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Walter and Eliza Hall Institute
(WEHI)

Customer Insights

Proteomics Technology Advances Discoveries in Healthy Development and Ageing Research

Innovation with Integrity

Mass Spectrometry

Proteomics Technology Advances Discoveries in Healthy Development and Ageing Research

Researchers apply the latest proteomics methods to understand how protein changes in our body influence health and disease.

Working with Bruker
Dr. Andrew Webb of the Walter and Eliza Hall Institute (WEHI), Melbourne, Australia, studies how the biological foundations laid down during gestation and childhood affect development, and how longer life expectancy presents new challenges for an ageing population. His team uses Bruker's timsTOF Pro, impact II and other mass spectrometry (MS) software and instrumentation.

"The MS technology developed by Bruker has allowed us to take on the challenge of high-throughput proteomic datasets, thus harnessing the powerful potential of proteomics for medical research."

About the Author

Dr. Andrew Webb is an associate professor and head of the WEHI Proteomics Laboratory. After receiving his PhD in Biochemistry at Monash University in 2005, Dr. Webb was trained in immunology, virology (Imperial College London) and mass spectrometry (University of Melbourne). He has helped to establish the laboratory as an integral collaborative resource, with the specific aim of applying the next generation of proteomics to important biological questions relevant to human health. In 2019, Dr. Webb also established the new Colonial Foundation Healthy Ageing Centre, a collaboration between WEHI and the Royal Melbourne Hospital. In pursuit of its objective to support medical research designed to find solutions to the disabilities of ageing, the Colonial Foundation has committed to grant $15 million over five years.

Biological Foundations of Ageing

The Walter and Eliza Hall Institute (WEHI) is one of Australia's leading biomedical research organizations, with an international reputation for performing highly influential basic and translational research. Among these research areas is the study of how the biological foundations laid down during gestation and childhood affect development, and how longer life expectancy presents new challenges for an ageing population.

WEHI's Advanced Technology and Biology division uses advanced and emerging technologies such as protoeomics to accelerate scientific discoveries for healthy development and ageing research. These technological advances and powerful computational resources enable

Dr. Webb and his team to obtain new insights for advancing medical research, to explain how diseases develop, spread, and respond to treatment. Dr. Webb explains:

"MS based proteomics is one of the most powerful scientific tools we have. As we're measuring the protein, metabolites and lipid molecules directly, we can see them misbehaving in action in the context of disease; we can see what happens through the design of our experiments. This technology also allows measurement of these molecules over time, giving us insights into the fundamental molecular causes of disease. As a result, our approach has evolved over the years. As students, we were generally taught that disease comes from genetic mutations.

We now know genetics is just half of the story, it's ultimately our genetics in combination with how they interact with our environment.

These two things come together to cause disease, and that's what proteomics enables us to measure."

Dr. Webb leads a team of researchers at the WEHI Proteomics Laboratory working to develop new techniques to advance basic laboratory research and clinical studies. The proteomics team is directly engaged in the conception, design, and implementation of collaborators' projects, and the application and further improvement of cutting-edge proteomics techniques that will drive and facilitate new discoveries in biology. Current research involves developing both accessible wet and dry lab tools with a focus on automation to further proteomics research and, in doing so, drastically increasing throughput and lowering the entry barriers for medical researchers. Dr. Webb explains:

"A big part of my job is to explain to other scientists what they can achieve with the inherent potential and limitations of proteomics technology. The complexity of MS and MS based proteomics is a major barrier. By nature, it is a very multi-disciplinary approach and multiple facets of science are needed to understand it well. It's my long-term dream to create the tools and approaches that will enable us to break down the barriers of this complexity, and enable many more researchers to access this incredible technology. Today, we continue to support our collaborators that come to us with biological and medical based questions, and we work through the scientific challenges and solve them together."

In 2015 Dr. Webb began building the WEHI Proteomics Laboratory from the ground up. Today it employs 14 researchers and encompasses 6,500 sq. ft. of laboratory space and 11 mass spectrometers, including 5 Bruker timsTOF Pro mass spectrometers. The establishment of the laboratory provided a unique opportunity for WEHI to invest in advanced proteomics technology. Dr. Webb describes this evolution:

"Proteomics can be done in a lot of different ways, and there's lots of breadth to the technology. We developed our laboratory to support the Institute through collaboration. My research covers a wide range of medical conditions because we're a collaborative laboratory for basic science and medical research."

