



Customer Insights

- Innovating new drugs using Fragment Based Drug Discovery combined with Native Mass Spectrometry

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Extreme resolution MRMS technology enables scientists to see “hidden gems” of information in fragment based drug discovery research at the Griffith Institute for Drug Discovery



Working with Bruker

Sally-Ann Poulsen's laboratory at the Griffith Institute for Drug Discovery (GRIDD) uses advanced MRMS technology to support their research in small molecule drug discovery.

“The instrument from Bruker brings high performance and flexibility for faster analysis, saving time and money while producing richer datasets for small molecule analysis.”

Strategically investing in drug discovery

Griffith University is located in Brisbane, Queensland, Australia and was founded over 40 years ago. The university is ranked among the top 3% of universities globally with over 50,000 students and 4,000 staff across its five campuses. The university has a large focus on research with an extensive network of research centers and institutes across a range of disciplines, including drug discovery. Sally-Ann Poulsen, Professor of Chemical Biology at Griffith Institute for Drug Discovery (GRIDD), at Griffith University, explains further:

“Griffith University is committed to areas of strategic investment to help facilitate research excellence and drive positive solutions. Drug discovery was identified as one area of interest and this is where the Griffith Institute for Drug Discovery (GRIDD) originated from. The institute was formerly known as Eskitis and was renamed to GRIDD two years ago.”

Internal work

At GRIDD, there are over 120 members of staff and students focusing on drug discovery across four main areas of interest: cancer; infectious diseases; neurodegenerative diseases and spinal cord injury repair. Cancer is Sally-Ann's laboratory's main area of focus.

Chemistry and biology – A collaborative approach

The institute is distinctive in its collaborative approach between chemists and biologists. This is particularly unusual in Australia, as the country has a large number of medical research institutes with few or no chemists on site. GRIDD is among only a handful of such institutes that operate with chemistry and biology under the same roof.

Furthermore, GRIDD uses High Throughput Screening (HTS) robotics for both phenotypic screening, where the drug target is unknown, and target based screening, where the protein of

interest is known and the user is trying to affect how it behaves, either by inhibiting or activating it.

GRIDD is unique in Australia for its NatureBank facility, a drug discovery platform based on natural product extracts and fractions that have been derived from Australian plants, fungi and marine invertebrates, from Australia to Papua New Guinea. These samples have been processed into two libraries (a 10,000 natural product extract library and a 50,000 natural product fraction library), which are ready for HTS against any disease. NatureBank also holds more than 30,000 archived biota samples.

Compounds Australia, of which Sally-Ann was academic lead from 2013 – 2016, located at GRIDD, is the premier compound management facility in the Southern Hemisphere. The facility provides access to critical infrastructure and expertise to ensure flexible, efficient, reproducible and cost-effective compound management. Compounds Australia makes compounds available in sophisticated assay-ready formats to researchers worldwide, addressing the demands

of bioactive compound discovery throughout the drug discovery pipeline.

Sally-Ann has three main research areas. The first is medicinal chemistry, for making bioactive compounds, completing the assays and elucidating structure activity relationships. Her flagship discovery in this area is compounds that reverse drug resistance in glioblastoma, the most common and lethal adult primary brain tumor.

The second research area is making chemical probes – molecules for biologists to use as tools to better understand their biological systems. Examples include molecules made for diseases such as Leishmaniasis, caused by the Leishmania parasite which lives inside cells. Sally-Ann describes how scientists are looking under the microscope to stain for DNA, but are struggling to confirm if the DNA is from the parasite or the host cell. Her team is making compounds to help biologists improve how they work in this area, alongside the commercial tools they can purchase.



The final research area is mass spectrometry (MS), and Sally-Ann describes her journey in how she came to be an advocate of this technique:

"I completed my PhD in Australia before completing my postdoctoral research in the UK. After a year in Big Pharma I moved to the University of Cambridge Chemistry Department, which is where I first started using Magnetic Resonance Mass Spectrometry (MRMS), formerly known as Fourier Transform Mass Spectrometry (FTMS). Following a talk from Prof Dame Carol Robinson, renowned for pioneering the use of mass spectrometry as an analytical tool and for her ground-breaking research into the 3D structure of proteins, I felt I could do this for a different application and at Cambridge I had the opportunity of working with an early 4.7 Tesla Bruker APEX MRMS system."

Introducing mass spectrometry to GRIDD

Following her return to Australia and with MS identified as a technique for use, Sally-Ann successfully applied for funding from the Australian Research Council and had the first system installed in 2003, only the 6th to be installed in Australia.

