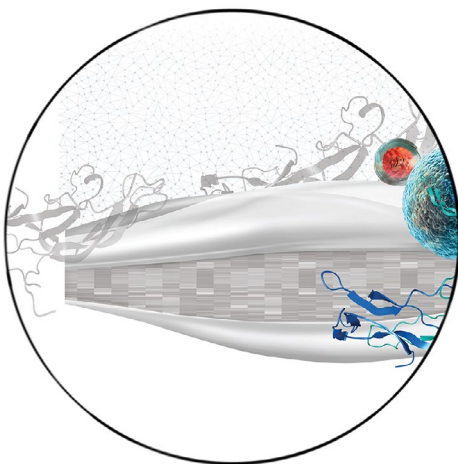


## Proteomics

## The evolving role of mass spectrometry in proteomics research

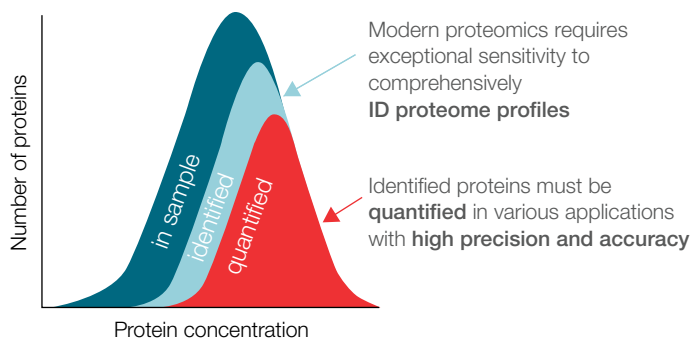
### Introduction: The evolution and impact of Orbitrap technology in proteomics

Thermo Scientific™ Orbitrap™ mass spectrometers have revolutionized proteomics, establishing themselves as essential liquid chromatography-mass spectrometry (LC-MS) platforms. Used in over 135,000 peer reviewed articles, their impact on the field is undeniable. These instruments are renowned for their high resolution and mass accuracy, making them indispensable for proteome exploration, enabling precise identification and quantification of proteins, analysis of post-translational modifications (PTMs), and study of protein-protein interactions.



The evolution of Orbitrap-based mass spectrometers has been marked by continuous innovation, with each generation bringing enhanced capabilities. Modern proteomics has transformed from purely qualitative analysis to a comprehensive approach combining both qualitative and quantitative measurements. This advancement ensures precise protein identification and high-quality quantitative data, essential for meaningful biological insights.

The Thermo Scientific™ Orbitrap Exploris™ Series instruments have been cornerstones in many proteomics laboratories, offering robust and reliable performance for various applications. For instance, the Thermo Scientific™ Orbitrap Exploris™ 480 Mass Spectrometer is crucial for quantitative proteomics due to its exceptional sensitivity, resolution, and mass accuracy. It allows precise and accurate quantification of proteins in complex biological samples, enabling in-depth proteomic analyses. Advanced features such as high-resolution accurate mass (HRAM), fast scan rates, and robust data acquisition capabilities facilitate the identification and quantification of thousands of proteins in a single experiment. This capability is vital for understanding protein expression patterns, PTMs, and protein interactions, advancing research in disease mechanisms, biomarker discovery, and therapeutic development.



**Figure 1. The paradigm has shifted in proteomics.** While it began as an almost exclusively qualitative technique, modern proteomics has evolved to span a continuum of qualitative and quantitative approaches.

Thermo Scientific™ Orbitrap™ technology is pivotal in both targeted and discovery proteomics. The Thermo Scientific™ Orbitrap™ Excedion™ Pro Hybrid Mass Spectrometer excels in both areas, offering high sensitivity and dynamic range for targeted proteomics, while its advanced fragmentation techniques and HRAM capabilities are ideal for comprehensive discovery proteomics.

