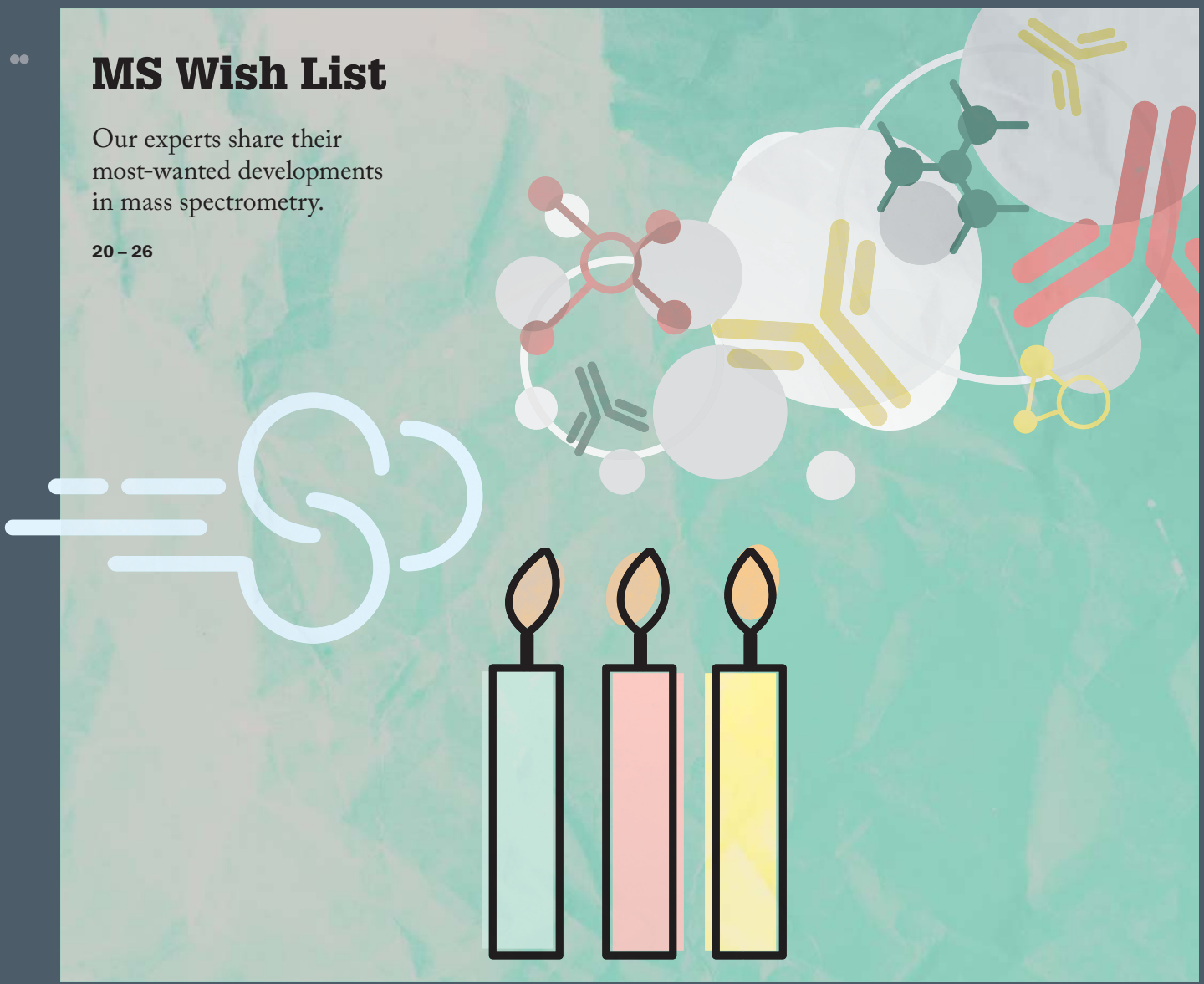
MAY 2019 # 76

the Analytical Scientist™

MS Wish List

Our experts share their most-wanted developments in mass spectrometry.