The primary focus for this was to study proteins in their native state, where they are folded as they would be in a cell, and so have the required 3D shape they need to bind to a small molecule. The key reason behind this was to use proteins as a template to help develop dynamic combinatorial libraries (1). These are mixtures of compounds that promised to improve aspects of drug discovery. Historically, however, scientists would complete the library synthesis, screen it and then have to go back to deconvolute, which was difficult and circumvented the benefits of working with the libraries, as Sally-Ann explains:



“As there were no available tools on the market, by using MS, I was able to observe the small molecule protein interactions and avoid the need to deconvolute, as well as confirm the quality of the analysis.”

This original system (decommissioned in 2017) was replaced by the Bruker solariX XR MRMS in 2014. The team at GRIDD led the application that secured funding through the highly competitive Australian Research Council for infrastructure.

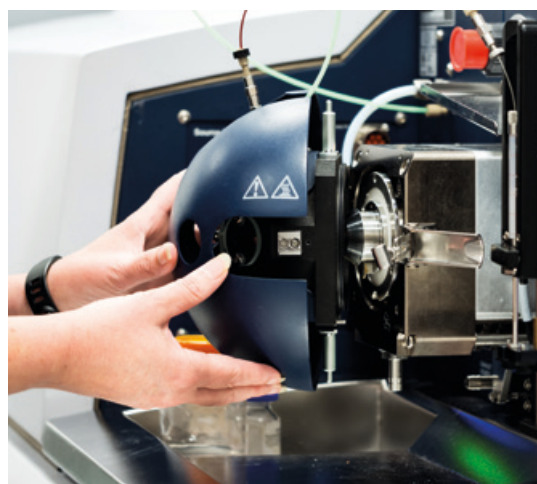
The team at GRIDD successfully presented the need for the new MRMS for its improved screening capabilities, as the solariX XR mass spectrometer has an increased upper size limit of proteins. This is due to the magnets being further developed – from 4.7 Tesla to the solariX XR’s 12 Tesla magnet. The upper size limit for the proteins Sally-Ann and her team at GRIDD can study has increased from 50 kilodaltons (kDa) to 150 kDa. The difference has meant that the gap between 50 – 150 kDa, which includes a high proportion of proteins that are of interest for drug discovery, would be accessible and this advanced capability provided a compelling case on its own. More specifically, within drug discovery, is the tool of fragment based drug discovery (FBDD). FBDD has emerged as a powerful tool for discovering drug leads. The approach first identifies very small molecules (fragments) that are about half the size of standard drugs, and these fragments are expanded or linked together to generate drug leads.

Another key benefit of the new system is its lower running cost. The previous system required cryogen (liquid nitrogen and liquid helium) to maintain the super conducting magnet, which is a very expensive process for refilling. The old system required refilling liquid helium five times a year at a cost of \$3,000 per refill. The new system recycles the helium and, therefore, it only

requires refilling once a year, a key selling point for the solariX XR.

Additionally, ease of use for those operating the instrument has proven a key benefit, as Sally-Ann explains:

“We had originally intended to use both the old and new systems but as the new system performed so well, the old system was no longer the ‘go to’ instrument.”



“The capabilities and ease of use of the solariX XR were so much greater, we retired the original instrument after 14 years of tremendous service that put GRIDD at the forefront of study drug-protein interactions.”

The solariX XR is equipped with extreme resolution and gives an extra layer of confidence in screening for weak interactions of very small molecules. Therefore, accurate identification of the binding and molecule mass for fragments is very important as a starting point in drug discovery.

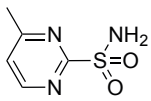
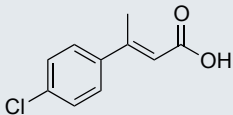
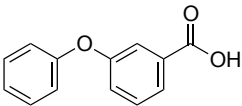
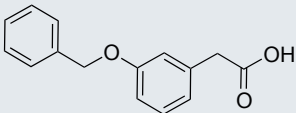
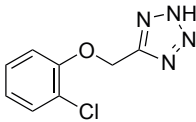
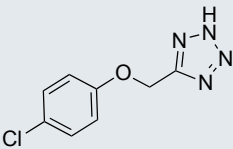
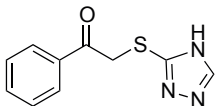
Compound No.	Fragment/Chemotype	SPR [Ⓐ] K _D (μM)	ESI-MS	nanoESI-MS [Ⓑ]	X-ray
1	 primary sulfonamide	1.35	✓	✓ (1:7.1)	✓
2	 cinnamic acid	905	✓	✓ (1:0.9)	✓
3	 benzoic acid	1280	✓	✓ (1:1.4)	✓
4	 phenylacetic acid	1080	✓	✓ (1:0.9)	✓
5	 5-substituted tetrazole	631	✓	✓ (1:1.7)	✓
6	 5-substituted tetrazole	709	n/a	✓ (1:1.4)	✓
7	 1,2,4-triazole	194	n/a	✓ (1:0.5)	✗

