MARCH 2021 # 95

the Analytical Scientist®

The Call of Coronal Duty

How proteomics became a crucial weapon in the fight against COVID-19

Have the Confidence to Load Your Sample and Walk Away

Enhanced Features of the Vi-CELL BLU Cell Viability Analyzer include:

- Expanded capacity (24-position carousel or a 96-well plate)
- Small sample volume
- Walk-away operation with on-the-fly sample login
- 50% faster than the Vi-CELL XR in FAST Mode
- Small laboratory footprint
- User-friendly reagent system
- Life-cycle service and support

LEARN MORE about the **Vi-CELL BLU Cell Viability Analyzer** at **www.beckman.com/blu**

BECKMAN COULTER
Life Sciences

For Beckman Coulter's worldwide office locations and phone numbers, please visit Contact Us at beckman.com

Always Look on the Bright Side of Life

A mantra to live by in the age of COVID-19

Focusing on the positives is often easier said than done – especially during a pandemic. But I wanted to use this editorial as an opportunity to encourage us all (myself included) to do so.

A prime example: most people will recognize the immortalized lyrics from the legendary Monty Python in the title. The same song was played at my grandad's funeral. Does it upset me? A little. But it also fills me with hope. As my grandad would have wanted, it forces me to reflect on the joy we shared with one another – and the joy of life itself.

It's been a long year. Much of the world entered lockdown or other restrictions in early 2020. Some of us have lost loved ones. Many have suffered in other ways. All of us are missing friends and family.

But there are reasons to be positive. The first: vaccines have landed! Designing these miracles of medicine in response to a threat has never happened so quickly – that's a reason to smile! And, here in the UK, we are already seeing hospitalizations drop (another frown deterrent!). Accordingly, this issue celebrates pharmaceutical feats, from the first instance of subcellular drug quantitation (see page 34) to Davy Guillarme's own forays in the field (on page 51).

Reason two: movement towards equity in science and beyond. In our field, coalitions have formed to support groups traditionally disadvantaged in the sciences. The Coalition of Black Mass Spectrometrists is a great example – as is the Females in Mass Spectrometry community.

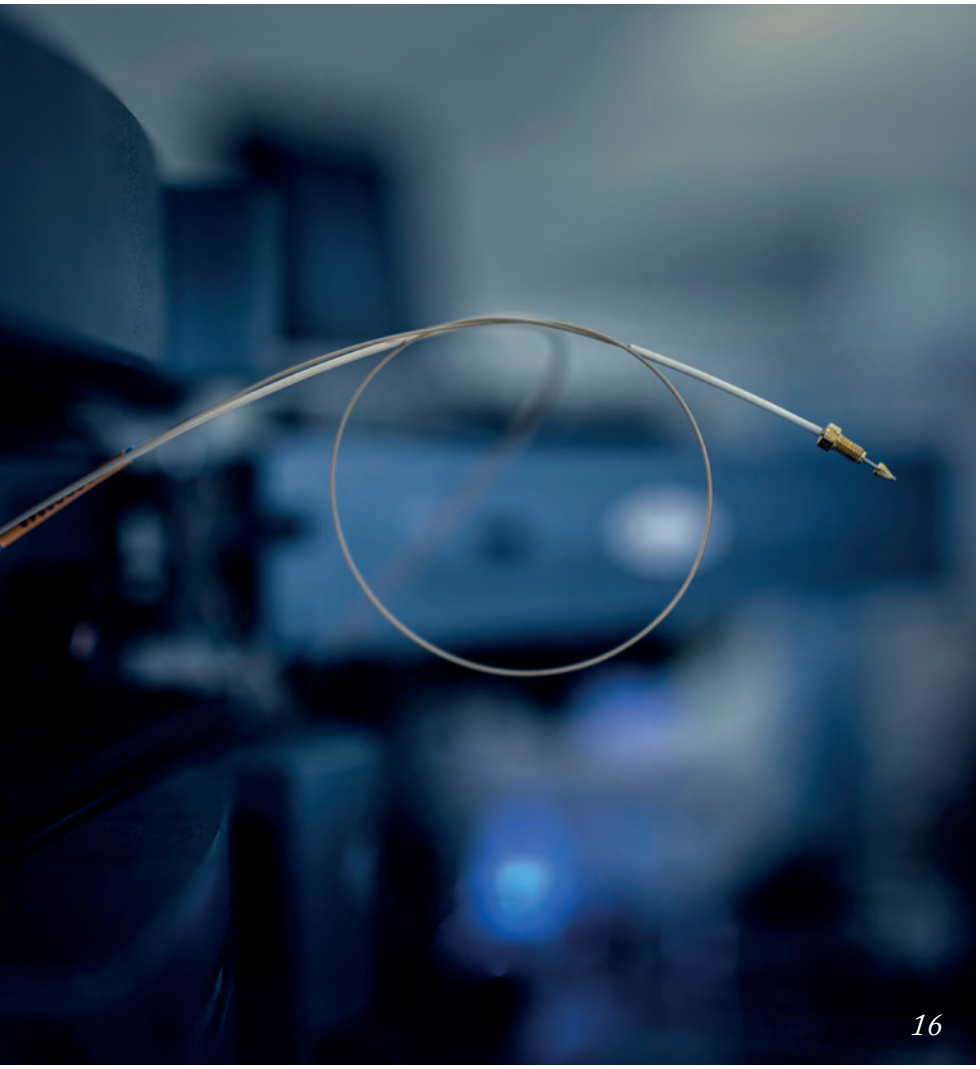
Groups like these are a driving force for positive change and equity – something we can all smile about! They also champion a message aligned with the theme of this year's International Women's Day theme: "Choose to Challenge." We're marking the occasion (and the wider Women's History Month) by shouting about the historical successes of women in analytical chemistry (see pages 6 and 7) and the need for female representation and role models (page 12).

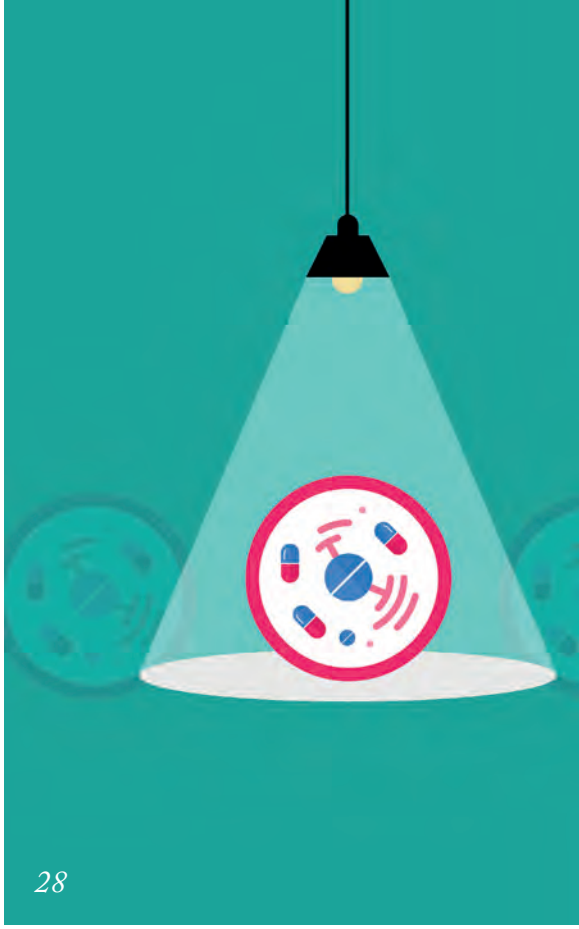
And a third reason? Normality is returning! The "new normal" will soon be the "no thank you," and we will be able to celebrate with the people we love most. If my grandad's passing taught me anything, it's how important those moments are. Until then, I hope our publication can at least put a smile on your face.

And remember: always look on the bright side of life (da-dum, da-da da-da da-dum)!

Matthew Hallam
Editor

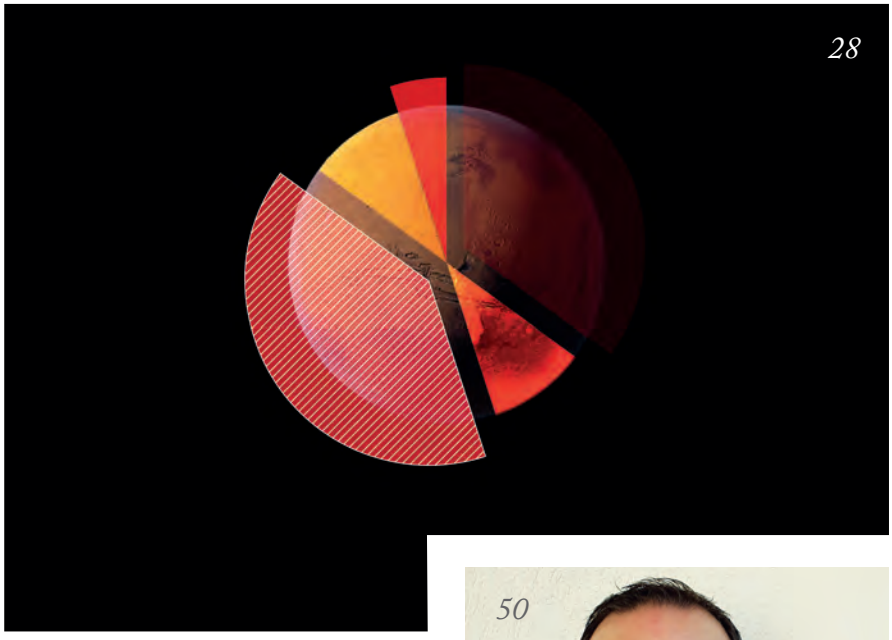
Contents





the Analytical Scientist

ISSUE 95 - MARCH 2021



Features

Departments

Sitting Down With

Feel free to contact any one of us: first.lastname@texerepublishing.com

Content Team
Editor - Matthew Hallam
Charlotte Barker (Associate Content Director)
Lauren Robertson (Deputy Editor)
Frank van Geel (Scientific Director)

Commercial Team
Publisher - Lee Noyes
Gaurav Avasthi (Business Development Manager)
Nick Ransom (Business Development Executive Americas)

Design Team
Head of Design - Marc Bird
Hannah Ennis (Senior Designer)
Charlotte Brittain (Designer)

Digital Team
Digital Team Lead - David Roberts
Peter Bartley (Digital Producer Web/Email)
Abygail Bradley (Digital Producer Web/App)

Audience Team
Audience Growth Strategy Manager – Brice Agamemnon

CRM & Compliance
CRM & Compliance Manager - Tracey Nicholls
Hayley Atiz (CRM Assistant)

Commercial Support Team
Internal Systems Manager - Jody Fryett
Dan Marr (Campaign Reporting Analyst)

Commercial Services
Commercial Service and Social Media Manager - Matt Everett
Kevin O'Donnell (Marketing Executive)
Alice Daniels-Wright (Video Project Manager)
Jess Lines (Project Support Coordinator)
Lindsey Vickers (Sales Support Project Manager)
Jennifer Bradley (Sales Support Coordinator)

Marketing Team
Marketing Manager - Katy Pearson
Jo Baylay (Marketing Executive)

Accounts Team
Kerri Benson (Accounts Assistant)
Emily Scragg (Accounts Apprentice)

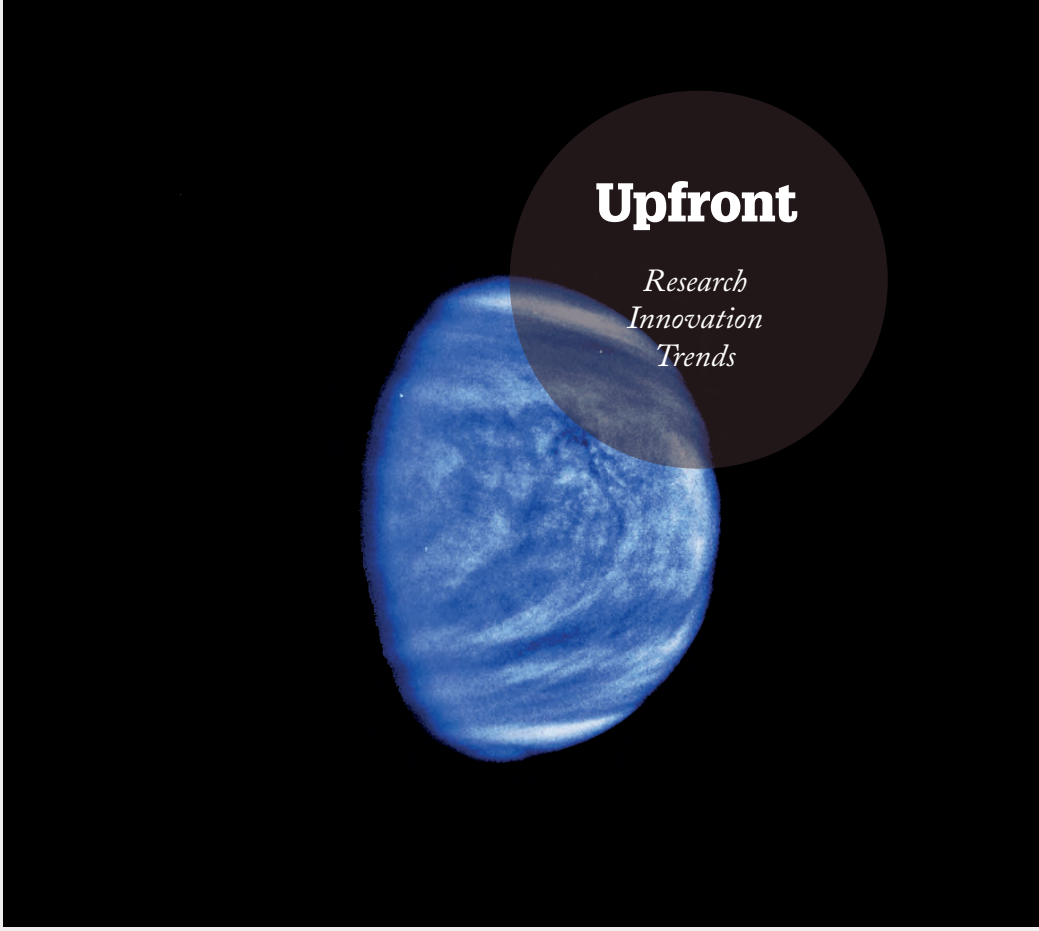
Human Resources
Human Resource Manager - Tara Higby

Management Team
Chief Executive Officer - Andy Davies
Chief Operating Officer - Tracey Peers
Senior Vice President (North America) - Fedra Pavlou
Financial Director - Phil Dale
Commercial Director - Richard Hodson
Content Director - Rich Whitworth

Change of address info@theanalyticalscientist.com
Hayley Atiz, The Analytical Scientist, Texere Publishing Limited, Booths Park 1, Chelford Road, Knutsford, Cheshire, WA16 8GS, UK

General enquiries
www.texerepublishing.com | info@theanalyticalscientist.com
+44 (0) 1565 745 200 | sales@texerepublishing.com

Distribution: The Analytical Scientist (ISSN 2051-4077), is published monthly by Texere Publishing Limited, Booths Park 1, Chelford Road, Knutsford, Cheshire, WA16 8GS, UK. Single copy sales $15 (plus postage, cost available on request info@theanalyticalscientist.com). Non-qualified annual subscription cost is available on request.



Venusian Views

Six-wavelength spectroscopy unveils new details about the surface of Venus

Beneath a thick, caustic atmosphere that is hot enough to melt lead lies the surface of our planetary neighbor. Despite the proximity, very little is known about the composition of Venus, mostly because traditional imaging techniques are blocked by its thick carbon dioxide cloud cover. The only information we have comes from measurements of just a few landing sites. However, a team of researchers from the Planetary Spectroscopy Laboratory at German Aerospace Center Institute of Planetary Research in Berlin have managed to discern the iron content of Venus and produce a geological map of its surface (1).

"Until recently, there were no orbital spectroscopic data for Venus – as are common on other planets – because Venus is covered by thick CO_2 clouds," said M Darby Dyar, lead author of the associated paper, in a recent press release (2). "Visible and near-infrared (VNIR) light cannot penetrate those clouds except in some very small windows in the NIR around a wavelength of 1 micron," Dyar said.