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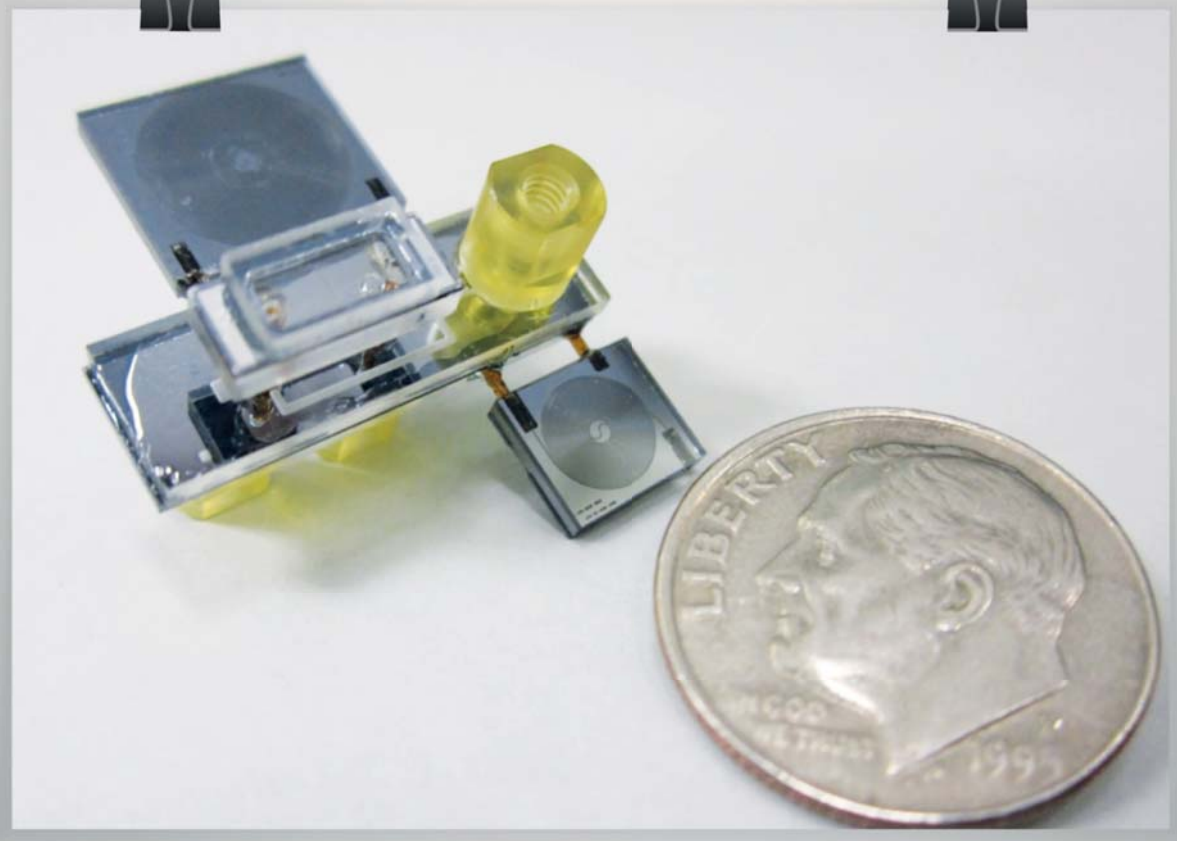


Small Dimensions

Researchers from Sandia National Laboratories (Albuquerque, USA) and collaborators at Caltech have developed a low-power, ultra-high-speed, comprehensive GC×GC system, consisting of microfabricated columns, nanoelectromechanical system cantilever resonators for detection, and a valve-based stop-flow modulator. The researchers demonstrated that the system had a peak capacity greater than 50 compounds per second.