The WEHI Proteomics Laboratory supports a diverse range of research that can be categorized into five key focus areas:

- **Structure**: Investigating protein structure using XL-MS, native and HDX MS
- **Interactions**: Elucidation of protein-protein and protein-drug interactions
- **Global Protein dynamics**: Investigating the mechanisms of protein function through quantitative measurements
- **Signalling**: Investigation of signalling pathways (using phosphoproteomics and ubiquitin enrichment strategies)
- **Biomarkers**: Identification of prognostic and diagnostic biomarkers of disease in biofluids.

Next-Generation Proteomics Technology

Clinical and biological phenotypes, including cellular responses to stimuli, arise from the composition of biomolecules and their organization in tissue and/or in cells. At present, understanding how these systems work at scale is neither comprehensive nor modellable, preventing prediction of phenotypes or cellular responses to stimuli.

This presents two related challenges; firstly in defining the type of molecular information needed to further understand, and secondly, creating the tools and methods required for measurement and analysis. Dr. Webb believes only through their combined development scientists will be able to make predictions from molecular measurements that move beyond what is achievable today.

The WEHI Proteomics Laboratory is tackling these challenges, working as a collaborative resource with the specific aim of applying next-generation proteomics to important biological questions relevant to human health. Advancements in sample preparation, instrumentation and bioinformatics are leading the team to a more comprehensive understanding of post-translational regulation, cell signaling and protein interactions. Dr. Webb describes the impact of next-generation proteomics technology on this research:

"One of the things that has emerged over time in my laboratory is the idea that we need to be interrogating and characterizing proteoforms; the forms of proteins that exist when you take into account post-translational modifications (PTMs) and other post-translational effects. We are heavily invested in understanding the physiological roles of proteoforms and developing techniques to better detect them. At the moment, we are using mass spectrometers, but we know that we're only just seeing the tip of the iceberg.

Whilst these instruments are incredibly sensitive, the dynamic range is the main limiting factor, as we know that the dynamic range that exists within cells and within many of the biofluids that we analyze is much larger than we can detect."

MS-based proteomics has become a powerful technology for the identification and quantification of thousands of proteins. However, previously the coverage of complete proteomes was challenging due to the limited speed, sensitivity, and resolution of mass spectrometers.

The Bruker timsTOF Pro introduced a novel design that allows for ions to be accumulated in the front section, while ions in the rear section are sequentially released depending on their ion mobility. This process, called Parallel Accumulation Serial Fragmentation (PASEF®), provides extremely high speed and sensitivity to reach new depths in shotgun proteomics and phosphoproteomics, using low sample amounts [1]. Dr. Webb describes the impact of the timsTOF Pro on his research:

"The Bruker timsTOF Pro has enabled me to tackle new research challenges head on.

We now have access to the technology that can generate very large-scale data sets in a much shorter time period, increasing the depth and quality of the experiments that we perform.

The timsTOF Pro's unparalleled long-term signal stability is essential. We have not seen this capability on any other instrument to date, where we can potentially run the instrument for 6 to 12 months, if not beyond, without anything more than a capillary clean. I truly believe that this is probably one of the most fundamental advances in the MS field in the past 15 years, which is going to have a significant impact on the clinical biomarker space, in particular for multivariate biomarker discovery."

The stability of retention time of the timsTOF Pro is demonstrated in Figure 1 [2].

