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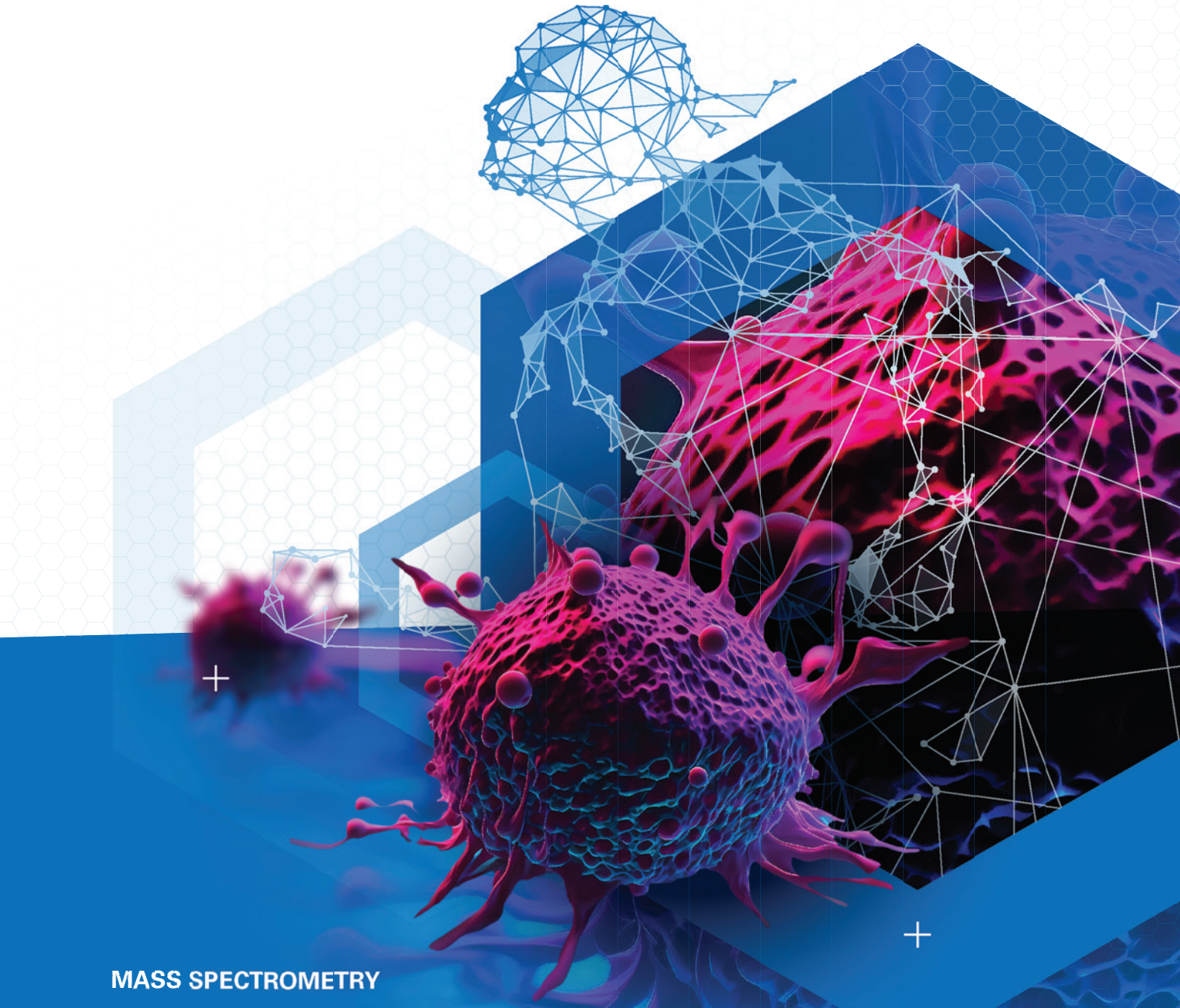

MASS SPECTROMETRY

Immunopeptidomics with TIMS and PASEF

Explore the immunopeptidome and discover novel neoantigens

Innovation with Integrity

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Low abundance targets? Unmask the immunopeptidome with timsTOF

Traditional methods stumble

- **Invisible targets:** Low-abundance MHC peptides hide, masked by overwhelming noise, hindering discovery
- **Quantity concerns:** Limited sample tissue? Existing methods struggle to see the full picture, missing crucial immune targets
- **Twin troubles:** Isobaric peptides? Traditional tools get confused by lookalikes. This is the reason we need improved selectivity and ion mobility, to discover hidden antigens
- **Singly charged species:** MHC class I immunopeptides typically consist of 8-14 amino acids. They frequently occur as singly charged species due to their nature
- **Complex data analysis:** Immunopeptidomics generates large amounts of complex data that are nontrivial to analyze and interpret. It is particularly challenging to address the largely increased search space when the source of peptides is unknown. This necessitates sophisticated bioinformatic tools, including de novo search algorithms, to identify MHC-bound peptides and potentially discover new immunotherapy targets

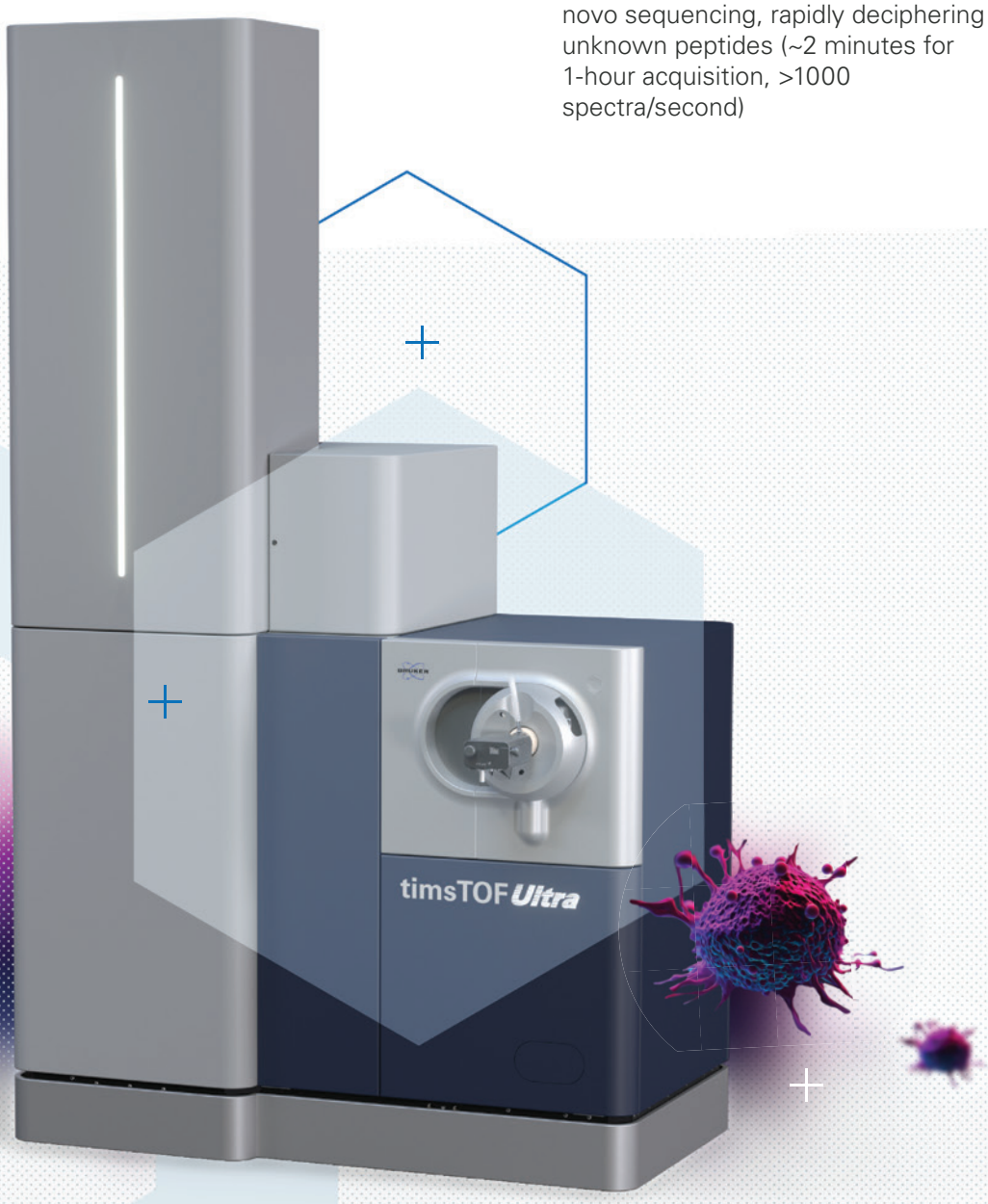
Hear from the expert

Nicola Ternette, Ph.D., Associate Professor, Antigen Discovery, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom

