JUNE 2017 # 53

the Analytical Scientist

Mass Spec Masterwork

Catherine Fenselau reflects on 50 years in analytical science.

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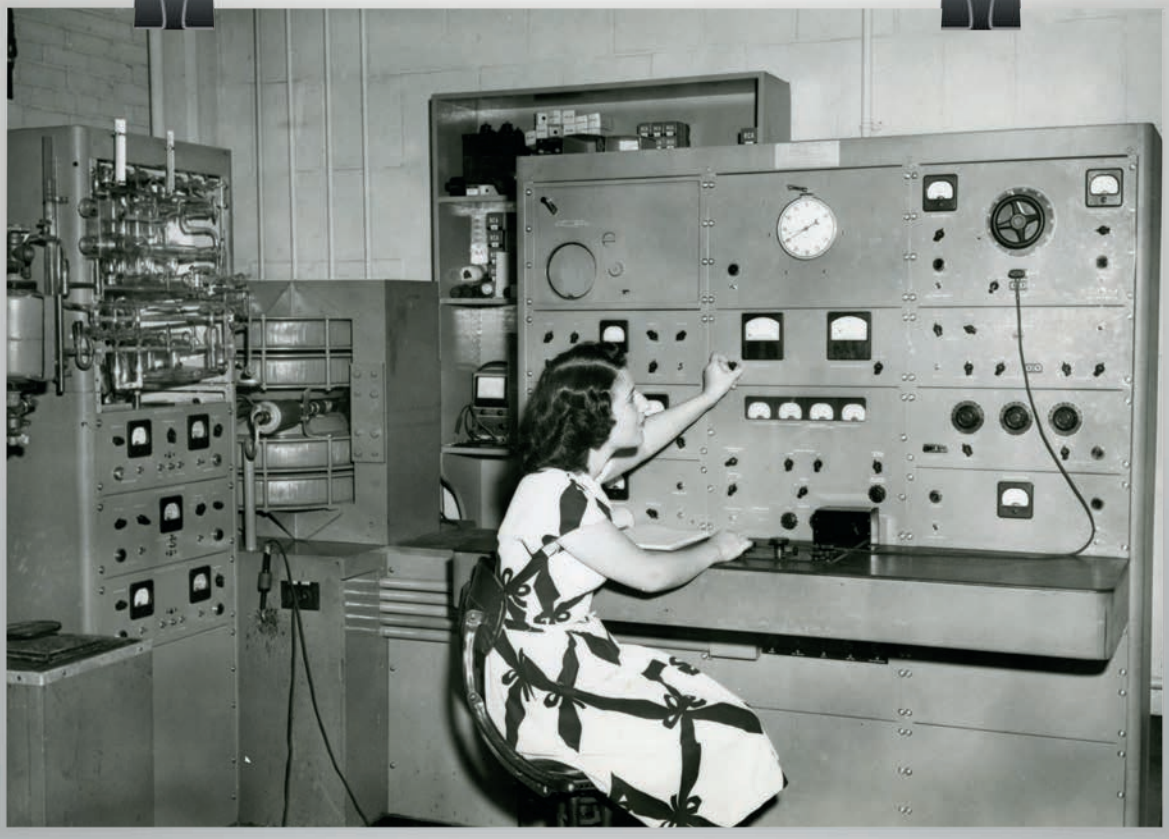

Mass Update

In this 1948 photo, a National Institute of Standards and Technology (NIST) staff member operates an early mass spectrometer. Today, the NIST mass spectral library is possibly the world's most widely-used database of mass spectra used to identify unknown organic compounds. NIST released a major update to that library on June 6, 2017. Find out more at: http://bit.ly/2iQDVBC

Credit: NIST

Would you like your photo featured in Image of the Month? Send it to charlotte.barker@texerepublishing.com

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Catherine Fenselau's 1961 yearbook photo from Bryn Mawr College – with a Warhol-esque twist.

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the Analytical Scientist

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nina.duffissey@texerepublishing.com
Nina Duffissey, The Analytical Scientist, Texere Publishing Ltd, Haig House, Haig Road, Knutsford, Cheshire, WA16 8DX, UK

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Onwards and Upwards

Exploring analytical science – past, present and future.

Catherine Fenselau is in the rare position of being able to reflect on more than a half-century in her field. In the late 1960s she made history by becoming the first mass spectroscopist hired by a US medical school. And despite the lack of precedent (and – in the early days – instruments... see page 24), she helped make possible a wealth of new insights into the proteins that shape us – in sickness and in health.

While Fenselau and colleagues were mining the secrets of the inner world, scientists across the world were joining forces to explore mysteries further from home. As Murray McEwan relates on page 32, the 1960s were also the era when space flight and radio telescopes led to fascinating glimpses into space, and subsequent advances in ion–molecule chemistry.

Of course, the story doesn't end there. We can certainly learn from the past, but at The Analytical Scientist we also want to keep you up-to-date on current developments and delve into likely future trends.

And so our sojourn into the past soon turns into a celebration of the present. Both the aforementioned research avenues bore fruit in exciting new applications or techniques. Early successes in applying mass spectrometry to biomedicine were frontrunners of the explosion in bioanalysis from the 1970s onwards, and the mind-boggling power of today's proteomic studies. And advances in ion–molecule chemistry eventually led to a new means for detecting volatiles in the air – SIFT-MS (see page 32).

Looking back, it's easy to see that analytical science – and technology particularly – has come a very long way in the past 50 years. Who knows what might lie in store over the next 50? After all, it's less than 15 years since Orbitrap technology hit the market, catching many people by surprise. Before 1994, no one had heard the word proteome, let alone glycome (late 1990s), microbiome (2001), or exposome (2005). In this issue alone, we feature the latest advances in everything from analyzing volatile organics (page 46), to the march of mass spec into routine clinical diagnostics (page 40), to new applications for SERS (page 50).

ASMS 2017 gave us a glimpse of the new products, ideas and faces that are already shaping the future of the field. The potential to control fragmentation, to analyze up to a thousand peptides per minute, to manipulate ions in entirely new ways... Whatever the future holds, there is one aspect of science that never changes, and that's the urge to go further, faster – to see beyond the horizon. As Fenselau so beautifully puts it: "One of the most delicious parts about being a scientist is the weekend when you know something that nobody else in the world knows."

Charlotte Barker
Editor

Upfront

Reporting on research, personalities, policies and partnerships that are shaping analytical science.

We welcome information on interesting collaborations or research that has really caught your eye, in a good or bad way. Email: charlotte.barker @texerepublishing.com

Sex off the Beach

Hold the sushi! Assessing safe levels of hormones in gender-switching eels with LC-DMS-MS/MS

There is an increasing demand for glass eels in Japanese cuisine, but when eels are grown commercially at a high density, they have a tendency to change sex from female to male – likely an evolutionary adaptation for population control. Not a problem in itself, perhaps, but since male eels don't tend to "measure up" (only female eels reach required market size), reversal of this natural masculinization is required. Sex-reversal can be induced by adding estradiol – a naturally occurring hormone – to eel feed; however, to ensure commercial growth, to meet regulatory approval from the FDA, and to guarantee consumer safety, levels of estradiol and its metabolites (estrone and estriol) in fish tissue must be monitored. To that end, aquaculture company NovaEel (based in Nova Scotia, Canada) partnered with researchers at Dalhousie University to develop an analytical platform that is up to the task.

Extracts of eel muscle were analyzed using reverse phase liquid chromatography coupled to differential ion mobility spectrometry/tandem mass spectrometry (LC-DMS-MS/MS) using a heated-assisted electrospray source. "In our lab, we've already been successful using this platform for proteomics and

metabolomics workflows, so it was just a matter of tweaking the instrument's LC and MS/MS acquisition parameters to produce satisfactory signals for the steroid hormones," says Alejandro Cohen, Scientific Director of the Proteomics Core Facility at Dalhousie University. "After a week or two of fine tuning our methods, we achieved sub-ng detection limits per gram of tissue."

DMS plays a key role in the platform by acting as an orthogonal pre-MS/MS separation mechanism and thus increasing the specificity of the method – something Cohen recommends when dealing with complex biological samples with 'noisy' backgrounds. But there can be a cost: "Optimal separation conditions for DMS does compromise the signal intensity somewhat," he says. "However, I suggest users evaluate carefully to what extent the selectivity of DMS affects the sensitivity of their assays."

Getting back to the eels... Estradiol was rapidly metabolized (50 percent depletion rate per day) and decreased to levels found in wild mature female eels (and the non-treated controls) five days after hormone treatment ended.

So feel free to re-join the line for the "eel-you-can-eat" buffet... JC

Reference

1. *A Cohen et al., "Analysis of 17β-estradiol, estriol and estrone in American eel (Anguilla rostrata) tissue samples using liquid chromatography coupled to electrospray differential ion mobility tandem mass spectrometry", Rapid Commun. Mass Spectrom., 31, 842–850 (2017).*

Eggs on Your Face

Could brill eggs pose a less expensive alternative to caviar in luxury cosmetics?

What?
Caviar has increasingly been used in cosmetic products as a source of lipids – marketed for their antioxidant, emollient and moisturizing properties. In a recent study (1), brill eggs were compared with sturgeon eggs, following determination of fatty acids, such as linoleic and linolenic acids and triacylglycerols (TAGs).

Who?
Federica Dal Bello and her team from the Department of Molecular Biotechnology and Health Sciences, University of Torino, Italy.

Why?
Simply put: sturgeon eggs are expensive. Finding a cheaper substitute and reducing cost of production would be of interest to both manufacturers and consumers.

How?
The levels of fatty acids (considered to "promote vitamin A and E activity," according to the published paper) were determined using GC-MS; HPLC coupled with high-resolution mass spectrometry (HMRS) was used to identify and characterize the triacylglycerol molecules (thought to enhance skin metabolism).

Findings?
It turns out that, when it comes to luxury skincare, nothing but caviar cuts it. The brill eggs showed very low concentrations or an absence of fatty acids, such as linoleic and linolenic acids – crucial for that sought-after skin "rejuvenation." Plus, caviar eggs had eight times the amount of TAGs. *JC*

Reference

1. *F Dal Bello et al., "Caviar versus brill eggs: A novel high performance liquid chromatography–mass-spectrometry application for evaluating cosmetic ingredients composition", Eur J Lipid Sci Technol, 118 (2016)*

CO_{too} Warm?

Optical spectroscopy uncovers increased carbon dioxide emissions on the Alaskan tundra

Temperatures in the Arctic have been rising at twice the rate of the global increase, especially in winter (1) – something that may be affecting carbon dioxide emissions and negatively affecting local ecosystems.

As part of the Carbon Arctic Reservoir Vulnerability Experiment (CARVE), a team of measurement, satellite and modeling specialists have spent three years measuring the regional carbon flux from the ecosystems in the Arctic that may be stressed as a result of climate warming. "Most studies in Alaska so far have focused on small areas – and even that is difficult to do in the harsh arctic conditions," says Róisín Commane, lead author and Research Associate at Harvard School of Engineering and Applied Sciences. "But as well as allowing us to assess the impact of climate change on the Arctic, understanding the regional effects of these increasing temperatures is very important for making accurate predictions."

The team used two cavity ring-down spectrometers (CRDS) from Picarro on board a NASA aircraft to sample and measure CO_2 and methane concentrations to calculate regional flux. They also studied the National Oceanic and Atmospheric Administration's 41-year record of carbon dioxide measured from ground towers in Barrow, Alaska. The outcome? Emission of greenhouse gases from these areas has increased by 70 percent in the last 40 years – implying that rising temperatures are driving early winter respiration and thus making Arctic ecosystems a net source of CO_2.

Commane continues to focus on understanding more about the drivers of carbon dioxide flux in the Arctic.

"For some analysis work, we're looking at using $^{14}CO_2$ isotopes to age the air coming from the tundra in the fall. We're also looking at how changes in hydrology might be driving those increases in CO_2 that do not have a matching increase in methane over the same time period," she says.

Commane is also involved in two other missions with similar goals. The first – ABoVE (The Arctic-Boreal Vulnerability Experiment), a NASA project – will involve flying over Canada and Alaska this summer. "We're following a similar approach to CARVE but for this project we have put one of the CRDS instruments in the back of a two-seater Mooney aircraft. My colleagues Steve Conley and Colm Sweeney will fly constant profiles over Alaska and Northern Canada six times between now and the end of October, so we can calculate the carbon fluxes over this larger area."

The second airborne project – called ATom (Atmospheric Tomography) – will measure the composition of the remote atmosphere aboard a NASA DC8 aircraft. "For ATom we're measuring over 300 gas concentrations and aerosol characteristics,"

Clockwise from top left: Colm Sweeney and Steve Conley with the Mooney. Róisín Commane on the DC8 during ATom. Steve Wofsy, Bruce Daube and Róisín Commane with the DC8.

she says. "This aircraft is a lot larger than either the Sherpa we used for CARVE or the Mooney being used for ABoVE, so we have more instrumentation and people aboard. We've traveled around the world twice on this project so far and have two more trips to go – so my next year will be pretty busy!" *JC*

Reference

1. *R Commane et al., "Carbon dioxide sources from Alaska driven by increasing early winter respiration from Arctic tundra", PNAS, 114, 5361-5366 (2017).*

The Power List 2017 – 10 Top 10s

The Analytical Scientist Power List is back. And this year, 10 is the magic number…

Got a great supervisor? A collaborator who goes the extra mile? A colleague you'd like to see recognized? You have just a few more weeks to nominate them for the "10 Top 10s" Power List.

For 2017, we want you to let us know the best and brightest analytical scientists across 10 categories. The nominees will be whittled down to the final Top 10s by our judging panel, made up of leading industry and academic scientists, and the editorial team. The categories are:

1. Separation Scientists: Cutting-edge chromatographers and electrophoresis experts
2. Spectroscopists: Leading lights in spectroscopy
3. Mass Spectrometrists: Masters of mass spec
4. Giants of Nano: Scientists advancing microfluidics and nanoscale science
5. Pharma Pioneers: Stars of pharma and biopharma
6. Omics Explorers: Researchers uncovering the secrets of life through genomics, proteomics, metabolomics, lipidomics and more
7. Public Defenders: Scientists protecting people and planet - in food, forensic and environmental analysis
8. Inventors: Clever minds whose breakthroughs are changing analytical science
9. Mentors: Supervisors, colleagues or teachers truly inspiring the next generation of scientists
10. Leaders: Analytical champions influencing the progress of measurement science

The Power List, now in its fifth year, has a simple yet important aim: to celebrate the great work of analytical scientists all over the world. As a previous entrant says, "Anything that draws attention to the profound influence analytical science has on our lives is a very good thing."

Generating excitement (and often controversy), the Power List has become an annual talking point within the field and the wider scientific community. For example, last year's inspirational Top 50 Women drew attention to underrepresentation in analytical science – and our Top 40 Under 40 shone a spotlight on up-and-coming talent.

You have until August 11 to nominate your analytical heroes – whether colleagues, mentors, friends or inspirations – for the recognition they deserve. You can make nominations in any or all categories.

From New Labs to New Solutions

What's new in business?