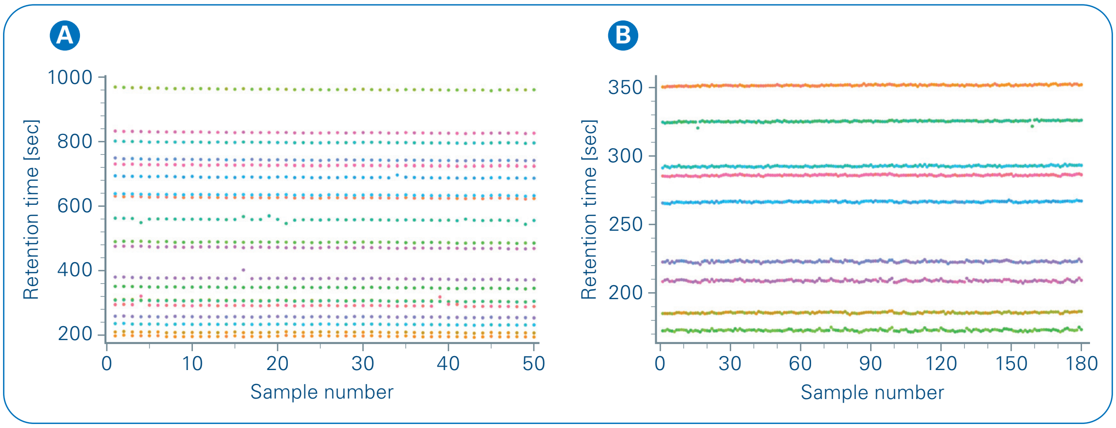

Figure 1: Short gradient methods allow reproducible analysis of samples. Retention time stability of selected peptides from (A) 200 ng (17 min, 50 samples) or (B) 80 ng (5 min, 180 samples) injections of a HeLa tryptic digest that were identified across all samples.

Trapped Ion Mobility Spectrometry (TIMS) is a separation technique in gas phase, which resolves sample complexity with an added dimension of separation in addition to high-performance liquid chromatography (LC) and MS, increasing peak capacity and confidence in compound characterization. Equally important, the TIMS device also serves to accumulate and concentrate ions of a given mass and mobility, enabling a unique increase in sensitivity and speed along with the additional dimension of separation. This fourth dimension, known as the collisional cross section (CCS), provides information about the size and shape of molecules, which enables more reliable relative quantitation information from complex samples and short gradient analyses. Peptide CCS measured at large scale and with high precision by TIMS technology, powered by PASEF, delivers increased confidence of identification in 4D-Proteomics™. Dr. Webb describes his experience with the Bruker timsTOF Pro:

"From the first time I saw the data the timsTOF was producing, I knew we had something special.

It was quite different from the MS data we were used to, adding a true fourth mobility dimension to the data. We could see the enormous potential and advantages associated with this new capacity. Additionally, having trapping in front of the time-of-flight (TOF) analyzer gives you extremely high sensitivity and, because of the way the instrument is set up, you can have extremely high acquisition rates. The acquisition time of samples using LC-MS ranges from 5 to 60 minutes in our experiments. With the addition of the timsTOF Pro, we now have the flexibility to control speed and sensitivity to accommodate this wide range of conditions.

Probably the most important feature however is that the timsTOF Pro offers incredible robustness. Taking all these attributes together, there's no other instrument we could conceive of using for large scale clinical cohorts."

Dementia Detection and Treatment

Advances in proteomics technology has also opened up new possibilities in dementia detection and treatment. In 2019, Dr. Webb led a team of collaborators who were awarded $15 million in funding over five years from the Colonial Foundation. The grant established the Colonial Foundation Healthy Ageing Centre, led jointly by WEHI and the Royal Melbourne Hospital. The center aims to develop diagnostic tests for the early detection of dementia in people as young as 40.

Dementia is a major health challenge in Australia, as well as around the world. In 2016, one in 10 Australians aged over 65 were diagnosed with dementia. Without breakthroughs in diagnostics and therapies, the number of dementia patients in Australia is expected to more than double by 2050 [3]. Early detection of the disease is crucial because by the time symptoms occur, most of the damage cannot be reversed.

The Colonial Foundation Healthy Ageing Centre is the first of its kind in Australia and provides a platform for harnessing the latest technology and collaborative power of experts from both WEHI and the Royal Melbourne Hospital. The goal is to provide doctors across Australia with accredited tools and tests that make a positive difference to the quality of life for patients and their loved ones. Dr. Webb explains:

"The centre is working with neurologists and neuropsychiatrists on early diagnostics for dementia. Most patients wait 4-5 years for a diagnosis. In most of the dementia and neurodegenerative conditions, by the time these patients have been diagnosed, a lot of damage has already been done. We need better methods for early diagnosis. Our funding will enable the Colonial Foundation Healthy Ageing Centre to acquire 12,000 biobanked samples from healthy cohorts, where some have progressed to develop dementia 8-10 years later. So, we can go back in time and make these measurements and hopefully predict the onset of disease. Then the next phase would be to move into proper clinical biomarker trials."

