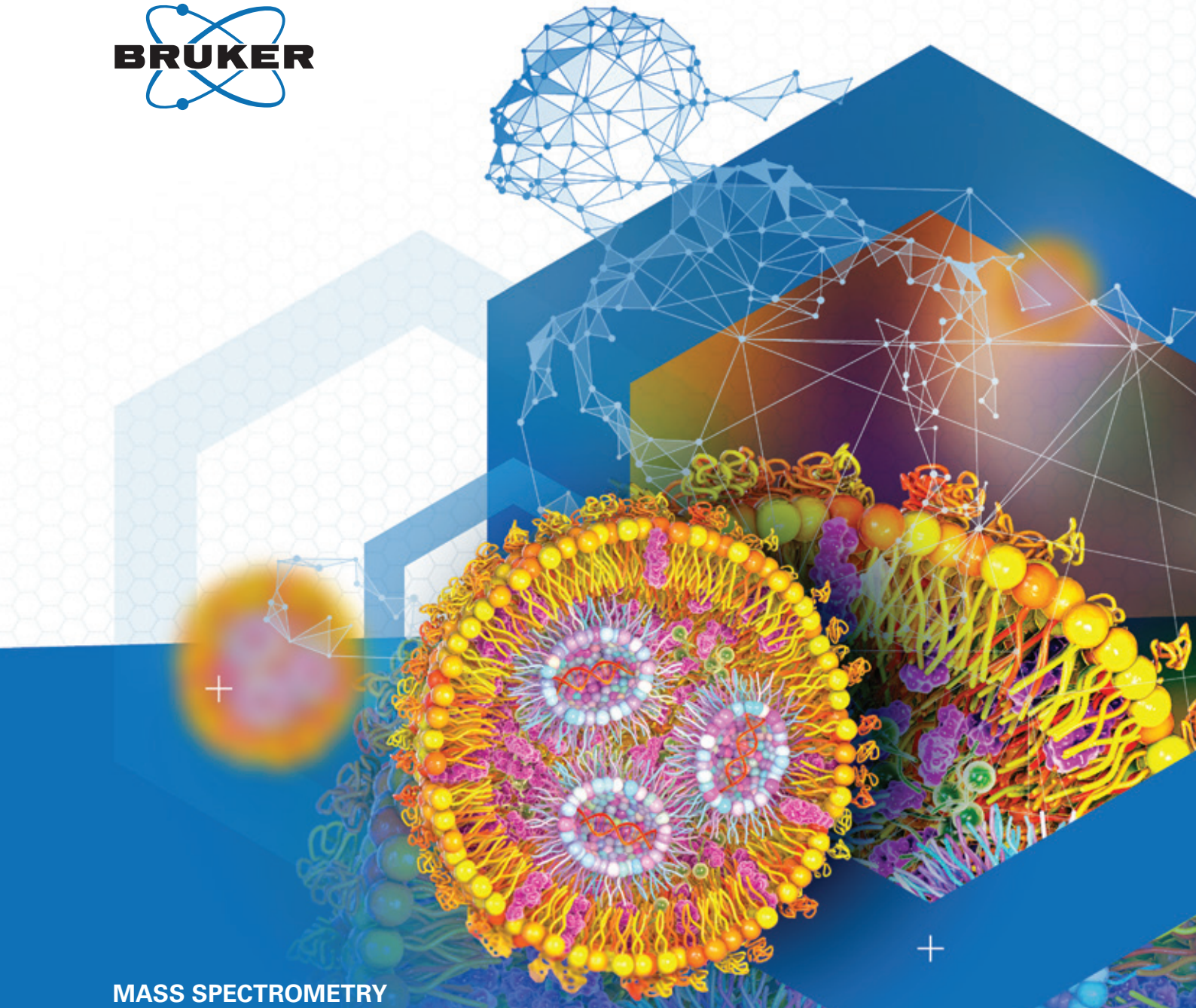
BRUKER



MASS SPECTROMETRY

4D-Lipidomics™ Powered by TIMS and PASEF®

Empowering researchers to decipher the complex language of lipids

Innovation with Integrity

Lipids: The unsung heroes of biological systems

Lipids are essential building blocks of cell membranes and play a critical role in signaling pathways. The field of lipidomics aims to develop a greater understanding of their complex biology and offers promise for breakthroughs in numerous application areas.

Transformative Bruker 4D-Lipidomics solution

Bruker's 4D-Lipidomics solution empowers researchers of all levels to explore the complex world of lipids with unprecedented ease. This revolutionary technique goes beyond traditional methods by incorporating trapped ion mobility spectrometry (TIMS) to enhance the selectivity and depth of lipidome analysis.

High capacity chromatographic separations are achieved using **UHPLC** and **Bruker Bio-LP columns.**

VIP-HESI enables deeper lipid profiling with **maximum sensitivity** and **robustness.**

timsTOF systems utilize TIMS and parallel accumulation serial fragmentation (PASEF) to enhance lipid separation, provide **CCS** measurement, and deliver MS/MS at **stunning speed.**

TASQ® RealTimeQC provides **in-batch monitoring of precision and other critical analytical parameters.**

MetaboScape® delivers **rigorous lipid annotations** with complete automation, leveraging TIMS-cleaned MS/MS data and lipid class-specific **CCS prediction** hyperplane models to ensure accuracy.

MetaboScape employs visualization of **Kendrick Mass Defect** (KMD), m/z, CSS and retention time for **verification of annotations, automated outlier detection,** and interactive exploration of the lipid-scape.

Hear from the expert

Michal Holčapek, Professor of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Czech Republic.