To overcome this issue, the team measured the emissivity of a range of igneous rocks at high temperatures in the lab, and showed that they matched surface spectra collected by the Venera 9 and 10 Soviet landers in the 1970s. "The new lab data allowed us to develop machine learning algorithms that can measure the iron contents of surface rocks from orbit with high accuracy," added Dyar. "This is important because key igneous rock types have distinctive iron contents, so we'll be able to distinguish basalt, andesite, dacite, and rhyolites on the surface. Knowledge of rock types informs our understanding of how the Venus surface evolved."

There are five of these small windows, where the CO_2 clouds are more transparent, and the team hopes their new data and six-spectral band spectrometer design could enable future orbital spectral observations as part of the VERITAS and EnVision missions.

References

1. *J Helbert et al., Science Advances, 7, eaba9428 (2021). DOI: 10.1126/sciadv.aba9428*
2. *Plantary Science Institute (2021). Available at: https://www.psi.edu/news/venusdyar*

INFOGRAPHIC

Women in Analytical Chemistry

A selection of key figures from our field's history

the Analytical Scientist

Kathleen Lonsdale

A pioneer in X-ray crystallography who helped uncover the structur of benzene

Stephanie Kwolek

Polymer chemist and long-time DuPont employee who is famous for inventing Kevlar

BUSINESS IN BRIEF

A roundup of this week's business news, from Martian lasers to MS-based COVID-19 testing

- Thales – a leader in the design, development and manufacture of high energy lasers – has made analytical history this month as part of the Perseverance rover mission. The company's laser is part of the rover's onboard SuperCam unit, which will look for signs of microbial life on Mars using Raman spectroscopy – the first time this technique has been tested on the Red Planet (1).
- Avacta Group and Bruker Corporation have entered into an agreement to evaluate the Adeptrix coronavirus antigen test, which is based on bead-assisted MS. This represents an exciting step towards implementing this high-throughput test in typical clinical microbiology lab workflows (2).
- A number of prominent analytical scientists were honored at the Pittcon 2021 virtual conference. Amongst the awardees are Isao Noda, Richard Yost, Robbyn Anand, Perdita Barran, and Ian Lewis (3).

- Thermo Fisher Scientific has launched a new range of capillary chromatography columns and emitters that promise high-resolution, low-flow separation of intact proteins, monoclonal antibodies and peptides (4).
- Waters recently launched the Acquity Premier LC solution, which incorporates their MaxPeak High Performance Surface technology to improve analytical data quality and simplify analyses (5).

References

1. *Business Wire (2021). Available at: https://bwnews.pr/3kkIXrb.*
2. *Avacta (2021). Available at: https://bit.ly/2NTUBgA.*
3. *Pittcon (2021). Available at: https://bit.ly/3aNokAK.*
4. *PR NewsWire (2021). Available at: https://prn.to/3bBBCPX.*
5. *Business Wire (2021). Available at: https://bwnews.pr/3dJrJTk.*

Scouting for Antimicrobial Morsels

Researchers develop new LC-MS method to track down traces of multiclass antibiotics in different foods

Even before the first antibiotic – penicillin – was used for therapeutic purposes, scientists were aware of the dangers of microbial resistance to these "wonder drugs." Their increased use in the agricultural industry has led to trace levels of these compounds in our food, which could increase the risk of pathogens developing resistance to them.

Until now, it has been difficult for scientists to analyze a large number of antibiotics in a variety of foods, largely because of their vastly different chemical structures and properties. However, researchers recently published a new approach based on HPLC-MS that enabled them to detect 77 distinct antibiotics in cereals, meat, eggs, milk, vegetables, and fruits – a breakthrough that should help further research on this pressing topic.

Reference

1. *M Hu et al., J Agric Food Chem, 69, 5 (2021). DOI: 10.1021/acs.jafc.0c05778.*

Dorothy Mary Hodgkin

Instrumental in the development of X-ray chrystallography to determine the structure of biomolecules

Erika Cremer

A German physical chemist who developed the theory and instrumentation for the first gas chromatograph

Yvette Cauchois

Pioneer in a number of X-ray spectroscopy techniques and the development of synchrotron research

The Tree of (Solar) Life

The analysis of radioactive carbon in tree rings provides insight into a millennium of solar activity

At the center of our solar system sits a hot ball of burning gas that provides the major source of energy for life on our planet. Sunspots offer us a precious glimpse at the level of solar activity beyond its surface, but our eyes have yielded little information over the past four centuries – and detailed, direct satellite observations of these temporary phenomena are limited to the last 70 years. Now, however, a team from the Laboratory of Ion Beam Physics at ETH has managed to look as far back as the last millennium by measuring radioactive carbon levels in tree rings.

"We analyzed nine different trees that had been dated by dendrochronology – using their growth rings as an indicator – at annual resolution with accelerator MS (AMS)," says Nicolas Brehm, lead author of the paper. "Using this technique, we were able to get the $^{14}C/^{12}C$ ratio of each year," he adds. Any change in ^{14}C levels would reflect a change in the level of protection offered by the Sun – which usually guards the Earth from radioactive cosmic particles through its magnetic field.

"The analysis of the 11-year solar cycle before the observation of sunspots is of particular interest because, until now, we have been limited to analyzing the amplitude and length of just 25 cycles – since precise measurements are only available from about 1750 onwards," says Brehm. "We've shown that amplitudes – how much the sun's activity fluctuates – of these cycles are significantly reduced during grand solar minima (periods of low activity) compared to maxima (high activity)."

Not only does this work provide new insight into solar dynamics, but their high-temporal-resolution record of atmospheric carbon provides a whole new data set for improving radiocarbon calibration procedures. "Our next steps are to extend the record further back in time to reconstruct solar activity in annual resolution and search for more energetic particle events," adds Brehm.

Reference

1. *N Brehm et al., Nat Geosci, 14, 10 (2021). DOI: 10.1038/s41561-020-00674-0.*

Spectroscopy on the Brain

Combined spectroscopy techniques aid accurate glioblastoma identification in mice

Glioblastoma accounts for over 60 percent of brain cancers, but survival five years beyond diagnosis is still rare. Raman and reflectance spectroscopies may allow us to distinguish healthy from diseased brain tissues with relative ease, improving our ability to detect and resect these tumors – and save patients.

After implanting murine glioma cells expressing enhanced green fluorescent protein into mice, Enrico Baria and colleagues used fluorescence microscopy to spot malignant tissue. Raman and reflectance spectroscopies aimed at the area of peak fluorescence intensity then informed a tissue classification algorithm, facilitating 97 percent identification accuracy when combining the two techniques.

The approach could provide a powerful tool for further animal studies – and could eventually complement preoperative MRI in humans.

Reference

1. *E Baria et al., Neurophotonics, 7, 045010 (2020). DOI: 10.1117/1.NPh.7.4.045010.*

IMAGE OF THE MONTH

Old Mold

A team of researchers have used a combination of analytical techniques (including secondary ion MS and Raman spectroscopy) to characterize a 635 million-year-old microfossil – the oldest terrestrial fossil ever found (1). The image above shows the filamentous structure of the fossil, which has led the team to conclude that a fungus is the most likely origin. This finding could be crucial in helping scientists better understand the timeline of our planet's terrestrialization – the emergence of organisms from the sea onto land.

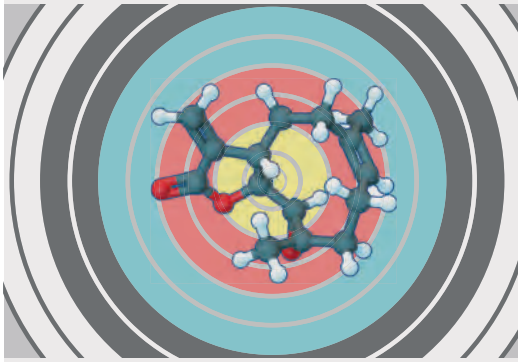
Image credit: Andrew Czaja of the University of Cincinnati.

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

QUOTE OF THE MONTH

"All sorts of things can happen when you're open to new ideas and playing around with things."

Stephanie Kwolek (1923-2014), an American polymer chemist who is famous for inventing Kevlar.



Target Practice

A new native MS method may help overcome the bottleneck of drug target identification

Identifying the biological targets of small-molecule drugs remains a bottleneck in drug discovery efforts – but is it one we can overcome? A new method that capitalizes on the specific detection of protein-drug complexes by native MS looks favorable.

Miaomiao Liu and colleagues applied their native MS method to investigate known interactions between parthenolide (a natural product of antimalarial drug artemisinin) and thioredoxin (a redox protein present in all living organisms). To their joy, binding of parthenolide to thioredoxin binding was identified in untargeted fashion in two samples: a mixture of five proteins from malarial parasite *Plasmodium falciparum* and a bacterial cell lysate.

The implications of this finding? The preliminary data indicate that native MS could be used to identify binding targets for any small molecule. If future studies realise this potential, pharmaceutical researchers may be in luck!

Reference

1. *M Liu et al., Sci. Rep, 11, 2387 (2021). DOI: 10.1038/s41598-021-81859-4.*

In My View

Experts from across the world share a single strongly held opinion or key idea.

Bringing Employability to the Classroom

How can we ensure our graduates develop a wide range of transferable skills that will boost their chances of securing a job?

By Fiona Ponikwer and Bhavik Patel

All analytical chemistry graduates should leave university with a wide array of practical and scientific skills that will allow them to pursue a number of careers across a broad range of sectors. We must not forget that many of these graduates will pursue roles outside of the traditional laboratory setting – and we need to ensure we provide them with the skillset to match. Creating educational activities that place the emphasis on employability has always been a challenge in our field. One solution is to offer short experiential visits or internships, but these are not always inclusive and are difficult to offer on a larger scale.

To tackle the issue, Bhavik decided to develop the "Analyst Laboratory Challenge" – an academic activity at the University of Brighton with an entertaining twist. Based loosely on The Apprentice (the hit TV show where aspiring entrepreneurs demonstrate their skills – or lack thereof – through a series of tasks), the initial Analyst Laboratory Challenge placed students into small teams or companies. Each company had to assess an analytical chemistry column to demonstrate their practical and data analysis skills. They were then asked to produce technical data sheets and pitch their product – as a company – to a panel who awarded a prize to the best performing team.

Though the project was effective, the skills required were quite narrow. Fiona, a senior lecturer in Learning and Teaching Technology with business experience, had some interesting ideas on how to enhance the activity. She felt we had a unique opportunity to develop students with skills that would transfer into a wider job market. To create a unique work-integrated learning (WIL) activity – the practice of combining traditional academic study or formal learning with student exposure to the world of work – we ran the programme in intensive mode over a week, and adjusted the assessed elements: individuals received scores for scientific and practical knowledge of chromatography, but there was greater emphasis on teamwork, time management, and various forms of scientific and non-scientific communication.

We also changed the individual written reflections at the end of the challenge to video diary entries throughout the week, and moved from static posters to a short (under 3 minutes) trade-fair style infomercial. Although this was one of the most challenging aspects for each team,

"Collaboration with colleagues from different disciplines is also crucial and can lead to creative partnerships."

the finished films were creative and, in several cases, of near-professional quality. The video diaries allowed them to reflect on the skills used to deal with various challenges that had arisen during the week, which they could later evidence when applying for jobs. The group presentation was later used as a professional pitch to a panel who took up roles of scientific/technical managers, as well as marketing and business managers.

Over several years, we surveyed the graduates who took part to discover the potential impact it may have had on their employability, and our results were recently published in the Journal of Chemical Education (1). In brief, we found that 74 percent of respondents felt that all aspects of the activity reflected the working environment. The survey also indicated that 60 percent of responding students felt that this activity enhanced their employability, and 44 percent felt it had been key in supporting their application for a job. Success!

So, what have we learnt from conducting the Analyst Laboratory Challenge? It is essential to start with a smaller, manageable activity, and to evaluate and adjust where necessary each time you run it. Be bold with your developments, but make them SMART (specific, measurable, achievable, realistic and time-framed). Collaboration with colleagues from different disciplines is also crucial and can lead to creative partnerships.

Finally, it is worth noting that there are obviously many different approaches to embedding employability into the analytical chemistry curriculum. We truly believe this educational WIL, which can be modified for any discipline of chemistry, provides a unique and inclusive way to broaden skillsets and should be a key part of the employability toolkit at universities.

Reference

1. *Ponikwer, F; Patel, BA, 'Work-Integrated Learning: A Game-Based Learning Activity That Enhances Student Employability,' J Chem Educ, (2021). DOI: 10.1021/acs.jchemed.0c00919*

Mind the (Gender) Gap

In the run up to International Women's Day, we should reflect on the impact of role models and consider how we can work together to increase their presence in the field of analytical science

By Isabelle Kohler, Assistant Professor, Division of BioAnalytical Chemistry, Amsterdam Institute for Molecular and Life Sciences, Vrije Universiteit Amsterdam, The Netherlands

February 7, 2021, marked fifty years since women gained the right to vote in federal elections in my home country, Switzerland. For the women of my generation (or younger), it is hard to imagine a time when we would not have been allowed to vote or even open a bank account without the approval of our husbands. Half a century later, we are still far from equality. Women still struggle to get the jobs they want, are concentrated in lower-paying jobs, face additional hurdles on their path to leadership positions, and suffer from multiple motherhood penalties – not to mention possible workplace harassment.

How does this translate to academia? In my adoptive home of the Netherlands, the "Women Professors Monitor" is run every year by the Dutch Network of Women Professors (Landelijk Netwerk Vrouwelijke Hoogleraren) to review the differences between men and women at the academic level. In 2020, they highlighted the strong decline in the percentage of women at each step of the academic ladder (1). Indeed, despite 53 percent of graduates at Dutch Universities being women, only 30 percent of associate professors are female and this falls to a mere 24 percent for full professors. Furthermore, the growth rate of female professors was just 1.1 percent in 2020. To put this into perspective, if we continue at the same speed, it will take until 2041 to reach a balance in male versus female representation among full professors.

"I am concerned that female professors are leaving academia because they cannot find suitable and supportive places for them.

As a woman in academia, and currently at the assistant professorship/tenure track level, I am deeply worried about these numbers and trends. I am concerned that, despite the huge amount of work carried out by both my female and male predecessors to encourage and support women in academia, progress is very slow. I am concerned that female professors are leaving academia because they cannot find suitable and supportive places for them. I am concerned that I am one of the few women in my institution. Most pertinently, I am concerned that we are unconsciously displaying the wrong academic picture to our talented and motivated students.

How can we expect to significantly improve representation of women and minorities in academia when the major role models are white men who regularly work at nights and during weekends?

I'd argue, we can't. The impact of role models has been extensively studied; they play a crucial role in the development of young people and the career advancement of junior professionals (2,3). Importantly, gender- and race-matched role models can lead to better professional performance and an overall higher level of satisfaction at work compared with non-matched role models.

Interestingly, I was recently discussing the results of the Women Professors Monitor with my female colleague (and friend) Lotte Schreuders, who is a Junior Teacher at the University of Amsterdam. She asked me about my own role models – who they were and in what ways they had inspired me. I fell silent. I've certainly had a number of people who have supported me and helped me to find opportunities in academia, which I'm extremely grateful for, but role models? I told her I'd never met a person on my career path that I view as an example to be imitated (the very definition of "role model.")

It was then I realized I have – somewhat

"At the Centre for Analytical Sciences Amsterdam (CASA), we believe that building communities among students and professors is crucial for students' professional and personal development."

surreptitiously – become my own role model. I wish I had encountered more women who I could have turned to for professional advice, that the institutions I worked for had mentoring programs I could have benefited from, or that I had met female mentors that had overcome some of the challenges I was similarly facing. But I didn't, so I became my own role model. And it worked fine for me, but what about others?

At the Centre for Analytical Sciences Amsterdam (CASA), we believe that building communities among students and professors is crucial for students' professional and personal development. With the community we have built and the mentoring program we are currently implementing, we want to offer students the possibility of not only finding role models, mentors, and peer mentors, but also being part of an inclusive and diverse community of scientists.

In the run-up to International Women's Day, I want to encourage all my fellow colleagues worldwide – both male and female – to reflect on the impact of role models and mentors on the younger generation. Stand up, and act for diversity and inclusion in science. We need a broader range of role models in academia to show the younger generation what is possible. As the proverb says: "If you want to go fast, go alone. If you want to go far, go together." Let's work together to ensure we create the most inclusive place for everyone in the field of analytical science and in wider academia.

