Cutting-Edge Metabolomics

As new technology platforms push us to the limits of what's possible, the metabolomics community is closing in on the future of the field: routine and rapid quantitative analysis.

By Karl Burgess, Head of Metabolomics, Glasgow Polyomics, University of Glasgow, Scotland.

Believe it or not, I started out as an undergraduate in computer science and cybernetics. Unfortunately, the world of robotics involved a lot more mathematics than I expected. And so after a year of computer programming, I switched to pathobiology. But I never lost my interest in computers and programming, and that has been invaluable as I've progressed through my career; during my postgrad days I moved into bioinformatics and molecular modeling, which brought my two halves together. I soon realized that I wanted more time in the lab, which led me to do a research-based masters degree in biological and biomedical science.

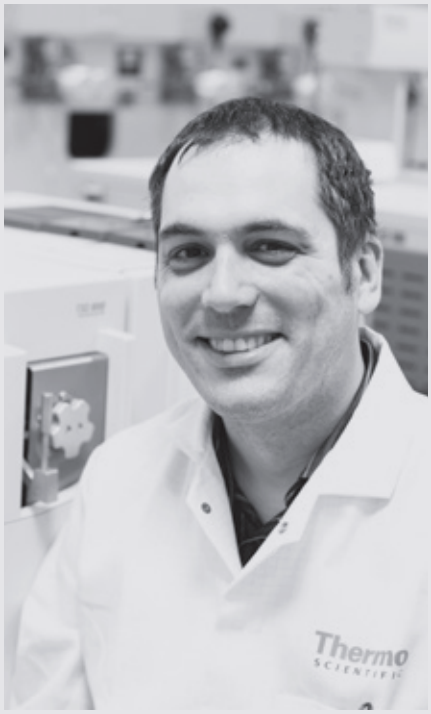
I ended up in the proteomics lab at the University of Glasgow in a world where robotics, wet-lab work, biology and computer happily co-existed. I'd found my calling – at least for a while. Using mass spectrometry coupled to computational techniques that make sense out of the biological data is where my broad interests now lie. In many cases, it's not about creating new algorithms, it's about processing data and presenting them in a useable format that biologists can understand.

Orbitrap temptation

So, why the shift from proteomics to metabolomics? One of the reasons was to get my hands on an Orbitrap instrument to be honest... I actually started out uncomfortable with the idea of metabolomics – it's a completely different ball game. In proteomics, we could use Mascot to provide a probabilistic score for a given protein based on the mass spectra. You can use a cut-off system and, much like a court of law, you end up with an innocent or guilty verdict on the identity. In metabolomics, we were working entirely on mass and retention time – it's a very binary way of working and felt quite limiting; it was a "yes" or "no" answer to identification without knowing how certain you were in either case. Now, we're building fragmentation libraries and the requirements for supporting metadata in studies are increasing all the time.

Indeed, metabolomics is now very rigorous – and it's been a big learning curve for me in terms of quality control. Excellent reproducibility is key; dozens of replicates may be necessary to get the statistical quality for quantitation. And that's the point where clinicians start to become very interested – robust, quantitative data on biomarker-style molecular relationships they are used to working with.

I did most of my PhD work on a relatively fast-scanning but pretty low-resolution ion trap instrument. When I first got an Orbitrap instrument (an XL), I was showing my boss the data at 100,000 resolution, and he actually thought it was centroided – I had to zoom in about 20 times before I could demonstrate the reality of the peak widths. It was a really great moment! I've also done some work on high-resolution QTOFs, but stability of mass accuracy was a problem. The Orbitrap has always been rock solid in that regard. In fact, when we bought our ex-demo XL, it had been boxed up in the demo lab, left in a crate for three months, unboxed outside the building and bumped up a rough slope into the lab. After pumping the instrument down we found that it was still within 3ppm...



Metabolomics today

Heading up metabolomics at Glasgow Polyomics means that I get to work on some really diverse projects – all sorts of crazy samples. Indeed, the whole facility is geared up to apply state-of-the-art technologies to investigate biological systems by combining multi-level, multi-omics datasets.

As an example, we've had a lot of success partnering with Matt Dalby's group on the analysis of stem cell differentiation and interaction with surfaces. With Matt, we've got some fantastic collaborations (Nikolaj Gadegaard and others, who make nanopatterned materials) where we explore how different nanostructures promote different kinds of differentiation. Obviously, if differentiation occurs, there are lots of complex modifications to the metabolome. Tracking these changes over the course of differentiation on different surfaces is enormously powerful.