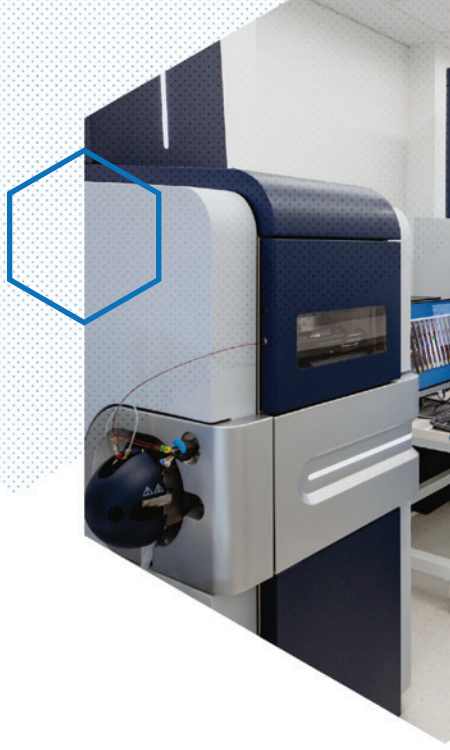
WATCH PODCAST

A paradigm shift in mass spectrometry

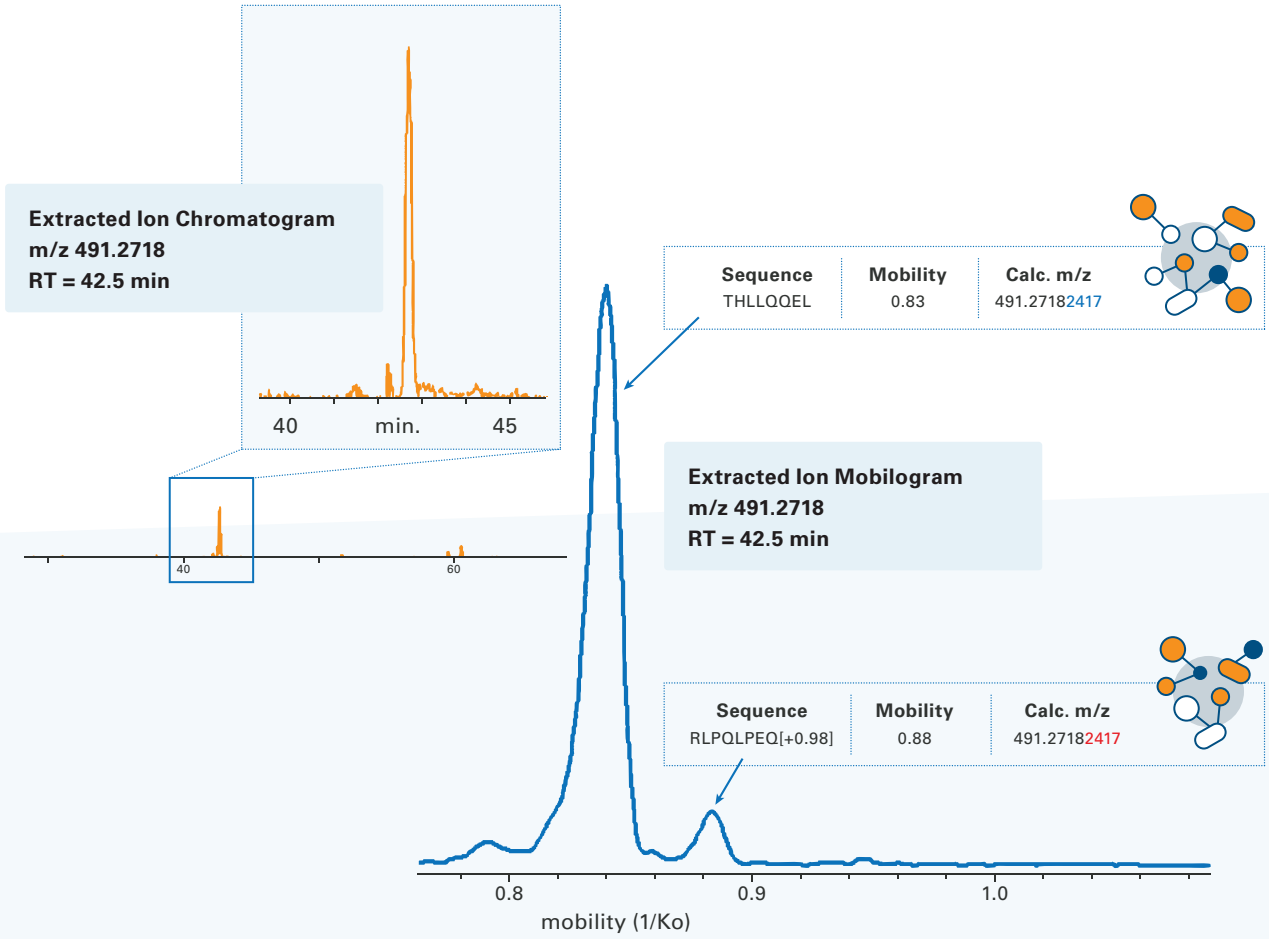
- **Exclusive sensitivity:** timsTOF's unique separation power reveals hidden low-abundance peptides
- **Less tissue, more data:** Even precious, scant samples unlock rich immunopeptidomic insights
- **Sharper focus:** Accurate targeting of MHC peptide properties makes sure no ions are missed
- **No more isobaric shadows:** TIMS combined with advanced data analysis unveil hidden gems, separating true hits from imposters
- **Precision single charge targeting:** Unique polygon filtering only includes singly charged peptides masses of interest, not unwanted noise
- **Bioinformatics powerhouse:** Bruker ProteoScape™ with its integrated BPS Novor module unleashes the power of advanced de novo sequencing, rapidly deciphering unknown peptides (~2 minutes for 1-hour acquisition, >1000 spectra/second)



MOMA: Address highly similar peptides in MHC analysis



- Separate and quantify isobaric immunopeptides with ultra-accuracy
- Boost sensitivity for reliable detection of near-identical peptides
- Unravel complex MHC patterns hidden by sequence similarities



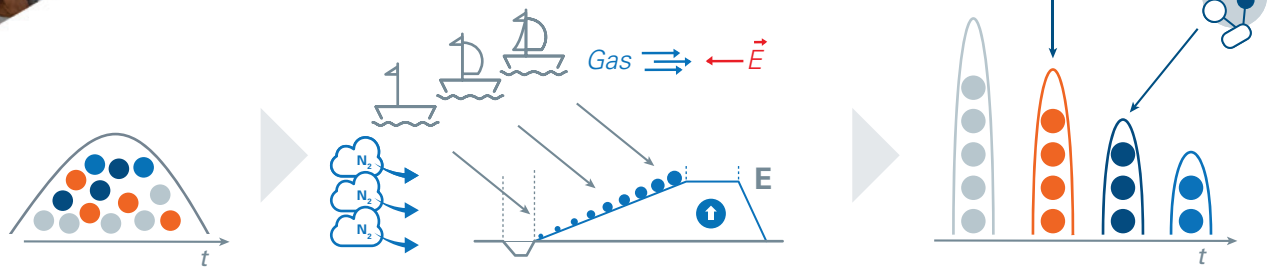
Did you know?

- ***MOMA means accuracy:*** *MOMA (Mobility Offset Mass Aligned) leverages the fourth dimension of separation to reduce chimeric spectra and isolates isobaric peptides, ensuring accurate peptide identification and quantification*

TIMS and PASEF

Ultra sensitivity for immunopeptides

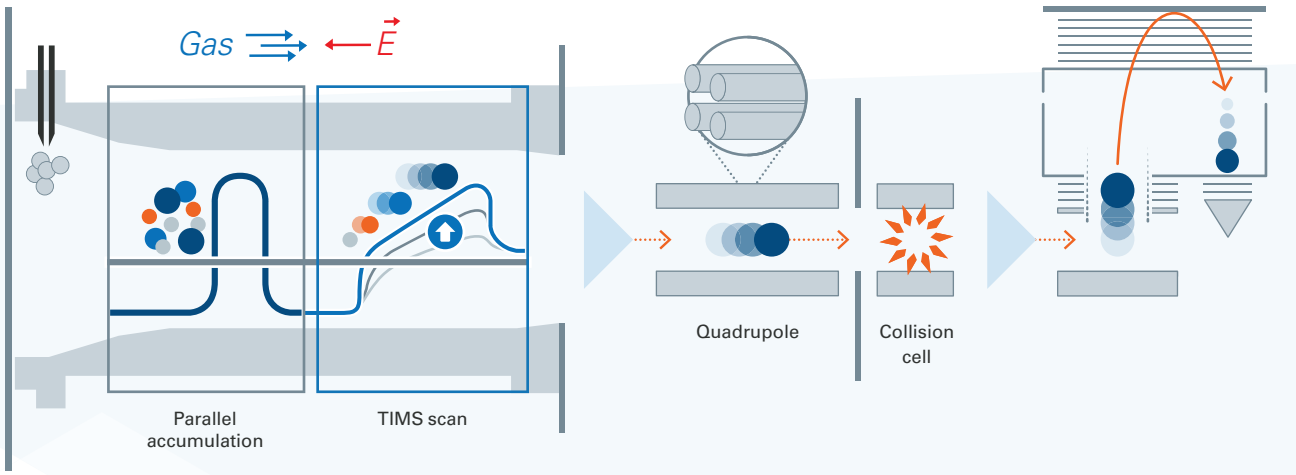
- **Unmatched sensitivity:** Space focusing on ions amplifies signal, revealing even the faintest MHC signatures
- **Precise precursor control:** Target short, singly charged MHC Class I peptides with pinpoint accuracy, no longer masked by larger molecules
- **Deeper MHC insights:** Uncover hidden ligands and subtle peptide differences, unlocking a wealth of immunological information



Time focusing of ions (accumulation)

Ion seperation based on mobility - collisional cross section (CCS)

Space focusing of ions with similar mobilities (CCS)