WATCH PODCAST

**The HPLC system shown is manufactured and supplied to Bruker Daltonik by Agilent Technologies*

Roadblocks in conventional lipidomics

Traditionally, unraveling the mysteries of lipids has been a laborious and intricate process. Conventional lipidomics techniques face several key hurdles:

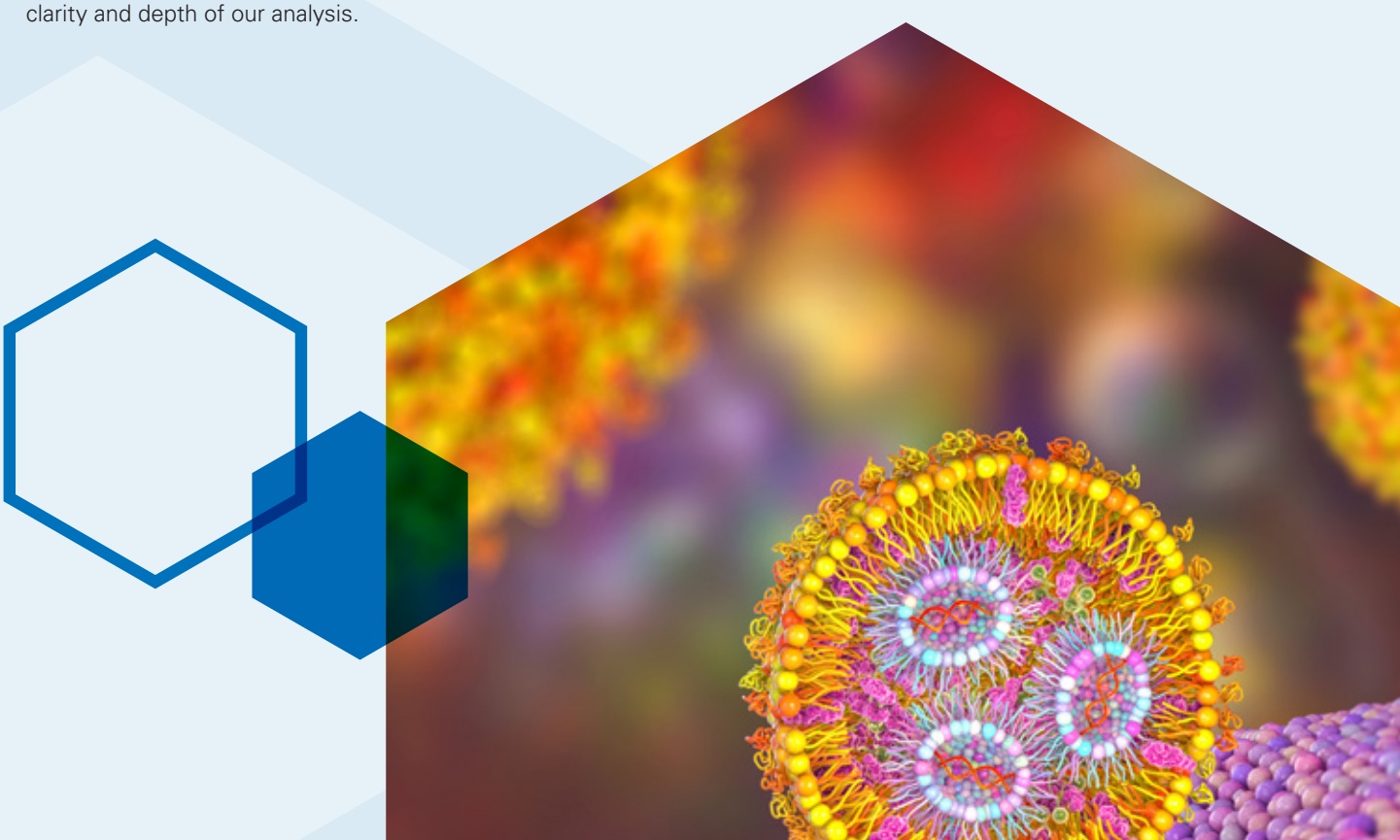
- **Limited sensitivity:** Current methods often miss the mark when it comes to detecting less abundant lipids. This restricted view can mask crucial biological processes, hindering our understanding of the complete lipid picture.
- **Diversity dilemma:** The sheer number and complexity of lipids present a formidable challenge. With numerous isomers and structural variations, confidently identifying specific lipid molecules becomes a complex task. This ambiguity can lead to misinterpretations of the data.
- **Need for speed:** Large cohort analyses require high sample throughput. Since annotation confidence in lipidomics depends on fragment information, fast MS/MS acquisition is essential, but not all instrument types can provide this.

Beyond the numbers: Challenges in quantitation and identification

The vast array of lipids within a sample further complicates analysis:

- **Precise quantitation:** Selectively and accurately measuring the abundance of each unique lipid remains a significant challenge. This precise quantification is crucial for understanding the relative importance and biological significance of different lipids within the system.
- **Annotation ambiguity:** The limited availability of reference material makes unambiguous molecular species identification even more challenging. Annotations, which assign identities to the lipids, rely on comparisons to known standards. A lack of these standards hinders definitive identification, limiting the clarity and depth of our analysis.
- **Inconsistent workflows:** Fragmented workflows relying on multiple instruments and software platforms are prone to errors and inconsistencies.

Further reading: Murphy RC. Challenges in Mass Spectrometry-based Lipidomics of Neutral Lipids. Trends AnalytChem. 2018 Oct;107:91-98. doi: 10.1016/j.trac.2018.07.023. Epub2018 Aug 4. PMID: 31031456; PMCID: PMC6483396.



The complete solution for 4D-Lipidomics:

Easy as one, two, three!

The Bruker 4D-Lipidomics solution offers a user-friendly, three-step approach for both untargeted lipid profiling and targeted lipid panel analysis with exceptional performance in both positive and negative ionization modes.