References

1. *Landelijk Netwerk Vrouwelijke Hoogleraren (LNVH), Monitor Vrouwelijke Hoogleraren (2020). Available at: https://bit.ly/301EU9N*
2. *S Zirkel, "Is There A Place for Me? Role Models and Academic Identity among White Students and Students of Color," Teachers College Record, 104, 357 (2002). DOI: 10.1111/1467-9620.00166*
3. *W Levinson et al., "Mentors and Role Models for Women in Academic Medicine," West J Med, 154, 423 (1991). PMID: 1877183*

Ethical Considerations in Clinical Proteomics

Conversations about ethical issues with proteomics data need to be had – and we are here to start the conversation

By Sebastian Porsdam Mann, Philipp Geyer, Peter Treit, Matthias Mann

Proteins exert most biological functions in the body – so it's no surprise that their levels vary over time and between environments, adjusting to the needs of the organism. Proteomics studies these fluctuations to determine which proteins are responsible for specific functions. Why? The main role of clinical proteomics is to identify proteins correlating with health and disease states in humans.

These proteins are so called biomarkers, and can streamline disease diagnosis and personalize treatments. To this end, state-of-the-art workflows aim to assess all proteins in a sample in an unbiased way – a difficult task, given the sheer number of proteins and their vast ranges of abundance in samples. Realizing the promise of clinical proteomics is one of the great challenges we face in analytical science.

Bringing the benefits of proteomics into the clinic requires us to attend to issues that extend beyond technology. As with most activities with implications for health and wellbeing, the broad inferential powers of proteomics come with related ethical responsibilities. Many of these, including the importance of informed consent and data security, are familiar from other biomedical and human subject-related fields. However, the capacity of proteomic profiles to broadly reflect an individual´s biological state refashions old questions relating to the types of information that can be derived – regardless of the purpose for which the information was originally collected.

We set out to illustrate this ethical dilemma by re-analyzing a previously conducted weight loss study from MS-based proteomics of human plasma (1). Protein levels varied widely between people, but were stable between sampling periods in individuals. So we were able to identify individuals in our cohort by their characteristic protein levels and alleles. We were also able to measure levels of proteins that vary between different ethnicities and genders, as well as proteins associated with pregnancy. Clearly, this is all potentially sensitive information.

We also found that proteomic profiles contain "incidental findings" – clinically relevant information on disease states other than the one for which the sample was taken. For example, glycation patterns and levels of apolipoproteins could indicate somebody's risk of developing diabetes or Alzheimer's disease.

The ease with which we obtained this information from existing datasets convinced us of the need to discuss the ethical implications of proteomics. To kickstart this conversation, we applied systematic review methods adapted for qualitative data to capture nascent discussions of these ethical issues in the literature (2). Of the 16 relevant articles we identified, most were out of date or contained only cursory references to ethical topics.

We also looked to the literature on clinical genomics. How did that field deal with similar issues? First, there appears to be consensus in clinical genomics that actionable information relevant to a person's health should – or must – be returned to them. Moreover, worries about how well sample genomes represent global human diversity appear to be just as prevalent in genomics as in proteomics.

But there are also clear differences between the fields that prevent us from adopting genomics' guidelines wholesale. Most important in our eyes: the potential for proteomic profiles, in contrast to genomic data, to capture phenotypic information in samples and track changes over time. This offers clear opportunities, such as periodic proteomic profile collection with healthcare providers for diagnostic, treatment, and prevention purposes. Such preventative measures give us a chance to maximize the benefits of our science (for example by empowering us to enhance disease resilience) – another ethical consideration we must bear in mind.

There are clear potentials and pitfalls when we think about proteomics from an ethical perspective. Our main contribution, we hope, is stimulating an early and comprehensive conversation on the topic. The experiences of clinical genomics and other biomedical fields show that legislation- and guideline-based regulation will eventually come. And, though this may come from external sources, we argue that clinical proteomics would benefit from self-regulation through open discussions and dissemination of consensus-based professional guidelines. This is the wisest way by which we can fulfil the promise of clinical proteomics while protecting patients from ethical issues in the long term.

References

1. *PE Geyer et al., "Plasma proteomes can be reidentifiable and potentially contain personally sensitive and incidental findings," Mol Cell Proteomics, 100035 (2021). DOI: 10.1074/mcp.RA120.002359*
2. *S Porsdam Mann et al.m "Ethical principles, opportunities and constraints in clinical proteomics," Mol Cell Proteomics, 100046 (2021). DOI: 10.1074/mcp.RA120.002435*

Let There be Light

The award-winning LUMOS II is an FTIR microscope with an edge. We caught up with the instrument's experts – Peng Wang, Ph.D., and Tom Tague, Ph.D., – to find out what makes it so special.

Why was the LUMOS II developed?

Tom Tague: The LUMOS II was developed with one goal in mind: to create the easiest-to-use microanalysis tool that has ever existed, while incorporating state-of-the-art capabilities. How? In part, by including a video wizard in our software. Did we succeed? Even the newest users can use the LUMOS II and obtain great results without apprehension or daunting learning curves. In fact, I did a demonstration out in Utah and my host, Carol, asked if she could hang onto the system for a few days to show some colleagues. Having only used it for an hour or two herself, she felt perfectly comfortable demonstrating it to others and ended up selling two more systems for us!

Could an untrained member of our editorial team operate it?

Tom: Absolutely. If one of your editors were to visit, we would run one sample for you and then give up the pilot's seat completely. I have no doubt that you'd be able to use the machine from that point forward – you just can't get lost in the software! This is the way we always demonstrate this instrument, and the key advantage of doing so is that customers feel comfortable operating the system and obtaining high-quality data from the moment the service engineer installs it.

It seems you wowed the crowd at PITTCON, too – what was it like to win the 2020 Gold Award?

Tom: In short – very exciting! There is only one Gold Award each year, so it was a wonderful recognition of the great effort that our development team put into developing a truly unique product. Our conference booth showed a short video explaining the system's features and capabilities, and hundreds of people stopped by to find out more.

What types of samples is the LUMOS II most useful for?

Peng Wang: Multilayer and multicomponent samples like packaging materials and paint chips are ideal for analysis using the LUMOS II. These would typically require preparation by microtome and mounting before microscope analysis. Using our instrument, however, you're able to obtain data from multiple layers quickly and easily using just the reflectance mode. Depending on the number of layers present, you may also need to conduct ATR measurements. The LUMOS II is equipped with a dedicated ATR crystal to deal with these samples.

I'm also excited by the potential for LUMOS to support the study of microplastics. The instrument facilitates screening of plastic residue particles for rapid microplastic detection and identification. This capability could help researchers trace the origin and distribution of microplastics – and their impact on our lives and environment.

Who should use the LUMOS II?

Peng: Definitely those studying particles less than half a millimeter or so. IR microscopy is one of the most widely used techniques for materials identification at the micrometer scale, underscoring its importance in key areas like pharma and forensics. Key features of the LUMOS II (including high performance, accessible imaging capability, and sample accessibility) ensure its potential to become the next truly universal tool for microanalysis – both for routine analyses and challenging applications.

Tom: Peng is right! The LUMOS II is useful for anyone trying to identify small parts of samples of any size. The microscope's working distance is the longest in the market, making it the ideal choice for large and small samples.

Meet the LUMOS II Experts

Hi! I'm Peng Wang, Ph.D., and I am an Applications Scientist with Bruker. I'm proud to say I've been with the company for more than 12 years. My role includes helping potential customers find the best possible solutions for their applications, conducting software and instrument training with existing customers, and providing applications support, as well as discovering new applications fields for our instruments.

Hi, I'm Tom – the Applications Manager for Bruker Corporation. I am also a member of the Visiting Advisory Committee of the Metropolitan Museum of Art in New York's Advisory Board of Amplified Sciences, and also a member of the American Chemical Society, Society for Applied Spectroscopy, American Physical Society, and the Optical Society of America. I am active in developing new methods and instrumentation, with the goal of improving the sensitivity and detection limits of spectroscopy-related applications. I have more than 80 publications and 5 Patents.

BRUKER

www.bruker.com/LUMOS

The Call *of* Coronal Duty

An insider's perspective on the proteomic battlegrounds of COVID-19

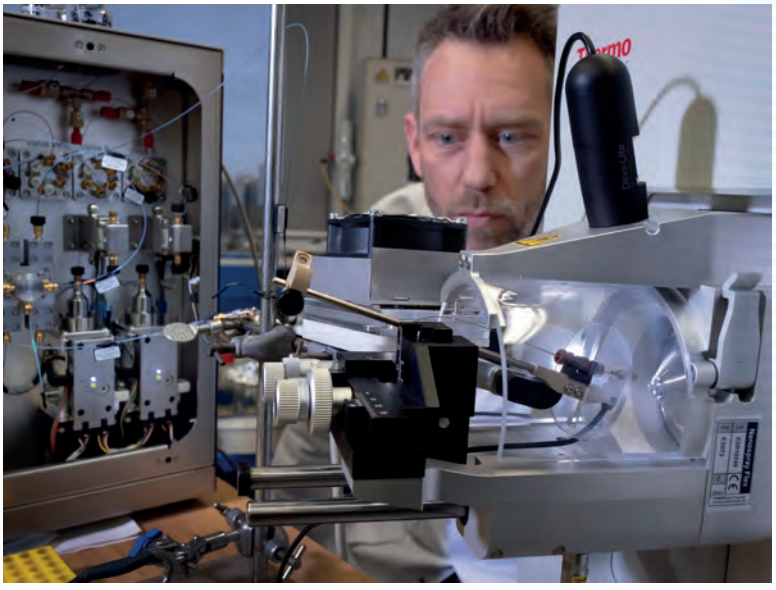
With March 11, 2021 marking one year since COVID-19 was officially classified as a pandemic by the WHO, it goes without saying that many of us – not least those on the frontline of the fight – are feeling a bit war weary. And yet, for so many in the analytical chemistry community who answered the call of duty back in 2020, the battle rages on.

With so much uncharted ground still to cover when it comes to this novel coronavirus and our response to it, there is an abundance of work ongoing across all sub disciplines of our field. For now, we decided to share some of the spoils from one battlefront in particular: proteomics. Here, experts Jeroen Demmers, Perdita Barran, and Manfred Wuhrer tell us about their work in the fight against COVID-19, and provide an insider's perspective on some of the developments we can expect to see in the coming months.

COVID-19 Detection: *Hitting the Mark*

How we successfully used targeted proteomics for the detection of SARS-CoV-2 proteins

By Jeroen Demmers, Director of the Proteomics Core Facility and Associate Professor of Proteomics, Erasmus University Medical Centre, the Netherlands



Early last year, once it became clear that COVID-19 had started to spread out across the world, there was a general sentiment in Europe that it wouldn't happen that easily here. Just like the SARS and MERS coronavirus pandemics that came before (in 2003 and 2013, respectively), many people thought that this novel virus would be kept out of the region as well. Soon enough, the pandemic hit northern Italy hard, and it wasn't long before there were messages of infected people in the southern province of Noord-Brabant in the Netherlands.

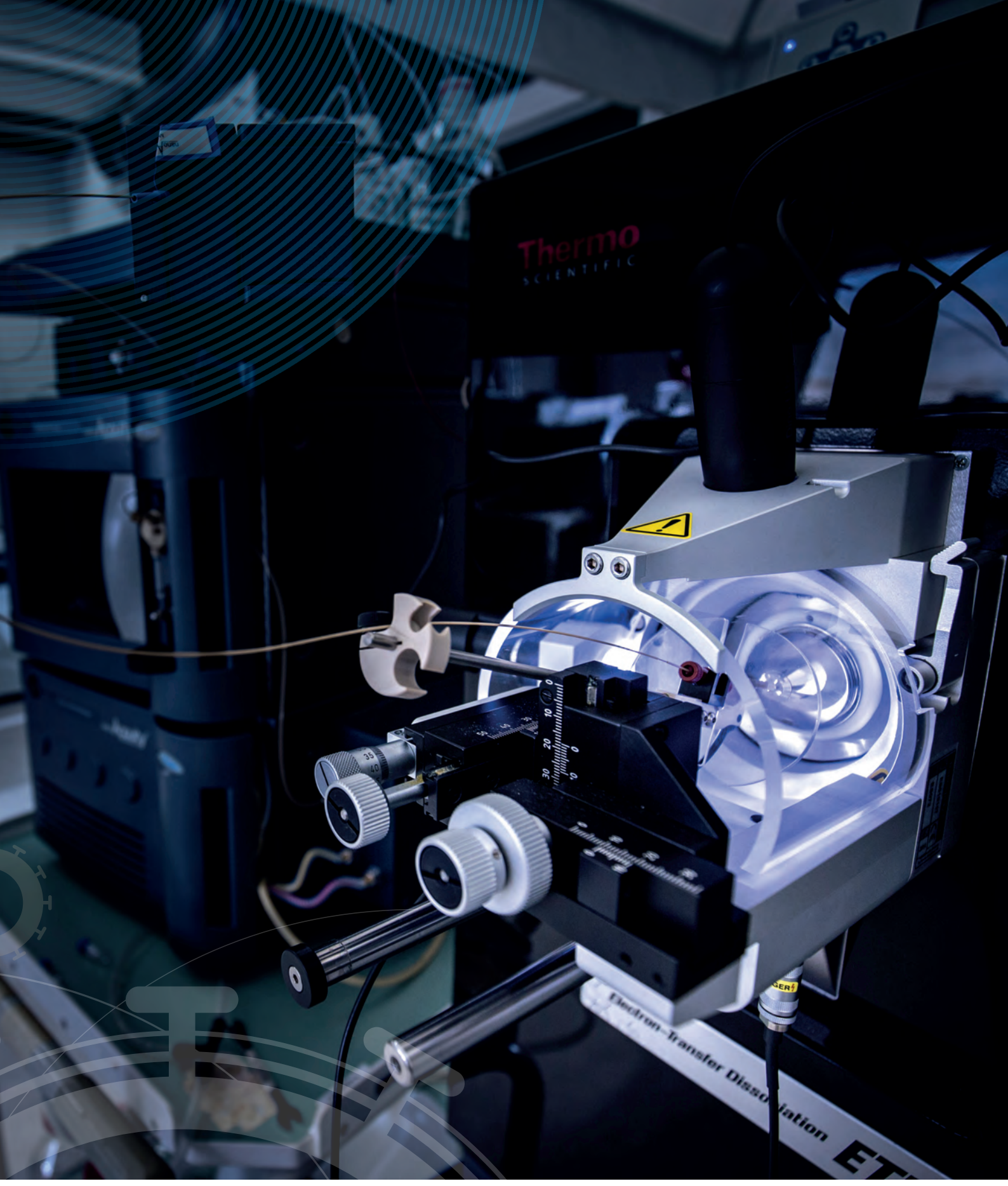
The first official outbreaks were reported in early March, and things developed quickly from there. In the second half of March our institute was shut down – like many others across Europe – and only research on COVID-19 was allowed to continue. For us, this work was happening at the Viroscience department at Erasmus MC, where several research groups had been focusing on coronaviruses for decades. My research lab and core facility had a choice: shut the lab, or grab this opportunity to contribute to SARS-CoV-2 containment by adapting our technology for use in virus detection and – if successful – diagnostics.

The journey to discovery

Thanks to our previous work during the MERS coronavirus pandemic (our lab had identified the MERS-CoV human receptor protein using MS-based proteomics; see our "Gone Fishing" sidebar on page 23 for more information), we had already established connections with the Viroscience department. I decided to contact coronavirus specialists Bart Haagmans and Mart Lamers as I knew they were working day and night on SARS-CoV-2 assays to answer questions about the mechanism of infection. For one of their assays, they were interested in analyzing the response of the host cell proteome to viral infection in a recently developed organoid-derived bronchioalveolar tissue culture. Using our technology, we were able to help them monitor up- and downregulation of large numbers of proteins upon viral infection and learn more about the intracellular pathways that are turned on or off as a result of infection. Because of this work, and our connections with the Viroscience department, we were also granted access to some of their interesting SARS-CoV-2 samples – meaning we could test whether it was possible to measure viral proteins in complex samples, such as cell lysates.

We started off with samples from an infected Vero E6 cell line derived from the African green monkey – this cell line is used to propagate viruses and serves as a rich source of viral material. A dilution series was then created to demonstrate the limit of detection of specific viral proteins. As the virus was already genotyped, the protein amino acid sequences that we needed for the analysis of proteins based on peptide fragmentation or MS/MS data were already available. Also, we were quite lucky (or unlucky?) in that just a few days before most of the institute was shut down, a brand new Orbitrap Eclipse MS was installed in the lab. The first proteins analyzed on that machine were SARS-CoV-2 proteins!