I'm now trying to tie up my interests in infectious disease with the surface attachment work in the area of bacterial biofilms. Infection of medical implants is a

really significant problem, especially with antimicrobial resistance increasing. We're looking into novel antimicrobials that modify biofilm formation with endogenous metabolites and repurposed drugs. I've got a great collaborator: Gordon Ramage, who works in the Dental School, and has been analyzing multispecies biofilms for many years. With his expert clinical microbiology knowledge, and the three PhD students we've got on the project, we're now starting to get some interesting results.

On the software side, we're working on probabilistic annotation of metabolites from data using a Bayesian clustering approach. This is part of the drive towards providing a meaningful probabilistic analysis of identification. In many ways, it's a first step towards creating a framework in which we can slot multiple measures of physicochemical properties to determine the likelihood of a particular ID.

GC Orbitrap joins the party

We've already put GC-Orbitrap technology to the test in a really cool project called 'the way of all flesh' with Richard Burchmore, which is essentially analyzing the decomposition process of of dead bodies. Time of death is really tricky to work out once liver temperature has dropped to ambient. And so, the search is on for biomarkers of death, using metabolomics and proteomics. First, we let a big piece of steak decompose over a 12-day period, taking MS datasets as time went by. We got some very interesting leads in terms of amino acid biomarkers.

Whilst at Thermo Fisher Scientific in Runcorn, UK, we were able to move onto rat models. First of all, the data reproduced the work we'd done on LC-MS previously, but the added resolution and the presence of the NIST libraries allowed us to distinguish things like sugar isomers that we have difficulty with on our untargeted LC-MS method. In fact, the software on the GC-Orbitrap system allows us to automate metabolite identification using enhanced spectral deconvolution, NIST library candidate searching and accurate mass filtering. Sensitivity was phenomenal; with a 1 µL injection we were overloading the system, so we had to move to split injections.

In the final stage of the project, we managed to acquire samples over various time periods from a body farm (or more correctly, a forensic anthropology research facility) in Texas. We are gearing up to run the human work on the freshly installed GC-Orbitrap system in our lab right now – exciting stuff. We're hoping that GC-Orbitrap technology can deliver better coverage of the biomarkers we've discovered, as well as the opportunity to perform good quantitative measurements.

We'll be presenting all of our findings at the 11th International Conference of the Metabolomics Society in San Francisco bay area towards the end of June.

In the near future, I'm also looking forward to doing a lot of biofilm work on the instrument. I actually started this research area as it provided a platform for pushing metabolomics innovation, but once you've got your own bit of biology to investigate, it all gets quite exciting. High-resolution separations and mass accuracy are really key to analysis of biofilms.

Moreover, the GC Orbitrap enables untargeted metabolomics because it provides accurate mass full scan data rather than targeted transitions, as you would get on something like a triple quad. The array of quorum sensing molecules that bacteria use to communicate with each other, triggering, for example biofilm adherence and dispersal, are very diverse, and not yet well characterized. An untargeted approach gives us the potential to identify new compounds; accurate mass EI fragments allow us to characterize them. Additionally, high GC resolution allows us to separate isomeric compounds and, with some extra chemistry, even chiral compounds, which are extremely important in bacterial signaling and peptidoglycan synthesis.

In metabolomics, we're essentially looking for everything. Therefore, access to NIST libraries is enormously powerful as it allows us to make unexpected discoveries in a non-targeted fashion. Targeted metabolomics by definition narrows the field.

Metabolomics of 2025

In my view, GC-HRMS is fast approaching the point of being the ultimate metabolomics platform. And LC-MS is catching up rapidly. In 10 years, I predict that metabolomics will be easy (!) You'll buy an instrument and a set method, and advanced software will do the work for you. In an ideal world, we'll have contributed heavily to the development of that software. We've got quite a few publications in software and algorithm development for MS, and they're beginning to coalesce into one single web-based platform. Once again, it's about providing people with useful, interesting data. I would say software is the biggest challenge right now; the hardware tools we need are here.

As far as GC-Orbitrap technology goes, I'm deliberately trying to keep my acquisition a bit of a secret (this article won't help). The people I have told are extremely excited about the prospect of running samples and, candidly, I don't want a never-ending backlog just yet.

Even if I'm 10 percent more confident in the data, it's really important – and in reality, it's a lot more than that because I can provide compound matches to fragment patterns in percentage terms and then use accurate mass to really drill down into specific fragments. To put it simply, GC-Orbitrap technology gives us extra confidence. And confidence is an extremely important asset in our field.

Video interview with Karl Burgess: tas.txp.to/0515/KarlBurgess
To find out more: thermoscientific.com/HRAMGCMS