Ion accumulation and **serial elution** of seperated ion packages in parallel

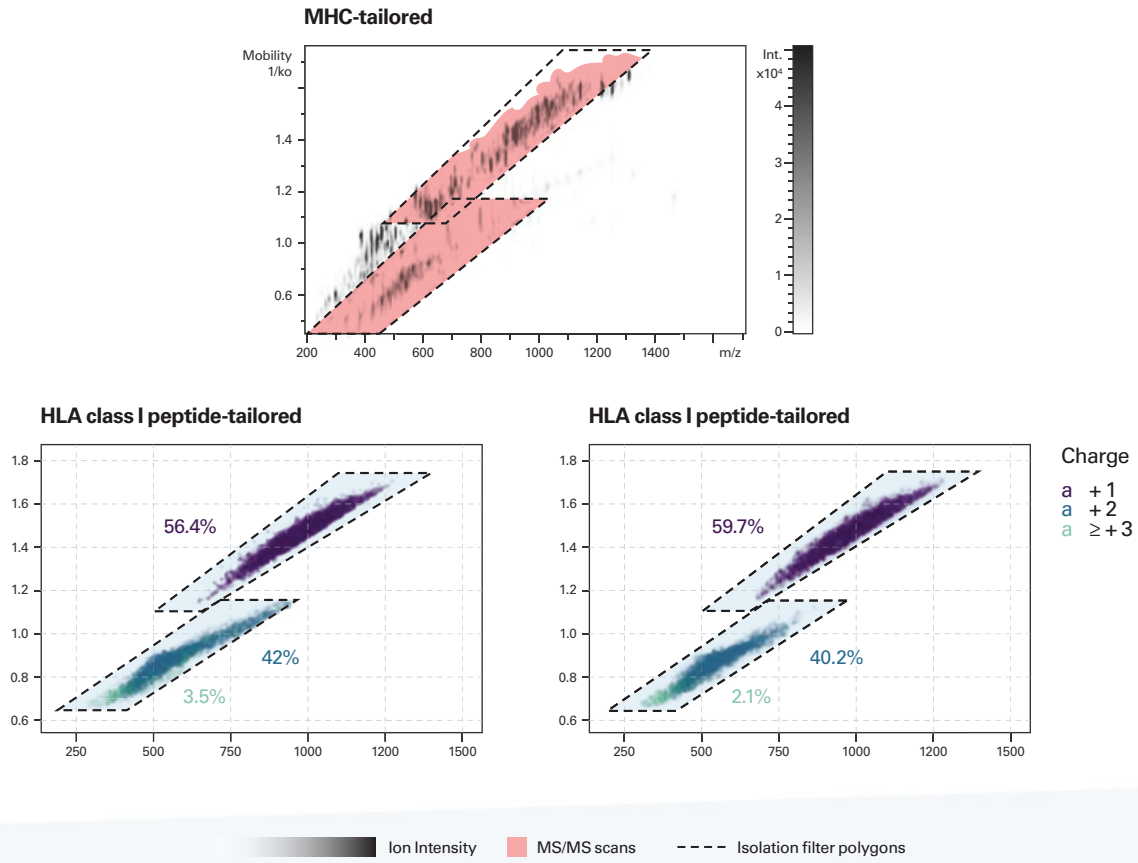
Fast switching, **synchronized** quadrupole

Time-of-flight (TOF) analyzer at **µsec speed**

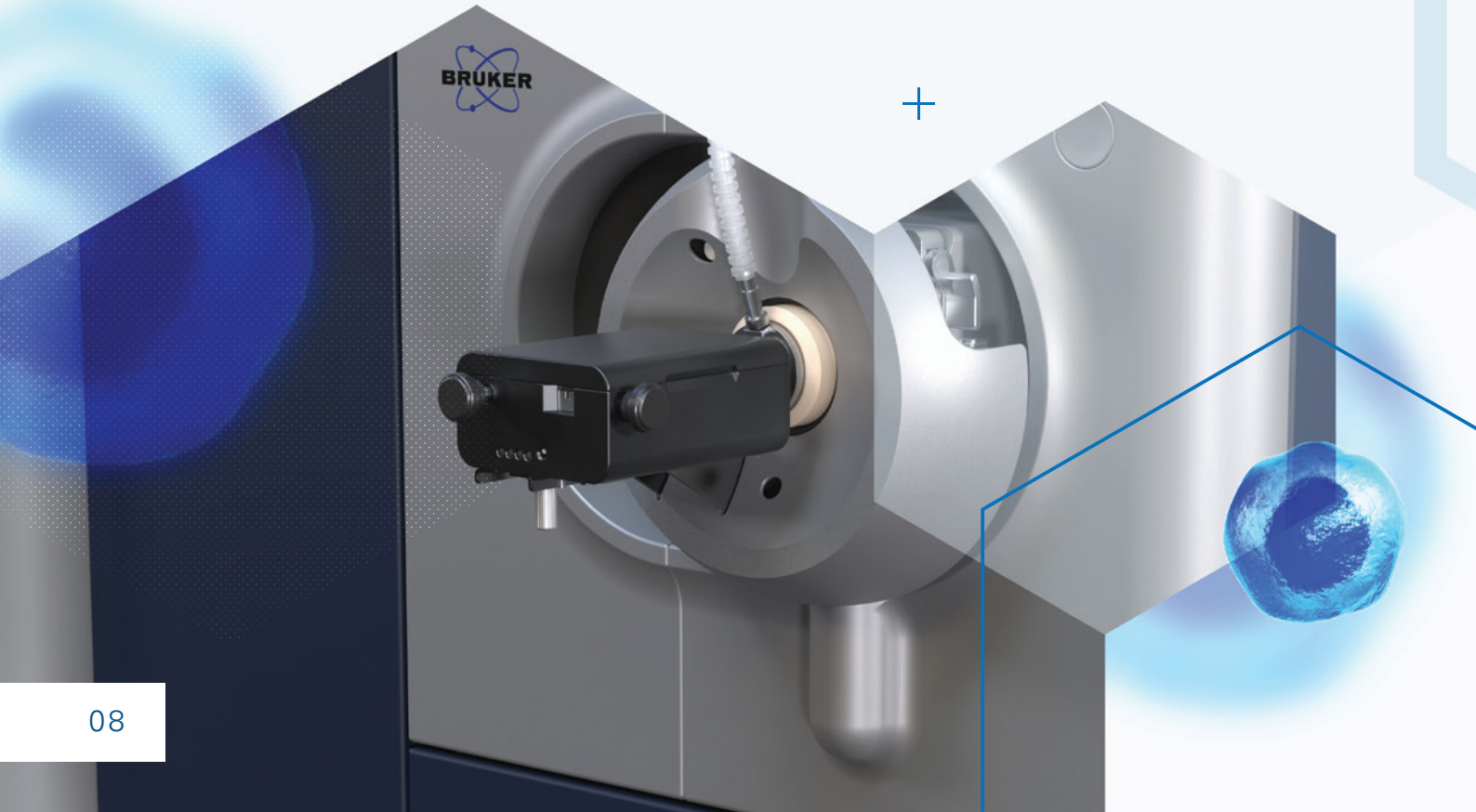
Did you know?

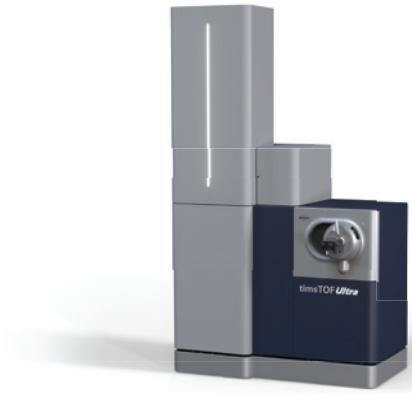
- ***CCS molecular fingerprints:*** *Measures a peptide's Collision Cross Section (CCS) with TIMS, like a molecular fingerprint, showing its size and shape. This can be used to gain insights into peptide conformation and aid peptide identification*

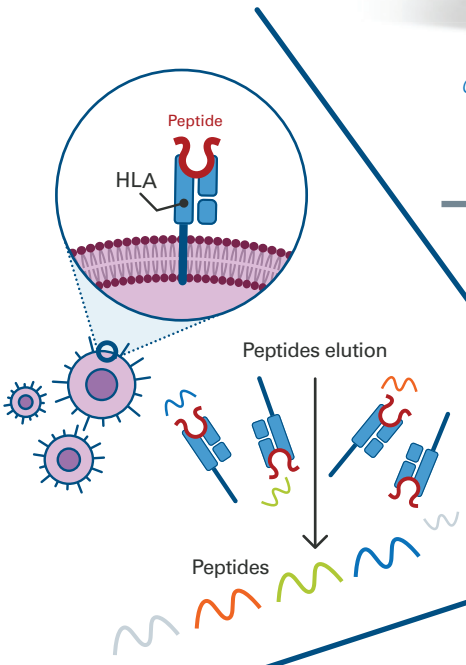
TIMS enabled polygon filtering scan

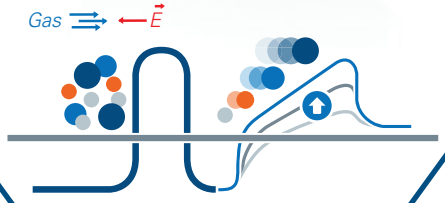


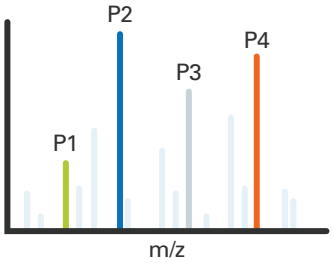
Immunopeptidomics polygon filtering: Exemplary heatmaps of ions of specific charge state distribution and masses are targeted.[1]