The output of a standard proteomics experiment is usually a table of identified proteins, which is generated in the final step of a database search using software tools that may take up to several hours. The progression of this process (at least in the tool that we use) is indicated by a green bar. I clearly remember the anxiety and excitement that we felt when, after the very first database search, we saw the bar hit 100 percent and the list of identified proteins popped up: the first time we identified SARS-CoV-2 proteins really felt like looking the monster directly in the eye.


Thermo
SCIENTIFIC

How can proteomics help *in the fight against COVID-19?*

Understanding the role that proteins play in the SARS-CoV-2 infection process and disease progression is vital to the development of therapeutic and preventative strategies. In this way, proteomics has proven to be an indispensable tool in COVID-19 research, and its role will no doubt be expanded in the future.

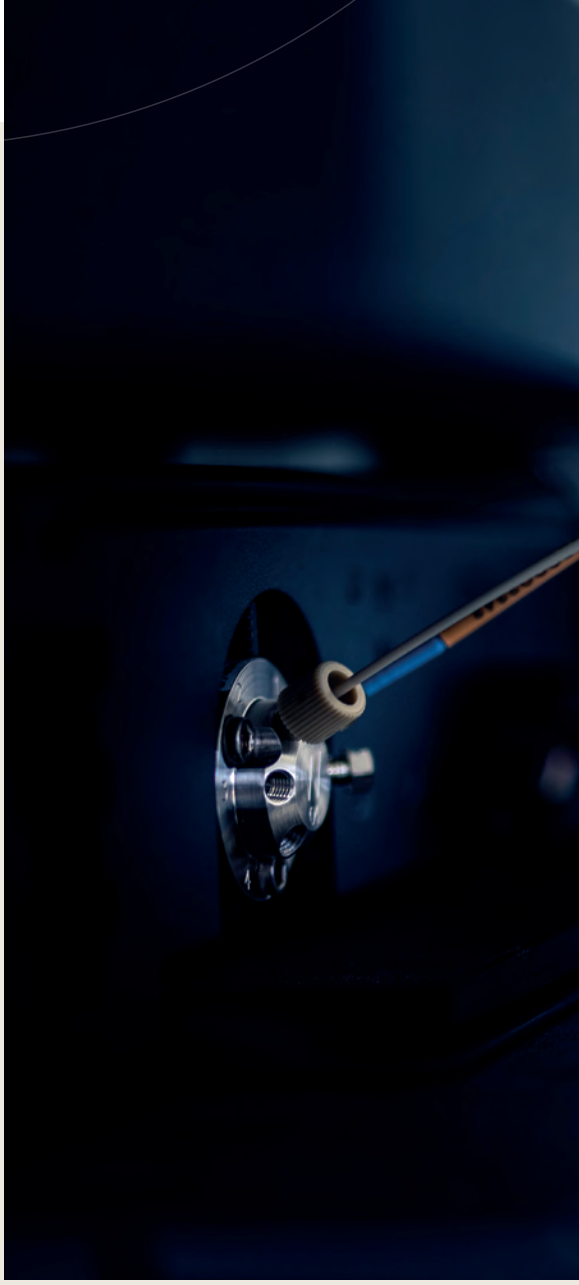
Firstly, MS-based detection of SARS-CoV-2 proteins and their proteolytic peptides offers a simple and rapid virus detection assay. Using targeted proteomics, peptides of the SARS-CoV-2 nucleocapsid and spike proteins can be detected with high sensitivity and specificity in research samples and clinical specimens. This opens up the possibility of taking this technology to clinical diagnostic labs and translating it into point-of-care devices as alternatives for nucleic acid-based methods, which could be particularly interesting from a cost-effective healthcare perspective.

Proteomics could also be used to develop approaches capable of predicting COVID-19 cases that might later progress into clinically severe disease. In fact, several studies have already identified potential protein biomarkers that are differentially expressed in COVID-19 patients and could be used to predict viral infection at early stages. (See the sidebar "Collaboration and Determination" to learn more about Perdita Barran's work around targeted proteomics and biomarkers).

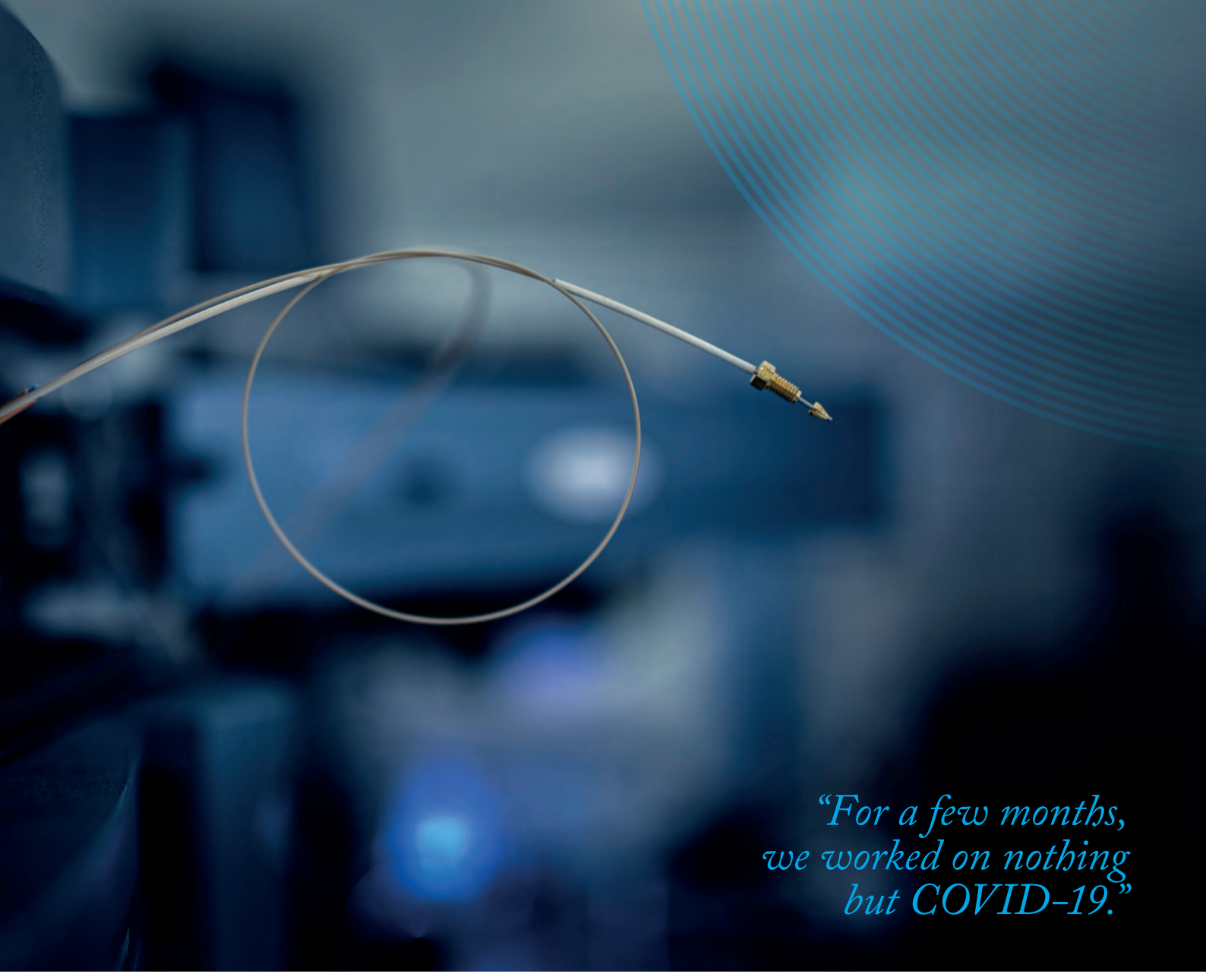
In other areas, investigation of the humoral antibody response to SARS-CoV-2 proteins has aided the development of antibody-based assays for diagnostic and therapeutic purposes. Recently, a comprehensive SARS-CoV-2 human protein–protein interaction map was generated using affinity-purification (AP) MS. Several hundreds of specific interactions between SARS-CoV-2 and host cell proteins were defined, and it was discovered that, for some of the involved human proteins, several existing FDA approved drugs were already available. Other proteomics-based research on the host cell response has shown that the complement system and metabolic pathways are severely affected in COVID-19 patients.

Unbiased, explorative proteomics has also been used to define the proteomes of autopsy samples from COVID-19 patients. For instance, it was shown that cathepsin L1, rather than ACE2, was significantly upregulated in the lungs of COVID-19 patients. In addition, systemic hyperinflammation and dysregulation of glucose and fatty acid metabolism was detected in multiple organs, which shows how the multi-organ proteomic landscape of such autopsies may help in our understanding of the biological basis of COVID-19 pathology.

Crosslinking MS has been used to study the interaction sites between antibodies and the spike protein in detail. Such studies, often combined with protein structure elucidation by tools such as cryo-EM are crucial in the development of antiviral therapeutics. In research studies, huge non-covalent assemblies of proteins – such as intact virus particles several 10s of Megadaltons in mass – can be analyzed by MS. This way, conformational dynamics of viruses and viral proteins can be uncovered and this can yield valuable information on the stability and topology of macromolecular assemblies in general and virus capsid structure in particular.



Viral proteins, in particular those in the viral envelope (such as the spike protein), are extensively decorated by protein glycosylation. To understand how this post-translational modification influences spike-ACE2 interactions with the host cell membrane, these glycan structures have been characterized in detail by (glyco) proteomics. Detailed analyses of the impact of emerging variants in spike and natural or designed-for-biologics variants of ACE2 on glycosylation and binding properties are important next steps in developing therapeutics.



The right sample

For a few months, we worked on nothing but COVID-19. Virtually all other projects were put on hold and since most meetings at work were cancelled and there were no teaching duties, it really felt like a postdoc project where the full focus is on basic science. I truly relished this lack of distraction, despite the troubling situation we – as citizens of the world – were in. On a personal level, we were building a new house and we weren't sure whether we could still sell our old house – what with the threats of a housing market collapse together with the crashing stock markets and other doomsday scenarios that circulated. It was truly both an exciting and troubling time.

We soon identified a set of proteolytic peptides that could serve as the target peptides in follow-up experiments. Also, we were able to calculate limits of detection for viral proteins in our proteomics assays. Under ideal conditions, we could go down to the mid- to low attomole range in targeted experiments, just like the numbers we had seen before in another project on non-related proteins.

While setting up these assays, I had already started asking around for patient material to see whether we could detect proteins in clinical specimens (such as nasal swabs) and to determine if it could be used as a diagnostic tool. However, getting patient samples turned out to be more difficult than I had anticipated. For conventional PCR-based testing, samples are usually stored in a "transport medium." This medium contains a lot of protein, the signals of which dramatically mask the signals of viral proteins in our assay. Unfortunately, adaptation of standard protocols in diagnostic departments is virtually impossible, and as research scientists who are used to changing protocols if something doesn't work, this was quite frustrating.

But one day we got a message from a collaborating clinical virologist who had collected a different type of sample from a COVID-19 patient. This was a sputum sample, deposited on a little glass slide with no addition of transport medium or any other buffer solution. Upon inactivation of the virus in 80 percent acetone, we could take the sample from the BSL lab to our own lab and subject it to our standard bottom-up proteomics protocols – which basically means digesting all the proteins into peptides. This sample was in fact the first clinical specimen in which we could clearly detect SARS-CoV-2 peptides.

We used a targeted proteomics assay, which means that we set the MS in such a way that it only detects viral peptides that were selected a priori. The quadrupole in the Orbitrap hybrid MS then acts as a filter that lets only the peptides (or m/z values) of interest pass through. Upon fragmentation of the peptide to determine the amino acid sequence, the fragment ions are measured in the Orbitrap with high selectivity and sensitivity – the high mass accuracy of the Orbitrap is a clear advantage over such targeted methods in a triple quadrupole instrument here. The fragment ion fingerprint that is thus obtained is highly specific for the selected peptide. These fingerprints are then computationally compared with the specific fingerprints that were defined in the experiments on infected Vero E6 cells. Using targeted MS, the sensitivity can be increased and the limit of detection is at least 10-fold lower compared with data-dependent acquisition (untargeted) MS.

Next, we contacted clinical virologists from a hospital in the south of the Netherlands, which was located in the center of the area that was hit by the first COVID-19 wave in the spring of 2020. The clinicians there used so-called Eswabs, for which no protein rich transport medium is necessary. This results in much less background in our analyses and therefore increased sensitivity. Despite the excess of red tape, we managed to get an Eswab sample cohort to our lab. This sample set contained various swabs within a wide range of PCR Ct values and we could see a nice inverse correlation between Ct value and peak intensities of target peptides in the mass spectra, reflecting the abundance of proteins. Later, in a second sample cohort, we managed to get similar results and could detect SARS-CoV-2 peptides at fairly high Ct values (i.e. low viral counts).

Winning the war…

Where are we now? We have established the proof-of-concept and have shown that it is definitely possible to detect SARS-CoV-2 proteins using MS. The challenge now is to translate this methodology from the R&D stage to the clinical diagnostic lab. For the analyses we have performed so far, we used state-of-the-art, ultra-sensitive Orbitrap mass spectrometers – which are typically not present in clinical diagnostic labs. Still, the basic technology is comparable to triple quad MS and these are readily available in many clinical labs.

There is still a debate around the level of sensitivity we really need in COVID-19 testing. The limit of detection of PCR based methods is unsurpassed, but do we really need that sensitivity? It is unclear whether infected individuals, whose nasal swab PCR Ct values are in the high 20s or low 30s, are infectious. Although no viral proteins could be detected in most swabs with associated Ct values of >26, we have to test whether the sensitivity that can be reached by MS-based approaches is sufficient to differentiate between infectious and non-infectious people. Only then will we be able to assess the potential value of MS-based COVID-19 testing.

One clear advantage of this technology over other testing methods however, is that proteins of multiple different viruses

Gone *Fishing*

For our work on the MERS-CoV human receptor protein, we designed a relatively simple "fishing" experiment that was performed using in vitro synthesized MERS-CoV Spike 1 protein immobilized on magnetic beads. The spike protein was used as bait, and we went fishing in a pond of human proteins – or "cell lysates," prepared by crushing cells that were cultured in a petri dish in the presence of detergents. We found one human protein that showed a very specific interaction with the bait protein, suggesting this was a receptor protein present on the outside of the human host cell – for example an epithelial cell in the lung that the virus grabs and uses to enter the host cell.

The identification of the receptor protein was not only crucial in understanding how the virus infects a host cell, but also for the development of antiviral therapeutics and vaccines. For instance, by blocking a receptor using small molecule drugs or antibodies, infection of the host cell can be prevented. We identified the protein DPP4 as the receptor for MERS-CoV, and this finding was confirmed by in vitro and in vivo experiments (see the online article for a reference!). Since it is different from the ACE2 receptor that SARS uses to enter the host cell, this was a somewhat unexpected finding at the time.

From a proteomics point of view, this study is the ultimate example of the importance of identification of proteins in an unbiased manner – the core of MS-based proteomics technology. If more conventional experimental methods, such as screening assays, had been used at that time, the identity of the receptor is unlikely to have been found so quickly. One would have to have made a selection of possible receptors a priori, and if the protein was not included in that selection, it would not have been found.

Collaboration *and* Determination

Perdita Barran, Professor of Mass Spectrometry at the University of Manchester, UK, shares a targeted approach to SARS-CoV-2 proteomics

How can targeted proteomics help in the fight against COVID-19?

Targeted proteomics can help diagnose whether someone has the virus, but it can also help to determine the effect of the virus on a given individual by providing biomarkers that can predict the course of the disease. I am working on projects in both of these areas. Ultimately, targeted proteomics (and indeed metabolomics and lipidomics) could provide the cheapest and most robust methods to determine the course of infection – and to help doctors decide how to treat individuals.

Can you tell us a bit more about your own work?

We have found that the NCAP protein in SARS-CoV-2 is very amenable to fast digestion, and that it can be detected at 100 attomol level by UPLC-MS. This means we can determine how much viral protein is present in any individual sample. MS directly measures the viral protein, without any labeling or the need for many additional reagents.

What is interesting to me in this area at the moment is comparing the abundance of viral protein (as found by MS) to viral RNA (as detected by RT-PCR). It may be that the viral protein abundance is a better indication of infectivity, as there are lots of reports of RNA hanging around much longer than an individual is infectious. Maarten Dhaenens has been one of the pioneers in translating a method for clinical diagnosis of COVID 19 with MS.

