BRUKER

timsTOF Pro/fleX

● The new standard for shotgun proteomics

Innovation with Integrity

Four reasons to switch to 4D-Proteomics™ on the timsTOF platform

To provide concise arguments for replacement of older 3D mass spectrometers with the 4D capable, timsTOF platform and its unique TIMS (Trapped Ion Mobility Spectrometry) capability which adds the CCS dimension to retention time, precursor mass, and MS/MS.

The timsTOF platform is the new standard in shotgun proteomics with its proprietary TIMS/PASEF® (Parallel Accumulation – SErial Fragmentation) technologies, and an open architecture data-file format that enables easy input for the various bioinformatics approaches. With unmatched sequencing speed, uncompromised sensitivity, and ultra-high resolution (UHR) it delivers the highest depth and quality, while maintaining robustness. The timsTOF platform is uniquely equipped with state-of-the-art dual-TIMS funnel ion optics that sorts and time-focuses ions before they enter the quadrupole-time-of-flight (QTOF) mass analyzer (Figure 1). This exclusive combination delivers best-in-class sequencing speeds (>100 Hz), simultaneously retaining UHR at both the MS and MS/MS levels. Unlike other MS platforms, scan speeds (MS and MS/MS) are uncoupled from mass resolution, so every acquisition proceeds with maximum sensitivity and robustness.
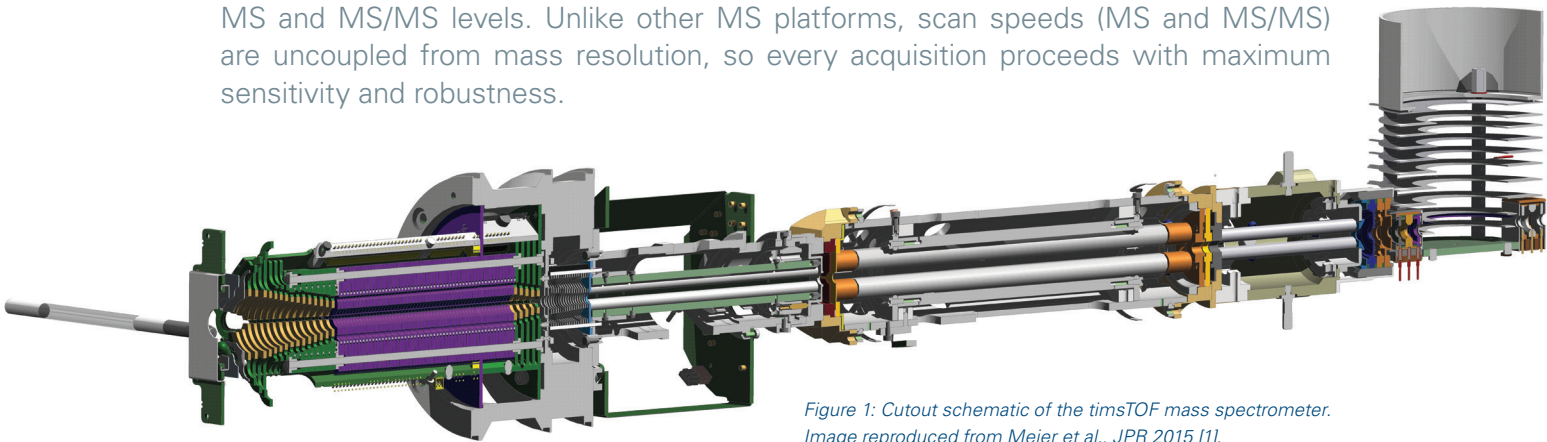

Figure 1: Cutout schematic of the timsTOF mass spectrometer. Image reproduced from Meier et al., JPR 2015 [1].

Robustness comes from the orthogonally positioned glass capillary ion source. It is perpendicular to the mass analyzer ion path and allows for ions to enter the resolving mass analyzer, while contaminants continue along the straight line and are pumped out. This critical design feature delivers significant improvement in analytical robustness. The dual-TIMS funnel further allows for contaminating gas loads to be pumped out between the stacked elements, and additionally improves ion focusing 20 to 30-fold, allowing for lower sample loads with uncompromised sensitivity. This triple combination of (i) orthogonally positioned capillary, (ii) dual-TIMS funnel, and (iii) lower sample loads, translate to industry leading analytical robustness that maximizes instrument up-time [2], especially important to large cohort studies.

Introduction

Fundamental challenges in MS-based proteomics include the high sample complexity, the large dynamic range in protein concentration, and the resulting big-data computational analysis. Development in mass spectrometers towards higher sensitivity, faster sequencing speed and larger peak capacity address many of these challenges. With the introduction of the timsTOF platform the next evolution in technology is achieved by adding an additional dimension of gas phase ion separation via the use of TIMS coupled to UHR-QTOF technology. This results in the transition from 3D-Proteomics (retention time, *m/z* and MS/MS fingerprint) into 4D-Proteomics™ (addition of intrinsic CCS values). 4D-Proteomics™ enabled by PASEF® allows scientists to dig deeper into their sample, provides more confidence in data, and increases data completeness by reducing the number of missing values. It is now possible to measure hundreds and thousands of omics samples due to the extremely high sequencing speed and proven robustness.

Benefits of the timsTOF platform in 4 key points:

1. **Increased Sensitivity and High Acquisition Speed at Ultra-High Resolution:**
   PASEF enables MS/MS acquisition at >100 Hz, making high-throughput measurements using **short gradients** possible [2] while maintaining **deep proteome coverage** or **high protein depth** measurements in **less time**. The time-focusing benefit of dual-TIMS funnel technology increases sensitivity thereby allowing for loading of **lower sample amounts** and simpler sample preparation techniques.

2. **Mobility Offset Mass Aligned (MOMA):**
   The MOMA benefit provided by TIMS technology identifies more near-isobaric peptides and post-translationally modified (PTMs) peptides per unit time. **PTM-positional isomers**, a common occurrence, are not only **detected** but can be **quantified**.

3. **CCS-Aware Workflows and Open Data Format:**
   CCS-Aware analysis software **increases** the **confidence** of identifications and quantitation, while **increasing data completeness**. Open data format easily allows timsTOF data to be used in custom developed bioinformatics solutions.

4. **Increased Robustness:**
   The orthogonal geometry of the inlet glass capillary to the axis of the mass analyzer combined with dual-TIMS funnel technology, greatly increases robustness, allowing for sustained MS performance over **large sample cohorts**, **maximizing** instrument **uptime**.

Driving innovation with 4D-Proteomics™

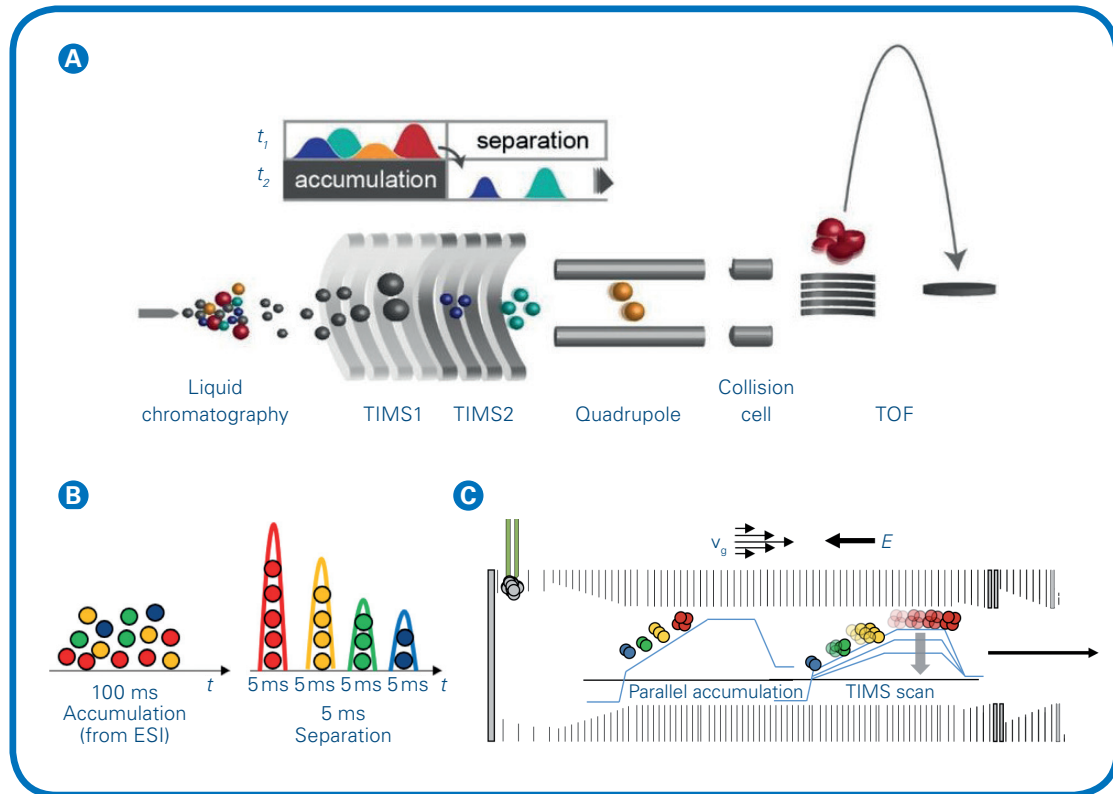## PASEF® acquisition modes for speed and sensitivity

