TIMS-MS

timsOmni™

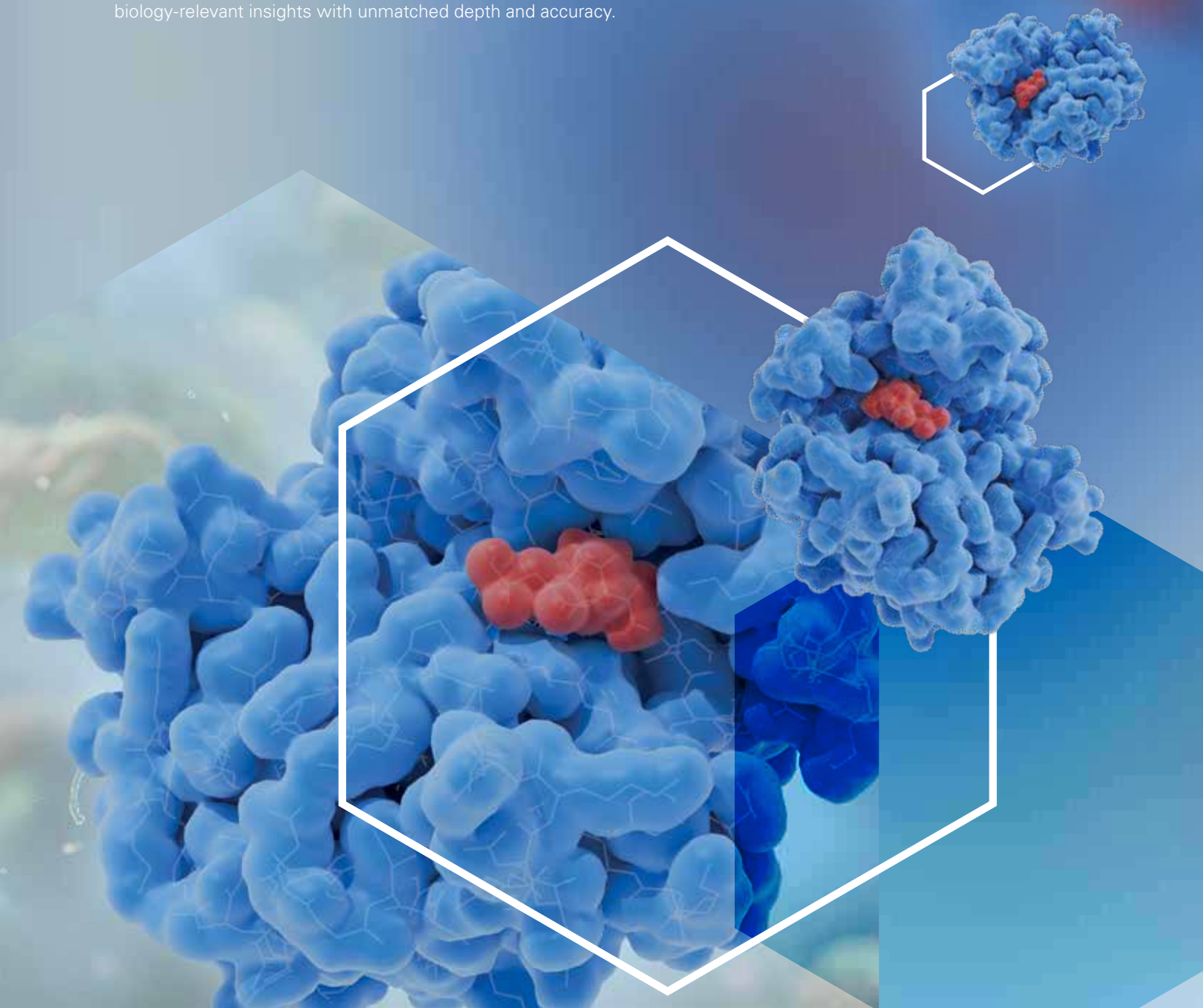
An **eXtreme** leap in deep proteoform sequencing and advanced structural elucidation

Innovation with Integrity

An eXtreme leap for electron-based fragmentation techniques

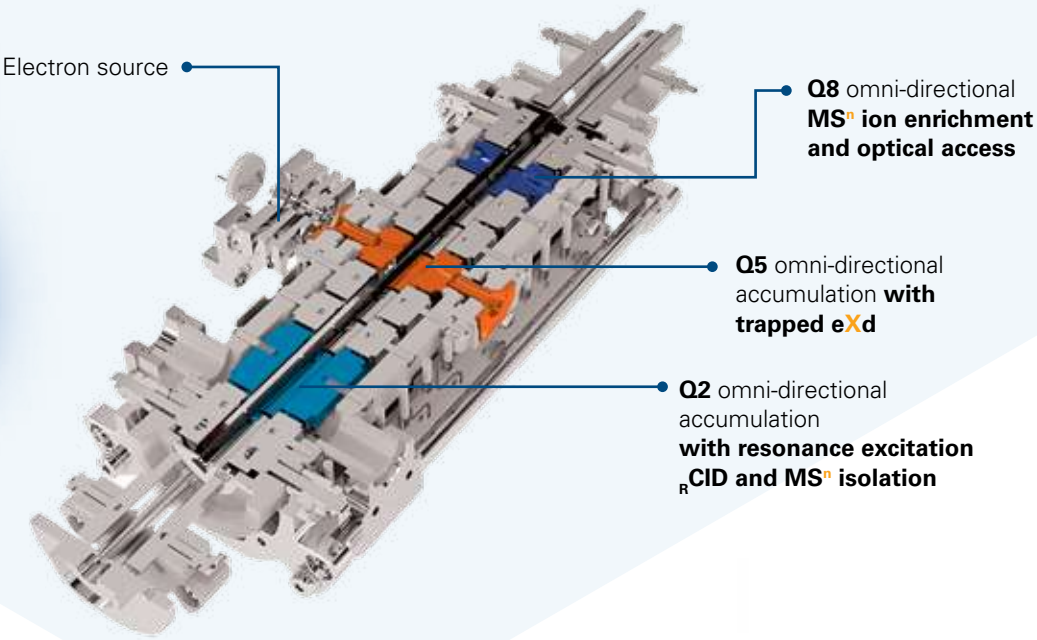
Welcome to the timsOmni™, a transformative instrument poised to revolutionize mass spectrometry in systems biology. Designed to push the boundaries of scientific discovery, the timsOmni achieves extraordinary sensitivity, advances deep proteoform sequencing and delivers unprecedented precision in structural elucidation. Discovering the intricacies of proteoform diversity and gaining new insights into the functional plasticity of proteins is now within reach. The timsOmni further enables detailed structural elucidation of potential mutagenic impurities, revealing how subtle chemical modifications can transform benign small molecules into carcinogenic threats, corroborating the superior versatility of this groundbreaking technology.

The timsOmni embraces the power of top-down mass spectrometry by harnessing the complete spectrum of electron-based fragmentation schemes, underscoring the value of complementary dissociation techniques. Empowered by advanced MS^n eXd and ion enrichment methods, this transformative platform detects labile post-translational modifications (PTMs), produces complementary fragment ions to resolve ambiguous spectra, and reconstructs complex molecular information with OmniScape™ software, delivering biology-relevant insights with unmatched depth and accuracy.

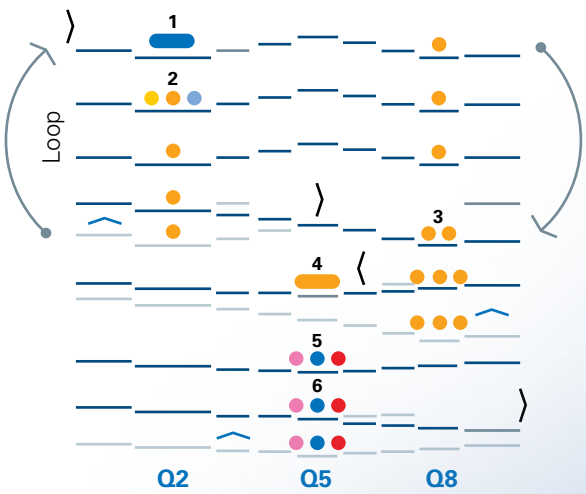


Experience unmatched MS and MS^n sensitivity

Powered by proprietary omni-directional lossless transfer and ion enrichment, signals for even the lowest-abundance ions are dramatically amplified. Through precise modulation of selected ion packets, any ion, regardless of its intensity, can be selectively targeted for electron-based fragmentation.



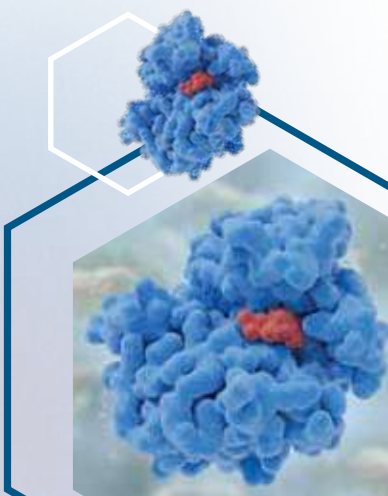
Omni-directional MS^n eXd with ion enrichment



1) Quad isolation and direct omni-accumulation of precursor ions in section Q2.
2) Resonance excitation Collision Induced Dissociation ($_R$CID) and isolation of target fragments for further interrogation.
3) Signal amplification via ion enrichment of selected MS^2 CID fragments in Q8.
4) Transfer of the accumulated MS^2 CID ion population in section Q5.
5) MS^3 trapped eXd fragmentation of the enriched ion population
6) Ejection of MS^3 product ions to TOF.