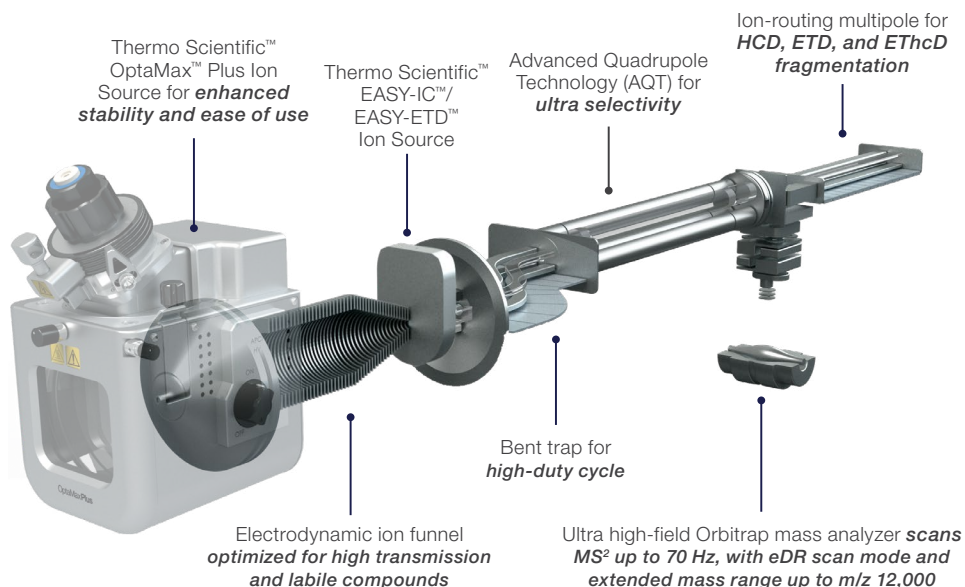
### Higher sensitivity and new capabilities

- Higher sensitivity and enhanced dynamic range with eDR scan mode
- Hybrid DIA workflows allow for combination of discovery and precise quantitation in a single workflow
- Increased sequence coverage with alternative EASY-ETD (electron-transfer induced dissociation) / EThcD (electron-transfer/higher-energy collision dissociation) fragmentation



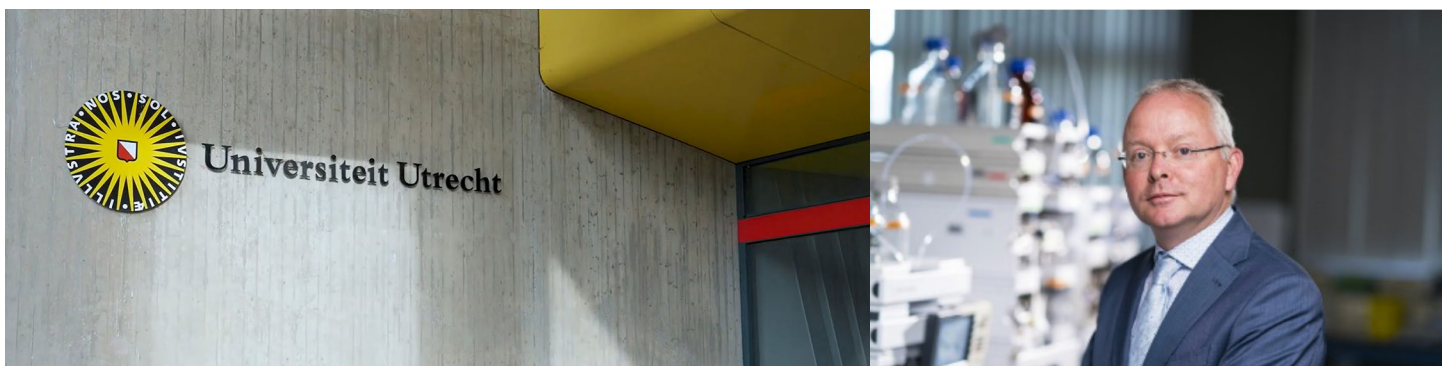
**Figure 2. The Orbitrap Excedion Pro mass spectrometer includes enhanced capabilities for proteomics including accurate quantification with extended dynamic range, Hybrid-DIA supported by an adaptive RT routine, deep proteome coverage, and PTMs and immunopeptidomics analysis with EThcD fragmentation.**

These advancements make the Orbitrap Excedion Pro mass spectrometer an invaluable platform for next-generation proteomics studies, enabling comprehensive characterization of protein-ligand and protein-protein interactions, improved detection of low-abundance peptides, and deep insights into complex biological samples.



