[CASE STUDY]

Waters™

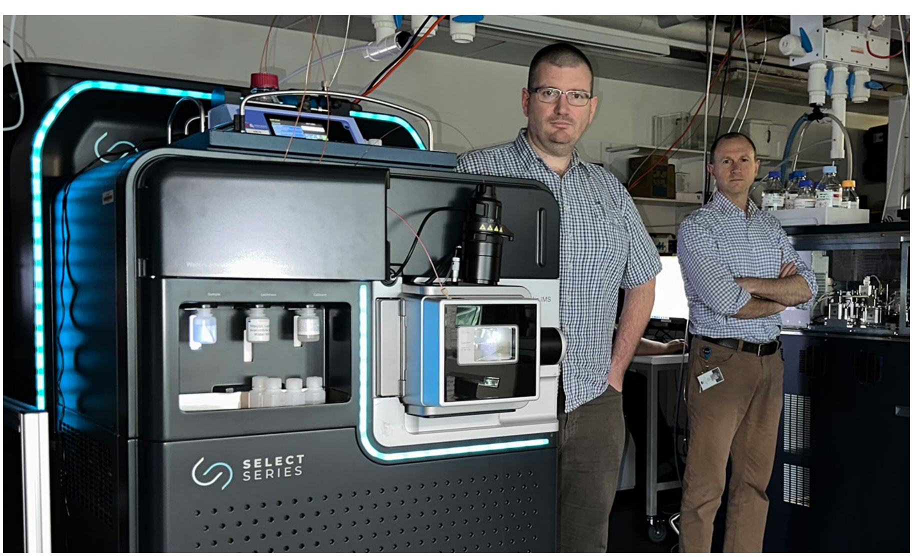
Cyclic IMS Instrumentation Enables Innovative Lipidomics Research in Australia

The Queensland University of Technology invested in cutting-edge IMS technology to not only expand its work with their patented ozone-induced dissociation (OzID) technique, but also embark on other ambitious research projects.

ION MOBILITY MASS SPECTROMETRY RESEARCH AT QUEENSLAND UNIVERSITY OF TECHNOLOGY

Ranked in the world's top 200 universities, the Queensland University of Technology (QUT) has established itself as "the university for the real world" by charting transformative education and research that is relevant to Australian communities. With more than 50,000 students across two inner-city campuses in Brisbane, QUT offers academic programs in fields spanning business, creative industries, education, engineering, health, law, science, and social justice across five faculties.

The university's Central Analytical Research Facility (CARF) was established in 2014 to provide specialist equipment and expert scientists to support the analysis needs across the University, irrespective of discipline or department, and to push forward with new technology and groundbreaking research. Prof. Stephen Blanksby joined QUT to serve as the first CARF director before being promoted to his current position as the Pro Vice-Chancellor Research Infrastructure. His research focuses on advances in mass spectrometry (MS) to empower molecular discovery in chemistry and biology with a particular focus on overcoming the challenges in structural lipidomics.
Part of his research also included developing the ozone-induced dissociation (OzID) technique. OzID is a gas-phase oxidation reaction that can be integrated into ion mobility spectrometry (IMS-MS) instruments to produce distinct lipid fragments. This integration enhances the structural characterization of lipids, including additional isomer separation and the confident assignment of double bond positions.[1]



Working with Waters, Prof Blanksby (right) and Dr Poad (left) led a team that acquired the first Waters SELECT SERIES Cyclic IMS instrument in Australia, in 2021.

WORKING WITH WATERS

QUT's relationship with Waters™ started with a SYNAPT™ G2-S*i* Instrument, which was later customized by Dr. Berwyck Poad, Senior Research Fellow at QUT, to operate beyond the standard instrument parameters. This included implementing OzID on the SYNAPT, which is used for analysis of lipid isomers. Waters supported the team in pushing scientific boundaries and facilitating QUT's discoveries.