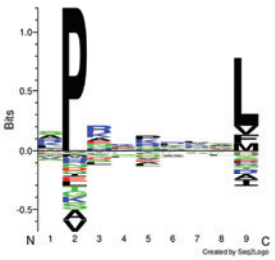








Data visualisation for further bioinformatics analysis



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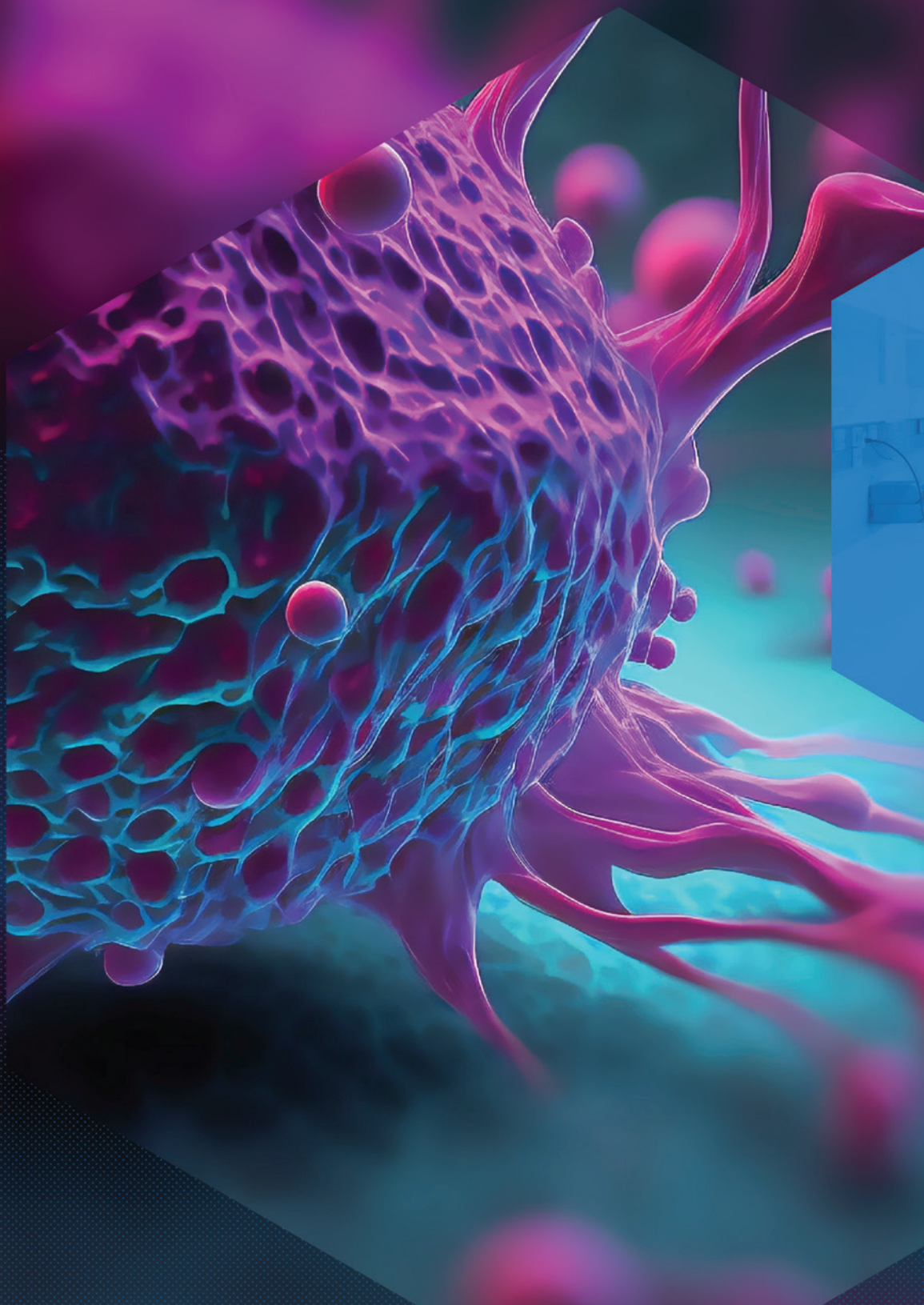
G P A L G R S F L

BRUKER ProteoScape™

rapid novor

“De novo searches have been a critical aspect in my labs research for many years...”



Prof. Anthony W Purcell, Head of Immunoproteomics Laboratory, Monash University

"...the ability to have an algorithm that delivers results with accuracy and precision at the speed of acquisition means we can assess immunopeptidomics samples at scale and in real time. This has major implications in how fast my group can translate research findings into actionable information, and it pushes this workflow even closer to delivering clinical impact of these analyses on an unprecedented timescale."

BRUKER

Innovation with Integrity

Characteristics of peptides presented by MHC class I or II

Characteristics	MHC class I	MHC class II
Location	Cell surface of nearly all nucleated cells including infected or abnormal cells (e.g. cancer cells)	Cell surface of antigen-presenting cells, such as dendritic cells and macrophages
Peptide source	Intracellular proteins processed within the cytosol by the proteasome	Extracellular proteins that are endocytosed by professional antigen-presenting cells and processed via the endosome and lysosome
Peptide length	8 to 14 amino acids	15 to 25 amino acids
Binding groove	Closed, more compact, typically more hydrophobic peptides	Open, more flexible
T-cell recognition	Cytotoxic T-cells, triggering the elimination of infected/abnormal cells	Helper T-cells, regulating the immune response and activating other immune cells
Immune responses	Eliminate infected or abnormal cells	Regulate other immune cells

Online information
bruker.com/immunopeptidomics


timsTOF Ultra

Advancing immunpeptidomics with quantitative mass spectrometry

A note from the Tenzer lab

At the Tenzer lab, we focus on the development, optimization and application of mass spectrometry (MS)-based workflows for quantitative immunoproteomics to facilitate a detailed understanding of the interplay between tumor antigenicity, tumor microenvironment and the anti-tumor immune response. Currently, the research group harbours several high-resolution mass spectrometry instrument platforms, including two timsTOF Pro 2, one timsTOF HT and one timsTOF SCP (Bruker) platforms. With these high-performance instruments, the Tenzer research group investigates the contribution of proteases involved in MHC-I-restricted antigen processing and develops high sensitivity methods and workflows for the in-depth characterization of the immunopeptidome.

The High Throughput Immunopeptidomics Platform (HTIP) aims to identify cancer antigens triggering the immune response, as potential targets for immunotherapy. Most immunopeptidomics workflows are not adapted to the throughput and response times required for clinical studies or patient monitoring. To meet these needs, we are developing high-throughput immunopeptidomics workflows by optimizing parallelized sample preparation, high-sensitivity LC-MS methods, and streamlined data analysis pipelines.

Dr. David Gomez-Zepeda, Head of the High Throughput Immunopeptidomics Platform (HTIP), Helmholtz Institute for Translational Oncology (HI-TRON), Mainz, Germany

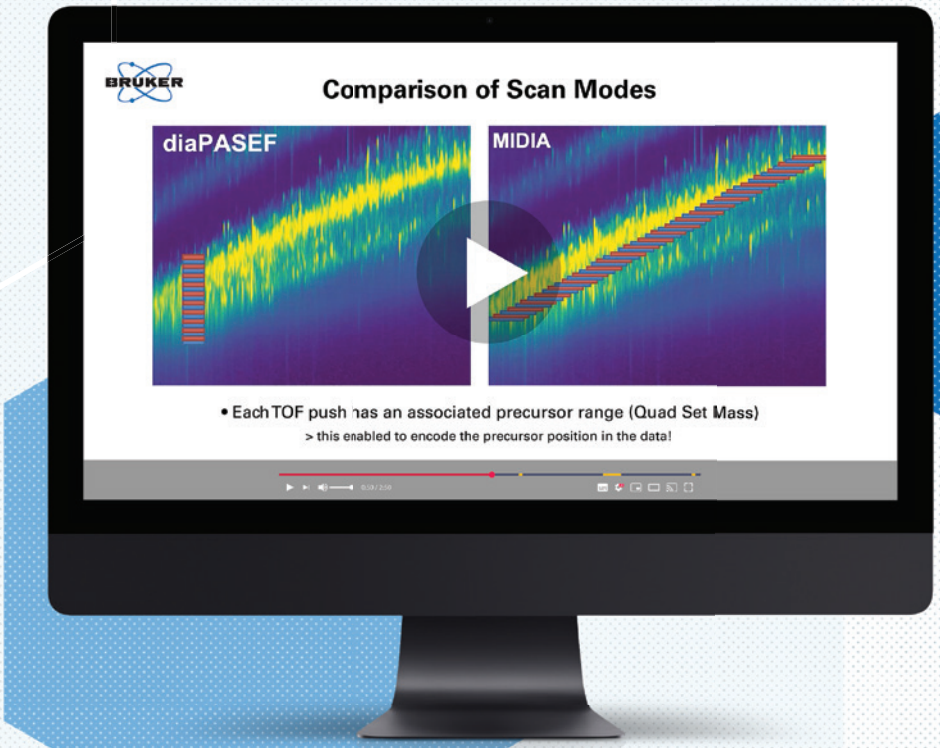
"Unlocking the potential of therapeutic antigens demands highly optimized immunopeptidomics workflows capable of analyzing even the smallest samples with precision and speed. In response to this challenge, we have recently established the HTIP. Implementing high sensitivity methods, such as Thunder-DDA-PASEF, on our Evosep One coupled to timsTOF Ultra system will provide the optimal sensitivity and sample turnaround required to discover therapeutic antigens."

Prof. Dr. Stefan Tenzer, Immunoproteomics Unit,
Helmholtz Institute for Translational Oncology
(HI-TRON), Mainz, Germany

"The identification of tumor-specific neoantigens is a highly promising strategy to develop and improve personalized cancer immunotherapies leveraging T-cell mediated recognition and elimination of tumor cells. T-cells differentiate between self and non-self-cells by specifically recognizing peptides bound to the major histocompatibility complex (MHC), which define the MHC ligandome. High sensitivity mass spectrometry-based immunopeptidomics enables the detailed characterization of the MHC ligandome, including unique tumor antigens. This information enables the design of personalized immunotherapies tailored to specifically target the patient's cancer cells, thus potentially enhancing treatment efficacy and reducing adverse effects."

Discover the power of midia-PASEF

WATCH WEBINAR



Dissecting the powerhouse: PASEF and TIMS

PASEF® a key technology in timsTOF instruments, uses Trapped Ion Mobility Spectrometry (TIMS) to boost sensitivity and selectivity. Unlike conventional methods that struggle with weak signals, PASEF amplifies ions for clearer identification. Additionally, TIMS separates peptides based on shape and charge, adding another layer of separation beyond mass-to-charge ratio. This combined approach addresses two major challenges in immunopeptidomics.