In our regular column, we partner with www.mass-spec-capital.com to let you know what's going on in the business world of analytical science. This month sees the introduction of several new tech solutions at ASMS, and the European Food Integrity Research Project gains an industry boost as Thermo Fisher Scientific joins the growing list of participants.

Phenomenex has opened a new facility for GC R&D and manufacturing.

Products

- Waters introduces LiveID Software for food analysis
- SCIEX: FDA clears Vitamin D assay kit for mass spectrometry and announces new solutions at ASMS 2017
- Biotage launches Isolera Dalton 2000 Mass Detector
- Microsaic enhances capabilities of its 4000 MiD Miniaturised Mass Spectrometer for bioprocessing applications
- Bruker introduces novel mass spectrometry solutions at ASMS
- Agilent showcases technology solutions at ASMS 2017

Investment & Acquisitions

- Eurofins to acquire GATC Biotech AG in Germany and buy Finnish environmental testing firm Nab Labs
- Thermo Fisher Scientific to acquire Patheon for $7.2b
- Merck acquires Grzybowski Scientific Inventions
- Bruker acquires German microscopy company Luxendo

Collaborations

- Waters and Wyatt announce SEC co-marketing agreement
- Thermo Fisher Scientific joins European Food Integrity Research Project, and shares new TMT licensing program for service providers
- Fluidigm: Seven Helios mass cytometry systems for MRC Consortium

People

- Tecan appoints Erik Norström Head of Corporate Development

Organizations

- Phenomenex opens new GC column research and manufacturing facility

For links to original press releases and more business news, visit the online version of this article at: tas.txp.to/0617/BUSINESS

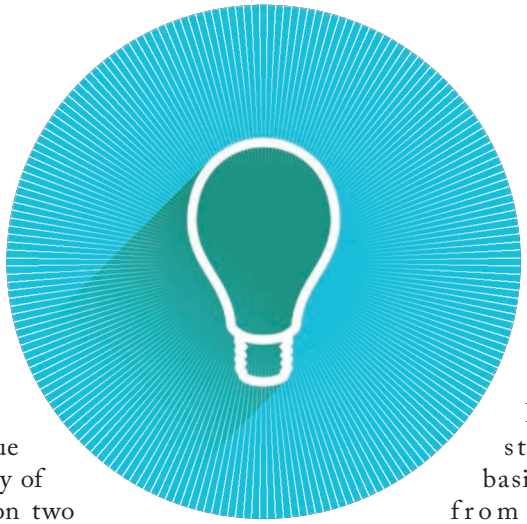
Bright Young Things

Can a UK-wide science festival hook the next generation of analytical scientists?

Throughout May and June 2017, high school children across Bristol have been taking part in the University of Bristol's Festival of Science – part of a wider initiative established by the Salters' Institute. Tim Harrison (University of Bristol Chemistry School Teacher Fellow) tells us about the fun experiments the students got stuck into and why it's important to promote analytical science among young people.

What experiments were the students doing, and what analytical skills they were testing?

The competitions are still ongoing around the country so I can only give a general answer! The students were given a crime scenario and made use of both chromatographic techniques and 'spot tests' to identify the culprit. The tasks were designed to test measuring, equipment handling, graphing and



observation – all skills required by analytical chemists. We also held a competition that was unique to the University of Bristol, based on two tasks. The first required students to create sugar solutions and use gravimetric analysis to create a calibration curve of sugar concentration against mass before determining the concentration of an unknown solution. The second task was based on an old favorite – an iodine clock reaction – where students had to work out how much of each reagent solution was required to make a color change at prescribed times.

All the sessions began with an exciting demonstration by you...

I showed the students some basic experiments from my lecture demonstration "Gases in the Air," which included experiments with liquid nitrogen and dry ice. I also showed them a comparison between the chemical reactivity of helium and hydrogen, and a catalytic decomposition reaction.

How did students react?

They were nervous at first but quickly settled down to the numerous tasks in hand, working steadily and, for the most part, accurately! Every school worked safely, demonstrated good general laboratory techniques and showed admirable levels of teamwork.

Why is this kind of initiative so important?

Analytical chemistry skills are very important to society in general, from the checking of food and water safety to the branch usually referred to by the general public as "forensic chemistry." This chemistry competition gave the students chance to demonstrate the skills they had learnt from their own teachers in a school setting. We just provided the opportunities for these students to show off what they can do!

www.saltersinstitute.co.uk/festivals/

In My View

In this opinion section, experts from across the world share a single strongly-held view or key idea.

Submissions are welcome. Articles should be short, focused, personal and passionate, and may deal with any aspect of analytical science. They can be up to 600 words in length and written in the first person.

Contact the editors at edit@texerepublishing.com

The Importance of Chiral Metabolomics

Chiral amino acids, metabolites long overlooked as "unnatural," are now under the spotlight – as biomarkers for kidney disease.

By Tomonori Kimura, Department of Nephrology, Osaka University Graduate School of Medicine, Osaka, Japan.

Chronic kidney disease (CKD) is a highly-prevalent, global health problem; for example, in Japan, it is estimated that about 10 percent of the population have CKD. The number of patients with worsening kidney functions, eventually requiring costly kidney replacement therapy or transplantation, is increasing. In addition, the risk of life-threatening cardiovascular diseases increases with the progression of CKD stages. Preventing CKD patients from progressing to end-stage kidney disease is therefore critical, but unfortunately there are no effective methods to predict the progression of CKD. Currently, prediction relies on kidney functions estimated from serum creatinine and some additional information, such as proteinuria, but these are insufficient. Naturally, nephrologists are earnestly searching for better biomarkers.

Could amino acids, those vital components of human bodies, help provide the answer? The levels of amino acids (which comprise 20 percent of the body) are influenced by the functions of many organs, including the kidneys; kidneys regulate the body's amino acid balances via reabsorption. Scientists have been studying amino acids ever since their discovery – for more than a hundred years. But because people only detected L-forms in nature, D-amino acids were regarded as unnatural and have not been studied vigorously. The presence of D-amino acids started to be reported sporadically, including in the blood of patients with kidney diseases. Some studies also indicated the physiological roles of D-amino acids in bodies (for example, D-serine is also known as a neurotransmitter of NMDA receptors in neurons) but once again these reports were sporadic – mainly because of the measurement challenge; typically, the amount of D-amino acids in human bodies are present at trace levels, and the chemically similar nature of amino acid enantiomers makes it difficult to separate them and measure them simultaneously. And because reliable methods to measure D-amino acids are lacking, their functions and presence in tissues have remained a mystery.

It is only recently that methods have been devised to measure D-amino acids precisely via a metabolomic approach. Kenji Hamase of Kyushu

"I believe that the mysterious world of D-amino acids will turn out to be a fruitful one for clinicians."

University in Japan and his colleagues went to great lengths to develop a metabolomic platform – based on micro-2D-HPLC – that can detect whole sets of chiral amino acids from human samples with precision. In the first dimension of HPLC, 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F)-labelled amino acids are separated by reverse-phase separation. Then, the fraction of each NBD-derived amino acid is automatically transferred to the enantioselective (chiral-selective) column for chiral separation. The 2D-HPLC system is powerful enough to detect all amino acid enantiomers from clinical samples ranging from around 1 fmol to 100 pmol – quantitatively.

Our research group from Osaka and Kyushu University searched for prognostic biomarkers of CKD by using such chiral amino acid metabolic profiling. Our study revealed that D-amino acids, particularly D-serine and D-asparagine, were robustly associated with the progression of CKD to end-stage kidney disease. The risk of progression to ESKD was elevated from two- to four-fold in those with higher levels. What is more interesting is that this trend is only seen in D-amino acids, and not in L-amino acids. The fact that just a trace portion of amino acids have a stronger relationship with disease processes and prognosis strongly supports the importance of chiral separations.

A D-amino acid test could provide a powerful tool for clinicians, helping them identify high-risk CKD patients for intensive care. The development of a device suitable for clinical use – designed to increase throughput – is currently under way. Another important direction for the future will be undertaking further detailed research to study the physiology and metabolism of D-amino acids, both of which are poorly understood, so that we can enrich our understanding of kidney diseases. Through chiral metabolomics, I believe that the mysterious world of D-amino acids will turn out to be a fruitful one for clinicians.

References

1. *T Kimura et al., "Chiral amino acid metabolomics for novel biomarker screening in the prognosis of chronic kidney disease", Scientific Reports, 6, 26137 (2017).*
2. *K Hamase et al., "Simultaneous determination of hydrophilic amino acid enantiomers in mammalian tissues and physiological fluids applying a fully automated micro-two-dimensional high-performance liquid chromatographic concept", J Chromatogr A 1056-1062 (2010).*

Analytical Assistance, "Pro Humanitate"

When it comes to technology in developing countries, it's about time we asked ourselves what more we can do to help.

By Norman Fraley, Senior Researcher at the Frolich Institute and Instructor – Sustainability Graduate Program, Wake Forest University, North Carolina, USA.

At Pittcon 2017, 14 strangers from 11 countries met to discuss the topic of analytical technology in developing countries. The insights and experiences were both fascinating and compelling. The discussion about the role we could play in supplying or developing that technology, in particular, was long overdue.

Instrumentation is big business. C&E News reported that for combined instrumentation sales in 2015, 25 companies accounted for nearly 60 percent of the global market for analytical and life sciences laboratory equipment, with US$23.6 billion in sales (1). Four of the big dogs – Thermo Fisher Scientific, ABB, Siemens and Agilent – combined have a market capitalization of over $224 billion, up over 20 percent in the past year. About 7 percent of sales is spent on R&D for new products, roughly $2,800 million. That's a substantial amount of money.

Over the past few years, new instrument developments have primarily been focused on incremental increases in sensitivity and software upgrades. In many cases, these improvements have been the result of corporate acquisitions. With these improvements comes the need for ultrapure reagents, stable power, clean laboratory environments and, of course, significant additional capital budgets. Now don't get me wrong, I am a fan of better sensitivity. My question is this: How low is low enough? It seems that the answer is, "it depends."

For example, at the Frolich Institute, we have been working with farmers and food producers in Ethiopia for several years to increase yields and food production to meet the needs of their rapidly growing population. They have seen nearly 50 percent increase in grain yields in the past ten years. Farmers are producing enough specialty crops to export to the rest of the world, and with this comes the promise of becoming a middle-income country. But they

cannot. The best customers – the "first world" countries – demand certification, HACCP, data on pesticides, aflatoxins, GMO and allergens analysis from certified laboratories as a condition of importation. Again, don't get me wrong – I am a fan of safe food and animal feed. But the developing countries do not have access to such levels of analytical capacity and technology, either because of cost, time or accessibility. So although they have safe food to sell, they are rarely able to comply with our restrictions.

My questions are these: at what point does it make sense for instrument manufacturers to invest a few hundred million dollars in manufacturing devices that are not "superior" but simply "good enough"? What would it take to design and build analytical devices that can work in environments that do not have reliable power, water, reagents and skills? When would be a good time for us to start doing our part as clever innovative scientists and engineers and help the developing world to pull themselves into middle-class countries?

I believe that it is way past that time. We have the opportunity to create 1950s-era analytical devices that use today's technologies to solve everyday analytical challenges in the developing world. The scientists in these countries truly want to be green, sustainable and self-sufficient, but cannot afford $300,000 for the latest quaternary LC-MS/MS to run aflatoxin tests. Many have tried equipment donations only to discover the gifts remain unused because of unreliable power, lack of pure reagents and gases, limited knowledge and skills and general logistical challenges.

What happened to sufficient? pH meters that read to three decimal places are certainly impressive, but how about one that reads to one decimal, but is wind-up or solar powered, self-calibrating and only costs a few dollars? I believe that tools that can work over there will also be of great benefit over here.

The scientists at Pittcon and many others have agreed to continue the discussion we had at the conference. The next step is discovering whether deeds can back these words in a way that can provide meaningful assistance, "pro humanitate."

Reference

1. *http://cen.acs.org/articles/94/i17/Top-instrument-firms-2015.html*

Peeling Back the Layers

Pulsed glow discharge TOF-MS allows fast, direct analysis of complex thin-coated materials.

By Jorge Pisonero Castro, Department of Physics, University of Oviedo, Oviedo, Spain.

A plethora of innovative materials are produced by depositing thin and ultrathin coatings on different substrates. For instance, hard disks able to store terabytes of information are based on multiple magnetic and non-magnetic nano-layers. Similarly, the latest photovoltaic cells consist of nano and micro layers of different elements.

The mechanical, optical, magnetic, and/or thermal properties of these materials are directly related to their chemical composition, including the elements used, their distribution within the layers, and the presence of disruptive trace elements. To make sure the layers are as they should be, we need solid analytical techniques that are able to provide fast multi-elemental chemical analyses with high depth resolution (to monitor the different layers) and high sensitivity (to detect major, minor and trace elements).

Reference techniques, such as secondary ion mass spectrometry (SIMS), have high sensitivity and spatial resolution, but the sputtering process to remove and analyse the different layers is slow, and requires ultra-high vacuum conditions. Long analysis times and low sample throughput are the result, limiting its appeal for routine analysis. Plus, SIMS is based on a single atomization and ionization step, which results in matrix effects that significantly affect quantification. Conversion of qualitative depth profiles (ion signal versus sputtering time) into quantitative depth profiles (concentration versus depth) are tedious and require matrix-matched calibrating samples.

Glow discharges (GDs) appear to provide a faster option. GDs are low-pressure plasmas produced by a voltage difference between two electrodes immersed in an inert gas (generally Ar), where the sample forms the cathode. Sputtering is produced by the bombardment of the material with Ar ions and fast Ar atoms, resulting in a relatively high sputtering rate. Moreover, sputtered atoms from the sample are then diffused into the negative glow region of the GD plasma, where they suffer different excitation and ionization processes. Therefore, atomization and ionization/excitation are temporally and spatially separated, lowering the potential for matrix effects.

In particular, glow discharge optical emission spectroscopy (GD-OES) has proved very effective for the quantitative analysis of thin and ultra-thin layers of diverse nature; for example, conductive and

non-conductive layers. Recent instrumental developments, such as differential interferometry profiling (DiP), allow online calculation of the sputtered depth, simplifying the quantification process (see https://theanalyticalscientist.com/issues/1216/depth-profiling-with-gd-oes).

However, GD-OES suffers from some limitations when it comes to trace elements. Converting emission signals into concentrations still requires the calculation of emission yields that could be affected by the presence of non-metals in the material (such as oxygen, nitrogen or hydrogen). These elements could produce significant quenching effects in the plasma so it's important to apply the appropriate correction in the quantification methods.

Would coupling glow discharge with mass spectrometry (GD-MS) overcome these problems? For the analysis of multi-elemental thin layers, very fast simultaneous or quasi-simultaneous mass spectrometers are required. In particular, GD has been coupled to time-of-flight (TOF) mass spectrometers, which can take a complete mass spectra every 30 µs. The operation of GD in a pulsed mode, using either direct current or radiofrequency, reduces sputtering and allows each ion signal to be integrated in the most appropriate temporal domain, which reduces the presence of polyatomic interference and allows the highest sensitivity.

