MAY 2016 # 40

the Analytical Scientist

Is Analytical Science Sexist?

Six (female) chemists tackle equality, glass ceilings – and the prospect of an all-women Power List.

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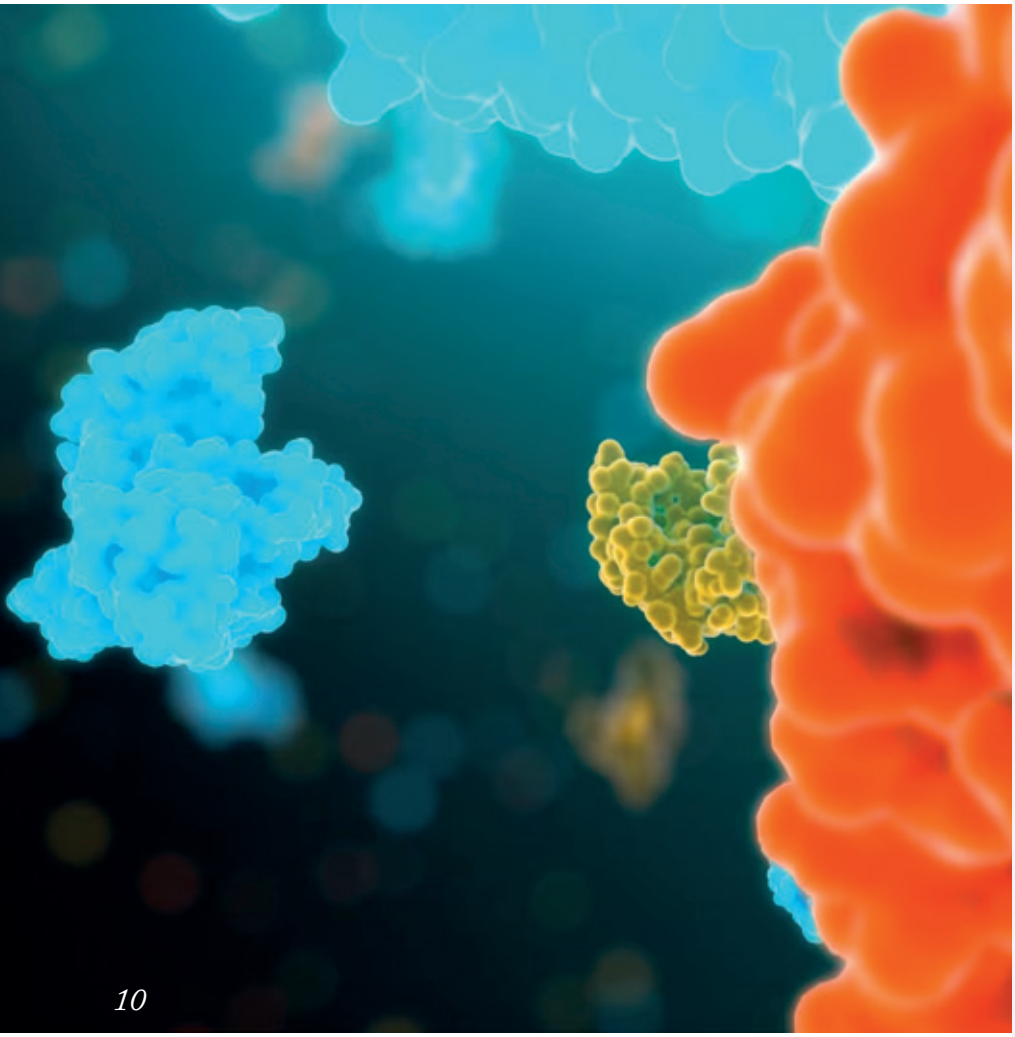

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On The Cover

Women get vocal about (in)equality. Pastiche of Franz Ferdinand's album "You Could Have It So Much Better" (itself a pastiche of Rodchenko's iconic constructivist poster).

the Analytical Scientist

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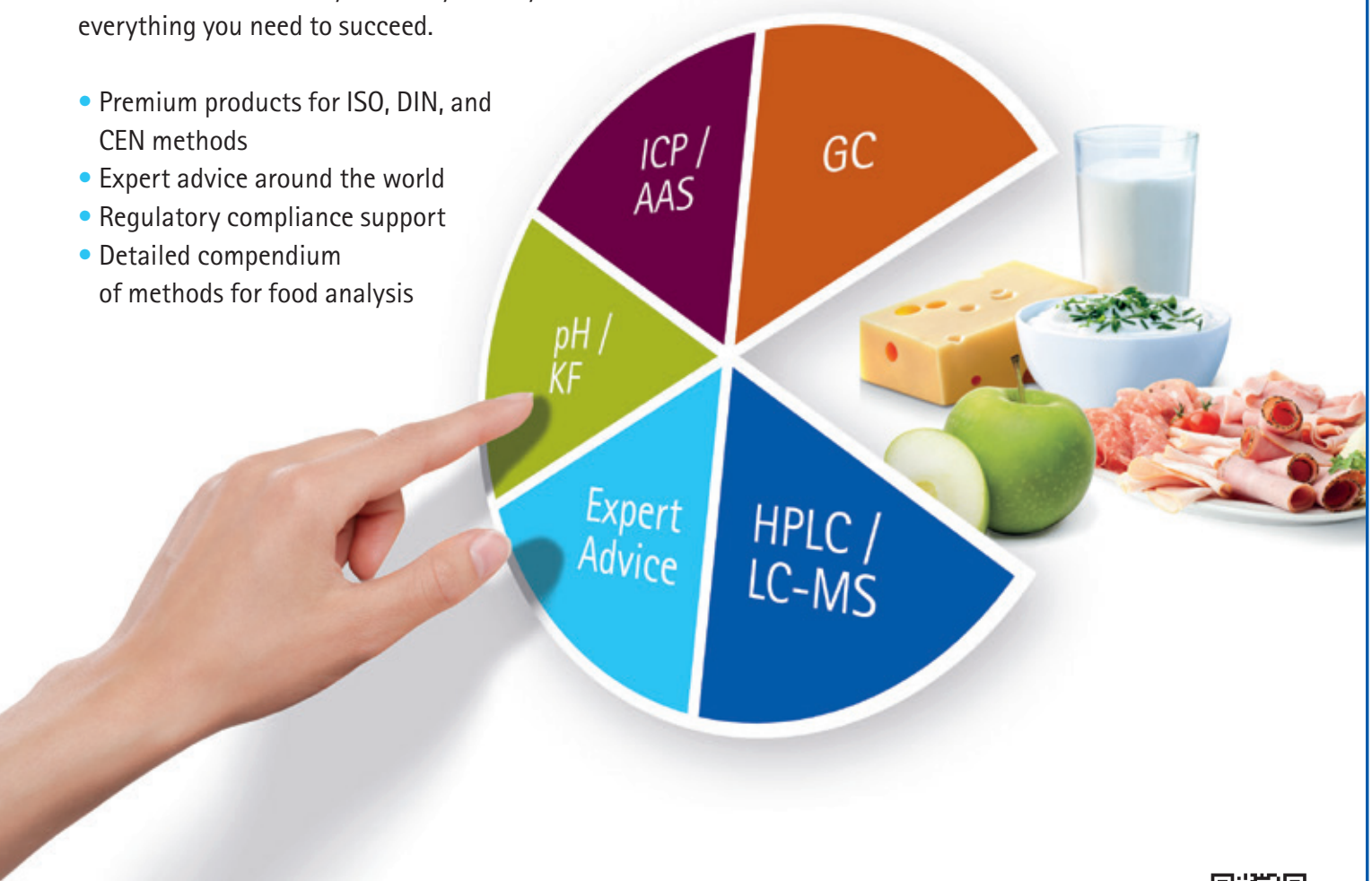

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The Dogmatic Scientist

A vitriolic attack on astrophysicist Neil deGrasse Tyson sparks controversy – and acts as a stark reminder of the need to love science but refute 'scientism.'

Neil deGrasse Tyson is, supposedly, an educator and a populariser of science; it's his job to excite people about the mysteries of the universe, communicate information, and correct popular misconceptions. This is a noble, arduous, and thankless job, which might be why he doesn't do it. What he actually does is make the universe boring, tell people things that they already know, and dispel misconceptions that nobody actually holds."

So writes Sam Kriss in an opinion piece published by Wired (1). Harsh words – but it gets quite a bit worse. In fact, the 'click-bait' headline is worse: "Neil deGrasse Tyson Is a Black Hole, Sucking the Fun Out of the Universe."

Unsurprisingly, below the article is a growing list of comments – 280 on the last count (April 25). There, you can find two running battles. On the one hand, there is a fight between scientists and non-scientists; this group appears to have missed the deeper thread of the article, perhaps thrown off by the occasionally juvenile prose. On the other hand, those who have considered the bigger picture struggle over the desire (or need) to educate the general public about the beauty of science (and the scientific method) and the dangers of pedantry, bigotry or dogmatic "scientism" – a worldview that gives science godlike status. I watched as the arguments ricocheted like shrapnel into sub-squabbles that inevitably descended into religion, politics, and even racism.

I clicked away from the page mindful of the true heart of science (and its methods): to ask questions while striving to find new or better answers. But that's not always as easy as it sounds. As analytical scientists, are you immune to dogma? On page 50, Chris Pohl warns against "taking as gospel what people think about a particular system."

But scientists should know better – what about the general public? Albert Heck (page 10) notes the importance of pitching science at the right level for a wider audience – in his case a video promoting proteomics. "It was important for us not to make it too complex – but also not to oversell it," he notes. The latter point is an important rule that is often forgotten in attempts to make discoveries stand out from the crowd.

I hope that the majority of analytical scientists, by definition, have less trouble in separating, with excellent resolution, science from scientism. If you know otherwise, I'd love to hear from you.

Rich Whitworth
Editor

Reference

1. *www.wired.com/2016/04/neil-degrasse-tyson-black-hole-sucking-fun-universe/*

Upfront

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

We welcome information on interesting collaborations or research that has really caught your eye, in a good or bad way. Email:
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Proteomics to the People

A beautiful video 'sells' the fascinating world of large-scale proteome studies

For Albert Heck, public outreach is important – especially when it comes to proteomics. In collaboration with the Netherlands Proteomics Center, he produced a video with key information on the subject – something he feels the public need to be aware of. "People know what their genes and genomes are, and are starting to think about what's possible in genome sequencing and screening," he says. "Proteomics people and biochemists know that proteins represent what actually really matters, but society doesn't realize that yet." He believes part of his job is to "reach out" to society and share this vital information. "They need to know that it's the world of proteins that causes our phenotypes, causes our diseases, and affects our health."

The team wanted to create something that would attract people's attention, but that would also hit the right technical note. "We said it should be relatively simple but it should also be beautiful," says Heck. "It was important for us not to make it too complex, but also not to oversell it. Plus, we wanted to keep it short – and to make sure it added to what's already out there."

Communicating the more complex information was a challenge: "We wanted to get to the nitty gritty of how you sequence proteins and how mass spectrometry works, which was what we had the longest discussions about. We didn't want it to be too simplistic for experts or veer too far from the truth."

The team worked with the video designers (sensu.org) and Utrecht University's communications department to devise a clear strategy for 'selling' proteomics – an approach rarely heard of in academic spheres. "We made sure we launched it on a set day and that it would be shared not only on YouTube, but via Twitter and Facebook," Heck says. But according to Heck, the strategy was more about sharing information with the public than about 'marketing' their own research. "As an academic research group you need to make every Euro you don't put into your research or teaching count – so we had to aim to get it to society at large. I also insisted that it should be open-access in the hope that it would be used by the whole community. Until the last 20 seconds, we don't even mention that it was made by us."

The video is already garnering attention both from within and outside the scientific community. "We have had close to 5,000 views already," Heck says. "Considering the topic, I'm really happy with the exposure we've had so far – if you look at other technical videos on YouTube, they've had 50 or so hits after a year, and that was what I was afraid of. I just hope that it gets the attention that it deserves."

The icing on the cake, Heck feels, would be scientists using the video to teach the subject – and some are keen to do so. "Some of my MS colleagues have reacted well, saying they can use the video to explain what they do. That makes me really proud – and really glad we did it."

As yet, there are no firm plans to make any more videos, but Heck says the exposure and appreciation has given the team an 'appetite'. "I think this is the future. We have to explain our science in modern parlance," he says. "It depends how successful we are with this one. We are all paid by taxpayers, so we should also give something back. On the other hand, if we bring something out, we want it to be of the highest quality, and that means it takes effort – I won't be making a video every month!" *JC*

For more information and to watch the video, visit: www.uu.nl/en/news/the-fascinating-world-of-proteomics

Protecting Beauty from the Beasts

Using an optoelectronic nose to 'sniff out' the pollutants attacking Disney artwork

Many of Disney's characters are a little long in the tooth, but the artwork shouldn't look that way. Chemists from Illinois University have been working in collaboration with the Getty Conservation Institute and the Walt Disney Animation Research Library to devise a means of protecting museum artworks from damage and discoloration. The result? Cost-effective optical sensors that are capable of monitoring low-level contaminants with sensitivity 500 times that of current sensors used by conservators.

Lead researcher Kenneth Suslick said there has been an entrepreneurial aspect to the team's research for many years, but that they built on their previous research (1) to create sensor arrays that could be used to monitor original Disney artworks on a journey to China. The sensor arrays were attached to the outside of each piece, as well as inside each sealed frame. In addition to external pollutants, the team were surprised to discover that the artworks were also being exposed to low-level sulfide contamination from within – specifically, from the acrylic window inside the container – a good demonstration of the power of such sensor arrays.

"There are many dyes that change color depending on their chemical environment," says Suslick. "And if you put these into an array, the combination of color changes turns out to be a molecular fingerprint that is unique to any odorant or any mixture of odorants. In essence, it's a digital, multi-dimensional extension of litmus paper." The device uses RGB imaging of the colorimetric sensor array before and during exposure to analytes, before generating a difference map (red minus red, green minus green, blue minus blue). Each difference map vector is compared with a pre-existing library, using standard chemometric techniques. For semi-quantitative analysis, difference vectors are collected over a range of analyte concentrations; the accuracy of quantification can be within 20 percent and will improve as the library becomes more extensive, according to Suslick.

Next step – commercialization. iSense, a company located in Silicon Valley and co-founded by Suslick, will drive the sensor technology into several application areas, including the biomedical field. *JC*

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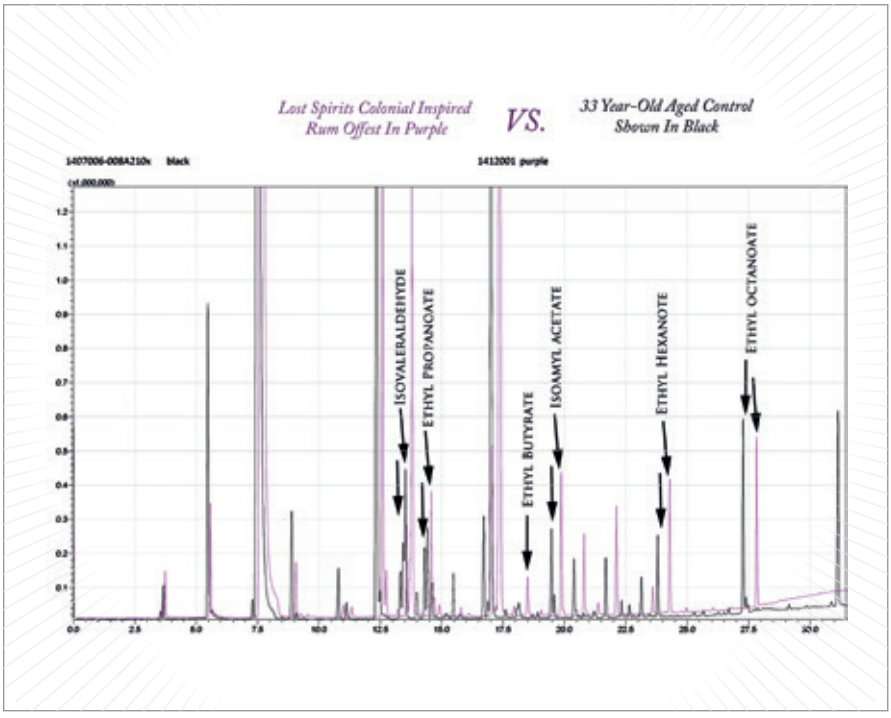
Shaken and Stirred

GC-MS helps to create aged spirits… in only six days

The aged spirits market is a lucrative one, attracting reverence and loyalty from liquor enthusiasts in equal measure. The flavor profile of distilled spirits is enhanced by the length and method of storing – and the price tag tends to correlate with the length of time in the barrel. But Bryan Davis may have found a shortcut. Davis, founder of the Lost Spirits Distillery and described as "one of the super-stars of Mad Scientist Distillation" (1), has developed a reactor that mimics the aging effect – cutting the processing time by up to 20 years.