The other role for targeted MS will be to determine the presence of mutations in the virus. This diagnostic capacity could be extremely helpful in surveillance testing, as we will need to know if the vaccine continues to provide immunity – especially as new strains emerge.

How has MS added value to the pandemic so far – and what about its future impact?

To date there are 244 published papers on PubMed that have COVID-19 and MS in the title or abstract. This is likely an under-representation of the role that MS has played in this pandemic. Increasingly, scientists are using MS to study the progression of the disease with renewed focus on understanding the effects of long COVID. I think MS will have an important role in the development of therapeutics to treat people with the virus, as well as contributing to vaccine development.

More importantly, the fact that so many scientists have been willing to work together and share knowledge has been incredible. The COVID-19 MS coalition, which I helped initiate, is a great example of this – within a few weeks we had more than 800 members. Actions like this, in the face of the threat of the virus on all of us, will lead to more collaborative science and allow us to develop public health that is less competitive.

I also hope that any new resource being purchased for coronavirus research will benefit the diagnosis and treatment of other diseases. The data being collected now all over the world will be a great future resource. The way we're accelerating rapid diagnostic tests to the point of having them validated and being used by clinicians is a real celebration.

Reference

1. *M Larsen et al., Science, eabc8378 (2020). DOI: 10.1126/science.abc8378*

"The challenge now is to translate this methodology from the R&D stage to the clinical diagnostic lab."

can be targeted in one assay. If peptide signatures for a given virus are defined, these can be relatively easily included in the target list. This way, samples can be screened for multiple viruses simultaneously. This is not only useful now, but also in the future when differentiation between different pathogens will be needed.

One challenge to overcome will be improving the analysis time: the sample preparation for proteomics assays takes a while, mainly because of the protein digestion step. This can be dramatically reduced by microwave irradiation. Furthermore, LC gradients could be much shorter than they are now: we have managed to reduce the gradients lengths threefold and could still detect most of the SARS-CoV-2 peptides. Running clinical samples using LC gradients of only up to a few minutes should be possible.

As a final note, I'd like to mention that part of our early work was published on biorXiv. Although manuscripts are not peer reviewed, they can be downloaded by the scientific community and the general public for free. For COVID-19 research, this has been a tremendous help in the dissemination of data, knowledge and protocols. Even though our manuscript has not yet been published in a scientific journal, our selection of target peptides and MS data sets have been used by others and already proven useful. I believe this is a beautiful illustration of the power of open-access scientific knowledge – a trend I hope to see continue in the future.

I've demonstrated my own work using MS to detect viral proteins, but this is just one application of this versatile technology. It is clear to me that by studying proteins, both from SARS-CoV-2 and the human host cell, proteomics has profoundly changed the way we study viral infection and disease progression at the molecular level. I am excited to see the many potential novel applications that will no doubt come to fruition in this fast moving field.

A structural and systems *biology view*

Manfred Wuhrer, Professor of Proteomics and Glycomics at Leiden University and Head of the Center for Proteomics and Metabolomics in the Netherlands, shares his view on how MS-based proteomics can contribute to COVID-19 research in the clinical lab

What's the role of MS in structural research around SARS-CoV-2?

MS largely contributes to the structure elucidation of the spike protein. Initial bottom-up proteomics studies showed that the spike protein is heavily glycosylated. This did not come as a surprise, as SARS-CoV-2 shares this feature with many other viral surface glycoproteins – it was remarkable how quickly different laboratories then performed in-depth analyses of the glycosylation of the S protein! These studies provided key insights into how glycans shape the viral surface and influence the interactions with host cell factors and the immune system.

Do you think MS could make a useful (and realistic) diagnostic tool for COVID-19?

Current COVID-19 molecular diagnostic assays focus on the detection of parts of the viral genome (PCR-test) or protein antigens ("quick" or even "self-test"). At the moment, MS does not play a role in diagnostics, but it certainly has a lot of potential in this direction. Various efforts are ongoing to establish assays for the low-resolution MS detection of viral proteins to obtain a molecular fingerprint of diagnostic value from, for example, nasal swabs or even gargle solution. These attempts build on the previous success of whole-cell MS and intact mass analysis of major microbial proteins – something that is now widely used for diagnosing bacterial and fungal infections. I am curious to see whether the MALDI-TOF-MS platforms that are widely established in medical microbiology laboratories will find their way into the diagnosis of viral infections, including SARS-CoV-2.

The bottom-up approach chosen by Jeroen Demmers likewise has good potential for translation into clinical laboratories. After transfer of these assays onto triple quadrupole LC-MS platforms, they can certainly be established in clinical chemistry laboratories, which often have the necessary hardware and increasingly also the protein expertise available.

What about the use of biomarkers in clinical diagnostics?

COVID-19 often has a huge, systemic impact on infected people, and there's a range of immunological, cell biological, and metabolic effects with biomarker potential. Due to the enormous impact of the pandemic, an array of omics technologies have been applied to COVID-19 patient materials which has led to a range of promising biomarkers. Using MS, my team has recently helped to define a specific glycosylation switch on antibodies against the viral spike protein in COVID-19 (1). This switch appears to initiate inflammation, and we are now looking at whether it can serve as an early marker predicting the development of severe COVID-19. I think a key challenge will be to integrate and scrutinize this wealth of information using a meaningful, systems biology and systems medicine approach.

Getting Lipid Nanoparticle Production Right

The rise of mRNA-based vaccines for COVID-19 has thrust lipid nanoparticles (LNPs) into the limelight. We talked to KNAUER's Matthias Luebbert to learn about the company's recent move into this exciting space...

Tell us a bit about LNPs and how they relate to current COVID-19 vaccine development…

LNPs – nanoparticles with an outer shell of lipids – have been increasingly used as a novel drug delivery system for the last few years, where their ability to encapsulate and protect a range of active ingredients, including mRNA, and deliver it to target cells in the human body is highly valuable. LNPs have been used in several of the COVID-19 vaccines that have already been rolled out or are in development, representing the first large-scale application of this platform technology.

What are some of the challenges associated with the production of LNPs?

LNPs are a relatively new technology for everyone – including the pharma companies and the companies providing the relevant systems; one clear challenge is that there is no pre-configured production equipment available on the market. And that means companies have had to not only find suppliers that are capable of designing a suitable hardware solution, but also software that is compliant with pharmaceutical production guidelines. On one hand, the industry needs time to properly implement this new technology and work through the challenges, but on the other hand we simply don't have the luxury of time – the world is waiting on these vaccines!

Why did KNAUER decide to move into this area?

Going back to spring 2020, there was significant uncertainty about the economic impact of the pandemic. We started looking for new application areas, and LNP production became part of our strategy. High pressure metering pumps and valves for liquids have been core development competencies of KNAUER for decades, and individual system engineering has always been something we offer. We also have a lot of experience around high pressure dosing and laboratory systems engineering. When a vaccine manufacturer contacted us, we were able to quickly and efficiently come to them with a proposal by building on our relevant experience; after all, many aspects of fluid systems are similar; for example, choice of piping, control systems, fluid contact materials, and so on... And I'd add that our experience in managing customer projects of a comparable size was particularly helpful.

With that first project underway and some solid lessons learned, our activity in LNP production quickly blossomed!

How do you approach each project?

As you may know, KNAUER offers a whole range of equipment – from small accessories for flexible laboratory setups to cleanroom compatible production skids – and though there are some core aspects of these systems that remain the same, each application is a little different. And so the real focus is working closely with customers to fully tailor the solution to the need. Starting from a general "sketch," we have regular phone and web meetings to discuss every single aspect of the project in detail. Finally, we propose a solution for the customer's particular application that seamlessly aligns with their plant design, control system, cleaning procedures, and space constraints.

Meet Matthias

After studying chemical engineering and gaining a PhD focused on supercritical fluid chromatography, Matthias Luebbert joined KNAUER in 2005 as a product specialist for preparative and simulated moving bed chromatography. Since then, he's held various roles with associated product and project responsibility, but currently works within the business development group.

Since early 2020, Matthias has been the project manager for lipid nanoparticle (LNP) customer projects, which includes system definition, technical documentation, and regular discussions with clients.

Who should consider your services?

Who shouldn't?! Our range of systems spans the spectrum from small laboratory set ups for academia and industrial R&D to production facilities for the mass production of vaccines. We are able to supply everyone active in LNP research and production with a system optimized for their individual application.

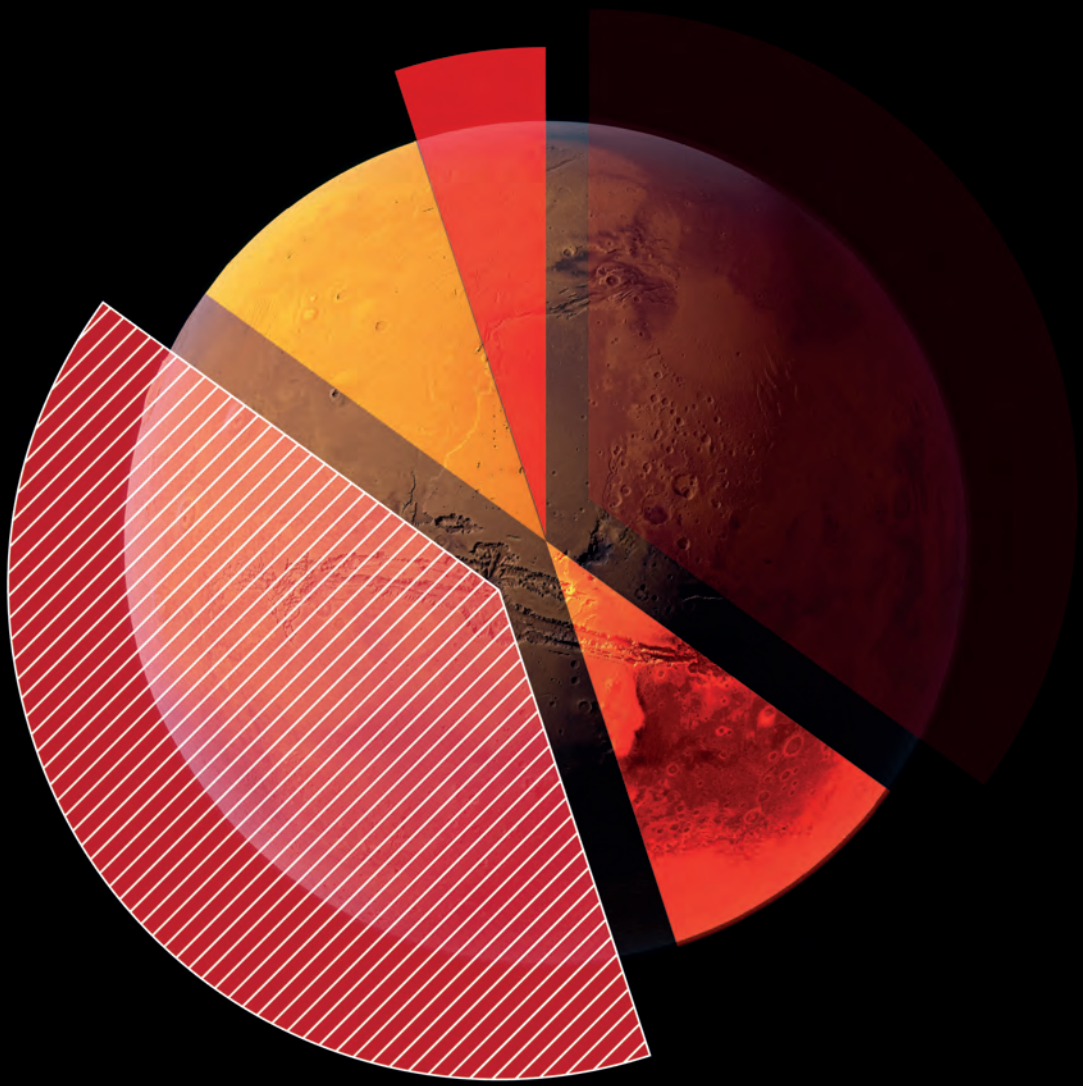
It's a high-pressure area to be involved in right now…

I'll freely admit the workload is quite high! But despite the high pressure and the hard work, contributing something very real to the pandemic response makes us all feel extremely proud. Seeing the vaccine now being rolled out – well, there really is no better reward for all the effort over the past months. To all my fellow colleagues and partners working on the pandemic response: you're doing a great job! Let us continue with the same strength and conviction moving forward.

In SEARCH of SULFATES – and ALIENS!

The story of our temperature-controlled experiments to study the formation of mixed sulfates – a key indicator of possible biological processes on the Red Planet

By Juan Manuel Madariaga and Duncan Stacey



NASA's Mars 2020 Perseverance rover landed in the Jezero Crater on Feb 18, 2021. Part of its mission: to search for signs of life and to explore the planet's geology.

The rover will characterize the planet's ancient climate and geology, paving the way for human exploration of the Red Planet. It will also be the first mission to collect and cache Martian rock and regolith (broken rock and dust). Subsequent missions, currently under consideration by NASA in cooperation with ESA, will then send spacecraft to Mars to collect these cached samples from the surface and return them to Earth for in-depth analysis.

Fast-forward one year. The ExoMars programme 2022 mission plans to deliver a European rover, Rosalind Franklin, and a Russian surface platform, Kazachok, to the surface of Mars to continue the search for life (or signs thereof) by collecting soil samples with a drill and then analyzing those samples using next-generation instruments – no need for return to Earth! This task is a very special – not only because it may provide answers to some of humankind's greatest questions, but also because ExoMars will be the first mission to combine our ability to move across the surface of Mars and to study the ground at depth.

A common question at this stage is typically: "What are you looking for in the soil?" Well, we're not expecting living organisms or burrowing green men – that's for sure. One apple of our eye is sulfates. These minerals have already been discovered on Mars and act as reservoirs of organic remains and past microbial life.

"The general scientific consensus is that geochemical activity on Mars and Earth were similar at one time."

SEARCHING FOR SULFATES

The most advanced research on Mars to date was conducted by the Curiosity Rover, which has worked tirelessly since it landed in 2012. One of the instruments on the Rover (an X-Ray diffraction instrument) has reported the presence of three calcium sulfates: gypsum, basanite and anhydrite. Coupled with other findings, such as pyroxenes, olivines, clay minerals, and hematite, but – most importantly – other sulfates like jarosite, these discoveries suggest that Mars once had a wet environment (around 3.8 billion years ago, to be precise). This is another key indication that the planet was once capable of supporting life.

Looking more closely at the elements in these minerals is the next step towards understanding how some of these calcium sulfates were formed. Simple sulfates of this kind are found on Earth (along with other mixed sulfates), but does this mean that Mars once enjoyed similar conditions to those in which life flourishes here?

The general scientific consensus is that geochemical activity on Mars and Earth were similar at one time. In fact, when Mars harbored liquid water billions of years ago, its temperature is also estimated to have been between -10 to +30–40 °C. Sound familiar? Considering both these aspects, it may come as no surprise that we expect to find further mixed sulfates on Mars that can also be found here on Earth.

These shared qualities also mean we are able to identify the places where biosignatures are most likely to be preserved on Mars, based on our searches for similar compounds on Earth. On Mars, this location is the Jezero Crater. The landing and research site for Perseverance, this crater is one of the oldest on Mars and the remnants of a lake from over 3.5 billion years ago. Given that a river once flowed into this lake, it's the perfect site for the sulfate search.

And, with new instruments (Raman spectrometers) making their way to Mars on Perseverance and Rosalind Franklin, researchers will be able to identify sulfates with more accuracy and power than previously possible. The Raman spectrometers aboard the Perseverance can analyze samples remotely (the remote sensing instrument SuperCam can analyze at distances up to 5 meters) and mere centimetres away (via the SHERLOC, aka Scanning Habitable Environments with Raman and Luminescence for Organics and chemicals, spectrometer), while the Raman laser spectrometer aboard Rosalind Franklin will analyse drilled samples at the 50-micron scale, allowing it to identify both crystalline and amorphous mineral phases. These nifty instruments will also be making their way to the Moons of Mars in upcoming missions – so keep an eye out for that.