Analysis time with pulsed-GD-TOFMS is almost two orders of magnitude lower than with SIMS, with similar depth resolution. And sample throughput is significantly improved, as ultra-high vacuum conditions are not required and the samples can be easily exchanged. A relatively accurate quantification can be achieved using matrix and non-matrix matched calibrating samples (1–6).

But before pulsed-GD-TOFMS can fulfil its potential, we must improve on the reduction of background signals from non-metals, increase sensitivity, gain a full understanding of the physical and chemical plasma processes of the pulsed GD plasma, and develop more accurate quantification methods based on online determination of sputtered depth. If we can achieve all this, pulsed-GD-TOFMS will become a powerful method for fast multi-elemental analysis of thin and ultra-thin layers.

References

1. *C González-Gago et al., "Analytical potential of rf-PGD-TOFMS for depth profiling of oxidized thin film composite membranes", J Anal At Spectrom, 31, 288-296 (2016).*
2. *J Pisonero et al., "Critical evaluation of the potential of radiofrequency pulsed glow discharge time of flight mass spectrometry for depth profile analysis of innovative materials", Anal Bioanal Chem, 405, 5655–5662 (2013).*
3. *J Pisonero et al., "Minor elements determination and evaluation of diffusion/segregation effects on ultra-thin layers using pulsed-RF-GD-TOFMS", J Anal At Spectrom, 26, 1604–1609 (2011).*
4. *M Bustelo et al., "Pulsed radiofrequency glow discharge time of flight mass spectrometry for nanostructured materials characterization", Anal Chem, 83, 329–337 (2011).*
5. *J Pisonero et al., "Quantitative depth profile analysis of boron implanted silicon by pulsed-radiofrequency-glow discharge-time of flight mass spectrometry", Sol Energ Mat Sol Cells, 94, 1352–1357 (2010).*
6. *J Pisonero et al., "Critical revision of GD-MS, LA-ICP-MS and SIMS as inorganic mass spectrometric techniques for direct solid analysis", J Anal At Spectrom, 24, 1145–1160 (2009).*

Embracing the Proteogenomic Toolkit

To win the war on cancer, we need to put proteomics on an equal footing with genomics.

By Andreas Hühmer, Director, Proteomics and Metabolomics Marketing, Chromatography and Mass Spectrometry, Thermo Fisher Scientific, USA.

Advances in our understanding of cancer biology through gene expression analysis have resulted in major steps towards the goals of reliable and effective cancer diagnosis, prognosis and treatment. But despite the progress we've made over the past few decades, many would justifiably argue that genomics has not fully lived up to its promise.

Although a number of cancer-driving gene mutations have been identified through the genomic characterization of tumor tissue by large-scale projects such as the Cancer Genome Atlas, the widespread identification of targetable cancer drivers remains a significant hurdle. For metastatic breast cancer, for instance, few validated oncogenic drivers exist (1). Moreover, establishing whether gene mutations are cancer "drivers" or "passengers" continues to be a challenge – and is difficult to determine based on genomic assessment alone.

Genomics has taught us that cancer is far more complex than we previously thought. The tumor microenvironment is highly heterogeneous, with significant variability even between individual cancer cells (2). This complexity is compounded by the fact that cancer is dynamic; taking a tumor sample and sequencing its genetic contents merely produces a snapshot, not the blueprints for future tumor growth. The apparent lack of correlation between the genome and phenome highlights the need for a complementary proteomic approach to unravel cancer's complexity.

Meanwhile, our ability to map out the proteomic landscape within tumor tissue has steadily grown over the past two decades. Advances in mass spectrometry and informatics now allow us to study protein samples on an unprecedented scale. The latest liquid chromatography-mass spectrometry (LC-MS) technologies, coupled with new multiplexed proteomics approaches based on isobaric labeling and advances in data processing, are leading to improvements in the depth and speed of quantitative proteome profiling – all while using ever smaller sample volumes (3).

But it's when these two approaches are used in combination that we can make the most progress. Proteomics techniques are now being used alongside genomic analysis to help unlock new cancer immunotherapies far more quickly than conventional approaches (4). Traditionally, the search for targetable cancer antigens was a time-consuming and error-prone process, involving DNA sequencing and mutation prediction algorithms, followed by large-scale immunological assays. Using mass spectrometry to profile peptides directly, we can reduce that timeline to a matter of weeks.

Advances in proteomics technologies are also driving improvements in cancer biomarker discovery. Recently, groundbreaking research by the Karolinska Institute in Sweden demonstrated the potential of integrating both protein and genetic markers in a single test for prostate cancer (5). And one goal of the US' National Cancer Moonshot program's Blood Profiling Atlas project is to develop a readily accessible database of blood biomarkers that will make it easier for oncologists to diagnose patients using liquid biopsies.

Genomics will continue to play an important role in cancer research. However, it is becoming clear that gene expression analysis alone is unable to sufficiently advance our understanding of cancer biology necessary for truly effective patient stratification and personalized therapy. A decade of technological development has made proteomics research-ready; we must now fully use the whole proteogenomics toolkit to truly make inroads on the fight against cancer.

References

1. *M Arnedos et al., "Precision medicine for metastatic breast cancer – limitations and solutions", Nat Rev Clin Oncol, 12, 693–704 (2015). PMID: 26196250.*
2. *N Navin et al., "Tumour evolution inferred by single-cell sequencing", Nature, 472, 90–94 (2011). PMID: 21399628.*
3. *GC McAlister et al., "MultiNotch MS3 enables accurate, sensitive, and multiplexed detection of differential expression across cancer cell line proteomes", Anal Chem, 86, 7150–7158 (2014). PMID: 24927332.*
4. *M Bassani-Sternberg et al., "Direct identification of clinically relevant neoepitopes presented on native human melanoma tissue by mass spectrometry", Nat Commun, 7, 13404 (2016). PMID: 27869121.*
5. *FH Schröder et al., "Screening and prostate cancer mortality: results of the European Randomised Study of Screening for Prostate Cancer (ERSPC) at 13 years of follow-up", Lancet, 384, 2027–2035 (2014). PMID: 25108889.*

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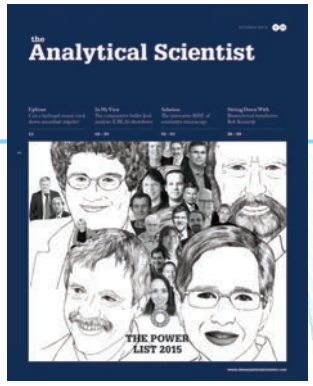

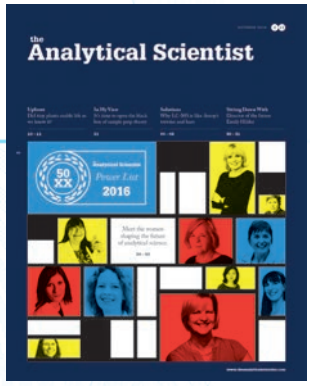

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Driving GC-VUV Applicability

Introducing VUV Analytics' newest recruit – Jack Cochran – who brings 30 years of gas chromatography experience as Senior Director of Applications.

Tell us about your journey into VUV Analytics...

I'll start at the beginning (but not as far back as when I was born) by stating that I've always had a love of gas chromatography. I grew up as a gas chromatographer at EPA in Oklahoma and so GC really was my entry into the wider analytical world. And if GC has been the heart of my whole career, detectors have been the 'brain', so I've always maintained a strong interest. I've done a great deal of mass spectrometry – both as a user and on the vendor side – but I've worked with all GC detectors over the years, and I've always been keen to follow promising advances.

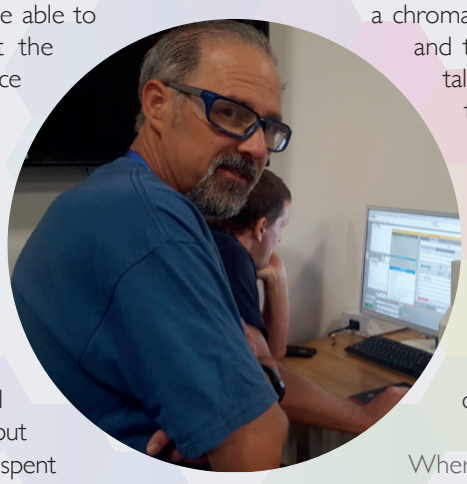
When the opportunity to move back into the detector game came along (and in a much more pleasant climate), I jumped at the chance. I could see that the VUV detector offered the potential to speed up analyses in ways that weren't possible with non-selective detectors – or with MS in some cases (co-eluting isomers, for example). Another big reason for my move is that the detector is so darned cool; it's unique – and given that so few detectors come along for GC, it's also hard to ignore. Consider that vacuum ultraviolet absorbance spectroscopy was historically considered to be too difficult – the materials engineering solutions that were applied to allow us to collect absorbance spectra in the VUV range are tremendous.

Finally, I have to say that the attraction of a successfully growing start-up company also pulled me in the right direction. Now that I'm later into my career, I want to have more and more of an impact. Who doesn't want to go out in a blaze of glory?

Let's dwell on the "uniqueness" – how great is it to see VUV spectra for the first time?

We recently discussed methanol and ethanol in transformer oil as a headspace application – and when we acquired the data for the first time, I saw a good oxygen peak, but I was blown away by the fact that I was looking at a brilliantly shaped water peak. Given my background – environmental, food, and so on – I'm used to looking at pesticides, PCBs and PAHs, but to chromatograph water like that and gain an authoritative spectrum... it's just not something I'm used to.

It's great to still be able to enjoy the thrills at the bench. My new office is just across from the lab – and call me the lurker or the kibitzer, if you will, but I love being in there, working with the talented young chemists. I'm there to offer advice and support, of course, but most of our time is spent sharing in the excitement of seeing something new. The absorbance spectra of compounds for the first time, the different spectra of isomeric compounds, the ability to compress chromatography through high flow rates – all of these things are spellbinding to me. And I've only been here three months...



How did you perceive VUV detection before you joined?

Most of all, I was impressed by the fact that they were actually able to do it! As I mentioned, you don't see new detectors come along for GC very often, so I remember wanting to find out what it could do. I arranged a 'lunch-and-learn' seminar for my colleagues to hear about VUV detection. CTO Dale Harrison gave an impressive presentation on the technology and how it could be applied. He actually downplayed the team's GC skills – I was impressed not only with the detector, but also what they were achieving with it from a chromatography perspective.

I also started to see VUV detection popping up in the programs of various chromatography meetings. At Restek, we were developing a new GC column for PAH separations, where there are some important isomers – chrysene and triphenylene being a notable confounding pair that can't be resolved by mass spectrometry. We spent quite some time working on a chromatographic solution... and then I listened to a talk by Kevin Schug on the deconvolution of chrysene and triphenylene without the need for separation. I thought, "Wow." It made me re-think the potential to simplify chromatography.

Where do you see VUV detection heading from an applications point of view – what are the killer apps now and in the future?

One of our "killer apps" – the relatively new ASTM D8071 method for the determination of hydrocarbon group types, and select hydrocarbon and oxygenate compounds in finished gasoline. It's getting a lot of interest right now, especially because PIONA analysis typically requires a reformulyzer, which is expensive, not to mention hard to operate and maintain. I foresee us continuing to make progress in fuel analysis applications.

We are also working extensively in areas where formaldehyde determination is important. Formaldehyde is almost invisible to FID and requires derivatization, but it has

a very nice absorbance spectrum.

Residual solvents in pharmaceuticals is likely to be another killer app. Here, our compression of the chromatography is attractive and made possible by time-interval deconvolution techniques for analytes of interest, which make co-elutions less of a concern. And there is an exciting possibility of determining water, formaldehyde, and residual solvents in pharma samples – simultaneously.

Water determination in solvents (as an alternative to Karl Fischer titration) is another application that we're assessing; we think it looks very promising.

Further into the future, there are many areas that stand to benefit from the advantages of VUV spectroscopy. Petroleum applications will continue to play to our strengths – in particular, the fact that aromatics absorbance is so strong and can be distinguished from saturates will allow us to offer process stream analyses that wouldn't be accessible without more complex equipment. And that's echoed in MOSH/MOAH applications in food packaging analysis. (I remember a fantastic talk from Koni Grob many years ago at an ISCC meeting; I think it was called, "Lubricated Packaging, Lubricated Food, Lubricated People.") Grob's method for the determination of MOSH and MOAH components involves an extraction from the material of interest followed by online LC-GC-FID (classic Grob!). We think we can make headway here and remove the need for the silica gel LC step...

References

1. *J Schenk et al, "Analysis and deconvolution of dimethylnaphthalene isomers using gas chromatography vacuum ultraviolet spectroscopy and theoretical computations", Anal Chim Acta 945, 1–8 (2016).*

**Results come from a short survey of 91 readers working with applications in agriculture, chemicals, environmental, food/beverage, forensics/toxicology, life science/clinical, materials, natural products, petrochemical, and pharmaceutical sectors.*

Reader GC Survey*

>50% of respondents would consider an alternative method with a reduction of 10–30 mins in chromatography runtime/analysis time.

Jack says... "From this result, I'd say that people have become more moderate in their thinking than I would expect. Chromatographers have spent a lot of time over the years talking about how we were going to employ 0.1 mm ID (0.1 µm film thickness) columns to do everything ten times faster. But that's just not realistic from a sample loading capacity standpoint. When it comes to runtimes, I believe it is often possible to reduce them 2–4x or more with the technology we have; for the most part, we're not flow limited and nor are we isomer co-elution (or other co-elution) limited because of the uniqueness of VUV absorbance spectra.

"Going back to the method for methanol and ethanol in transformer oil, we actually went up to 8 ml/min carrier gas flow to shorten the runtime – but that meant the GC was actually waiting for autosampler headspace equilibration. At that point, it doesn't make sense so we backed off to 4 ml/min, which is still much too high for most mass spectrometers. Not only did the higher flow allow us to reduce runtime, but it increased detectability because we could take the split ratio down to as low as 2.5. As a chromatographer who looks at these things in detail, that's extremely exciting to me. Given the many advantages of increased flow, it will be a strong focus for us."

>68% of respondents found that differentiation of isomers was a significant time burden.

Jack says... "The first thing I would say is that the potential to separate positional isomers by their VUV absorbance spectra is way higher than with mass spectrometry, which almost always yields essentially the same mass spectra. The mass-to-charge peak heights and ratios may be different, but overlap of ions is inevitable, meaning that deconvolution is not possible. Numerous papers show the utility of VUV when it comes to isomers; Schug's group published a great paper on deconvolution of dimethylnaphthalene isomers, for example (1).

"Terpenes and turpentines are challenging from a co-elution perspective simply because there are so many of them. And where previously we might need to use a less thermally stable but more selective GC phase in the hope of separating them, we can now turn to the detector for selectivity and use more robust columns. That's a huge mindset change for me – but one that I am enthusiastically adopting! Of course, you could always keep your selective column and gain from compressing the chromatography..."