The system can build spirits with the same chemical signature seen in classical aging – but with certain added advantages. "With proper tuning, it can do this without the hindrance of excess ethyl acetate build up, a 'solventy' aroma flaw sometimes caused as a consequence of age," Davis says. "It can also build a slightly denser concentration of long-chained esters, responsible for the distinct finish or aftertaste found in the most prized spirits." To gain a better understanding of the process, mass spectrometry was used to produce a chemical fingerprint of the volatile range compounds in aged rums.

Davis was responsible for designing the reactor, but the analytical work was done by a local environmental lab. "We used direct injection mass-spec on the volatile side, with the semi-volatile organics done using GC-MS," he says. According to Davis, the chromatograms became a very useful tool at the last stage of the process for fine tuning the results: "They provided empirical evidence of the system's efficacy in the volatile organic compound (VOC) range, but also its capability not only in building the density of semi-volatiles, but in producing a nearly identical fingerprint to 20 years in the cask (2)."



An offset chromatogram overlay of the Lost Spirits Colonial Inspired Rum compared against the 33 year-old control rum. Image courtesy of Bryan Davis, Lost Spirits Distillery.

Davis, a former art teacher, says he took to chemistry "like a duck to water" despite a lack of formal education in the subject. "I never actually did high school chemistry. Our partner, a PhD chemist, did a great job of giving me a quick education in everything I needed to know about organic chemistry and booze." But when Davis needed more information, he turned to PubMed: "I remember, for example, looking up what happens to the yeast derived ester profile of the spirit when fermentation characteristics change..."

Davis' new chemistry knowledge and existing familiarity with the alcoholic beverage industry allowed him to find a niche area in a competitive market. "We had an absinthe lab facility that did well, but the market crashed. So we decided to leverage our relationship with the distributors," he says. "There are already a lot of craft facilities in the US where vodkas, gins and absinthes can be made overnight, but aged products are simply not there – or aren't very good."

The developments have stirred up strong reactions – from disbelief to horror – and left some people shaken. "People initially asked if my work should be banned, if it was 'fair', or if I was undermining God's will!" Davis says. The company has wisely avoided antagonizing certain market segments, taking a more tactical approach: "We haven't done a Scotch whisky or a bourbon, and that's no accident," he says. "Bourbon and Scotch in particular are more religions than beverages!" *JC*

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2. *Lost Spirits White Papers: www.lostspirits.net/#!science/nj82e*

LC-MS in the US

Are you hitting San Francisco? Or taking a San Antonio stroll? Here are our top picks for ASMS and HPLC 2016

June is a big month in the analytical science calendar, with both the ASMS and HPLC conferences showcasing hundreds of renowned speakers and attracting visitors in their thousands. Whichever you're attending, you're sure to find plenty of LC, MS, or LC-MS presentations to get your teeth into. Need help selecting among such stiff competition? Look no further – here are a handful we've saved to our schedules.

ASMS 2016

Sunday, June 5
Forensic Mass Spectrometry: Tell Me Something I Don't Know (Glen Jackson and Facundo Fernandez, Hall 1, Level 1, 5.00pm)
See Glen's article in The Analytical Scientist in our March issue: tas.txp.to/0516/Myths
Monday, June 6
Forensic Analysis of a Mass Poisoning in Mozambique Associated with a Homebrewed Beverage using LC-HRAM MS and DART-MS (Sara Kern, Hall 1, 4.10pm)
Tuesday, June 7
On the Application of Electrochemistry-Mass Spectrometry to Study the Biotransformation of UV Blockers in the Environment (Pedro Segura, Hall 1, 4.10pm)
Wednesday, June 8
Imaging Mass Spectrometry Identifies New Markers in Prostate Cancer Pathology (Kristina Schwamborn, Stars 4, 9.10am)
Thursday, June 9
High Quality Estimation of False Discovery Rate for Proteoform Identification with Top Down Proteomics (Richard LeDuc, Hemisfair 3, 2.50pm)

The 64th ASMS Conference will be held in the Henry B. González Convention Center, San Antonio, Texas from June 5–9, 2016. www.asms.org Twitter: #ASMS2016

HPLC 2016

Tuesday, June 21
Fast, Accurate and Unrestricted Mass Spectrum Interpretation: A New Vision of Proteomic Landscapes (Arun Devabhaktuni, Golden Gate Ballroom C, Floor B2 level, 5.25pm)
Wednesday, June 22
Characterization of Monoclonal Antibodies and Antibody-Drug Conjugates using LC-MS and 2D-LC-MS (Koen Sandra, Golden Gate Ballroom B, Floor B2 level, 1.55pm)
Thursday, June 23
Novel Multidimensional Liquid Chromatography Strategies for Top-down Proteomics (Ying Ge, Golden Gate Ballroom C, Floor B2 level, 1.55pm)
Friday, June 24
Separation Science and Analytical Chemistry: Past, Present and Future. (Barry Karger, Golden Gate Ballroom, Floor B2 level, 3.15pm)
Also recommended:
Tuesday Free Tutorial: Preparing Your Manuscript and Publishing it from an Editor's Perspective. (Jonathan Sweedler, Yerba Buena Ballroom, Salons 5/6, Floor Lower B2 level, 1.30 – 3.00pm)

The 44th HPLC conference will be held at the Marriott San Francisco Marquis, San Francisco, California from June 19–24, 2016. www.hplc2016.org Twitter: #HPLC2016 @HPLC2016

Pass (on) the Popcorn

Tracking the scent – and appeal – of bearcats

Binturongs – also known as bearcats (but related to neither) – are elusive creatures from the forests in southeast Asia with a distinctive feature: their urine smells like buttered popcorn. Five researchers – Christine Drea (a professor in the Department of Evolutionary Anthropology at Duke University), Tom Goodwin (a distinguished professor of chemistry at Hendrix College), Anneke Moresco (a postdoctoral fellow at Cincinnati Zoo and Botanical Gardens), Lydia Greene (a graduate student at Duke) and Tim Wallen (an undergraduate student at Hendrix) – combined forces to find out why a mammal that works so hard to hide manages to smell so… appealing.

The researchers took samples of urine during standard medical examinations, and subsequently identified 29 chemical compounds – including the one responsible for the popcorn scent. "We found that 2-acetyl-1-pyrroline (2AP) was a major volatile compound emanating from both male and female binturong urine," says Drea. Its correlation with the reproductive hormone, androstenedione, a precursor both of testosterone and of estrogen, led the team to conclude it may be a pheromone used to indicate that a binturong is in the vicinity. Furthermore, 2AP was among the few compounds that were resistant to decay and became more dominant over time – an advantage for solitary animals roaming through dense vegetation. "From the scent profile, other binturongs would be able to tell what sex the depositing animal is, how long ago it was there, and what reproductive state it might be in," Drea says. In other words, key information for a binturong looking for an amorous encounter.

The team used solid phase dynamic extraction (SPDE) GC-MS (Agilent Technologies 6890N and 5973N). "These techniques can detect very small concentrations of each compound – and no chemicals or solvents have to be added, making it an environmentally benign method of analysis," says Goodwin. "For most of the compounds we identified them with the NIST library. For 2-acetyl-1-pyrroline, however, we synthesized it using a literature procedure, and compared its mass spectrum and retention time to the peak from the binturong urine. The method was qualitative in the sense that we did not determine the exact concentration of each compounds. We made qualitative comparisons of the peak areas in the mass spectral data."

What isn't clear is how the binturong produces 2AP. "If you were to make this compound, you would have to use temperatures above what most animals can achieve physiologically," says Drea. After finding no trace in their food, the researchers believe the most likely explanation is that 2AP is produced when binturong urine comes into contact with bacteria and other microorganisms.

Drea's own interest was sparked when she realized that the Carnivore Preservation Trust (CPT – now called the Carolina Tiger Rescue) was near Duke University – and by the fact that female binturongs were reputed to be dominant to the males. "I'm particularly interested in understanding 'exceptional' species in which females rule the roost, so I made a trip out there to have a look," she says. "That's when I met with Anneke (who was the main veterinarian at the time) and we devised a plan to study reproductive hormones." Drea had already been collaborating with Goodwin, researching chemical communication in other species, a joint venture that allowed them to look at the links between reproductive hormones and volatile chemicals.

"They are solitary animals, but are great to work with," says Moresco. "Everything we learned from their reproductive to nutritional habits was new in a sense, because there is so little known about them. Did you know they are one of only two carnivorous mammals with a prehensile tail? That's important to take into account when trying to catch and handle them!" *JC*

Reference

1. *LK Greene et al., "Reproductive endocrine patterns and volatile urinary compounds of Arctictis binturong: discovering why bearcats smell like popcorn", Sci Nat, 103(37):1-11 (2016). DOI 10.1007/s00114-016-1361-4*

Getting Down to Business

What's going on in the analytical sciences?

Each month, in collaboration with www.mass-spec-capital.com, we'll be looking at four different areas of business – collaborations, acquisitions, products and appointments – so that you can keep abreast of what's going on in the world of analytical sciences. As a taster, we've compiled the Top Five of each – from ASMS 2015 to ASMS 2016.

You can find "Collaborations" here, and all four lists – plus more detail – online.

To give you food for thought – and to invite you to take part in the discussion – we offer a few questions: What will be the role of miniaturized mass spectrometers? Chinese company Bohui has acquired Advion; what about companies like 908 Devices and Microsaic? Will the big mass spec players enter the game with their own developments or simply buy the technology (or both)?

What about the shopping sprees of Thermo Fisher Scientific and Danaher? Will they have an impact on the world of mass spec and chromatography or will it make no difference to players like Waters and Bruker, who stick more closely to analytical roots?

Finally, can young entrepreneurs rise to the top of fast-growing start-ups or established players? Or is senior level management reserved for those with decades-long experience?

Collaborations

1. Agilent & Waters (OpenLAB/Empower)
2. OmicScouts & Pelago Bioscience (Cellular Thermal Shift Assay/MS-based proteomic test)
3. Thermo Fisher Scientific & Gladstone Institutes, Thermo Fisher Scientific Proteomics Facility (mass spectrometry/drug target identification)
4. Sciex & Metabolon (Lipidyzer Platform/lipidomics)
5. Agilent Technologies & Thermo Fisher Scientific (OpenLAB/Chromeleon)

For Top Five lists of acquisitions/ investments, products, and appointments from ASMS 2015 to ASMS 2016 (plus more details and links to press releases) visit: tas.txp.to/0516/BUSINESS

In My View

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

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

Contact the editors at edit@texerepublishing.com

Garbage In, Garbage Out

High-quality, automated sample preparation can help you take out the trash.

By Guenter Boehm, Vice President Applications and Customer Communications, CTC Analytics AG, Zwingen, Switzerland.

It's all too easy to get excited by the launch of new gadgets and gizmos. Certainly, most chromatographers hanker after the latest cutting-edge hyphenated chromatography system that promises increased functionality, speed and sensitivity. But there is a time-tested adage: 'garbage in, garbage out' – and it's probably more pertinent today than ever before, especially as the boundaries of performance are continually pushed. The bottom line? You can have the best chromatography system in the world, but if you inject poor quality and/or inconsistent samples, your results will be trash.

It is widely recognized that sample processing and preparation creates a bottleneck in lab workflows. It affects labs all over the world and accounts for around two thirds of the overall time spent on chromatographic analysis. Indeed, a 2015 survey established that 60 percent of chromatography users (in a range of industry sectors) believe the biggest challenge in sample prep is the time and labor intensity of the procedures required (1). The number of steps involved before a sample is ready to analyze can be extensive; just over half of those questioned regularly undertake three or more prep techniques per sample, and around 5 percent use seven or more.

But resources are not the only problem. The multifactorial nature of sample preparation means that it harbors potential for error at any one of its multiple steps. From poor sample storage to the addition of impure solvents and inconsistent dilution techniques, it's no wonder sample collection, preparation and processing is by far the largest source of error in analytical laboratories (2).

Finally, as detectors get more and more sensitive, the susceptibility to sample quality and variability increases significantly. It stands to reason that any interventions that minimize error during preparation and ensure as consistent a sample as possible will have a positive impact on data quality. So, what can be done?

To reform sample quality, we must challenge the traditional view that pre-analytics are a series of separate processes. Despite the fact that many instrumental chromatographic techniques have matured (and automation of some kind is now relatively commonplace), I believe there is much more that can be done to address the remaining sample prep logjam. To date, automation has tended to take two separate paths; namely, robotic sample preparation or automated sample injection. Whilst there can be advantages to automating these two processes in isolation, the entire sample preparation workflow still includes numerous manual steps, most notably the need to transfer samples from one system to another. This 'loophole' in the system consumes analyst time and leaves the door wide open for potential errors to creep in.

With the emergence of fully integrated automated systems that combine sample preparation (for example, standard addition, liquid/liquid extraction, SPE) and injection in a single platform, the intermediate error-prone, time-consuming manual steps can be eliminated, improving accuracy and repeatability. Moreover, because such

systems can run 24/7 without human intervention, such 'smart' automated systems can facilitate high sample throughput, allowing scientists to focus on the skilled analysis and interpretation of the results rather than on time-consuming wet chemistry or sample injection.

For example, we recently worked on a metabolomics study of algae cell cultures using a PAL RTC platform, which involved a pretty complex pre-analytical process (3). We combined Bligh and Dyer (LLE) extraction with dual-column UHPLC-MS/MS separation and detection in an automated setup (which included adding the set reagents and splitting the aqueous and organic fractions prior to injection into the UHPLC system). Even with an initial manual step, the automated method proved significantly less labor-intensive than the manual technique and also allowed the results to be directly subjected to a library search using LC-MS/MS data in SWATH mode (SCIEX), meaning that further targeted experiments to identify unknowns were unnecessary. Moreover, when compared with the manual method, the automated setup had better repeatability for both aqueous and organic fractions.

Analytical scientists, regardless of industry or sector, are all aiming towards a common goal: achieving the best quality results as efficiently as possible. Taking a smart approach to automating the sample preparation process cuts the 'garbage' being fed into the sample analysis process and improves data quality and speed of delivery to researchers – and thereby reduces the 'garbage' output from the lab.

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On the –Omics Journey

Profiling and fingerprinting in food analysis signposts a route to a better life.

By Chiara Cordero, Associate Professor of Food Chemistry, Department of Drug Science and Technology, University of Turin, Italy.

Modern '-omics' disciplines applied to food (foodomics, sensomics) focus analytical efforts on elucidating bioactive compounds (nutrients and non-nutrients, active secondary metabolites, pre-biotics, odorants and tastants) – essentially, all possible chemical stimuli for food-body interactions (1–3). The ultimate aim is to understand the intriguing – though rather complex – crosstalk between 'what we eat and why, and what we are'. Such investigation requires a comprehensive and integrated approach that will detail a food sample's constituents, in terms of physicochemical properties, concentration in the matrix, distribution throughout the body and, within the "-omics" concept, the physiological activity or sensory properties (odor/taste quality, perception threshold). These comprehensive analyses require multidimensional and hyphenated

analytical platforms capable of exploring all the chemical information dimensions with a detailed profiling approach (4, 5) and all the inter-connecting sample information dimensions (for example, those related to the biological phenomenon being studied) using advanced fingerprinting techniques (6, 7).

'What we eat' is one side of the coin that we need to understand in (chemical) detail so that we know the exact composition to i) differentiate high quality from mass produced products, ii) enable the authentication of a specific botanical/geographical origin or iii) assist technologists during industrial processing with a view to defining a quality benchmark. 'What we are' relates mainly to the interaction of food components within our body. It goes beyond the nutrition domain and includes the effect of non-nutrients and bioactive compounds that may promote health and wellness (nutraceuticals).

Over the last ten years, our research group has dedicated a lot of effort to exploring these concepts and implementing "simple" comprehensive two-dimensional gas chromatographic (GC×GC) platforms and further orthogonal dimensions, including mass spectrometry (MS), olfactometry, automated sample preparation and advanced data mining (8–10). The latter hyphenation, which is fundamental when exploring and interpreting complex data, was realized thanks to the collaboration with experts in other fields. Stephen Reichenbach (Computer Science & Engineering Dept., University of Nebraska–Lincoln, USA) has taken on this challenge and has developed intuitive, effective software solutions for 2D data mining.