When the time came to expand the center's IMS capabilities, CARF turned again to Waters to test a seed SELECT SERIES™ Cyclic IMS™ Instrument, which was later purchased via grant funding. However, the timing was challenging, as the entire process took place during COVID-19 lockdowns.
Dr. Poad explains:

"Our installation engineer wasn't even allowed into the state to pump the system down due to the border restrictions . When we successfully installed the instrument, Waters held remote training sessions. Thanks to the efforts of the Waters team, we all made it work."

Now Prof. Blanksby is leading a $1.52 million project with CARF and collaborating universities to make next-generation ion-mobility mass spectrometry (IMS) available for both research and services in Australia. The CARF infrastructure ensures accessibility and routine analysis for all, while the research team focusses on specific departmental interests.

> "We see IMS as a tool that can enable exciting science. With the improved resolution and functionality of the Cyclic IMS instrumentation, we can resolve features that would have previously been hidden in our analyses. It is a game changer for our mass spectrometry workflows."
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> DR. BERWYCK POAD
> *CARF Senior Research Fellow*

Working with Waters, Prof Blanksby and Dr Poad led a team that acquired the Waters SELECT SERIES Cyclic IMS Instrument in 2021, the first installation of this major new class of IMS instrument in Australia. This cutting-edge IMS technology has contributed to the expansion of QUT's ambitious research, particularly for projects where resolving isomeric complexity is important.

IMS RESEARCH AT CARF

It's common in university settings to have instruments and expertise spread out among different groups or departments. As a result, there's often no commonality for shared knowledge about instruments or service contracts to maintain them. QUT's goal for CARF was to bring analytical tools and expertise under one banner and make the technology more accessible to researchers.

The facility's services range from basic research support to commercial research, and they are offered to QUT staff and students, external research collaborators, and commercial organizations. Specialist staff can perform the analyses or provide training on the instruments for research partners who prefer a hands-on approach. The state-of-the-art equipment and expert technologists at CARF offer transdisciplinary analytical support, including sample preparation, data collection and interpretation of results.

That involves a range of different scientific and analytical equipment, including seven mass spectrometers. It was the facility's growing interest in IMS that sparked the interest in the Waters SELECT SERIES Cyclic IMS instrument. However, the varying COVID-19 lockdown policies in place at the time provided significant barriers to overcome. The biggest challenge was everything needed to happen remotely via Zoom.

While not ideal, the team appreciated the way Waters support personnel from the UK, Australia and Japan worked to make it happen. Even watching the analyses remotely, they could tell that the Waters SELECT SERIES Cyclic IMS could greatly enhance their research. Dr. Poad explains:

"We remotely ran some samples that we sent to Waters Japan (where an instrument was located in the Demo Center), and we started to see that the Cyclic had a lot of separation power that we could exploit."

At the time, the team were working on projects where the separation and identification of lipid isomers were a significant challenge. These samples were used to evaluate the potential of the cyclic IMS. Dr. Poad said:

"The isomeric changes we were exploring are pretty subtle and it was impressive to see that these tests indicated that we could use the Cyclic to tease them apart, a result that we thought would certainly impact our research."

On the back of the results of those initial tests, Prof. Blanksby and Dr. Poad applied for funding from the Australian Research Council (ARC) via a Linkage Infrastructure, Equipment and Facilities (LIEF) grant to purchase the system. The QUT-led grant was applied for in cooperation with the University of Queensland, Griffith University, and the University of Wollongong. Prior to the grant funding being announced, Waters installed a SELECT SERIES Cyclic IMS instrument at CARF to allow Dr. Poad and the team to further evaluate the potential of the technology. Dr. Poad explains:

"We have a history of collaboration with research colleagues at these universities, and we were able to build a compelling case for funding. Waters helped us obtain the seed system before the grant was awarded because they saw an opportunity in Australia to showcase the technology, given researchers involved in the grant application and how their groups could explore this high-end instrumentation for their research."