- **Untargeted lipid profiling using dda-PASEF:**
  - Simultaneous quantitation and characterization with high sensitivity and selectivity using dda-PASEF with up to 300 Hz acquisition speed.
  - Separation of isomeric and isobaric lipids using the power of TIMS for enhanced selectivity.
  - Additional annotation confidence with automatic CCS measurement enabling comparison to predicted or empirical reference data.
- **Targeted lipid panel analysis using prm-PASEF:**
  - Outstanding positive and negative mode performance for development of the most sensitive assays for ID and quantitation of lipid profiles.
  - Routine analysis of known lipid panels using automatically generated target lists from untargeted lipidomics experiments.
  - Selectivity and sensitivity of prm-PASEF provides highest MS/MS data quality even for low concentration lipids.

Selective measurement meets confident characterization

1. **Chromatography and ionization**

Agilent 1290 Infinity II UHPLC

Bruker Bio-LP Columns

VIP-HESI

2. **TIMS-enabled high resolution mass spectrometry**

PASEF

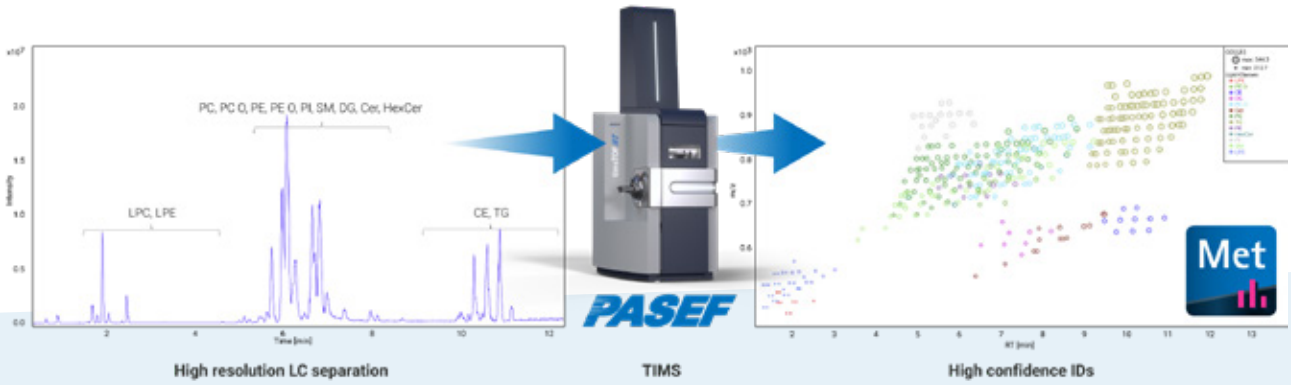
3. **Automated lipid annotation and data analysis**

ASQ Met

Unlock the secrets of lipidome with the Bruker Bio-LP column, which provides rapid, high-capacity separations of lipid [molecular] species in complex matrices. Paired with PASEF technology, even the smallest LC peaks unveil their secrets, offering enhanced sensitivity, robustness, and an additional dimension of separation for improved peak capacity. These advancements simplify data analysis by improving chromatographic peak shapes, resolutions, and retention times, ultimately boosting confidence in lipid annotations and quantification using the MetaboScape and TASQ software solutions.

1. Exploration with depth: High capacity separations with Bruker Bio-LP column chemistry

The bonded C8 phase facilitates excellent retention and separation of a broad range of lipid species found in biofluids and tissue extracts, while also enabling their clean elution from the column with a lower proportion of highly viscous solvent (versus the 90% Isopropanol formulation conventionally used for C18-based reversed-phase lipid separations). Together with a lower overall system pressure, these benefits yield higher peak capacity separations with greater reliability for precision lipid profiling.



Discover lipidome secrets with the Bruker Bio-LP column and PASEF technology, offering fast, high-capacity separations, high sensitivity, and simplified data analysis for confident lipid annotations using MetaboScape.

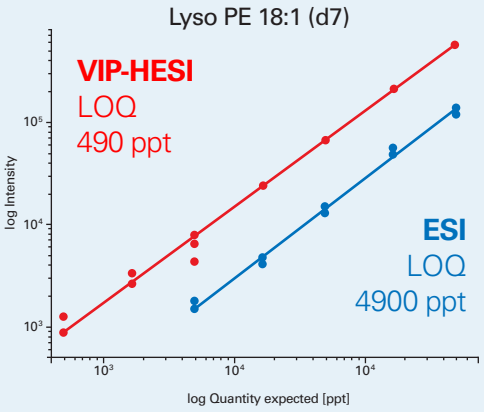
Enhanced ionization with VIP-HESI

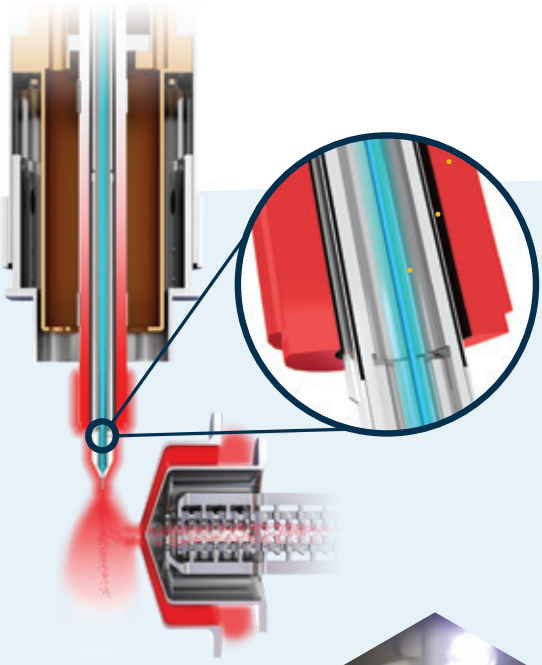
The VIP-HESI (Vacuum-Insulated Probe - Heated ESI) source delivers a revolutionary leap in sensitivity, empowering researchers interested in both broad lipid profiling and targeted analyses. These gains provide more structurally relevant fragments which gives higher accuracy in lipid annotations down to the species level. VIP-HESI captures those low abundant lipid species in negative mode for greater depth of coverage.

