Broad Institute
of MIT and Harvard

Customer Insights

Driving advances in immunopeptidomics research to understand disease pathways

The Broad Institute's Proteomics Platform is using trapped ion mobility spectrometry (TIMS) for the technical advancement of proteomics.

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Working with Bruker
Researchers at the Broad Institute of MIT and Harvard are developing and applying advanced proteomics methods to gain further understanding of disease pathways, targets and drug effects, using TIMS.

"Having early access to the latest technology developments means we can really push the boundaries of what can be achieved."

The Proteomics Platform

Claudia Ctortecka Ph.D. is a Postdoctoral Fellow at the Broad Institute's Proteomics Platform, led by founder and Senior Director Steven Carr Ph.D., an internationally recognized leader in the development and application of novel proteomics methods. The 5,000 sq. ft. wet chemistry and instrument laboratory Proteomics Platform houses TIMS time of flight (TOF) systems including the Bruker timsTOF Ultra, timsTOF single cell proteomics (SCP) and the timsTOF high-throughput (HT) instruments.

Dr. Carr and his team of dedicated staff scientists, postdoctoral fellows, computational scientists and students forge collaborative partnerships across the institute's expansive network to provide mass spectrometry (MS)-based proteomics analysis capabilities that leverage cutting-edge technology to answer pressing questions in biology, chemistry and clinical sciences. A core aspect of the group's work includes the quantification of proteins and their modifications from bulk cells, tissues and biofluids down to the single cell level. To achieve this, they apply cutting-edge high throughput and highly sensitive mass spectrometric methods and enrichment approaches to enhance peptide and protein identifications with the goal to understand disease biology and drug effects at the mechanistic level.

At the Proteomics Platform, Dr. Ctortecka's work is focused on workflow development. She is driving the technical advancement of proteomics, post-translational modifications (PTM) analysis and immunopeptidomics MS acquisition towards sensitive profiling of small samples, like tissue biopsies. Based on her doctoral work, Dr. Ctortecka continues to move into a more applications-focused approach to lower input requirements with the eventual goal of resolving cell signaling for a better understanding of tumor development and progression.

Tackling the immunopeptidomics challenge

The study of immunopeptidomics focuses on the identification and quantification of immunopeptides, protein fragments displayed by major histocompatibility complex (MHC) molecules to the immune system. Immunopeptidomics probes the cellular mechanisms involved in peptide antigen processing and presentation, which is crucial for the advancement of T cell-based therapies and to study immune homeostasis. Immunopeptidomics offers the potential to uncover the extensive catalog of peptides that can be recognized by T-cells, uncovering insights into the immune system's recognition of these molecules. Additionally, immunopeptidomics research could help identify peptides that trigger an immune response, for the development of immunotherapies against cancer, autoimmune disease, and infectious diseases.

Typically, the main challenge posed by immunopeptidomics are the intricacies of capturing the relevant biochemical context extracting and identifying peptides presented by MHC molecules from complex biological samples such as tumor tissue or cells. Achieving this selective extraction while preserving the integrity and representation of the peptides is a daunting task. Tumor tissue, for example, is heterogeneous and contains a multitude of cell types with varying MHC expression profiles [3, 4, 5].

Furthermore, the abundance of MHC-bound peptides relative to the total cellular proteome is typically low, which poses a sensitivity challenge. PTMs amplify the difficulty of isolating and characterizing the immunopeptidome accurately. Researchers must employ sophisticated enrichment strategies and sensitive analytical techniques such as timsTOF MS leveraging its efficient ion utilization enabled by trapped ion mobility spectrometry to overcome this limitation.

Sensitive high throughput immunopeptidomics

At the Proteomics Platform, Dr. Ctortecka performs in-depth MS-based profiling of human leukocyte antigen (HLA) peptides, immunopeptidomics, as part of the 'HLA team' led by Dr. Jenn Abelin and Dr. Steven A. Carr (see in group photo below), a task that requires a large amount of input material in order to achieve the required depth of analysis to detect clinically relevant T cells antigens like neoantigens. Off-line fractionation can be used to improve coverage and immunopeptidome depth, but clinical samples are often input limited and therefore not amenable to such workflows [4]. The need for fractionation and associated increase in instrument analysis time is also a challenge for applying immunopeptidomics on large cohorts of samples.