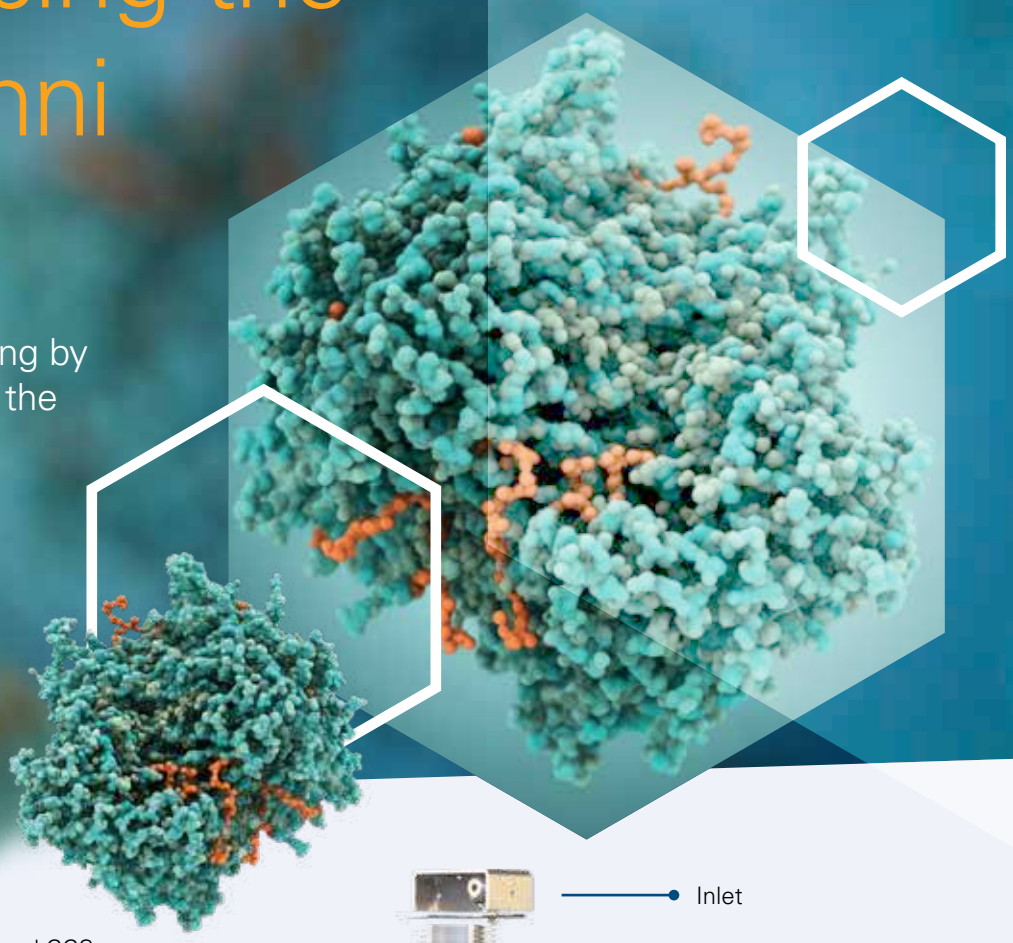
Designed for results

Ion enrichment is applied for PTM characterization of protein complexes sprayed under native conditions using the new NEOS source.

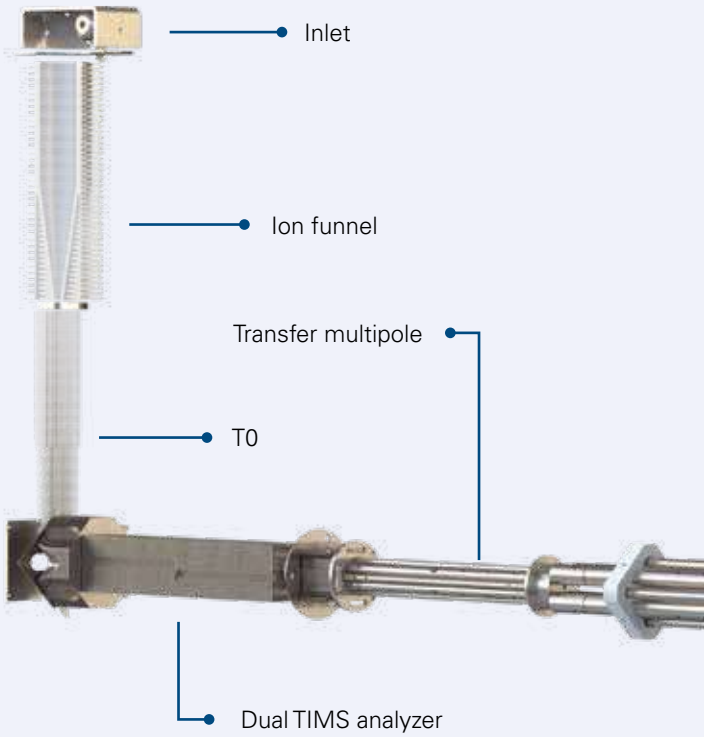


Introducing the timsOmni

Advanced structural elucidation and deep proteoform sequencing by **MS^n eXd** coupled to the power of TIMS.

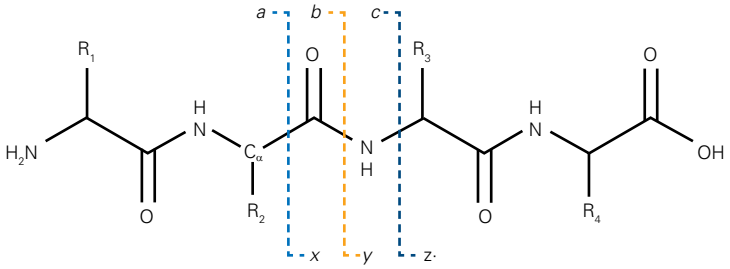


- TIMS separation to determine conformational heterogeneity and CCS to reveal structure
- Trapped eXd for optimal precursor utilization boosting fragment ion yield
- Precise control of electron-based fragmentation for detailed molecular profiling
- Omni-directional MS^n and trapped eXd with ion enrichment for unmatched sensitivity
- Utilization of all PASEF modes for bottom-up proteomics and multiomics



Explore the conformational landscape

Collision induced unfolding (CIU) experiments are performed in T0 upstream of the TIMS. TIMS separation followed by eXd of selected conformations provides insights into higher order structure of proteins.

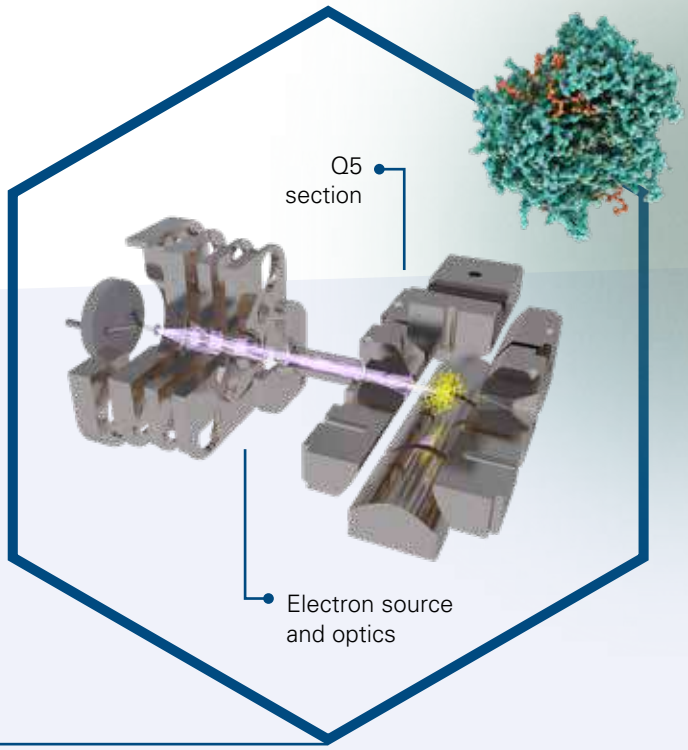


Nomenclature for the primary fragment ions types observed for peptides and proteins in tandem mass spectrometry.

In ECD, when a protein or peptide ion captures a free electron, bond cleavage occurs rapidly and locally at the N–Cα bond, producing primarily c/z fragments. This non-ergodic process preserves labile post translational modifications like phosphorylation, glycosylation, sulfonation, etc.

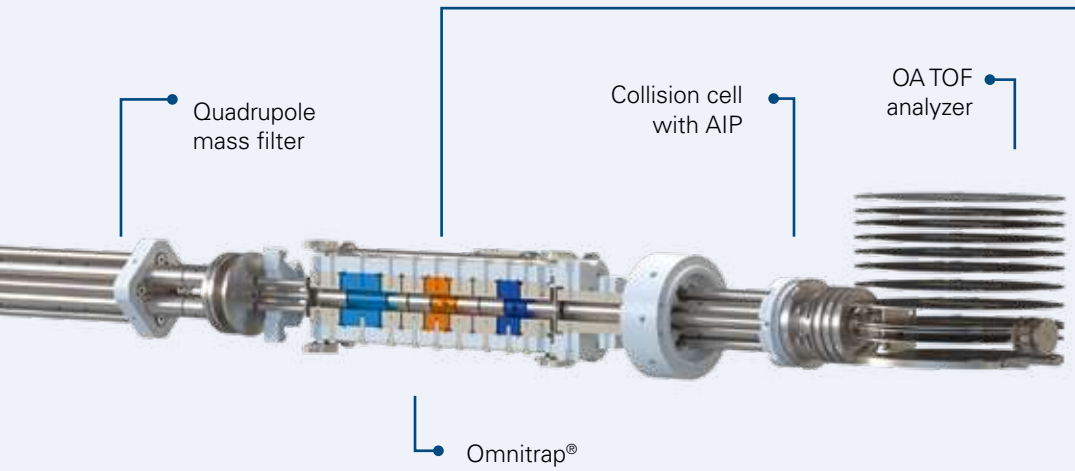
In CID, ions are gradually heated through multiple collisions, spreading energy throughout the molecule (ergodic). This causes only the lowest-energy bonds to break, mainly producing b/y fragments with limited sequence coverage and often poor PTM characterization.

Combinations of ECD and CID using **MS^n eXd** can be used to further enhance protein sequence coverage through formation of a/x, b/y and c/z ions.



A small detail with a big impact

A hot cathode produces a high-density electron beam, guided by a lens system into section Q5 for high efficiency eXd reactions with trapped ions, increasing product ion yield compared to other soft fragmentation methods.

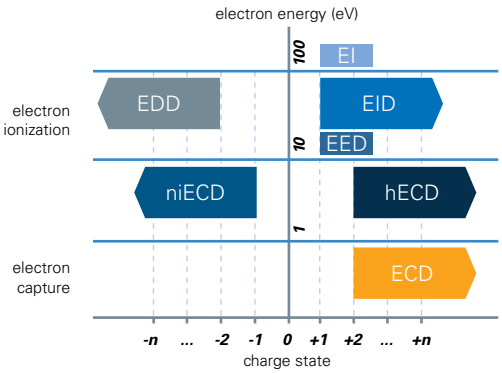


Why AIP is a game changer

The AIP addresses mass discrimination effects in transferring ions to the TOF and results in a much broader mass range of ions being observed in the mass spectrum.