Table 1: Correlation of screening results for fragment hit chemotypes with surface plasmon resonance (SPR), electrospray ionization-mass spectrometry (ESI-MS), NanoESI-MS, and X-ray crystallography (green tick = hit, red cross = not a hit, n/a fragment not tested). **A** Dose-response experiment performed at 25 °C with a 5-point fragment concentration series range. **B** nano MS hit with ratio of unbound CA II:fragment bound CA II peak intensities in brackets.

As well as the MRMS system installed, GRIDD also has a Bruker quadrupole time-of-flight (QTOF) maXis II. Sally-Ann and her team have plans to use this instrument on future projects as another filter of obtaining data and ruling out ineffective compounds quickly. Sally-Ann's team would then take those compounds with binding potential across to the MRMS for higher resolution analysis.

Reducing false positives

A huge advantage of the MRMS system is its minimal false positive results. Other techniques suffer from producing high false positives but this is not an issue with the solariX XR, as Sally-Ann explains:

"Fragments at high concentrations can aggregate, or stick together, and do not behave as they should. Other fragment screening techniques may suffer from high false positives as they are having to deal with the fragments at higher concentrations. In our method, the fragment is at the same concentration as the protein, therefore we do not have these aggregation issues so false hits are much less likely to occur. This is a huge advantage, because if you have 1000 compounds screening in the library, you might only filter to 200-300 if the false positives are high in number rather than 10-30 true hits."

The MRMS method means you avoid wasting valuable time on compounds that will not advance drug discovery."

Lowering attrition with fragment based drug discovery

Sally-Ann is specifically focused on the area of FBDD. It is getting harder to progress new drugs through the drug development process, and the rate of acceptance is declining. The investment by the pharmaceutical industry in small molecules to get them to phase 1, 2 and 3 clinical trials, before failing late in the process, is referred to as attrition, and is extremely costly. The mantra in

the pharmaceutical industry is if you are going to fail, fail early. Therefore, the industry has to look at how to avoid attrition and make the process less costly, leading to more attention for FBDD.

The difference between fragment and traditional HTS is the size of the molecule. A fragment, or small molecule, is typically under 200 Da in molecular weight, whereas a HTS compound is between 400-500 Da. The ability of the fragment to interact with a protein is less and the binding affinity is weaker, but if they do interact this indicates they fit perfectly. This is why it is an area of great interest for drug discovery, as it is less likely to be one of those compounds with high attrition later down the pipeline.

Sally-Ann explains the challenges associated with screening hits:

"If you identify a HTS screening hit, it can be very difficult to develop into a drug, as its potency can be made up of both good and bad interactions, which can compensate for each other. Therefore, as chemists try to improve the hit compound, the data can often lead them to change into a larger molecule with worsened drug-like properties and with a higher chance of failure. This is because as they get to the stage where they enter the human body, toxicity levels or associated off-target effects become visible."

Fragment screening offers a potentially much better starting point for drug discovery and better overall prospects that a compound will move along the drug discovery pipeline towards FDA approval or exit early to limit the investment knock as much as possible."