37% of respondents spend 10–30 min/sample on interpreting data and reviewing results

Jack says... "People considering VUV spectroscopy may be concerned about data review with an unfamiliar technique. Consider the ASTM D8071 method, where data processing really is almost fully automated. In brief, retention index values are being used in the background for peak finding and so the detector cruises along finding the appropriate compound of interest for spectral library matching; time-interval deconvolution and absorbance responses are automatically tied into response factors that have been generated for each compound class – and you get presented with a report at the end. Each application is different, but a similar approach could be applied to any sample using our VUV Analyze tool. And if you've optimized your runtime by compressing the chromatography, wouldn't it be kind of silly to immediately lose the time saved on data review? We want to reduce that burden."

76% of respondents use mass spectrometry
3% of respondents use VUV spectroscopy

Jack says... "Oh – that's easy to comment on... Both numbers should clearly be closer to 100 percent! And we'll be developing more and more real-world applications to convince people about that from a VUV spectroscopy point of view..."

DIGGING DEEPER, BUILDING BETTER

AN INTERVIEW WITH CATHERINE FENSELAU

Catherine Fenselau's impressive analytical science career has taken her from ancient ruins in Colorado to the analysis of lunar rock samples, from the early introduction of MS technology to the biomedical lab, to the heady days of fledgling mass spec journals. Here, Catherine reflects on the past, present and future of mass spec and explains why analytical science deserves more respect.

When I was a child, I wanted to be a "lady archaeologist". We went to Mesa Verde National Park many times on family vacations, and I thought the archaeology there was just wonderful. One of the big mysteries of the Mesa Verde ruins is where the people went; they had a tough couple of decades with drought, inter-tribal warfare and even cannibalism... Then, after 1,300 years or so, they all left. Where did they go? I thought it would be great to find out – form a hypothesis and then dig out the proof. And I suppose that's what really got me thinking about being a scientist.

FROM TITRATION TO **SPACE STATION**

As I got older I took the biology–chemistry–physics sequence that most American high schools provided around that time, but wasn't sure which would be the best way to spend the rest of my life. When I got to Bryn Mawr College in Pennsylvania, I majored in chemistry – the curriculum captured my attention, but it was the engaging chemistry faculty who really hooked me in. Why not biology or physics? Perhaps I "titrated" myself into the right level of quantitation; at that time at least, chemistry was much more quantitative than biology, but physics was more quantitative than chemistry...

I was fortunate to work with Carl Djerassi at Stanford University for my PhD. And it just so happened to be one of the first labs to apply mass spectrometry to structure elucidation. You may know Djerassi's name – he's on the patent for the birth control pill – so you could say he changed western civilization. He was a natural products chemist, but also someone who really believed in the power of technology.

He had been impressed with Klaus Biemann's success in applying mass spectrometry to undeciphered alkaloid structures. As someone interested in steroids, Djerassi wanted to use this fast, highly sensitive method to help elucidate steroid structures, so he got a mass spectrometer, hired two postdocs from European labs (where there were physical chemists using the technology) and, over the next two decades, took on 10–15 graduate students to develop the technology for his interests. I recognize a good opportunity when I see one.

In 1967, I moved to the NASA Space Sciences Laboratory for a postdoc with Melvin Calvin, where we practiced mass spectrometry techniques on rocks, in preparation for the analysis of lunar soil samples. NASA had a whole international consortium of labs to prepare for that project – and it seemed glamorous despite the analytical chemistry being pretty simple. Calvin and I published a paper in Nature (1), reporting on the behavior of olefins, and how they squeeze into the holes in model rocks. We were working with high-end equipment, which was exciting – it was a very successful experience for me. However, I only stayed two years before moving on to my own position. My father always told me I should have waited till the moon rocks came back...

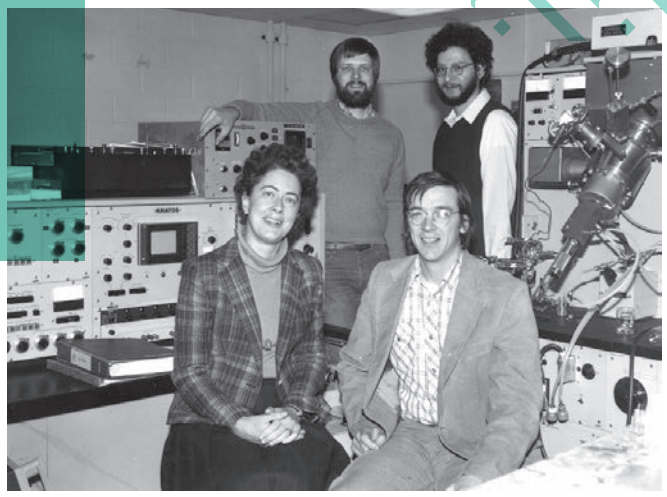
My husband and I both received job offers from Johns Hopkins School of Medicine, so I packed up my cats (and my husband), got in the car and drove across the country to Baltimore, where I have been ever since.

FORGING **AHEAD**

At Johns Hopkins, I was the first trained mass spectroscopist to join a US medical faculty. The problem? When I started, there was no mass spectrometer! I had to travel to the National Institutes of Health (NIH) labs to use their instruments until we were granted the funding to obtain our own. Though mass specs were a novelty for clinical applications, they had found real utility in monitoring the production of aviation fuel. Herbert Hoover's grandson founded a company in southern California to manufacture instruments during the Second World War, so that all oil companies could use the same criteria. We bought an instrument made for this purpose, modified it and applied it to biomedical problems.

For me, one of the most delicious parts about being a scientist is the weekend when you know something that nobody else in the world knows; you made a finding in the lab on Thursday or Friday, but you have yet to communicate it. I was lucky enough to have a number of those weekends in this period of

> "ONE OF THE MOST DELICIOUS PARTS ABOUT BEING A SCIENTIST **IS THE WEEKEND WHEN** YOU KNOW SOMETHING **THAT NOBODY ELSE IN THE WORLD KNOWS.**"



Clockwise from top left: Original team of the Middle-Atlantic Mass Spectrometry Lab in 1985 (Catherine Fenselau, husband Robert Cotter, James Yergey and David Heller). With sons Andy and Tom on Maui. Lecturing in Kuala Lumpur.

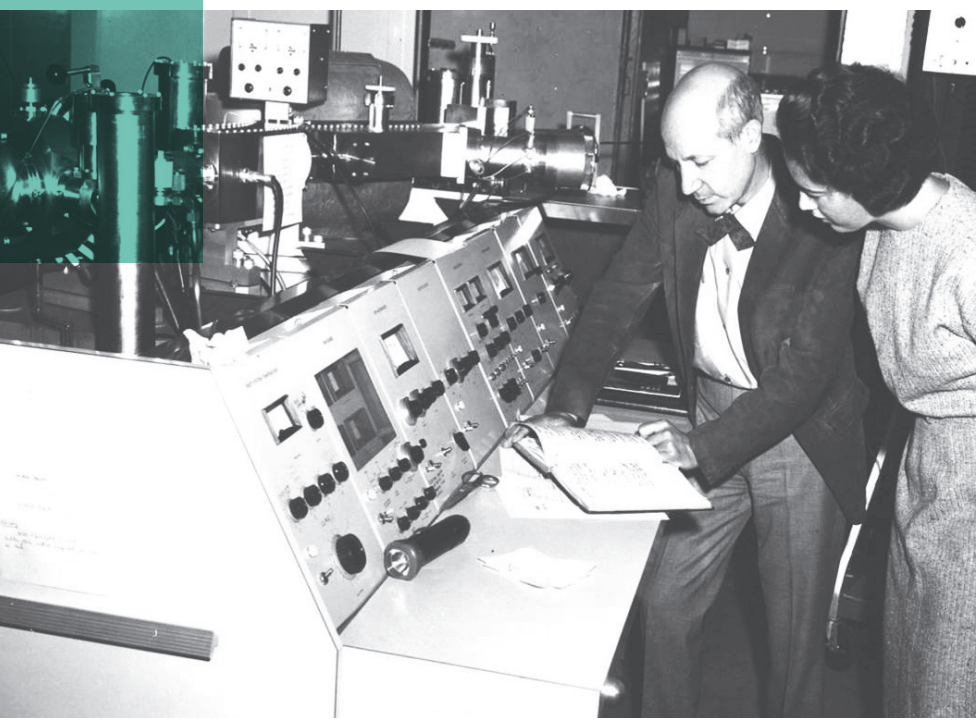
my career. One was our discovery that a common class of drug metabolites called carboxyl-linked glucuronides could alkylate proteins. When we eventually published our findings (2,3), pharma companies took notice, because it's a clear mechanism for toxicology. And so this new knowledge meant they could reformulate drugs to make them safer.

Some of the samples we analyzed back then were quite amenable and we got good answers. Others were not; for example, anything that had a phosphate group on it wasn't volatile enough. With our particular instrument, the sample had to be converted to the gas phase, but often it just burnt rather than vaporizing. I spent a lot of time at Johns Hopkins talking to other scientists to try and find a new way to bring these involatile molecules into the gas phase. A lot of folks recognized the limitation. And several of those scientists finally provided us with (Nobel-prize winning) solutions...

EXCITING TIMES

They were heady days. Mass spectrometry was so new that those of us who moved into medical schools at that time found that every analytical endeavor was novel, publishable and of considerable interest. At the University of Maryland, where I moved in 1987, we had a four-sector JEOL mass spectrometer (everyone who had one of these was totally thrilled with the engineering and capability, but the technology quickly became obsolete after the introduction of electrospray ionization empowered smaller instruments).

We used it, among other things, to measure the basicity of amino acids. Much of the fundamental work on peptide sequencing was dependent on understanding which parts of the



Clockwise from top left: With Melvin Calvin at UC Berkeley in 1966, examining a CEC 21-110 double focusing mass spectrometry system. Hiking in Colorado. Being presented with the 2012 ASMS Award for Distinguished Contribution in Mass Spectrometry by Scott McLuckey.

peptide were more basic – where the protons would be localized. The basicities of most amino acids had been measured; however, there was no value for arginine – nobody had found a standard compound to compare it with and thus to make an estimate of its basicity. I was at a conference in Europe, and saw a poster showing studies on some very basic compounds, one of which was strong enough to be used as a standard to quantitate arginine's basicity.

We went home, ordered it from a chemical supplier, popped out the measurement and shared it immediately with the community (4). Folks who needed that measurement were very pleased and referenced us, while some physical chemists complained – probably rightly – about the method we'd used; it was an estimate after all – but it was a pretty good estimate! It allowed analytical folks to develop their theories and their own methods. And it's one of my proudest and most pleasurable research memories.

> "IN THE MASS SPEC FIELD, I WOULD SAY THE MOST RECENT RAPID ADVANCE WAS THE ORBITRAP – I DON'T THINK ANYONE SAW THAT COMING."

SHAPING **THE FIELD**

In 1973, Carl Djerassi recommended me to be the co-editor of a new mass spectrometry journal called Biomedical Mass Spectrometry (now called Journal of Mass Spectrometry). Several journals started up around that time – mainly because JACS got tired of publishing the prolific output of the new mass spectrometry community! I worked on that journal for sixteen years. Then I became Associate Editor for the ACS journal, Analytical Chemistry. Importantly, that journal had decided that mass spectrometry was important and was expanding its coverage. I left ACS in 2015 – another 26 years was long enough!

It was an exciting time. I'd go to talks and conferences, find good papers, encourage their authors to write them up and publish them in peer-reviewed journals. I also spent a lot of time looking for reviewers, making sure the papers got corrected, resubmitted and published. There's a famous quote from Djerassi: "The research isn't finished until the paper's been peer reviewed and published." I have also always made an effort to encourage, include and honor women scientists in the field.

Editing journals gave me an invaluable opportunity to nourish the growth of the field, both with a new journal and then with a prestigious journal that was newly interested in mass spectrometry. It was a time when that area could be nurtured and shaped, and I was one of the lucky folks who got to do it. That was an exciting position to be in.

There's now a journal for everything and maybe too much gets published; for example, this last decade, the proliferation of electronic journals has made it possible to publish any observation – and they're not all interesting. But we work in a capitalist economy – the journals that don't get many papers submitted or that only publish boring papers are probably not going to make much money. The scientists themselves will select the winners and losers.

There has been a great deal of controversy around reproducibility or authenticity of work within peer-reviewed papers, and though I wouldn't say there's never been any false data in mass spectrometry, I'm not aware of it personally. Frankly, I don't think our rewards are high enough. There's probably more false reporting in fields where there's a very high payoff – where the secret you kept all weekend is going to get you a Nobel prize, not just delight the pharmaceutical industry. Analytical chemistry is a solid discipline that happens to be very useful across the whole spectrum of science – but it's not like discovering CRISPR. For me, it's satisfying when some other lab reproduces your work or takes the next step, because it demonstrates that your work was correctly reported.

SEPARATING PROTEINS, INTEGRATING COMMUNITIES

Currently, the research area I'm most excited about is the search for a mass spectrometry-based method for structural elucidation of polyubiquitins and ubiquitinated proteins. Ubiquitin is an 8,500-dalton protein that polymerizes and also attaches to other proteins in the cell, often determining the fate of both the proteins and the cell. In essence, it sends a message about where a protein should be moved and what it should do. The messaging seems to be dependent on the structures of mono- and polyubiquitin sidechains. So far, biochemists haven't had sensitive physical methods to characterize these branched proteins and read the ubiquitin code. So we've been working to develop a mass spectrometry approach. It

> "EACH OF US CAN ONLY DO SO MUCH WITH ONE CAREER SPAN, BUT BY TRAINING OTHERS, YOU CAN MULTIPLY THAT IMPACT."

involves ionizing the intact branched protein, and then fragmenting it. The fun part is interpreting the spectrum! We've adopted some computer programs to help us locate how many branches there are and where they're attached to each other to unravel the structural puzzle (5). And I'm pleased to say that our approach is getting a lot of attention from the community.

Technologically, protein separation is still a major challenge in (biological) mass spectrometry. In the next decade or less, we need to see better separation techniques for proteins. That's not necessarily going to come out of the mass spec community, but whether it's a HPLC method, or a capillary electrophoresis method, we need something that will fractionate with higher resolution than what we have now.

In the mass spec field, I would say the most recent rapid advance was the Orbitrap – I don't think anyone saw that coming. I'm hoping we'll continue to see new ionization techniques and instrumental developments. But I don't ever want mass spectrometry to be a black box. Though there are some valuable applications where the instrument functions that way, ultimately if we're going to continue to evolve, we need to know what's inside and how it works. We need more sensitivity and we need more mass range. I can carry out a top-down analysis of a polyubiquitin that weighs 50,000 – but not 100,000... yet. I'm optimistic that as biology itself evolves, we'll be able to work on increasingly important problems, and thus make more and more impact – most likely in partnerships and collaborations.

Science always advances when we communicate with each other and I've always practiced collaborative science. Bioinformatics or computational science is helping to drive us forward and be more productive, so we now need programmers and bioinformaticists in our field. It's fair to say the field is data rich – and computing continues to have a massive impact. One person cannot know everything. I don't know in depth about the cell biology of a tumor-bearing mouse, and I'm actually not very good at programming, so I work with collaborators. I also notice that the federal funding agencies appear to be thinking along similar lines – it seems easier to get money from the NIH if you have a team applying, rather than a proposal from a single laboratory.