VIP-HESI is designed for…

…highest sensitivity

- **High sensitivity owing to brightest ion source**
- **Provides improved lipidome coverage**
- **VIP-HESI lowers LOQ 10x e.g for Lyso PE 18:1**



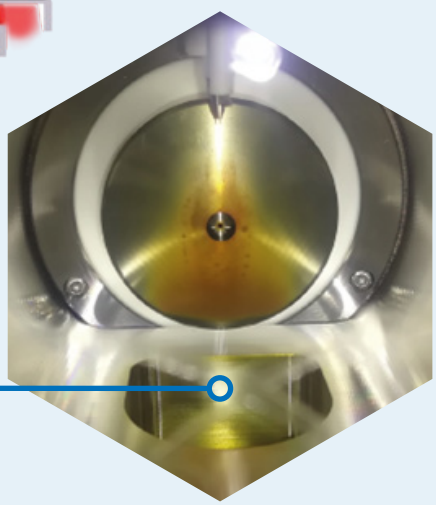


Read the Application Note to Learn More:

…and robustness

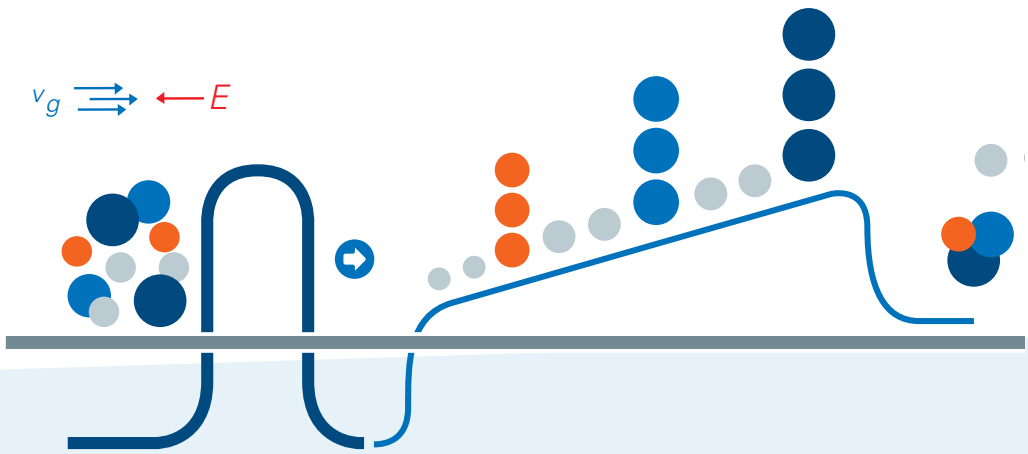
The **large exhaust outlet** captures and diverts the gas:

- Reduced matrix effects
- Less source contamination and reduced memory effect



2. The heart of 4D-Lipidomics: Bruker's TIMS technology

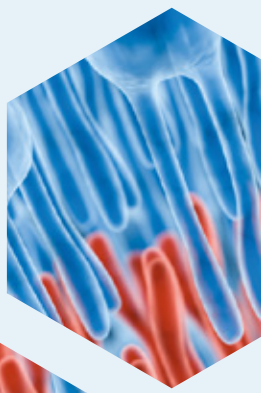
Bruker's timsTOF mass spectrometer takes lipidomics to a whole new level with its innovative TIMS technology. This revolutionary approach goes beyond traditional mass-to-charge ratio (m/z) analysis, adding a fourth dimension based on a molecule's size, shape, and charge, offering unparalleled insights into the intricate structure of lipids. This information complements the traditional m/z data, providing a more comprehensive picture of the lipidome represented in a biological sample.



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."