BENEFITS OF IMS

The biggest benefit of expanding the facility's IMS instrumentation was the technology's ability to better separate and measure molecular structure, which is expected to drive biomolecular discovery across both plant and animal kingdoms as well as accelerate advances in materials science. CARF researchers saw an opportunity for the Waters SELECT SERIES Cyclic IMS to support ongoing research with its OzID technique, which was developed using a customized Waters SYNAPT G2-S*i* Instrument. This alternative ion activation method relies on the gas phase ion-molecule reaction between a mass-selected target ion and ozone in a mass spectrometer.[2]

Prof. Blanksby and his team wanted to use the capabilities of the Cyclic to expand on their use of contemporary ultra-high performance liquid chromatography (UPLC) to separate and uniquely identify isomeric lipids that differ only in their position(s) of unsaturation using the team's patented OzID technique.[3] OzID is particularly useful for studying lipid biomolecules, and provides complementary structural information to traditional collision-induced dissociation (CID) and generate fragment ions that identify positions of unsaturation (carbon-carbon double bonds) within the lipid.

The addition of the Waters SELECT SERIES Cyclic IMS Instrument enables the team to expand their ground-breaking work by providing additional structural data that could take the technique to the next level. Dr. Poad explains:

"Our lipid research benefits from that structural elucidation. For example, one isomer might be an indicator for inflammation response, and another might not. Just moving one double bond might be all it takes, but it has a profound impact on the biological role of that lipid. We wouldn't have been able to see that before, which is why we used these samples for our initial tests of the Cyclic. We were very interested in the capabilities of the Waters SELECT SERIES Cyclic IMS."

But lipidomics was only one area where the addition of the SELECT SERIES Cyclic IMS could expand CARF's research capabilities[4], with inorganic chemistry[5] and polymer science[6] also seeing benefit. Dr. Poad works closely with QUT-based inorganic chemists on coordination cages using ultra-high-resolution cyclic ion-mobility mass spectrometry (cIM-MS). Coordination cages, also known as supramolecular coordination complexes or metal-organic cages, are three-dimensional structures formed through the self-assembly of metal ions and organic ligands.

The design and synthesis involve the use of coordination chemistry principles, where specific metal-ligand interactions determine the overall structure and stability of the cage. The choice of metal ions, ligands, and their spatial arrangement play a crucial role in determining the shape, size, and properties of the resulting cage. Dr. Poad describes the impact of the new Waters SELECT SERIES Cyclic IMS on this research:

"Our inorganic colleagues had existing workflows on the Waters SYNAPT G2-S*i*, but they were interested to see what extra structural detail they could get with the Cyclic. These self-assembled cages have multiple ways they can fit together. You can get isomeric forms that have slightly different Collisional Cross Sections (CCS), and they behave differently in the ion mobility cell. We've been able to leverage the ion mobility on the Cyclic and the SYNAPT to disentangle some of the structural complexity of these inorganic cages. That's a use for IMS that people wouldn't have necessarily thought about. They'd use NMR for structural analysis, but IMS is complementary, and you can acquire an IMS trace that allows you to pick out proportions of isomers that are in the mixture, which helps in understanding differences in reactivity and structural integrity. There are even situations where we have found IMS can provide this detail where NMR can't, such as some of the cages containing certain metals."

CARF was established in 2014 to provide specialist equipment and expert scientists to support the University's analysis needs, providing a foundation for groundbreaking research.

BENEFITS OF THE WATERS SELECT SERIES CYCLIC IMS

IMS provides significant benefits in multiple applications, including the ability to separate isomers by shape as well as mass-to-charge ratio, obtain significantly cleaner mass spectral data, and measure the ion's CCS. One of the most appealing advantages for Prof. Blanksby and his team was the combination of DESI XS with Waters SELECT SERIES Cyclic IMS to provide levels of specificity for MS imaging approaches. DESI imaging produces a visual map of the spatial distribution of a wide range of analytes — for example small molecule drugs, metabolites and lipids across the surface of a tissue sample — and the unique capabilities and modes of acquisition of Waters SELECT SERIES Cyclic IMS enable comprehensive in-depth experiments to be performed.