WHERE SOCIOLOGY MEETS SCIENCE

I've had some exciting experiences in my career, but looking back, I think training 160 students and postdocs has been the biggest highlight. Each of us can only do so much with one career span, but by training others, you can multiply that impact. Several of my former PhD students have helped open new application areas for mass spectrometry. For example, Igor Kaltashov is one of the leaders in biophysical mass spectrometry, and Richard van Breeman has been a leader in the use of LC-MS to study metabolism of food additives and nutritional supplements.

An engaging professor needs to enjoy their subject, and they need to be interested. A sense of humor helps too. Djerassi was a terrific mentor, and I like to think that one of the major lessons I learnt from being in his lab was how to manage and run a research group. He engaged us intellectually in conversation and in how he wrote his papers. My own teaching style is probably very old fashioned; I still write on the chalkboard, I still stand up and lecture.

I think when students join the lab, there's an unspoken contract – they're supposed to work hard on my project, and I'm supposed to work hard to get them a job at the end. I think as educators it's our responsibility to teach the students to do lab work, but also to communicate their work. We need to teach them a little bit about sociology: if they go to a conference, everybody they meet is potentially a peer reviewer. Every introduction is an opportunity. I try to teach teamwork as well; some of the most difficult aspects of my research involve collaborations, as I mentioned. The grad students and postdocs involved in that work have to learn to communicate across scientific boundaries, which isn't always easy. You have to be patient. There are things that seem obvious to us, but not to our cell biology colleagues. And we also have a lot to learn from them.

I'm currently teaching a graduate course in biological mass spectrometry; there's widespread interest in the technology (I have students from three different colleges on campus, and five or six departments). A high percentage of the new students my department recruits actually want to be trained as bioanalytical chemists, because they see that we tackle important problems. They also appreciate that there's a lot of flexibility within a career in that area – you could end up being a government

Clockwise from top: With Bob Cotter at a rally in Washington DC. With grandchildren Nick and Rebecca in 2000. Sunny Wang receives her PhD.

regulator, go on to law school or pursue bioanalytical research...

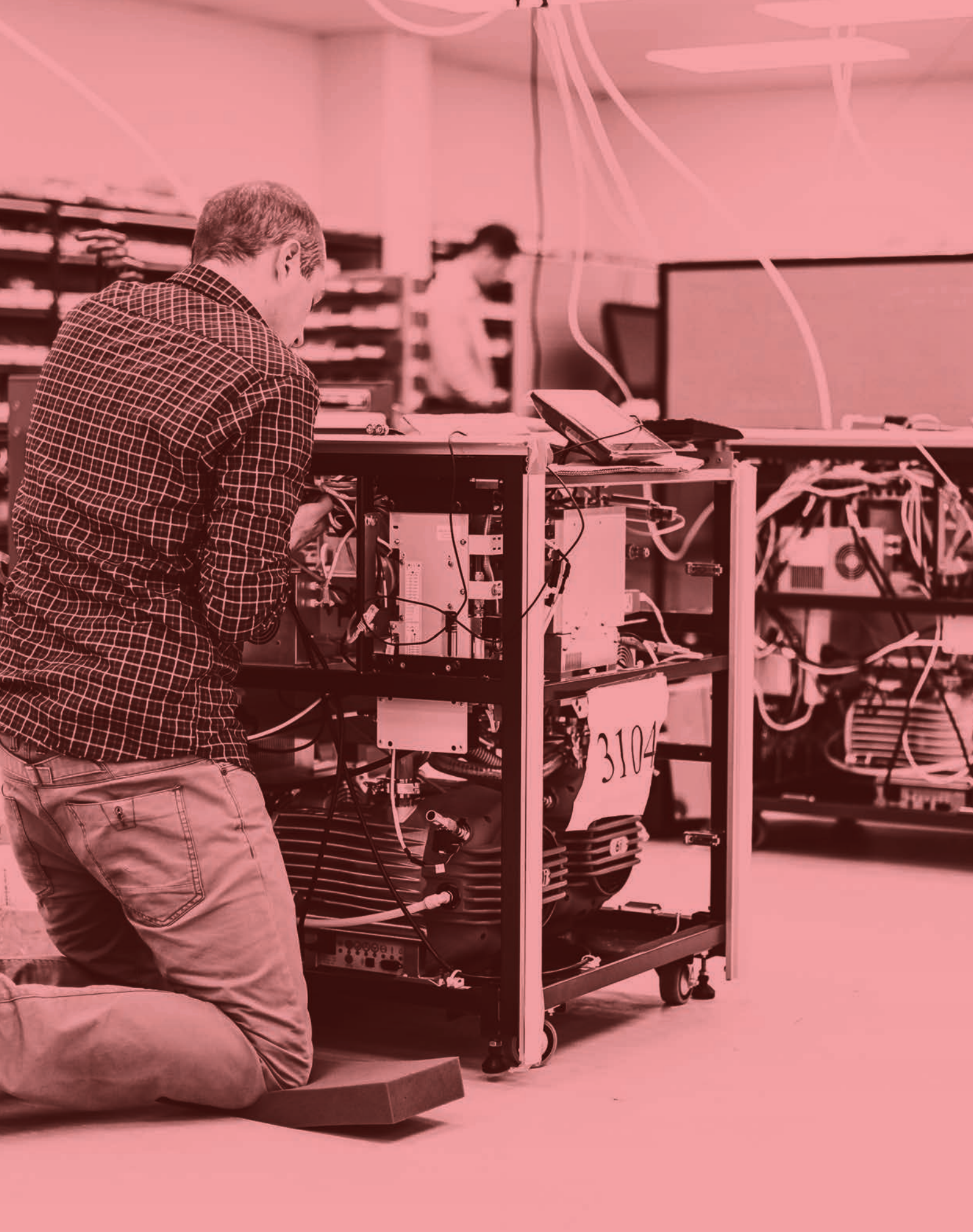
The challenge for analytical chemistry as a whole is to be accepted as part of the frontline of chemical research. Often it is considered a supporting science, which suggests it's additional rather than essential. There's no official analytical chemistry position at Stanford, but there are two or three faculty members in chemistry and chemical engineering who are leaders in analytical chemistry. I trained in organic chemistry, came into mass spectrometry and essentially ended up in biochemistry. And I got my first job because somebody wanted to bring mass spectrometry into a medical school. In any case, I think my career shows just how helpful analytical chemistry can be – as well as how much fun an analytical chemist can have!

I often think about the fact that I didn't go into archaeology, but I don't regret it – because I plan to go into the field when I retire. Now I understand a little more how science works, I plan to check with archaeologists to see what advances they have made in understanding the migration of the lost tribes of Mesa Verde. I want to build on what's already known.

Catherine Fenselau is Professor in the Department of Chemistry & Biochemistry at the University of Maryland, USA.

References

1. *C Fenselau, M Calvin, "Selectivity in zeolite occlusion of olefins", Nature, 212, 889 (1966).*
2. *M Salmon et al, "Rapid transesterification of bilirubin glucuronides in methanol", Life Sciences, 15, 2069 (1974).*
3. *R van Breemen, C Fenselau, "Acylation of albumin by 1-0-acyl glucuruonides", Drug Metab Disp 13, 318 (1985).*
4. *4. Z Wu, C Fenselau, "Proton affinity of arginine measured by the kinetic approach", Rapid Commun In Mass Spectrum, 6, 403 (1992).*
5. *AE Lee et al, "Preparing to read the ubiquitin code: top-down analysis of unanchored ubiquitin tetramers", J Mass Spectrometry, 51, 629 (2016).*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Sifting for Gold

Like many start-ups, Syft Technologies hasn't had the easiest of rides. In fact, there were times when we came close to admitting defeat. But our sheer belief in the potential of SIFT-MS kept us aloft – and now we're going from strength to strength.

By Murray McEwan

Back in the early 1970s (after gaining my PhD at Canterbury University here in Christchurch, New Zealand) I set off for a post-doctoral position at the Center for Research into Experimental Space Science at York University in Toronto, Canada. My goal? To study the ion–molecule reactions that were attracting such a lot of interest at the time.

Stellar beginnings

In the late 1960s, the advent of the smaller quadrupole mass spectrometer meant that it could be loaded onto a rocket; the US promptly did just that, and as it returned to Earth from the ionosphere, they began recording ions in the atmosphere. The results were unexpected in that different ions were observed than anticipated. A laboratory was established at the National Bureau of Standards in Boulder, Colorado, to investigate ion–molecule reactions and to explain how the ions in the atmosphere were interacting with atmospheric gases.

At almost at the same time, radio astronomy was coming into its own. Optical telescopes couldn't tell us much about so-called interstellar clouds because they were opaque, but radio telescopes could look into their interiors. And they found that molecules existed there – another puzzle. There is very little material inside an interstellar cloud. On earth you might get 10^{19} molecules per cm^3, while interstellar clouds contain a maximum of 10^6 molecules per cm^3. They are also very cold – around 10 degrees above absolute zero. At that temperature, all chemistry stops, so how were the molecules being formed? Scientists soon began to speculate that ion–molecule reactions were behind the unusual results. Ion–molecule reactions are very fast and most have no activation energy – very different to neutral–neutral chemistry.

The lab at York University was one of only a few around the world beginning to investigate ion–molecule chemistry. The very first reaction I studied was one of the most important in interstellar clouds – that between H_3^+ and CO. When I returned to the University of Canterbury, I continued to work on ion–molecule reactions.

Whether we were studying atmospheric or interstellar chemistry, we were all asking the same questions: how does an ion react with a molecule and what are the products? The instruments we used to get the answer were large and cumbersome, with huge vacuum pumps and reaction tubes where the chemistry took place, and a quadrupole mass spectrometer to detect the ions formed. Initially, we used the flowing-afterglow technique: we flowed helium through large reaction tubes at low pressure, generating the ions at the upstream end of the tube, which were carried along by the helium to react with neutral molecules added at the downstream end. We would add our ions to the stream of helium gas in the flow tube, and study the resulting reaction. Later, we adopted the selected-ion flow tube (SIFT) technique – generating ions in a separate vacuum chamber and running them through a mass spectrometer to ensure that only the chosen ions made it into the reaction tube. To measure the speed of the reaction, we measured the concentration of the reactant and used it to determine the rate constant. Between the 1970s and 1990s, thousands of ion–molecule reactions had their rates and products determined in such a manner.

Flipping the concept

But in the mid-1990s, there was an important development. By now, a large database of ion–molecule reactions had been established. In 1996, two colleagues in the UK – David Smith and Patrick Španel at Keele University – decided to make use of that information to do something different. If we can measure the concentration over time to work out the rate constant, they reasoned, why not use the rate constant to determine the concentration of analytes in a sample? We thought this was a great idea and soon adopted the concept, using a microwave discharge of moist air to generate three main reagent ions – H_3O^+, NO^+ and O_2^+ – to react with the analytes, and using mass spectrometry (MS) to detect the "product ions".

It was immediately clear that there was commercial potential for this work. The fast rate constants of ion molecule chemistry enable us to determine very low concentrations of analytes in air, and do it in just a few seconds; conventional techniques, such as GC-MS, take much longer and require highly skilled operators.

To demonstrate the technology to visitors to our lab at Canterbury University, we would analyze their breath metabolites on the spot. On another memorable occasion, we had a visit from a researcher who had been working in the Antarctic, taking blood samples from seals. Her group was trying to determine how much fish the seals ate – with a simple headspace analysis of the blood samples we could measure the trimethylamine metabolites (essentially measuring how "fishy" the blood smelt), and so answer their question.

"It was immediately clear that there was commercial potential for this work."

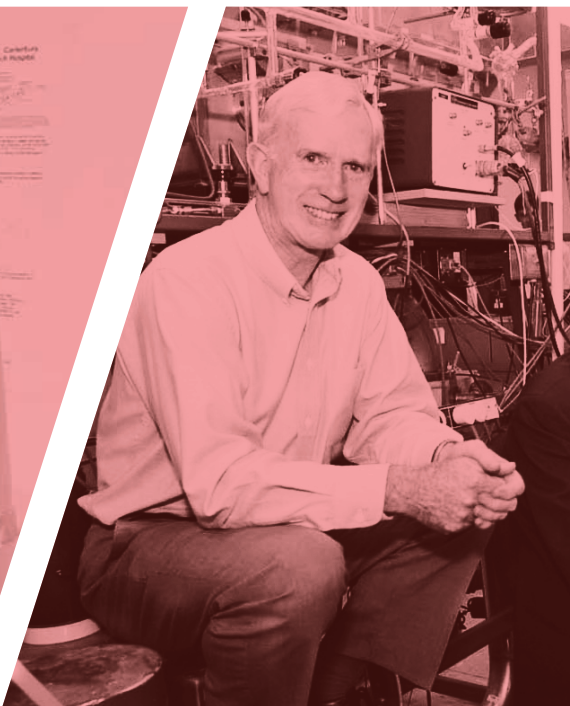
Ready for lift off

In the early 2000s, the university felt the time had come to commercialize the technology and, along with private investors, set up Syft Technologies Ltd at the end of 2002.

The first thing we needed was a great team. We have found over the years that our very best students often take up positions overseas after graduating; Syft was an opportunity to get them back to New Zealand. New Zealand scientists returned from the US, Europe and Australia to work with us, and immediately set to work on making the technology commercially viable. Given that our laboratory instrument was over 5 meters long, weighed several tons and required a number of highly trained operators, the task was not a small one. Our ultimate goal was to create a device that could be operated even by completely untrained staff. The first instrument was the Voice 100, released in 2004. At 500 kg and around waist height, it did everything that the lab instrument did, was simple to use, and gave immediate results.

Right after the Voice100, was released, we had an opportunity to prove the worth of the technology in the real world. Several shipyard workers in ports around the world had tragically died after entering shipping containers contaminated by toxic chemicals used for fumigation. The Australian government were having trouble monitoring these fumigants to ensure safe working conditions. They had been using colorimetric


Alcohol in Breath and Blood
first aid



tubes that change color when fumigants are present, but they have a very high consumable cost, and workers found them too subjective. When they evaluated GC-based devices for frontline use they found that they couldn't analyze all of the fumigants in question with a single chromatographic column; they had to use three. They heard about the capabilities of SIFT-MS and became our first customers, followed by the Canadian government and a number of major European ports. Whereas GC-MS typically needs trained operators, our instruments could be operated by staff working on wharfs, with virtually no scientific knowledge. The instrument is push-button operated, and the results are displayed on-screen as easy-to-understand concentrations, with an alert to flag dangerous levels of fumigants. It was a huge boost for a young company, and defined ease of use as a major selling point for SIFT-MS.

The Voice100 was still rather unwieldy though, so we set about creating the next iteration – the Voice200, which was released in 2007. Not just smaller, the new instrument was also significantly more sensitive, and has since been further updated externally and internally. Until recently, there were some notable analytes that we couldn't measure by SIFT-MS. For example, one of the fumigants that is used in shipping containers is vikane (sulfuryl fluoride; SO_2F_2). This molecule doesn't react with any of our positive reagent ions, so we couldn't measure it using our traditional approach. Another example is hydrogen chloride gas (HCl), an important contaminant in the electronics industry. Around 18 months ago, we made a change in the way we operate our ion source, allowing it to generate negative ions – O^-, OH^-, O_2^-, NO_2^- and NO_3^-. Those ions react with many of the molecules we weren't previously able to measure, filling an important gap.