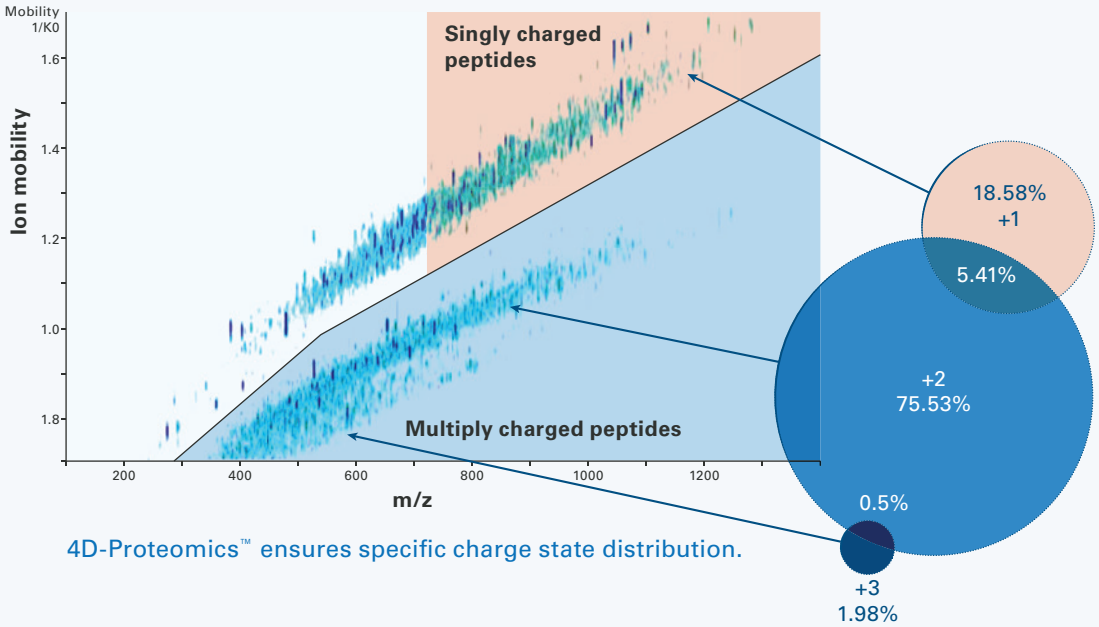
- **Isomeric ambiguity:** TIMS-based CCS differentiation resolves isobaric and near-isobaric peptides, eliminating spectral overlaps and ensuring confident identification of closely related molecules
- **Low-abundance elusiveness:** PASEF's accumulation capability empowers the detection of previously invisible low-abundance MHC peptides, opening a window into unexplored immunological territories

Polygon filter: Customizing the searchlight

Unlike a one-size-fits-all approach, timsTOF's polygon filter empowers researchers to tailor their investigation. This innovative feature allows custom selection of precursor m/z and CCS ranges, focusing MS/MS acquisition on ions of interest. This individualized approach offers several advantages:

- **Enhanced sensitivity for singly charged MHCs:** Focusing on +1 charge state expands the detectable repertoire of MHC peptides, without having to include low m/z +1 impurities
- **Reduced spectral complexity:** By excluding irrelevant precursors, the polygon filter minimizes background noise, leading to cleaner spectra and more accurate identifications
- **Confident quantification with MOMA:** With MOMA (Mobility Offset Mass Aligned), the polygon filter enables precise quantitation even for highly similar peptides. Utilizing the additional dimension of CCS, MOMA eliminates residual ambiguity, ensuring reliable quantification of subtle immunological variations

~20% or more of neoantigens are only found as +1 precursors.[3]



4D-Proteomics™ ensures specific charge state distribution.

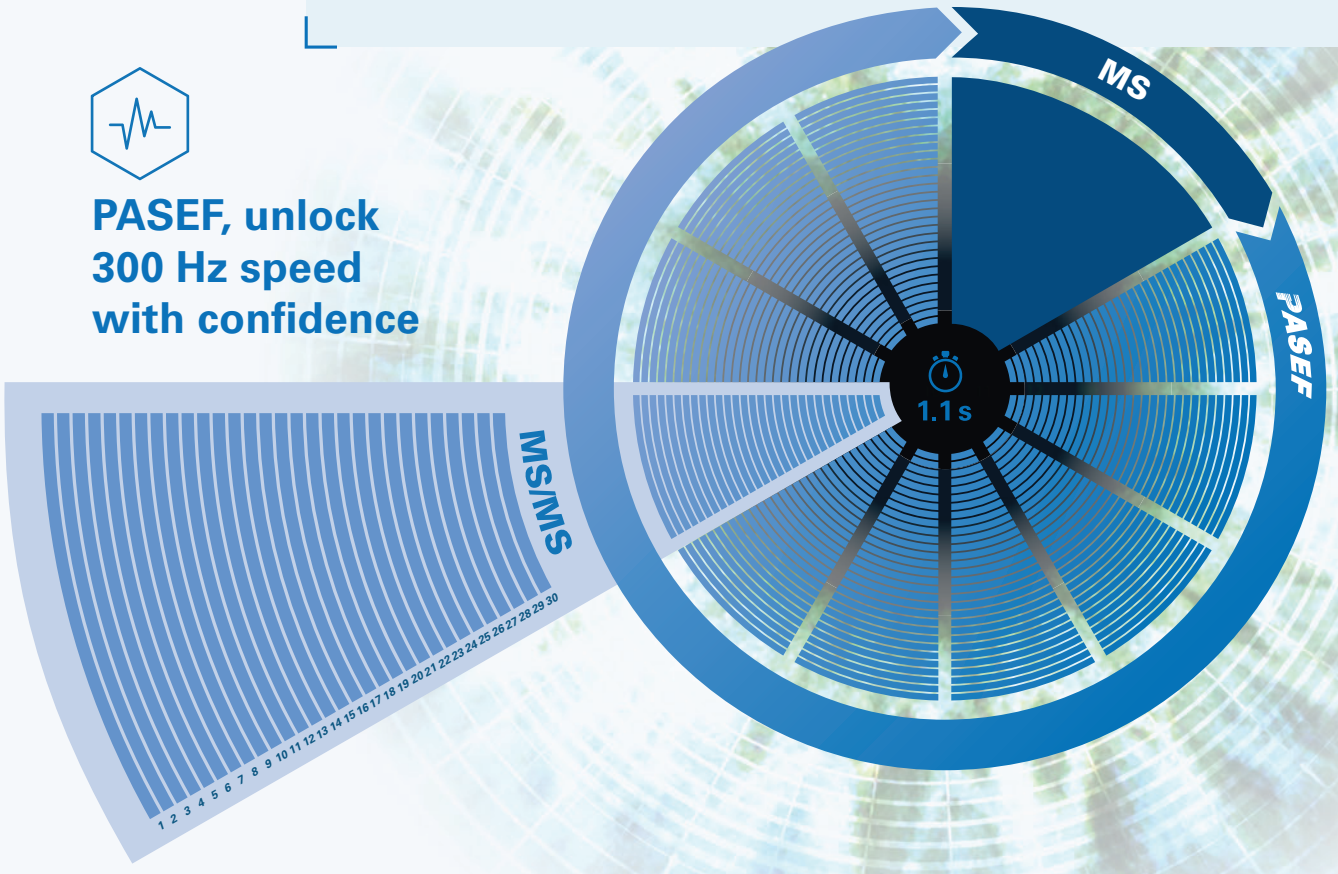
Benefit from the unique polygon filtering and PASEF

- **Improved selectivity:** Custom polygon filters efficiently trap and fragment key ions of interest, included often ignored +1 peptides. Reduce false positive identifications that enhances the accuracy and reliability of immunopeptidomics data.
- **Increased sensitivity:** PASEF increases the number of ions available for fragmentation. This enhancement in sensitivity is particularly advantageous in immunopeptidomics, where MHC-bound peptides are often present in low quantities.
- **Faster data acquisition:** PASEF's parallel accumulation capabilities allow for faster data acquisition, up to 300 Hz, enabling high-throughput analysis of immunopeptidomes. Speed is crucial when dealing with complex samples or for high throughput analysis.
- **Deeper analysis:** PASEF is used to perform in-depth analysis of immunopeptidomes, ideally identifying all peptides that are present in a sample. In immunepeptidomics, finding "the one" is key.

Did you know?

- ***Target what matters:*** *Forget generic peptide searches! timsTOF's polygon filter allows you to hone in on relevant precursor ions, maximizing your capture of MHC class I peptide ions, even those that don't play by the usual rules*

PASEF, unlock 300 Hz speed with confidence



See beyond what's visible. Discover PASEF sensitivity and speed

Dr. Torsten Müller, Business Development Manager Proteomics

"Joining Bruker nearly two years ago, I continue to be amazed by the impact of PASEF in proteomics. The company's commitment to innovation and active engagement with the scientific community make every day an exciting journey of discovery and advancement."

Did you know?

- ***Speed up discovery:*** *Blast through research bottlenecks with timsTOF's PASEF technology, acquiring data at a blazing 300 Hz. Get deeper analysis, faster*

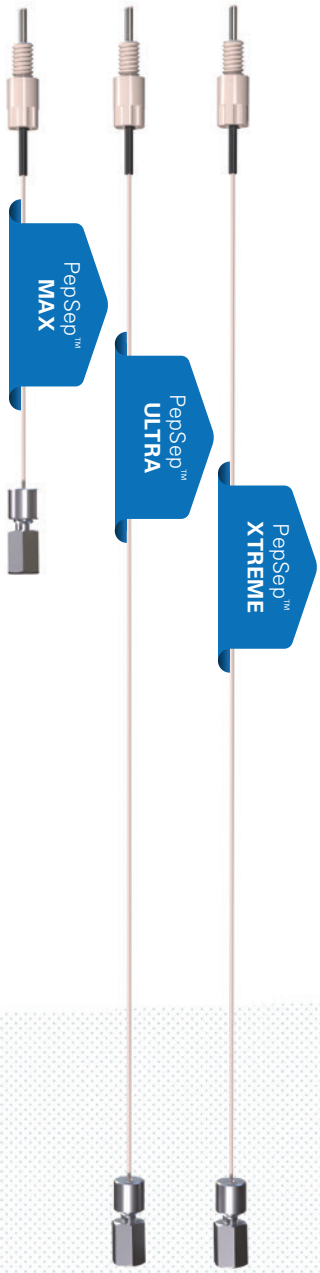
Driving separation with PepSep HPLC columns

