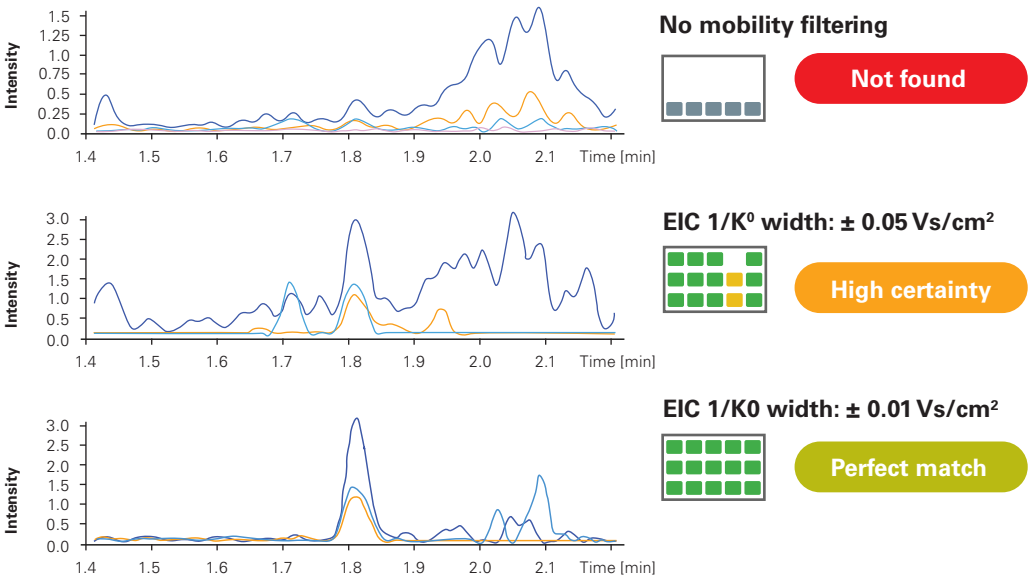
CCS hyperplane used for the automatic prediction of CCS values.

CCS-enabled tools for confident lipid annotation

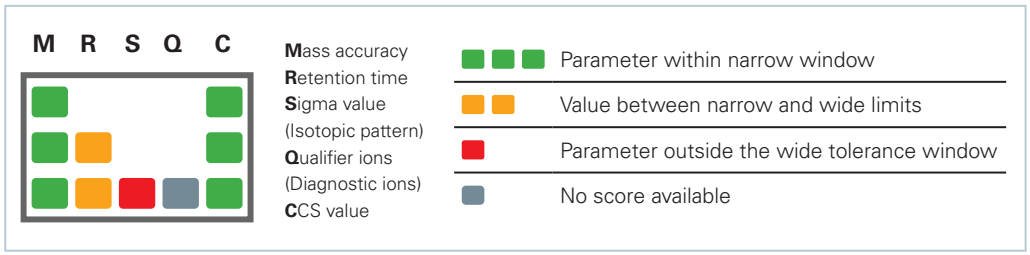
MetaboScape is a fully CCS-enabled solution with multiple tools utilizing CCS values to improve annotation quality. Besides MS/MS spectral libraries with integrated CCS values, e.g. the LipidBlast library for ½ million lipids, a new tool for the automatic prediction of CCS values based on CCS hyperplanes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

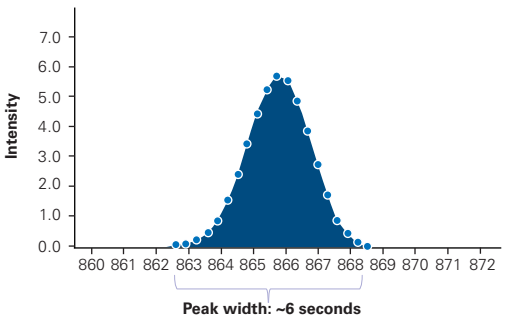
Ultrahigh-throughput confident screening and quantification in complex matrices

The exceptional speed of the timsTOF Pro 2 system allows full unknown screening with either GC-APCI or UHPLC VIP-HESI data acquisition. In combination with TASQ® software, Bruker offers a complete solution from data acquisition to automated data analysis.



Applying post acquisition mobility-filtering of EICs increases sensitivity in complex matrices by removing interferences from both chromatograms and spectra. This can clearly be seen in the trace for Thiacloprid in onion at a concentration of 1 ng/mL. The lower traces have been filtered at progressively narrower mobility ranges, allowing a perfect match to the TargetScreener database, as indicated by the green color of the MRSQC score.

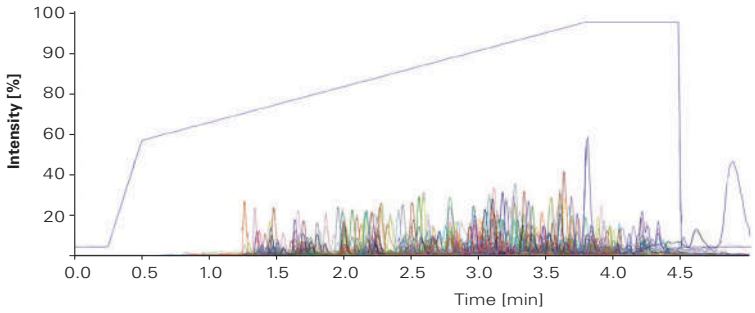




Exceptional speed of the timsTOF Pro 2 allows to achieve a high number of data points obtained over a 6 second peak of 2,3,7,8-TCDD acquired using GC APCI. This allows accurate quantification and low RSD values even for low concentration compounds.