Credit: Submitted by Ron Manginell. Reprinted from JJ Whiting et al., "A high-speed, high-performance, microfabricated comprehensive two-dimensional gas chromatograph," Lab on a Chip (2019). DOI: 10.1039/c9lc00027e

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

Contents

30

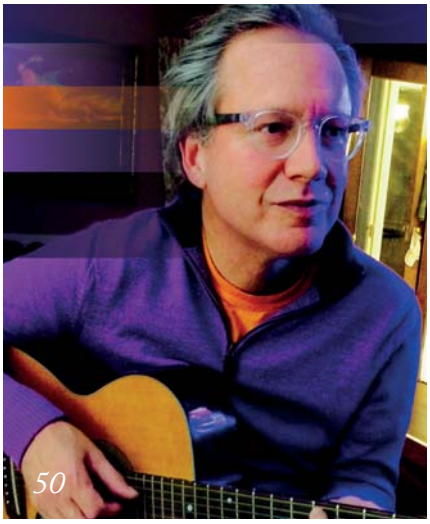
On The Cover

We're blowing out the candles and making a wish, as our experts discuss their most longed-for MS developments.

Upfront

In My View

Features



Reports

Application Notes

Spotlight On... Technology

Departments

Sitting Down With

the Analytical Scientist

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Power for Change

As you make your nominations for the 2019 Power List, consider the wider impact of your choices.

Nominations are now open for this year's celebration of the heroes of analytical science – the Power List. After last year's focus on up-and-coming researchers – the Top 40 Under 40 – for 2019 we're returning to the full Top 100. The brief is simple: nominate the world's most influential analytical scientists – across all application areas and technologies. Submitting nominations is easy – just visit tas.txp.to/powerlist2019 and propose as many names as you like for consideration.

While you are considering the worthiest candidates, I do have a favor to ask: though merit must, of course, be taken into account, please don't forget to at least acknowledge the issue of diversity.

The concept of meritocracy, a term coined by Michael Young (a British egalitarian who later became entirely disillusioned with the idea), is now regarded by many as a myth. Cultural, social and educational opportunities must be taken into account if we are to talk about individual achievements.

As in many scientific fields, the upper echelons of industry or academia in chemistry (and consequently our own power list) remain disproportionally white and male (1). Most of us would like to see greater diversity, and as Laurie Locascio pointed out in our In My View section, that requires all of us to take positive steps to make sure the achievements of underrepresented groups are recognized and their voices heard (2).

Visible role models are an important part of encouraging a diverse workforce, as Locascio notes, "When members of under-represented groups see people like themselves in top positions in government, industry, and academia, they believe that they, too, can succeed as leaders."

To see the positive effects of a more diverse workforce, an immeasurable number of small steps must be made. One of those steps might just be taking diversity into account when nominating an esteemed colleague to our 2019 Power List.

Charlotte Barker
Editor

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2. *L Locascio, "Why science depends on diversity", The Analytical Scientist, Available at: tas.txp.to/locascio. Accessed 7 May 2019.*

Upfront

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

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

As Smooth as Tennessee Whiskey

Researchers sniff out the complex and distinct flavor profile of Tennessee whiskey with SAFE, SIDA and GC-olfactometry

Tennessee whiskey's smooth flavor is the stuff of songs, but its production is a mysterious art form. Like Bourbon, Tennessee whiskey is made from 51 percent corn and aged in charred oak barrels, but Tennessee whiskey has to be filtered over sugar maple charcoal using the Lincoln County Process (LCP). Results presented at the American Chemical Society Spring 2019 National Meeting and Exposition (1) shed light on the effects of the LCP and could ultimately help distillers dial in the flavor profile their customers want.

John Munafo, leader of the study at the University of Tennessee, and student Trenton Kerley worked with the Sugarlands Distilling Company (Gatlinburg, Tennessee) to determine how the LCP affected the flavor of their Roaming Man Tennessee whiskey. First, Munafo and Kerley identified all the odorants in unfiltered whiskey using GC-MS and GC-olfactometry. Next, they worked out which ones were key to the unfiltered whiskey's flavor using aroma extract dilution analysis and quantified these by stable isotope dilution. Analysis of whiskey that had been filtered by the LCP revealed dramatic changes in the levels of some of the odorants. After investigating different LCP parameters, Munafo says, "I was surprised by just how much leverage you have […] and how changing the parameters results in different flavor chemistry in the distillate."