Precise modulation of electron kinetic energy and reaction time

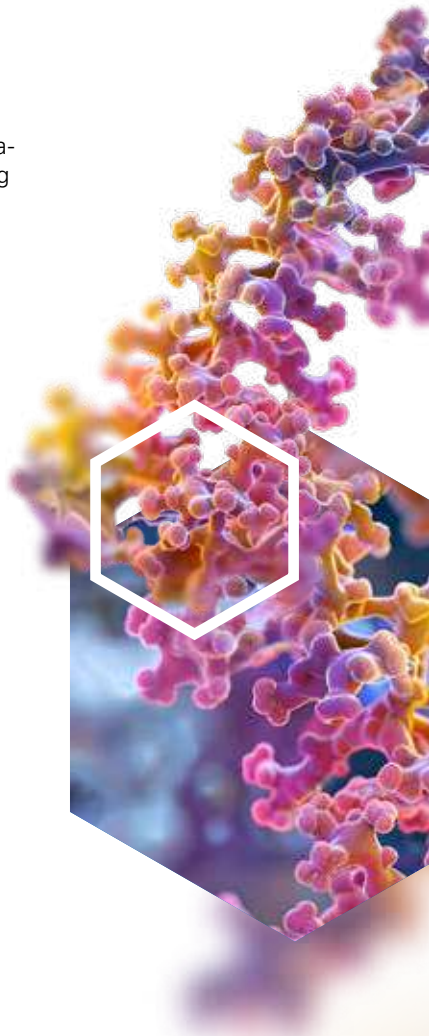
Precisely tune electron energy to access diverse fragmentation regimes. Adjust eXd reaction times and explore uncharted electron energy levels for different classes of analytes to achieve optimal product ion coverage.



eXd Landscape

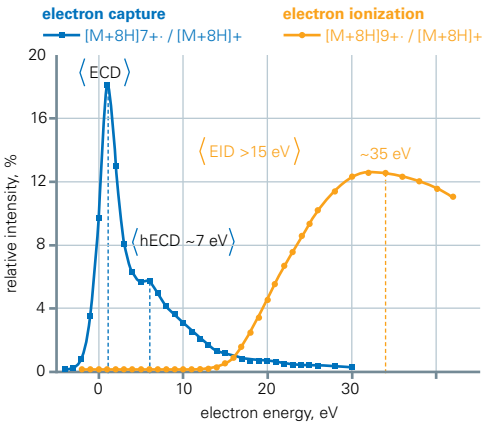
Access to different electron-based fragmentation regimes becomes available by fine tuning electron energy from 0.1eV to >100 eV

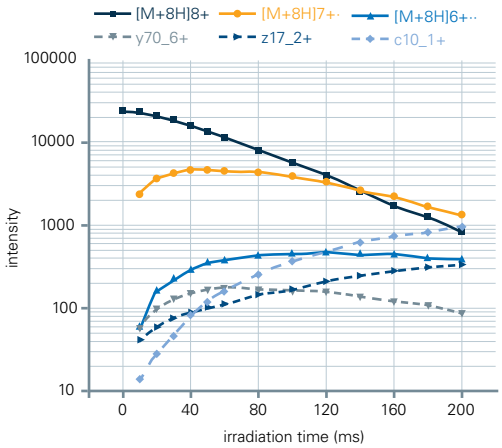
ECD – Electron Capture Dissociation
hECD – hot Electron Capture Dissociation
EED – Electron Excitation Dissociation
EID – Electron Induced Dissociation
EI – Electron Ionization
niECD – Negative-Ion ECD
EDD – Electron Detachment Dissociation



Electron energy optimization

Optimum electron energies are identified for electron capture and ionization of ubiquitin $[M+8H]^{8+}$ ions by tracing the signal of the charge-reduced $[M+8H]^{7+\cdot}$ and the charge-increased $[M+8H]^{9+\cdot}$ product ions respectively as a function of electron energy.





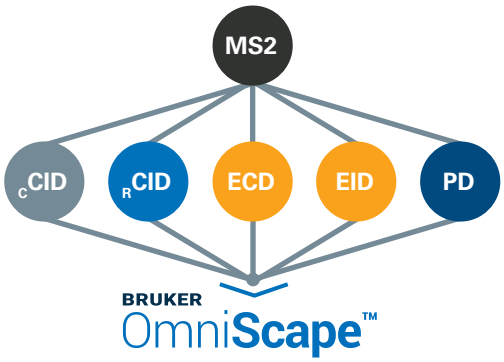
Trapped ion eXd

In trapped ion eXd, precursor ions are confined and rapidly fragment upon electron irradiation. Adapting the trapping time enables it to boost the fragment yield and reach optimal precursor consumption (>90%). This unique capability differs from traditional in-line electron-based fragmentation techniques.

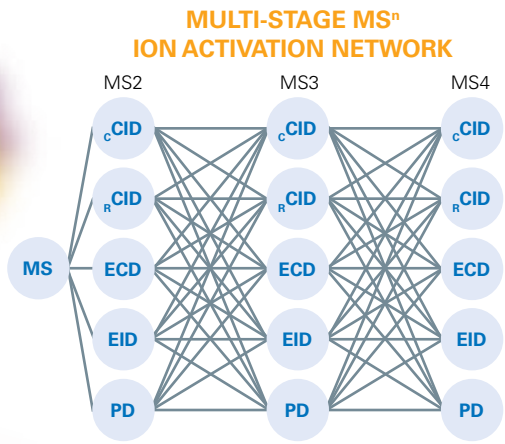
Powerful multimodal and multi-stage MS^n

Online multimodal fragmentation

Multimodal fragmentation relies on combining information from first-generation fragment ion types, produced by complementary ion dissociation methods, to drastically increase sequence coverage.

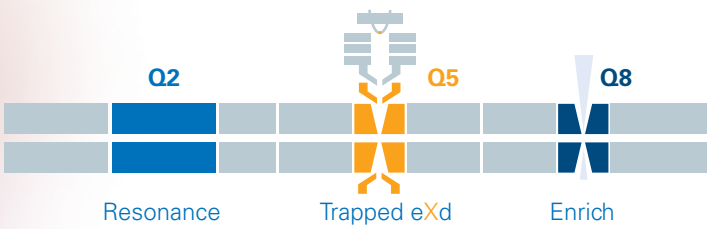


cCID – Collision-cell Collision Induced Dissociation
rCID – Resonant-excitation Collision Induced Dissociation
ECD – Electron Capture Dissociation
EID – Electron Induced Dissociation
PD – Photo-dissociation



Successive stages of ion activation enable first- and second-generation fragments to be selected for subsequent analysis. Distinct dissociation techniques can be employed at each MS^n stage, allowing for highly customizable workflows. These tailored MS^n strategies are well-suited to explore a broad range of analytes with diverse structural and chemical characteristics.

Ion enrichment provides unprecedented levels of sequence and structural interrogation through signal amplification, leveraging the high-capacity trapping sections across the Omnitrap platform.



Let's break it down!

cCID occurs by axial acceleration of ions into the collision cell

rCID occurs by resonant excitation in section Q2 of the Omnitrap platform

eXd occurs in section Q5, receiving variable energy electrons from an external hot cathode

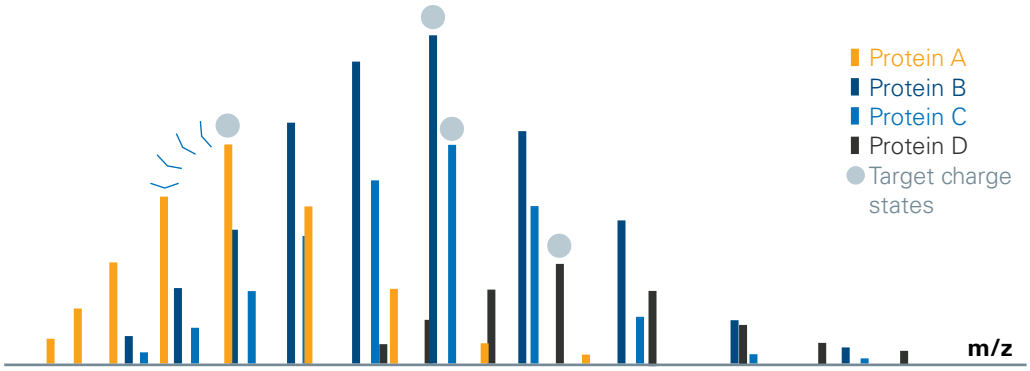
Signal amplification via ion enrichment and also optical access is provided in section Q8

Top-down proteomics with charge DDA

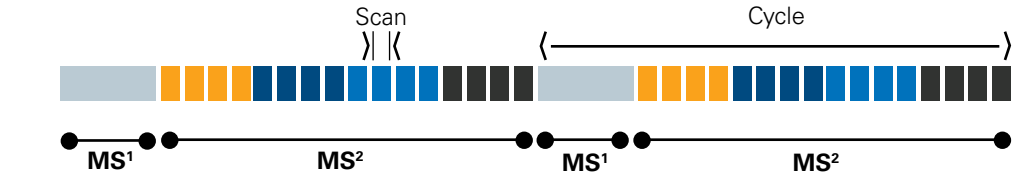
Charge DDA (cDDA) enables on-the-fly charge-state deconvolution during LC–MS analysis, allowing precise targeting of coeluting proteoforms across a wide dynamic range and eliminates redundant fragmentation of highly abundant species. By dynamically shifting the isolation window to non-overlapping regions of the m/z spectrum, cDDA significantly reduces chimeric spectra.

Key Features

- Ultra-fast on-the-fly charge-state deconvolution with intelligent m/z selection criteria.
- Precursor selection targets non-overlapping charge states producing high fidelity MS^2 data.
- Dynamic precursor accumulation optimizes ion fill times in the high-capacity sections of the Omnitrap platform for enhanced signal-to-noise MS^n spectra.
- Automatic control of trapped eXd reaction times based on precursor charge state and user-defined target count criteria deliver optimal reaction efficiency.



cDDA is based on ultra-fast on-the-fly identification of charge-state envelopes corresponding to individual proteoforms.



Averaging multiple TOF spectra sequentially at kHz rep rate produces high signal-to-noise ratio mass spectra.