**Figure 3. The advanced features of Orbitrap Excedion Pro mass spectrometers provide improved sensitivity, an extended dynamic range, a broader mass range, and exceptional mass accuracy, building upon the capabilities of the legacy Orbitrap Exploris 480 mass spectrometer.** These features work together to ensure the precise identification and quantification of analytes, making Orbitrap Excedion Pro mass spectrometers the definitive choice for cutting-edge mass spectrometry in their class.

# Case study: Albert Heck's lab and its cutting-edge proteomics research

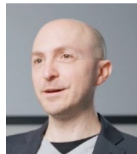


## Meet the featured researchers from Utrecht University



**Albert Heck**

*Professor, Utrecht University, the Netherlands  
Scientific Director of the Netherlands  
Proteomics Center*



**Fabio Marino**

*Assistant Professor  
Utrecht University, the Netherlands*



**Amy Kessler**

*Postdoctoral Researcher  
Utrecht University, the Netherlands*

Albert Heck is a professor at Utrecht University and the Scientific Director of the Netherlands Proteomics Center and leads a research group in Biomolecular Mass Spectrometry and Proteomics.

The lab's overarching goal is pushing the boundaries of proteomics capabilities through innovative mass spectrometry methods, enabling deeper biological insights. Recently, the team integrated the Orbitrap Excedion Pro mass spectrometer into their workflows, significantly enhancing their research capabilities. The broad research scope of the lab necessitates versatile tools to investigate a wide range of protein-related phenomena.

Learn how he and other researchers are leveraging the advanced benefits of the Orbitrap Excedion Pro mass spectrometer. Their research focuses on various aspects of proteomics, including:

- Protein interactions and PTMs.
- *De novo* sequencing of proteins and peptides, including modified peptides.
- Immunopeptidomics, particularly in accurately characterizing MHC peptides for therapeutic applications.
- Detailed characterization of glycoproteins and glycopeptides.
- Application of advanced mass spectrometry technologies such as electron-transfer induced dissociation (ETD), and combined fragmentation techniques (ETHcD) for high-quality sequencing.
- Intact protein analysis, including antibody biology, lipoproteins, antibody-drug conjugates, and protein-centric proteomics.
- Exploration of previously neglected biological interactions and modifications, such as those involving lipids, drugs, DNA adducts, and novel post-translational modifications.

“The Orbitrap Excedion Pro MS has significantly improved our ability to identify novel targets and explore previously neglected areas of biology.”

–Albert Heck, Professor, Utrecht University, the Netherlands

The Heck group benefits from the Orbitrap Excedion Pro MS for several key reasons:

- 1. Advanced fragmentation capabilities:** The instrument enables efficient EThcD, which combines higher-energy collision-induced dissociation and electron transfer dissociation simultaneously. This provides higher quality MS<sup>2</sup> spectra and better sequence information on biomolecules.
- 2. De novo sequencing:** The instrument allows the sequencing of proteins without using genome templates, which is crucial for understanding glycobiology and antibody biology.
- 3. Modified peptide analysis:** The enhanced capabilities enable better analysis of modified peptides, glycopeptides, MHC peptides, and intact proteins with improved accuracy and depth coverage.
- 4. Novel research areas:** The instrument enables them to investigate:
  - Unexpected protein modifications
  - Glycoproteomics
  - Protein-centric proteomics
  - Lipoproteins
  - DNA-protein adducts
  - Antibody-drug conjugates
  - Crosstalk between PTMs
  - Interactions between proteins and other biomolecules