"At one point we were down to 17 employees. We now have over 45 permanent staff."

Dark days

When the company was set up in 2002, I was a university professor and had precisely zero experience of start-ups. It's fair to say it's been a steep learning curve.

Initially, we thought that we could stay in New Zealand because the power of the technology would be enough to bring people to us. But of course, people had never heard of us. Chromatography has a long pedigree – it's well-established and people understand it. To move to a completely different technique is a real leap, and we faced a lot of skepticism. We were making heavy losses, and questioned whether we could survive.

Things looked grim. Fortunately, some of our investors stood by us, saying, "We believe in this technology." Seeing our instruments being used by customers in wonderful ways – especially some of the exciting clinical diagnostic applications that were emerging – kept us going.

In 2009, some of the major shareholders invested in the company to keep it afloat. In 2012, we gained a new CEO (Doug Hastie) and management team, who brought a new approach to sales, and the company has gone from strength to strength ever since.

We now have agents all over the world selling our instruments – partners with greater visibility than we could hope to achieve. Agents like Anatune, who are experts in GC-MS, were able to talk knowledgeably to their customers about the deficiencies of GC-MS and why SIFT-MS is an attractive alternative. We get many repeat customers; Samsung now have more than 20 of our instruments.

At one point we were down to 17 employees. We now have over 45 permanent staff, plus around 30 students over the southern-hemisphere summer vacation – all working hard to further develop the technology. Their talent, enthusiasm and commitment are the biggest thing we have going for us, and we're proud to think that we're bringing talented scientists back into New Zealand who might otherwise be working overseas.

What's next?

People are beginning to recognize the opportunities that rapid monitoring of volatiles opens up, and our customers are finding all sorts of uses for SIFT-MS, some of which we had never even dreamed of (see "From Garlic Breath to Clean Rooms").

As we become better known – and as customers approach us with exciting new applications – there are a lot of conversations to be had (and significant air miles for our staff!). We are exploring the potential for automating the technology, along with our UK partners Anatune Ltd, which we believe will open up new opportunities in the pharma industry.

Murray McEwan is Chief Technical Officer at Syft Technologies Ltd, and Professor Emeritus at the University of Canterbury, Christchurch, New Zealand.

From Garlic Breath to Clean Rooms

SIFT-MS isn't a universal replacement for chromatography, but when customers need to do things faster – or analyze compounds that don't lend themselves to existing techniques – SIFT-MS can have a real impact.

By Vaughan Langford

Protecting workers

As noted by Murray, our very first customers were port authorities, who used SIFT-MS to detect toxic fumigants in shipping containers. The technology is now being applied in manufacturing settings too; for example, in clean rooms where semiconductors are manufactured. As semiconductors have become more sophisticated, it's become crucial to ensure that VOCs and other gases are kept to a minimum in the clean room. Gases can impact on the quality of the semiconductor films that are being laid down, so companies are increasingly adopting continuous low-level monitoring. In the past, if they monitored VOCs at all, companies used thermal desorption GC-MS and a multi-port sampling system – using a single instrument and cycling through different work areas, typically measuring two points per day per site. SIFT-MS allows you to monitor continuously, pick up problems faster and so save money through avoiding loss of product.

Another fast-growing application is vehicle interior air quality. Around 20 million cars are made in China every year. The plastics used inside the vehicles produce volatile chemicals, some of which have been shown to be harmful (the coveted "new car" smell may have a sting in the tail). Governments are now starting to pass legislation to limit the concentration of some of these chemicals. Legislating is one thing, but how do you measure it? Prior to SIFT-MS, companies were reliant on GC-MS and other off-line techniques, which are difficult, time consuming and expensive (up to US$1,500 per car).

SIFT-MS is also well adapted to environmental monitoring, and Samsung use our instruments to monitor a range of pollutants and odor compounds at the boundary of their semiconductor factories in South Korea.

The smell of success

Odor analysis is another application area where I believe SIFT-MS can have a significant impact as it gains wider acceptance. In 2008, two professors at Ohio State University – Jim Harper and Sheryl Barringer – starting using one of our instruments for their studies into food odors. Barringer has since published more than 30 academic papers using the technology for a whole range of different food products, probably the most significant of which has been on garlic malodor on breath. Using SIFT-MS, Barringer was able to show that eating apple, mint or lettuce can help neutralize volatiles responsible for "garlic breath" – including diallyl disulfide, allyl mercaptan, allyl methyl disulfide, and allyl methyl sulfide – a finding that gained her a great deal of attention in the press (1). Our instruments have also been used at the University of Ghent for a number of applications, including determining fish freshness.

Odor compounds are notoriously difficult to analyze by chromatography. The best detectors are human noses, but odor panels are expensive, human senses vary, and people get fatigued. SIFT-MS can detect the key compounds in a single analysis; from the odorous aldehydes, the volatile fatty acids, right through to organosulfur compounds, all of which require derivatization or other chemical pretreatment prior to chromatography. Various different analyses for chromatography can all be performed simply and quickly with one instrument, and that's likely to be a big draw.

The promise of breath analysis

One of the reasons that Syft Technologies attracted investment was the hope of developing breath diagnostics. It's tough to identify biomarkers in the low ppb, and breath analysis – whether by SIFT-MS or other means – has been slower to deliver results than many people had hoped. But we have several customers doing good work, and researchers recently reported promising results describing SIFT-MS analysis of several compounds that are diagnostic for esophageal and stomach cancers (2). Differences in levels of butyric, pentanoic and hexanoic acids, plus butanal and decanal, had an overall accuracy of 85 percent in identifying patients with stomach or esophageal cancers.

Vaughan Langford is Director of Applications at Syft Technologies Ltd, Christchurch, New Zealand.

References

1. *R Mirondo, S Barringer, "Deodorization of garlic breath by foods, and the role of polyphenol oxidase and phenolic compounds", J Food Sci, 81, C2425–C2430 (2016).*
2. *NHS Choices, "Breath test shows potential for detecting cancer" (2017). Available at: http://bit.ly/2jx5G2c. Accessed 25 May 2017.*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Taking MS into the Clinic

Two clinicians share their 16-year journey to bring MS-based screening for newborn hemoglobinopathies into routine use – and explore what's next for MS in routine diagnostics.

The "-omic" era promises huge opportunities in personalized medicine. We should, however, remember that the real aim of this rapidly expanding field of research and analysis is to develop accurate, specific and cheap clinical tools that actually improve the treatment pathway. Translating discovery and validation efforts into clinical practice is challenging; nevertheless, it is possible, and we believe it is something that is being accelerated by the general introduction of mass spectrometry (MS) technology into clinical diagnostics.

DISPELLING PRECONCEPTIONS

There's long been a perception that immunoassay technology is superior to MS for routine diagnostics, not only with regard to sensitivity, speed, and cost, but also with regard to the perceived technical challenges associated with the latter, such as laborious sample preparation. But MS has advantages, and these are less often appreciated. In particular, it is much more specific than immunoassay; in fact, you may find a difference

of three orders of magnitude between the MS-measured concentration of a given analyte and the immunoassay value. There are a lot of non-specific metabolite/protein immunoassays out there! This represents a potential problem for validation of MS-based protein quantitation where one approach is to assume that the molar concentration of a tryptic peptide represents the original protein concentration. Another, and we would argue more pragmatic approach, is simply to validate any peptide on the basis of intrinsic diagnostic specificity and sensitivity. In reality, it is the multiplexing potential of protein MS that provides the major advantage, obviously economic, over immunoassay by significantly reducing the cost per result.

We have long believed in the potential of protein MS in clinical diagnostics; even so, we knew that if we were to get it more broadly used in clinical practice, we would need to simplify each step in the process – sample preparation, tryptic digestion, LC-MS/MS, and data analysis.

WHERE COULD MS PROVE USEFUL?

First of all, we needed to identify potential clinical applications of protein MS. Based on our experience with MS/MS-MRM newborn dried blood spot (DBS) screening for inherited metabolic diseases, there was an obvious opportunity in newborn screening for sickle cell disease.

It is worth remembering that, back in 1999, when we started our MS/MS-MRM work in this area, multiple reaction monitoring (MRM) techniques were not part of the toolkit; class compound scans, e.g. neutral loss for amino acids and parent ion for acylcarnitines, were seductive and had become the norm in newborn metabolite screening. This was despite the professional analytical view that quantitation of small molecules was best achieved using MRM analysis. More significantly, butylation of samples prior to MS/MS scanning was the accepted standard.

Our first contribution to the routine use of MS/MS in newborn screening was to dramatically simplify the sample preparation process by demonstrating that the metabolites of interest could all be measured without butylation, i.e. non-derivatized metabolite screening. All that was required was simple elution from the DBS with methanol:water containing the appropriate stable isotopes, followed by flow injection. The second contribution was to directly target the metabolites of interest using MRM scanning. This represented two significant advantages. Firstly, inherited disorders could be targeted specifically, removing the ethical constraints imposed in the UK regarding class compound scans, i.e. the detection of disorders not sanctioned by the Newborn Screening Committee. Secondly, the precision of metabolite measurement was enhanced by a factor of 10 when compared with an equivalent data acquisition time for a class compound scan.

The initial analytical validation was straightforward, but we needed to prove that underivatized MS/MS-MRM metabolite analysis was sufficiently robust for routine newborn DBS screening. To test this, we prepared 2,000 blood samples each from ourselves and a colleague, S Bird, and analyzed about 300 samples, including standards and controls, each working day for a month. The method proved to be exceptionally robust and overall coefficients of variation for metabolites, e.g. phenylalanine, were less than five percent. So we'd shown that MS/MS-MRM could be used in screening DBS for metabolites – and that presented us with a further huge opportunity.

At that time, newborn hemoglobinopathy screening was just starting, using techniques that may appear archaic but are still in use in the majority of hematology laboratories today, i.e. isoelectric focusing (IEF) and HPLC. Both techniques can perform excellently in routine diagnostic laboratories but become challenging in the context of high-throughput newborn screening. However, from a protein MS perspective, newborn hemoglobinopathy screening appeared to be "low-hanging fruit," because hemoglobin is a hugely abundant protein. Furthermore, we knew that if we developed something that worked, we had a market for it: about 750,000 newborns in the UK each year, all participating in an established screening program. Moreover, we didn't need to invest in discovery work, because we already knew what the targets were; the genomics had already been done. It was time for us to put it to the test.

GATHERING THE EVIDENCE

To begin with, we simply analyzed diluted whole blood by electrospray MS. This gives a beautiful charge series envelope, with the MS spectrum totally dominated by hemoglobin alpha and beta chains. Unfortunately, we considered the MS backgrounds to be too high and, because MS only reveals mass/charge (m/z) differences in the globin chains, not sufficiently mutation-specific. In addition, although sickle protein with a m/z shift of 30 daltons is relatively easy to detect, all the other mutations that combine with it to result in sickle cell disease differ by only 1 dalton in ~16,000. We could not differentiate these mutations on a standard triple quadrupole instrument. Our conclusion was that MS analysis was neither sufficiently sensitive nor specific for routine newborn DBS hemoglobinopathy screening.

Our next step drew on our experience in the quantitation of small molecules. The idea was to employ tryptic digestion of

blood followed by analysis of the resulting peptides in MRM mode. Trypsin, like other endopeptidases, preferentially acts at specific recognition sites in any protein, and therefore generates a consistent and reliable population of peptides – it liberates 15 peptides from human beta globin, for example. The brief, specified by the NHS Sickle and Thalassaemia Screening Programme, was to be able to detect hemoglobins S, C, DPunjab, OArab, and E. Earlier publications had demonstrated that the unique beta-chain peptides specific to these variant hemoglobins could be identified using chromatography and MS of tryptic digests of blood. This was reassuring, but we needed to demonstrate that we could detect each of the variant hemoglobins by MS/MS-MRM, using flow injection analysis, with sufficient sensitivity and specificity to provide a practical and robust solution for routine DBS newborn hemoglobinopathy screening. At least as important was the need to simplify traditional tryptic digestion procedures, which still are far too onerous to fit into any clinical process. Many laboratories complain if they have to add only one additional reagent to their existing process, so we knew we would have to keep the sample preparation simple.

IT WAS A REVELATION!

Solving these problems involved a lot of trial and error. With regard to sample preparation, initially we wanted to demonstrate the concept in whole blood, without any sample cleanup. It was also important to demonstrate that we could release the informative peptides, at least semi-quantitatively, within a one-hour trypsin incubation. We considered these to be the minimum criteria for a subsequent high-throughput clinical assay. In addition, if this were to be applied to newborn screening, flow injection would be an essential prerequisite.

When it came to the actual mass spectrometry, we didn't even know that we'd be better working with doubly charged rather than singly charged peptides. Our lack of understanding of peptide ionization and fragmentation, combined with an absence of peptide standards, meant that MS tuning using patient samples was challenging. Our initial full-range scans of whole blood tryptic digests looked horrible; there were plenty of signals, too many, and even when we zoomed in on the expected single- and double-charged m/zs expected for the wild-type beta chain T1 and sickle T1, the signals were not convincing and offered no prospect for formal tuning. Finally, in frustration, we programmed MS/MS product ion scans based on the theoretical single- and double-charged m/zs. It was a revelation! It was instantly obvious that doubly charged peptide fragmentation was much more informative. All of a sudden we could see a classic peptide spectrum, showing all the peptide signals as amino acids are lost from the N and C terminal ends.

"ALL OF A SUDDEN WE COULD SEE A CLASSIC PEPTIDE SPECTRUM, SHOWING ALL THE PEPTIDE SIGNALS AS AMINO ACIDS ARE LOST FROM THE N AND C TERMINAL ENDS."

Progress was immediate: the most informative MRM for sickle peptide, i.e. adjacent to the mutation site, was identified, together with an equivalent MRM for wild-type peptide. Within an hour we demonstrated that, using rapid tryptic digests of whole blood, direct flow injection, and MRM mode analysis, we could differentiate normal wild-type beta T1 subjects, heterozygous sickle carriers, and homozygous sickle cell disease patients. (see Figure 1). That very same afternoon we were able to optimize the tuning parameters and set up the same procedure for each of the other sickling mutations. Subsequently, we collected a couple of hundred highly informative blood samples and analyzed them with our optimized method. We demonstrated 100 percent sensitivity and 100 percent specificity. The concept was patented by King's College, London, and we eventually published the work in 2005 (1).

DID WE ANSWER OUR QUESTIONS?

So we'd answered one of our primary questions: with minimal sample clean-up and a short tryptic digestion, the variant peptides can be detected sensitively and specifically using flow injection electrospray MS/MS with MRM data acquisition. But, had we done enough with regard to minimizing sample preparation and tryptic digestion in order for the assay to be acceptable in a routine newborn screening laboratory? Probably not! In fact, we addressed this by posing a new question: how would our assay perform in real-time neonatal screening?