Multiple sequential MS^2 scans result in high-fidelity isotopic distributions of fragment ions

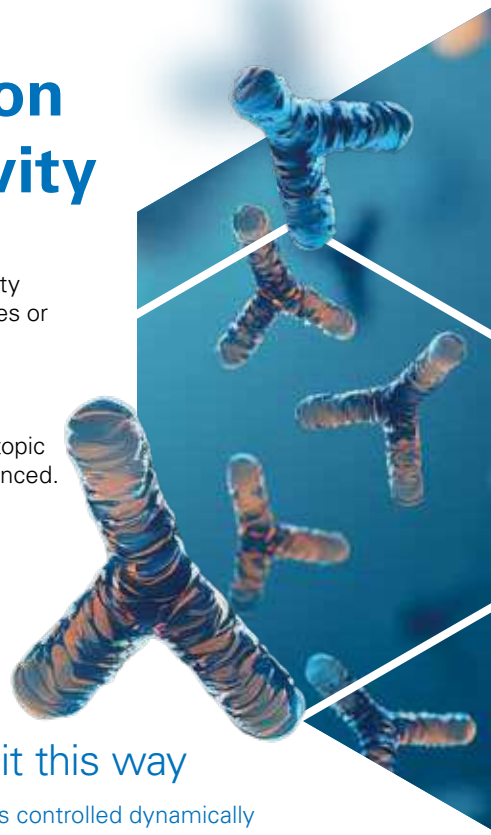
A quick fact

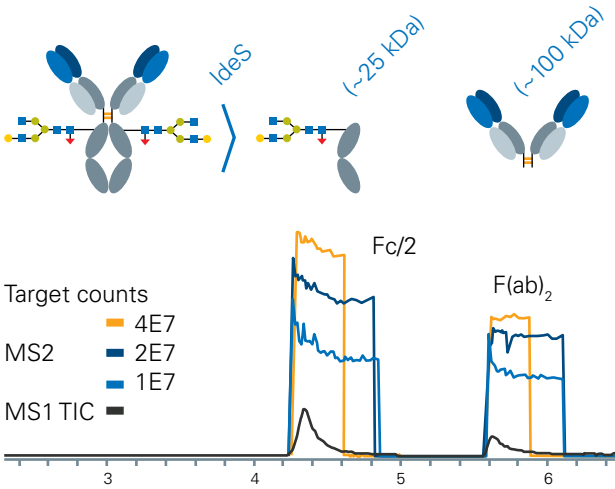
cDDA is tailored to top-down proteomics and differs from a standard top “n” method applied in bottom-up by using a more sophisticated precursor selection algorithm.

Dynamic precursor accumulation for superior LC-MS/MS sensitivity

Top-down and middle-down LC-MS approaches enable rapid analysis of recombinantly expressed biologics. Targeted methods are particularly advantageous when high specificity and maximum sensitivity are required for protein sequencing, whether in complex matrices or purified samples typical of biopharmaceutical workflows.

To further advance such analytical strategies, dynamic precursor accumulation actively regulates ion flow in MS/MS scans to optimally fill and compress the ion cloud within the trapping sections of the Omnitrap platform. By optimizing ion statistics, the fidelity of isotopic distributions is preserved with sequence coverage and analytical confidence further enhanced.



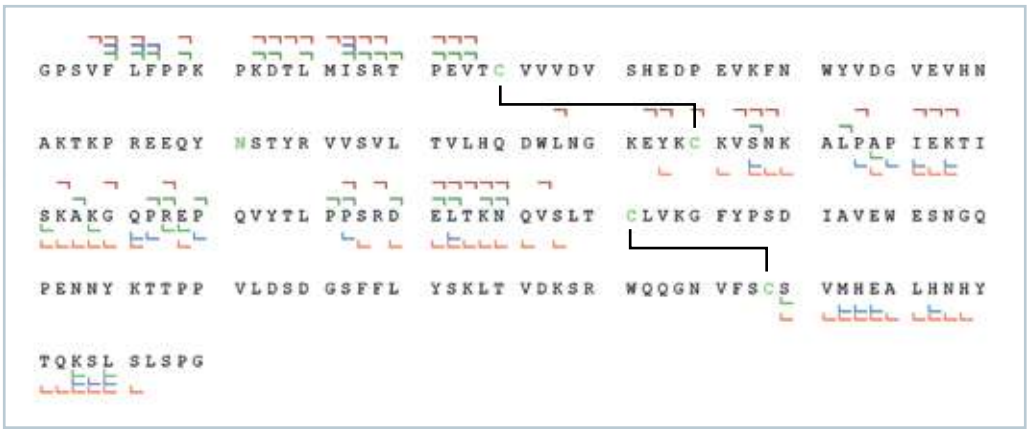


Think of it this way

Ion fill time is controlled dynamically in MS^2 scans.

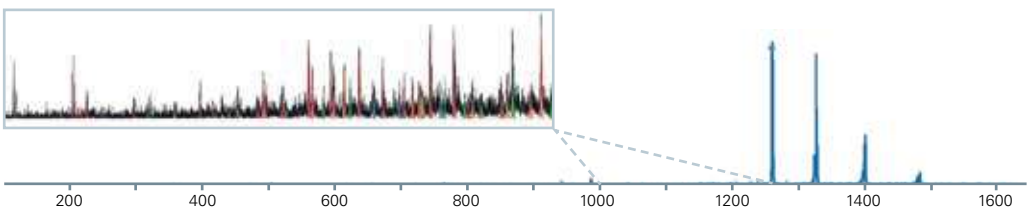
Trapped eXd reaction time is adjusted according to precursor intensity and charge state.

Dynamic precursor accumulation can also be applied in cDDA.



33% sequence coverage and 71% outside of intrachain disulfide bonds

a x
b y
c z

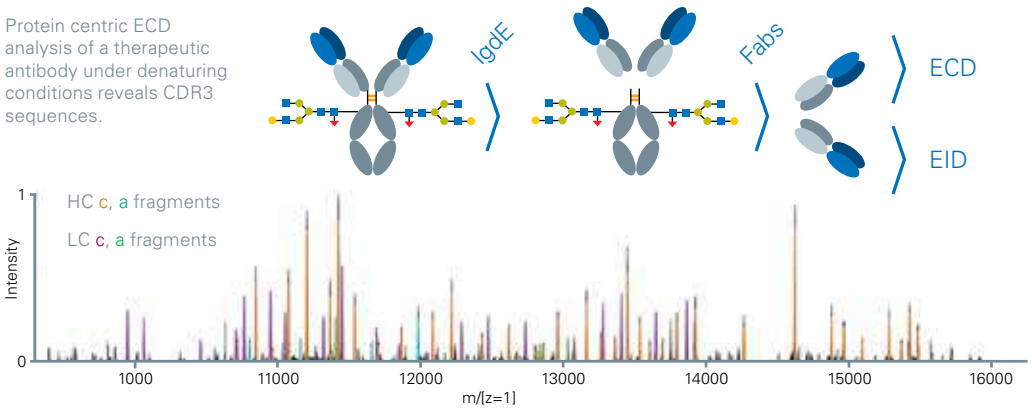


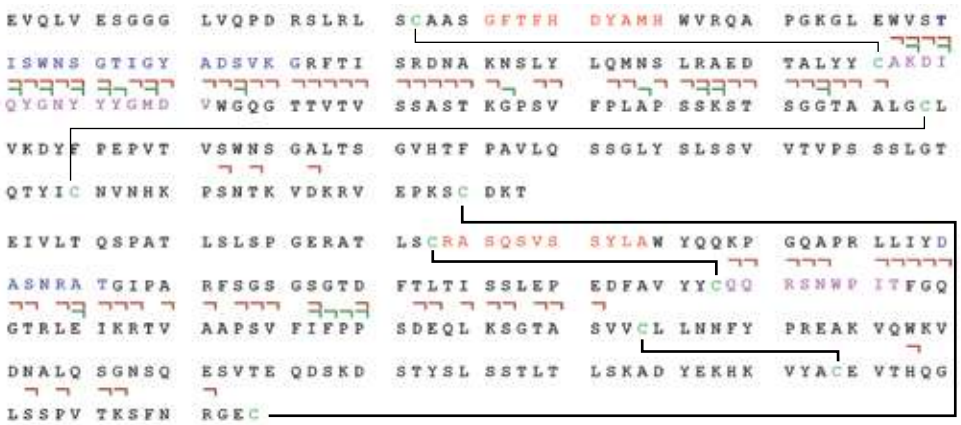
Sequencing Antibody Complementarity-Determining Regions

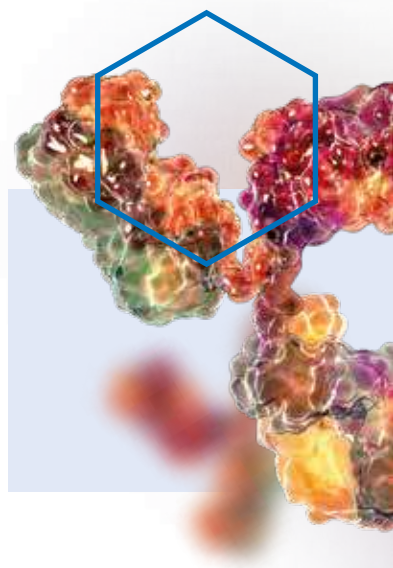
Analyzing and monitoring circulating antibody levels is critical for characterizing the progression of a disease, identifying patients with delayed symptom onset and predicting potential long-term immunity.

- Complementarity-Determining Regions (CDRs) are the hypervariable loops within the variable domains of the light and heavy chains primarily responsible for selectivity and affinity towards a specific antigen. Comprehensive ion sequence ladders are essential to characterize the unique CDRs.
- CDR sequencing refers to the process of identifying the exact amino acid sequences within these loops to confirm identity, assess potential modifications, or facilitate antibody engineering. The superior efficiency of trapped eXd ensures comprehensive coverage of the critical functional regions in antibodies.

Protein centric ECD analysis of a therapeutic antibody under denaturing conditions reveals CDR3 sequences.







Comprehensive ion sequence ladders are ideal for *de novo* sequencing and human plasma antibody repertoire profiling.