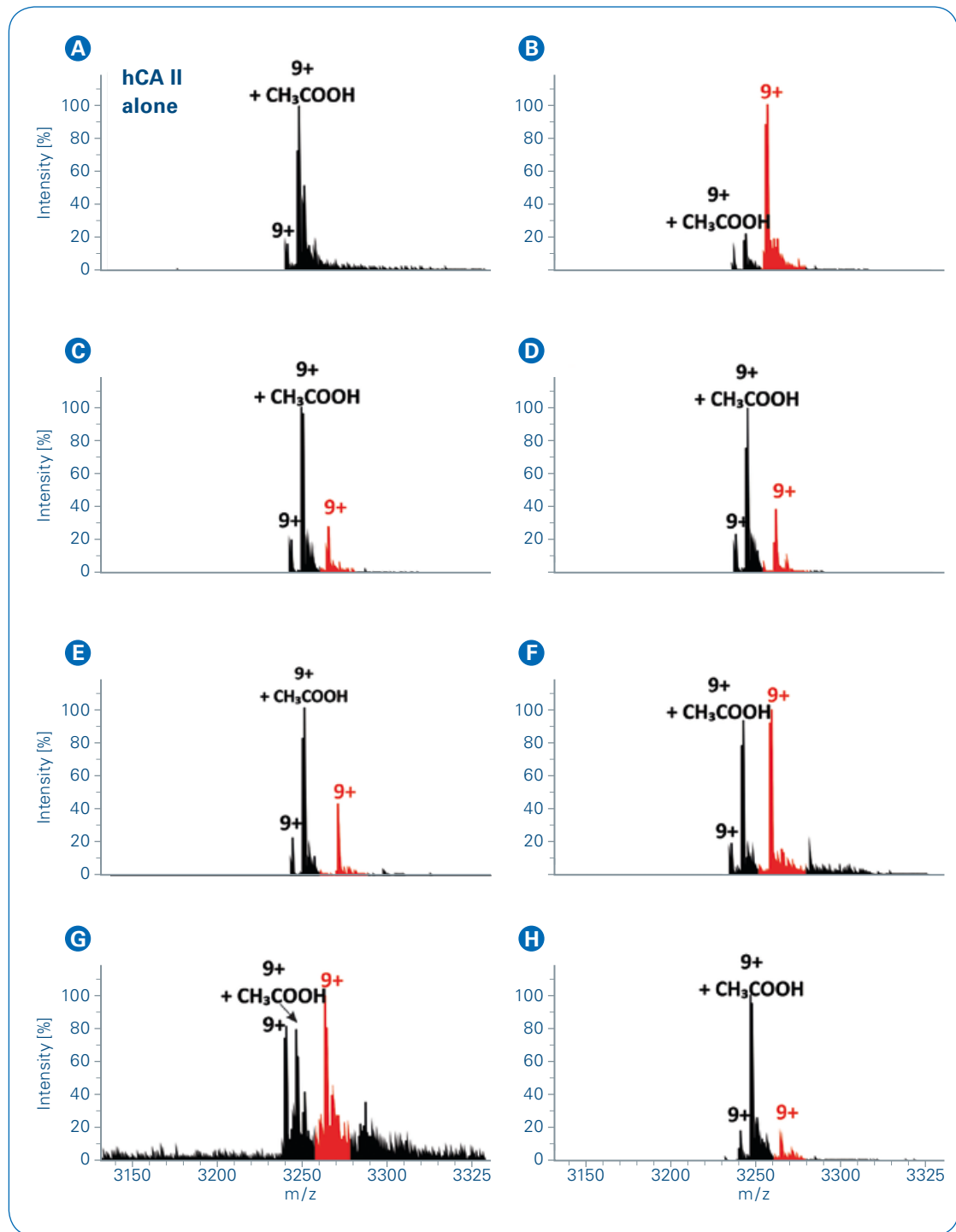


Figure 1: NanoESI mass spectra of hit fragments with carbonic anhydrase II (CA II). The 9+ charge state is shown, with [protein + fragment] 9+ peaks in red. **A** human CA II protein only **B** primary sulfonamide chemotype **C** cinnamic acid chemotype **D** benzoic acid chemotype **E** phenylacetic acid chemotype **F** tetrazole chemotype **G** tetrazole chemotype **H** 1,2,4-triazole chemotype.



Fragment screening combinations

Fragment screening has been a successful approach for FBDD researchers as there have been two FDA approved drugs from the process. It is proving an area of great and growing interest for the pharmaceutical industry and in academic research. FBDD is, however, contingent on the development of analytical methods such as MS to be able to identify the weak binding fragments. As such, researchers at GRIDD are optimizing their workflows and combining techniques together with collaborators in order to give the best chance of accelerating drug discovery. Techniques for FBDD, in addition to MS, include Surface Plasmon Resonance (SPR), X-ray Crystallography and Isothermal Titration Calorimetry (ITC). Each method has pros and cons but there is a need for multiple (orthogonal) methods, in order to be confident that a hit is a true hit.

Sally-Ann and her team have combined native state MS with two proven and popular fragment screening methods, SPR and X-ray crystallography, in a fragment screening campaign against human carbonic anhydrase II (CA II). (2)

The research recognized native state MS as a rapid, sensitive, high throughput, and label-free method to directly investigate protein–ligand interactions. However, there were few studies using this approach as a screening method to identify relevant protein–fragment interactions in FBDD. The results showed the first fragment screening analysis of electrospray ionisation (ESI)-MS and NanoESI-MS using a high resolution Fourier-transform ion cyclotron resonance (FTICR) instrument (Bruker solariX XR 12.0T MRMS) in parallel with SPR as shown in figure 1 and table 1.