Thanks to the superior informing power of GC×GC-MS, we have studied complex fractions of volatiles from high-quality hazelnuts, raw and roasted coffee, green and black tea, dried milk, plant extracts and essential oils. Every analytical run provides a deep insight into the chemical complexity of each sample and provides new perspectives in food chemical investigations that, just a decade ago, were inconceivable – or at least not possible without laborious and time-consuming pre-concentration steps. Looking at the food component distribution and interaction with the body, metabolomics is encouraging collaborative and fascinating studies on the effects of diet and physical exercise on Type 2 diabetes in mice models and in humans (11, 12).

The multi-multi dimensional platforms we have experimented with, thanks to the inspiration of our "boss" (Carlo Bicchi) and the creativity of new generations of scientists, have both intrigued industry and initiated further collaboration. The primary objective? To transfer new concepts in chemical measurements to 'real-world' applications. The vision? To see -omics concepts applied to routine analysis. I believe this will enable quality food chemical characterization, by-product valorization, and bioactive compounds profile activity to be controlled and monitored – all of which will have a positive effect on our lives and society. To that end, academic research must take advantage of the support made available by instrument companies to help direct research effectively through prototyping new analytical solutions for QA and QC laboratories. For example, our collaboration with Agilent and SRA Instruments (Italy) enabled us to transfer most of the "-omics" applications developed for thermal modulated GC×GC-MS platforms to the simpler and cost effective differential flow modulated systems (13–16). Those of you at Riva 2016 will have (had) the chance to see me deliver this work in person (tas.txp.to/0516/riva2016). A prototype is now ready to launch and we are all looking forward to where it will take us on our –omics journey!

References

1–15 Available only online: tas.txp.to/0516/Cordero

16. *C Cordero et al., "Potential of the reversed inject differential flow modulator for comprehensive two-dimensional gas chromatography in the quantitative profiling and fingerprinting of essential oils of different complexity", J Chromatogr A, 1417, 79–95 (2015). PMID: 26387790.*

Two Words: Food Scanners

H2020 wanted to put food authentication into the hands of the consumer. What impact will the outcome have on our field?

By Yannick Weesepoel, researcher, Authenticity and Nutrients Business Unit, RIKILT Wageningen UR, The Netherlands.

Two years ago (in June 2014) and by mere coincidence, I entered the field of vibrational spectroscopy. I was starting my first 'real' job as a young researcher at RIKILT in Wageningen. My business unit deals with food authenticity and fraud, multivariate statistics, analytical techniques and many different food types. We looked at what we were doing in the lab and reassigned the tasks, and then I found myself leading the near-infrared, mid-infrared, and Raman spectroscopy division. At that point, I hadn't fully appreciated the revolution going on in the

"It seems that vibrational spectroscopy is being driven into new areas that we may never have considered even a few years ago."

field, which was elaborated upon by Heinz Siesler (University of Duisburg-Essen, Germany) in the July 2015 issue of The Analytical Scientist (1).

To get to the point, it is clear that miniaturized equipment is being developed at a rapid pace for professional usage in all subdivisions of vibrational spectroscopy. This miniaturization, in combination with a considerable database and some advanced chemometrics (such as machine learning), has resulted in powerful easy-to-use tools for food prescreening. At the NIR 2015 conference in Brazil last October, oral and poster sessions were replete with new applications using miniaturized NIRS instruments. And we presented our first work on non-destructive, through-package authentication of chicken meat. Although our meat authentication was quite an out-of-the box idea, responses were positive. And it seems that vibrational spectroscopy is being driven into new areas that we may never have considered even a few years ago.

But what about consumer applications for food authentication? Back in July 2015, the European Commission offered a €1 million award in its H2020 research and innovation program for a "food scanner" that "analyses precisely, quickly and efficiently food composition, nutrition facts, and potentially harmful ingredients such as allergens" (2). The winner will certainly need an even more out-of-the-box approach than those I saw at NIR 2015. But if it is possible to develop a universal food scanner, it will almost certainly lead to a revolution in food traceability and fighting food fraud. Wouldn't it be convenient for food researchers to get spectral data linked to GPS coordinates? The competition is now closed, but judging will run until autumn 2016.

Surprisingly, one of the key H2020 food scanner challenges was solved early on: cheap hardware. Almost as soon as the H2020 challenge was issued, a startup company made my day by sending me a $250 NIR spectrometer. Linked to a smartphone using Bluetooth and equipped with a simple cloud interface, it offers an almost Apple-like experience. Although it will take a while to test and validate this new hardware properly, you can see that it represents an exciting new era in hardware and big data. Funnily enough, when visiting the Recent Advances in Food Analysis conference in Prague back in November 2015, surprisingly few posters or talks were dedicated to portable food scanners. Perhaps in our field, the technology has not yet advanced sufficiently for proper scientific presentation – or those working on such solutions were simply holding cards close to chests and holding out for a big win! In either case, I'm very much looking forward to the outcome of the H2020 competition; and I'd be surprised if the term 'food scanner' doesn't become familiar language in the near future...

Reference

1. *H Siesler, "Spectroscopy Brought Down to Size", The Analytical Scientist, 0715 (2015). https://theanalyticalscientist.com/issues/0715/spectroscopy-brought-down-to-size/*
2. *https://ec.europa.eu/research/horizonprize/index.cfm?prize=food-scanner.*

Diagnosing Lung Cancer Earlier

Can comprehensive two-dimensional gas chromatography breath analysis lead to better lung cancer diagnostics?

By Romain Pesesse, PhD student, Organic and Biological Analytical Chemistry Laboratory, Department of Chemistry, University of Liège, Belgium.

Globally, lung cancer kills more than 1.5 million people a year, according to the World Health Organization. Compared with other high incidence cancers, it is a silent killer because very few symptoms are present in the early stages of the cancer process, which makes validating specific and sensitive screening challenging. Currently, late diagnoses, poor prognoses, and low survival rates are the norm.

In an effort to develop robust non-invasive early screening methods, the diagnostic potential of breath analysis has been investigated for a couple of decades; the hope is to isolate volatile organic compound (VOC) patterns that could be correlated to lung cancer. Despite our capability to isolate, separate, and identify VOCs, the lack of consistency between studies prevents breath analysis to be fully validated and used at the clinical level.

Our work in breath analysis is based on comprehensive two-dimensional gas chromatography (GC×GC) coupled to high-resolution (high-accuracy) time-of-flight mass spectrometry (TOF-MS), a technique that could be considered one of the most powerful tools in separation science. The high analytical resolution of GC×GC-HR-TOFMS relies on the proper combination of GC column phases for enhanced chromatographic resolution, but also on the efficiency of mass spectral deconvolution and the accuracy of the MS measurement. Such a multi-dimensional collection of data (1tR, 2tR, pure mass spectra, accurate mass) can improve our ability to differentiate between VOCs that find their origin inside the body versus VOCs issued externally by the patient and the environment.

In our approach, breath samples are collect using Tedlar (polyvinyl fluoride film) bags that we later empty into the thermal desorption tube. The sampling process is crucial, as the collection of human breath is subject to several interfering and confounding factors (food regime, smoking habits, and so on). Also, because exhaled air is a mixture of alveolar and ambient air, both endogenous substances and exogenous contaminants are sampled. Although lung washout using controlled medical air has been considered, our current strategy considers the collection of paired patient and control, together with environmental air samples and further comparison and subtraction between them. The idea is to minimize the effect of the presence of contaminants on class (patient versus control) segregation, while maintaining a simple and rapid sampling for patients.

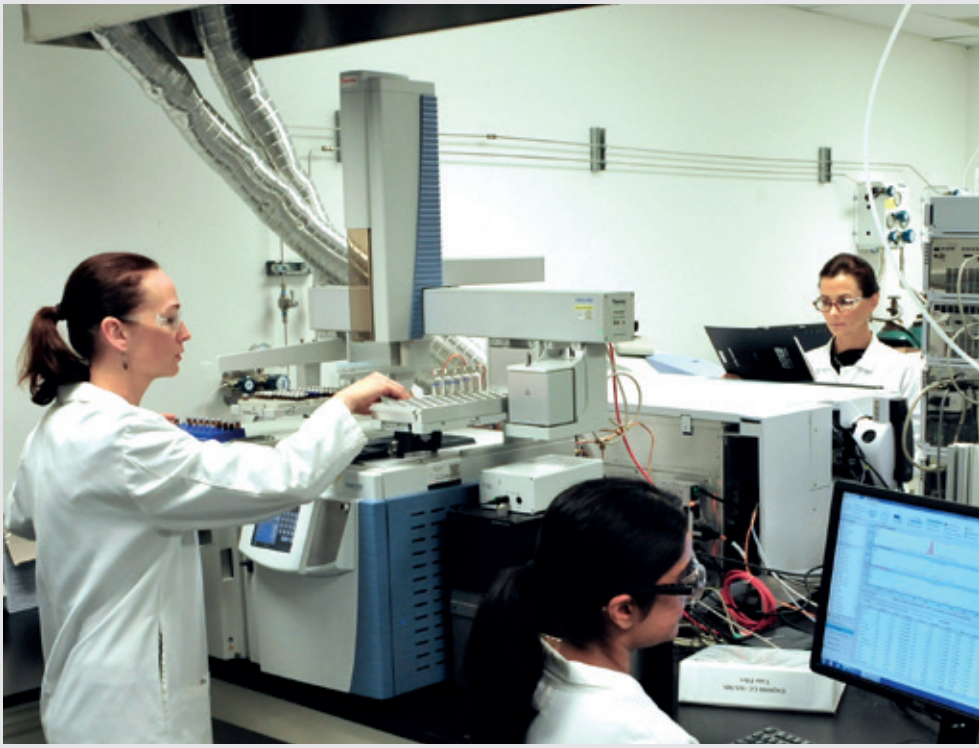
After GC×GC-HR-TOFMS separation and tentative identification of breath VOCs, large data sets are organized in classes. Next, we apply several statistical tools and processes (Fisher ratios, principal component analyses, dispersion boxes) to reduce the data and extract a list of peaks that appear to be showing a certain level of specificity to samples included in the lung cancer class. We replicate measurements to create a composite chromatogram image that includes all compounds found in a given class. This data reduction allows the isolation of a limited set of peaks ($n<20$) from the original list of more than one hundred peaks. At this stage, the two-dimensional chromatograms are revisited to ensure that the highlighted peaks are properly shaped and that signals are free from chromatographic artifacts. The corresponding spectral information, including fragmentation patterns and accurate mass of parent ions, is then used to identify the peaks based on mass spectral library searching and molecular formulae calculation. These putative suspects can later be used to create a VOC profile that can be compared with VOC profiles of patients to contribute to diagnosing lung cancer sooner.

In parallel, we also developed an approach that is dedicated to trapping VOCs emitted by lung cancer cell cultures. Some preliminary tests have demonstrated that VOC profiles from the headspace of lung cancer cell media, isolated at various stages of cell growth, are different from VOC profiles of control cell cultures. Our aim is to see if these lung cancer cell culture head space VOCs can be compared, at least in terms of chemical families, to the putative biomarkers found in exhaled human breath.

In our view, the consideration of these two different aspects – breath analysis and cell culture headspace analysis – could enable better understanding of unknown pathophysiology that affects the VOC pattern in lung cancer. The use of state-of-the-art separation science tools for such an integrated approach will hopefully contribute to further demonstrating the early diagnostic potential of breath analysis – and get it one-step closer to clinical application.

Taking Analytical Control

Then & Now, with Katie Banaszewski, Method Development Scientist III at Now Foods, Bloomingdale, Illinois, USA.



Katarzyna "Katie" Banaszewski (left) takes the new instrument for a spin.

Then: one cloudy day in 2014...

We're a dietary supplement company and we're naturally very conscious about the safety of our products, so it's tough having to rely on contract analytical laboratories for our pesticide residue analysis. Our samples are highly complex (multivitamin products can have 40 compounds!) – and we simply don't have the instrumentation needed to perform such analyses. Unfortunately, I don't feel like we're fully in control of our data and I also don't know exactly what kinds of methods are being used by the contract lab. I know the contract lab will be doing their best – we chose them carefully. Nevertheless, it makes me feel a little uneasy – especially, given the finicky nature of pesticide residue analysis in botanical matrices (by far the most difficult analysis there is!). We want to continue to be the best, so we need to look at other options.

Bringing pesticide residue analysis in house makes great sense, but we need to make sure we invest in the right instrumentation. Of course, sufficiently sensitive hardware is important, but we also need software that is powerful enough to help us process the data efficiently; analyst time is valuable to us! Importantly, we also need flexibility; we may want to use the system for other kinds of analyses at a later date.

It's a competitive market out there for analytical systems; we need to do some solid research...

Now: May 3, 2016

Having reviewed the systems on the market, we finally decided on the Thermo Scientific™ TSQ™ 8000 Evo Triple Quadrupole GC-MS/MS. There are a couple of points that made this particular system stand out from the crowd. We are especially impressed with the versatility of the system; though pesticide residue analysis was a primary driver, we also needed to be able to accommodate other kinds of analysis; for example, headspace analysis for residual solvents or analysis of essential oils. Moreover, we needed the ability to make that switch quickly, avoiding downtime. Another appealing aspect was the ability to remove the source without breaking vacuum, which also allows us to maximize uptime of the instrument.

I remember when we were first considering our investment, good software was also high on our priority list. We are now using the Chromeleon™ 7.2 Chromatography Data System, which I would say is currently the most powerful software for navigating MS data. The fact that the qualitative and quantitative capabilities are integrated is fabulous. Plus, our analysts find it easy to use – they were very excited to start working with the new system.

Now that we've moved testing in house, we are in control of every aspect of our trace analysis program – and that means we're also in control of the data, and in a better position to defend that data. Before, we could only trust the contract labs to do the best they could with our very difficult matrix sets.

We've always had very talented science teams, and now I feel like our laboratories are world class to match. And as a company, we can fully focus on the safety of our products. The dietary supplement industry is dynamic and vibrant – and our customers demand quality supplements. To stay ahead of the game, it's clear that we need the most advanced technologies out there.

Is (analytical) science still sexist? Six chemists contemplate the 'glass ceiling' for women in science: does it exist and if so, can it be broken?

By Joanna Cummings

Under-representation of women in high-level positions is a cross-industry problem, and the scientific community is no exception. A recent Future Science Group (FSG) survey stated that across the European Union, only 11 percent of senior-level academic positions in science are held by women, while worldwide, women researchers comprise only 28 percent of the R&D community (1). Meanwhile (and as recently as 2014), almost 2,000 theoretical chemists called for a boycott of the International Congress on Quantum Chemistry because the speaker list was entirely male (2).

At The Analytical Scientist, we are aware of (and affected by) the issue, but pleased to report that the number of women on The Power List almost tripled from eight in 2013 to 23 in 2015. In 2014, women comprised 32.5 percent of the "Top 40 Under 40". But is that truly representative? Across the globe, awareness of gender inequality is rising, with firm steps being taken to boost the status of women – and, importantly, other minority groups. Indeed, university "Equality Champions" are attempting to redress the balance in world of academia; another example is this year's University in Delaware conference, which raised awareness and provided a valuable networking experience for "women of color" in STEM careers (3). The ferocious Twitter backlash against Tim Hunt suggested that for some, there is no place for sexism in science – whether real or perceived.

In 2015, Nicola Gaston (who shares her views on page 27) published her book "Why Science is Sexist". In it, she details the ways in which unconscious bias is still holding women back. Going back to the FSG survey, 88 percent of respondents felt that young women are dissuaded from advancing in STEM careers by the lack of female role models. To that end, 2016's Power List will celebrate the skills and careers of women in analytical science – 50 of them to be precise (nominate now: tas.txp.to/0516/Top50Women). Importantly, we hope that our efforts are not seen as tokenism or condescension, but rather an attempt to balance the scales – at least until meritocracy becomes more practice than theory.

Will it be difficult to find 50 inspiring women? We don't think so, and nor do the following six people. All of them are currently working in analytical science – some in labs, some with technology, some teaching the next generation. Some have experienced overt sexism; some have progressed through their careers free from discrimination. All of them are women. Here, they share their experiences – and flag up some of the reasons why an all-women Power List is a positive step forward.