CDR1, CDR2, CDR3,
S-S bridges
a c

Albert Heck

Professor of Chemistry and Pharmaceutical Sciences,
Utrecht University and Scientific Director of the Netherlands Proteomics Center

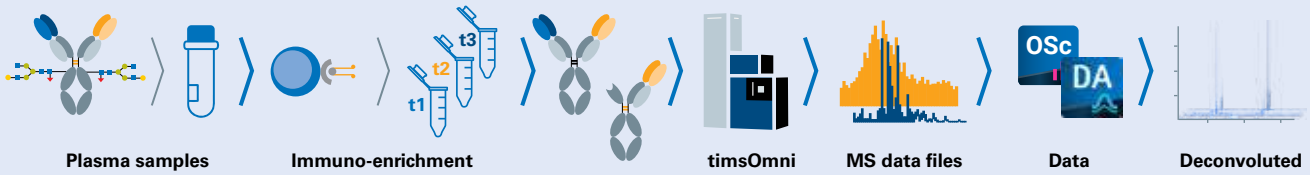
"Proteomics will finally go Protein-Centric by using the timsOmni"

Monitoring *in vivo* biotransformation products for **improving the efficacy of biotherapeutics**

Multispecific antibodies are rapidly emerging as a leading class of biotherapeutics, capable of simultaneously binding to multiple target antigens. These innovative modalities offer novel mechanisms of action, with enhanced efficacy, reduced risk of resistance, and fewer side effects compared to traditional therapies. However, unlike monoclonal antibodies, multispecifics are more prone to degradation *in vivo*, requiring thorough metabolite characterization.

Top-down and middle-down MS approaches are particularly well-suited for such detailed structural analysis, as bottom-up strategies often fall short in capturing the complete proteoform landscape. Compounding this challenge is the extremely low abundance of these metabolites in *in vivo* samples, which makes their analysis especially difficult. The introduction of the timsOmni marks a significant advancement, ushering in a new era for the comprehensive characterization of multispecific antibody modalities.

Analytical strategy for characterizing *in vivo* biotransformation of biotherapeutics



Plasma samples (collected at different time points after administration)

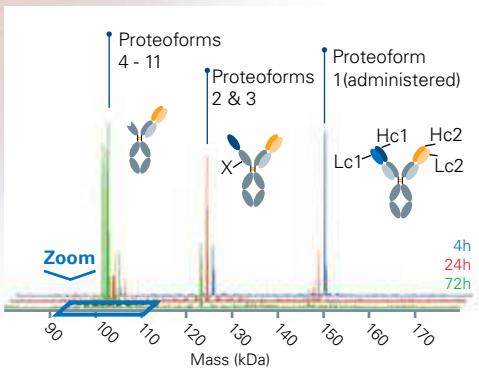
Immuno-enrichment (magnetic beads)

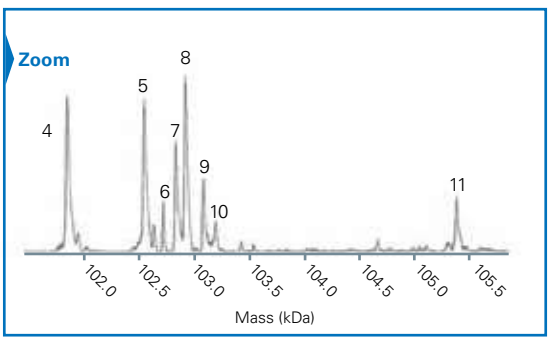
timsOmni and nanoElute 2

MS data files

Data processing

Deconvoluted mass spectra





Time-resolved monitoring of *in vivo* biotransformation products highlighting preferential clipping of the LC1 and HC1 chains, responsible for reducing biotherapeutic efficacy.

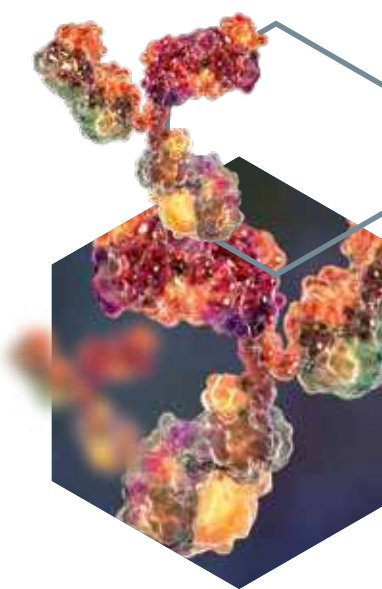
Detection of very low abundance biotransformation products, ranging from 3 to 30 nM, is essential for investigating the *in vivo* stability of next generation biotherapeutics.

Julia Chamot-Rooke

CNRS Senior Scientist, Head of the Mass Spectrometry for Biology Unit at Institut Pasteur Paris, France

"The timsOmni delivers unmatched sensitivity and sequencing power - key to decoding multispecific antibody metabolites."

*Data collected by Lucile Kogey-Fuchs PhD Student CIFRE #2023/0657 between Sanofi & Institut Pasteur

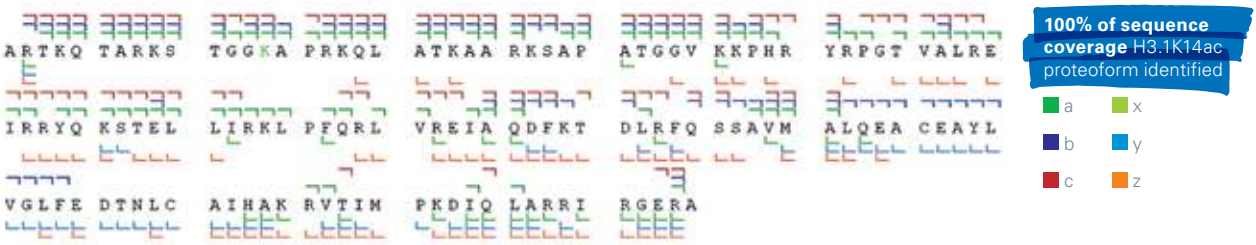


Decrypting histone modifications is key to understanding disease

Histones are crucial for DNA packaging, with their post-translational modifications (PTMs) playing a significant role in regulating gene expression by modifying chromatin structure. Understanding these histone modifications is paving the way for insights into diseases such as cancer, neurodegenerative disorders, cardiovascular diseases, and metabolic conditions.

The high variability of modification sites in histone structures demands advanced analytical tools for accurate and reliable characterization and this can be achieved using the state-of-the-art fragmentation capabilities of the timsOmni along with sophisticated algorithms in OmniScape software to enable highly confident positional PTM assignments.

Multimodal eXd (EID, ECD, CID, and ECD/CID) on charge states 19+ and 20+ provides complete sequence coverage for unambiguous localization of PTMs:



Ole N. Jensen

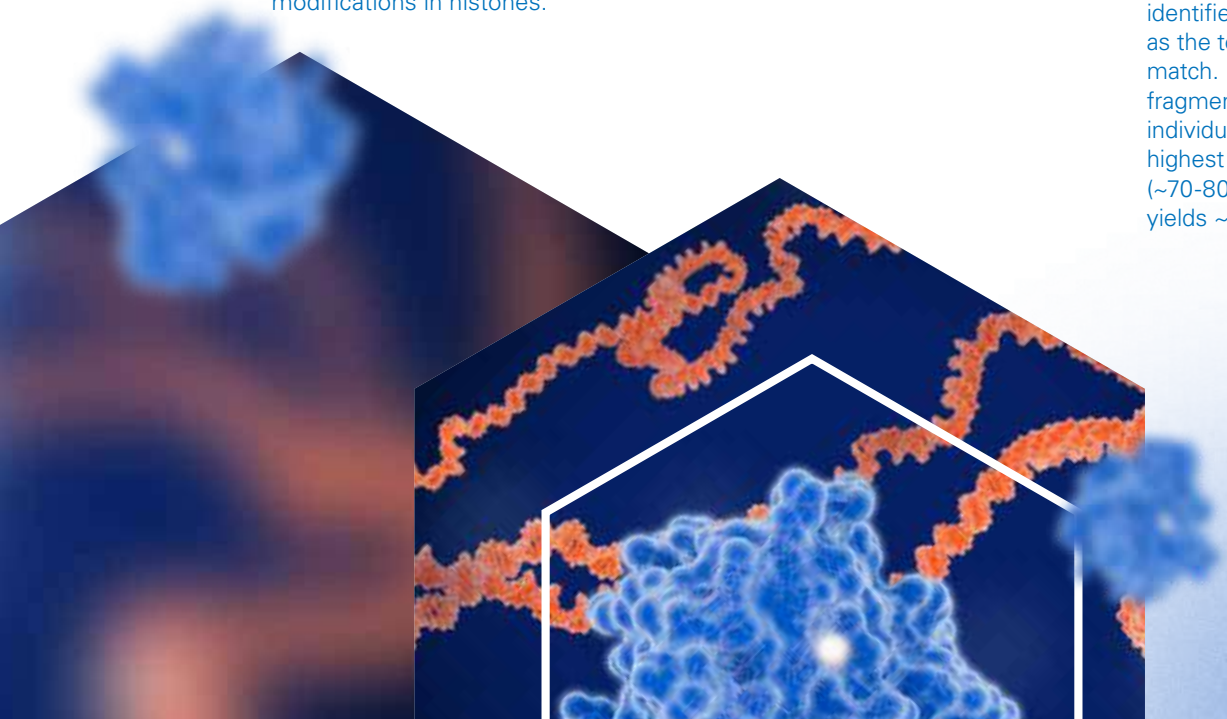
Professor Biomedical Mass Spectrometry and Systems Biology, Univeristy of Southern Denmark

Director, PRO-MS, Danish National Mass Spectrometry Platform for Functional Proteomics

"The timsOmni technology and OmniScape software already impacted our strategies for intact protein and proteoform analysis. Multimodal MS/MS fragmentation and MS^3 affords very high amino acid sequence coverage and accurate localization of post-translational modifications in histones."

From complexity to clarity

OmniScape consistently identifies H3.1K14ac as the top proteoform match. Electron-based fragmentation methods individually provide the highest sequence coverage (~70-80%), while CID yields ~40%.