MS has a huge number of advantages over other techniques as Sally-Ann explains:

“The advantages of MS are due in part to the speed, and it doesn’t use too much sample. Therefore, in a workflow, I envisage this technique as the front end of the fragment screening cascade of methods to try. I see MS as the starting point and the other methods can come in later as you bring the compounds screened down from, for example, 1000 to 50.

It is almost a pre-filtering tool in the process but can be used as a standalone in the workflow.”

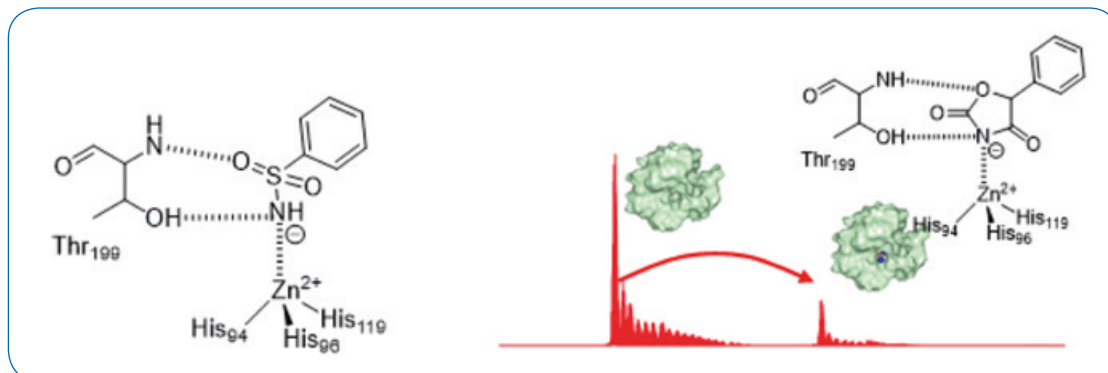


Figure 2: Binding interactions of the classic primary sulfonamide chemotype **A** with carbonic anhydrase II (CA II) are emulated by the oxazolidindione chemotype **B** discovered by fragment screening using magnetic resonance mass spectrometry (MRMS).

Identifying a new zinc binder by quantitative native MS

Another piece of published research by Sally-Ann and collaborators at Commonwealth Scientific and Industrial Research Organisation (CSIRO) reports the identification of a new zinc-binding fragment. (3) The researchers reveal an additional fragment that is both a novel and potent inhibitor of CA II. SPR and native ESI-MS identified compound 10, which has an affinity and ligand efficiency approaching that of sulfonamides, a well-known class of zinc binder for CA II. The researchers were so astonished by the findings that upon first reflection, it was thought that the samples had been mixed up.

The researchers determined the crystal structure of compound 10 bound to CA II, confirming the binding pose of the new fragment to CA II, which

included a primary interaction with the zinc and a couple hydrogen bonds with the protein, helping to explain the high affinity, as seen in figure 2.

The research used a series of 18 analogues of compound 10 to assess the structure–activity relationship (SAR) using both SPR and MS. The researchers were able to obtain quantitative MS data by holding protein concentration constant (at 14.5 μ M) and varying the fragment concentration from 0.5 to 120 μ M. Plotting the percentage of protein bound and curve-fitting revealed dissociation constants remarkably similar to those determined using SPR. Nine of the new fragments showed at least some activity, though none were significantly more potent than compound 10. Crystal soaking experiments led to seven new structures, with all the fragments binding in a similar manner as compound 10.

FBDD in the future

Sally-Ann is looking to further collaborate with industry for FBDD in the future, as there is a growing interest from the pharmaceutical sector to aid drug discovery.

"The research landscape in Australia is changing, with the impact on working in industry really encouraged. This has timed well with the pharmaceutical industry growing its interest in FBDD, so I hope to work more with industry to use these applications for industry drug targets. This is possible as our method is unbiased, therefore, we can screen for different proteins."

For more information on the Griffith Institute for Drug Discovery, please visit <https://www.griffith.edu.au/institute-drug-discovery> or follow on twitter @GRIDD_GU. For more information on the solariX XR, please visit <https://www.bruker.com/products/mass-spectrometry-and-separations/ftms/solarix/overview.html>.

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