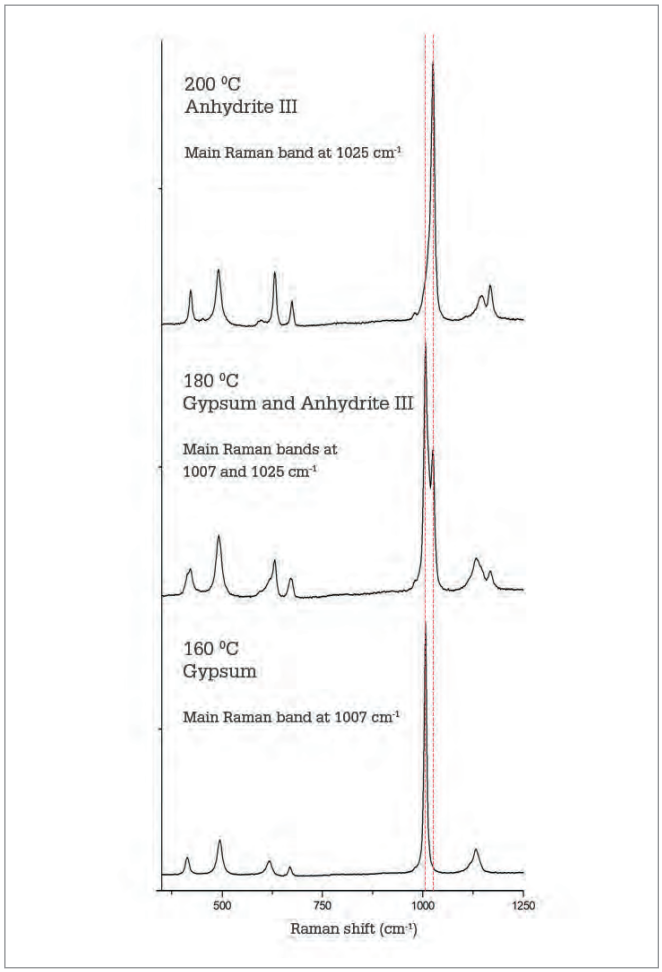


Figure 1: Raman spectra from our synthesized sulfates, acquired at different temperatures using a temperature control chamber. At 160 °C, the calcium sulfate Gypsum is still stable with its main Raman band at 1007 cm^{-1}. However, when the temperature reaches 180 °C, Gypsum starts to lose the hydration molecules; this loss is reflected in the Raman spectrum, with the appearance of the Anhydrite III band at 1025 cm^{-1}. The transformation from Gypsum to Anhydrite III finishes at 200 °C.

OUR CONTRIBUTION

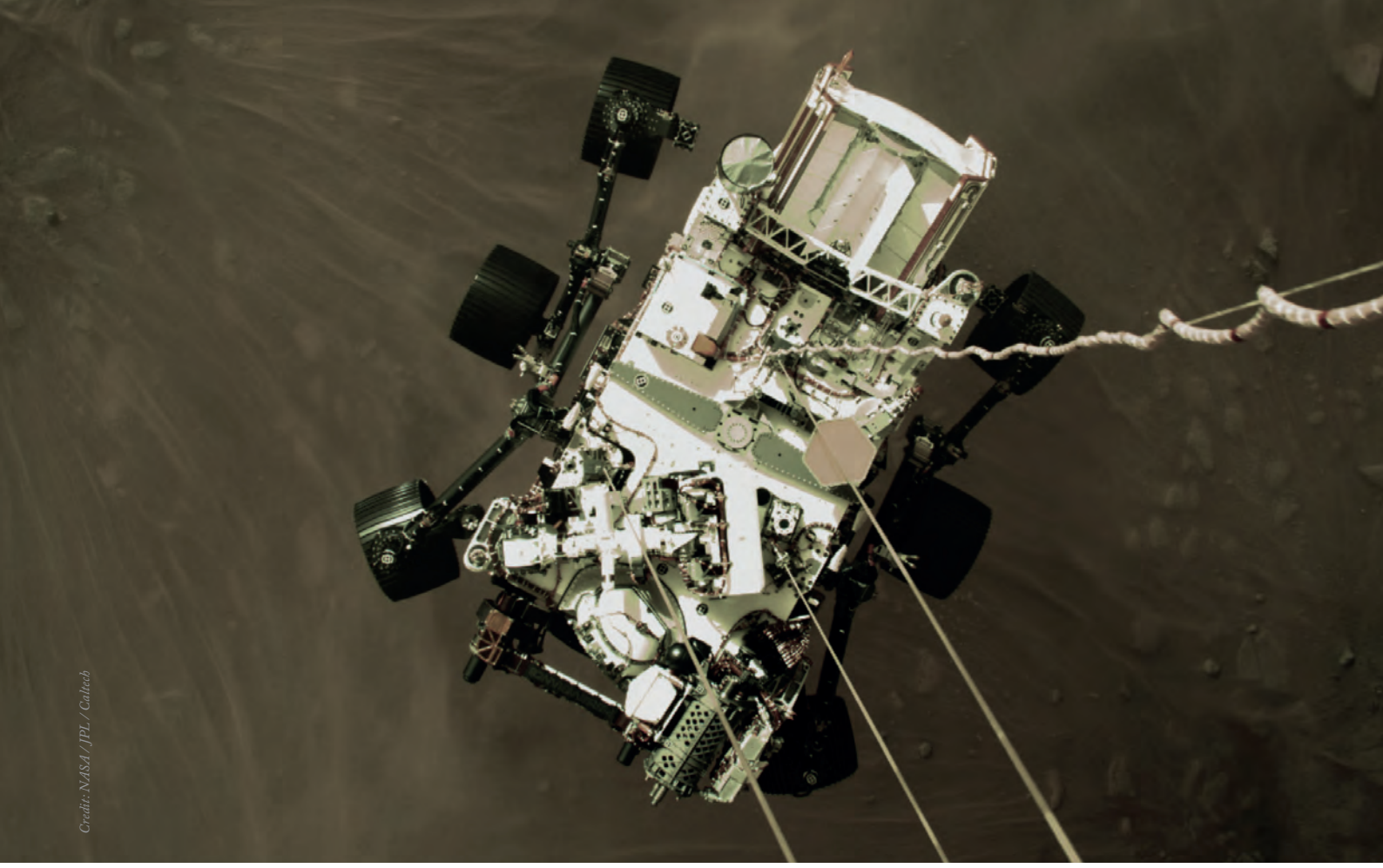
Our research, which we presented at the virtual Europlanet Science Congress (EPSC) 2020 (1), informs experiments at home (on Earth) that aim to understand the geochemical processes that lead to the formation of key minerals – including sulfates.

As part of this, we synthesize pure minerals to facilitate access to high-quality standards materials for Raman and infrared. These reference spectra can then be used to interpret

A BIT ABOUT JUAN

I have worked as a Professor of Analytical Chemistry at the University of the Basque Country in Leioa, Spain, since 1993. I'm proud to lead a research group of 34 researchers, 16 of which are permanent staff members. Together, we play a valuable role in the European Network for Research in Heritage Science. My research has focused largely on environmental issues around cultural heritage assets (archeology, artworks, and buildings) as well as extraterrestrial materials. In both of these arenas, I lend a significant focus to the development of analytical procedures and instruments to overcome new challenges.

I'm also the coordinator of the Spanish Network for Geochemical Studies of Mars, which includes five Spanish research institutions. As part of this role, I have the pleasure of contributing to research teams supporting both of the upcoming missions to Mars! At the moment, researchers in my lab are conducting experiments that simulate the processes that result in the formation of different sulfates. The goal: to identify the sulfate compounds that can be found on Mars, and then work with specialists in geophysics and geochemistry, hydrogeology, and sedimentology to propose the processes that may have given rise to them.



Credit: NASA / JPL / Caltech

"Though it may sound strange, our research on Mars mineral candidates will also be applied in our stone building research."

the unknown Raman and infrared spectra delivered by the instruments onboard Perseverance. Furthermore, these materials will also help in designing protocols to characterize the samples taken by Perseverance when they return to Earth (in approximately 2028–30).

Building on prior research where we investigated the behavior of sodium and potassium sulfates, we performed Raman analyses of three sulfates, one known to be present on Mars – gypsum [$CaSO_4 \cdot 2H_2O$] –, and two other mixed potassium-calcium sulfates – syngenite [$K_2Ca(SO_4)_2 \cdot H_2O$] and görgeyite [$K_2Ca_5(SO_4)_6 \cdot H_2O$] – using a micro-Raman spectrometer with a 532 nm excitation laser and a highly sensitive CCD detector with a mean spectral resolution of 1 cm^{-1}. The spectrometer was also coupled to a temperature-controlled stage for automatic temperature control because of the ability of these sulfate minerals to contain crystallized water; the Raman responses of compounds are sensitive to decreasing and increasing temperatures.

Overall, we tested temperatures ranging from -100 to 400 °C (using a temperature control chamber attached to the Raman spectrometer), allowing the minerals to restabilize after each 20 °C shift (see Figure 1). This range was chosen as these temperatures cover the range we might expect on Mars; the temperature on the planet itself ranges from around -100 to 20 °C, but meteorites ejected from Mars can reach up to 400 °C. As was expected, the tested temperature changes were apparent in the vibrational modes (indicative of molecular changes) in the minerals. More specifically, temperature increases caused a shift in the Raman bands from lower to higher wavenumbers, indicative of our hydrated sulfates becoming anhydrous. Conversely, dropping the temperature did not move the bands, but changes in the form of the bands was observed. This suggests that minerals were not transformed, but rather the crystallinity in the water molecules of the hydrated minerals increased. This information will help us to differentiate between sulfates on Mars – and it could help us to predict the minerals present in meteorites from Mars prior to their ejection from the surface.

A BIT ABOUT DUNCAN

DUNCAN STACEY

I gained my PhD in Optics and Spectroscopy from the University of Liverpool, UK, in 1993. I have worked with scientific instrument manufacturers for microscopy and spectroscopy ever since. Since 2014, I've acted as a Sales and Marketing Director for Linkam Scientific Instruments, where I spend much of my time in conversation with leading companies in imaging, spectroscopy, and microscopy to develop our products. I am focused on the development of new markets and research solutions for temperature and environmentally controlled experiments. I am also a fellow of the Royal Microscopical Society and a member of their corporate advisory board.

EARTHLY APPLICATIONS: FOCUS ON STONE BUILDINGS

Such Raman analysis can also be used to study other types of mineral to gain information about, for example, ancient buildings. Research into mixed sulfates at the Department of Analytical Chemistry at the University of the Basque Country focuses on cultural heritage – in particular, stone buildings (2).

Many different sulfates have been detected in buildings. Typically, these are the result of chemical reactivity between compounds in the atmosphere – sulfuric acid, for instance – and the alkaline compounds common in stone-built walls. The presence of these sulfates, along with nitrates, mean that the walls are – in essence – "washed" when it rains, leading to loss of material. The newly revealed layer beneath can then be attacked by the chemicals in the atmosphere like the previous layer, leading to eventual loss of more and more material.

The problem is clear – and it is a particular challenge in areas close to the sea or industrial ports. The impact of temperature on this process needs to be considered, too. In parts of central Europe, wall temperatures can range from -10 °C in winter to 55 °C at the height of summer, changing the hydration form of soluble sulfate salts present in the efflorescences (the salt deposits left behind by water on stone surfaces) and subefflorescences. Changes from hydrated to anhydrated minerals is also accompanied by changes in volume; for example, mirabilite ($Na_2SO_4.10H_2O$) has 5 times the volume of thenardite (Na_2SO_4). As such, these transformations also generate a physical stress in the pores of walls, promoting the formation of cracks and eventually detachments. Our studies on the Raman response of mineral transformations are contributing to a database of enormous importance when it comes analyzing the nature of efflorescences and predicting the associated risks for buildings.

WHAT COMES NEXT?

The lab is now on a mission to continue synthesizing stable mixed sulfate minerals to cover the whole scope of mineral candidates that we might expect to find on Mars. And what comes after that? Well, we will continue synthesizing and analyzing other minerals, but this time focusing on perchlorate and chlorate compounds. The hope here is that this knowledge will help us (along with geophysicists, geochemists, sedimentologists, and so on) to elucidate the inner working of Mars' chlorine cycle.

Though it may sound strange, our research on Mars mineral candidates will also be applied in our stone building research. Why? Because some of the minerals we expect to find on Mars will appear in buildings affected by extreme environmental conditions. Such findings have already been confirmed over the past few years.

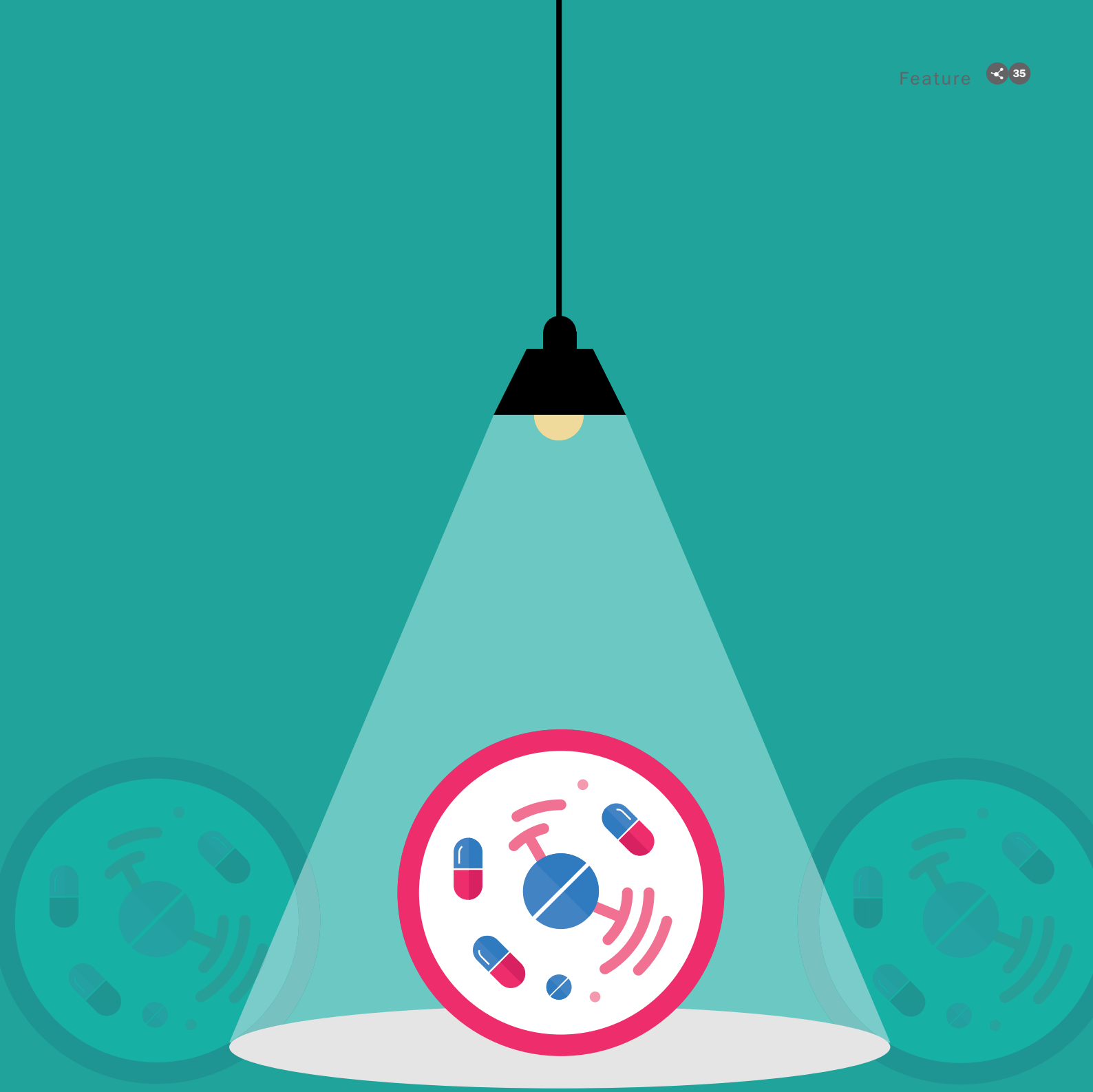
Improvements in temperature-controlled and environmentally controlled Raman spectrometers with better detection capabilities also promise a bright future for our research, including (but hopefully not limited to…) high-resolution Raman images and the simulation of extra-terrestrial atmospheric conditions, such as temperature, chemical composition, pressure, and humidity. We are now working on a custom stage that we hope will allow us to recreate the environment on Mars, providing further support to our research. Such capabilities will aid our mission to detect minor and trace mineral phases and their spatial distribution, increasing our chances of predicting where we can detect biosignatures, organic molecules, or water reservoirs. All of this information is invaluable in our hunt for knowledge about habitability – and the search for alien life.