To unravel the complexity of the samples, the researchers had to dig deep into the analytical toolbox. Munafo offers an example, "Labeled isotopes were used to quantitate the odorants in the distillate, using stable isotope dilution analysis (SIDA). Many of the deuterium- or C13-labeled odorants were not commercially available so had to be synthesized in-house. In addition, we used a state-of-the-art high vacuum distillation technique, called solvent-assisted flavor evaporation (SAFE), which requires close attention and about half a day to prep one sample for analysis." Ahead of the quantitative work, the team had to ensure that they were measuring the correct odorants in the distillate, for which GC-olfactometry was critical. "It is a laborious technique that requires a highly skilled technician," says Munafo.

The ultimate aim? Munafo is confident that they will be able to advise distillers on what changes to make to the production process to obtain their desired flavor profile. And although water is Munafo's beverage of choice, he likes whiskey a lot more now that he's been studying it: "The flavor chemistry is fascinating," he says. Controversially, Kerley prefers Bourbon (2)!

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1. *American Chemical Society, "Understanding what makes Tennessee whiskey unique" (2019). Available at: https://bit.ly/2CIGli7. Accessed April 16, 2019.*
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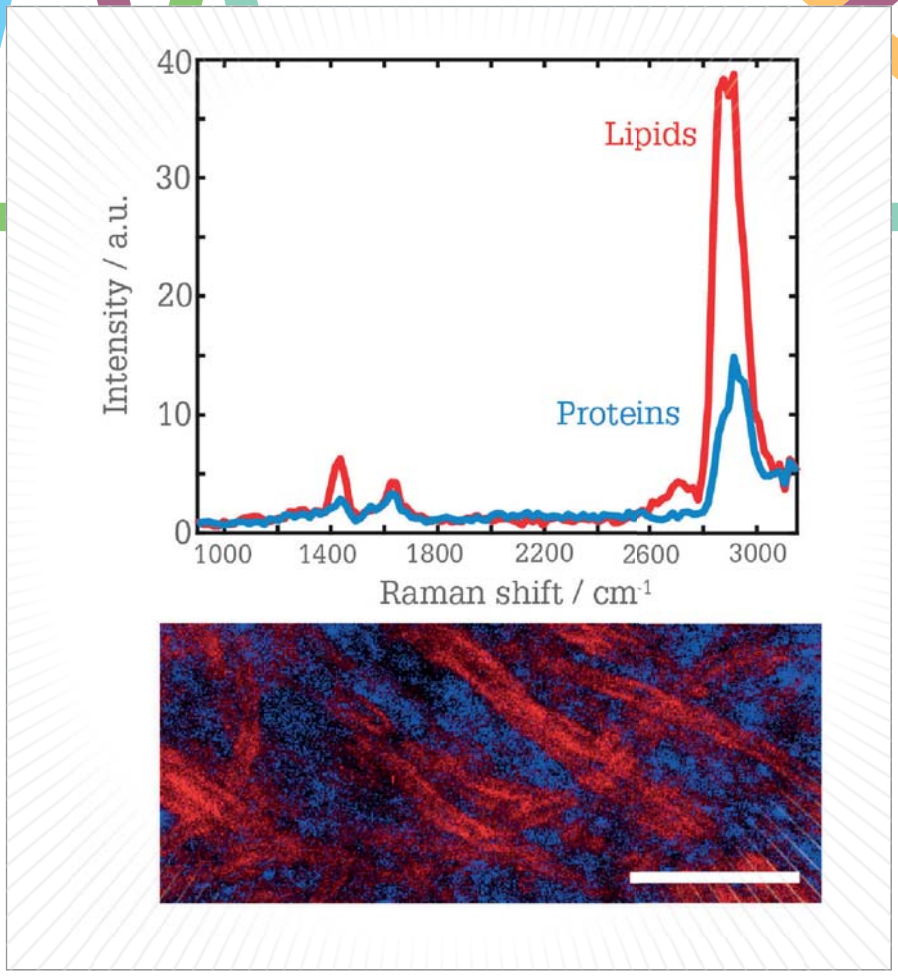
Now Streaming: Spectral Simplification