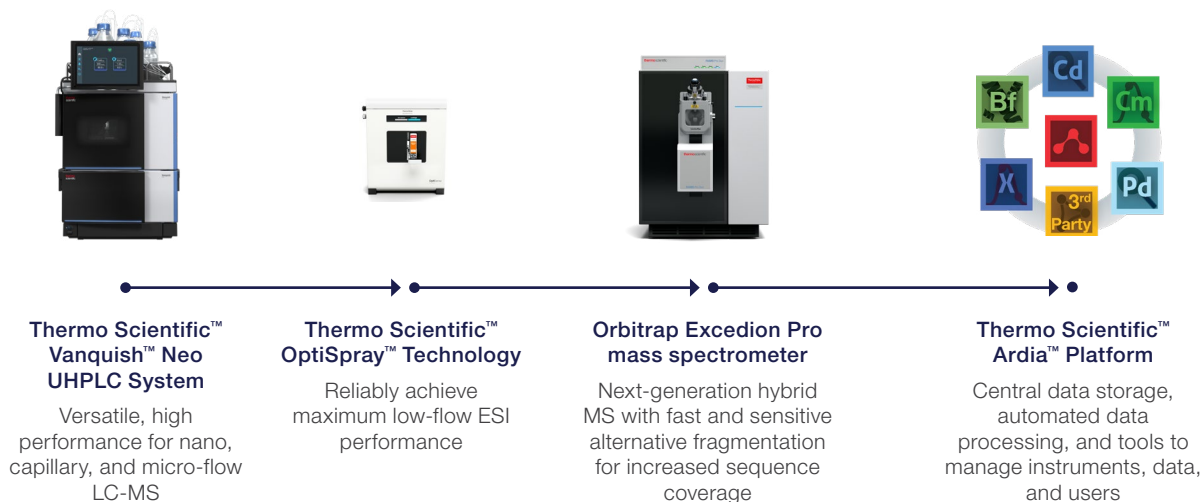
The Orbitrap Excedion Pro mass spectrometer's combination of robust performance and advanced capabilities allows the Heck group to push the boundaries of proteomics research into previously unexplored areas of biology.

The research group faced several pain points with their previous mass spectrometry tools. Former instruments often fell short in providing comprehensive data, especially for complex protein modifications and interactions. Incomplete sequence coverage sometimes led to false positives, which could be costly in the context of therapeutic development. Additionally, traditional instruments did not efficiently combine multiple fragmentation techniques or incorporate automatic reaction time scaling.

The Orbitrap Excedion Pro mass spectrometer addresses these challenges by offering advanced capabilities that align with the researchers' needs. In addition, the instrument allows flexible workflows with multiple fragmentation techniques without compromising the duty cycle of the instrument.

### New capabilities for targeted and discovery proteomics

- Accurate quantification with extended dynamic range
- Hybrid-DIA, parallel reaction monitoring (PRM), and DIA in a single experiment
- PTMs analysis with EThcD fragmentation
- Immunopeptidomics with EThcD fragmentation
- Deeper proteome coverage



**Figure 4. The Orbitrap Excedion Pro mass spectrometer is more than an instrument that provides extraordinary results.** It is part of a next-generation end-to-end Thermo Scientific™ workflow that brings together best-in-class components covering everything from sample preparation, liquid chromatography, and ion mobility to data acquisition and interpretation. In addition, our Unity™ Lab Services team delivers world-class service solutions to support your instruments.

“The Orbitrap Excedion Pro MS has been truly transformative for our research. Its advanced fragmentation capabilities and flexibility have allowed us to push the boundaries in proteomics and detect sequences that so far have been elusive.”

–Amy Kessler, Postdoctoral Researcher, Utrecht University

## Q&A with Albert Heck and other researchers at the University of Utrecht

Q: What challenges did you face with your previous mass spectrometry tools?



**Albert Heck:** Our previous instruments often fell short in providing comprehensive data, especially for complex protein modifications. We struggled with incomplete sequence coverage, which sometimes led to false positives, and the inability to efficiently combine multiple fragmentation techniques limited the scope of our research.

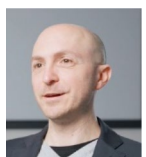
Q: How has the Orbitrap Excedion Pro MS addressed these challenges?

**Albert Heck:** The Orbitrap Excedion Pro MS has significantly improved our ability to identify novel targets and explore previously neglected areas of biology. Its hybrid fragmentation capabilities with HCD and ETD have provided us with high-quality spectra and comprehensive sequence coverage, allowing us to study novel proteins and complex modifications more effectively.

Q: Can you describe the impact of the Orbitrap Excedion Pro MS on your research?