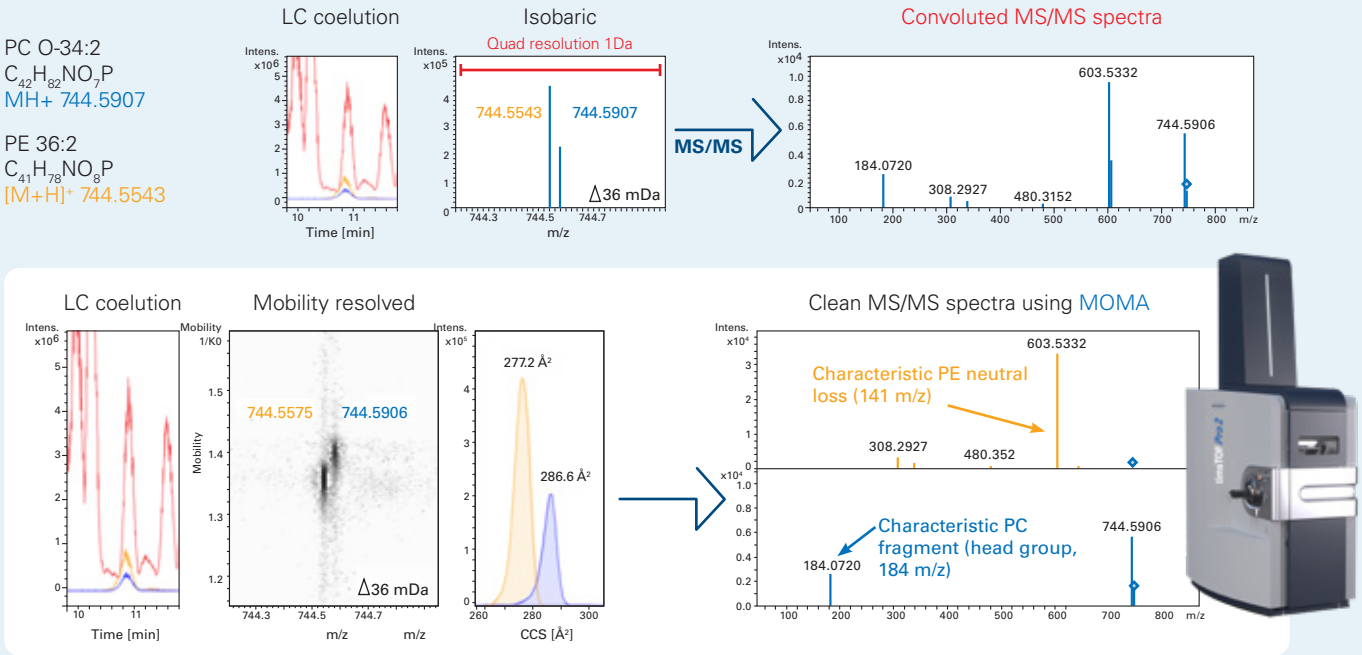


The power of TIMS in 4D-Lipidomics

TIMS goes beyond separation, offering several advantages that enhance your lipidomics workflow:

- **Enhanced sensitivity:** TIMS utilizes ion trapping to boost sensitivity, allowing detection of even low-abundance lipid species.
- **Superior selectivity:** Separate certain isomers and isobars (e.g., PC-O & PE) by Mobility Offset Mass Aligned (MOMA) data acquisition for more selective and accurate MS1-based lipid quantitation.
- **Simplified spectra:** TIMS reduces the complexity of LC-MS/MS spectra by minimizing "chimeric" spectra, leading to improved automated lipid annotation.
- **Speed:** With up to 300 Hz, PASEF acquires significantly more MS/MS data in a single run than traditional methods. This means deeper exploration of your lipidome, uncovering even low-abundance species.
- **CCS measurements:** TIMS provides collision cross section (CCS) measurements, a valuable tool for confident lipid identification and characterization.

MOMA for cleaner, unambiguous MS/MS

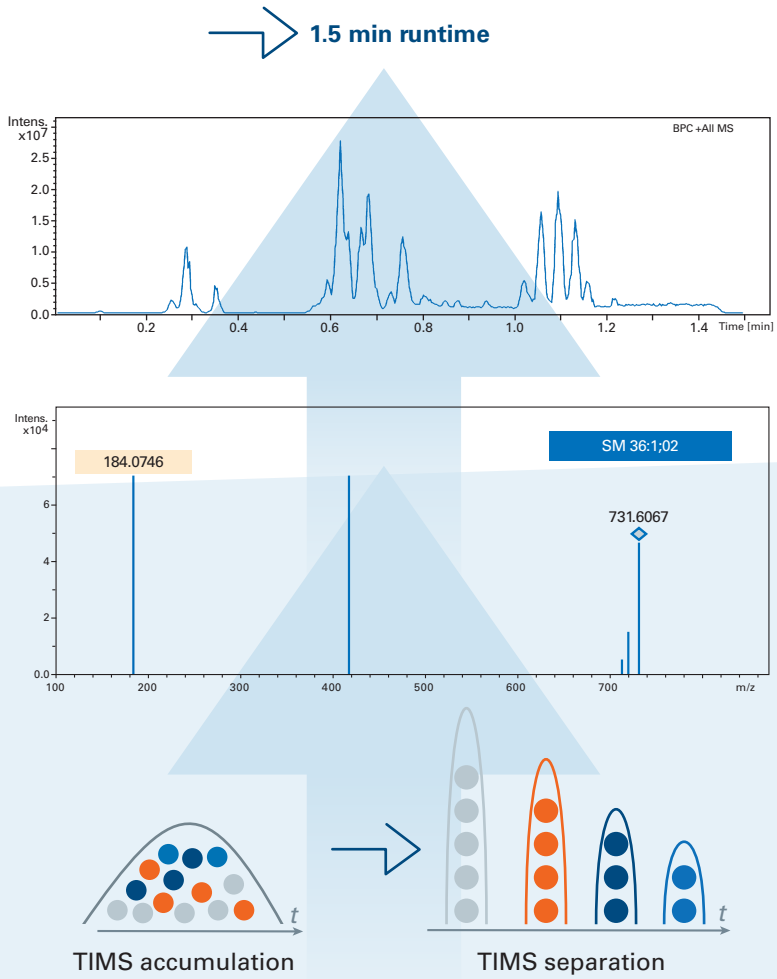


*Dual advantage of TIMS: **Selective MS1 quantitation:** TIMS separates challenging isomers and isobars, like PC-O and PE. This Mobility Offset Mass Aligned (MOMA) data acquisition of isobaric lipids allows for more selective and accurate quantitation directly from MS1 data, streamlining your workflow. **Enhanced automated annotation:** TIMS also reduces the complexity of LC-MS/MS spectra by minimizing "chimeric" spectra. These simplified spectra lead to improved performance for automated lipid annotation software.*

Enhance your discovery capabilities with improved lipidome coverage

PASEF acquires TIMS-cleaned ("MOMA") MS/MS and CCS measurement at **stunning speeds of up to 300 Hz**. Perform faster, more complete lipidome measurements:

- 300 Hz MS/MS permit **short gradients** making **high throughput** possible
- **PASEF** spectra acquired in **1 millisecond**
- **PASEF** data acquisition ensures selective measurement and **TIMS-cleaned, rapid MS/MS**



Eduardo Sommella, Ph.D.