Figure 2: Ⓐ *The timsTOF platform's dual-TIMS analyzers allows for parallel accumulation followed by high-resolution ion mobility separation. The ion cloud enters TIMS1 over a 20-100 ms accumulation time but elute from TIMS2 as discrete ion packets separated by CCS and compacted into 2-5 ms segments before entering the TOF.* Ⓑ *This temporal preconcentrating effect of dual-TIMS funnel results in up to a 30x signal-to-noise improvement compared with other continuous acquisition instruments.* Ⓒ *Simultaneous filling of TIMS1 while eluting from TIMS2 delivers 100% duty cycle. Adapted from Meier et al., Biorxiv 2020 [4].*

PASEF synchronizes MS/MS precursor selection with TIMS separation (Figure 2). This allows fragmentation of more than one precursor per TIMS scan and increases the sequencing speed several-fold without loss of sensitivity. The patented PASEF acquisition mode is only available on the timsTOF Pro and timsTOF fleX instruments.

Unparalleled MS/MS acquisition speed: A PASEF data dependent acquisition (DDA) consists of a single MS frame, followed by 10 MS/MS PASEF frames (Figure 3A). Since each TIMS frame requires only 100 ms a total duty cycle of 1.1 s is achieved. The precursor selection engine dynamically selects precursors based on intensity, *m/z*, and ion mobility. On average 12 precursors are selected per MS/MS PASEF frame (Figure 3B), resulting in a MS/MS acquisition rate of >100 Hz [2].

The dual-TIMS funnel implementation of the timsTOF platform (Figure 2A&C), spatially separates ion accumulation and ion mobility analysis into two sequential sections of the TIMS tunnel, occurring in parallel. Up to 100% of the ions that enter the mass spectrometer are utilized. This enables PASEF to overcome the diminishing returns of increasingly fast detector sampling, which otherwise necessarily implies loss of sensitivity (less and less ions per spectrum).

This high MS/MS acquisition speed can be harnessed for in-depth proteomics analysis with LC gradients as low as 5 min achieving sample throughput of 180 samples/day while identifying >10,000 unique peptide sequences and 3000 protein groups [3]. Or with a more modest throughput of 50 samples/day (17 min gradient) identify >36,000 unique peptide sequences from >6000 protein groups [3]. Deep proteome coverage can be achieved with fractionation, such as high-pH reversed-phase fractionation, typically at the cost of sample throughput. On the timsTOF platform, more than 9000 protein groups in HeLa and > 10,000 protein groups in mouse cerebellum were identified in 24 fractions with <12 hours of MS acquisition time.
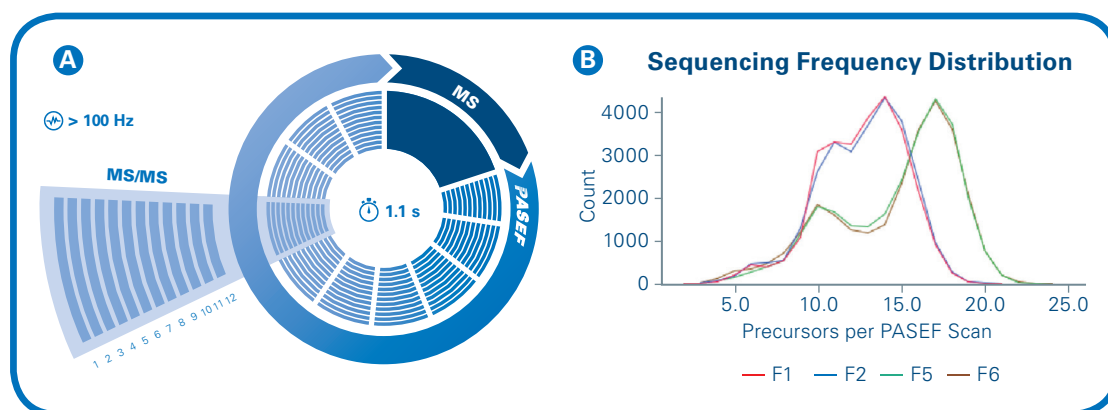

Figure 3: (A) Standard PASEF cycle depicted pictorially. One MS TIMS frame is followed by 10 MS/MS PASEF frames resulting in a duty cycle of 1.1 s. (B) Histogram of precursors per MS/MS PASEF frame. On average 12 precursors are targeted per MS/MS PASEF frame during the analysis of a trypsin-digested whole cell lysate, such as HeLa, resulting in an MS/MS acquisition speed of >100 Hz.

dia-PASEF®: Data independent acquisition (DIA) modes isolate and concurrently fragment populations of different precursors by cycling through predefined precursor *m/z* ranges. In traditional DIA or SWATH workflows the large isolation windows result in noisy fragment ion spectra from co-isolation of unwanted charge states (1+, ≥4+) and chemical noise. Collectively, the DIA selection windows cover the entire mass range of interest, but only a few percent of the ion current is sampled due to the consecutive scanning of the acquisition window. dia-PASEF is both more sensitive and selective as it applies the PASEF principle to data independent acquisition, combining the advantages of DIA with the inherent ion efficiency of PASEF (Figure 4A). Making use of the correlation of molecular weight with ion mobility in the dual-TIMS funnel, dia-PASEF samples selectively along the ion cloud at up to 100% of the precursor ion current [4]. Over the entire LC-MS/MS dia-PASEF run a perfect data cuboid is created containing *m/z*, ion mobility (CCS), retention time and intensity.

The standard dia-PASEF scheme utilizes 16 PASEF frames to capture 64 dia-PASEF windows within a 2 s duty cycle (Figure 4A). The extremely high ion sampling fully translates to higher sensitivity [4], over 3000 proteins can be identified by dia-PASEF from just 10 ng of HeLa digest or the complete yeast proteome can be acquired in 30 min (Figure 4C). The acquisition speed and sensitivity gains do not compromise the reproducibility and quantitative accuracy. Utilizing the well described human, yeast and *E.coli* mixed sample [5], we observe median CVs of 6.7% and 7.2% on protein group level and 8.1% and 8.6% on peptide level for sample HYE-A and sample HYE-B, respectively and excellent correlation between samples and technical replicates (Figure 5A&B). Furthermore, we observe very good global accuracy on the timsTOF platform with regulation ratios measured close to the theoretical ones (Median ratio sample HYE-A/HYE-B for yeast: 1.9 (expected value: 2.0) and for *E. coli*: 0.3 (expected value: 0.25), Figure 5C).
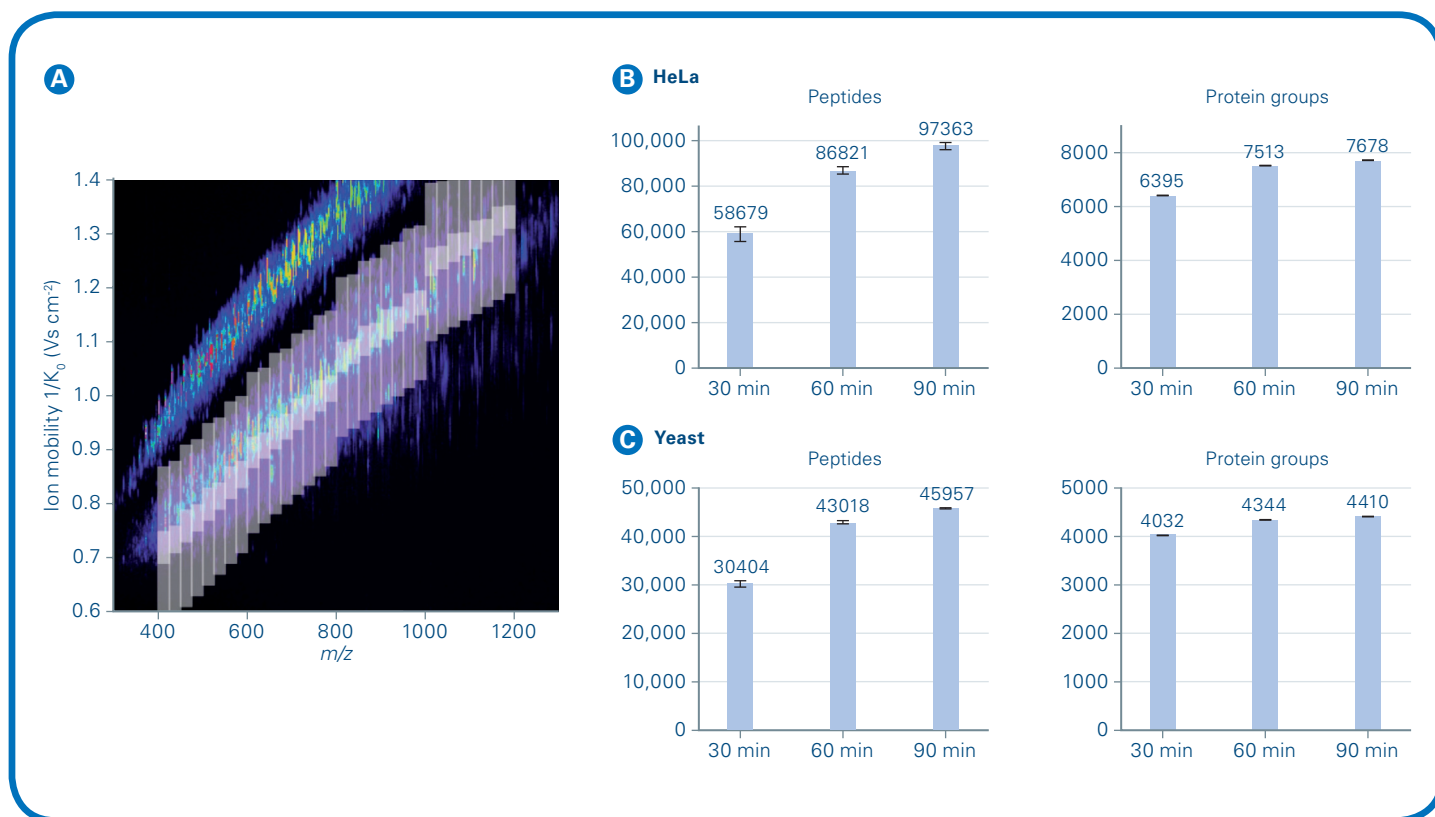