References

1. *J.M. Madariaga et al. "Temperature transformation of calcium and potassium Martian sulfates as seen by an Exomars 2022 RLS-like Raman instrument" EPSC Abstracts 2020, 14, EPSC2020-1063. https://doi.org/10.5194/epsc2020-1063*
2. *N. Prieto-Taboada, S. Fdez-Ortiz de Vallejuelo, M. Veneranda et al. "The Raman spectra of the Na2SO4-K2SO4 system: Applicability to soluble salts studies in built heritage" J. Raman Spectrosc. 2019 50:175–183. doi.org/10.1002/jrs.5550*

Seeing the Smaller Picture

We invited Michael Kurczy and Alison Hulme to talk about imaging drugs at the subcellular level



The importance of the global pharma industry has rarely been more evident. Though much of the work happens behind closed (high security) doors, exciting advances are transforming the way we can create and study drug molecules – from inception to injection.

Admittedly inspired in part by the attractive pictures they produce, we set out to explore the application of modern analytical techniques to imaging drugs, not just in the body or tissues, but at the subcellular level. Please welcome Michael Kurczy and Alison Hulme, leading minds from the realms of MS imaging (MSI) and spectroscopy, respectively, who were kind enough to let us pick their brains on the matter! Now let's get to the interviews…

Michael Kurczy

DMPK, Research and Early Development, Cardiovascular, Renal and Metabolism, BioPharmaceuticals R&D, AstraZeneca, Gothenburg, Sweden

What's the focus of your research?

Our main focus is to design a reliable method to localize and quantify drug candidates at the subcellular level. We are developing our platform using a Cameca NanoSIMS 50L, so I feel the need to mention how unique our situation is. We work in an incredibley collaborative environment as part of the Chemical Imaging Infrastructure (CII) at AstraZeneca, which is supported by Gothenburg University and The Chalmers University of Technology. These institutes are in turn hosted by the AstraZeneca BioVentureHUB – an open innovation framework that makes infrastructure operation possible at our Gothenburg site. In addition to researchers at AstraZeneca and the CII, we have also had postdocs funded by the AstraZeneca postdoc program and an industrial PhD student working on this endeavor.

Why image drugs at the subcellular level?

Because that is where the action is! As we say in the paper, the cell may be the basic unit of life, but disease pathways are regulated at the subcellular level. The real question is thus: "how well does a measurement at the plasma or tissue level reflect the situation inside a cell?" Our approach analyzes individual cells rather – not a tissue homogenate. We knew that the spatial resolution of the NanoSIMS was adequate to image drugs at the subcellular level, but we felt driven to report absolute concentrations to give meaning to the resulting data. We spent a lot of time validating the idea that we could translate isotopic ratios into the concentrations of isotopically labeled drugs.

Is this a relatively new concept?

The idea of transforming an isotopic ratio to a concentration is certainly a new concept. It's a strange idea – and one that would only come from collaboration between a bioanalytical chemist and a geologist. People conducting biological SIMS don't typically report limits of detection (LODs), but geologists do! We wanted to understand our LOD in more depth, and – luckily – our facility manager (and lead author on the resulting paper) Aurélien Thomen has a geology background.

How did you do it?

We decided to treat biological samples as homogeneous material. This works well because, when we analyze cells with NanoSIMS, they are embedded in an epoxy that replaces the intracellular water. All we had to do then was calibrate the isotopic enrichment of 13C to the carbon density of the epoxy. This was fairly easy to calculate, and easy to test!

But we also needed a real cell sample with a with a known concentration of an isotopically labeled compound. There are not many examples of such a system. Fortunately, Andrew Ewing (Gothenburg University and Director of the CII) had characterized the dopamine concentration contained in exocytotic vesicles in rat pheochromocytoma cells (PC12) – just what we needed! It was somewhat straightforward to load these vesicles with 13C-labeled dopamine. In fact, we jokingly call this sample the Rosetta Stone, because it allowed us to confirm the translation of isotopic ratios into concentrations.

This is all very exciting! What now?

This is really the first step, but we have now shown that we can speak the language of DMPK (drug metabolism and

"This is really the first step, but we have now shown that we can speak the language of DMPK"

pharmacokinetics). Researchers can use bioanalysis to measure drug concentrations in plasma or tissue to create a profile of concentration over time. This approach is used to model how a drug moves though the body. That's where our research comes in. We anticipate that adding a subcellular component to these kinds of models will be possible in the near future. And the result? Increased knowledge and an accelerated drug discovery process.

Where do you see this approach in 20 years' time?

It's my dream that this technology become a routine and mainstream method – every pharma company could have a NanoSIMS in the lab! At that point, we hope to have a suite of standards to cover an array of labeling strategies, as well as standardized sample prep protocols and instrument parameters. But the biggest hope is that people working in pharma will be comfortable collecting and understanding NanoSIMS data and using it to make decisions that help us develop effective therapies more quickly.

Are there any obstacles we must overcome?

The CAMECA NanoSIMS is a big investment, so it the biggest obstacle is access. We were lucky enough to use a machine that had been purchased by a nearby university – maybe collaborative frameworks like this will be important for improving access. But the key, as I said before, will be making those working in pharma comfortable with the data that his approach produces. I believe that reporting an absolute concentration is a big step in that direction. As things stand, however, we're the only company with this instrument available in one of our buildings! We're privileged in this way. Best of all, it means that we have a lot of time to test and learn - and to create new methods!

Reference

1. *A Thomen et al.,"Subcellular mass spectrometry imaging and absolute quantitative analysis across organelles," ACS Nano, 14, 4316 (2020). DOI: 10.1021/acsnano.9b09804*

Alison Hulme

Professor of Synthesis and Chemical Biology, School of Chemistry, University of Edinburgh, Scotland, UK

Tell us about yourself and your research!

I trained as a synthetic organic chemist, but I was never entirely happy with just making molecules. I have always been fascinated by the biology of the molecules I make, as well as their chemistry – an interest that found its roots in my mother (a biology teacher). She would bring high school experiments home to show us, such as dogfish (very stinky!) and cheek cells under a microscope – fascinating! My group's research integrates synthetic methodology development with chemical biology to provide molecular-level insight into challenges in biology and medicine. I collaborate with academics working in the fundamental biosciences right through to hospital clinicians.

We use the standard range of analytical tools for synthetic chemistry, including LC for purification, and NMR and MS for characterization of the compounds we make. Over the past seven years, we have combined our synthetic expertise with that of Val Brunton (Professor of Cancer Therapeutics at the Cancer Research UK Edinburgh Centre) and Martin Lee (Institute of Genetics and Molecular Medicine Advanced Imaging Resource, University of Edinburgh) to develop an emerging technology, stimulated Raman scattering (SRS) microscopy, for biomedical applications.

What is the state of SRS microscopy today?

Leica have just introduced the first SRS microscopes to the market, but, not wishing to boast, these commercial instruments aren't quite able to produce the resolution or quality of image that we can produce in the laboratory – after all, we are able to control all the component parts. People are now exploring applications for the instruments, for example, in histopathology, around the world – largely in the UK, US, Germany, and Japan – but the number of these microscopes in routine use is still somewhat limited. Our aim is to encourage uptake of the instrumentation by showcasing its potential in biomedical applications.

Tell us about your work using SRS microscopy to image drugs in living cells…

Our research in this space has focused on imaging drugs that have an inherent vibrational motif in the cell silent region of the Raman vibrational spectrum, as well as developing new tags that can track drugs lacking these inherent vibrational features. In both cases, we have focused on alkyne motifs because these give spectral peaks that are approximately 100 times narrower than fluorescence peaks, and occur in a spectral region with low background noise. We image the cellular structure and the drugs by using the inherent vibrational spectra of biomolecules (such as proteins and lipids), which can be obtained without any fixing or staining. The result: live-cell studies!

Our team has acquired some of the highest resolution live-cell images of intracellular drug distribution to date. A great example is our 2020 study published in the Journal of Medicinal Chemistry (1), in which we used SRS microscopy to image the tyrosine kinase inhibitor ponatinib at biologically relevant concentrations without labeling. This study also provided insight into changes in uptake and sequestration of the drug that occur during the development of drug resistance – informing us how the drug worked initially, and the mechanisms by which its function was interrupted. These promising results have placed the field of SRS microscopy firmly in view of the pharmaceutical industry as a means to improve drug development.

How can this technique help improve drug development?

Despite the identification of an unprecedented number of potential new drug targets over the past two decades, and accompanying investment in the generation of new chemical entities with improved potency and selectivity, only 10 percent of clinical candidates progress to regulatory approval. Incorporating imaging into complex drug screening models has the potential to improve the robustness of preclinical studies of drug uptake, retention and metabolism. The insight gained from such studies could enable earlier removal of ill-fated compounds from the development cycle, leading to improved drug quality and lower financial risk.

And why use Raman over other imaging methods?

Though there are a number of imaging methods available for the analysis of drug uptake, distribution, metabolism and mechanism of action, Raman presents with a number of advantageous traits. An example: the lack of need for external labels to image cellular structure, as Raman relies on vibrational frequencies in functional groups! Raman also exhibits higher spatial resolution and weak water interference, which makes it suitable for biological applications. Other imaging methods, such as PET and MRI, suffer from low spatial resolution, and MS requires extensive sample preparation – limiting our ability to image in live samples.

There are limitations in our current capabilities too, though. For example, even with these coherent Raman microscopy

techniques, we obtain comparatively weak signals, meaning we rely on relatively high local intensities to obtain our results.

What about the future of SRS microscopy?

One area that has been being explored, but not really exploited yet, is the use of multiple spectral frequencies simultaneously. I expect that we will see more of this multiplexing in the future – as well as a greater focus on this technique (and other imaging techniques) in general. The pharma industry (and AstraZeneca and GlaxoSmithKline in particular) is already becoming increasingly involved in imaging networks that will allow them to capitalize on these emerging technologies.

I also anticipate that current challenges, such as the need to develop vibrationally intense chemical tags to amplify signals that are also metabolically stable and exert minimal impact on the drug being studied, will be overcome in innovative and ingenious ways. And a last exciting prospect is the development of approaches that incorporate multiple imaging modalities; these will allow researchers to study drug effects at multiple levels, from the whole body down to specific tissues, cells, and organelles.

Reference

1. *K. Sepp et al. "Utilizing stimulated Raman scattering microscopy to study intracellular distribution of label-free ponatinib in live cells", J. Med. Chem., 63, 2028 (2020) DOI: 10.1021/acs.jmedchem.9b01546*

Solutions

Real analytical problems
Collaborative expertise
Novel applications

From Big Pharma to New Green Horizons: Lessons Learned with Ross Burn

With just a three-day crash course in business finance under his belt, analytical chemist Ross Burn co-founded CatSci in 2010. Here, he talks about surviving and thriving as a new company focused on process chemistry, alongside more recent moves into industry 4.0 and collaborations in the contract research space.

By Ross T. Burn

Let your profession choose you

Having an interest in chemistry and being someone who enjoys solving problems meant that analytical chemistry was a great fit for my MSc. That's what I studied at Strathclyde University – along with forensics. This was back in 1998-2003 before forensic chemistry hit the mainstream with CSI! Even then, some people had this idea that they'd be helping to solve crimes or giving evidence as an expert witness in court, but the day-to-day job of a forensic scientist tends to be quite routine.

I was always more inclined towards the analytical side – I especially enjoyed separation science and investigative analysis. And that led me to study for a PhD, funded by Pfizer, based on analyzing the human proteome and trying to find new biomarkers of disease. There, I was exposed to the workings of drug development within a large pharmaceutical company and began to appreciate the role analytical science plays in bettering human health. So, while I couldn't see myself going down the forensic side, you could say pharma chose me!

A big pharma company allows you to develop into a top-class scientist

After my PhD, I was fortunate to get my first role at AstraZeneca. After having spent several years at Pfizer during my PhD and then at AstraZeneca as a process chemist, I realized that large pharmaceutical companies know how to develop top class scientists. Not only are you working with cutting edge equipment, but you're learning from experienced professionals who are brilliant at following the science towards answers to complex practical problems. Big pharma gets a lot of bad press, but my experience was that they empowered us to be the best scientists we could be. You can see why many are happy to spend their entire careers within a large pharma company, but that wasn't my path…

You need thick skin to build new relationships from scratch

In 2010, AstraZeneca decided to reduce its R&D footprint and shut down several sites in the UK. But they wanted to retain what we were doing well in the Bristol lab: catalysis screening. So, four colleagues and I decided to set up an independent lab and service AstraZeneca's portfolio until they were able to recreate the facility in Macclesfield.

Although for a period of time we were carrying on the work we were doing as AstraZeneca employees, I noticed some significant changes when trying to find new contracts. Without a big pharma name, building new relationships from scratch wasn't easy and we had to grow thick skin to handle rejection. I also


DANGER
ACETONE

took on the financial responsibilities of the company from the start, in addition to working on the science, business development, and leadership. Finding the right work/life balance was tough while we honed our entrepreneurial skills and fought to create a sustainable business.

Fortunately, the UK government at that time ran a three-day crash course in business skills that taught you the essentials as a business owner. And AstraZeneca were kind enough to allow us to take some time during our final months to educate ourselves. They did that for anyone looking to change careers as part of their winding down process.

A 40-second elevator pitch is worth more than a 100-page business plan

I realized early the importance of having a good value proposition – and that's what really got us going. My advice to anyone looking to set up a business is, don't expect it to be something like Dragon's Den! Ensure you thoroughly carry out your market research, validate that your product provides a value-add or a solution to a current problem or need, and make sure your product or service is market ready at launch. There are startup networks and accelerators available that function as sounding boards for testing your hypotheses and prepare yourself for many years of hard graft to build a sustainable business.

And as a new business you will need funding – cash is king, as they say. Traversing the entrepreneurial valley of death from startup to breakeven isn't easy. We're a bootstrap business, which means that we are owned by the co-founders and we don't have any capital partners that inject cash into our business. The upshot is that we started the business with bucket loads of sweat equity, and still to this day reinvest as much of our profits as possible to fuel our business to grow. We also take on significant risk accelerating growth by leveraging additional bank debts into the business.

We decided to focus on sales and marketing from the beginning and assemble the best team we could. The goal was to get beyond the breakeven point as quickly as possible and to plan our funding needs from there. There are a number of great schemes available in most countries (in the UK, one can take advantage of the Entrepreneur Investment Scheme) that allow companies to raise external finance quickly. Remember, there's trillions of dollars in the ecosystem waiting to help a business start and scale up – you just need the right business and team to make it happen.

"I believe we should do our best, as a company and an industry, to reduce our carbon footprint and minimize the use of finite materials."

I wouldn't recommend spending weeks writing a 100-page business plan in the early stages. Instead, make sure to refine your sales and marketing strategy so that you can clearly explain your value proposition – one that will resonate with stakeholders – in 10 slides or so. Having a 40-second elevator pitch to grab people's attention is vital.

Don't be afraid to re-evaluate when things don't go to plan

We were lucky enough to have our first client, AstraZeneca, in the bag at launch and we managed to win business quite quickly. But that turned out to be a false dawn. Many of our initial customers were intrigued to see how we were using AstraZeneca's honed methodology, which we owned, to solve catalysis problems. They wanted to see what sorts of processes AstraZeneca was using and perhaps didn't intend on giving us repeat business.

In the end, we decided to shift our business model from catalysis screening to pharmaceutical process research and development. Many of our initial projects were in other sectors such as chemicals and agro. We decided to use catalysis as a tool to solve problems in the pharma industry and go back to what we were always good at: developing chemical processes for small molecule medicines. Since we made the change, we've grown an average 50 percent year-on-year.

Consider the environmental impact early or risk trouble down the road

Another key decision we made was to focus on green chemistry. I believe we should do our best, as a company and an industry, to reduce our carbon footprint and minimize the use of finite materials. Many processes use platinum group metals that predominantly come from just two countries: South Africa and Russia. Our approach is to use iron or base metal catalysis, which are far more abundant. The environmental case is clear but there's also an economic argument. Such rare materials will continue to increase in price and you are limited in terms of building redundancy into your supply chains – something people are increasingly concerned about due to COVID-19, Brexit and global trade negotiations.

We passionately believe that companies need to be thinking

about the environmental impact of their processes early. We see a lot of emerging companies developing new chemical entities. Often, they aren't looking to commercialize the process themselves so environmental concerns aren't near the top of their priorities. But companies must be aware that, even if you're making kilos of API, the process mass intensity could be huge – especially if you have a high number of steps. We try to explain that companies need to be thinking about the process at the candidate selection stage because you don't want an inefficient process with considerable environmental impact down the road.