"We were particularly interested in the DESI XS. We already have a DESI with the SYNAPT, but it's a lot fiddlier to set up, whereas the DESI XS is turnkey. This new imaging capability can really help us differentiate isomers in tissue sections with a lot less time involved in optimization of the source conditions."

CARF researchers deployed the Waters SELECT SERIES Cyclic IMS to support ongoing research with its OzID technique.

The instrument design and control software of the Waters SELECT SERIES Cyclic IMS enables a range of experiments, including acquisition of time-of-flight data, single pass ion mobility, and the ability to scale mobility resolution to match the complexity of a sample. Additionally, the combination of CID, Electron Capture Dissociation (ECD) and Surface Induce Dissociation (SID) with cyclic ion mobility provides a significantly enhanced tool set.

> "We were excited about the improved ion mobility separation, the extra ion activation techniques and the ability to play around with those tools. We also preferred the Waters SELECT SERIES Cyclic IMS instrument's geometry, which performs mass selection before ion mobility, which is much more appealing to us. We were also surprised by how good the mass resolution of the TOF was. Our Cyclic can easily do 70,000,[7] versus around 20,000 that we routinely get on the SYNAPT. So, we get a more accurate mass at a higher mass-to-charge range. This hadn't been something we considered before the instrument was installed."
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> DR. BERWYCK POAD
> *CARF Senior Research Fellow*

MassLynx™ supports interactive control of the Waters SELECT SERIES Cyclic IMS and facilitates set-up of IMS methods, giving the user full control and allowing set-up of sophisticated experiments:

"We've found that it's easier to train people on the Cyclic and get them to the point where they can acquire publishable data in a short amount of time. It is much easier to use. That really speaks to the efforts of the Waters software team on making using the instrumentation user-friendly.

NEXT STEPS

As their research projects on the Waters SELECT SERIES Cyclic IMS continue, the CARF team now also turns to opportunities provided by another newly acquired Waters SYNAPT G2-S, which was obtained pre-used from another laboratory. After the instrument was thoroughly refurbished by Waters and shown to meet specification, CARF now plans to further customize this new SYNAPT with the support of Waters. Dr. Poad explains these plans:

"We would like to modify this SYNAPT so we can do photodissociation on it. These modifications will be substantial, and it will take a fair bit of effort. This will require us to go outside of the normal operating parameters of the instrument, as we did with our original SYNAPT instrument for our OzID work. We really appreciate the help and expertise of the Waters team to make this possible."

Dr. Blanksby and the team also plan to continue to share the advantages of OzID for researchers looking to gain insights into the structural properties of lipid molecules.

> "We're working to spread our patented OzID technique worldwide. We've conducted several proof of principle experiments with global collaborators to see how OzID can complement the capabilities they already have."
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> DR. BERWYCK POAD
> *CARF Senior Research Fellow*

The bigger picture, however, is how the investment in Waters SELECT SERIES Cyclic IMS serves as the latest example of the university's dedication to scientific freedom that allows researchers to pursue new ideas, explore uncharted territories, and push the boundaries of existing knowledge.

This approach encourages the CARF team's curiosity-driven investigations that can lead to breakthrough discoveries and innovative solutions to societal challenges because QUT researchers have the freedom to explore unconventional ideas and approaches. Dr. Poad explains:

"Our research is driven by the questions that we want to answer. Many of our projects and collaborations have originated from conversations with colleagues around the coffee machine in the tearoom. This allows us to go after the things that sound interesting, which can result in groundbreaking discoveries and disruptive technologies. Our Waters IMS instruments will certainly contribute to our efforts in pushing the boundaries of scientific knowledge."

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7. Specification is >60,000 FWHM (Specification Document 720006590EN)

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