**Albert Heck:** The Orbitrap Excedion Pro MS has enabled us to delve deeper into the crosstalk between post-translational modifications and interactions between proteins and other biomolecules. This has opened up new possibilities for studying modifications that we hadn't looked at before, such as those between drugs and proteins or lipids and proteins.

Q: What specific features of the Orbitrap Excedion Pro MS have been most beneficial for your work?



**Fabio Marino:** The instrument's high efficiency and beautiful spectra have been particularly beneficial. We can now sequence peptides without relying on DNA templates, which has expanded our research capabilities. Moreover, we can identify with certainty modifications that we had previously missed. The flexibility to use multiple fragmentation techniques without compromising the instrument's duty cycle has been a significant advantage.

Q: How does the Orbitrap Excedion Pro MS compare to previous mass spectrometers you have used?



**Albert Heck:** Compared to previous models, such as the Orbitrap Exploris and QTOF mass spectrometers, the Orbitrap Excedion Pro MS offers significant improvements in data quality. It provides deeper protein sequence coverage and improved PTM characterization.

Q: Can you share any specific examples of how the Orbitrap Excedion Pro MS has impacted your research?



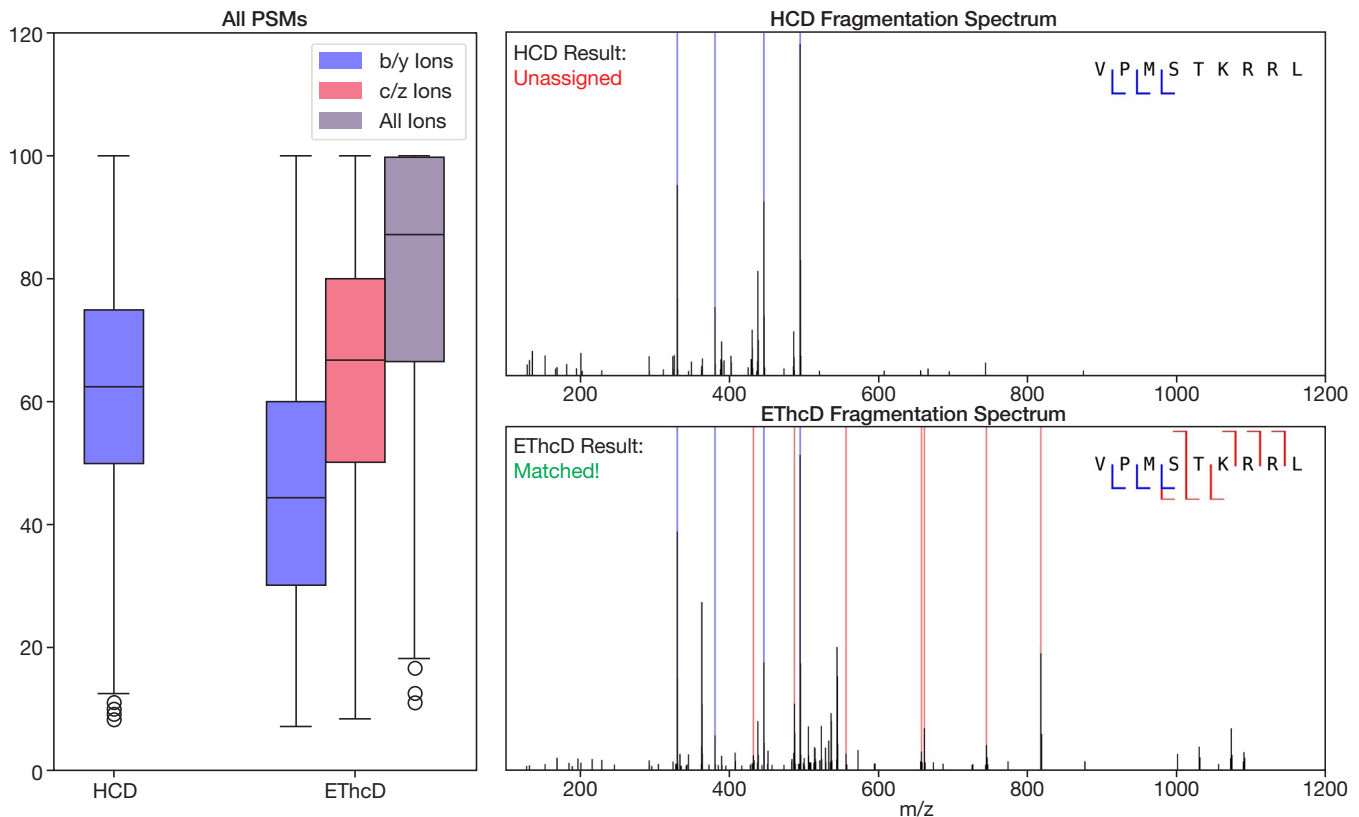
**Amy Kessler:** The Orbitrap Excedion Pro MS has been truly transformative for our research. Its advanced and efficient fragmentation capabilities have allowed us to push the boundaries of what is possible in proteomics. This instrument provides us with the tools we need to make groundbreaking discoveries and advance our understanding of complex biological systems.