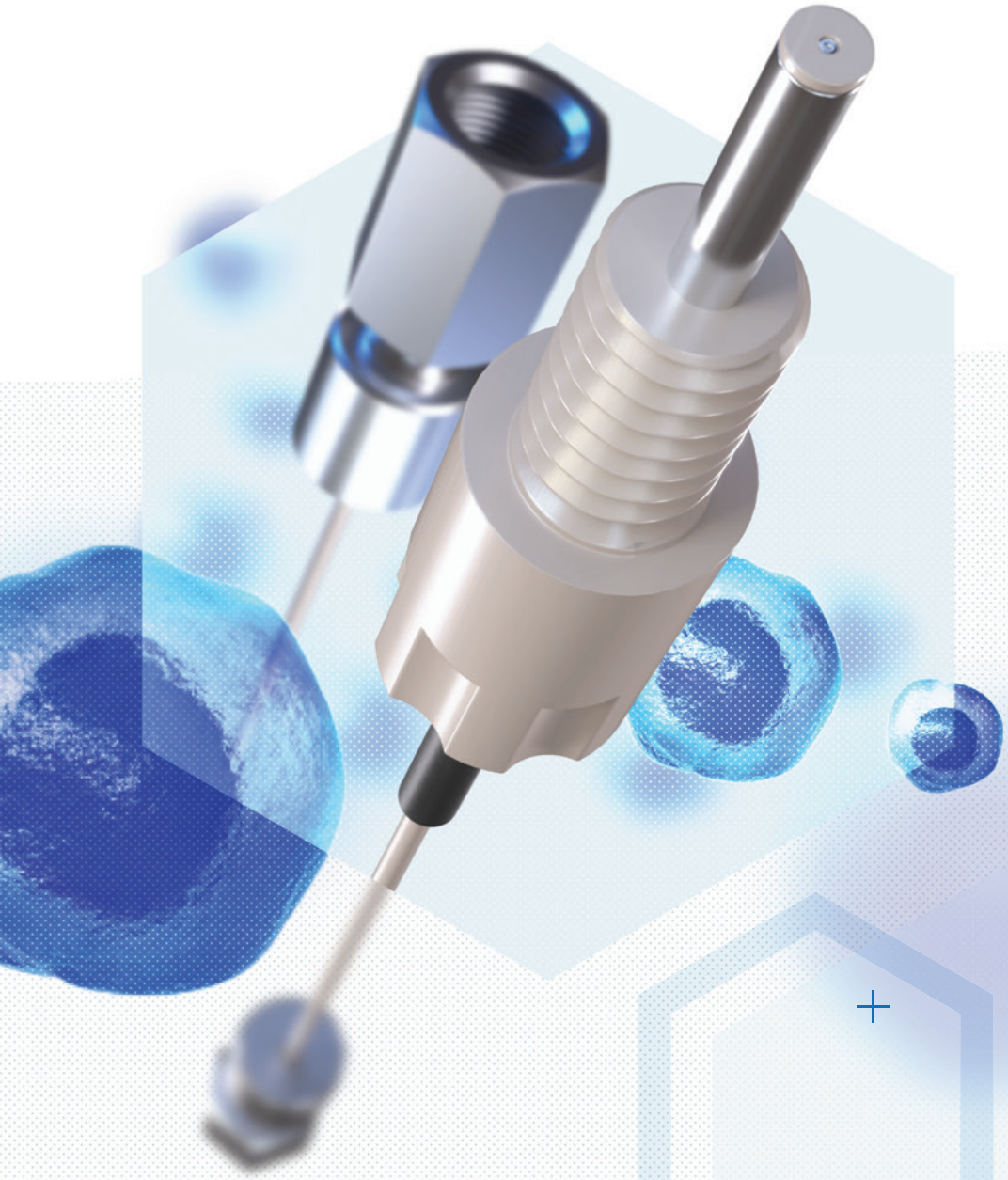
Go deeper into the immune landscape with unparalleled sensitivity. Unlock hidden targets: Detect up to 2.5x more MHC peptides, even with limited samples.

Made for immunopeptidomics: PepSep™ ULTRA columns make ultra sensitivity in immunopeptidomics more attainable, aiding in breakthroughs in immunotherapy development, biomarker discovery, and personalized medicine research with high robustness and confidence.

Get to know PepSep

LEARN MORE



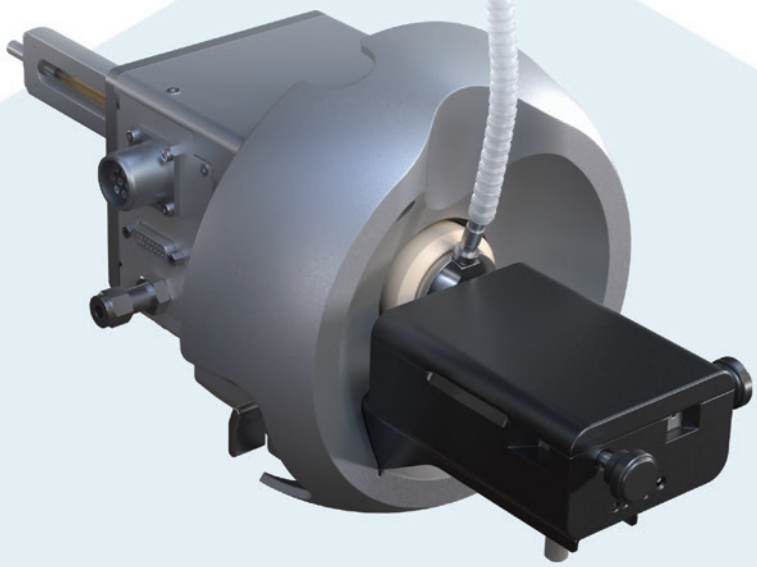


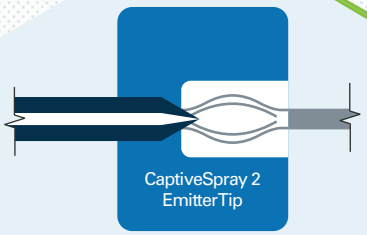
Nano-flow sensitivity made easy and robust

CSI Ultra is a next-generation ion source designed for the timsTOF Ultra, providing great sensitivity for challenging immunopeptidomics applications. CSI 2 is a simple, robust, and easy-to-use ion source designed for all other generations of timsTOF systems. Both ion sources feature cutting-edge vortex-generating airflow technology that ensures optimal performance.

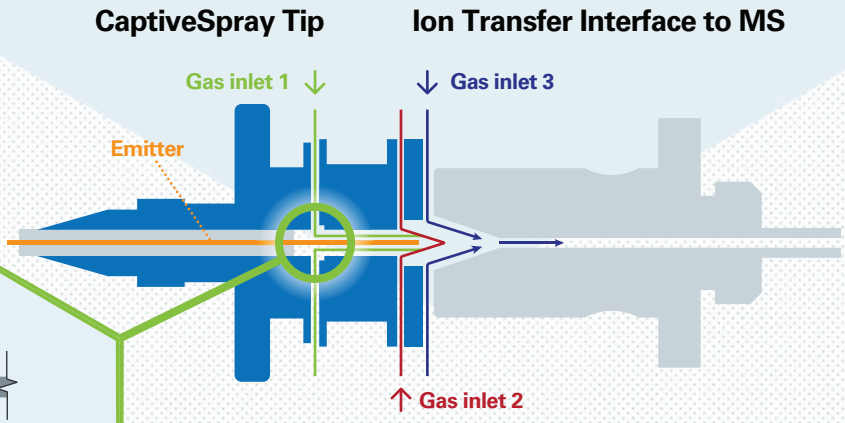
Discover next-gen ion sources

LEARN MORE





CaptiveSpray
Vortex around spray concentrates and focuses spray into the MS source



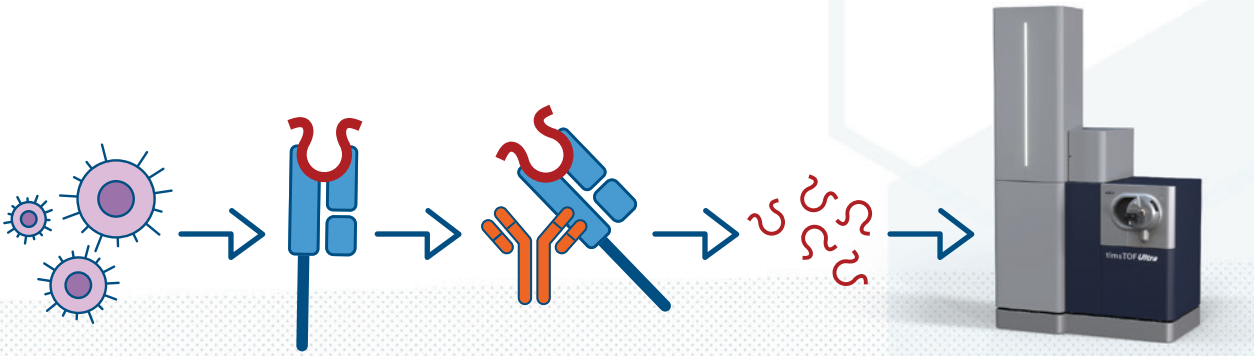
Effortless precision and optimal sensitivity: Experience zero-adjustment positioning for a stable spray, enhanced by vortex-driven nebulizing gas flow, ensuring consistent performance across a wide range of nanoflow rates.