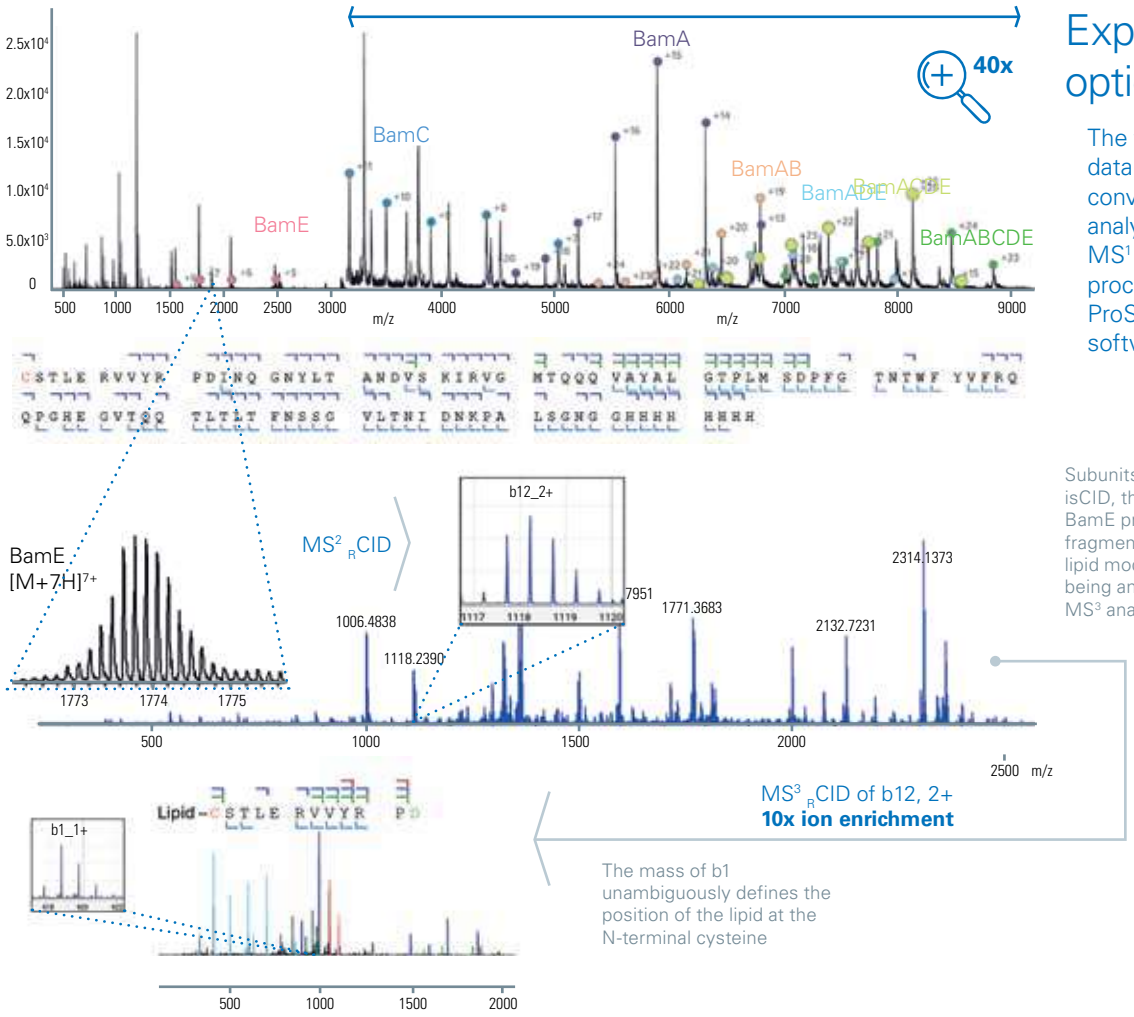


Beyond native MS, deep sequencing and PTM characterization of protein complexes

Understanding how membrane proteins assemble to function in health and disease state is critical for drug development. This necessitates multi-level analysis of protein oligomeric states, subunit interactions, posttranslational modifications, and their binding to ligands and inhibitors.

We illustrate how the integration of native and top-down mass spectrometry, with MS^n and ion enrichment, offer direct and detailed structural insights. The analytical power of the timsOmni is exemplified using the β-barrel assembly machinery (Bam), a heteropentametric protein complex that facilitates the insertion of β-barrel proteins into the outer membrane of Gram-negative bacteria.

Native MS spectrum of Bam membrane protein complex



Subunits are released by isCID, then MS^2 RCID of BamE produces abundant fragments carrying the lipid modification, with b12 being an ideal target for MS^3 analysis

The mass of b1 unambiguously defines the position of the lipid at the N-terminal cysteine

Expand your options

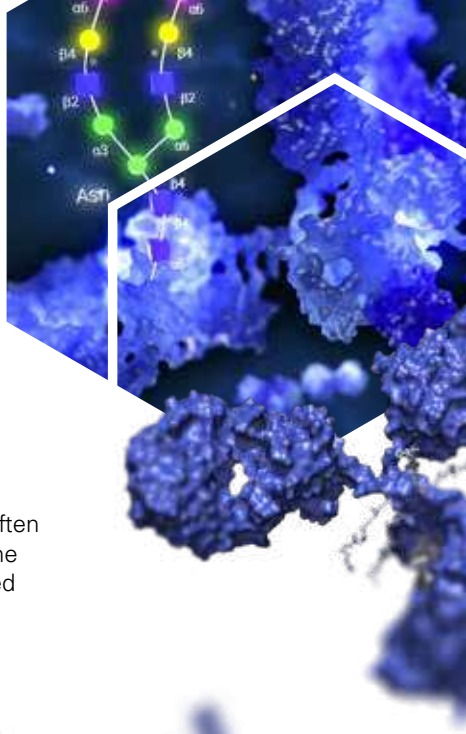
The timsOmni open data format allows convenient data analysis of native MS^1 spectra, here processed with ProSight™ Native software

Abraham Oluwole

Postdoctoral researcher, Carol Robinson lab, University of Oxford, UK

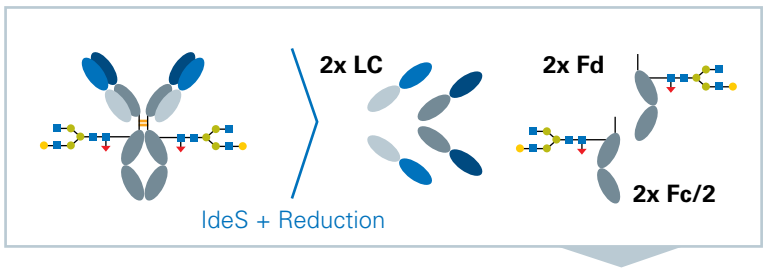
"The ion enrichment mode in the timsOmni will facilitate increased scrutiny of low abundant fragments by MS^n to achieve complete characterization for deeper insights"

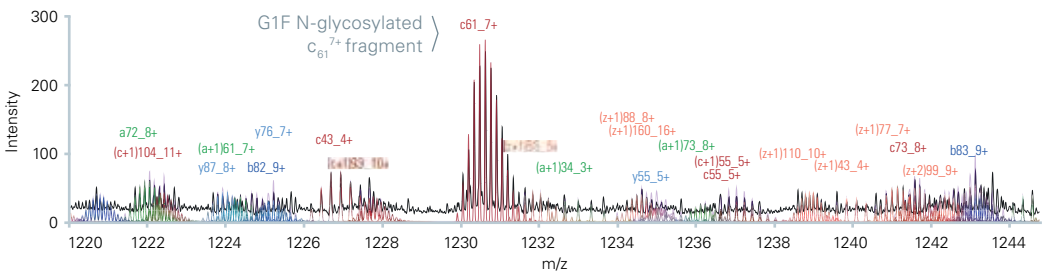
Precision mapping of antibody glycosylation using eXd



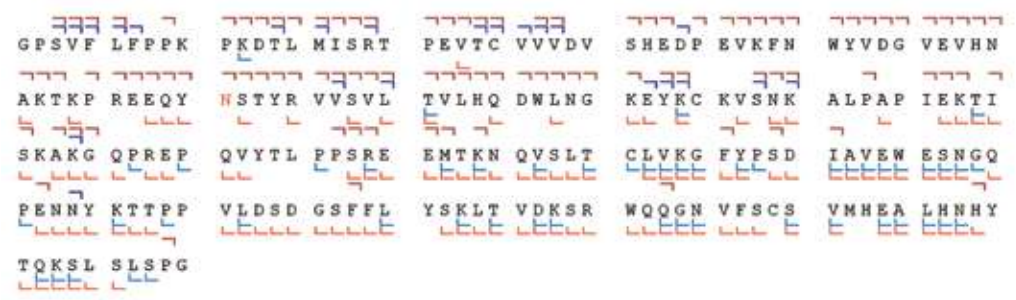
Glycans are critical for modulating protein structure and function, including efficacy as well as immunogenicity/toxicity of biopharmaceuticals. Variations in glycan structures can significantly impact a protein's function, making their precise characterization essential in biopharmaceutical development.

High-resolution characterization of glycans is vital, and difficult to achieve with CID, which often disrupts their fragile bonds. In contrast, eXd preserves glycan linkages while fragmenting the peptide backbone, enabling detailed mapping of glycosylation sites and providing unmatched structural resolution for therapeutic development and quality control.





MS² ECD of the Fc/2 subunit of the NIST IgG produces informative fragment ladders, with high abundance c_{61}^{7+} and c_{61}^{6+} fragments yielding the expected position and composition of G1F. The position of G0F modification was likewise confirmed by MS² ECD on the corresponding glycoform.



Sequence Coverage 91%

a y
b z
c

Precision redefined

Human glycans are built exclusively from only nine monosaccharides. eXd can be used to identify the site of the glycosylation and subsequent MS^n yields high resolution information on composition, topology, and structure.

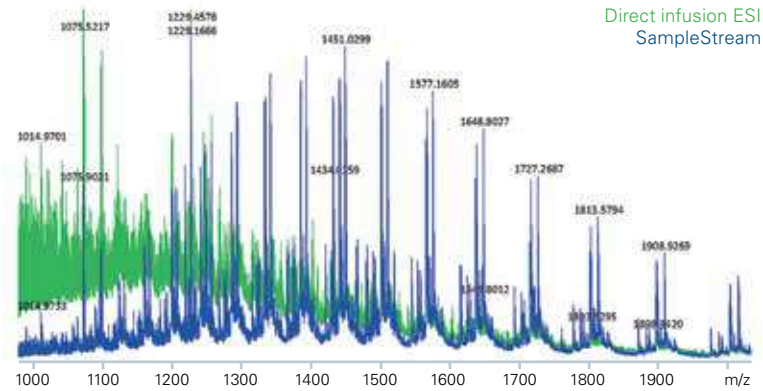
Advancing the Applied Proteomics field with multimodal fragmentation and MS^n

Bacterial enzymes are transforming industrial processes with their exceptional efficiency, specificity, and cost-effective production. In food manufacturing, these enzymes enhance fermentation, improve texture and clarify products by breaking down starches, proteins, and pectins. In pharmaceutical manufacturing, high-efficiency enzymes streamline drug synthesis, resulting in improved yields and higher product quality.