A standard 20 minute method for 240 pesticides can be shortened to 5 minutes when combining TIMS bbCID for increased peak capacity and the highly sensitive VIP-HESI source.

One source for two ionization modes



Where samples contain co-eluting isomers, CCS data becomes invaluable in assigning the correct identification. Tramadol and *O*-Desmethylvenlafaxine are impossible to correctly assign using retention times, molecular ion, isotope pattern, and MS/MS information. Hence, if either compound is present in a sample, the identification result will return a positive identification for both.

Whereas performing retrospective data analysis and including CCS information for both compounds clearly identifies the peak as *O*-Desmethylvenlafaxine with the measured CCS value being within 0.2% of the expected value and the MRSQC score showing a perfect match to information within the TargetScreener 4D database thus allowing unambiguous identification.

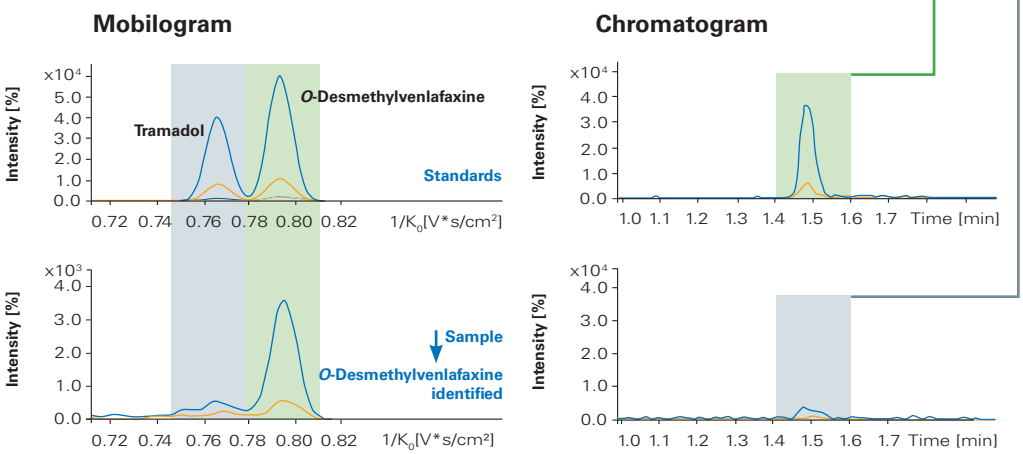
While *O*-Desmethylvenlafaxine and Tramadol cannot be distinguished using conventional LC-MS/MS, TIMS separation enables a distinct identification, in this case of *O*-Desmethylvenlafaxine.

TIMS OFF

Analyte Name	RT [min]	*m/z* meas.	MRSQC
O-Desmethylvenlafaxine	1.48	264.1959	
Tramadol	1.48	264.1959	

TIMS ON

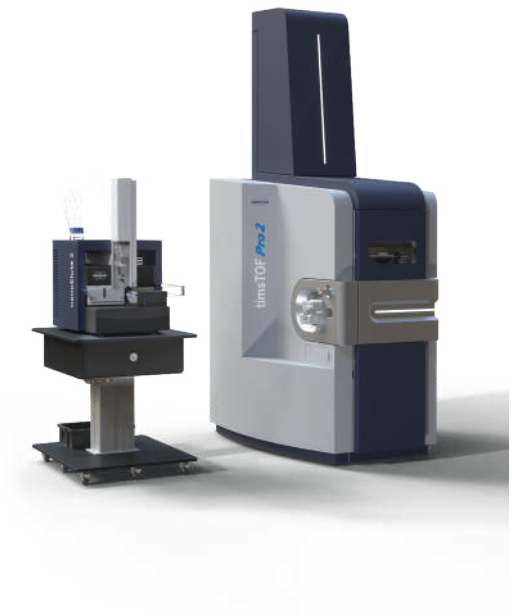
Analyte Name	RT [min]	*m/z* meas.	CCS [A²]	CCS [A²] meas.	CCS [%]	MRSQC
O-Desmethylvenlafaxine	1.48	264.1959	166.80	167.13	0.20	
Tramadol			161.20			



Mobility filtered EIC showing presence of *O*-Desmethylvenlafaxine (top) and absence of Tramadol (bottom).

timsTOF Pro 2 and PaSER

Run & Done! Results in hand, seconds after the acquisition ends.



PaSER™ (Parallel Search Engine in Real-time) is the combined hardware and software solution enabling fully integrated GPU database search results and results based sample queue management for the timsTOF family (Pro 2, HT, SCP, and fleX). PaSER delivers results for dda-PASEF and dia-PASEF with blazing speeds and quantitative capabilities. The best-in-class search speeds of PaSER allow you to have results in hand as soon as your experiments have run, or Run & Done.

Professor 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."

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

Bruker Switzerland AG

Fällanden · Switzerland
Phone +41 44 825 91 11

Bruker Scientific LLC

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

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

Online information
bruker.com/
timstof-pro-2