Alithea Bio's breakthrough with Bruker timsTOF Ultra

Alithea's vast experience in immunopeptidomics has fueled an array of specialized technologies and HLA-Compass, Alithea's extensive quantitative atlas of HLA peptide presentation in health and disease. Alithea partners with global BioPharma to:

- **Accelerate timelines:** HLA-Compass and in-house HLA-typed biorepository samples fuel drug development timelines
- **Novel targets:** HLA-Compass data in conjunction with HLA binding prediction, allele clustering and spectral verification pinpoint the selection of ideal targets for HLA peptide-targeted therapeutics
- **Safety assured:** HLA-Compass guides risk assessment for potential on- and off-target toxicity for HLA peptide-targeted therapeutics
- **Neoantigen detection:** Uncover novel neoepitopes with the highly sensitive NeoZoom platform and proteogenomics

Navigate with confidence: HLA-Compass powered by timsTOF Ultra



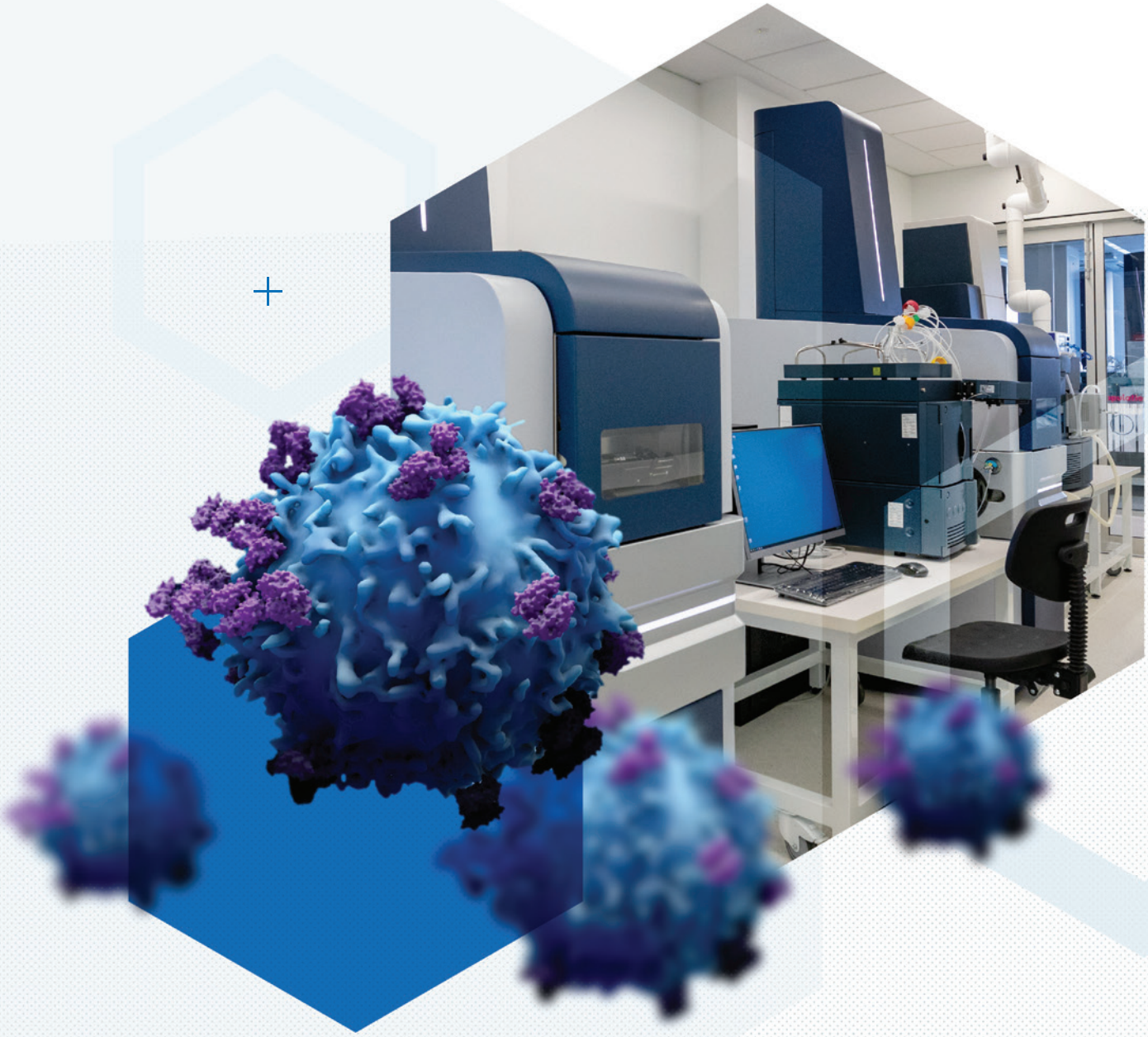
Alithea bio

Fanny Giannou, Founder and CEO of Alithea Bio, Freiburg, Germany

"The power of the timsTOF at Alithea bolsters our dominant position in the HLA peptidomics domain and underscores our commitment to pushing the boundaries of this technology for diagnostic applications. Aligning forces with Bruker is a testament to our dedication to fostering the global development and realization of safe and effective immunotherapies."

Dr. Tim Fugmann, Co-Founder and CSO of Alithea Bio, Freiburg, Germany

"By harnessing the unprecedented sensitivity and speed of Bruker's timsTOF systems, coupled with AI-powered Bruker ProteoScape and rapid novor HLA peptide search engines, we are set to achieving the profiling of complete HLA peptidomes in single runs within this year. This ambitious goal signifies a monumental stride forward, not only facilitating the discovery of novel drug targets but also furnishing indispensable safety information."



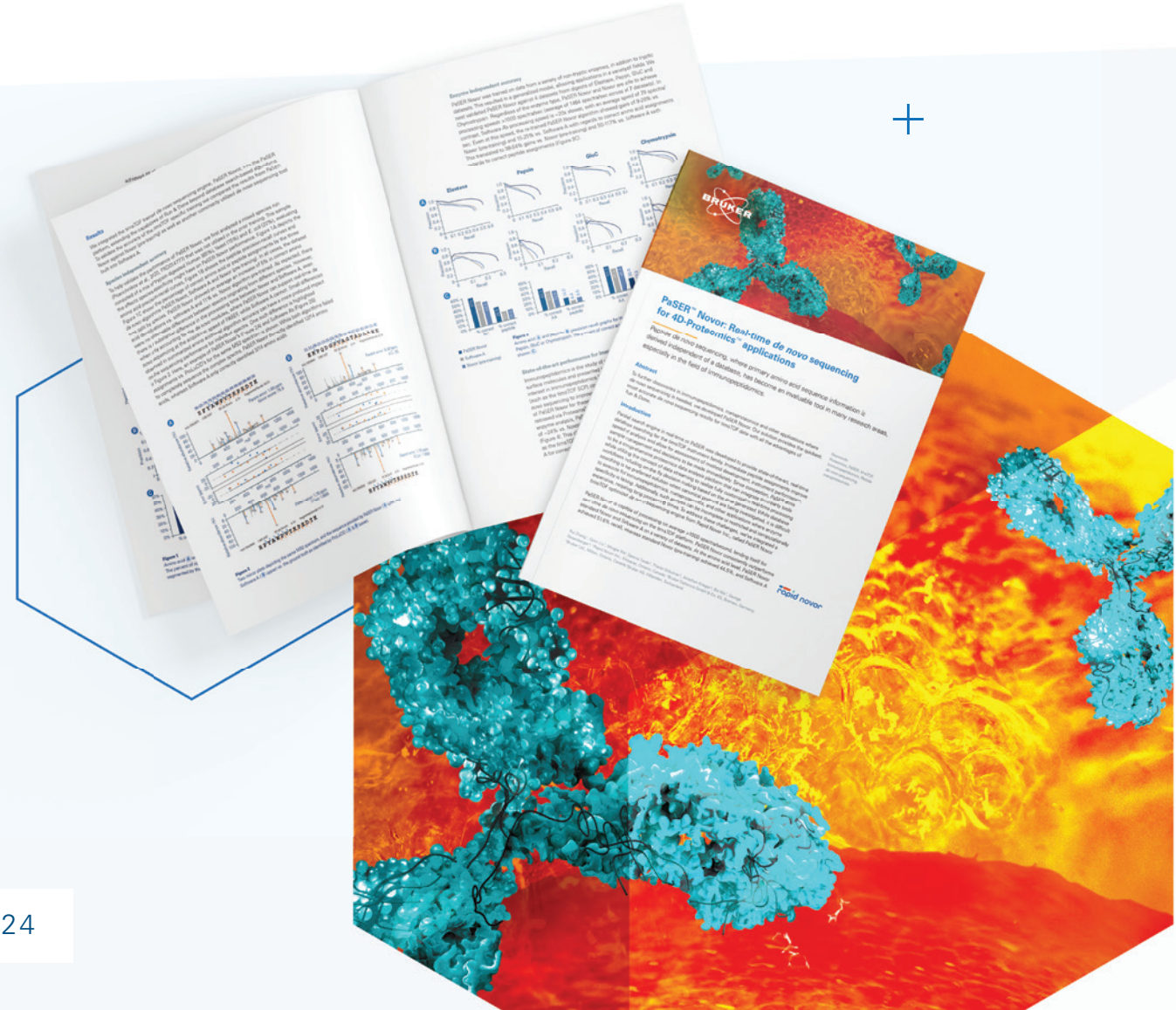
Master immunopeptidomics with Bruker ProteoScape and BPS Novor

Immunopeptidomics relies on advanced bioinformatics tools to decipher the complex data generated by LC-MS. Explore deeper insights with Bruker ProteoScape, a real-time search engine optimized for CCS. Now featuring de novo sequencing (BPS Novor) and seamless integration with Mass Dynamics' data analysis and visualization tool, BPS empowers genuine discovery within the 4D-Proteomics Ecosystem by Bruker.