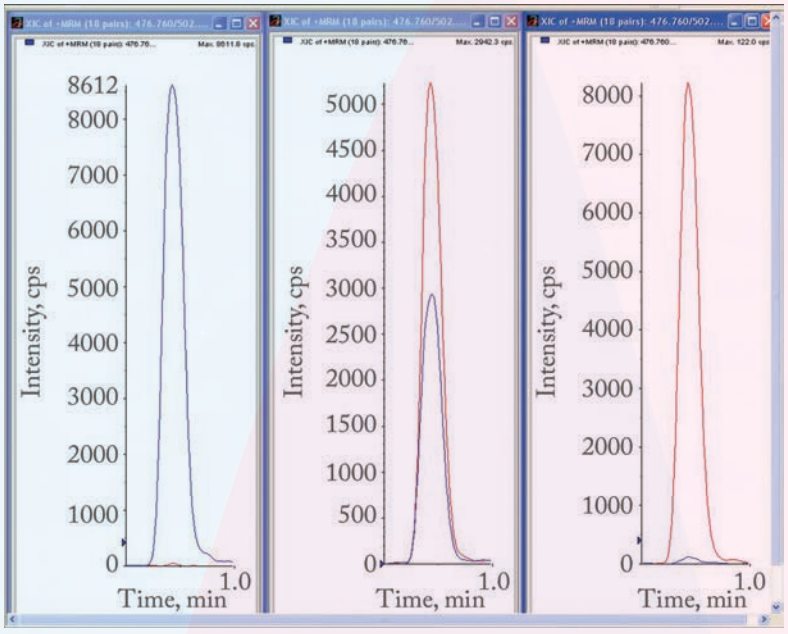

Figure 1. MS-MRM analysis of trypsinized hemoglobin detects, left, normal homozygote (AA), center, sickle heterozygote (AS), and right, sickle homozygote (SS).

This required a real-world technical evaluation, and we were fortunate to receive funding for this from the NHS Sickle Cell and Thalassaemia Screening Programme. The evaluation was undertaken in collaboration with St James' Hospital in Leeds, the idea being to compare our method with the IEF technology that they were using at the time.

Duplicate punches were taken from neonatal DBSs in Leeds; one was analyzed by IEF, and the other was transported to us overnight, dry, in 96-well plates. When we received the samples we just added reagent, incubated for 30 minutes at 37° C, added stopping/dilution reagent, and injected 2 µL of sample using flow injection. The MS/MS-MRM analysis data acquisition time was 0.6 minutes. In effect, we had truly minimized sample work-up and data acquisition. The results were generated blind and were available within 24 hours, and they were excellent. From 40,000 neonatal DBSs, we identified all the sickling variants correctly, including 15 individuals with mutations associated with clinical disease. Just as important, in the context of newborn screening, there were no false negatives. It is worth emphasizing that in newborns, the beta chain is largely switched off, so as little as one percent of total hemoglobin may be beta chain. This means you need exquisite sensitivity, i.e. good signal to noise ratio, and the beauty of the MS/MS technique, in contrast to our original MS analysis, is the excellent signal to noise ratio.

WE HAD VALIDATED THE METHOD – WHAT NEXT?

This was in 2008, and we now felt that we had validated our method, but how to get it into the routine newborn screening laboratory? We concluded that the easiest way forward was to develop a kit. We eventually produced a kit containing two reagents, one of which was a stable isotope internal standard, to provide a check, in every sample, on trypsin activity and instrument sensitivity. This was aimed at de-risking the process and ensuring that we would not miss any clinical cases. The stable isotope reagent also acts as a pre-analysis system suitability check of the MS/MS instrument. The concentration of the internal standard is set quite low so that if there are any instrument sensitivity problems, they can be identified before starting an analytical run. In summary, it's a CE-marked kit,

MS/MS for Biomarker Detection in Urine

Application
Longitudinal measurement of proteins in urine – for example, assessment of the albumin/creatinine ratio (ACR) to identify and monitor cardiovascular risk.

Utility
The urine ACR test can identify those at risk of cardiovascular disease 10–20 years before it becomes a clinical problem, and is increasingly claimed to be a highly useful early warning test for diabetic complications and general cardiovascular risk (5).

Barriers
Cost is perceived to be a barrier to implementation of urine ACR as a population screening tool, but may be manageable if we use a sample collection system similar to the DBS system.

you only need two reagents, it takes only 30–45 minutes, it incorporates peptide standards for instrument setup, and it utilizes fully electronic data analysis with Chemoview on Sciex and NeoLynx on Micromass instrumentation, respectively.

At this stage, all we had to do was to convince potential clients to change to our kit. It is true, no one likes change! Although the NHS Sickle Cell and Thalassaemia Screening Programme had funded the successful study, it was only just over a year ago that the technique was officially designated as an acceptable test that could be purchased by NHS commissioners. Fortunately, in the interim, a far-sighted biochemist based in Cardiff, Stuart Moat, decided that the method was ideal for the Welsh Newborn Screening Programme. Stuart has now published NHS Wales' experience with this kit (2) and, as a result, several other newborn screening laboratories in England have made the change. The kit is currently being used in Cardiff, Leeds, and Great Ormond Street Hospital, with significant interest from other laboratories in the UK, Europe, USA, Middle East, and India. We estimate that newborn DBS hemoglobinopathy screening, using MS/MS-MRM, will be performed on 250,000 babies next year in the UK.

SIXTEEN YEARS OF WORK; WHAT NEXT?

So yes, we've managed to get from basic science to the routine clinical laboratory – but remember it took about 16 years to do it! Nevertheless, we have shown that it is possible, and I expect to see other protein MS/MS-MRM based tests in routine clinical diagnostics in the next few years. A really important example, that has enormous potential, is measuring the protein content of urine – in particular the albumin/creatinine ratio (see Sidebar "MS/MS for Biomarker Detection in Urine"). This story started with the pioneering work of Harry Keen in the sixties, which showed that some patients with diabetes have increased protein excretion in their urine, specifically albumin (3). Importantly, Keen realized that the increased urine albumin was associated with the subsequent risk of developing diabetic nephropathy. More significant, but difficult to explain, was that increased urine albumin also identified the patients at risk of cardiovascular disease, 10–20 years before it became clinically evident.

We now have large epidemiological studies, over 1.1 million people in the general population (~5 million subject years), which show that the risks of cardiovascular events are related to the urine protein (4). More recently, in a meta-analysis of 24 cohorts of about 600,000 people with no history of cardiovascular disease (median follow-up 4.2 to 19 years), the risks of cardiovascular mortality, coronary heart disease, stroke, and heart failure are all directly related to the urine albumin/creatinine ratio (ACR) (5). It really poses the question, as stated in the Lancet paper last year (5): when will clinicians appreciate the importance of ACR, as a very early warning test, in determining diabetic complications and general cardiovascular risk?

Some clinicians are skeptical because of the relatively high biological variability of ACR tests, but this can be addressed with a multiple sampling approach: we always take the geometric mean of three early morning urines, which smooths out the natural variation. Transportation and storage of urine samples is costly, but the sensitivity of MS/MS-MRM means that urine samples can be collected on filter paper – just as blood is collected for newborn screening – or other micro-sampling devices. This raises the possibility of developing personalized medicine, where individuals take control of monitoring their own health by collecting micro blood and/

or urine samples at home and posting to a referral laboratory. If this is done regularly, e.g. annually, within a few years, the intrinsic individual variation of any diagnostic test can be assessed and a within-individual normal range established. The result is very early warning of developing disease including diabetes, cardiovascular, renal, liver, and many cancers. Early detection means that lifestyle changes can be made or appropriate therapies started, with the potential to reverse, stop, or ameliorate disease progression.

In conclusion, the introduction of routine clinical protein MS has taken many years, but in the future, now that the MS hardware is more established, the research bench to routine clinical diagnostic timeline should be shorter. We believe that tryptic peptide analysis by electrospray MS/MS-MRM has significant clinical utility and will play a key role in both population screening and personalized medicine: sample collection is easy, the patient pathway can be optimized, and it can be made cost-effective by simplifying the analytical logistics and exploiting the multiplexing capability of MS.

Neil Dalton is Professor of Pediatric Biochemistry, King's College London; Director of the WellChild Laboratory, Evelina London Children's Hospital and SpOtOn Clinical Diagnostics Ltd.
Charles Turner is Deputy Director of the WellChild Laboratory, Evelina London Children's Hospital and a Director of SpOtOn Clinical Diagnostics Ltd.

References

1. *YA Daniel et al., "Rapid and specific detection of clinically significant haemoglobinopathies using electrospray mass spectrometry-mass spectrometry", Br J Haematol, 130, 635–643 (2005). PMID: 16098080.*
2. *SJ Moat et al., "Newborn blood spot screening for sickle cell disease by using tandem mass spectrometry: implementation of a protocol to identify only the disease states of sickle cell disease", Clin Chem, 60, 373–380 (2014). PMID: 24158758.*
3. *H Keen et al., "The concomitants of raised blood sugar: studies in newly-detected hyperglycaemics", Guys Hospital Reports 118, 247–254 (1969). PMID: 5804067.*
4. *K Matsushita et al., "Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis", Lancet, 375, 2073-2081 (2010). PMID: 20483451.*
5. *K Matsushita et al., "Estimated glomerular filtration rate and albuminuria for prediction of cardiovascular outcomes: a collaborative meta-analysis of individual participant data", Lancet Diabetes Endocrinol, 3, 514–525 (2015). PMID: 26028594.*

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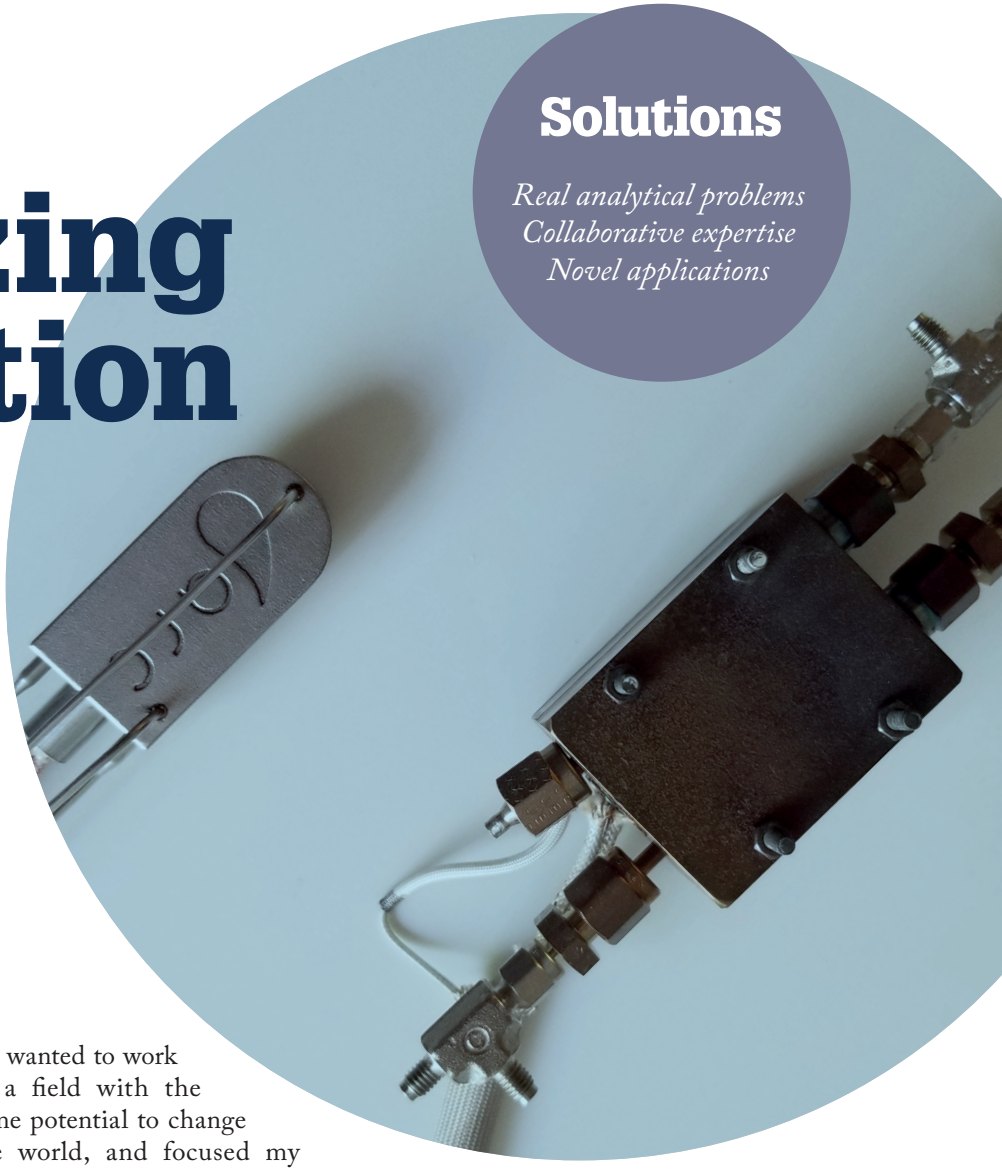
Solutions

Real analytical problems
Collaborative expertise
Novel applications

Catalyzing Innovation

The story behind the Polyarc® system – and how it sparked a discovery to make car tires from sugar.

Paul Dauenhauer was interviewed by Charlotte Barker



The Problem

Our group aims to produce renewable chemicals from biomass, which involves analyzing complex organic molecules with a lot of heteroatoms (atoms that aren't carbon or hydrogen). Heteroatoms change the response rate, and so GC-FID of these molecules necessitates careful calibration. For each compound, you have to buy a standard (if one is available – many aren't) to define the response rate – or use a crude estimate. We needed to find a better way.

Background

The first chemical engineering lecture I ever attended was on the Haber process, which combines nitrogen from the air with hydrogen to produce ammonia. Before its discovery in 1904, the world was heading for mass starvation because agriculture was failing to keep up with population growth. Easy access to ammonia-based fertilizers gave us the means to grow enough food for everyone, but it has also had serious environmental consequences. Nevertheless, it's a chemical technology that has truly changed the world. I was inspired.

I wanted to work in a field with the same potential to change the world, and focused my attention on renewable chemicals. There is enough biomass available, the economics make increasing sense as glucose prices fall, and I saw a chance to get ahead of the wave.

My group is made up of engineers, but we're also analytical chemists by necessity; the analytical challenges in producing renewables are immense. One of those problems is the difficulty of analyzing heteroatoms by GC-FID. Because our sample mixtures contain a large number of different heteroatoms, you can end up with a chromatogram with anything from 20 to 200 peaks. The time and expense of all that calibration meant that it just wasn't feasible to study the hundreds of compounds we were interested in. Instead, we were forced to

"My group is made up of engineers, but we're also analytical chemists by necessity; the analytical challenges in producing renewables are immense."

work with very simple systems followed by extrapolation. But given that the response factor of these heterogeneous molecules can vary from 0.01 to 1.2, estimates are not very effective!