Dr. Claudia Ctortecka, Ph.D., Postdoctoral Fellow, Proteomics Platform, Broad Institute of MIT and Harvard.

Dr. Ctortecka has been conducting research in MS for over eight years and has been fascinated with the technology since early in her career. She studied SC proteomes with Karl Mechtler and Sasha Mendjan at the Vienna Biocenter, focusing during her doctoral studies on dissecting the proteome of single mammalian cells. In one of her projects, Dr. Ctortecka studied early cardiac development and associated diseases resolving related processes at the level of individual cells [1]. Dr. Ctortecka also worked on automation and low volume pipetting and sample preparation for SCP together with a diverse team of specialists driven by Dr. Anjali Seth to help develop high-throughput processing at single cell level [2]. She explains:

"When I first started out, we relied on multiplexing to enable a high enough throughput which meant sacrificing sensitivity. Our research required a lot of technology and workflow development – tweaking instruments, breaking equipment apart and putting it back together again to try out new ideas. It was exciting to be involved in the early stages of advanced SCP research."

Dr. Ctortecka chose to move to the Carr proteomics group at the Broad Institute:
"I felt I had learned a lot and, as a next step, wanted to move into a more applications-focused role. Dr. Carr's lab is a key center for application-focused proteomics. We are fortunate to be constantly approached by diverse collaboration partners who ask interesting biological questions on a daily basis."

Carr and his team therefore developed a highly sensitive MS workflow for direct identification of HLA peptides from patient-derived tumor samples or cell lines [5].

In this paper, Dr. Ctortecka together with another postdoctoral researcher, Dr. Phulphagar investigated the use of a high-throughput, sensitive, and single-shot MS-based immuno-peptidomics workflow that leverages ion mobility separation. It demonstrated >2-fold increase in identified HLA-I peptides and propose multiple collision energy slopes for samples with different HLA-I peptide-binding motifs [2].

Dr. Ctortecka explains:

"The goal was to push the sensitivity limitations of what was possible. For this we invested a lot of time into optimizing acquisition parameters and in-depth data analysis to get the best out of the data."

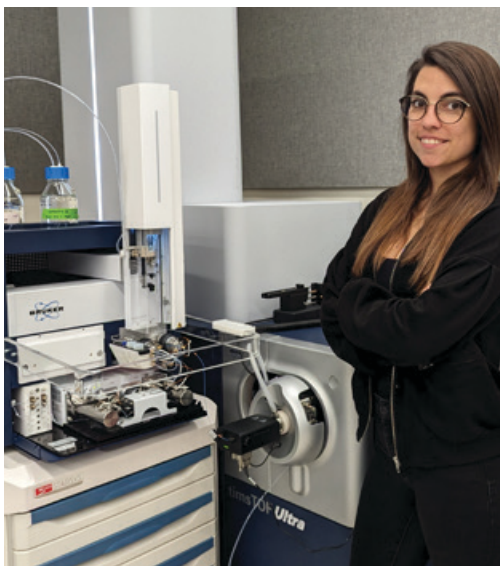
"We have demonstrated that we can increase throughput by overcoming the need to fractionate the samples. I think one of the reasons for this besides the increased scanning speed, is that we can efficiently leverage singly charged precursors. We are still investigating how well we can do this and how much information we can get out of them, but it is the selection of peptide-like singly charged species using their collisional cross section (CCS) that is helping us unlock this new potential."

New discovery through scientific collaborations

The Proteomics Platform is regularly approached by collaborators in its network to engage in new research. Dr Ctortecka describes one of her current projects:

"I am currently working with a neurobiology group at Harvard to perform sub-cellular proteomics of axons - something which I didn't think would ever be possible. With a couple of modifications to the workflow we were using I was able to push the proteome depth to get really good coverage.

