
**Roberts Laboratory**

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# Customer Insights

## Collisional cross section (CCS) – an essential element in advancing proteomics research at Emory University

Data from Bruker's timsTOF fleX is enabling advances in disease-related biology that look set to improve diagnosis and therapeutic decisions for patients with neurodegenerative conditions.

**Working with Bruker**

As a cutting-edge research lab that relies on best-in-class instrumentation for all of its daily work, the reliability of the instrumentation – and the relationship between the lab and a vendor – are critically important. Dr. Blaine Roberts explains:

*" Bruker's timsTOF is so easy to use; we train postdocs and graduate students and they can basically just walk up and use it. There aren't a million buttons to press or choices to make. It's just, load your liquid chromatography (LC) method, load your mass spectrometry (MS) conditions, hit run, label everything appropriately and you're done. So that's been great."*

## Introduction to plasma proteomics

The first studies of proteins that could be regarded as proteomics began in 1975, when researchers initiated mapping the proteins from the bacterium *Escherichia coli* using 2D gel electrophoresis. However, the term 'proteomics' was coined in 1994 by then-PhD student Marc Wilkins at Macquarie University [1] who went on to establish the first dedicated proteomics laboratory in 1995.

Interest grew, not only in establishing the significance of proteomics for basic research into diseases, but also in its use as a tool for developing biomarkers and informing drug discovery.

In 2003, the Human Protein Atlas (HPA) program started with the goal of mapping all the human proteins in cells, tissues and organs using various omics technologies, including antibody-based imaging, MS based proteomics, transcriptomics and systems biology. The resulting knowledge resource allows scientists both in academia and industry to freely access the data for exploration of the human proteome. Twenty years on, in June 2023, version 23 of the HPA was launched where a new 'interaction' section was introduced containing human protein-protein interaction networks for more than 11,000 genes that will add new aspects in terms of protein function.

The potential dataset is vast so, as a result, researchers have often narrowed their focus to work on a specific subset of the proteome or a particular disease state. For example, in recent years, characterizing the human plasma proteome has become a major goal. It is considered to be one of the most challenging proteomes to decipher as it contains immunoglobulins, cytokines, protein hormones, and secreted proteins indicative of infection on top of resident, hemostatic proteins. It also contains tissue leakage proteins accumulated as the blood circulates through different tissues in the body. As a result, the blood can be considered to contain information on the physiological state of all tissues. The depth of the plasma proteome covers a dynamic range of more than $10^{12}$ between the highest abundant protein (albumin) and the lowest (some cytokines). In addition, temporal and spatial dynamics further complicate the study of the human plasma proteome.

Of course, other sample types – tissue extracts, cerebrospinal fluid (CSF), and urine, for example – are also interesting material for investigation, and each poses individual analytical challenges. Importantly, as analytical and informatics technologies have advanced, scientists have gained additional tools and significant progress has been made in many areas of research, with proteomics in neurodegenerative diseases arguably at the forefront of this work.

## Driven by a need for understanding

Against this background, Dr. Blaine Roberts, Associate Professor and Principal Investigator, Roberts Laboratory, Department of Biochemistry and Department Neurology at Emory University School of Medicine, Atlanta, Georgia, developed a long-standing interest in understanding the role of metals in biology. His recent research has focused on developing new proteomics technologies to measure metalloproteins in order to better understand Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS). In addition, his research encompasses the use of proteomics to discover and characterize new blood-borne biomarkers for Alzheimer's and Parkinson's disease.

Since 2019, Dr. Roberts has led his own research group of seven scientists at the Emory University School of Medicine, but his interest in the field started back in 2007 when he was completing PhD work in in Prof. Joseph Beckman's lab at Oregon State University.

He explains: *"Our goal then was to investigate the protein structure of the superoxide-scavenging enzyme copper-zinc superoxide dismutase (SOD) and look for features that might contribute to the dysfunction of the enzyme in patients with ALS. We found that the mutations cause a loss of zinc from the SOD enzyme which led to the reaction going backwards. The enzyme will actually produce superoxide instead of scavenging it. The central nervous system (CNS) produces a lot of nitric oxide, which reacts with superoxide at diffusion limited rates – as fast as any two molecules can – to produce peroxynitrate and that can go on to induce cell death."*

A post-doc position with Prof. Colin Masters at the University of Melbourne, Australia not only allowed Dr. Roberts to develop his interest in metalloproteins further by developing tools and techniques for metalloproteomics, but also initiated a new line of research that would subsequently form an important theme when he established The Roberts Lab. He began work on blood-based diagnostics for Alzheimer's disease using samples collected as part of a study called the Australian Imaging Biomarker and Lifestyle Study of Ageing.