The problem with FID when it comes to quantification is that its response factor is dependent on molecular structure. I was chatting to one of my graduate students about the problem one day, when we hit upon a solution. It occurred to us that we could identify and quantify the compounds separately. For quantification, we only needed to figure out the amount of carbon, while ignoring the structural information – and we could use an independent method, such as mass spectrometry, for identification. We figured that if we converted everything to methane before the FID, we would eliminate the impact of molecular structure on quantification and end up with a linear response. Although it was a very simple idea, putting it into practice was a real engineering challenge.

The Solution

We start with a mixture of complex molecules, and pass them through a GC column. After the separation has occurred, instead of the column feeding into the FID, each molecule goes into a microreactor that converts every carbon-containing molecule to methane via dual reactions of combustion followed by reduction to methane. The second half of this process (CO_2 to methane) is similar to commercial methanizers; however, the initial combustion step means you can convert all organic compounds to methane, whereas a methanizer can only convert CO and CO_2. The system can be used to replace existing methanizers, but it has significantly more capabilities. The reaction can be represented as:

Organic compounds + Air + H2 → Methane (CH_4) + Non-carbonaceous byproducts

Sugar Rush

For us, the technology behind Polyarc has made a huge difference to our work. In fact, it helped us on the way to a major breakthrough – a process for manufacturing isoprene (the principal component of car tires) from glucose, rather than from petroleum.

It's a three-step process:

1. Glucose > Itaconic acid
2. Itaconic acid > 3-methyl-tetrahydrofuran
3. 3-methyl-tetrahydrofuran > Isoprene

The first two steps are simple enough – itaconic acid is already commercially available from glucose fermentation, and sequential hydrogenation produces 3-methyl-tetrahydrofuran (3-MTHF).

The final part (dehydra-decyclization of 3-MTHF) was the real challenge. First, we assessed all the conventional catalysts – none could get over 40 percent selectivity. The breakthrough came when we identified a new catalyst with a 90 percent selectivity. The resulting isoprene is identical to that found in synthetic rubber tires.

The Polyarc system greatly enhanced this research in two ways. First, our initial experiments produced many side products, each of which was readily quantified with the Polyarc system. Second, our experiments were conducted using a high-throughput experimental system that could measure up to 100 data points per day. The Polyarc system enabled rapid screening and analysis of a large number of complex mixtures to help us determine the conditions which gave 90 percent yield.

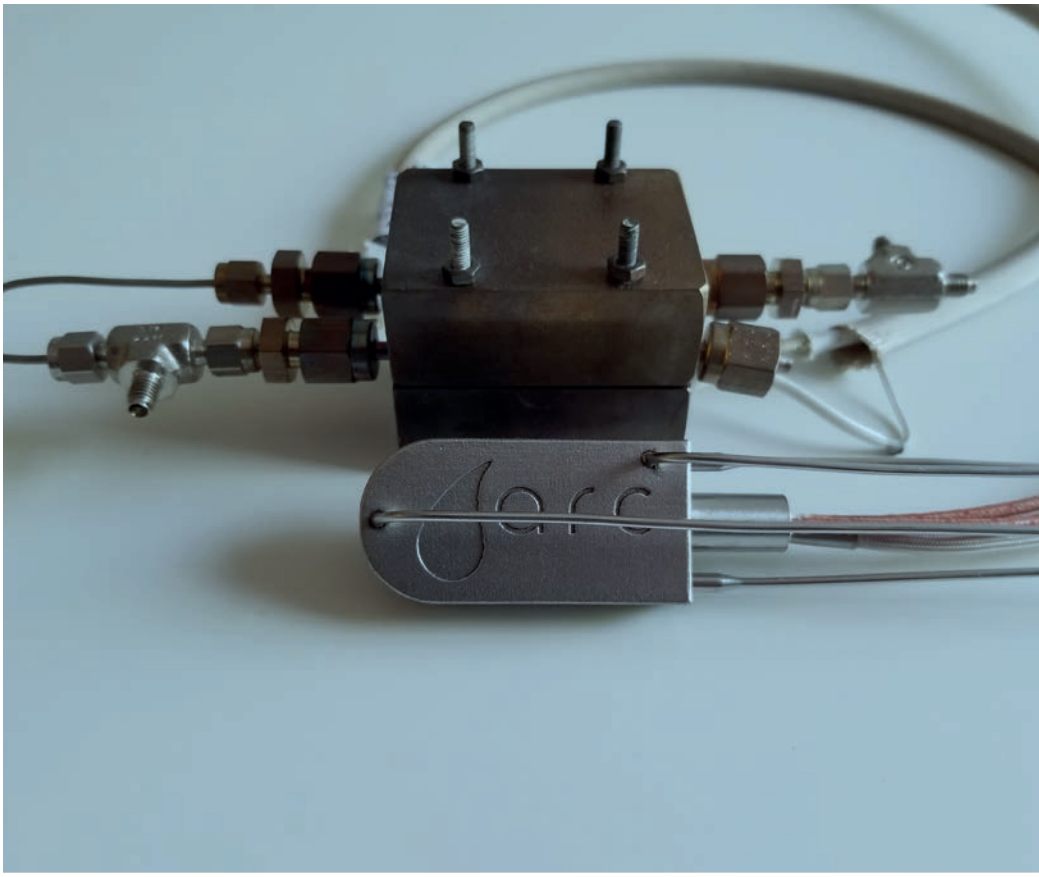
The ability to produce isoprene from a renewable source comes with many benefits. We know that there is a finite amount of petroleum, and as supplies run short, costs will rise. By using grass, corn or wood to produce isoprene, we essentially take CO_2 out of the air and into the product – a form of carbon sequestering. Domestic production is another big advantage. I'm from Minnesota, where the two big industries are corn and forestry. Both industries are under pressure, and keen to find new and profitable uses for their crops.

Our detailed techno-economic analyses reveal that the most important cost driver is selectivity, so we are putting the majority of our efforts into pushing selectivity as high as we can. The process is now up to 90 percent selectivity and we continue to work on that, while we talk with several companies about commercializing the technology. Moving up to an industrial scale will be a challenge, but we're engineers, so that's our bread and butter.

The Polyarc: Applied

The Polyarc system has now found applications well beyond its original use for analysis of renewables. For example:

- Flavor and fragrance analysis of compounds where commercial standards are unavailable or prohibitively expensive.
- Extractables and leachables analysis using mass spectrometry and a Polyarc/FID to expedite analysis while increasing accuracy.
- Forensic analysis of synthetic drugs where the addition of Polyarc has enabled rapid quantitation in addition to the existing qualitative analysis.
- Bulk and specialty chemical analysis of complex mixtures to reduce calibration procedures, due to many of the compounds of interest having boiling points below room temperature.



The original prototype (top) that eventually became the Polyarc (bottom).

The methane goes into a conventional FID detector, where the response is proportional to the amount of carbon. Now that all molecules have been converted to methane the response rate is the same across the board, so there is no time-consuming calibration. Non-carbonaceous byproducts elute as volatile compounds. For example, sulfur will first be oxidized to sulfur dioxides and then reduced to hydrogen disulfide. For this reason, the Polyarc catalyst cartridge is designed with a long lifetime, to account for the impact of poisoning over time.

Conceptually, it's very simple – but there were two major challenges in producing a useable system. First, we had to identify the thermodynamic conditions that would ensure a greater than 99.9 percent conversion to methane. This was addressed using a global thermodynamic calculation of all possible molecular structures. Second, we needed a sufficient catalytic conversion to approach equilibrium conversion to methane. To achieve this we combined catalyst and reactor design for maximum catalytic activity. We created a prototype – an ugly, Frankenstein's monster of a machine made up of a steel block with straight holes connected with tubing and fittings. Despite problems with mixing and long residence time, the prototype proved very useful in our work.

I have known Activated Research Company (ARC) CEO Andrew Jones for around 10 years and when he visited my lab in 2014 our "monster" caught his eye. After I'd explained how it worked, Andrew pointed out that others might find our approach useful. We naively thought it was just a relatively simple solution to our specific problem and so it hadn't occurred to us that there might be a commercial success story waiting to unfold.

To cut a long story short, ARC turned our prototype into a slick device – the Polyarc® system. The principle is the same, but ARC introduced many improvements; in particular, using 3D metal printing to produce the microreactor – a world first. ARC also focused on minimizing dead-volume in the microreactor to ensure the chromatography is maintained as much as possible, and designed a

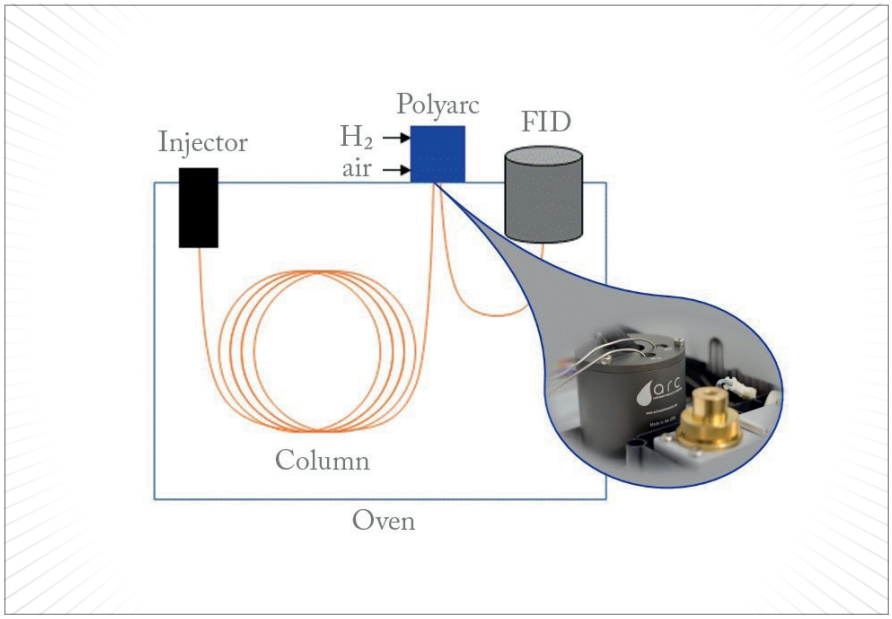


Figure 1. How the Polyarc system works.

"It hadn't occurred to us that there might be a commercial success story waiting to unfold."

system that can be easily integrated into any GC set-up. The elimination of dead volume and mixing with the 3D printed metal microreactor helps to maintain chromatographic resolution, retaining nice, sharp peaks that are easy to integrate and quantify.

Beyond the Solution

The Polyarc system has been commercially available for nearly two years now, and I regularly get calls from people asking for advice on applications or operation. People are finding new applications that I had never thought of (see Sidebar: The Polyarc: Applied). I initially conceived of the technology as a means to analyze compounds with no reference standard, but there are other labs who would prefer not to store standards; for example, forensic labs analyzing illegal drugs.

Award-Winning Innovation

The Polyarc system was recognized in the 2015 Analytical Scientist Innovation Awards (TASIAs), with the judges commenting: "it solves a problem as old as GC."

The Innovation Awards were set up to celebrate innovation from companies big and small, during the preceding 12 months. Have you got a breakthrough you'd like to shout about? Contact the Editor (charlotte.barker@texerepublishing.com) to find out how you can enter this year's TASIAs.

To SERS with Love

Sitting Down With… Duncan Graham,
Head of Department for Pure and Applied Chemistry, University of Strathclyde, Glasgow, UK.

How did you get into science?

It may sound strange, but it all started with a bird. I grew up in the Scottish Borders, which is very rural and was an environment dominated by wildlife and farming. One day I found yet another dead bird and decided to dissect it to understand what was inside. I looked into how to preserve organs with vinegar and formaldehyde, and started to get into chemistry from there. Skip forward a few years, and during my PhD in organic/synthetic chemistry I was offered a three-month postdoctoral position at the University of Strathclyde – this turned into six months, then I got a year's contract, then a five-year fellowship, and finally I was made permanent.

How has your research developed over time?

I started off doing a lot of synthetic chemistry for surface enhanced Raman scattering (SERS) – mainly making new reported molecules with strong surface affinity for silver, and modifying DNA to make it SERS active. Now, we've moved more into nanoscience, where we can still make new materials but focusing less on the specific molecular functionalities and more on the optical properties achievable by altering the synthesis of nanomaterials. The work is allowing us to interface with biological systems and biomolecules, as we move towards more challenging environments, including in vivo.

Why is SERS important?

SERS can provide advantages, both in terms of sensitivity and discrimination of signal – but not in every scenario. For example, if fluorescence can be used, why use SERS? However, in situations where fluorescent backgrounds interfere with the fluorescence measurement, SERS can be advantageous. In vivo measurements, in particular, benefit from a SERS base as opposed to fluorescence. SERS has some unique advantages – but have they translated into practical use? We're getting there; we see SERS being used in a number of lateral flow immunoassays, tagging applications for security and a lot of research into in vivo imaging, as some examples. More scientists are researching SERS and its use and I think more examples will arise soon of where it is addressing unmet needs. Instrumentation has developed incredibly rapidly over the last few years, and the ability to make different enhancing surfaces is now pretty well developed. The biggest challenge is getting people to understand what the technique can do and why you would want to use it; getting it out of the spectroscopic community and into the hands of other people. I think that's really where it can start to explode.

What led you to found the Centre for Molecular Nanometrology in 2005?

We were looking for a way to harness the relationship between physics and chemistry at Strathclyde, using spectroscopy as a "glue". In chemistry, we specialized in Raman and SERS, and fluorescence was the mainstay of physics – so we felt we could focus on on optical spectroscopy. We have since made several new appointments and we moved into new laboratories two years ago as a consequence of our growth. The aim of the Centre is to explore the growing area of nanotechnology and its role in molecular biology and medicine. In particular, though, I think the Centre has been a really useful mechanism for bringing on young scientists starting out; people are brought together based on their potential and their "skills fit". It's a way of giving appropriate credit, flexibility and support to new staff developing their own careers.

Your career seems to typify multidisciplinary...

The long-term direction of our research is set through the discussions and collaborations we have with the potential end-users of our research – clinical practitioners or researchers. That's where a lot of our insights into opportunities for analytical chemistry in the healthcare sector come from. We work with Edinburgh University to run a joint center for doctoral training with 60 PhD students, and every student has both a physical sciences and a clinical supervisor, so it naturally brings together those two disciplines. It's really exciting.

Who do you work with most?

I do most of my collaboration with Karen Faulds. She's an attention-to-detail sort of person, while I'm more "big picture" and worry about the details later – so it's a good partnership! She comes from the analytical, spectroscopic side, whereas I started out as an organic chemist, so approach things from the bio/organic side of things. Between us we have a broad knowledge base for this type of research. "Two heads are better than one" has never been a truer adage and allows us to really be creative and innovative.

Are you a big believer in teamwork?

I've always been someone who prefers to work with people rather than alone. You need someone to have a laugh with, after all! I've captained several rugby teams, and one thing I learned was: you can never ask someone to do something you wouldn't be prepared to do yourself. I always try to lead by example, which means I have to try to publish the best papers I can, and get the best research funding to keep the group going, to keep the research dynamic, to keep coming up with research ideas... It's a perpetual wheel of activity.

What motivates you?

Seeing other people come up with ideas that you can help formulate and then carry through to successful outcomes – that is really motivating. And cheese. I love cheese.

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