Figure 4: (A) Position of the precursor isolation windows in the dia-PASEF acquisition scheme overlaid on the average precursor ion intensity in a 120 min LC-MS experiment. 16 PASEF frames are utilized to capture 64 dia-PASEF windows within a 2 s duty cycle. Number of identified peptides and protein groups at an FDR of 1% for different gradient lengths for HeLa (B) and yeast (C). See application note LCMS-167 for more details.

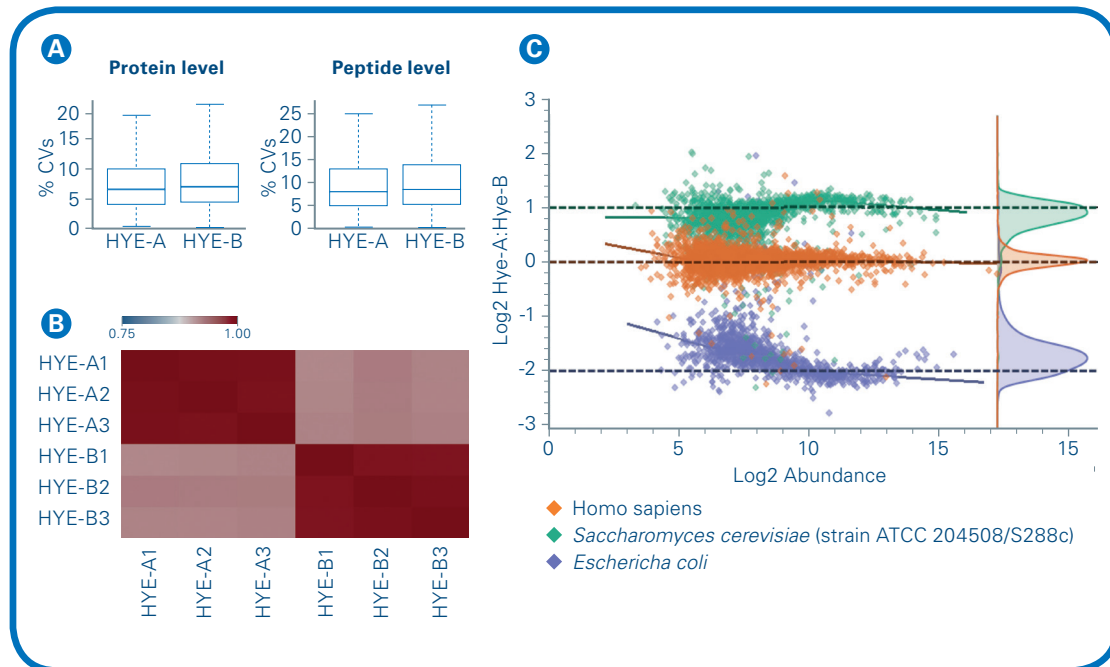Figure 5: Reproducibility and normalized quantitation of dia-PASEF measurements. Boxplots show the CV distribution in % per condition on protein group and peptide levels (A), revealing the excellent reproducibility with median CVs below 8% for both sets of samples. (B) Sample correlation matrix of precursor quantities between the two samples, HYE-A and HYE-B, together with technical replicates. (C) Log-transformed ratios of proteins (human in orange, yeast in green and E. coli in purple) were plotted over the log-transformed intensity of sample HYE-B. Dashed colored lines represent the expected values. See application note LCM-160 for more details.
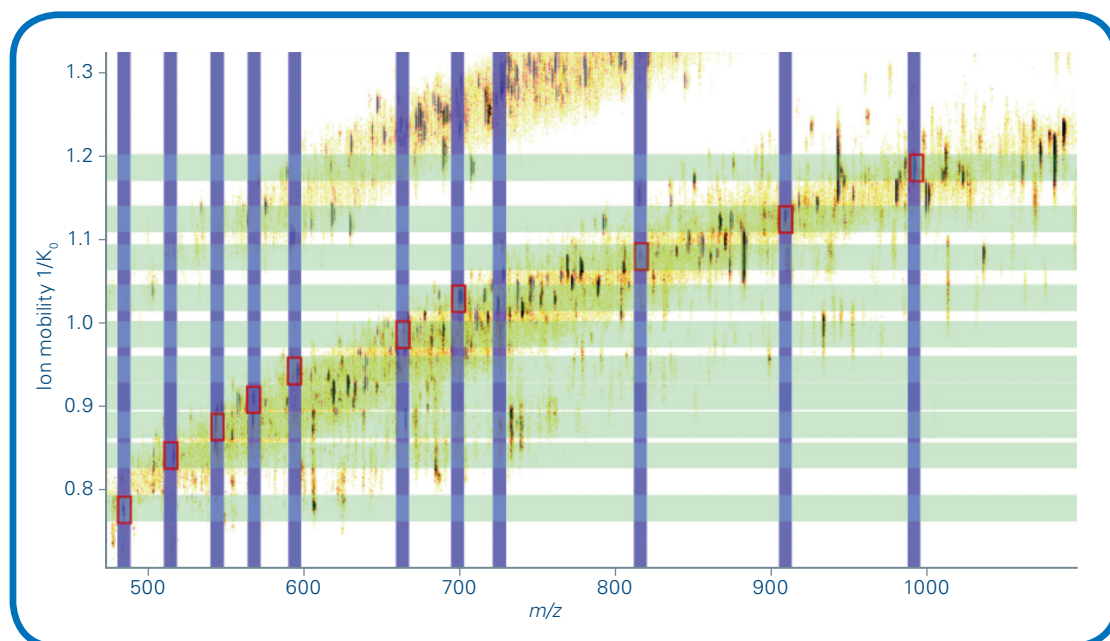

Figure 6: Illustration of one TIMS elution (100 ms) during which multiple targets (10 shown) can be simultaneously targeted with no loss in sensitivity or selectivity. In violet: narrow quadrupole isolation windows (scaled up for visibility). In light green: Ion mobility isolation windows. The targeted species are highlighted in red.

prm-PASEF®: The PASEF principle can also be applied to targeted proteomics workflows, in the form of parallel reaction monitoring (PRM). prm-PASEF preserves the benefits of increased sensitivity, specificity, and inherent robustness of the timsTOF platform while **maximizing the number of precursors that can be targeted per unit time without losing sensitivity**.