A preference algorithm originally developed for Netflix could speed up Raman imaging, readying the tool for potential clinical use



The researchers demonstrated their new methodology by using a Raman microscope to obtain spectroscopy images from opaque brain tissue. Scale bar: 20 microns. Credit: Hilton De Aguiar, École Normale Supérieure

Raman imaging has already shown promise as a diagnostic tool, both to identify cancer cells in tissue samples and to detect disease biomarkers. But slow imaging and the production of unwieldy amounts of data have thus far hampered its use in clinical settings, where speed and efficiency are essential. In an effort to solve the problem, a team of researchers at École Normale Supérieure in Paris has turned to a most unlikely source: Netflix.

Repurposing an algorithm originally developed in 2009 as part of a competition to develop more accurate movie preference software for the streaming giant (1), the group hoped to make use of the substantial predictive power to "fill in the gaps" in spectroscopic images. By predicting the make-up of unimaged sections of samples, the analysis time and volume of spectral data necessary to determine a samples chemical composition can be reduced.

"We combined compressive imaging with fast computer algorithms that provide the kind of images clinicians use to diagnose patients, but rapidly and without laborious manual post-processing," said team leader Hilton de Aguiar (2). The team also tackled cost by replacing the camera normally associated with Raman imaging with a spatial light modulator. "The device we used is orders of magnitude less expensive and faster than other options on the market," says Aguiar.

To test the camera's ability to distinguish high levels of chemical complexity, the team prepared samples of brain tissue and single cells, and were rewarded with their newfound ability to acquire spectral data – compressed by 64 times – in tens of seconds as opposed to the minutes or hours taken by other approaches (3).

If further testing on other biological samples proves successful, clinicians may one day gain access to a rapid new diagnostic tool, which – much to the relief of patients everywhere – will presumably not require a monthly subscription.

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2. *The Optical Society, "Researchers Use Algorithm from Netflix Challenge to Speed up Biological Imaging" (2019). Available at: https://bit.ly/2WhB5tS. Accessed May 3, 2019.*
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Ice Cream Bean Dream

Metabolomic investigation of the "ice cream bean" sheds light on diversity in tropical ecosystems

Metabolomic data from the Smithsonian Tropical Research Institute's Barro Colorado Island Research Station in Panama show that pressure from hungry insects is a driver of diversity in tropical environments (1). By studying the chemical defenses of nine different species of Inga ("ice cream bean" trees) and recording their insect herbivores, the authors showed that trees with similar phytochemistry and shared herbivores were less successful – important proof that insect herbivores drive diversity in rainforest plants. Lead author Dale Forrister (University of Utah) tells us more about analytical chemistry in the jungle.

What was the study goal?

The question of how so many species can coexist in tropical forest has been debated for decades. In this study, we attempted to leverage our unique dataset on resources and defensive traits and herbivore–host associations in order to more directly test the role of herbivores. We chose to focus on plants in the Genus Inga, commonly known as "ice cream beans" in reference to their sweet fruit.

What did you find most challenging?

Characterizing the chemical profile of Inga species and quantifying the similarity between species was tough. Every step required some innovation and creativity, from engineering ways to dry leaf samples in an atmosphere of 100 percent humidity to developing the analytical methods for comparing chemical similarity between species.

How did you prepare and analyze the samples?

First, we find and identify our focal species. We then collect leaves that are still expanding and dry them using silica gel. Back in the lab, we grind the leaves and then extract them using organic solvents. LC-MS allows us to isolate and quantify the abundance of individual compounds within the complex mixture of secondary compounds in our plant extracts while the MS/MS spectra provide structural information. The end result is a comprehensive view of the chemical profile for each species.