Department of Pharmacy, University of Salerno, Fisciano, SA, Italy

Eduardo Sommella and co-workers conclude, "One of the main advantages of this method relies on hyphenation with trapped ion mobility. Operating in the PASEF mode adds an additional separation dimension, which, thanks to the speed and the mobility separation allows to obtain clean MS/MS spectra also for co-eluting lipids, which are separated based on the different CCS, increasing at the same time the annotation confidence."

Merciai, Fabrizio et al. "Development and application of a fast ultra-high performance liquid chromatography-trapped ion mobility mass spectrometry method for untargeted lipidomics." Journal of chromatography. A vol. 1673 (2022): 463124. doi:10.1016/j.chroma.2022.463124.

Laura Bindila, Ph.D.

Clinical Lipidomics Unit, Institute of Physiological Chemistry, University Medical Center, Mainz, Germany

Laura Bindila, Ph.D. and her team explain in their recent publication in Nature Communications:

"CCS values are leveraged for increased annotation confidence and throughput, where CCS values serve as an added data attribute for confidence score, to confirm lipid isomer annotations and/or reduce misannotations and hits."

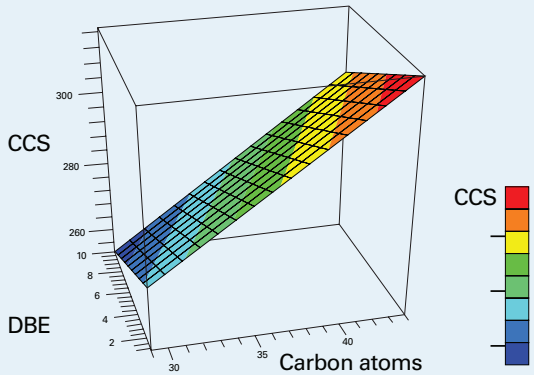
"With focus on the routine confident annotation in high-throughput profiling, where rather short chromatographic gradients are used, the added CCS data attribute was found useful for automatic and confident lipid annotation."

Lerner, Raissa et al. "Four-dimensional trapped ion mobility spectrometry lipidomics for high throughput clinical profiling of human blood samples." Nature communications vol. 14,1 937. 20 Feb. 2023, doi:10.1038/s41467-023-36520-1.

Observed CCS values

Predicted CCS values

Enhanced annotation confidence



Prof Zheng-Jiang Zhu

Shanghai Institute of Organic Chemistry Chinese Academy of Sciences, Shanghai

Prof Zheng-Jiang Zhu and his team conclude that, "altogether, TIMS technology significantly improved the separation of isomeric and isobaric lipids and increased the purity of isolated precursor ions, and as a result, achieved higher quality of MS/MS spectra for lipids."

Chen, Xi et al. "Trapped ion mobility spectrometry-mass spectrometry improves the coverage and accuracy of four-dimensional untargeted lipidomics." Analytica chimica acta vol. 1210 (2022): 339886. doi:10.1016/j.aca.2022.339886.

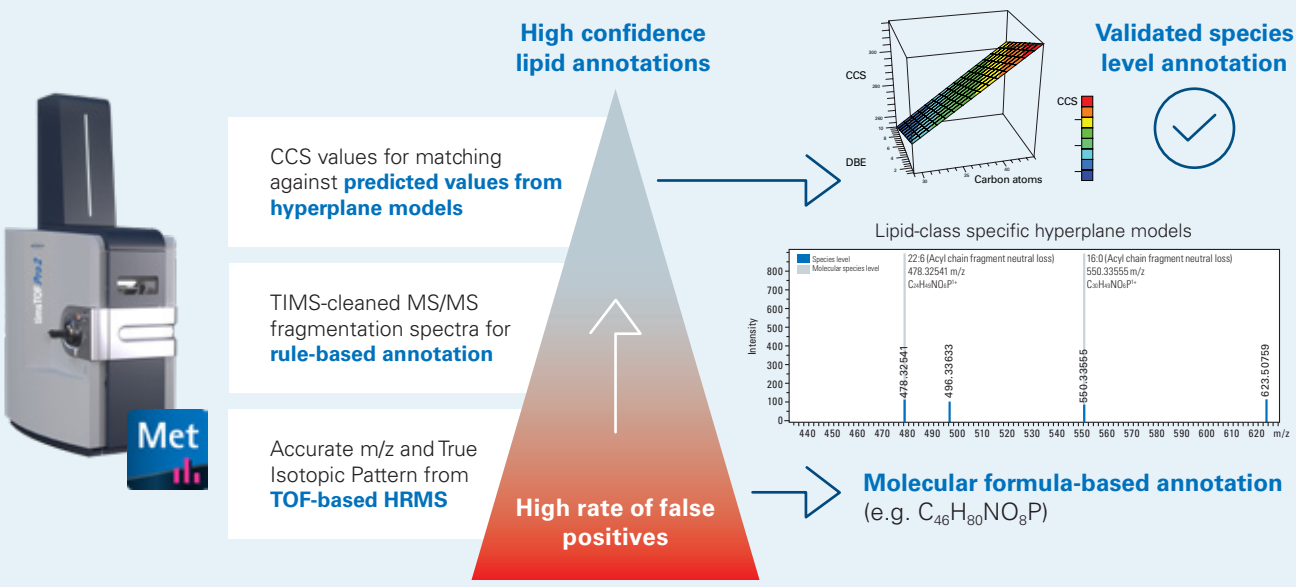
3. The key difference: High-confidence automated annotation in untargeted lipidomics

The Bruker 4D-Lipidomics solution tackles the challenge of high confidence annotation within one software workflow solution: MetaboScape®

MetaboScape is revolutionizing lipidomics by offering a more reliable, efficient, and objective multi-step approach to data analysis. By adopting this technology, you can streamline your workflow, gain deeper insights into your research, and achieve significant advancements in untargeted lipidomics. This includes tasks like:

- **Feature detection:** Identifying and classifying potential lipid signals in mass spectrometry data with automated retention time alignment, deisotoping and grouping of adducts and neutral losses.
- **Rule-based annotation:** Automatic annotation of TIMS-cleaned MS/MS fragmentation spectra using known lipid fragmentation rules prevents over-reporting of structural details when compared to fragmentation pattern (spectral library) matching.
- **Publish with confidence:** Lipid annotations reported in accordance with Lipidomics Standard Initiative (LSI) guidelines.
- **CCS-Predict Pro:** In the absence of reference standard data, predicted CCS values can be used to increase annotation confidence with machine learning CCS prediction hyperplane models.
- **Outlier detection:** Remove false positive annotations with automatic outlier detection in both the retention time and CCS dimensions.