prm-PASEF exploits all the benefits of 4D-Proteomics™ to overcome the limitations of traditional targeted proteomics acquisition strategies, such as multiple reaction monitoring (MRM) and PRM. Traditional PRM, using trapping instruments, can be extremely sensitive, however this sensitivity is obtained at the cost of reduced duty cycle necessitated by selective accumulation. prm-PASEF takes advantage of the temporal preconcentration in the dual-TIMS funnel to allow measurements of chromatographically co-eluting targets, separated in the mobility dimension, within the same TIMS elution without a loss in sensitivity (Figure 6). If co-eluting targets cannot be resolved in the mobility dimension, they are simply acquired in consecutive TIMS elutions. With a traditional MRM approach, measuring 10 targets in parallel results in 10x loss in sensitivity. Since the sensitivity gains in prm-PASEF are a product of time focusing rather than from increased accumulation time, the very high sensitivity level is obtained at no cost to duty cycle or parallelization. prm-PASEF is the ideal solution for targeted proteomics experiments with no compromises in the number of targets, throughput, selectivity, and sensitivity (Figure 7).
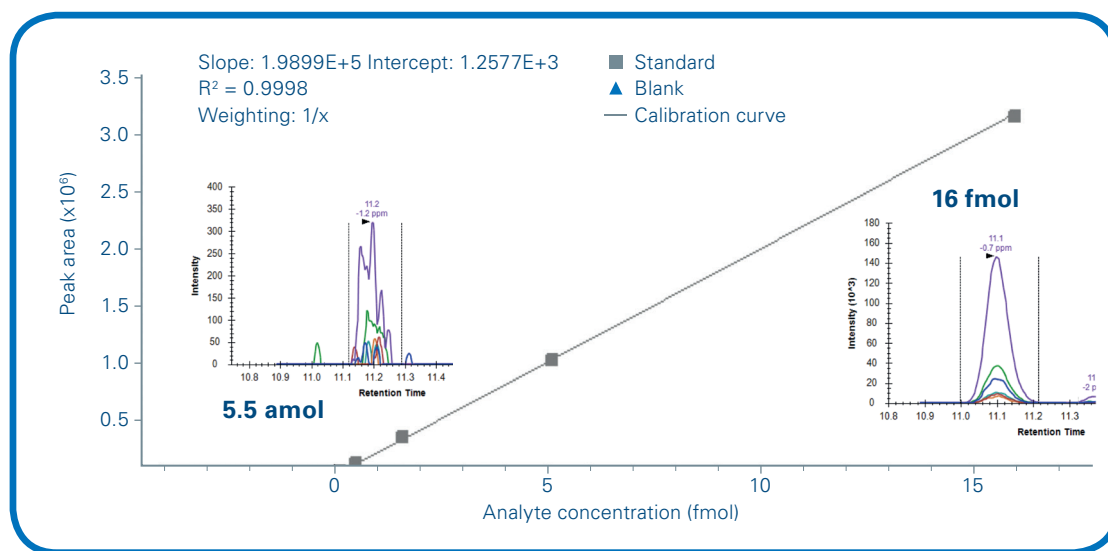

Figure 7: 256 aqua peptides were diluted in a K562 cell digest background and separated with a 30 min gradient. The linear calibration curve covers the concentration range from 5 amol -16 fmol. Data courtesy Dr. Antoine Lesur, LIH.

TIMS dramatically boosts sensitivity bridging the gap to translational studies

Cutting edge proteomics experiments demand the most sensitive mass spectrometer. Laser capture microdissection, small organs, and even single cells all require **very high sensitivity** with **limited sample material**. The dual-TIMS funnel in the timsTOF platform significantly improves sensitivity while maintaining unsurpassed sequencing speeds. The temporal preconcentration of ions in the dual-TIMS funnel results in up to 30x signal-to-noise improvement when compared with inefficient continuous acquisition instruments.

Low sample input: When low sample input is simulated by injecting dilutions of a sample, over 8000 peptides, representing nearly 2000 protein groups, can be identified with 1 ng of sample with 60 min LC gradient. At 50 ng, over 42,000 peptides representing nearly 5000 proteins groups can be identified (Figure 8).
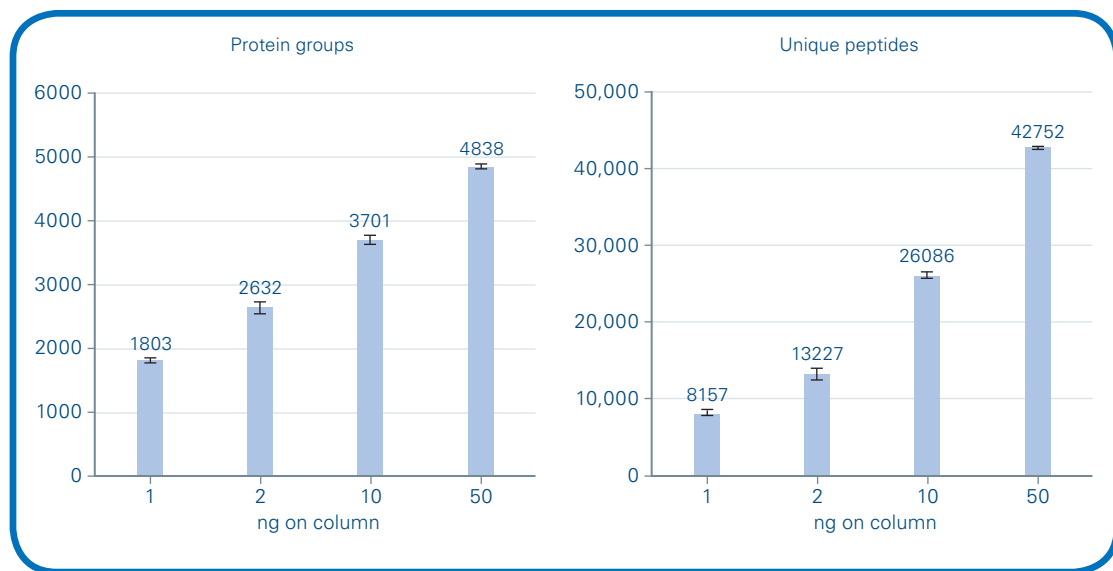

Figure 8: Number of identified peptides and proteins at an FDR of 1% for different amounts of sample injected on a timsTOF Pro platform.

SpatialOMx® - target proteome subpopulations[§]: The inherent sensitivity of PASEF bridges the gap between realizing translational research where other platforms have previously failed and makes possible the revolutionary technique of **MALDI-guided SpatialOMx®**. In SpatialOMx®, a section of tissue is segmented into sub-regions according to similarities in molecular fingerprints measured by MALDI Imaging. Often these sub-regions are undetectable by histology and thus offer extreme specificity for a particular cell phenotype. Sub-regions of interest are targeted for microextraction by Laser Capture Microdissection (LCM) and 4D-Omics™ analysis. Compared to traditional tissue homogenization, SpatialOMx® delivers the highest confidence that molecular changes detected are directly related to the cell phenotype being studied. Figure 9 summarizes a SpatialOMx® measurement made from a section of breast tumor biopsy using timsTOF fleX. First, MALDI Imaging of lipids is conducted. Subsequent analysis of the images using SCiLS™ Lab software identifies three sub-regions of differing molecular phenotypes within the region annotated as tumor by a pathologist. Locations of the three sub-regions are used to guide LCM in excising an area equivalent to ~2000 cells from each sub-region (~160 ng injected per sub-region), followed by microwell extraction and 4D-Proteomics™ analysis. As shown in Figure 9, each sub-region yields **deep and differing proteome subpopulations** that reflect unique GO annotations for each sub-region that would otherwise have gone undetected from analysis of larger pieces of the tissue. timsTOF fleX is the ideal platform for performing **spatially guided 4D-Proteomics™**, **SpatialOMx®**, as it combines 4D-Omics and MALDI Imaging on a single platform.

[§] *See 'Guide for writing successful proposals for SpatialOMx® and timsTOF fleX' for more details.*
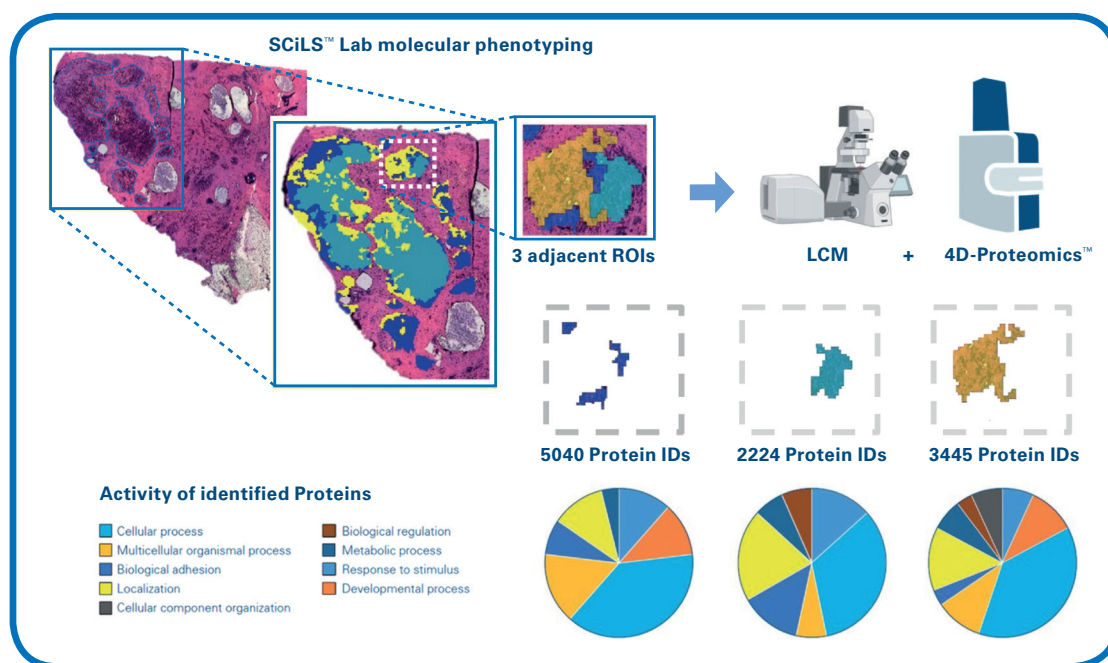