TOWARDS MERITOCRACY

By Ellen Miseo

Recently, I was at a reception at a conference and a young woman I know came rushing out, almost crying because a young man had said something incredibly inappropriate to her. Until that happened, I would have said that things are getting better for women – that they are being accepted and respected more. I'm certainly starting to see young women get more opportunities, though I also see them worrying more about job prospects, particularly at the graduate and postdoctoral level (but that's not just a concern for young women).

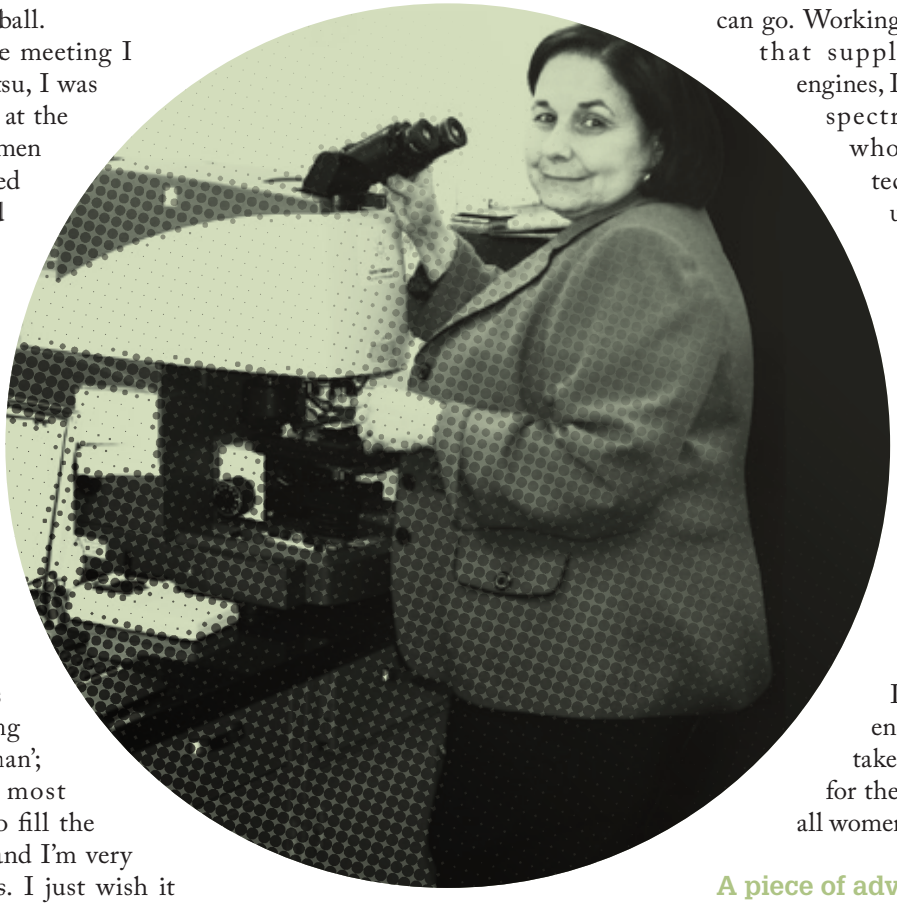
I didn't experience any sort of discrimination until almost 20 years into my career. After that experience, my radar just went off. I started to watch and think and talk about what the problems and issues are. I don't think overt sexism exists – at least not the way it did. Thinking back to the first Pittcon I attended, many companies had models wearing lab coats that barely covered their behinds. Their job? To attract people – men – into the booth. For the most part, that's disappeared in the field of analytical chemistry (thank goodness). But sexism still exists. There are still snide remarks.

I'm a middle-aged woman who grew up in New York City, so I make sure I am heard. But I've gone into meetings where a young woman comes up with an idea that no one is interested in, but then, all of a sudden, some guy says the same thing and now it's his idea. The fact that discrimination is society-wide makes it very hard to tackle in a specific industry. I think – based on what I've heard from people in other areas – that the analytical sciences are slightly better. Maybe more women are represented in analytical science in industry, because it's one of the chemistry fields that women have historically gravitated towards.

Women are also – let's be honest – often still responsible for childcare. And if you decide that your children are important, then you are not necessarily viewed as a fully-committed

professional. A couple of my very good friends are in senior-level positions with analytical instrument companies. I suspect that I have never been able to get that type of position (and some of them agree with me) because when my kids were young, I said, "My kids are important. I'm going to work four days a week. I'm always available, but I don't want my kids to think of me as an absentee mother". But how does that translate to getting a step up in your career? There's no way to put that in a resume. Nobody ever made a comment to my husband when he said he had to leave early to coach basketball.

The first corporate meeting I went to at Hamamatsu, I was downright surprised at the number of young women who were represented in technical sales and in our technology group. It was very different than in prior positions. But in Hamamatsu the senior management is all male. I've only been at Hamamatsu two years, but I suspect that there are at least a couple of senior managers who don't see 'a young man' or 'a young woman'; they see who the most competent person to fill the position. I know – and I'm very glad – that happens. I just wish it could happen more often...



"The fact that discrimination is society-wide makes it very hard to tackle in a specific industry".

What I do and why I love it:

I have worked in a lab, in a consulting environment, for instrument companies and as an adjunct professor. Every one of my positions relied on my knowledge of spectroscopy and my desire to interact with people.

My current position lets me talk to academics and start-ups and make connections. I enjoy the challenge of listening to someone talk about their research or a start-up idea and being able to think beyond the immediate need to where the idea can go. Working for an organization that supplies spectrometer engines, I find myself teaching spectroscopy to people who need to use the techniques but don't understand what they entail. I find that challenging.

The Top 50 Women Power List...

Lists should be meritocratic; everybody should be viewed the same. But clearly, that doesn't happen. Maybe an all-women Power List is one way of encouraging women to take the step up, advocate for themselves, and to give all women more exposure.

A piece of advice...

Keep your eyes and ears open, because a lot of discrimination is subtle. Don't feel that you have to take it. If you're in an intolerable situation, get out and say something; there's a legal system you can use. Make use of the tools that are available to you, and point out the discrimination. You can succeed.

Ellen Miseo works in Technology Development for Hamamatsu Corp, but has worked in software development for some years. An expert in FTIR, she is also President of the Society for Applied Spectroscopy.

MAKING GREAT STRIDES

By Heather Brooke

Honestly, it seems to me that women are making great strides in science and technical fields in terms of representation. Though we've not quite attained 50 percent of the demographic, I rarely feel like I'm in a gross minority and my ideas are generally taken seriously. I've been very lucky not to have experienced much resistance in my career, and haven't faced discrimination often – that I am aware of.

I did have an experience recently at a conference, when an application scientist for a vendor exclusively spoke to my male colleague, who is not a scientist, even when I was answering and commenting on his technical points. The majority of the way through the conversation, he looked at me with a bit of surprise and finally said, "Oh, are you the math genius?" Being a bit startled, I replied with, "Yes. Well, the chemistry genius actually." But that's an exception to the rule for me; it's the first time I have had to actively assert that I had real science knowledge.

I grew up in the 1980s, and distinctly remember having great women role models; for example, Sally Ride (the astronaut), my mom (who is a college professor in math and statistics), and a family friend who was a podiatrist. We need to stop telling girls that they aren't good at (and/or that boys are better) in certain subjects), such as math and science. And that's just the start – we also need to remove as much 'gendering' of interests, hobbies, and so on, as possible.

What I do and why I love it:

My current role is very dynamic; I do everything from highly technical consulting and training, to power networking at conferences, to creating marketing collateral. I love the opportunity to travel, meet interesting people, and actively use both my technical and social skills. I think the teaching/consulting may be my favorite part because I help make this really quite complicated topic (chemometrics and spectroscopy) accessible to people with all levels of technical skill. It is a great feeling to be able to directly affect someone's career.

The Top 50 Women Power List…

Women who are already in STEM careers simply need to be more visible – and this would definitely be a good start.

A piece of advice…

Pursue the things that interest you, because that's what you'll be good at, regardless of what anyone else tells you (and regardless of whether you are male or female). Also, networking is key, but don't make a job out of it; be personable, have fun, show real interest in the people you are connecting with – as opposed to being focused on what you can get out of knowing them.

Heather Brooke is Chief Chemometrician at CAMO Software Inc. She is also Workshops Chair for SciX, and co-chair of the networking committee for Women in Science and Engineering (WISE), which supports the advancement of women in technical fields.

Recommended Reads

"Why Science is Sexist", Nicola Gaston
Nicola Gaston asks why, in a field known for unbiased and objective enquiry, there is still so much unconscious bias against women scientists.

"A Chemical Imbalance", Polly L Arnold & Cameron Conant
Funded by the Royal Society Rosalind Franklin Award, this book and accompanying film tell the story of women scientists' fight for equality in the field of chemistry and the underrepresentation of women in STEM. Watch the film: http://chemicalimbalance.co.uk/project/watch-the-film/

"What Works: Gender Equality by Design", Iris Bohnet
Iris Bohnet discusses the difficulty in getting rid of unconscious bias, and provides solutions for tackling it at the organizational level.

"Lab Girl", Hope Jahren
A memoir by geochemist and geobiologist Hope Jahren, in which she shares the highs and lows of scientific research… And explains why she felt the need for a spanner in her pocket while working in the lab at night.

LEANING IN

By Mary Kate Donais

I think personality-wise, analytical chemistry sits well with some of the general characteristics that women tend to have. I teach at a small school where we have no graduate program, just undergraduates, and we have a higher percentage of men versus women in the college, yet there are slightly more women than men in my various degree programs. I think that in general – at least in the USA right now – the women who are applying have higher, better academic records, and that, if anything, it's harder to find the men to balance that out. But I also know there are other areas of science where that is not the case.

I've been exposed to a lot of different areas of the industry, and in some areas I was the only woman in a big group. I worked for an instrument company before coming to academia and that is much more male-dominated, but even there I was confident enough in my abilities: "OK. They hired me, so they must think I can do this." The people I worked with were some of my best early mentors and are some of my strongest supporters now, even since I left the instrument industry and came to academia. I've worked in some very good environments, even early in my career, but I realize not all women are so lucky – and it shouldn't be down to luck.

We have had more men attending 'women and diversity' sessions at SciX than we did initially. The trick is finding a balance between discussing topics in a productive and positive manner, and tackling the misconception that it's a "male-bashing session". Our Women in Spectroscopy group started as a subset of the Society for Applied Spectroscopy, and has broadened into under-represented groups within analytical science. For some of the younger women in the field, it can be hard – you can't just go to your male boss with questions like: how do you deal with having kids at the same time you're going through a tenure review? As a woman, how do you balance a professional job in which you're expected to travel, with a young family at home? You're going to get more 'real life' answers from a woman. Though of course, the balance of how families function these days makes it easier for women; I travel way more than my husband does and he is supportive of that.

The group had some online discussions about 'Lean in', written by Sheryl Sandberg, the woman who was COO at Google. She suggests that women are often hesitant to take chances – that it's part of our personality. All sorts of studies have been done about this: men assume that their skillset is better than it is and will take a chance, whereas women tend to think, "I don't have quite enough experience, I'm going to wait a little while longer". She thinks we're hurting women by taking that general philosophy and that we shouldn't be afraid to take a risk and do something new. That's how things have gone with my career – I have taken chances on certain things I didn't think were that big, but when I look back I think, wow, that was quite a leap!

What I do and why I love it:

I help shape the next generation of scientists. I love it because I am both a teacher in the classroom and a learner in research – it's the perfect balance.

The Top 50 Women Power List…

There are definitely some challenges unique to women – so why not? Hopefully, it will recognize people who are doing really great things but wouldn't have been on the list otherwise.

A piece of advice….

If you find you're not in a supportive environment with the specific people that you work with, find another one – whether that's a great group of people like those at the Society for Applied Spectroscopy, at a conference, or at a more personal community outreach. Just make sure you're finding support somewhere.

Mary Kate Donais is Professor in the Department of Chemistry at St Anselm College, New Hampshire, USA, and has worked as an analytical chemist in federal government, industrial, and academic settings. She is Chair of 2016's SciX conference.

AN ONGOING EFFORT

By Nicola Gaston

Sexism isn't necessarily about bad people; it's about bad judgments that are based on a cultural lack of understanding. Once you get that, the need for improved processes for decision making, such as gender-blind review of applications, start to make sense. I've been very lucky in my career, but I certainly recognize some of the mechanisms by which unconscious biases and stereotypes act to exclude women from science – and that led to me writing my book. The biggest shock for me was learning about the extent to which we can turn these biases against ourselves and affect our own performance.

I think we've irrevocably stepped beyond the old view of the problem, in which it was generally accepted that the representation of women was naturally getting better as they entered the workforce (but nevertheless that there might be limits on the extent to which we could get to equal representation, because of 'natural' differences). In a world in which we understand unconscious bias and the structural kinds of discrimination that exist, we need to act and think differently about gender bias. I'd hope that most women and men who learn about the problems of unconscious bias will find that knowledge empowering. There's no way around the fact that fixing gender inequality (as well as persistent racism and classism, for that matter) will take ongoing effort.

Two fabulous women from the University of Auckland set up a crowd-funded campaign to send copies of the book to senior academics in positions of power in New Zealand universities. They made it clear that they were sending the book to people on account of their institutional responsibilities, and got an amazing response – I think in the end there might be around 400 copies in the hands of heads of departments, deans and other senior university administrators. It's been a delight to see the emails come in expressing thanks and solidarity – it feels like we've managed to start a conversation that was desperately needed.

What I do and why I love it:

My research in the MacDiarmid Institute focuses on understanding how the arrangement of atoms in materials affects electronic structure, and from there determines functionality, such as whether a substance is metallic or insulating, or useful for solar cells. In retrospect, there are parallels between the way my research has taught me to look at the world and my conviction that understanding the structural basis of sexism is necessary to deal with – and change – the way it functions.

"Sexism isn't about bad people; it's about bad judgments".

The Top 50 Women Power List…

Raising the profile of women is necessary in order to compensate for historical (and current) inequalities in representation. Concerns about lists compiled 'just because they are women', are exactly why people need to understand the role of unconscious biases.

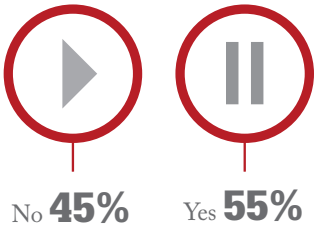
A piece of advice…

Feel free to get on with your science and try not to worry about sexism too much! However, knowledge is power, and understanding the origin of sexism in science can be useful, even if it only lets you put some of the second guessing aside.

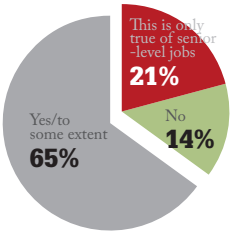
Nicola Gaston is Associate Professor in physics at The University of Auckland and Deputy Director of the MacDiarmid Institute, New Zealand. She has been a strong advocate for women in science, and explores the role of women scientists in her blog and subsequent book, "Why Science is Sexist".

PERSPECTIVES ON GENDER EQUALITY IN SCIENCE*

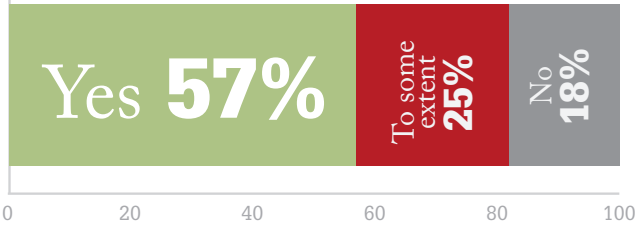
Have you ever felt held back in your career as a result of your gender?



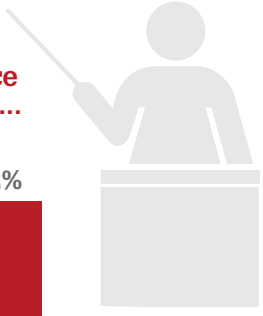
In general, do you think that women are underrepresented in science?