High confidence automated annotation
A systematic process of eliminating false positives



MetaboScape software: From discovery to targeted lipidomics

Beyond broad lipid discovery, prm-PASEF offers targeted analysis with exceptional sensitivity and selectivity. By combining annotations from untargeted dda-PASEF data into automated targeted prm-PASEF methods, MetaboScape unlocks this powerful technique for a deeper understanding of your lipidome, providing researchers with a more focused analytical workflow aimed at larger sample cohorts.

Translate discovery results to targeted analyses with unmatched selectivity

Untargeted 4D-Lipidomics with rule-based lipid annotation

MetaboScape creates a TASQ Method with characteristic MS/MS fragments for selected lipids.

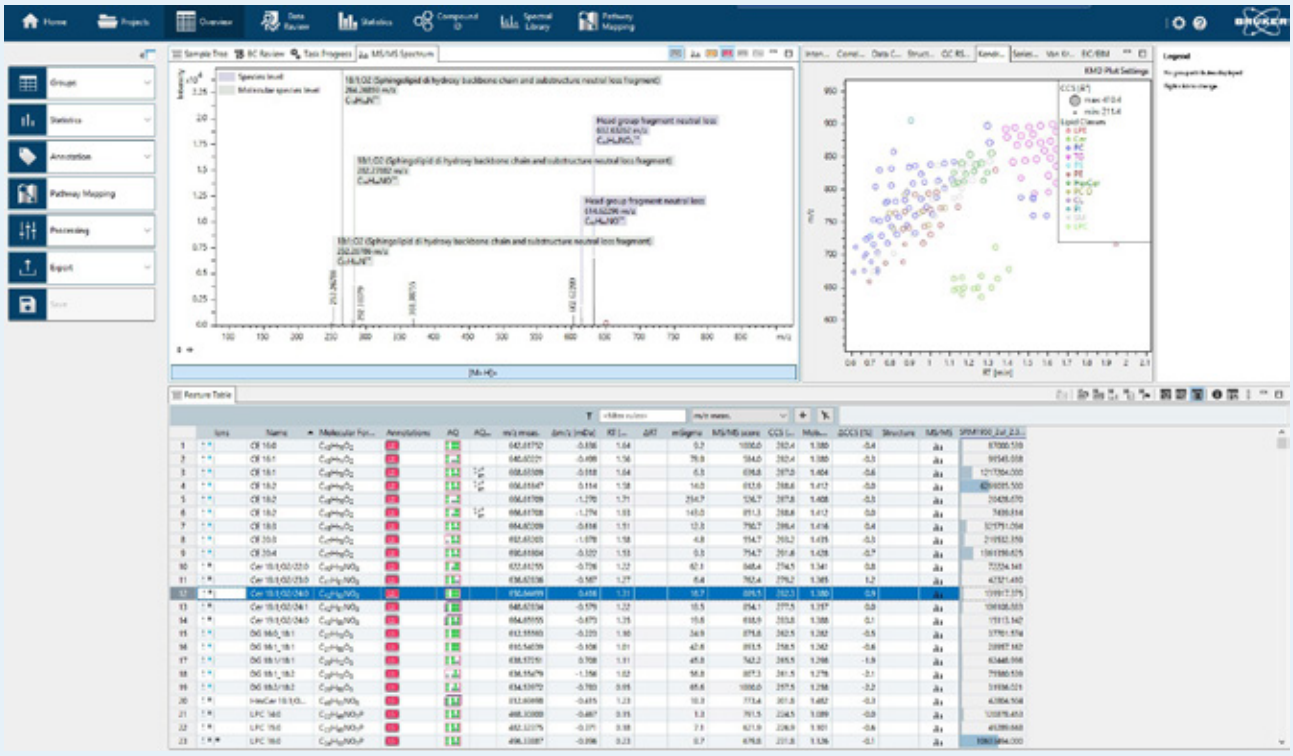
Method Management

Targeted analysis of **prm-PASEF** data

TASQ creates list of transitions to monitor in **prm-PASEF** run.

Import **prm-PASEF** data into TASQ.

prm-PASEF acquisition



Real-time monitoring with TASQ RealTimeQC

TASQ® RealTimeQC empowers you to take control of your experiments with a powerful suite of real-time data quality monitoring tools. This innovative software ensures the integrity of your data, saving you valuable time and precious samples.

Here's what TASQ RealTimeQC offers:

- **In-batch precision monitoring:** Keep a watchful eye on the consistency of your data throughout the experiment. RealTimeQC tracks the behavior of user-defined quality control (QC) analytes spiked into your samples. By monitoring these internal standards, you can identify potential issues like instrument drift or signal suppression early on, allowing for corrective actions before compromising your entire dataset.
- **Visualize multidimensional data on-the-fly:** Gain immediate insights into your data as it's acquired. RealTimeQC offers real-time visualization of key QC parameters. You can monitor trends, identify outliers, and assess the overall quality of your data acquisition process all within a user-friendly interface.
- **Automatic outlier detection:** Don't waste time sifting through mountains of data. RealTimeQC employs intelligent algorithms to automatically detect outliers in your QC analyte measurements. This allows you to focus on potential problems that require attention, saving you valuable analysis time.
- **Summary statistics at your fingertips:** RealTimeQC provides real-time access to essential summary statistics for your QC analytes. These statistics, such as average signal intensity and coefficient of variation (CV), offer a quick snapshot of data quality and flag potential inconsistencies that might warrant further investigation.
- **Informed real-time decisions:** With the wealth of information provided by RealTimeQC, you can make informed decisions on the fly. Identify potential issues early and take corrective actions to ensure the integrity of your data. This proactive action safeguards your precious samples and prevents wasted time on compromised experiments.