Figure 9: Illustration of MALDI Guided SpatialOMx analysis of breast tumor biopsy. Pathologist-annotated tumor regions are further segmented by SCiLS Lab based on molecular phenotyping derived from MALDI based lipid imaging. A very narrow region of tumor segmented into three molecular sub-regions is targeted for removal by LCM into collections of ~2000 cells from each sub-region. PASEF enables high sensitivity 4D-Proteomics™ from 160 ng of peptides injected into the timsTOF fleX to characterize the proteomes of each of the cellular subpopulations distinguished by GO annotation. See application note LCMS-166 for further details.

Glycopeptides: Glycopeptide ionization efficiency can be significantly improved by doping the nitrogen gas in the patented CaptiveSpray nanoBooster source (Figure 16). Doping with acetonitrile supercharges glycopeptide precursor ions and increases the number of charge states per precursor ion. Primary alcohols subcharge glycopeptide precursor ions and reduce charge state heterogeneity (Figure 10). Primary **alcohol subcharging** from glycopeptide ions can result in a **40-fold increase** in sensitivity compared with standard nano ESI sources (Figure 10A).

MS/MS spectra from high and low collision energies are combined to produce both glycan and peptide backbone specific fragments allowing comprehensive structural elucidation of glycopeptides (Figure 10C). The complementary sensitivity boosts from primary alcohol subcharging in the ion source and the dual-TIMS funnel benefit in elucidating the structures of many glycopeptides.
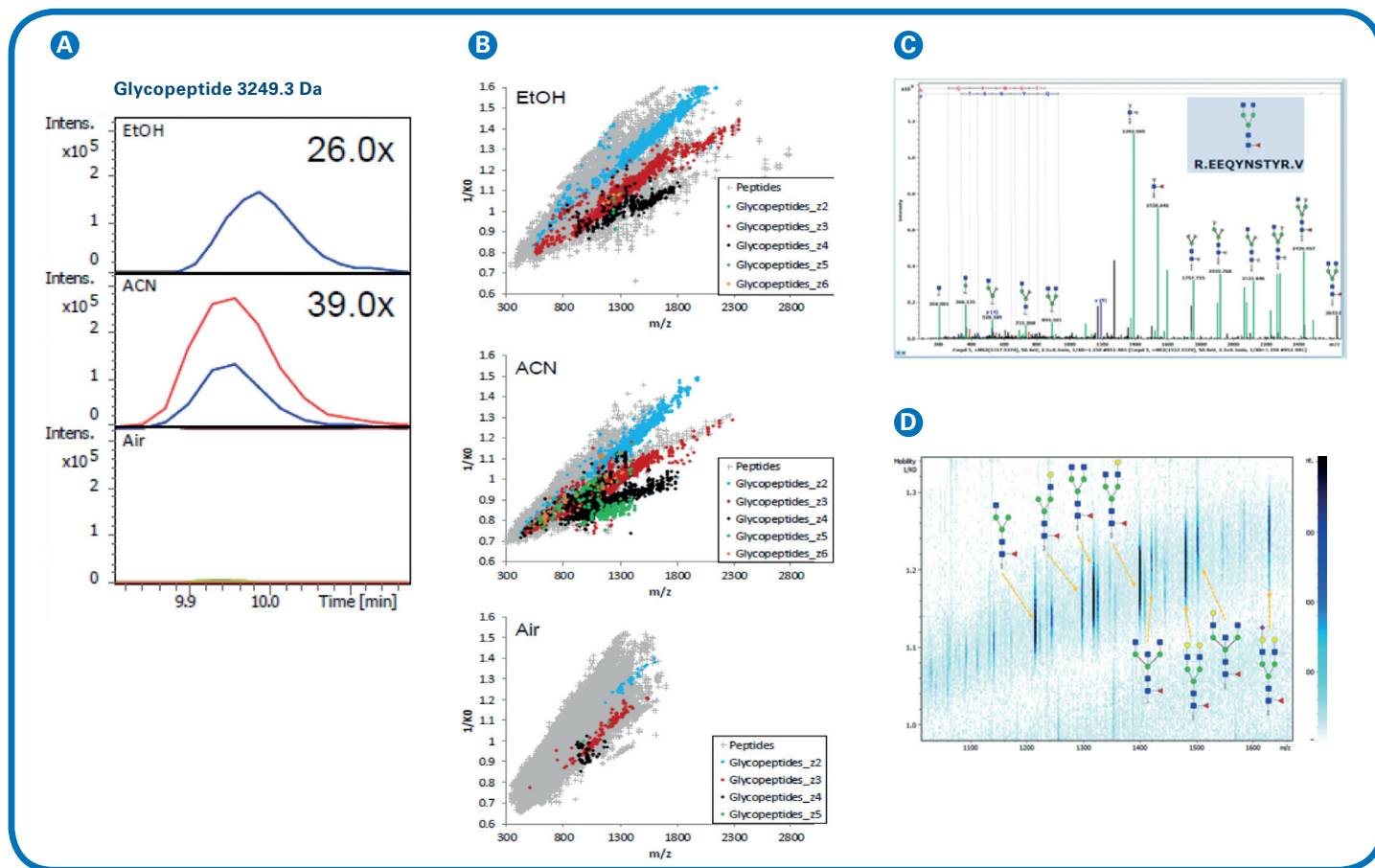

Figure 10: Ⓐ *Effects of supercharging (ACN) and subcharging (EtOH) of glycopeptides by doping nitrogen gas.* Ⓑ *Ion mobility vs. m/z distribution of glycopeptides with no doping agent (air) vs. acetonitrile (ACN) or ethanol (EtOH).* Ⓒ *Composite MS/MS spectra with high and low collision energies for the concurrent identification of glycan and peptide.* Ⓓ *Heatmap of ion mobility separated glycans and their annotated structure.*