Did you enjoy the fieldwork?

In many ways, the field work is the best part and fuels the many hours spent in the lab or on the computer. Working in these hyper-diverse tropical rainforests clearly shows how complex the web of interacting species really is.

It was fun to try the ice cream bean fruit too – they're really good and quite different from any other fruit I had tried before! They almost taste like cotton candy.

Where next?

We are currently working in the Yasuni National Park in Ecuador, a forest that harbors three times the diversity that we find in Panama. We want to see if similar patterns emerge and to test our hypothesis experimentally by creating "common gardens" in order to see if species with similar herbivore defenses are less able to co-exist.

Very little is known about natural products in nature. Despite the fact that nearly a third of drugs used by humans come from nature, many directly from tropical forests, the vast majority of species are untested. Metabolomics has great potential for exploring this untapped chemical diversity, offering scientist a powerful new toolbox. Chemicals play a huge role in nature, from defenses to communication they are the medium by which species interact. Being able to quantify this in a meaningful way provides a truly unique perspective.

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Splitting Hairs: Ingestion or Contamination?

How longitudinal sectioning and MALDI-MS can boost the reliability and sensitivity of drug determination in hair samples

When blood and urine tests are not possible (or suitable), hair analysis can be used to identify drug use. But a question mark lingers over a controversial topic: genuine ingestion versus external contamination. Various wash protocols were introduced to counter the problem, but how effective are they?

In an attempt to draw a line under the issue, University of Zurich PhD student Robert Erne and colleagues employed high-end techniques – namely, matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS) – to "systematically investigate the incorporation, decontamination, and extraction processes involved in hair analysis." The team acquired hairs from users and non-users of zolpidem, an anti-insomnia drug, and also zolpidem- and zolpidem-D6-soaked hairs, which were sectioned longitudinally and analyzed (1).

Longitudinal sectioning of hair samples increased the analytical sensitivity versus traditional horizontal sectioning because of the transfer of the drug from the circulatory system into the hair's inner compartment. In fact, even single zolpidem doses were detectable in single hairs. What's more, the method was also able to distinguish zolpidem users from zolpidem-soaked hairs through a decontamination protocol developed by the team; hairs from genuine zolpidem users could not be cleared of zolpidem, even when using the strongest protocol, while zolpidem was "almost completely" eliminated from soaked hairs.

Erne believes the work should help improve hair analysis, particularly in research contexts. In time, forensic applications of the method may also be possible, but such uses "are not the ultimate goal of the research." Next, the researchers aim to explore whether other substances that are known to cause contamination in hair analysis will lead to different results. "The best candidate for these studies is cocaine," says Erne – and experiments with the drug are already underway.

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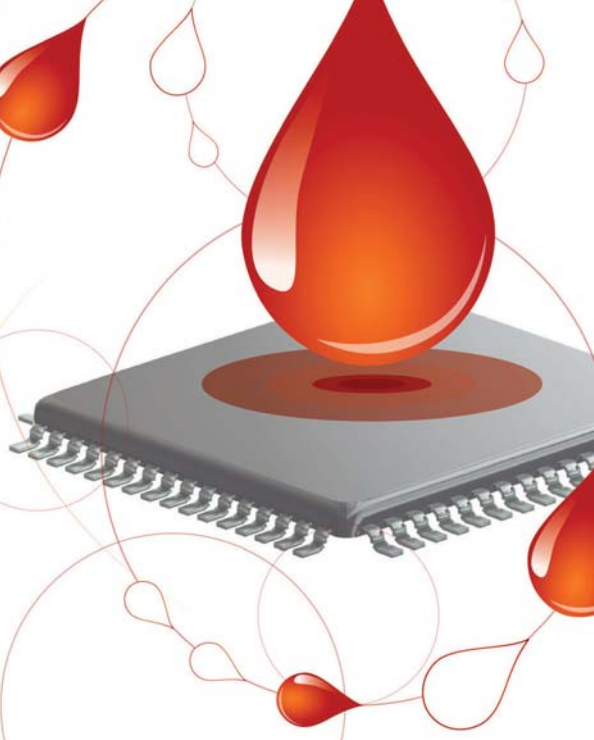