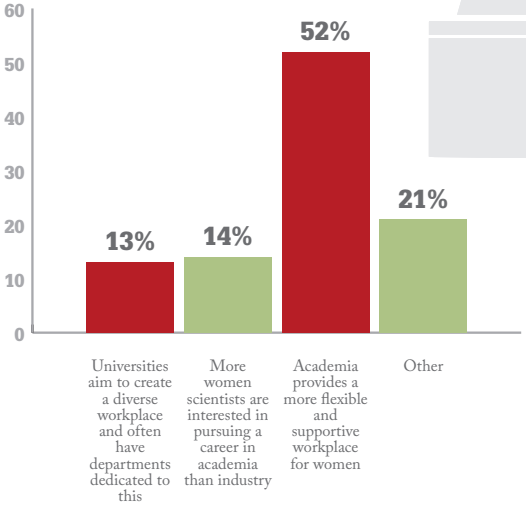


Do you feel that industry/academia tend to consider the impact of family and parental leave to a greater extent when hiring women?



Those who believed that academia was a more gender-balanced workplace felt that this was because ...



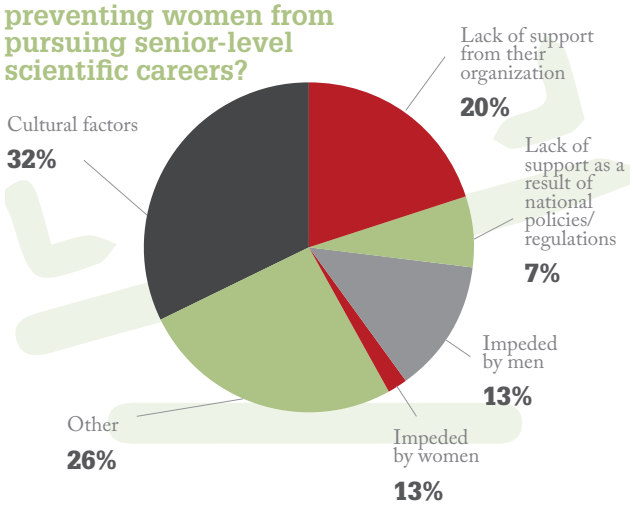


88%

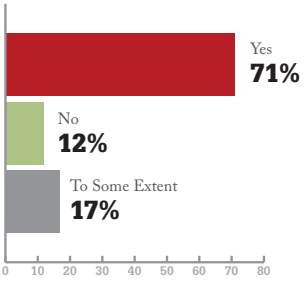
felt that, to some extent, the lack of female role models dissuades young women from advancing in STEM

Do you believe that awareness of gender inequality within science has increased throughout your career?

What is the main obstacle preventing women from pursuing senior-level scientific careers?



Do you feel that more could be done at a political/organizational level to aid women hoping to pursue senior-level scientific positions??



*Source: Future Science Group, Gender equality in science survey: www.future-science-group.com/rad-survey-infographic-2016

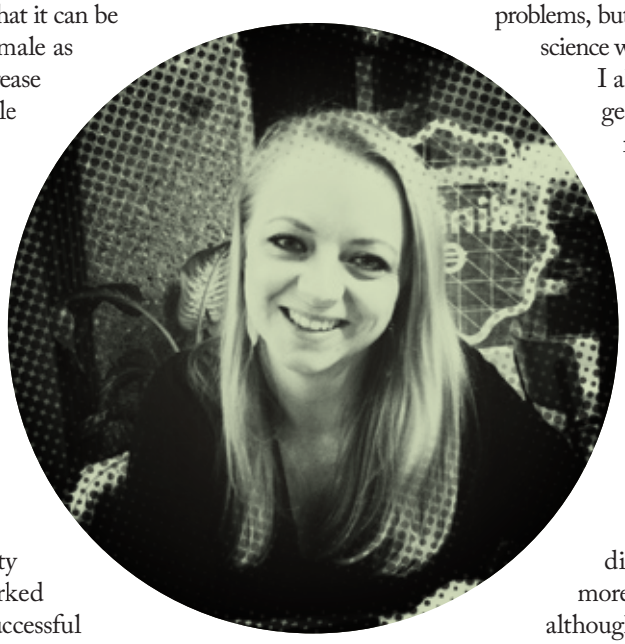
ON BALANCE

By Lisa Miller

I believe that the majority of young chemists do not see the gender gap. We have come through our undergraduate degree as a mix of men and women, all treated equally, and it has resulted in us expecting to be treated equally in future positions. During my undergraduate degree, the University of Strathclyde had a large number of women studying chemistry and my particular degree – Chemistry with Drug Discovery – was mostly female.

I have now spent over seven years in academia and during this time carried out secondments with Takeda Pharmaceuticals, GSK and the Netherlands Cancer Institute. The labs that I have worked in were all a mix of men and women, and I have not experienced gender discrimination so far in my career.

In fact, I have found that it can be an advantage to be female as there is a drive to increase the number of female scientists. Currently, I am planning ahead and looking at post-doctorate positions for after my PhD. These are often funded by fellowships, and there are a number of organizations aimed at funding female researchers.



Not everyone has experienced the equality that I have – or worked alongside as many successful female scientists. The staff of department I currently work in is heavily male-orientated. At present there are no female lecturers in the organic chemistry division, but the other areas are more balanced. With respect to the postgraduates it is again more balanced, which is hopefully a sign of things to come – where we will see more female scientists in academic roles. However, it is well known that many women don't wish to stay in academia after their PhD due to the difficulties of balancing work with starting a family.

Sharing examples of successful female researchers with young people will help to encourage more women to pursue science as a career. If they are told from an early stage that gender is not a problem, then they won't expect it to be a problem. I believe anyone who has good intentions and strong motivation will get what they aim for. But telling someone that they may experience discrimination will plant seeds of expectation, which could lead to them not pursuing their dreams for fear of failure. Obviously, we shouldn't ignore the problems, but spreading the good news of successful women in science will help to encourage those with any doubts.

"I get a feeling of pride when I see a female chemist at the top of their field. It gives me encouragement".

I always enjoy meeting other chemists, regardless of gender, but I do get that feeling of pride when I see a female chemist at the top of their field. It gives me encouragement that I can achieve all that I aim to.

What I do and why I love it:

I am a medicinal chemist, designing and synthesizing novel compounds. I love it because it challenges me every day, and also because I am creating new molecules that will hopefully one day help people.

The Top 50 Women Power List…

It would be great positive reinforcement for female researchers who are concerned about discrimination. And we might end up with a more current poster girl chemist than Marie Curie – although we do all love her, of course!

A piece of advice…

Do not fear a glass ceiling – in my experience, it isn't there. Chemistry is a small world with a great network, so speak to other scientists about what you would like to do, and they will help you to find the right person to speak to.

Lisa is a PhD Student at the University of Strathclyde. She is currently working on a medicinal chemistry project with Allan J. B. Watson in collaboration with GlaxoSmithKline that focuses on lead optimization of targets for fibrosis drug discovery.

BEYOND THE 'OLD BOYS CLUB'

By Ingeborg Petterson

In academia, I see women who are interested in analytical chemistry, who get to a PhD or post-doc level and still encounter issues around continuing into more senior positions. I have seen numerous PhD students and postdoc colleagues become very frustrated by the inflexibility of trying to balance a family with a fast-paced research career working on fixed-term contracts. About the time I was trying to decide whether or not to do a PhD, a supervisor flatly told me, "You have to decide whether you want to do a PhD or get married and have kids." Either/or.

My mom was a physics professor and a single mom for a long time. She made me realize what is possible. Somebody without a role model may have been very easily discouraged by a comment like that. In fact, such conversations could (and do) make or break careers.

We need men and women in supervisory roles to be sensitive to these issues, and realize the impact that such comments could have on their students. It's important to have women role models as a woman scientist, but men can be fantastic and supportive role models to women as well. Aside from that one negative incident, all of my supervisors were very supportive. My PhD supervisor was crucial in my career development, taking me to international conferences and helping me to become involved in the scientific community by encouraging me to join the Society for Applied Spectroscopy.

At SciX, we hold conference sessions on women and diversity in analytical science with different women representatives – from academia, industry, government researchers, and at different career levels, speaking about their career experiences. We called the first one 'Women in Analytical Chemistry', and had an all-woman audience and speakers. We've been wondering if that's the best approach, because everyone needs to be in the discussion.

"We need men in analytical science to be sensitive to these issues – everyone needs to be in the discussion".

Last year, we had two male panel members and a male speaker in a management position. The latter spoke of his challenges in giving career advice to women and men, and how he navigated that. To be honest, I don't know if you should give the same career advice to female and male students. You may better serve women by telling them that these issues exist and that they have to be aware of them.

The conference session has faced criticism, mostly from those saying that a scientific conference is not an appropriate place to talk about these issues and that we should 'stick to the science', but in my opinion it's exactly the right forum. That's the point of a conference: people in the field coming together to discuss relevant topics. The network that has formed from this conference session and the online Women in Spectroscopy group has been just amazing.

I do think people are becoming more aware, but we are often still in the 'old boys club' mentality. I have witnessed plenty of sexist jokes in professional settings like conferences and award ceremonies. I'm sure these aren't meant to be malicious, but there's still a general lack of thought about how something like that might affect people in the audience. Senior scientists, conference organizers, and others in positions of power should advocate for those in weaker positions who may not be able to speak up for themselves, and help build a community in which this behavior is not appreciated or acceptable. Additionally, you don't always have to pick the same old Caucasian males as plenary speakers; you can use it as an opportunity to highlight the careers of women scientists, and others who could really benefit from such an opportunity.

What I do and why I love it:
I am an analytical chemist, specializing in Raman spectroscopy. I love working to solve analytical challenges in terms of developing new technology and new tweaks on established techniques.

The Top 50 Women Power List…
It's important to highlight the careers of women and what they're doing. If you look at the lists of people who receive awards, it's mostly men. The argument is that there is not a huge pool of women with successful, lengthy careers to select from. To that I would say: you need to look a little bit harder.

A piece of advice…
Find some mentors or a network of people who will be supportive. The Internet has made that less difficult for those around the globe. Join a group, do your best to make it to a conference and make some friends in the field.

Ingeborg Petterson is Postdoctoral Fellow in Chemistry at Goucher College, Baltimore, USA, specializing in Raman spectroscopy in biomedical applications. She is Membership Chair of the Society of Applied Spectroscopy, and was recognized in The Analytical Scientist's 'Top 40 Under 40' in 2014.

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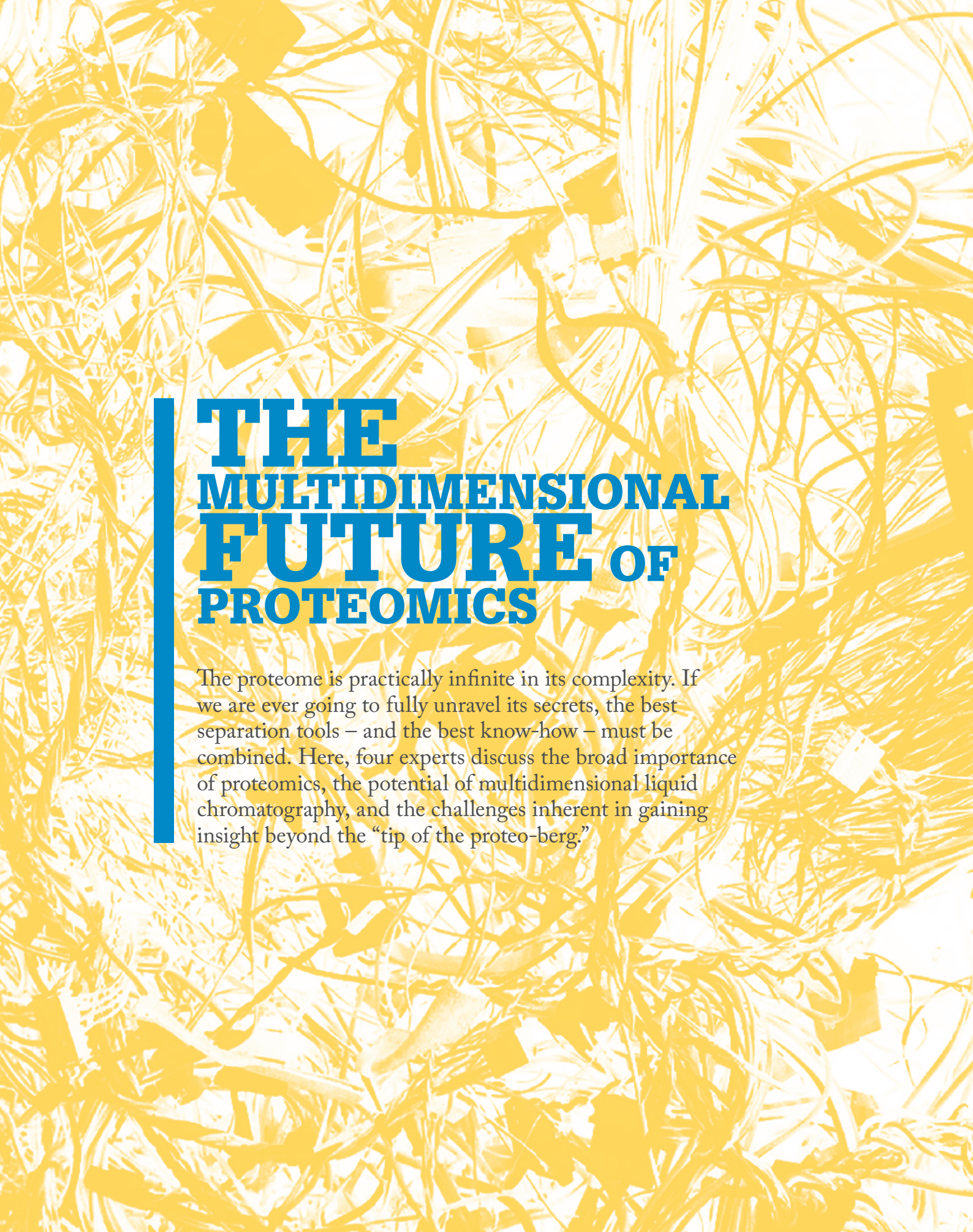
2. *http://www.rsc.org/chemistryworld/2014/02/chemists-call-conference-boycott-over-all-men-shortlist*
3. *http://sites.udel.edu/advance/conference/*



THE MULTIDIMENSIONAL FUTURE OF PROTEOMICS

The proteome is practically infinite in its complexity. If we are ever going to fully unravel its secrets, the best separation tools – and the best know-how – must be combined. Here, four experts discuss the broad importance of proteomics, the potential of multidimensional liquid chromatography, and the challenges inherent in gaining insight beyond the "tip of the proteo-berg."

Why does the analysis of proteins remain so important?

Andrea Gargano: The analysis of proteins is essential for understanding the complexity of the communication that takes place in our bodies. Improving tools for protein analysis has important implications for medical science, where we aim to understand the mechanisms of action of bioactive molecules, find (bio)markers for diseases, and characterize new classes of pharmaceuticals (for example, antibodies). Developments in protein analysis promote research at the boundary between biology and chemistry; namely, biochemistry, system biology and bioengineering. Moreover, recent progress in proteomic research has demonstrated that advanced analytical tools for protein analysis open up new possibilities in fields beyond protein science, such as polymer and biopharmaceutical research.

In essence, protein analysis is important because it is an analytical challenge with big implications.

Koen Sandra: Proteins have many functions – structural (keratin in hair, collagen in bones, skin), mechanical (myosin/actin in muscle movement), transport (hemoglobin for oxygen transport in blood), defense/immune (antibodies), biochemical reactions (enzymes), hormones (insulin regulated glucose metabolism) – and the list goes on. Amongst many other benefits, analysis of proteins can lead to the discovery of novel drug targets and biomarkers for disease diagnosis, prognosis, and prediction, and is key in the concept of personalized medicine. Proteins themselves are also on the rise as therapeutics; hence, from a biopharma perspective, accurate analysis is essential.

Shabaz Mohammed: If it's not already clear from Koen and Andrea's answers, I'll add that a significant number of diseases, including many types of cancer, can be related to the dysfunction of proteins and their interactions. Thus characterizing their structure, function and interactions is of the utmost importance.

John Yates III: Proteins are the operational agents of cells. They form structures, they transmit signals, they catalyze reactions to form metabolites, they form protein complexes. If you want to know how cells work, you have to study proteins.

How far are we from characterizing the proteome of complex organisms?

AG: We are a long way off – potentially 100,000 proteoforms (90 percent) away from the entire proteome of a complex living organism (including ourselves). However, important results have been achieved with current technology, supporting genomic and transcriptomic results and enabling discoveries in biomarker research. So far, we are just capable of characterizing the tip of the proteo-berg. To better grasp the proteome complexity, we need better analytical as well as chemometric and statistical tools that are capable of refining and rationalizing the large amount of data that we are collecting.