Mobility Offset Mass Aligned (MOMA): separate isomers

Proteolytic digestion of complex matrices (i.e. whole cell lysates, primary tissues) results in the generation of thousands of unique proteotypic peptides. High performance liquid chromatography (HPLC) is a powerful tool used to increase peak capacity when coupled to a mass spectrometer. In most workflows, co-eluting analytes with near-isobaric masses results in chimeric MS/MS spectra as the specificity of the quadrupole is finite. The dual-TIMS funnel first accumulate and simultaneously separates ions by mobility before delivering them into the mass spectrometer. The 5cm long, compact TIMS device can achieve IMS resolution up to 200, equivalent to a drift tube length of over 2 meters, thus co-eluting ions can be Mobility Offset Mass Aligned (MOMA), as demonstrated in Figure 11,
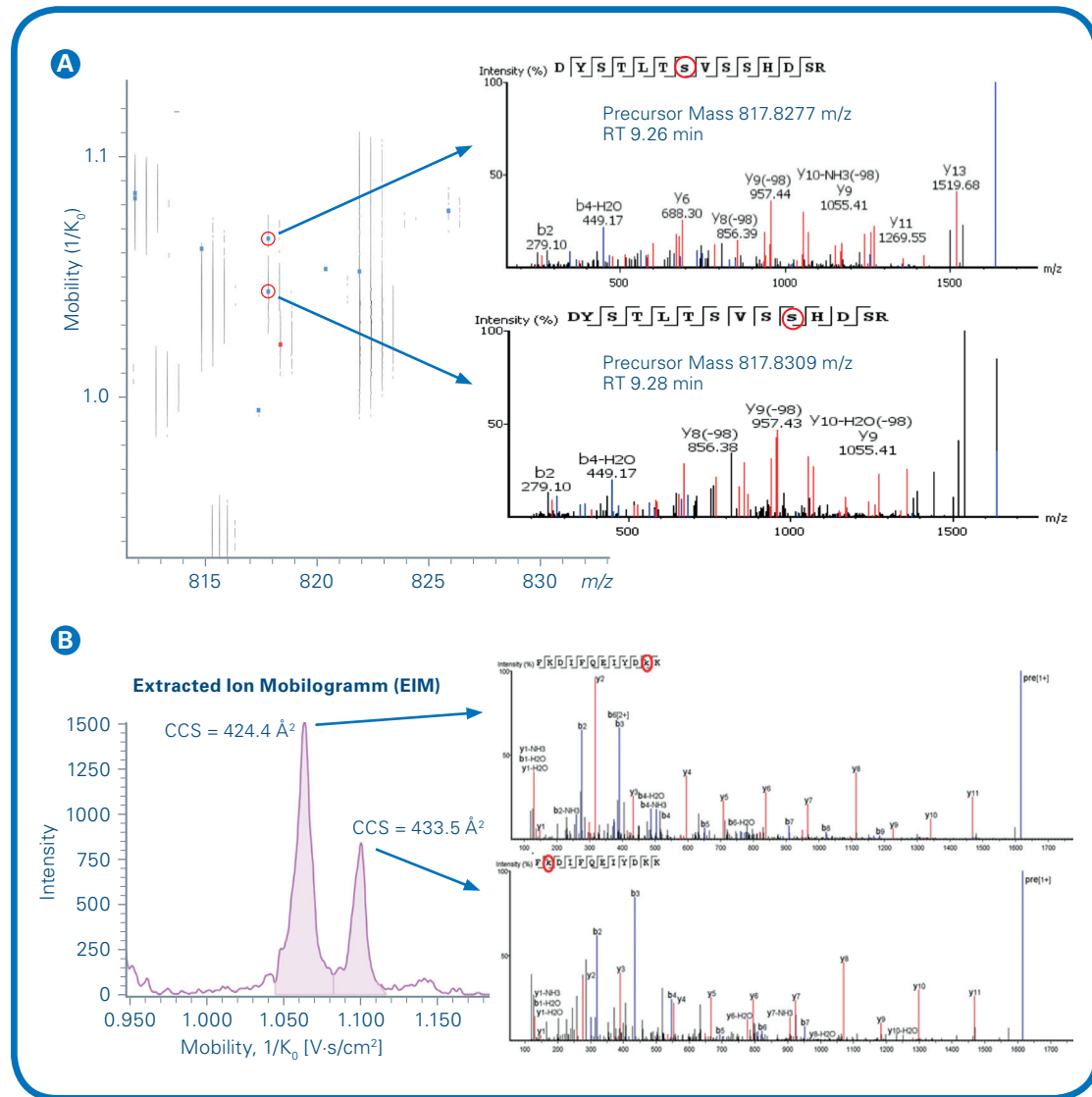

Figure 11: Ⓐ, A heatmap of the co-eluting, isomeric mono-phosphorylated peptide DYSTLTSVSSHDSR where mobility offset mass alignment (MOMA) discerns the two positional isomer forms and PASEF acquires non-chimeric and high scoring spectra for each peptide (inset). Ⓑ, Extracted ion mobilogram (EIM) of a co-eluting positional isomer of acetylated-Lysine and their corresponding fragmentation spectra, the baseline resolved CCS-Aware capabilities of MOMA enable quantitation. The red circled lowercase amino acid residues indicate the site localization for phosphorylation Ⓐ and acetylation Ⓑ.

where the **mobility difference** allows both **positional isomers** to be uniquely assigned. The capabilities of MOMA are **unique** to the **timsTOF platform** and result in a noticeable improvement in the number of peptides analyzed in complex samples.

The MOMA principle is exceptionally beneficial in the study of post translational modifications (PTMs), as PTM-positional isomers are a common occurrence and go undetected and unquantified in most current proteomics platforms. In Figure 11, we show two such cases: a positional isomer of phosphorylated-serine and acetylated-lysine. Note in both cases the positional isomers are well resolved by MOMA and PASEF acquires non-chimeric high-scoring spectra. As another example, the isomerization of aspartic acid to isoaspartic acid (isoAsp) occurs spontaneously in proteins and plays a role in cancer, neurodegeneration, aging, and other diseases. Figure 12 shows the resolution of two isobaric peptides containing aspartic/isoAsp at position 6 in the ion mobility dimension.
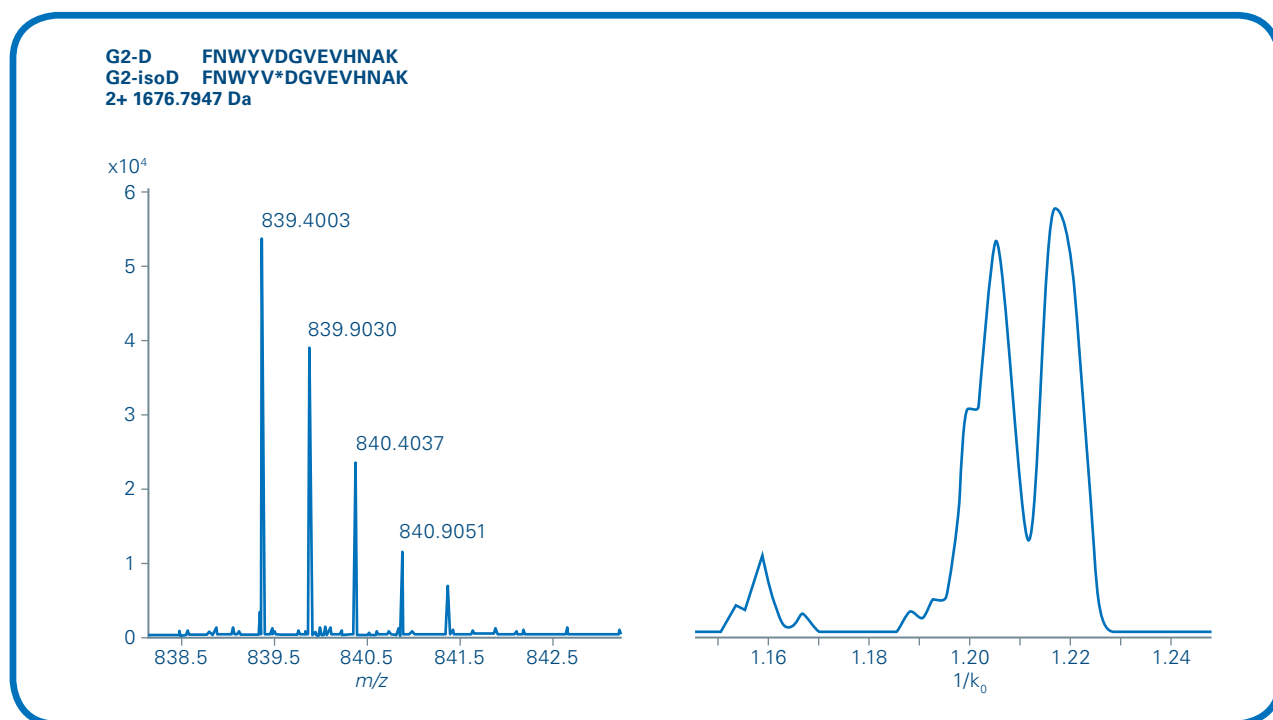

Figure 12: MS spectra and extracted ion mobilogram (EIM) of two isobaric peptides containing aspartic/isoaspartic acid at position 6. The two peptides are nicely resolved in the ion mobility domain providing a reproducible mobility (CCS) value and allowing quantitation based upon AUC of the EIM.

CCS-Aware: Bringing your workflow to another dimension

Just as retention time (RT) has been harnessed as an analyte attribute within the liquid phase, the gas phase structure is reproducible and intrinsic in nature. The timsTOF platform measures mobilities using first principles for all analytes, assigning a collisional cross section (CCS) value for small molecules, metabolites, lipids, peptides, and protein molecules. Experiments are enhanced with the 4th dimensional CCS value, improving the fidelity of protein and peptide assignments, quantification completeness (Figure 5 and Figure 13), deciphered isomeric species and predicted CCS term to aid database searching. The 4th dimension can be used to selectively evaluate analytes of unique interest within the CCS space.
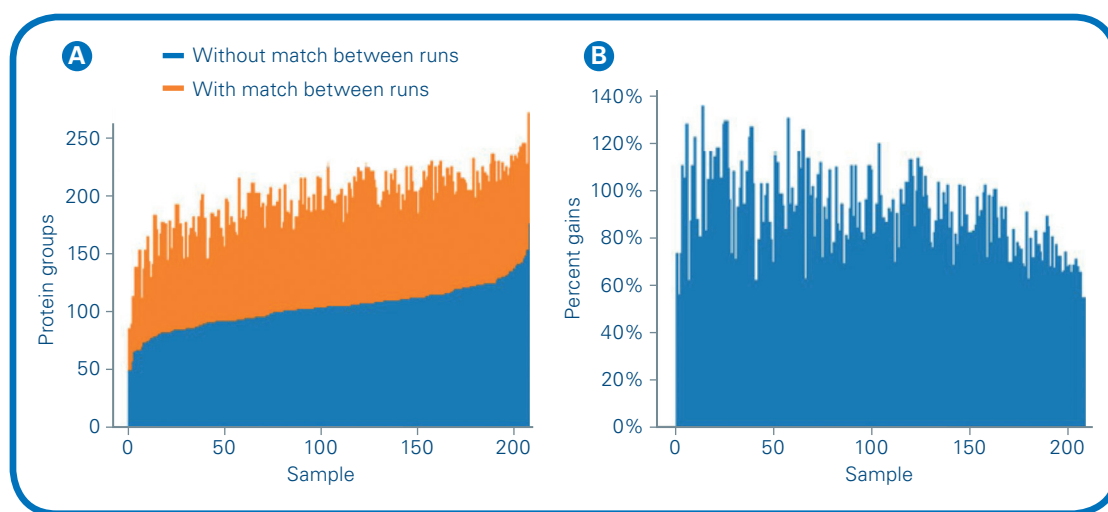

Figure 13: (A) Protein groups identified in 200+ clinical plasma samples without match between runs (blue) and protein groups identified and quantified when using 4D MBR (orange). (B) The average percent gain for each of the 200+ samples run collectively, averaging a 90% gain.