These results were beyond anything that had been previously achieved, even from bulk samples. If you combine the right technologies, it is incredible how far you can actually push the boundaries of research."



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In her work, Dr. Ctortecka together with a postdoctoral researcher Dr. Dominguez Iturza from the group of Dr. Paola Arlotta, is looking into the specific interactions between axons and oligodendrocytes, to study how the two cell types interact. The Arlotta research group explores the establishment and maintenance of cellular diversity and their contribution to cortical function. They primarily focus on gaining a fundamental understanding of the principles that govern normal cortical development and study mechanisms of human neurodevelopmental diseases. Neurodegenerative diseases and motor disabilities have been linked to abnormal myelin sheath formation around axons by oligodendrocytes in the central nervous system. A sub-cellular resolution on the proteome level is therefore required to study how these interactions occur and how myelin patterns appear.

Using data independent acquisition for complete datasets

Dr. Ctortecka describes how data independent acquisition supports her research:

"The only way to reduce missing data across large datasets is by using data independent acquisition (DIA). If you have very low sample input, this is currently the best option."

TIMS-TOF MS separates ions according to their size and shape, known as their CCS values, to capture an additional physicochemical dimension of biomolecules, enhancing sensitivity and peak capacity. This enables accurate monitoring of low-abundance molecules such as HLA bound peptides distinguishing even highly similar peptides exploiting mobility offset mass aligned (MOMA) capabilities [3, 4, 5].

Parallel accumulation serial fragmentation (PASEF) is a TOF MS-based acquisition method enabled by TIMS and employed for fast peptide separation and more confident identification. Together, the specificity, sensitivity, and high speed of MOMA and PASEF results in confident identification of HLA-I and HLA-II bound peptides though TIMS in a single run.

"We wanted to achieve the best immunopeptidomics depth possible at high confidence and with high throughput. We therefore embarked on a process of technology and method development. Throughput is one of our biggest bottlenecks as many of our projects are in the range of 100-200 patient samples where we are processing and analyzing HLA class I and class II. This results not only in a lot of measurement time but also many relative comparisons between the patients. Using TIMS-TOF technology allowed us to speed up acquisition even further, reproducibly boosting our identifications."

"We push the boundaries in sample data acquisition, conducting thorough method development, we are involved in early testing of new options, always with the goal of achieving high-throughput and high reproducibility in a robust analysis.

We understand that our samples are often very different from normal tryptic peptides or standard cell lines, especially as we are working with rare patient samples. We feel the need to also focus the method development on real samples rather than over optimize methods that won't help once they are translated into the clinical environment."

What's next?

Dr. Ctortecka's hope for the future is to see MS integrated in the clinic to take a tissue biopsy and process it directly with a standardized workflow. However, in immunopeptidomics this would pose a major challenge, particularly in data analysis. She describes the right instrumentation as a crucial step:

"To have the combination of highly sensitive instrumentation, and thorough data analysis that provides information useable by clinicians directly in the clinic would be the ideal end goal."

Dr. Ctortecka also identifies data integration as a challenge for future research, as the volume of data generated has jumped from volumes in the megabytes (MB) to levels in gigabytes (GB):

"There is still so much potential to be explored with the instruments.

We have just begun some work on more diverse PTM analysis that is not yet routine and we have been able to run shorter chromatographic gradients and achieve higher depth – with minimal optimization but rather informed acquisition parameter selection based on our experience."

With recent advancements in TIMS and PASEF researchers now have the ability to explore the immunopeptidome with minimal sample quantities, such as clinical material from fine needle biopsies, paving the way for groundbreaking discoveries that have the potential to drive personalized medicine forward. PASEF technology, facilitated by TIMS, streamlines the isolation and detection ions, providing substantial benefits for acquiring immunopeptidomics data. Benefits of this include enhanced peptide separation, differentiation of closely resembling peptides and comprehensive profiling of the peptidome. Together, these capabilities will propel the understanding of intricate biological systems and hold promise for advancements in immunotherapy.

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