The long-term goal of the center is to generate new health strategies that address the growing burden of dementia on communities. It is a multi-disciplinary approach that brings together a variety of experts, including biologists, clinicians, pathologists, executives and consumer organisations, such as Dementia Australia. The combination of these different perspectives is advantageous for making new discoveries in the treatment of dementia. Dr. Webb explains:

"Dementia Australia brought an awareness of the true burden of dementia — the importance of early diagnosis to the patient, and of facilitating clinical research and trials for potential therapeutics. Pathologists have brought a new appreciation for clinical pathology — how they must work in a clinical environment and how that differs from our historical academic environment.

What this means now is that we're learning how to achieve discovery through the lens of clinical use, i.e., how we design experiments that can more naturally translate into clinical use.

Then our clinicians provide a clear understanding of the true needs of patient care. Physicians know what they need to better treat and care for their patients, but there's a large gap between what they can currently provide and what they want to provide. While they know the technology is available, there are technical, logistical, and structural issues at play. Our centre ultimately aims to bridge that gap."

Working with Bruker

Dr. Webb said he originally became acquainted with Bruker instrumentation after visiting the company's headquarters. Although he and his colleagues were looking at different instrumentation, the Bruker impact II QTOF mass spectrometer caught their eye. Dr. Webb saw the potential of the impact II for enhanced analytical performance levels for applications where trace analysis from complex, high-background matrices is a challenge — such as biomarker research. He explains:

"Throughput is something that is crucially important to these studies. And the stability of Bruker's instruments was one of the selling points — it's something that most mass spectrometers have historically found challenging. Long periods of consistent signal are important.

Bruker has stood apart because they have a QTOF instrument with an incredibly robust transmission efficiency. For me, that was one of the driving forces for working with Bruker."

The WEHI team began collaborating with Bruker and testing the capabilities of the impact II mass spectrometer on their research. Later, Dr. Webb and his research team became early adopters of the timsTOF Pro. He describes his first impressions:

"We had one of the first timsTOF Pro instruments. We had seen the data, and we knew what it could do. It coincided nicely with the need in our laboratory for a really robust system.

One big advantage is the willingness of Bruker to have a flexible environment that is open for academics to work.

The data is in an open format and this access to the raw data is important. Now there's new instrument control software that's allowing us to control the acquisition of the instrument. Bruker is open to working with academics, building this amazing hardware, and allowing scientists to mature and grow the applications around this technology. Using the timsTOF Pro as the underlying platform, our goal was to focus on quality control, system suitability and reproducibility. We now have five instruments, three of which operate in the Colonial Foundation Healthy Ageing Centre, set up in a dedicated high-throughput mode."

Bruker and WEHI developed a close working relationship over time, and it also extended to the Bruker support team. Dr. Webb explains:

"It has been a really enjoyable experience working with the Bruker software and hardware teams. Bruker's service team is great, and we get the help we need. It's been a very positive experience. The Bruker engineers also have been very helpful. They really understand the technology and can diagnose it in a timely fashion."

Future Steps

The Colonial Foundation Healthy Ageing Centre represents a shift in thinking about the scientific and societal challenges in the diagnosis and treatment of dementia. Dr. Webb sees two major long-term outcomes: 1) taking the discoveries as a result of this working collaboration to translate biomarkers to clinical utility, and 2) feeding these discoveries and biomarkers back into patient data-directed research to help diagnosis and treat underlying disease. Dr. Webb explains:

"In medical research, we look at disease and compare it to healthy controls. It's done in a very binary fashion, such as case control studies. As researchers, we think about disease, but we don't really think about health. The Colonial Foundation Healthy Ageing Centre is trying to explain what health means — for example, what does your phenotype of health actually look like — and place disease in that context.