SM: The completion of genomes and the massive improvements in the last decade in cell manipulation, protein chemistry, chromatography and mass spectrometry allow one to identify pretty much any protein in a cell. There are still a few exceptions, such as very low copy number proteins or those with 'difficult' physicochemical properties (for example, hydrophobicity in the form of transmembrane domains) – and there are some proteins that suffer from both sets of problems, such as olfactory receptors. Given enough time and resources, one can generate evidence of presence for pretty much all the proteins in a particular type of cell. However, it isn't something that is often performed because the resources and time required to carry out such a feat are huge – and there aren't really any good reasons to carry out such an experiment. We often limit ourselves to a few days' mass spec time for an experiment and the depth level achieved (approximately 8-10,000 protein families for human cells) is sufficient for most biological questions. Consequently, the jump to characterizing all the distinct types of cells in an organism, of which humans have hundreds, is a far off dream.

JY: I'd say we're pretty close to being able to identify the presence of all proteins in a mammalian cell. But the difficulty is knowing how many proteins are really there. After all, protein expression varies with conditions. Additionally, the complete proteome will encompass protein isoforms and modified forms for all expressed genes – and that is a pretty large set of proteins.

What are the strongest arguments for using 2D-LC in proteomics?

KS: Why has proteomics historically been performed using 2D-PAGE instead of 1D SDS-PAGE? Because one can identify and quantify many more proteins. The same conclusions can be drawn when evolving from 1D to 2D-LC in proteomics. The inability of 1D-LC to adequately resolve real-world mixtures of high complexity is the driving force behind using multidimensional separations. It is no surprise that the field of proteomics is widely adopting the technology given the enormous sample complexity encountered. Simple unicellular systems already contain thousands of proteins, and for now, one can only speculate on the complexity of the clinically valuable and most complex human serum/plasma proteome. Due to the preferred handling of peptides over proteins and the consequent proteolytic digestion of proteins prior to downstream processing, every protein is represented by dozens

Multidimensional Masters

Andrea Gargano is a post-doctorate researcher at the University of Amsterdam, specializing in two-dimensional liquid chromatography. In the summer of 2015, Gargano was awarded a Veni grant from the Netherlands Organization for Scientific Research (NWO) and he is currently working on the development of (multi-dimensional) separation strategies for the characterization of intact proteins.

Koen Sandra is Scientific Director at the Research Institute for Chromatography (RIC), which provides world-class chromatographic and mass spectrometric support to the chemical, life sciences and (bio) pharmaceutical industries. As a non-academic scientist, Sandra is author of over 40 highly-cited scientific papers and holder of several patents related to analytical developments in the life sciences area.

Shabaz Mohammed, after finishing his PhD in mass spectrometry, moved to Denmark to work with Ole Jensen in the field of proteomics and, in particular, the development of techniques for improving protein information. In 2008, Mohammed became group leader and Assistant Professor in Utrecht and worked with Albert Heck at the Netherlands Proteomics Centre. In 2013, he moved to the University of Oxford where is now Associate Professor.

John Yates III is Ernest W. Hahn Professor of Chemical Physiology and Molecular and Cellular Neurobiology at The Scripps Research Institute, LaJolla, California, USA. Yates was recently named Editor of the Journal of Proteome Research.

of peptides. It is not unheard of to be confronted with thousands of peptides, spanning a wide concentration range, that have to be introduced into the mass spectrometer in a way that allows successful qualitative – and quantitative – measurement. Multidimensional LC possesses the additional resolving power to substantially reduce the complexity of such peptide mixtures and, therefore, to increase the number of measurable peptides, to widen the overall dynamic range and consequently to increase proteome coverage.

In addition, the implementation of an extra separation dimension has also been shown useful in the targeted analysis of proteins. In contrast to the above described discovery proteomics approach, targeted analysis does not require the analysis of the entire first dimension.

AG: State-of-the-art 1D-LC chromatographic setups, using long columns (0.5 to 1 m), packed with materials of small particle size (2 µm) and shallow gradients (from 1 to 6 hrs) can provide peak capacities up to 1500. However, the components of proteomics samples are hundreds of thousands (if not millions) and thus, during LC-MS analysis, mass spectrometry instruments have to deal with complex mixtures of peptides present in vastly different concentrations. Co-elution leads to ion-suppression effects that, together with MS dynamic range limitations, compromise the analysis of low-abundant species and thus limit the depth of proteomic investigations.

As Koen notes, online comprehensive two-dimensional liquid chromatography (LC×LC) enables deeper investigations of the proteome because of its higher resolving power. Moreover, LC×LC can combine chromatographic methods with different retention mechanisms (such as ion exchange, hydrophilic interaction LC, or high pH reversed-phase × low pH reversed-phase). As a result, sample components can be separated with orthogonal selectivities. In addition, the fast and efficient second dimension separations enabled by UHPLC technology significantly reduce the peak widths (typically ranging from below 1 to 10 s) enabling higher peak capacities with respect to 1D-LC (more than 1000 in less than 1h).

SM: I can echo Koen and Andrea. The first step in most protein characterization experiments involves chopping up proteins into peptides. To comprehensively identify a human proteome, one needs to handle a peptide mixture numbering in the millions that span a dynamic range of 7-9 orders of magnitude. The pace of improvement in 1D LC-MS is astonishing; however, issues remain and there are certain hard limits that haven't been addressed. The latest mass spectrometers have (successful) sequencing rates of around 10-20 peptides per second and, with state of the art UHPLC systems, one can hope to identify 20-40k peptides in a single analysis. Increasing those numbers won't be easy since the dynamic range of a mass spectrometer is (at best)

five orders and current MS systems can just about sample peptides at the bottom of its restricted dynamic range. Increasing speeds don't really improve the situation since the mass spectrometers can't collect sufficient populations of the low abundant peptides for a successful sequencing event because of the limited dynamic range. It's also been predicted that the resolving power of current UHPLC systems improvements would plateau at around double current values. Thus, the only way to increase capacity is to fractionate and the most powerful and sensitive (by far) method of fractionation is an additional round of chromatography.

JY: To get the complete mammalian cell proteome as described above will require a tremendous peak capacity and it is unclear if 1D LC can deliver that level of performance. Moreover, developments occurring in ultra-high resolution 1D LC, of course, can be adapted for 2D LC.

Where has 2D-LC had the largest impact?

SM: There are a significant number of examples demonstrating the power of multidimensional chromatography and it's quite difficult to highlight a particular example. There are entire fields that depend upon it. Characterizing how signals are transported through a cell often requires an understanding of the behavior of proteins being phosphorylated. The presence and absence of this small molecule on proteins can determine if a protein is active, where it is in a cell, and with what it interacts. Phosphorylated proteins are low in abundance (occupying the lower levels of protein dynamic range) and such events are thought to number in the hundreds of thousands if not millions in a cell at any time. Identifying these events and their meaning often leads to multiple rounds of chromatography for enrichment and complexity reduction.

AG: Offline 2D-LC has been widely used as a pre-fractionation strategy (prior or after protein digestion), to reduce the complexity of single shotgun RPLC-MS analysis. However, the long times required and big advancements in UHPLC-HRMS limited the spread of such workflows. An area where 2D-LC will continue to expand its impact is the selective analysis of part of the proteome, enriching certain protein or peptide species using affinity tags and/or special sorbents.

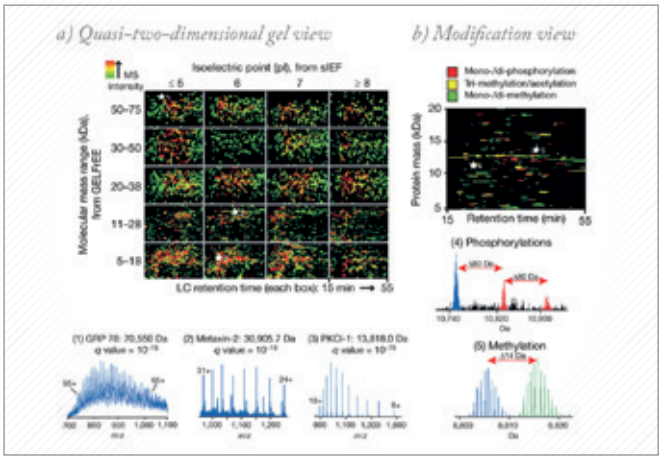


Figure 1. Four-dimensional separation of proteins from HeLa cell lysate using isoelectrofocusing, SDS-based nano-RPLC and mass spectrometry. (JC Tran et al. Nature 000, 1-5 (2011) doi:10.1038/nature10575)

KS: I have worked at a molecular diagnostics company for several years, where we were applying proteomics to discover and verify disease biomarkers. Using 2D-LC, we could mine the otherwise hidden proteome (hidden biomarker candidates), which came in particularly handy in the discovery programs. 2D-LC, yet in another format (targeted), was also successfully implemented in our biomarker verification workflows. Now that my focus has in recent years shifted more to biopharmaceutical analysis, 2D-LC based proteomics technologies again come in very handy to identify and quantify host cell proteins (HCPs). While in a 1D chromatographic set-up, the separation space is dominated by peptides derived from the therapeutic protein, the increased peak capacity governed by 2D-LC allows one to look substantially beyond the therapeutic peptides and detect HCPs at low levels (sub ppm relative to the therapeutic).

We have also used 2D-LC in the quantification of therapeutic proteins in blood plasma to support (pre-)clinical development (pharmacokinetic studies), which is technically identical to biomarker verification. We have even validated these methods according to EMA guidelines. In these projects, the first dimension is used to reduce the matrix complexity prior to second dimension LC-MS analysis using multiple reaction monitoring. Because of matrix effects associated with 1D-LC-MS, one only obtains sensitivities in the high ng/mL range. Incorporating that extra dimension allows one to reach the low and even sub ng/mL levels in blood plasma/serum.

Are there any shortcomings to the technique?

SM: Coupling multiple rounds of chromatography is still not a trivial task. Multidimensional chromatography is still, mostly, used by committed analytical chemists. Sensitivity is of paramount importance in proteomic experiments. HPLC columns often lead to unacceptable losses unless attention is paid to how the sample is brought to each chromatographic system and how it is treated after fractionation. Miniaturization plays a huge role in proteomics and it is often not straightforward to reduce dimensions and flow rates while increasing column pressures. A significant amount of know-how about the various flavors of chromatography and the underlying science is required to pick and build multidimensional systems for certain types of proteomic experiments. That said, for unmodified 'regular' proteins, a consensus is being reached and so certain two-dimensional configurations have now hit mainstream science.

AG: The major drawbacks of comprehensive 2D-LC (LC×LC) are: long analysis time (typically several hours), increased dilution (thus reduced sensitivity) in comparison with 1D-LC, and the complexity of method optimization. In analytical-scale separations, the introduction of systems with reduced dispersion volumes and UHPLC technology has drastically reduced the analysis time of LC×LC, enabling second dimension separations of about 20 seconds for a full gradient elution run. This is not yet the case for nano 2D-LC setups that are used for sample-limited applications, such as proteomic research. Here, due to dead and dwell volumes, the speed of the 2D cycle is typically limited to longer cycles (more than 10 min). In the coming years, further advancement in the miniaturization of LC apparatus (e.g. chip-based chromatography) will enable faster 2D separation cycles and thus facilitate the development of faster LCxLC applications. Several groups are working on the reduction of dilution in LC×LC and promising results are coming from the use of trap cartridges to collect fractions from the first dimension (what we call "active modulation") and inject small volumes in narrow second dimension columns.

Method optimization remains a challenge. However, software solutions to reduce the time and effort required to optimize two-dimensional methods will help analytical scientists in this task.

KS: In addition to the technical challenges already mentioned, I believe one of the shortcomings is related to nomenclature. All kinds of different terms are being used to describe the way 2D-LC separations are performed (comprehensive, heart-cutting, LCxLC, LC-LC, off-line, on-line, automated off-line, and so on). In some ways, this is not surprising given that the technology has been developed from two different angles (proteomics and chromatography, respectively). In multidimensional GC, there are no ambiguities around nomenclature/terminology. People often contact us to ask which kind of 2D-LC they are actually performing. Importantly, depending on how 2D-

> "It is no surprise that the field of proteomics is widely adopting multidimensional LC given the enormous sample complexity encountered."

LC is performed, one is confronted with flow and mobile phase incompatibilities, reproducibility issues, immature data analysis software, and so on. In the early days of using 2D-LC in proteomics, repeatability and reproducibility were not considered to be important issues. Now that the technology is really being applied to solve problems, old figures of merit have become primordial.

JY: The biggest shortcoming is the time required to perform the analysis, which limits the number of experiments that can be performed. Let's hope higher throughput methods for 2D-LC can be developed.

What is the current– and the potential – use of 2D-LC in proteomics?

AG: From what I have experienced, 2D-LC is used in specific studies where it is necessary to reduce the complexity and/or select part of the sample components of complex protein digests (bottom-up proteomics). Two-dimensional separation is also common in the analysis of intact proteins from cell lysates (top-down proteomics, see Figure 1). However, the longer analysis time and more sample handling steps of 2D-LC restrict its use in routine analysis of large numbers of samples. Some laboratories apply the Multidimensional Protein Identification Technology (MudPIT), introduced by John Yates' lab at the beginning of this century. This separation method is the only online 2D-LC setup commonly adopted in proteomics research and many publications have demonstrated the advantages of its high separation power. Yet the longer analysis times and the fact that this setup is exclusively limited to ion-exchange and reversed-phase combinations have limited the spread of this technique. An interesting advancement of 2D-LC in proteomics may come from the development of

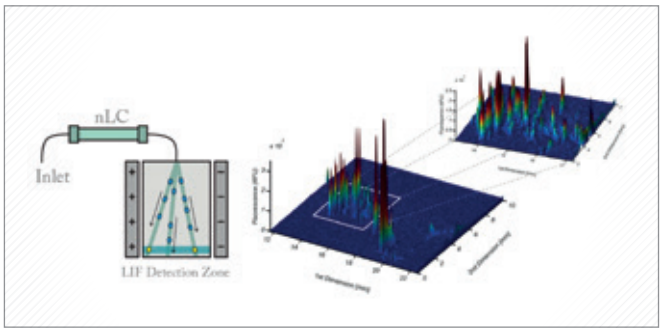


Figure 2. Coupling of nano-RPLC with micro free flow electrophoreisis for the analyisis of A Chromeo P503 labeled tryptic digest of biovine serum albumin. (M Geiger, NW Frost and MT Bowser; Anal Chem 86, 5136-5142 (2014))

> "The idea of 2D-LC as the best means to obtain a comprehensive proteome is pretty much accepted by the entire field."

online comprehensive two-dimensional chromatography (LC×LC), where independent chromatographic selectivities can be combined online, enabling more detailed analysis within times comparable to 1D-LC. Successful development in this area will greatly benefit research in top-down proteomics.

KS: As Andrea suggests, MudPIT made the large scale analysis of proteomes possible. Personally, I find it remarkable that the 2D-LC technology was independently developed by two research communities, which can clearly be seen in the different ways 2D-LC is performed nowadays. On the one hand you have the proteomics people who are historically more MS driven (Yates) and on the other hand the chromatographers (Jorgenson). Chromatographers want to maximize resolution while proteomics scientists want to maximize the number of proteins (and eventually PTMs) that can be identified and quantified.

The chromatographers, who typically did not develop the set-ups from a proteomics perspective, want to maximize resolution, which means that they have to maintain the first dimension separation upon transfer to the second dimension. To achieve that, peaks are sampled several times and stored in loops installed in between the first and second dimension column. Small internal diameter columns (1 mm or higher) are used in the first dimension and columns with wider internal diameter operated at high flow rates (mL/min) and high speed (< 1 min) in the second dimension. Indeed, from a theoretical perspective, this approach maximizes peak capacity (since first dimension separation is maintained upon transfer to the second dimension) but it is not at all compatible with mass spectrometry and proteomics. Indeed, flow rates in the mL/min range and second dimension run times below 1 min do not allow the detection of a substantial number of peptides. Multidimensional set-ups in proteomics typically use columns with small internal diameters in the second dimension (75 µm) that are operated in the nL/min flow regime. Second dimension gradients are also allowed to develop slowly (no real time constraints). As such, run times and flow rates are fully compatible with MS. The first dimension typically uses wider internal diameter so as to allow a high column load. Proteomics scientists do not worry about under-sampling the first dimension peaks. The number of samplings from the first dimension is typically low and the separated compounds are reunited, typically on an enrichment column. The two disciplines are evolving towards one another but, unfortunately, one now sees studies/papers appearing from chromatographers that were, in fact, already described 10 years ago in specific journals by proteomics scientists.