**For more information** on how the Orbitrap Excedion Pro mass spectrometer can enhance your proteomics research, please contact Thermo Fisher Scientific.

“The instrument's high efficiency and beautiful spectra have been particularly beneficial. We can now sequence peptides without relying on DNA templates, which has expanded our research capabilities. Moreover, we can identify with certainty modifications that we had previously missed.”

–Fabio Marino, Assistant Professor, Utrecht University



**Figure 5. Sequence coverage of all PSMs (left panel) from GR cell analysis using either HCD fragmentation or ETHcD fragmentation.**

Improved sequence coverage and increased selectivity demonstrated in GR cell analysis using ETHcD fragmentation. The sequence coverage of all PSMs for GR cell analysis with HCD and ETHcD is shown in the left panel. ETHcD shows higher sequence coverage due to the complementarity of b/y ions (blue boxplot) and c/z ions (red boxplot) when all ion types are included in the search (purple boxplot). This increased sequence coverage increases the selectivity for the analysis of immunopeptides. In the example shown here, HCD fragmentation (right panel, top spectrum) shows limited sequence coverage and the data analysis software is unable to assign the spectrum, i.e., a false negative. ETHcD fragmentation (right panel, bottom spectrum) shows a full sequence ladder of fragment ions for this precursor. These combined b/y and c/z ions provide full sequence coverage, allowing for confident spectral assignment. Moreover, these contiguous fragments and the resulting high sequence coverage they afford are vital for *de novo* sequencing. Annotated fragments were assigned by matching theoretical fragments with 10 ppm mass accuracy and confirmation of the fragment charge state. Sequence coverage was calculated using these annotated fragments.

Sample: Immunopeptides from GR cell and JY cells, courtesy of Professor Dr. Albert Heck

## Conclusion

The Orbitrap Excedion Pro mass spectrometer is a game-changer for researchers in proteomics. It meets the needs of the most demanding research environments by providing

advanced fragmentation techniques, user-friendly operation, and unparalleled flexibility. This instrument is poised to drive significant advancements in the field, enabling researchers to uncover new insights and make groundbreaking discoveries.

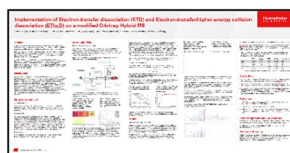
## Additional resources

Learn more about the work that Albert Heck and his lab are doing at the Netherlands Proteomics Center.



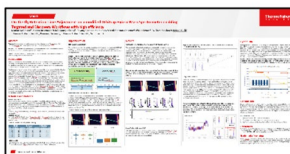
Watch video

Learn more about the benefits of Orbitrap Excedion Pro mass spectrometer for Proteomics, Metabolomics, and BioPharma.



Download technical poster

Implementation of electron-transfer dissociation (ETD) and electron-transfer/higher-energy collision dissociation (EThcD) on a modified Orbitrap Hybrid mass spectrometer.



Download technical poster

Capture scheduled retention time window shifts in large scale peptide quantitation using a modified Orbitrap hybrid mass spectrometer.



Visit website

Explore the enhanced features of the Orbitrap Excedion Pro MS that unlock new possibilities for omics analysis.



Download technical note

Hybrid DIA on the Orbitrap Excedion Pro mass spectrometer: Bridging global depth and targeted sensitivity for proteomic analysis



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