Later, and as a further adjunct to his original work with SOD in a subsequent publication in 2021 [2], Dr. Roberts and co-workers reported on research that had mapped the unfolding of the SOD enzyme molecule using trapped ion mobility spectrometry (TIMS).

Commenting on the obvious connections and sharp focus of his work, Dr. Roberts explains: *"We are always building on and making connections with existing knowledge and trying to extend our understanding, it's like unravelling a tangled ball of string, we keep pulling on the loose end to discover the next part of the challenge… that's what makes it fun!"*

## The Roberts Lab

Today, there are three main areas of active research in The Roberts Lab. One is a continuation of the work on metalloproteomics, the second is concerned with mechanisms in neurodegeneration where, for example, the group is investigating the role of amyloid beta in Alzheimer's disease. Finally, the third focus is on continuing and developing Dr. Roberts' original work on blood-based biomarkers and diagnostics, exploring further the use of red blood cells rather than plasma as the sample of choice as they search for diagnostic markers for Parkinson's disease, and working towards having a standard diagnostic enzyme-linked immunosorbent assay (ELISA) test format available for clinical use.

Dr. Roberts explains:

*"All of our current work, and work going forward, relies on the Bruker timsTOF fleX. It's a primary resource – not just because of its sensitivity and the fact that it's very easy to hook up and run, but also because we have the ion mobility spectrometry (IMS) and matrix assisted laser desorption/ionization (MALDI) imaging built seamlessly into the same system."*

He continues: *"If we are looking at isomers of amyloid beta for example, they are literally the exact same mass. Although MS can't differentiate them, we can rely on changes in chromatography and ion mobility to see the isomerization at two different positions within the peptide. Isomerization at one position puts a kink in the peptides that we can resolve using the collisional cross-section (CCS) measurement built into the timsTOF fleX."*

The next piece of the puzzle for Dr. Roberts is to image amyloid beta in tissue to be able to see how those isomers are localized. There are unanswered questions regarding if they are localised exclusively around plaques, or uniformly distributed in the grey matter, for example:

*"The timsTOF fleX's MALDI imaging capabilities is the only commercially available solution that provides ion mobility measurement with MALDI. This has allowed us to image amyloid beta isomers which is not possible without the combination of ion mobility and MALDI."*

## The impact of timsTOF fleX and CCS at The Roberts Lab

A comprehensive suite of analytical technologies – chromatography systems plus a range of spectrometers, coupled to MS and tandem MS (MS/MS) detectors, for example, make up The Roberts Lab infrastructure. In 2021, these were supplemented by the purchase of a Bruker timsTOF fleX, adding TIMS and MALDI imaging capabilities for the first time.

While native MS is an excellent tool for probing protein-ligand complexation or protein binding, MS alone lacks the ability to give definite measurements of the relative molecular size of those complexes. IMS has been widely applied over the last five decades, primarily in chemical physics and analytical chemistry applications. Only relatively recently has the potential of IMS coupled to MS (IMS-MS) been explored for the separation, identification and quantification of peptides and proteins. With the introduction of the timsTOF platform in 2017 Bruker introduced the next evolution in proteomics technology. The addition of CCS measurement drives the transition from 3D-proteomics (retention time, mass to charge (*m/z*) and MS/MS fragment ion spectra) to 4D-Proteomics.

Dr. Roberts comments:

*"IMS is a powerful complement to MS analyses, as the simultaneous measurements allow for an effective 4D separation of proteins based on size, charge, and mass in a millisecond time frame. The ion mobility measurements can be used to determine the ion specific CCS, a molecular descriptor that gives insightful information about the size of a molecule."*

The Bruker timsTOF platform uses TIMS, where ions are propelled through the TIMS tunnel by a gas flow. An electrical field controls each ion from moving beyond a position defined by the ion's mobility. Ramping down the electrical field allows the selective release of ions from the TIMS tunnel according to their mobility. The high degree of selectivity and the design of the TIMS allows for the enhanced speed through parallel accumulation and serial fragmentation (PASEF) of precursor peptide ions. With PASEF, the TIMS design enables the reproducible measurement of CCS values for all detected ions. These CCS values further increase the systems selectivity, resulting in more reliable quantitation in complex samples and short gradient analyses.