TASQ RealTimeQC: Your guide to high quality data acquisition

Prof. Thomas Moritz

Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Denmark

"Bruker's commitment to making data quality information immediately accessible is evident with TASQ RealTimeQC. Their innovative solution to quality monitoring provides assurance when it matters most and helps us make informed decisions during the analysis, before it's too late to act. With RealTimeQC, Bruker is uniquely and directly supporting lab-based metabolomics and lipidomics research."

Beyond the limits:
The future of lipidomics

Bruker is constantly pushing the boundaries of lipidomics research. Areas of advancement include:

- **timsTOF Ultra for single-cell lipidomics:** These advancements enable researchers to explore the diverse lipid landscapes within individual cells, opening new avenues for understanding cellular heterogeneity.
- **timsTOF fleX for SpatialOMx®:** Molecular changes originate at the cellular level and solution-based techniques lack sensitivity to capture changes produced from small cell populations. The SpatialOMx technology unlocks the potential of cell-based molecular discovery and enables probing lipid mechanisms and spatial distribution of lipids *in situ* using MALDI Imaging combined with a solution-based 4D-Lipidomics approach.

FURTHER READING
SpatialOMx lipid analysis of rat brain

READ TECHNOTE

Seul Kee Byeon, Ph.D.

Assistant Professor, Mayo Clinic, Rochester, MN, USA

"I have been able to detect more than 100 species of lipids from an isolated single cell in positive ion mode alone. My next steps include studying heterogeneity at the single cell level, which has not been possible before at this scale."

Watch the LabCast by Dr. Seul Kee Byeon, Mayo Clinic

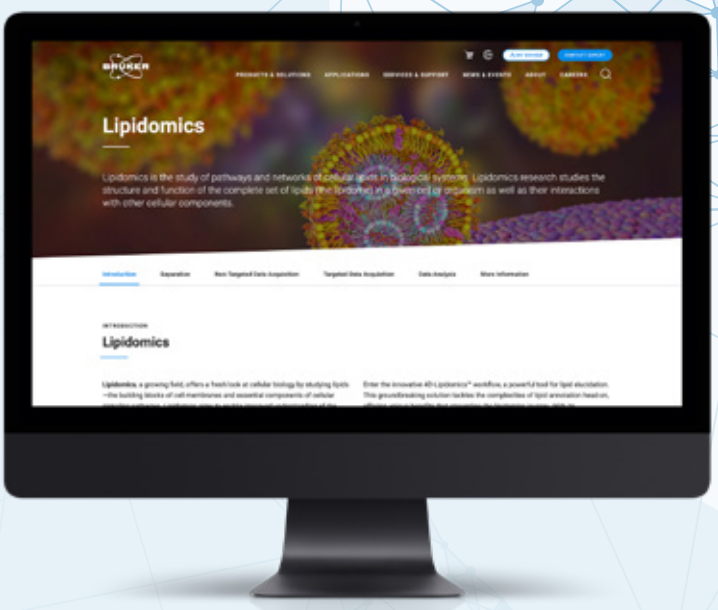
Conclusion: Empowering your lipidomics research

Bruker's 4D-Lipidomics solution featuring dda-PASEF for untargeted discovery and prm-PASEF for targeted data acquisition offers a powerful and versatile platform for unlocking the secrets of the lipidome. With its superior separation power, enhanced structural characterization, and robust quantitative analysis capabilities, 4D-Lipidomics empowers researchers of all experience levels to:

- **Achieve deeper insights into complex biological systems.**
- **Accelerate groundbreaking discoveries in lipidomics research.**
- **Push the boundaries of scientific understanding.**

Take the next step

Are you ready to unlock the full potential of lipidomics research? Contact Bruker today to learn more about our 4D-Lipidomics solutions and explore how they can empower your research.



For more information, visit:
bruker.com/lipidomics

4D-Lipidomics powered by TIMS and PASEF: Empowering researchers to decipher the language of lipids

Benefits	Features
Achieve deep coverage of the lipidome with high precision and selectivity	• Improved chromatographic peak capacity with Bruker Bio-LP column chemistry for faster high resolution separations. • Increased sensitivity and depth of lipid profiling with VIP-HESI. • TIMS-enhanced lipid separation for improved measurement selectivity. • Accurate CCS measurement for improved annotation confidence. • Fast, TIMS-cleaned MS/MS (up to 300 Hz) with dda-PASEF.
Increase efficiency and lower costs	• Unmatched instrument reliability maximizes up-time and minimizes operational and maintenance expense. • PASEF workflows enable high throughput data acquisition without compromise. • Seamless transfer of discovery results to PRM-based targeted analysis for validation and quantitation. • Downloadable acquisition methods to jump start your lipidome exploration.
Publish with confidence	• 4D-Lipidomics with TIMS and PASEF surpasses conventional workflows by combining multidimensional lipid separations with routine CCS measurement and ultra fast MS/MS. • Integrated QC tools for real-time monitoring of measurement accuracy and precision. • MetaboScape's automated pre-processing and annotation workflow leverages hard-coded expert knowledge. • MetaboScape provides stringent, rule based and CCS-enabled lipid annotations.

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

Online information:
bruker.com/
lipidomics

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