Proteomics is the best tool we have for getting a direct measurement of the phenotype. This direct measurement will enable us to understand what disease looks likes in the context of health. It's the basis for personalized medicine."

Future plans also include integrating data automation, including artificial intelligence (AI), which aids in making results more reproducible and reliable. Dr. Webb's team, in collaboration with Bruker, is leveraging AI technology to increase the speed and sensitivity of TIMS data analysis. Historically, CCS values have been viewed as a challenge in MS-based proteomics, but the development of AI tools is providing the opportunity to utilize CCS to its full extent in 4D-Proteomics. Dr. Webb is also beginning to implement new short-gradient methods such as the dia-PASEF workflow, which applies the PASEF principle to data-independent acquisition (DIA), making use of the correlation of molecular weight with ion mobility in the dual-TIMS funnel and sampling selectively along the ion cloud at up to 100% of the precursor ion current [4]. Workflows such as dia-PASEF leverage the open, flexible working environment of the timsTOF Pro that allows researchers to access the raw data and develop their methods.

The team at the Colonial Foundation Healthy Ageing Centre is working to develop novel statistical bioinformatic methods for processing, interpreting, and analyzing large-scale proteomic datasets to facilitate biological discoveries related to ageing and disease. Dr. Webb describes this challenge:

"At our center, our focus is on clinical validation with an eye toward clinical practice. Our goal is to reduce the barriers to translation. Proteomics as it stands is unlikely to make it into routine clinical use, as there are many issues related to the overall complexity of the workflows involved which lead to reliability and reproducibility issues. We're addressing these challenges by bringing our laboratory expertise to clinical pathologists and working together to identify and solve what's in front of us. We are working towards true automation of sample processing and data analysis.

This also relies on the stability of the timsTOF, which gives us the confidence that we'll be able to do that, and the instrument will likely play a key role in how we proceed in the future."

Dr. Webb and his team believe the strength of the Colonial Foundation Healthy Ageing Centre is the unique collaboration of clinicians, pathologists and researchers coming together to apply the latest knowledge and technologies to transform the clinical discovery process and rapidly translate discoveries into clinical utility. He explains:

"We're looking at why these technologies haven't been moved into clinical use and what problems we need to address. Translation is one of the main things we're trying to solve. I can see the potential of proteomics in the clinical space, and I truly enjoy solving these deep challenges along the way. Being a bit of a jack of all trades, with specialization across biochemistry, LC and MS, I'm good at bringing together the right minds and expertise required. Proteomics is a multi-disciplinary study. Moving into the clinical space, it's even more multi-disciplinary.

The most important aspect of the centre is the people it's bringing together – experts with a shared vision that are solely focused on addressing some of the biggest and growing health challenges facing humanity."

For more information about the timsTOF Pro, please click here.

References

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Researchers apply the latest proteomics methods to understand how protein changes in our body influence health and disease.

About the Walter and Eliza Hall Institute (WEHI)

WEHI is one of Australia's leading biomedical research organizations, with a national and international reputation for performing highly influential basic and translational research. Its vision is to be an innovative medical research institute that engages and enriches society and improves health outcomes through discovery, translation and education. The institute is at the forefront of research innovation, with a strong commitment to excellence and investment in research computing, advanced technologies and developing new medicines and diagnostics. WEHI is home to researchers who are working to understand, prevent and treat diseases including cancer — particularly blood, breast, lung, bowel and ovarian cancer; immune disorders such as type 1 diabetes, coeliac disease and rheumatoid arthritis; and infectious diseases such as malaria, HIV, TB and hepatitis.

About Bruker Corporation

Bruker is enabling scientists to make breakthrough discoveries and develop new applications that improve the quality of human life. Bruker's high-performance scientific instruments and high-value analytical and diagnostic solutions enable scientists to explore life and materials at molecular, cellular and microscopic levels. In close cooperation with our customers, Bruker is enabling innovation, improved productivity and customer success in life science molecular research, in applied and pharma applications, in microscopy and nanoanalysis, and in industrial applications, as well as in cell biology, preclinical imaging, clinical phenomics and proteomics research and clinical microbiology.

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