Fortunately, the industry as a whole is becoming more environmentally conscious. The big pharma companies all have green metrics to which they try to aspire – in turn, that's putting pressure on CMOs and CDMOs, as well as the smaller companies, to follow suit. Over the next 5 to 10 years, I believe most companies will have good green metrics in their objectives.

Never underestimate the importance of staff wellbeing

Of course, we, along with everyone else, are having to adapt to the new challenges associated with COVID-19. We were allocated key worker status and were open throughout lockdown and we never had any confirmed cases of COVID-19 within the company. We couldn't operate quite as effectively as usual but we managed to deliver on the projects we had booked. Like everyone else, we had to accelerate our digital strategy by probably 5 years, with managers and leaders working from home and interacting digitally with their colleagues. But we were already quite familiar with tools like Teams and Zoom because we have an international sales team.

The impact on the business wasn't as severe as other industries and companies. The real challenge for us was the wellbeing of staff. People react differently to an existential threat like a pandemic and many were anxious. Finding ways of making sure that everyone felt supported – with so much negativity in the press and people worried about their loved ones – was tough when working remotely.

In terms of our clients, they fell into two camps. In camp one, they knew how to proceed. They had their own milestones to hit and they decided to proceed as normal. In camp two, companies were more inclined to hold off on making decisions on repeat business until they had a better idea of when lockdown was going to end. This delayed decision making did give us some concern, but now we're back to business as usual.

Digital technologies are coming, but collaboration is also key to innovation

When we talk about the (digital) future, we're talking about CatSci 4.0. Phase 1 was the initial founding, phase 2 was the switch to process research and development, and phase 3 involved bringing in a new senior management team. Now we're planning on further embracing industry 4.0 concepts: using in-silico tools for chemistry, using bots for automated back end processes in the business, intelligent automation for parallel synthesis, and integrating artificial intelligence across the business. The idea is to free up the minds of the chemists to solve the difficult problems.

We're also seeing a trend away from the traditional contract research organization towards "innovation partners." Much of the innovation in the industry is coming from the smaller, emerging pharma companies and the supplier "CROs," with pharma companies outsourcing more and more – potentially to the extent that a candidate may be outsourced all the way through to the clinic. But many companies are thinking too bilaterally with their collaborations. For example, they might have an outsourcing manager that has conversations with parts of the value chain, but the niche CROs and CDMOs never work together. We believe we can disrupt that thinking and work more collaboratively. We're now forming partnerships with other niche organizations in the UK and overseas to bring new innovation to the industry. In fact, we recently announced new partnerships with three UK companies across the pharmaceutical supply chain – M2M Pharmaceuticals, New Path Molecular and Upperton Pharma Solutions. This collaboration will ensure that customers can access specialised capabilities and expertise throughout the journey from molecule to medicine.

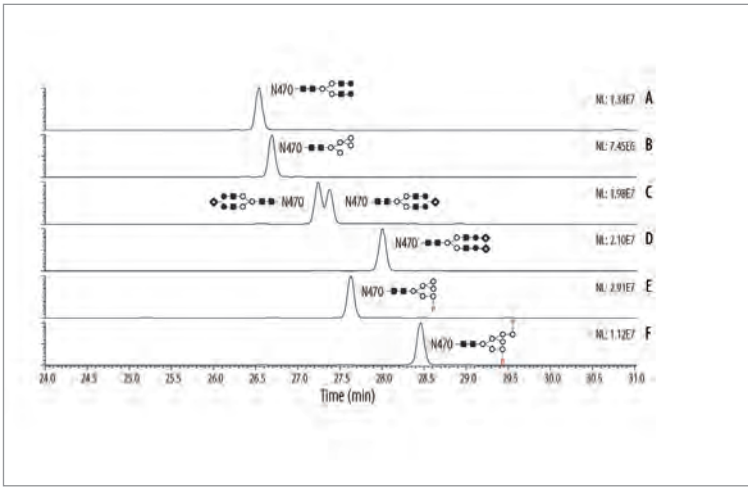
"Finding ways of making sure that everyone felt supported – with so much negativity in the press and people worried about their loved ones – was tough when working remotely."

Ross T. Burn is CEO at CatSci Ltd

Glycosylation analysis of therapeutic enzymes using µPAC™ capLC-MS

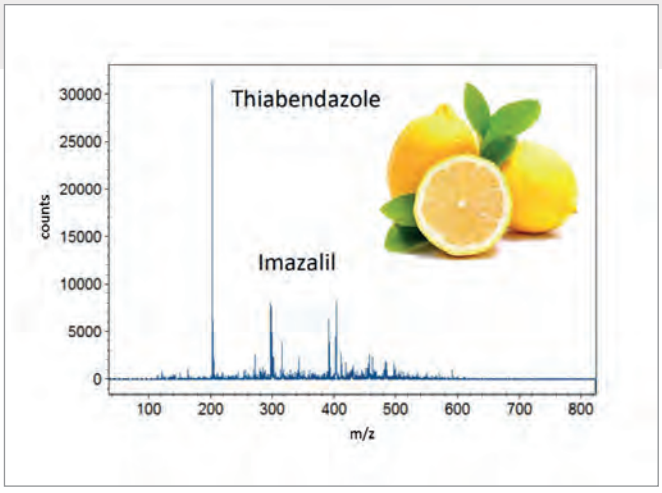
With the perfect order of its micromachined pillar structure, the µPAC™ capLC column is well suited for the separation of highly complex mixtures, which is needed in peptide mapping of glycosylated therapeutic enzymes. These tryptic digests result in many peptides with different glycan structures. The high resolution separation of the µPAC™ capLC column at flow rates ranging from 1 to 15 µL/min compromises sensitivity and robustness.

It allows the separation of glycosylated peptides in a complex mixture providing a wealth of information resulting in the elucidation of the glycosylation structure of the therapeutic enzyme. Figure 1 shows the XIC of a peptide with different glycosylation structures on a specific site of the protein (N470). Glycopeptides elute in the following order: neutral < mono-sialylated < mono-phosphorylated < di-sialylated < di-phosphorylated. Trace C shows the isomeric separation of a mono-sialylated bi-antennary glycopeptide.



Learn more about µPAC™ capLC-MS at www.pharmafluidics.com

Detection of Residual Pesticides on Fruits and Vegetables Using PortabilityTM Miniature Mass Spectrometer



Mass spectrometry can now be deployed for on-site pesticide screening in real time

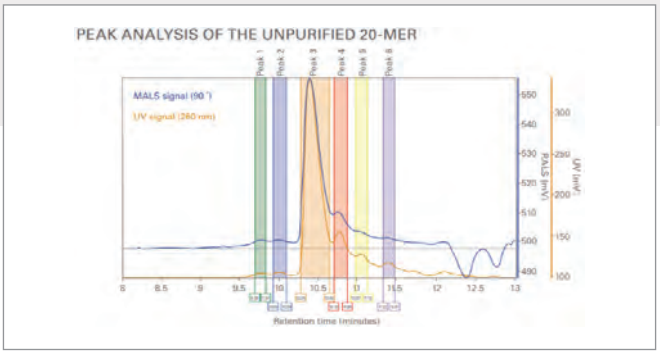
A case study for residual pesticide screening on fruits and vegetables is reported. All produce was purchased from a local market in San Jose, California and immediately analyzed by TD-ESI coupled to the PortabilityTM mass spectrometer without any sample preparation. The portable analyzer was able to detect ppm levels of pesticides such as thiabendazole, imazalil, flutolanil, and permethrin. Featuring light weight and compact size, BaySpec's novel mass analyzers based on linear iontrap technology are the most sensitive portable devices available on the market with parts-per-trillion detection sensitivity. These extremely compact instruments are simple to operate and maintain, and they are ideal for a variety of bulk or trace on-site detection in real time. Learn how you can bring the lab to the sample with portable analytical tools from BaySpec by reading our educational application note for pesticide screening of produce.

Read the full app note at http://tas.txp.to/1018/ANBaySpec

A perfect match for oligonucleotide analysis: size exclusion UHPLC and MALS detection

Oligonucleotide-based therapeutics such as antisense oligonucleotides and siRNAs are already being used as therapies for previously untreatable diseases and further molecules are being developed. The characterization of the product and its impurities is a challenge as both are structurally similar, while their effectiveness differs. Detection and characterization of oligonucleotides and impurities is simplified by combining the high resolution TSKgel® UP-SW2000 size exclusion column with the highly sensitive LenS3 multi-angle light scattering detector (MALS).

An unpurified oligonucleotide with a length of 20 bases was injected onto a UHPLC-SEC column (TSKgel UP-SW2000) resulting in a profile containing the product, shortmers as well as components larger than the product. Subsequent MALS detection with LenS3 determined the molecular weight of product and impurities for precise sample characterization.



Peak	Retention time	% RSD	MW (Da)	% RSD
1	9.774	0.1%	13,599	2.1%
2	10.012	0.0%	11,550	1.9%
3	10.398	0.1%	6,398	0.7%
4	10.776	0.1%	5,751	1.5%
5	11.053	0.1%	5,177	2.3%
6	11.422	0.2%	4,446	5.5%

Read the full application note here: http://bit.ly/Oligo-Analysis

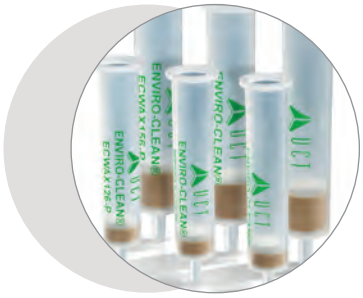
Spotlight on... Technology

µPAC™ capLC Column

PharmaFluidics µPAC™ capLC column is developed for high sensitive microflow LC-MS analysis. Taking advantage of its micromachined pillar structure, µPAC™ capLC columns offer high reproducibility, robustness and compatibility with LC instrumentation. The microflow rate enhances sensitivity with the possibility of high throughput.
For more information visit www.pharmafluidics.com

Bruker's LUMOS II- fully automated, stand-alone FTIR imaging microscope

The LUMOS II is a stand-alone FT-IR microscope that excels in failure analysis, material research and particle analysis. It is compact, precise and features ultrafast chemical imaging by FPA technology. The LUMOS II takes FTIR microscopy to the next level by improving the speed, ease of use, accuracy and reliability. Beginners get perfect results in no time, while experts are afforded almost unlimited possibilities.
Learn more about LUMOS ll: www.bruker.com/lumos



UCT's ENVIRO-CLEAN® WAX PFAS Extraction Cartridges

Your Go-To PFAS Clean-up Solution for EPA Method 533. These cartridges feature a weak anion exchange polymeric sorbent bed with maximum exchange capacity providing robust recoveries and overall reproducibility for diverse PFAS monitoring lists.
Contact info@unitedchem.com for more information or to request samples today!

ParticleScout Enhanced for 2021 – Raman-based particle analysis tool

WITec's ParticleScout now offers even greater speed, precision and user-friendliness. New features include acquisition time optimization, smart zoom, multiple sample area targeting, illumination options, smart separation of adjacent particles, wedge sectioning of round homogeneous samples and report formatting templates for data presentation.
www.witec.de/particlescout/

Turn Your GCxGC-TOFMS Data into Chemistry with LECO ChromaTOF® Tile

Revolutionize the analysis of GCxGC data with LECO's new ChromaTOF® Tile. By using tile-based Fisher ratios, statistically significant differences between sets of GCxGC data files rise to the top. Days and weeks of data sorting can be reduced to just minutes, so you can spend less time finding the differences and more time finding out what they mean.
https://eu.leco.com/product/chromatof-tile

Biologics Explorer

Sitting Down With… Davy Guillarme, Senior Lecturer and Research Associate, School of Pharmaceutical Sciences, University of Geneva, Switzerland

What's the focus of your research?

My main area of research has always been analytical chemistry, and in particular HPLC – a technique that has evolved rapidly since the turn of the millennium. UHPLC systems, core-shell column technology, and MS hyphenation are great examples of this evolution. Today, research in analytical chemistry is more often driven by applications than techniques, which is unfortunate; fundamental instrument research is important to make significant progress. I used to mix fundamental and applied aspects of HPLC, but now I apply HPLC (often coupled with MS) to the characterization of biopharmaceutical products, such as monoclonal antibodies, fusion proteins, and antibody-drug conjugates. More specifically, I focus on the development of innovative analytical strategies to improve speed, selectivity, and sensitivity.

What role does analytical chemistry play in the (bio)pharma industry?

In fact, analytical chemistry plays a critical role in almost every aspect of the drug development process, from discovery to development and commercialization, by providing assurances regarding medicine quality, safety, and efficacy. Constant improvements in analytical methods (for example, through improved selectivity and enhanced sensitivity to detect levels of impurities as low as 0.01 percent) are key to that mission. And that's why there is also a constant drive to develop new analytical tools for the rapid and accurate assessment of the safety of protein-based products. Ultimately, analytical science exists to protect patients.

What are the greatest challenges facing your field of research right now?

Limited selectivity and insufficient separation between biopharmaceutical isoforms – which can have differing toxicity profiles – (especially with HPLC-MS) is the greatest challenge we face. We can improve the characterization of complex drug products by increasing the number of dimensions in our analytical setup. Multidimensional LC and the addition of IMS before MS are fantastic ways through which we can achieve this; however, each is associated with shortcomings – the former can be difficult to use and the latter suffers from limited resolution. IM-MS instruments with increased resolution for reasonable costs could be transformative for the field!

What breakthroughs are you particularly proud of?

Working with colleagues from Genentech (Cinzia Stella and Julien Camperi) over the past two years, we have developed an automated, multidimensional LC approach capable of separating charge variants in ion-exchange chromatography for subsequent chemical reduction, trypsin digestion, peptide separation, and detection by Orbitrap MS. Our approach, involving four chromatographic dimensions and MS, allows us to rapidly identify and localize chemical modifications on proteins in biopharma and beyond.

Outside of our own research, I'm also very fond of the work of Therese Wohlschlager and Christian Huber at the University of Salzburg, Austria. They have developed a powerful analytical approach based on the combination of enzymatic digestion and high-resolution MS to discern the numerous glycoforms of fusion proteins. Fusion proteins are very complex due to their glycosylation profiles; the detailed characterization of these glycoforms is essential for ensuring product safety and efficacy.

You have a lot of different responsibilities! How do you juggle these with lab life – and family life?

It is true that my work at University is quite diverse, but I love that! No two days are the same. My agenda is a mix of teaching activities for pharmacy students, academic research, project management with industrial partners, consultancy for external companies, training courses for industry, and handling manuscripts submitted to the Journal of Chromatography B (I'm an Associate Editor!).

Of course, this can be tricky to squeeze in alongside spending time with my wife and kids, but I do my best. I have two daughters (ages 15 and 11) and one son (14), and we like to do all sorts of activities together: ski, bowling, pool, and travel. In a normal year, we like to take the kids to a foreign country at least once – but our holidays are on hold for the time being.

You've also won numerous awards…

I have indeed won several awards, including a spot on The Analytical Scientist's Power List a number of times over the years! I am particularly proud of being the recipient of the Jubilee medal from the UK Chromatographic Society in 2018, though. Previous great recipients of this award include Ian Wilson, Mary Wirth, Michael Lämmerhofer, Fabrice Gritti, and Gert Desmet; it's an honor to be put on a pedestal alongside them.

Who has been your most influential mentor? What made them so special?

I had two mentors in my career. The first is Sabine Heinisch, who taught me lots about core chromatography concepts during my PhD. She is both innovative and passionate in her work – that passion may have rubbed off on me a bit! My second mentor was then Jean-Luc Veuthey – an experienced and trusted adviser, who maintains a steady view of future trends in the field. He also has connections across industries and with many chromatography instrument providers, which is particularly valuable in our lab!

avantor™

Find out more at **vwr.com/chromatography**

Automated UHPLC method development solutions with novel stationary phases

Solve HPLC method development challenges systematically and efficiently – using Avantor® ACE method development kits – providing chromatographers with more choices for alternative selectivity, without compromising stability or robustness. Combined with ChromswordAuto 5 Hitachi Edition and VWR Hitachi ChomasterUltra Rs, maximise productivity in your method development processes.

VISIT OUR VWR CHROMATOGRAPHY SOLUTIONS WEBSITE

- Product pages, application areas and product links
- Knowledge Centre: Videos, white papers, technical papers
- Application library: Search for thousands of applications

vwr.com/chromatography
vwr.com/ace

02/2021-BB-KS
ZPROVWRI24887-EN