SM: The idea of 2D-LC as the best means to obtain a comprehensive proteome is pretty much accepted by the entire field. The exciting thing is that all the know-how built up over the last few decades now means that there is a much better awareness in the field on how to perform a 2D-LC experiment. Manufacturers are now providing far more appropriate and, more importantly, robust components to build 2D-LC systems. I think separation power will continue to improve but my expectation is that 2DLC will become the main approach ahead of one dimensional LC-MS for obtaining a comprehensive proteome analysis.

JY: The method is pretty well established as an off-line technology. You can also view methods such as IMAC in combination with 1D-LC as a 2D-LC method. I would say that most of the uses of 2D-LC are off-line. These methods are driving a lot of discovery in academic laboratories as the capability for more comprehensive discovery grows.

Who has played large roles in 2D-LC development, technically as well as theoretically?

AG: I have only been involved in 2D-LC research over the last three years. In this time my work has greatly benefitted from theoretical studies and technological developments resulting from the vison and efforts of many researchers. Certainly major

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developments have been driven by analytical "shrines" such as Jorgenson, Carr, and Yates' labs that practically realized online 2D-LC and participated in the development of the instruments that are now on the market.

KS: I agree with Andrea that there are certainly notable pioneers, such as Jorgenson (chromatography) and Yates (proteomics), but many others have substantially contributed as well – as with all questions of this type, it is too risky to forget important contributors with a definitive list. I would say many of the major vendors, including Dionex (now Thermo Fisher Scientific, but early developments took place at LC Packings/Dionex), Waters and Agilent are also at the forefront.

SM: As Koen notes, the number of people involved in 2D-LC development is far too long to list, although one of my fellow interviewees played a major role. John Yates' work on 2D-LC combined with his work on 'shotgun' proteomics helped lead to a sea change in how the field went about protein characterization. The importance of 2D-LC is such that all major LC and MS manufacturers are invested in active research and development in chromatography. One of the first dedicated solutions for nanoflow LC (a staple of proteomics) was developed by a little company called LC Packings/Dionex that, after a number of takeovers, became part of Thermo Fisher Scientific, as Koen says.

JY: Long before we tried 2D-LC for proteomics, I followed mostly the work of Jorgenson in this area. I was always impressed with the elegance and potential of the method. I know many others contributed to this area, but I was following the mass spectrometry and protein sequencing areas so my bandwidth was limited. Jorgenson was always pushing the outer limits of separations like CE at million volts, LC at 100,000 psi so his research was always "Gee Whiz" kind of stuff. It was fun to see what he would think of next!

What's the most important 2D-LC development of the last ten years?

SM: Tough question. It's been a very smooth evolution rather than a revolution. But the moment that sticks in my mind was when there was a collective realization that 2D-LC was the best way to obtain deep proteome characterization, which then led to many different chromatographic combinations. The need to find optimal complementarity led to new flavors of chromatography as well.

KS: For me, it's the move away from specialized labs using home-built systems to use by a broader audience, which has been facilitated by the introduction of commercial instrumentation.

AG: The use of UHPLC technology in two-dimensional separation systems following the research lead by Stoll, Carr and colleagues that resulted in the launch of robust systems capable of second dimension separation performed in cycles of 20 seconds.

JY: For me, the most important development has been combining 2D-LC with ultra-high pressure LC. The commercial availability of high pressure LC pumps opened up the use of this technique to a broader array of people and that helps to drive further innovation.

Where does multidimensional separation go from here?

AG: I'd like to think that in the future multidimensional systems won't exclusively be coupled in the time domain (where sample fractions are collected from a first separation and each of them is consecutively analyzed by a second separation) but will also use spatial dimensions. The possibility and power of this approach was shown recently by Bowser et al, coupling RPLC (time) with free flow electrophoresis (space) achieving an impressive peak production rate of 105 peaks/min (see Figure 2). More approaches to realize spatial × time based chromatography may arise from efficient devices for planar chromatography. Whatever form multidimensional separation takes, I look forward to highly sophisticated technologies that are fast and easy to use!

KS: Again speaking from a proteomics perspective (if you asked me the same question from a non-proteomics perspective, I would give a completely different answer!) I believe that both (or more) dimensions will be integrated in chip-like devices that act as sample introduction systems for MS. Once more, what is required in proteomics cannot be generalized, which is to say, low volume in the second dimension.

SM: Our understanding of manufacturing materials and refining selectivity is improving at a rapid rate. Our ability to operate at ever-higher pressures with columns packed with increasingly small particles is reaching an astounding pace. The performance of LC must go hand in hand with improvements in MS speed for the field of proteomics, but I am not concerned about that as an issue. I agree with Koen that there is a strong possibility that 2D-LC can be made modular and perhaps in chip form – at that point, the market will massively increase. Miniaturization and robustness is certainly the future.

JY: Put simply, it must be faster with greater peak capacity.

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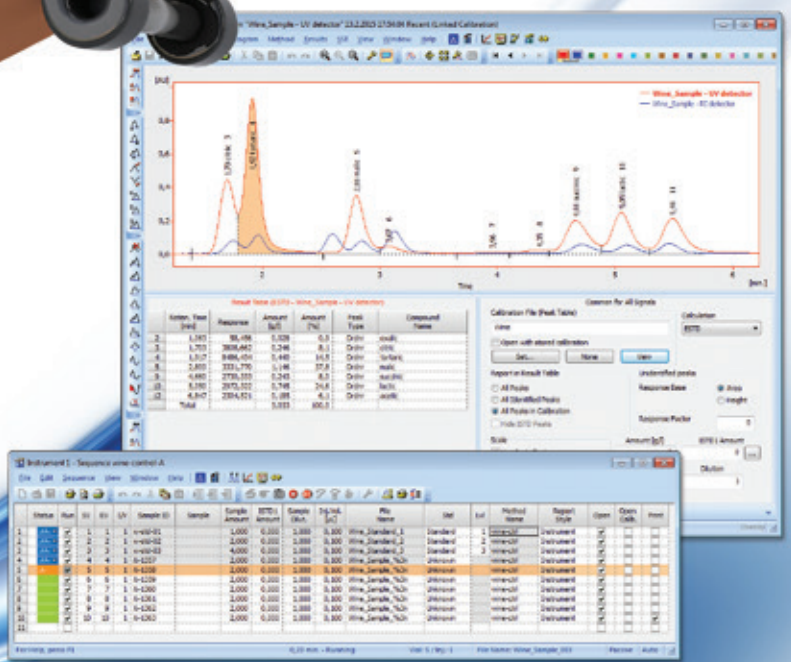


Rebooting Application Notes

App notes are undeniably useful, giving you access to expert knowledge in your field or helping you understand a new technique. But what if they went beyond simply providing static information? Enter AppsLab.

The Thermo Scientific™ AppsLab Library of Analytical Applications takes the next logical step in providing you with the information you need in your lab. Not only does it allow you free access – through an intelligent search and filter system – to nearly 2000 applications (and growing) from Thermo Fisher Scientific's huge team of scientists, it also enriches newer app notes with the eWorkflows used to create them. Here, we speak with Product Manager Susanne Kramer to find out more.

AppsLab has been your 'baby' for two years...

That's right. I joined Thermo Fisher Scientific to start working on AppsLab in 2014 and was immediately excited by the novel approach. It essentially follows many other trends in our increasingly online world. If you like cooking, you will have no doubt recognized the advantages of online recipes over traditional cookbooks; not only do they give you detailed access to ingredients and methods, but they also allow you to rate what you've tried and leave comments for others, meaning that recipes can be improved by the community. AppsLab offers that same level of interaction, but goes another step forward by providing users with eWorkflows that connect them directly to the actual methods and processes used – analogous to a recipe taking control of your food processor and setting the temperature and timing of your oven (although analytical 'recipes' are significantly more complex!).

As product manager, it is my role to constantly consider what our customers need and what they are interested in; in fact, my initial discussions with customers are still shaping ongoing developments. I also spend a lot of my time supporting our application chemists around the world, helping them add their content to the ever-growing library. And I listen to feedback – both internal and external – to ensure that we can continually improve AppsLab. I'm also responsible for expanding it into many more techniques and instrument types. It's a constantly evolving tool in that respect.

How would you describe AppsLab in a Tweet

"An online portal for easy access to rich application content with eWorkflow integration. Methods, workflows and more! #oneclickworkflow"

How can people interact with AppsLab?

A user would begin by searching for an application they are interested in. You could start by entering the name of a compound and a matrix, which would retrieve a list of relevant application notes that can be further filtered by other parameters, such as instrument type (currently LC, IC, GC, GC-MS and LC-MS) or even method run time – it's really very intuitive. Once you've found an application note, you gain access to a lot of information; for example, a list of compounds and retention times, a detailed method, the system setup (including columns and consumables), sample preparation steps. At this stage, you don't need to register – it's freely available to anyone who wants to use it. If an eWorkflow is available, you can register (for free), directly download the file, and be up and running with just one click.

In some cases, you may find an application, but still have additional questions, in which case you can "Ask the Expert." Your question will be forwarded to the author of the application or another member of the team. If you don't find a specific application, you can also use AppsLab to suggest one.

Finally, as noted earlier, you can also comment on applications – perhaps providing other users with useful advice – and even share applications (or searches) with colleagues through social media or email. We've tried to make it as open and useful as we possibly can.

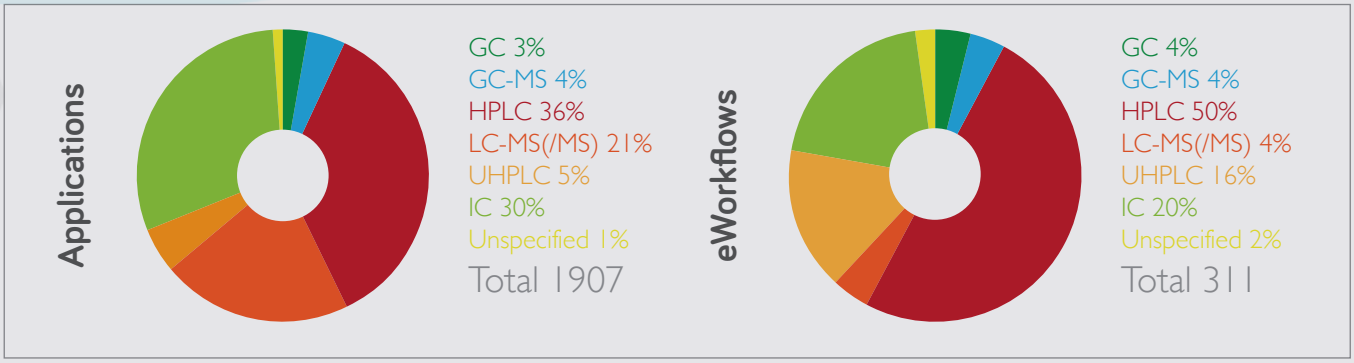
How will AppsLab evolve over the coming months?

We've very recently given AppsLab a new look and feel, which makes it even more user friendly. In the near future, I think users can also expect to see integration with eCommerce to make the purchase of columns and consumables in specific applications a much simpler experience (AppsLab already includes part numbers).

Right now, chromatography is very well represented, so another big focus area will be the inclusion of more mass spectroscopy information.

What, for you, is the 'wow' feature?

As much as I love AppsLab as a whole, I would have to say that it's the Thermo Scientific™ Chromeleon™ eWorkflow integration that really makes it stand out from the crowd. And I should add that an eWorkflow is much more than a method. In addition to the instrument method, eWorkflows can also include data processing steps and a report template that includes embedded calculations. eWorkflows hold the potential for huge time savings for our customers.



The Ambition of AppsLab

Christoph Nickel, Senior Director for Informatics and Chromatography Software, shares the concept behind AppsLab and how he sees it evolving.

How did AppsLab begin?

The initial idea was relatively simple. We have around 400 experts at Thermo Fisher Scientific, working on customer-relevant and interesting applications. Those application notes traditionally get turned into PDFs and hosted somewhere on the website – but the customer isn't always able to find relevant applications easily. We wanted to change that. We started thinking about the best way to put our application expertise into the hands of our customers. We recognized that some kind of online tool would help a great deal. And when we talked about the idea internally and sensed some excitement, it became clear that it could be so much more. I remember someone saying, "We should aim to be the iTunes of the application world!" An ambitious vision, and one that we are still working on – but I genuinely feel like we are moving in the right direction. The ability to rate applications – or even suggest new ones – offers a level of interaction that hasn't existed before.

If Susanne's 'baby' is AppsLab, yours is Chromeleon – eWorkflows appear to bring them together...

The eWorkflow integration really is the trump card of AppsLab. Chromeleon allows you to wrap an entire analytical method – from acquisition parameters through processing to the final report template – in a single file. They are easy to create, and even easier to use. Coupling those eWorkflows to specific searchable applications gives our customers a new way to interact with an outstanding Chromeleon feature. In routine environments, this is a huge benefit. It's also perfect for those labs with experienced staff who are introducing a new technique; ready made workflows can get you up and running almost immediately. And of course, all of the methods can be modified; if you're working on something new, you could at least use a similar application as a base, meaning that you don't have to start from scratch.

Where will AppsLab take us in the coming years?

One ambitious plan is to make another logical leap forward. In addition to searching and accessing application information and downloading eWorkflows in AppsLab, we'd like it to evolve into a predictive tool, which will be particularly useful for people working with novel compounds. If a compound isn't in the library, we can at least take a look at similar compounds and start to build a potential method around that. For IC, there is already a "virtual column" where a separation can be simulated – and while that will not be possible for certain techniques, I can see that we have a lot of room to grow in this area. It's ambitious, but achievable – and while it is unlikely to become fully predictive, some information is almost always better than none at all!

Another area that I would really like us at Thermo Fisher Scientific to push – and for the community to embrace – is the potential for sharing applications through social media. To that end, I call on everyone to explore what AppsLab has to offer, but then to share, comment and interact – with each other and with us. Together, we can make AppsLab shine!

Check out AppsLab at thermofisher.com/appslab for "Methods, workflows, and more"

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Advancing Enantiomer Analysis

The story behind direct mass spectrometric detection of chiral molecules using MS-PECD – a winner of The Analytical Scientist Innovation Awards (TASIAs).

By Maurice Janssen

The Problem

The measurement of chirality and enantiomeric excess (ee) of multi-component mixtures is of profound importance in pharmaceuticals, food, fragrance and agrochemicals. Normal mass spectrometry (MS) is chirally blind and prior enantiomeric separation (chiral GC, LC) or enantiomer selective chiral complexation is needed before MS detection. Can we eliminate these often quite elaborate prior chiral preparation phases on enantiomers? Or, put another way, how can we detect the two enantiomers and measure their ee in multi-component chiral mixtures directly by MS?

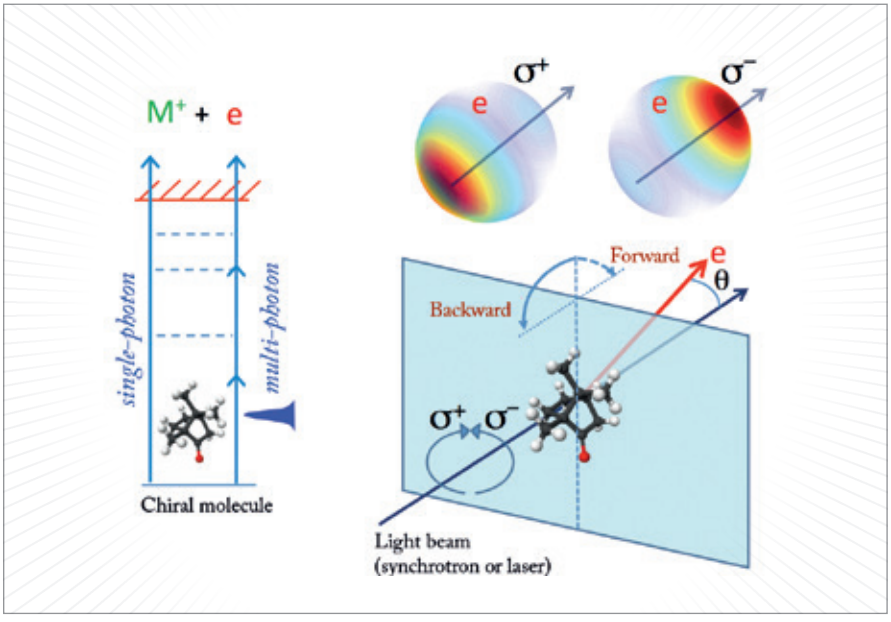

Figure 1. The concept of measuring PhotoElectron Circular Dichroism, PECD (adapted from Ref. 2). A circular polarized light beam (either from a laser or a synchrotron) ionizes a chiral molecule producing an electron and an ion. The three-dimensional angular distribution of ejected electrons shows a (strong) forward-backward asymmetry with respect to the plane of circular polarization, that switches sign when switching the enantiomer from R- to S, or switching the helicity of the light from left- to right circular polarization.