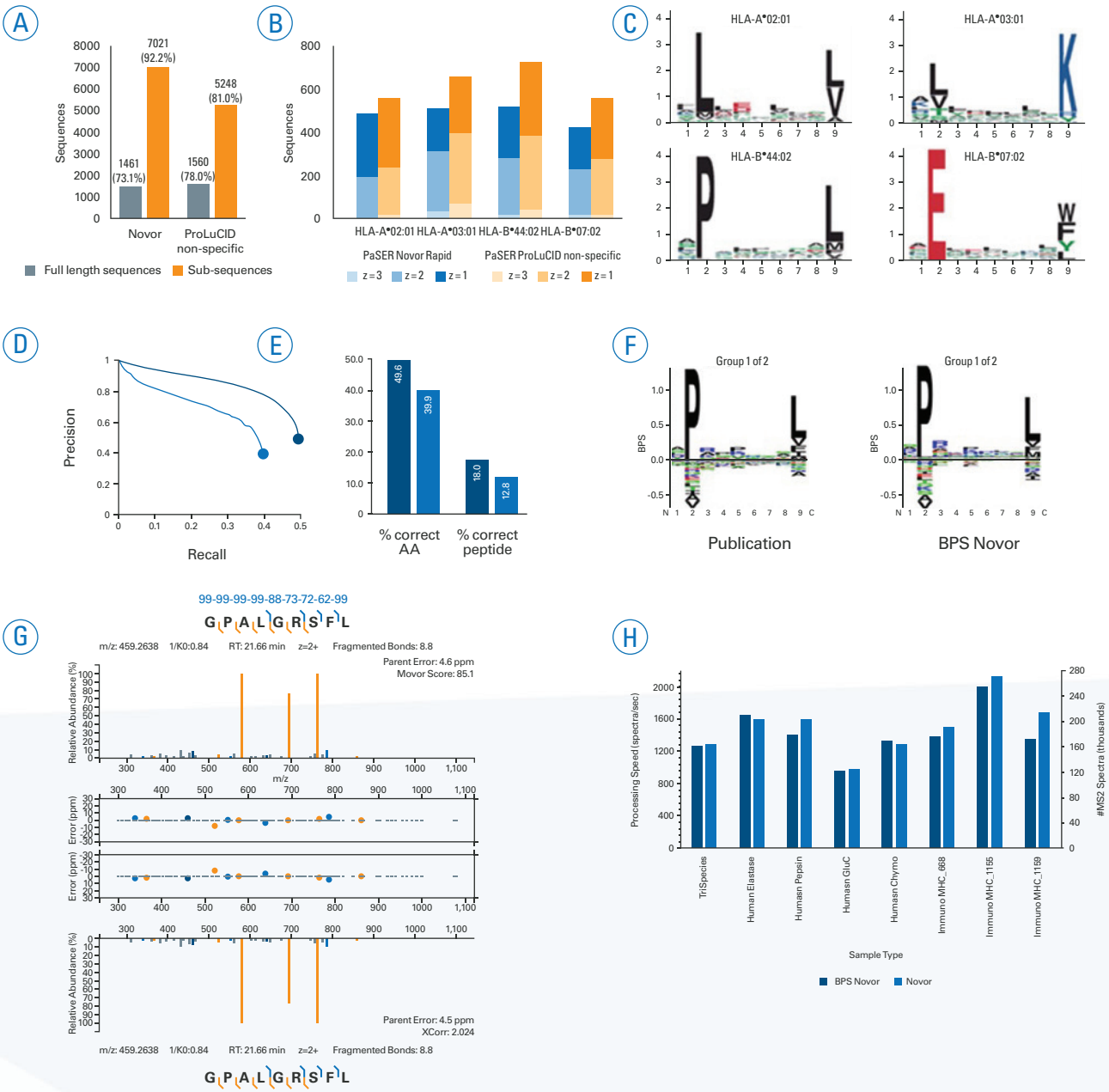
- **Real-time speed:** Process over 1000 spectra per second for immediate, precise results
- **De Novo deciphering:** Identify unknown peptides crucial for immunotherapy development
- **Advanced algorithms:** Leverage statistical and bioinformatics methods for accurate peptide identification from complex datasets
- **Customizable workflows:** Tailor your analysis to specific research goals

Accelerate discoveries with BPS Novor

DOWNLOAD PDF



Bruker ProteoScape BPS Novor: Your key to unlocking the full potential of your immunopeptidomics data

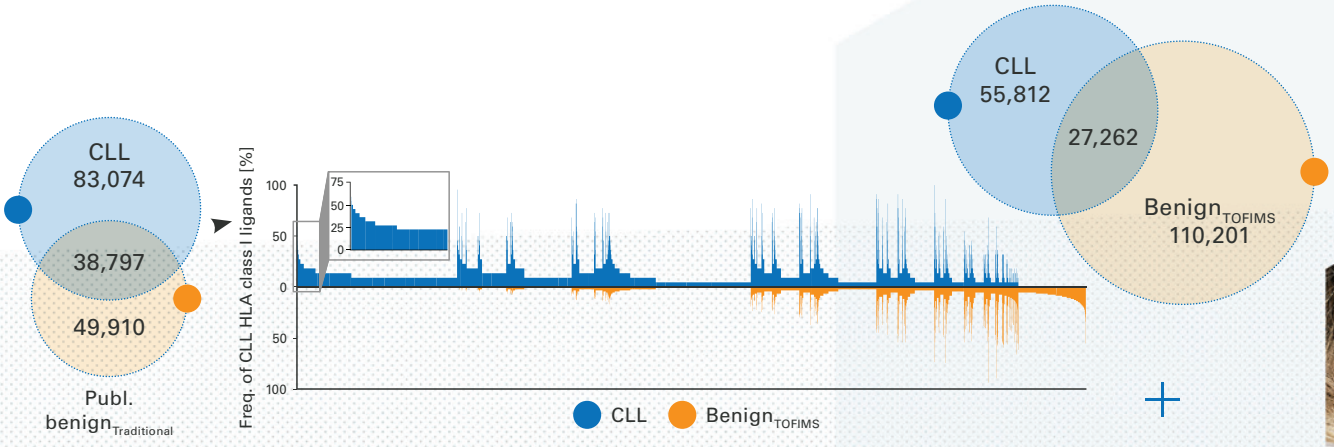


BPS Novor for fast, precise, and accurate de novo sequencing at PASEF speeds. A) Non-redundant full length peptide sequence and sub-sequences of 2000 synthetic HLA peptides identified using de novo peptide sequencing with BPS Novor or non-specific database search with ProLuCID in Bruker ProteoScape. Percentage of the 2000 synthetic peptides covered by the analysis is given in brackets. B) Precursor charge state distribution pattern (redundant sequences) of the synthetic HLA peptides grouped by presenting allele, blue BPS Novor, Orange ProLuCID non-specific search. C) Gibbs clustering analysis of the sequence motifs for the 4 alleles used in this study. In addition, when analyzing a subset of data from Feola et al., 2021, BPS Novor performed well in the precision-recall graph (D) as well as a E) noticeable increase of ~24% vs Novor (pre-training) for correct amino acids and >25% increase for correct peptides. This clearly delineates the utility of optimizing the algorithm for a given platform, such as the timsTOF series. BPS Novor also showed more modest gains of 3-10% vs Software A for correct amino acids. F) To define the consensus binding motifs for all 9-mer peptides (with Score >70) Gibbs clustering analysis was performed. Comparable results were observed between the analysis from Feola et al., 2021 (modified from Figure 3 in publication) and our BPS Novor results. Two distinct groups were formed and showed the same preferences for reduced amino acid complexity for residues at positions P2 and Ω as published. G) A representative 9-mer peptide spectrum as identified by BPS Novor and ProLuCID non-specific search. H) Processing time for BPS Novor for >100,000 spectra versus two commercially available timsTOF de novo sequencing packages.

From bench to bedside—translating novel immunotherapeutic strategies

The department of peptide-based immunotherapy at the University of Tübingen (Germany), is led by Prof. Dr. Juliane Walz and focuses on three main aspects:

- The analysis of the immunopeptidome using mass spectrometry and the further characterization of the antigen-specific T-cell repertoire of malignancies, infectious diseases, and inflammatory diseases
- The GMP production of peptides and vaccines
- The translation of novel immunotherapeutic strategies from bench to bedside with currently five clinical trials to evaluate self-developed peptide vaccines



Overlap analysis of HLA class I peptides of primary chronic lymphocytic leukemia (CLL) samples with benign reference data sets for the identification of high frequent tumor-associated antigens to be applied in peptide-based immunotherapy approaches.[4]

"Our overall goal is the development of peptide-based immunotherapy concepts for malignant and infectious diseases. Using high-sensitivity LC-MS based immunopeptidomics we work on rapidly transferring promising research findings into clinical application for patient treatments."

Prof. Dr. Juliane Walz, Department of Peptide-based Immunotherapy, University and University Hospital Tübingen, Germany

"Using timsTOF, we created a large-scale benign reference data set, which enabled the refinement of previsouly described tumor antigens, as well as the de novo identification of broadly off the shelf antigens and mutation-derived neoepitopes as targets for future peptide-based cancer immunotherapy development."

Dr. Otto Kari, VP and Head of Antigen Business Unit, and EIC projects lead, Valo Therapeutics (ValoTx), Helsinki, Uusimaa, Finland

"This amazing piece of top-of-the line kit (timsTOF Ultra) from Bruker, which has now been running in our R&D laboratory since August, allows us to identify thousands of antigens from each PeptiCHIP processed tiny tumor sample with unparalleled speed, sensitivity, and robustness."

"The timsTOF Ultra will be instrumental in the further development and maturation of our PeptiCHIP microfluidic chip technology, supported by the EIC Transition PeptiCHIP grant."

"We will also be exploiting its single-cell analysis capabilities to push the boundaries of research through our collaborations with leading researchers at the University of Helsinki and elsewhere."

VALOTX

Further reading and references

Immunopeptidomics published pre-prints and papers using the timsTOF instruments

1. *Adams, C., et al. "Fragment ion intensity prediction improves the identification rate of non-tryptic peptides in timsTOF." (2023) https://doi.org/10.1101/2023.07.17.549401*
2. *Feola, S., et al. "PeptiCHIP: A microfluidic platform for tumor antigen landscape identification." ACS Nano, 15(10), 15992–16010 (2021). https://doi.org/10.1021/acsnano.1c04371*
3. *Data and graphic kindly provided by TProf. Anthony W Purcell, Head of Immunoproteomics Laboratory, Monash University.*
4. *Hoenisch Gravel N, et al. "TOFIMS mass spectrometry-based immunopeptidomics refines tumor antigen identification." Nat Commun. (2023). https://doi.org/10.1038%2Fs41467-023-42692-7*
5. *Xu, P., et al. "Novel canonical and non-canonical viral antigens extend current targets for immunotherapy of HPV-driven cervical cancer." Cell Press (2023). https://doi.org/10.1016/j.isci.2023.106101*
6. *Gomez-Zepeda, D., et al. "Thunder-DDA-PASEF enables high-coverage immunopeptidomics and identifies HLA class-I presented SarsCov-2 spike protein epitopes." Research Square Preprint (2023). https://doi.org/10.21203/rs.3.rs-2625909/v1*
7. *Phulphagar, K. M., et al. "Sensitive, high-throughput HLA-I and HLA-II immunopeptidomics using parallel accumulation-serial fragmentation mass spectrometry." Molecular & Cellular Proteomics (2023). https://doi.org/10.1101/2023.03.10.532106*

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