Dr. Roberts confirms: *"The CCS values are really important both in our red blood cell work and the protein work we are doing. For our Alzheimer's disease research, having the CCS values helps to confirm what isomer or modification we're looking at. And similarly in the peptide space, having that extra characterization of the CCS with the mass and the fragmentation all adds up to give us higher confidence. Finally, what will be really interesting is using CCS in the imaging space as we start to image some of these biomolecules."*

In practice working with the timsTOF fleX, Dr. Roberts' team has found the system to be straightforward to use, sensitive, robust, and flexible. He explains:

*"The instrument was incredibly easy to set up and start running. Because the method is basically "baked-in" there aren't weeks or months of optimising methods to make sure we've got all the parameters perfect and we're getting the best out of our system and workflows."*

## Combining high MALDI sensitivity with TIMS capability

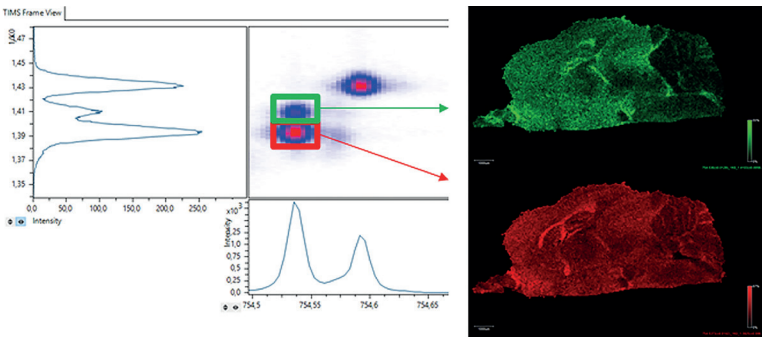
The timsTOF fleX provides users with all the analytical advantages of Bruker's timsTOF platform and in addition, integrates rapid, high-resolution MALDI imaging within the same instrument to give researchers the unique ability to accurately correlate measured results with morphological context.


The result is a true spatial 'omics platform. Applications are widespread. For example, the need to separate isobaric or isomeric molecules and get the true spatial localization of the analytes is a common challenge. The timsTOF fleX offers the only chance to differentiate isomeric distributions where high mass resolution fails. Using the CCS, the identity of your analyte can be validated with an additional quality criterion. CCS-enabled software intelligently matches spatial MALDI-TIMS imaging data with 'omics results and enables vital morphological context to ID-lists.

## Support from Bruker

Dr Roberts explains his experience working with Bruker:

*"As far as maintenance is concerned it's been a dream – we have had the timsTOF fleX for around three years now and maintenance-wise the instrument has been the best. We have had to do the least amount of maintenance on the timsTOF fleX of any of the mass spectrometers we have – and we have mass spectrometers from pretty much all the vendors."*




Isomer separation on the timsTOF fleX

Dr. Roberts enjoys a good working relationship with Bruker, commenting how the team has been very supportive:
*"Any of the challenges that we have had, particularly on the LC side, they've been able to respond quickly, and they've all been very helpful in getting things back on track. As far as supporting our research goes, all of our Bruker contacts are very enthusiastic to help. At a moment's notice we can get somebody on the phone to help us with a software issue or a query relating to how to process data for one of our workflows, for example."*

## Looking ahead

When asked to further comment on what is driving the next phase of his work, Dr. Roberts explains:

> *"With the timsTOF fleX we have not only the proven robust 4D Proteomics performance and the advantage of CCS measurement, but also the opportunity to add a spatial dimension to our analysis.*

*Now we know for sure that important molecular modifications are there we want to explore more; we want to discover how they're organized. Are they concentrated around the pathological structures and tissues? For example, looking at the brain, we want to know are they just in the grey matter? Are they just in the white matter? Can we somehow segregate spatially at a relevant tissue – or even cellular – level? This is very exciting work and we are looking forward to learning more."*

## References

[1] Wasinger VC, et al (1995). *Progress with gene-product mapping of the Mollicutes: Mycoplasma genitalium, Electrophoresis.* https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/elps.11501601185

[2] Butler K, et al (2021). *Utilizing ion mobility-mass spectrometry to investigate the unfolding pathway of Cu/Zn superoxide dismutase.* Sec. Analytical Chemistry, 09 February 2021. https://www.frontiersin.org/articles/10.3389/fchem.2021.614595/

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### About Roberts Laboratory

The Roberts Lab is part of the Emory University School of Medicine in Atlanta, Georgia. The lab's research is dedicated to using proteomics to understand Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. Specific interests include, understanding the role of metals in biology and developing new proteomic technologies to measure metalloproteins. In addition to this, the Roberts lab uses proteomics to characterize new blood borne biomarkers for Alzheimer's and Parkinson's disease.

For more information, please visit: **https://med.emory.edu/departments/biochemistry/research-labs/roberts/research.html**

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