Background

I spent some 35 years in academia. I was trained as an experimental physicist during my undergraduate and PhD studies at the Radboud University Nijmegen, the Netherlands. I moved further into the field of physical chemistry during my postdoc (around 1990) at the California Institute of Technology, Pasadena, and then moved to LaserLaB VU University Amsterdam, where I climbed the academic ranks (assistant, associate and full professor) until early 2015.

In 2008, we (my students, Arno Vredenborg and Wim Roeterdink, and our technician, Rob Kortekaas) had constructed a very exciting new lab in my group in Amsterdam to study the 'intimate dance' of electrons and nuclei in chemical bond breaking, using ultrafast lasers and advanced single particle coincidence imaging detectors. The research in our new coincidence lab was focused on advancing our fundamental knowledge of chemical photodynamics. And we also aimed to actively steer and control these photochemical reactions by playing with the colors and phases of our femtosecond laser. And thus we entered the young field of coherent control of chemical

dynamics, where we wanted to manipulate the outcome of chemical bond breaking with ultrafast shaped laser fields. In fact, lasers have been a recurring theme in my research since my first experimental physics projects in the mid-80s.

At the International Conference on Stereodynamics of Chemical Reactions in Dalian, China, during the fall of 2008, I presented some first results obtained with our latest 'toy' in Amsterdam. On the last day of the conference, I was inspired by a mostly theoretical talk on chiral discrimination in molecular collisions. I had never worked on chiral molecules and after the talk I discussed chirality in molecules with my longtime collaborator, Peter Rakitzis (University of Crete, Greece). Peter pointed me to recent work by someone else in our community, Ivan Powis (University of Nottingham, UK). In 2000, Ivan had published the first quantitative calculations of the asymmetry in the angular distribution of photoelectrons that are ejected following the single photon ionization (using circular polarized light) of chiral molecules, such as alanine. Some 25 years earlier, it was predicted theoretically by Burke Ritchie (Argonne National Laboratories, USA) that when you photoionize a chiral molecule with a circular polarized photon, the angular scattering distribution of electrons would be asymmetric along the propagation direction of the photon. Ritchie used a model potential that was not directly related to a real molecule. However, he predicted large asymmetries (~10 percent) and later this 'forward-backward asymmetry' was coined photo electron circular dichroism (PECD) by Ivan. After that publication in 1976 not much happened – Ritchies' prediction seemed almost forgotten until Ivan started some realistic calculations on real molecules in 2000.

In 2003, Ivan also started to measure this PECD effect experimentally in collaboration with a team headed by Laurent Nahon at the Soleil synchrotron facility near Paris. To photoionize a molecule you need an energetic photon (typically about 9-12 eV) and the Soleil synchrotron had a beam line where they could also control the polarization of that photon. In fact, they could switch the polarization of the beam of vacuum ultraviolet (VUV) photons from left circular polarized (LCP) to right circular polarized (RCP), and to linear polarized, all with good purity. Such a feat was far from trivial for synchrotrons around early 2000. Ivan and the Soleil team measured large asymmetries in the various chiral molecules they studied. The enantiomeric sensitivity of PECD that they observed in the electron angular distribution was typically around 1-10 percent, two to three orders of magnitude larger than the sensitivity of the conventional technique of absorption (vibrational) circular dichroism.

In another collaboration with Uwe Hergenhahn at the BESSY synchrotron in Berlin, Ivan demonstrated the same chiral phenomenon could be observed in X-ray core-level ionization. These exciting new developments certainly proved that PECD could be a very sensitive technique to detect molecular chirality; unfortunately, not many researchers have a synchrotron in their lab. Indeed, access to large-scale facilities that can provide the appropriate photon beam characteristics and the electron-imaging detector necessary is somewhat limited.

The Solution

During my stay in Dalian, I wondered what would happen with the photoelectron angular asymmetry, if you could use (for instance) three circular polarized photons (each with much less energy) to ionize a chiral molecule. I had ultrafast lasers in my Amsterdam lab and, because of my postdoc work at Caltech, I knew that it was very easy to ionize a molecule with such an intense laser through multi-photon ionization. With an intense pulsed laser, the molecule easily absorbs three 3 eV photons (~400 nm) and becomes ionized. The Monday after the Dalian conference of 2008, I was back in Amsterdam and excitedly emailed Ivan, asking if he had any idea how the photoelectron asymmetry was affected by the absorption of multi-photons instead of a single photon. Within a couple of hours, I received a very nice and comprehensive email about his work with single photon PECD and the fact that multi-photon PECD had not yet been demonstrated experimentally. His reply encouraged me greatly.

In our new lab in Amsterdam, we had built a powerful new electron-ion coincidence detector, so we could measure the electron angular scattering distribution in coincidence with the mass of the molecule on the opposing ion detector. So, for every molecule ionized in our set-up (even with different chiral molecules present simultaneously in mixtures), we could obtain the mass via time-of-flight, and the chirality by the coincident PECD of the electron angular distribution on the second detector. And all of this could be done with a commercial ultrafast laser. Suddenly, a trip to the synchrotron appeared to be no longer necessary – detection of enantiomers in chiral mixtures might be possible with our own tabletop instrument. And the idea of MS-PECD was born!

In reality, it took some time before we really started doing multiphoton chiral PECD experiments. But when a new postdoc from the renowned Tata Institute for Fundamental Research in Mumbai, India, arrived in my lab in 2010 – Bhargava Ram Niraghatam – and I began working with Stefan Lehmann, we were able to conduct our first laser-based chirality experiments. Bhargava and Stefan were a wonderful team and made rapid progress. We quickly got multi-photon PECD data on camphor; our tabletop MS-PECD technique worked! We took more time in the lab to improve the quality of the data and we worked hard on the proper analysis of our coincidence data. The multi-photon PECD effect that we measured was large, 8 percent forward-backward asymmetry in the photoionization of camphor at 400 nm.

We submitted our first MS-PECD results to Nature in September 2011, but the paper got rejected rather quickly. Looking back, this rejection was a blessing in disguise as it guided me to contact Ivan again. We needed a better theoretical understanding of multi-photon PECD. I invited Ivan to join us in our laser-based multi-photon PECD project to help us in understanding our results. Ivan accepted enthusiastically and from the fall of 2011, the laser-based MS-PECD project became a wonderful collaboration with Ivan. We published our results on camphor with solid data analysis, theoretical calculations and interpretation in the Journal of Chemical Physics (1) and were very pleased to receive the 2013 JCP Editor Award for groundbreaking research in chemical physics. In fact, we had successfully developed novel technology for tabletop, laser-based multi-photon MS-PECD detection of chiral molecules. If I do say it myself, our solution worked like a charm!

In early 2014, Ivan and I published an invited Perspective paper for Physical Chemistry Chemical Physics that reviewed both synchrotron-based single photon and laser-based multi-photon PECD (2). In it, we outlined the analytical potential of tabletop MS-PECD, especially in combination with the rapid advancements in ultrafast laser technology.

Experimentally, we wanted to demonstrate the potential of MS-PECD in a more analytical application, using various multi-component mixtures of chiral molecules. We wanted to measure the enantiomeric excess (ee) of the various molecules in the mixtures, without doing any prior enantiomeric preparations like chiral GC/LC enantiomeric separation or

Timeline

1976 Publication by Burke Ritchie on the theoretical prediction of a forward-backward asymmetry (PECD) in the photoelectron angular distribution from single-photon ionization of a chiral molecule by circular polarized light

2000 Publication by Ivan Powis of the first reliable calculations of the magnitude of the PECD asymmetry in single photon ionization of real molecules

2003 Publication by Ivan Powis, Laurent Nahon and the Soleil-team of the first measurement by imaging of the full angular distribution of electrons ejected after synchrotron-based single photon ionization

2008 Publication by Maurice Janssen and his Amsterdam group of a novel double Velocity Map Imaging electron-ion coincidence apparatus for laser-based photochemical research

First email discussion by Maurice

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selective chiral complexation (the Kinetic 'Graham Cooks method'). With a new graduate student from Iran in the lab – Mohammad Rafiee Fanood – we decided to make mixtures of limonene and camphor, and prepared mixtures with different ee.

The MS-PECD results turned out great. Of each chiral molecule in the mixture, we obtained the mass from the time-of-flight on the ion detector and the ee from the PECD asymmetry on the coincident electron detector. In June 2015, our results of the ee measurements in multi-component mixtures were published in Nature Communications (3). On the day that our paper was published, my new company – MassSpecpecD BV – was incorporated in Enschede, the Netherlands.

It was actually back in the spring of 2015 when I first took the leap of faith, leaving academia and transitioning to the world of technology transfer, innovation and (start-up) businesses to establish MassSpecpecD BV. We are now based on the campus of the entrepreneurial University of Twente.

Beyond the Solution

The mission of MassSpecpecD is simple: to drive MS-PECD technology into the laboratory-based analytical instrumentation market. We want to provide businesses and researchers who work with chiral molecules with an innovation that can help solve their questions and problems, aid research and development, and improve product quality control. Indeed, we believe that laser-based MS-PECD has unique advantages, capabilities and potential for end users.

The 20th century was the century of the electron. Discovered by J. J. Thomson in 1897, the electron literally electrified society and led to the relatively rapid integration of the transistor, integrated circuits, chips and computers into our lives. Electrical discharges and electron impact ionization have been part of MS technology since its employment in the first mass spectrometers by A. J. Dempster and F. W. Aston in around 1918. We are already some 15 years into the 21st century and for many it will be the century of the photon. When T. Maiman developed the first laser in 1960, some commented that it was a solution looking for a problem.

Fifty years later, it is clear that the laser 'solution' has found its problems. Photons, optics and lasers are rapidly proliferating in our (global) society, solving everyday problems, increasing human productivity, providing real-time communication around the world and opening novel applications that few even dreamt of. However, lasers and photons have found only limited employment in modern mass spectrometers. In fact, lasers have only been used in MALDI systems where few of the special features of photons and lasers are actually used; the tunability, frequency selectivity, short pulse duration, high fluence and high-repetition rate of modern lasers have all been largely ignored, so far, in commercial mass spectrometers – as has the potential for controlled soft-ionization coupled with the facile polarization control of photons. Until now.

The year 2015 was proclaimed by the United Nations as the International Year of Light and Light-based Technologies (www.light2015.org). MassSpecpecD BV was founded during that special year and we are very much looking forward to bringing the unique features and potential of photons and lasers as a novel solution in the analytical world of (chiral) mass spectrometry. In December of the Year of Light, our technology was recognized in The Analytical Scientist Innovation Awards (TASIAs). A good end to a good year!

Maurice Janssen is founder and CEO at MassSpecpecD BV, the Netherlands (www.massspecpecd.com)

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Janssen and Ivan Powis on the potential and merits of laser-based multi-photon PECD

2011 First experimental results of laser-based multi-photon MS-PECD in Camphor by Maurice Janssen and his Amsterdam group

2013 Full paper by Maurice Janssen, Ivan Powis and their team on the experimental results, data analysis and theoretical modelling of laser-based multi-photon MS-PECD

2015 Publication by Maurice Janssen, Ivan Powis and their team on the experimental demonstration of the direct quantitative MS-measurement of the enantiomeric excess of chiral molecules in multi-component mixtures by laser-based multi-photon MS-PECD

Maurice Janssen founds MassSpecpecD BV to introduce table-top laser-based MS-PECD spectrometers to the Laboratory Analytical Instrumentation Market

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Ion Legend

Sitting Down With... Chris Pohl, Vice President, Chromatography Chemistry, Thermo Fisher Scientific, San Francisco Bay Area, USA.

What first attracted you to science?

My dad was an engineer, so I was always interested in technology. But what really inspired me was a seventh grade science teacher. I did receive a chemistry set for Christmas one year (back in the day when you were allowed useful chemicals), but it was the short introduction at school that made me realize I wanted to be a chemist. I actually remember a specific triggering experiment; our teacher was explaining acids and bases with sodium hydroxide and hydrochloric acid, and did the classic titration with a pH indicator (phenolphthalein). She told us that at the end of the titration it was basically salt and asked for a volunteer to taste it. I was the volunteer... The fact that you could take two really poisonous chemicals and mix them together to form something as innocuous as salt really piqued my interest. Science almost seemed like magic. My father helped me build a small laboratory in the basement (complete with its own natural gas supply) and I had an electrolysis set up – but my main interest was explosives. I found that gunpowder is not that explosive – but I had a lot of fun with silver acetylide...

You're clearly an inventive and experimental chemist – does that explain your drive to publish patents?

I guess so! My original goal was to publish 50 patents by the time I retired. I'm now at 77 and don't feel like retiring yet; I've upped it to 100... for now. I remember the very first patent was based on work that I did at Clorox. As an analytical chemist, one of my roles was competitive product analysis – it was like being a detective. Many of the washing powders back then used sodium sulfate as diluent, and I had to use barium sulfate precipitation in a convoluted and, to my dismay, not very accurate analytical method. I became interested in analyzing inorganic ions with chromatography, and Hamish Small had published a paper on ion chromatography in Analytical Chemistry. When I showed the paper to my boss, trying to convince him to invest, he told me I should make one. We bought the components and, sure enough, we made a functioning ion chromatograph. But one critical aspect was a column that could function at high pH. I only had access to silica-based materials, which wouldn't last too long. One evening, when I was driving home from work an idea popped into my head (based on my experience with ion-pair chromatography): if I used a suppressor, I could use C18 silica rather than ion exchange material. I realized that probably wasn't even covered by the ion chromatography patent.

Clorox decided it was "unpatentable," but I ended up meeting with both the CEO and the head of R&D at Dionex, who later bought the rights to my invention (from Clorox) for the price of a single instrument. A few months later, they offered me a job. Dionex filed the patent, which was granted with 'no office actions'. The whole process fascinated me, and I wondered if I could do it again – or was I a 'one hit wonder'?

What about the fish that got away?

When you get a rejection letter from the patent office, in a weird way they are testing your determination – you have to be persistent. We gave up too early on one important potential patent, which was based on my invention of high pH anion exchange chromatography for the separation of carbohydrates. There was some prior art and the patent office argued that it was "obvious." But the previous work was done in the 1950s, and other papers noted that reducing sugars could not be separated at high pH because they would degrade – so it was clearly not so obvious. Nevertheless, we didn't know enough to push at the time. Funnily enough, we wrote "patent pending" in that paper (my second highest cited), and no one was brave enough to challenge it for nearly 10 years...

Which patent are you most proud of?

The first patent is always special but, to be honest, it wasn't all that successful. Two patents top my list. The first is a technique for accelerated (or pressurized) solvent extraction. I think it's the only patent where I did nothing other than come up with the idea. Nevertheless, it essentially kick-started an analytical industry by itself. The second is more recent and was based on hyperbranched chemistry that I originally dreamt up in 2003; it has proven to be very versatile in the manufacture of anion exchange columns. It's kind of like the chemistry equivalent of LEGO, and rather uniquely uses a HPLC pump to alternatively react a linear polymer with two reagents (diepoxide and a primary amine) to coat a resin in a column. In addition to being commercially successful, it's also a completely new synthesis method for anion exchange materials and a pure chemistry patent, which makes it special for me.

Your ideas often seem to stem from thought experiments...

In a way. A lot of my ideas come from keeping my eyes open to what's really happening in the chemistry, and not taking as gospel what people think about a particular system. People can sometimes make the mistake of turning speculation into fact... But it's also about testing the limits and curiosity. The work on my first patent stemmed from wanting to see how far I could push ion pairs – at what point are they no longer ion pairs? It also made me wonder whether there was some other interaction going on – something that was postulated much later on, but which is insinuated in the patent.

Sometimes it's simply about patience. Hamish Small talks of a "mental attic" where he stores ideas until the day he figures out a way to use them. I guess I'm similar in that way.

Full article online: tas.txp.to/0516/Pohl

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