Multimodal fragmentation and MS^n workflows enable precise mapping of protein sequences and modification sites on intact molecules, accelerating optimization in synthetic biology.

Combining results from multimodal and MS^n modes performed on different precursor charge states of an industrial protease increases the sequence coverage to 92.6%

a y
b z
c



Direct infusion ESI
SampleStream

Automate sample delivery, save time

The SampleStream™ platform (IPT) coupled to timsOmni runs through HyStar, enabling automated workflows with rapid online buffer exchange. Pure protein signals with enhanced s/n ratio can be targeted for MS^n eXd.

Anders Michael Bernth Giessing

Science Manager, Novonesis, Lyngby, Denmark

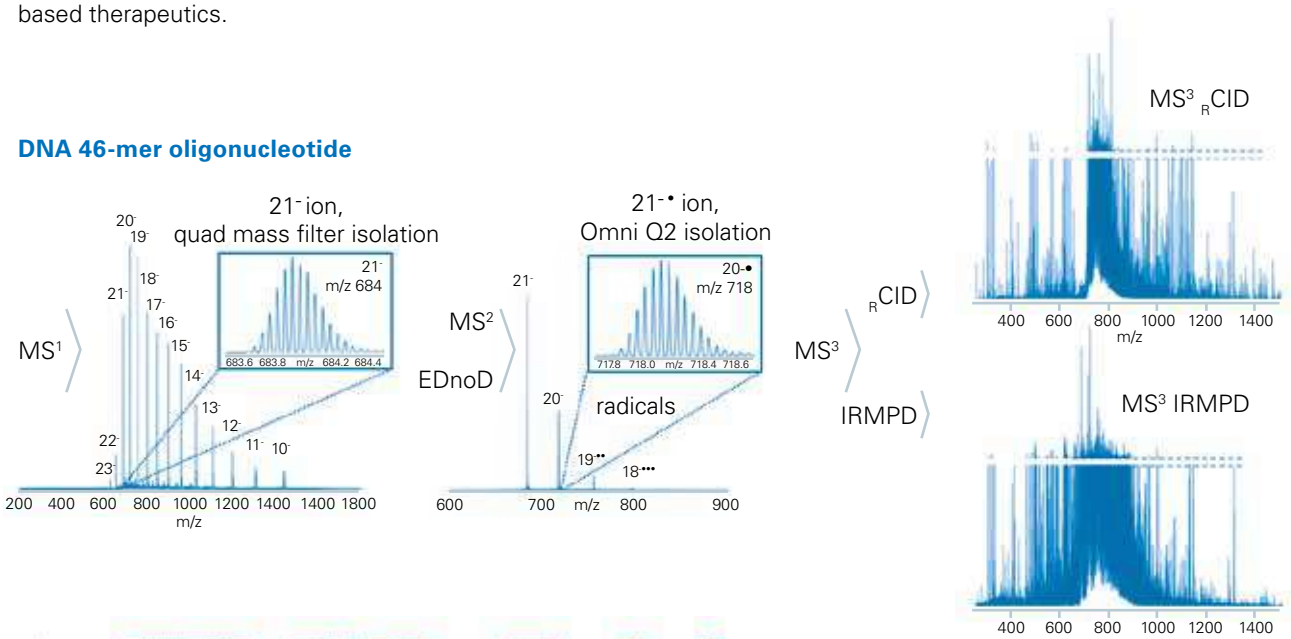
"We use intact protein mass analysis to ensure performance, stability, and consistency of our very diverse protein product portfolio. Introduction of the timsOmni, with its Swiss Army knife versatility, redefines intact mass and top-down analysis with the precision, speed, and confidence needed to provide definitive analytical support in development and production of industrial enzymes."

Deep characterization of oligonucleotides in negative ion mode by MS^n

DNA and RNA are vital for cellular homeostasis, driving protein translation and regulating genes through mechanisms like epigenetics and interfering RNAs, and are increasingly being applied as valuable drug modalities across a range of diseases.

Oligonucleotide characterization requires alternative fragmentation techniques because there are a limited number of canonical nucleotides and yet a diverse range of possible modifications. Mapping endogenous RNA modifications is key to understanding biological processes and top-down mass spectrometry with timsOmni is ideally positioned for precise sequencing. MS^n eXd enables EDD and is particularly effective for intact top-down characterization of oligonucleotide-based therapeutics.

DNA 46-mer oligonucleotide

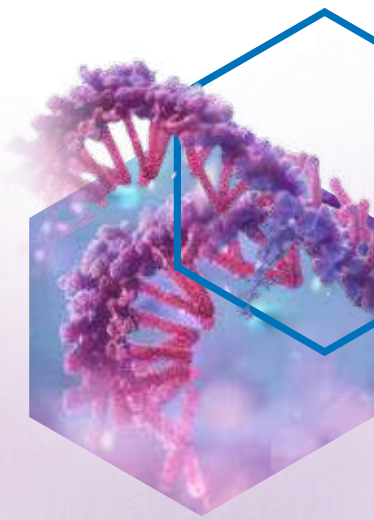


83% sequence coverage in MS^3 CID

Data analysis performed in OligoQuest™ software

Exploring uncharted opportunities

Leading researchers are also applying photodissociation in conjunction with EDD for deeper structural characterization of molecules and leveraging exotic fragmentation schemes



Valérie Gabelica

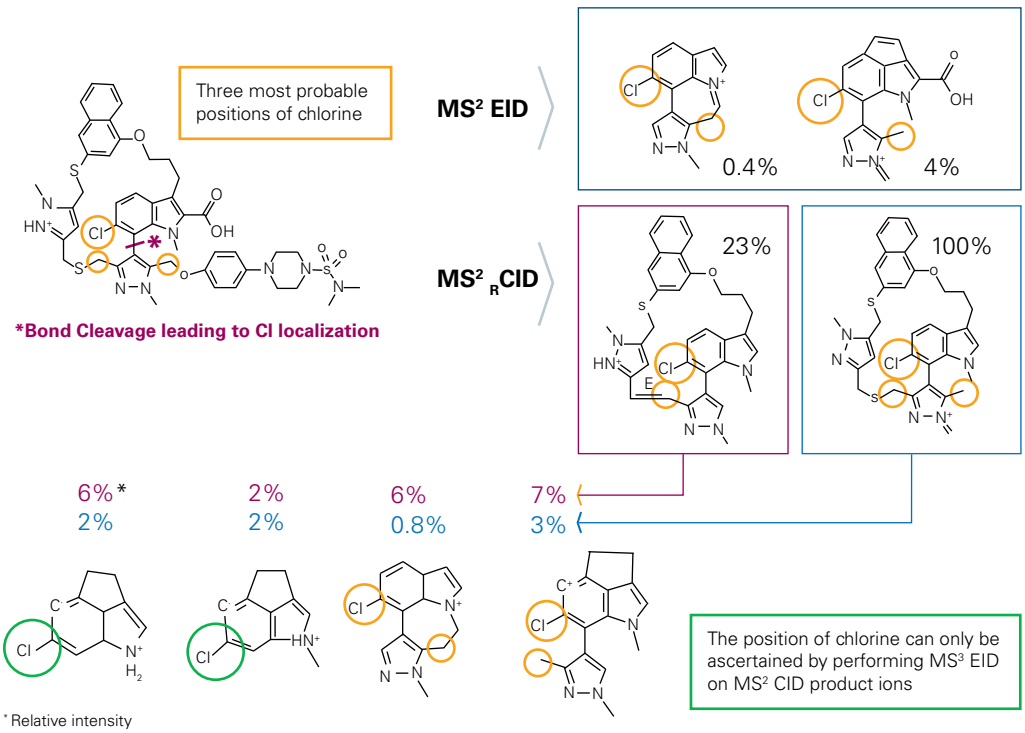
Full Professor, Analytical Chemistry, University of Geneva

"The timsOmni is our new Swiss-army knife for characterizing the structure and modifications of noncoding DNA, RNA, and oligonucleotide therapeutics"

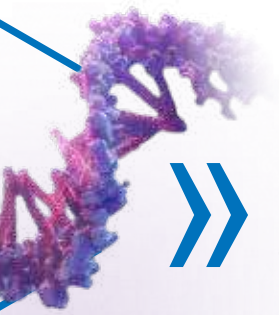
Advanced Structural Elucidation of Small Molecules with MS^n eXd

In pharmaceutical development, detailed information on chemical structures is crucial to assess toxicity of impurities often produced during degradation or synthesis. New analytical workflows providing detailed structural elucidation eliminate the need to synthesize impurity standards, considerably accelerating drug development.

Where is the chlorine? Aromatic chlorines are not reactive and are found in many drugs, while benzylic chlorines are potent alkylating reagents for DNA and proteins, rendering them carcinogenic. Locating the position of chlorination in trace impurities is critical as benzylic chlorines must be strictly controlled to minimize mutagenicity. The timsOmni goes beyond currently available MS^n CID and MS^2 EID activation methods, addressing such questions by accessing previously unreachable molecular bonds with MS^n eXd.



MS^2 CID and MS^2 EID can only eliminate one out of three possible chlorine positions.