CCS-Aware match between runs (MBR) helps complete datasets: Quantitative proteomics is the backbone of molecular biology; it is fundamental to know not only what proteins are present but how much of each species. Due to the stochastic nature of data-dependent mass spectrometry experiments, a level of data incompleteness exists when comparing multiple samples. One way to deal with these missing values, is to use an approach described as match between runs (MBR). In this approach, precursor mass and retention time, within a narrow window of tolerance, would be used to 'match' MS1 features without MS/MS identifications to those that have them. Having only two parameters (m/z and RT) has long exposed MBR to being error prone. CCS-Aware MBR allows the addition of the ion mobility dimension (CCS) to RT and m/z in building a library. In doing so the data becomes more selective and specific, greatly reducing false-positive and false-negative assignments [5]. As a case example, we looked at hundreds of short (11 min) LC-MS experiments run on clinically relevant plasma digests (Figure 13). Without MBR, on average 100 proteins are identified and quantified. When CCS-Aware MBR is enabled that number jumps to nearly 200 proteins, providing a 100% gain in the number of identified and quantified proteins [5].

CCSPredict: additional confidence for statistical significance and supercharging your database search

The pool of hundreds of thousands of peptides where RT, *m/z*, MS/MS and now mobility (CCS) are all accurately mapped, provides the foundation of a training dataset that can revolutionize the way proteomics is performed. From this it becomes possible to predict, a priori, the collisional cross section (CCS) of proteotypic peptide ions enabling **CCSPredict**. CCSPredict can help call the correct analyte and in addition boost the fidelity of the called analyte. CCSPredict will also **transform database search** algorithms, both **increasing** their search **speeds** but also **confidence** in the assignments. CCSPredict will also play a critical role in experiment design. As an example, we present prm-PASEF, where method building with CCSPredict can aid the PRM scheduler to maximize sensitivity without compromising duty cycle. The 4th dimension of information from CCSPredict can also aid real-time mass spectrometry approaches achieving higher sensitivity and specificity.

Exploit the 4th dimension – ROI polygons to boost selectivity: Data quality is paramount for your experiments. Most platforms confine the operator to select either a *m/z* or *m/z* range. The timsTOF platform allows you to selectively capture the most valuable information for your experiments. By constructing a region of interest (ROI) polygon, **users can control mobility** and ***m/z* that encompass the most critical pieces of information**. In the example below, low *m/z* and high mobility single charged precursors were discarded when targeting MHC immuno-peptides. MHC class I peptides are known to exist as high *m/z* singly charged ions and accessing the 4th dimension gives us the capability to view, capture and quantify this information.

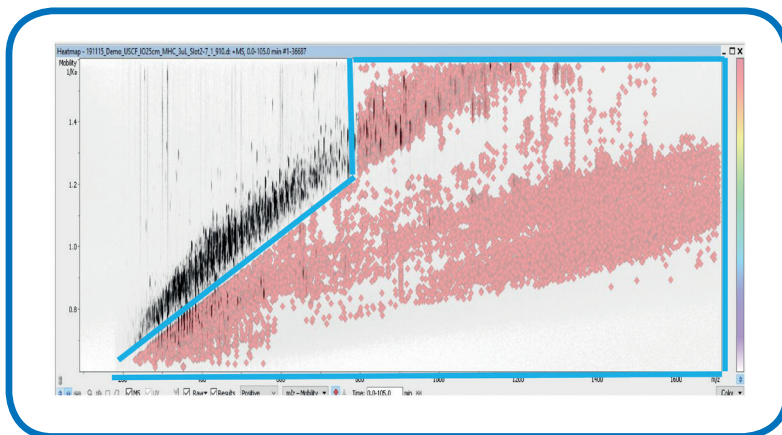Figure 14: Heatmap of MHC immunopeptide sample, highlighting where MS/MS were acquired (pink diamonds). Overlaid is the precursor selection polygon.

Freely access the 4th dimension – open file format: **timsTOF data** is stored in an **open** and versatile file format (*.tdf) based on SQLite. This allows for **direct access to the raw data** and for a wide variety of tools to be easily developed to analyze and visualize the 4D data. Many of the most popular proteomics tools already natively support timsTOF data, including MaxQuant, PEAKS, Spectronaut, Skyline, MSFragger, Byonic and the Integrated Proteomics Pipeline (IP2) to name a few.

Remarkable instrument robustness and ease-of-use

Robustness by design: Measurements from **large sample cohorts** (100-1000 samples) in proteomics research labs or MS service providers, such as core-facilities, necessitates robust instrument performance. Most of the mass spectrometers available on the market suffer from poor robustness compared to the timsTOF platform, resulting in instrument performance drops over time. Experiments then require enormous normalization during data analysis and system down time ranging between 12 to 24 h every 3 to 6 weeks. The orthogonal ion optics of the timsTOF platform (Figure 1 and 2C) together with its very high sensitivity, requiring less sample to be injected, minimizes the contamination of the mass spectrometer, and results in a system that provides **stable and reproducible** results over **several months** without the need for in-depth cleaning of the system (Figure 15).

Made easy for you: As mass spectrometers continue to evolve; the daily operations of the system have become more reliant on expert operators. The timsTOF mass spectrometer was designed with a focus on ease-of-use from up-front-setup including column and ionization source mounting (Figure 16) to the user interface. The maintenance procedure only requires calibration of mass and TIMS and can be done on the timsTOF platform in less than 5 min, without exchanging the source or disconnecting the column. Other MS instruments require a weekly maintenance, tuning, and calibration procedure of more than 45 min, including disconnecting the column and source exchange.
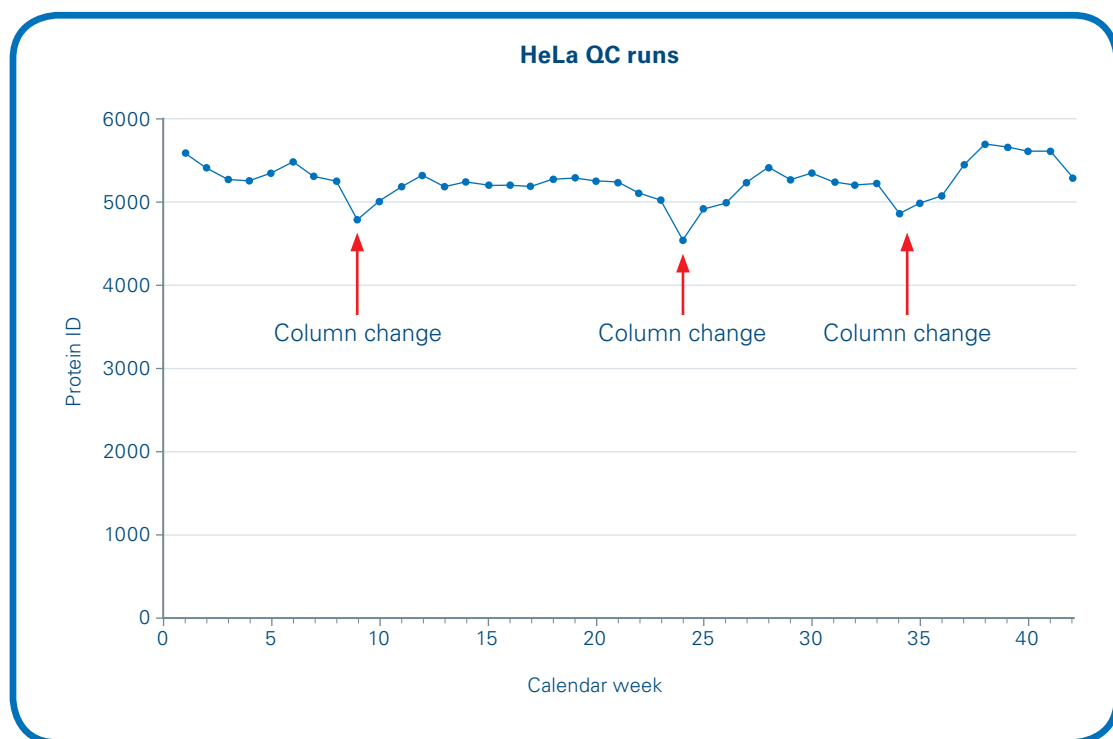

Figure 15: timsTOF Pro instrument performance over 40 weeks. On a weekly basis, a 200 ng Hela digest sample was measured using a 90-minute gradient. Observed drops below 5000 protein IDs were related to column aging and immediately after exchanging the column instrument performance was above 5000 protein identifications.