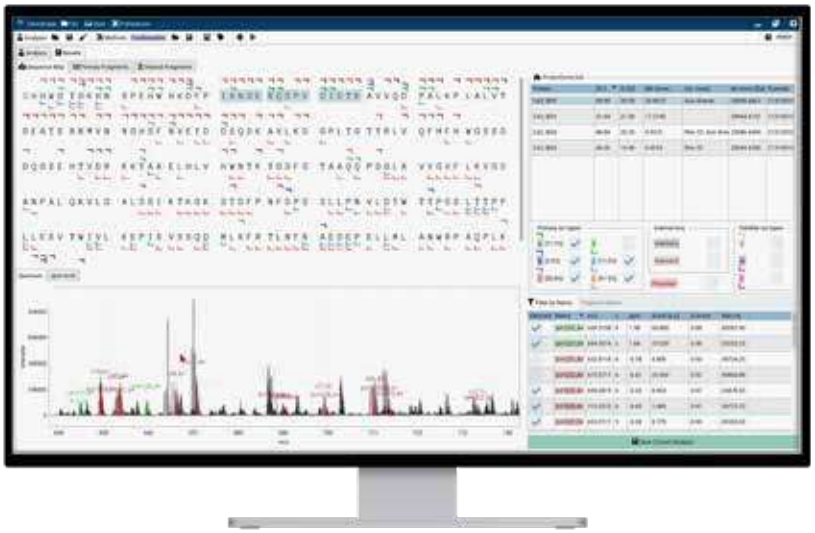
Gustaf Hulthe

Pharmaceutical Technology & Development, AstraZeneca R&D, Gothenburg, Sweden

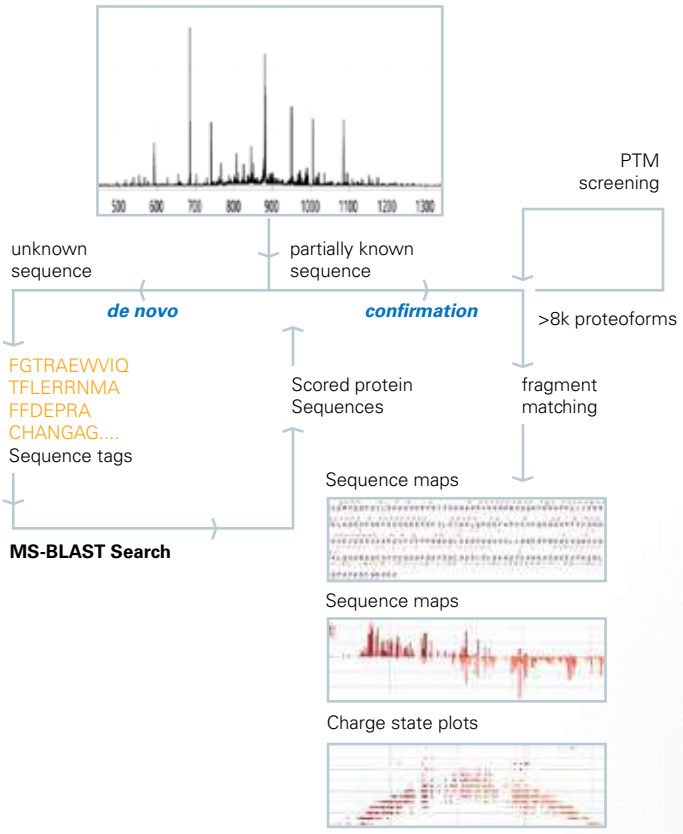
"In many cases MS^2 CID or MS^2 EID alone cannot resolve the structure of observed impurities. So far, the timsOmni in MS^n mode has solved the structural problems we have challenged it with, and without the need for synthesis. The versatile possibilities offered by this new platform may become a game changer."

OmniScape software

a new era of confidence in top-down sequence analysis



- **OmniWave™** for deisotoping highly complex mass spectra
- **Advanced scoring system** based on mass accuracy and isotope patterns
- **Confirmation workflow** applied to partially known protein sequences
- ***De novo* algorithm** for database search of top-down mass spectra
- **PTM Screening** for ultra fast proteoform identification of vast search spaces



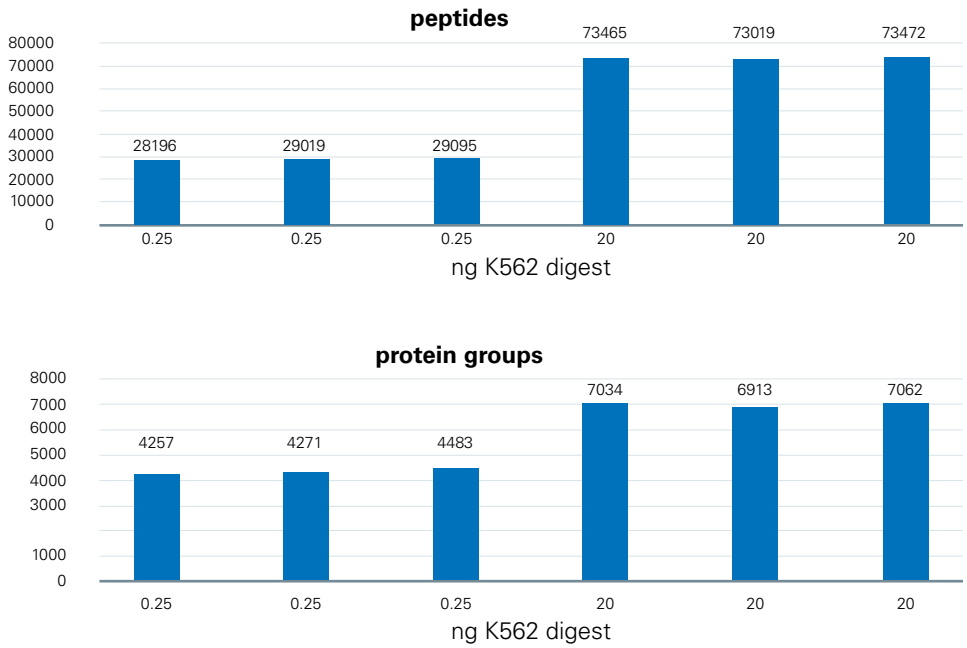
Innovation behind the solution

The OmniWave algorithm calculates theoretical isotopic distributions for all possible charge states and for every single isotope in the mass spectrum. The algorithm selects the optimal set of isotopic distributions that best explain the mass spectrum. Monoisotopic masses are consequently identified with high confidence, enabling the *de novo* and sequence confirmation workflows to be applied with high efficiency.

OmniWave™

timsOmni: Excellent Sensitivity for Bottom-Up Proteomics

Built on the robust foundations of the timsTOF Ultra 2, the timsOmni system seamlessly transitions to pass-through mode, delivering exceptional performance for bottom-up proteomics. This versatility allows for the analysis of single cells or other low input samples, down to a single immune cell, with unparalleled precision.



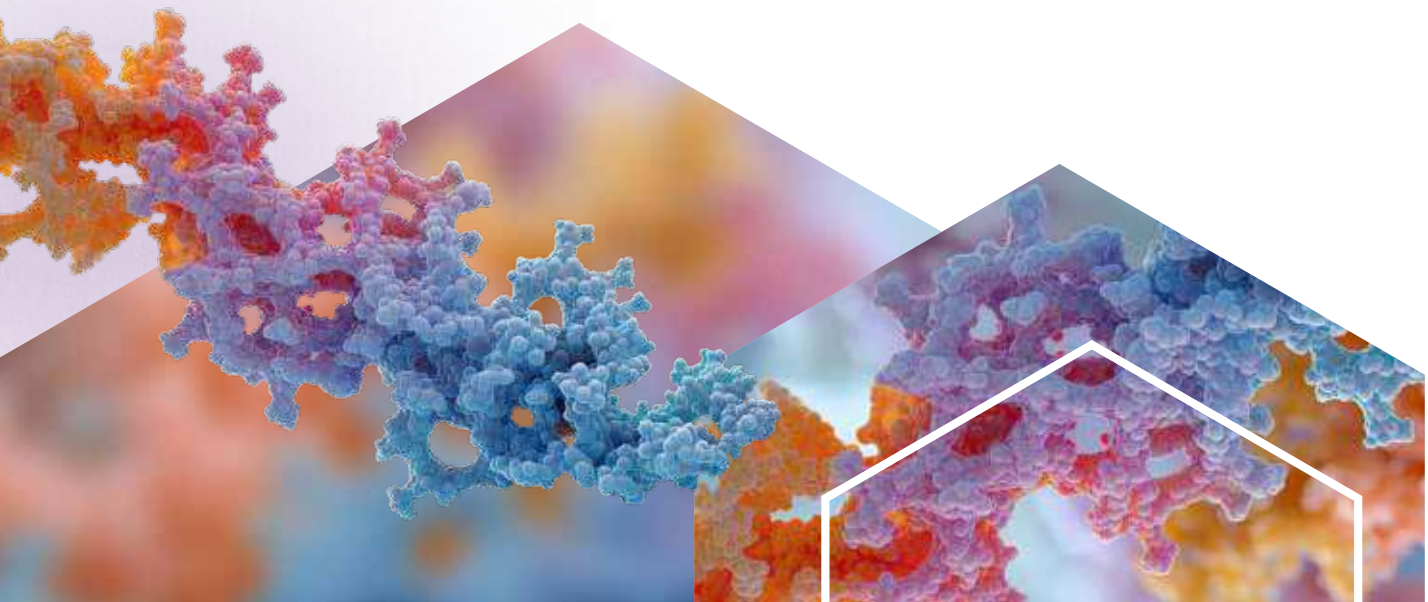
Peptides and protein groups in a Promega K562 digest measured by dia-PASEF. A 22 min gradient was used with an Aurora Ultimate column at 250 nL/min flow rate. Results were searched against the Swissprot human database using Spectronaut 19 and directDIA+, with no matching applied between runs.

Experience the best of both worlds:

- Ultra-high sensitivity for bottom-up and top-down proteomics.
- Switch effortlessly between advanced MS^n eXd fragmentation modes and standard PASEF® methods.
- Harness the diverse ion activation network for deep proteoform sequencing and advanced structural elucidation of a wide-array of biomolecules.

It's a fact!

The timsOmni provides access to all PASEF modes available on the timsTOF platform series.



The timsOmni - An eXtreme leap in deep proteoform sequencing and advanced structural elucidation

Feature	
Ion Sources:	• CaptiveSpray • NEOS (new off-line nanoESI source) • VIP-HESI (with built-in APCI)
MS^n eXd for multiple stages of ion activation and dissociation:	Multi-stage trapped eXd fragmentation with charge state dependent reaction times and precise modulation of electron energy to access diverse fragmentation schemes.
Ion enrichment technology for deep sequencing and structural elucidation:	Signal amplification via ion enrichment and MS^n eXd using omni-directional ion transfer for advanced ion processing workflows.
Athena Ion Processor (AIP) for broader m/z range detection:	Optimized release of ions depending on the application, addressing mass discrimination effects across a very wide m/z range and boosting sensitivity.
Charge Data-Dependent Acquisition (cDDA):	On-the-fly charge state deconvolution on LC time scales for intelligent quadrupole isolation of proteoforms across a wide dynamic range.
Extended m/z range quadrupole isolation:	<4500 m/z quadrupole isolation in transmission mode and 150 to >10,000 m/z omni-Q2 isolation in trapping mode

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