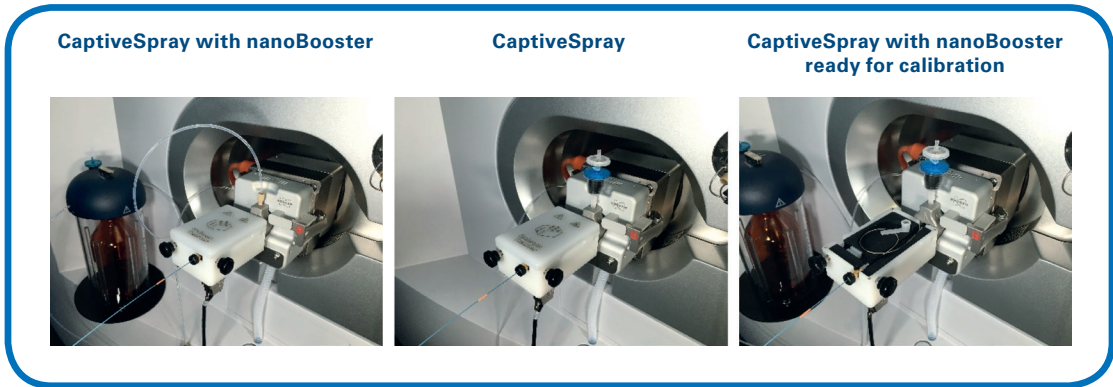| CaptiveSpray with nanoBooster | CaptiveSpray | CaptiveSpray with nanoBooster ready for calibration |

Figure 16: Simple upfront setup of the timsTOF platform including column, column oven and CaptiveSpray ion source with or without nanoBooster for added nitrogen gas doping capability.

Prof. M. Mann, Director of Max Planck Institute of Biochemistry and Director of Proteomics at The Novo Nordisk Foundation Center for Protein Research

"We observed that the re-designed ion transfer path – presumably mainly the 90-degree bend at the entrance of the TIMS funnel and the new quadrupole with increased inner diameter - had a positive effect on the robustness. This was evidenced by continuous operation of the instrument during its development for more than 1.5 years, in which time we only cleaned the ion transfer capillary but not the internals of the instrument."

Product Highlights

The timsTOF platform offers many unique advantages to today's proteomics MS related challenges.

1. **PASEF** enables MS/MS acquisition at >100Hz allowing for uncompromised depth of coverage. dia-PASEF exploits the correlation between molecular weight and ion-mobility to sample up to 100% of the precursor ion current.

2. **prm-PASEF** maximizes the number of precursors that can be targeted per unit time while preserving the increased sensitivity, specificity, and inherent robustness of the timsTOF platform.

3. The **dual-TIMS** funnel increases sensitivity while providing accurate and reproducible CCS values for all analytes.

4. Co-eluting ions can be Mobility Offset Mass Aligned (**MOMA**), allowing the acquisition of non-chimeric spectra and the unambiguous identification and quantitation of PTMs.

5. The **open-data file format** allows for transparent access to raw data and the rapid development of CCS-Aware bioinformatics solutions. **CCS-Aware** software increases the confidence of identifications and quantitation while greatly increasing data completeness.

6. The orthogonal geometry of glass capillary, to the axis of the mass analyzer, combined with the high sensitivity afforded by the dual-TIMS funnel, greatly increases the systems **robustness**, and **maximizes** instrument **uptime**.

7. Fully supported by PEAKS, MaxQuant, Skyline, Spectronaut, IP2 and Byos software packages. The timsTOF platform was 'designed-for-purpose' to overcome many of these challenges, including simple upfront setup and an open data-file format, that allows scientists to focus on their research interests rather instrument performance.

Required Instrumentation to fully equip a timsTOF lab

The timsTOF proteomics bundle includes the CaptiveSpray ion source with nanoBooster, the nanoElute LC system, 2x Ion Opticks Aurora columns, 2x Bruker Ten columns, mass calibrants and starter LC consumables package. Most common LC systems are also compatible, including the Evosep One. Peaks is available to be bundled in the package while other 4D-Proteomics™ software packages are also supported.

Instrument training and support

Bruker's extensive network of worldwide Service and Applications teams are available to provide support. Upon installation of the system, an engineer will qualify the system by demonstrating that the system meets all specifications and provides a familiarization training so that Users can begin measuring straightaway. Included with each system are certificates for Users to attend one of the many training sessions held throughout the year at Bruker's demo facilities. On-site training is also available. At any time, Bruker scientists or engineers are available for phone or online support at no charge.

Product specifications

Size	Floor standing: 98 x 141 cm (Footprint), 257 cm (Height)
Weight	350 kg
Vacuum System	5 stages, 100 m3/h rough pump
Apollo II ion funnel electrospray source	Flow rate: 1 µL/min-1 mL/min
Mass Range	20-40,000 *m/z*
Reproducibility of CCS value determination	<0.5% RSD
Quadrupole isolation	Up to 3,000 *m/z*
Quadrupole Mass Range	Up to 40,000 *m/z*
Mass accuracy in MS and MS/MS	With internal calibrant: better than 800 ppb RMS Error With external calibrant: better than 2 ppm RMS Error
Mass Calibration	ONE SINGLE calibration valid for MS and MS/MS analysis. Calibration is independent from charge state of calibrant mass
Mass drift over 8 hours with ΔT < 1K	< 2 ppm
Mass resolution	60,000 FSR (full sensitivity resolution) (@ 1222 *m/z*)
Isotopic pattern	The true isotopic pattern is maintained due to TIP™ technology (True Isotopic Pattern) and allows three dimensional chemical characterizations of analytes via SmartFormula3D algorithm using exact mass, TIP, and MS/MS fragment data.
SmartFormula3D	Enables unambiguous formula determination at "sub-ppm" confidence level up to 1000 Da.
Mass stability & dynamic range	hrXIC (high resolution Extracted Ion Chromatogram) technology with better than +/- 0.5-1.0 mDa stability on centroid data values over a typical LC peak.
Full scan sensitivity in MS	ESI: Reserpine 1 pg S/N>100:1 RMS With Ion-Booster (optional): Reserpine 100 fg S/N>100:1 RMS
Full scan sensitivity in MS/MS	The signal height obtained from a consumption of 2.5 fmol of Glu-Fibrino-peptide B will be better than 1000 counts on the most intense y' sequence ion from the MS/MS spectrum of the doubly charged precursor ion. This shall correspond to a signal to noise ratio better than 50:1. The MS/MS sensitivity specification is met while using quadrupole isolation of the precursor ion demonstrating that there is minimal transmission loss through the isolating quad. A solution of 100 fmol/µL Glu-Fibrinopeptide B shall be introduced at a flow rate of 3 µL/min.
Sensitivity TIMS – MS	ESI: Reserpine 50 fg/*µl* <15% RSD
TOF repetition rate	Up to 10 kHz
Temperature regulation	Yes
Temperature compensation	Yes
Digitizer	5 GSample/sec ADC with 50 Gbit/sec
Dynamic range	10-bit ADC for high quantitative dynamic range
Acquisition rate	>100 Hz in PASEF mode up to 50 Hz MS 50 Hz MS/MS (profile and peak detected spectra to disk)

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Patents Covering Unique Features

TIMS cell (BIL 031/08), Title: *Apparatus and method for parallel flow ion mobility spectrometry combined with mass spectrometry*.
Issued: US7838826B1; US 8288717B2

Temporal Zoom, IMEX (BDAL 293/11), Title: *Spectrum Acquisition Modes for Ion Mobility Spectrometers Using Trapped Ions*.
Issued: US8766176B2; GB2490387B
Pending: DE102012004398A1

Spatial Zoom (BRE 399/15, BRE 404/15), Title: *Spatial zoom mode for accumulative trapped ion mobility spectrometry*.
Issued: US9304106B1; US9546980B1
Pending: EP3054475A1; EP3165913A1; US20170125234A1; CN105869980A; CN107039231A

Parallel Accumulation TIMS (BRE 398/15), Title: *Trapping ion mobility spectrometer with parallel accumulation*.
Issued: US9683964B2
Pending: EP3054473A1; CN105869983A

Parallel Accumulation - Serial Fragmentation, PASEF (BRE 405/15), Title: "*Acquisition of fragment ion mass spectra of ions separated by their mobility*.
Pending: EP3165914A1; US20170122906A1; CN107037170A

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