Game of Exosomes

A new lab-on-a-chip device aims to facilitate rapid, noninvasive early cancer detection from a single drop of blood or plasma

Early cancer detection is an elusive, but highly appealing goal – especially in cancers that often go undiagnosed until the advanced stages. One example is ovarian cancer, in which well over half of women are diagnosed at stage III or IV (1). Now, a new lab-on-a-chip device can detect cancer quickly and noninvasively in a droplet of blood or plasma by identifying tumor exosomes (2) – extracellular vesicles that play an important role in cell-to-cell communication.

Although exosomes were historically thought of as cellular "trash bags," recent discoveries have revealed their unanticipated significance. "In the past decade, we have realized that exosomes deliver molecular instructions in the form of nucleic acids and proteins that affect the function of other cells," says lead author Yong Zeng (University of Kansas). When produced by tumor cells, exosomes stimulate tumor growth and induce metastasis, making them ideal targets for cancer detection. But their rarity during the early stages of cancer makes spotting them a challenge that requires an ultra-sensitive biosensor.

Existing methods for exosome detection are not only time-consuming, but also suffer from low sensitivity and poor isolation efficiency. How did Zeng's team overcome these issues? "When particles move close to the sensor surface in microscale channels, they're separated by a small layer of liquid. We used a 3D nanoporous herringbone structure to increase the surface area for exosome capture within the chip and physically push exosomes into contact with the chip's sensing surface, which contains antibodies specific to the proteins present on exosomes." Zeng likens this approach to draining a million tiny sinks to allow items floating on the surface to touch the bottom.

When the device was tested in ovarian cancer patients, it was able to detect tumor exosomes in miniscule amounts of plasma. Specifically, the team discovered that a protein called folate receptor alpha is present in ovarian cancer exosomes, but not in those from healthy controls. The fact that the device identified exosomal folate receptor alpha in the plasma of early-stage ovarian cancer patients underlines its potential suitability as a biomarker detector.

One of the most attractive aspects of the device is its accessibility; the lithography-free fabrication method permits low-cost, rapid, and large-scale production of 3D nanostructured patterns. Without the need for high-tech nanofabrication equipment, the device could easily translate into any clinical setting. So what's next? The team intends to pursue clinical applications by targeting multiple cancer types. Zhang says. "Although there is still a long way to go before we validate the device for clinical use, its potential adaptability to different diseases and biological targets – such as cells and viral particles – gives us great optimism for the future."

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Storming the Fort

We spoke to co-chair Kevin Schug about the 43rd International Symposium on Capillary Chromatography and the 16th GC×GC Symposium – aka "Riva in Texas"

How is the program looking?

ISCC and GC×GC is known for showcasing the latest advances in capillary and comprehensive separation sciences, and this year is no exception. We have worked hard to bring together a high-impact scientific program filled with the key opinion leaders you would expect, as well as a host of new faces.

Topics I'm particularly looking forward to include new GC detector technologies, portable microscale and microchip GC and LC platforms, cannabis analysis, on-line comprehensive LC×LC, bio/omics, and petrochemical analysis.

What's new this year?

The symposia will feature dedicated discussion sessions on GC×GC, state-

of-the-art capillary LC, and GC column selection. In GC×GC, one question to be answered is whether this rapidly evolving technology is ready for prime-time in routine industrial applications. For capillary LC, going smaller has some major benefits chromatographically, but few commercial sources of capillary columns exist – we want to explore why the technology appears to be taking longer to adopt than it should. The plethora of column choices can be bewildering in GC, with continual advancements in column chemistries. In this session, experts from industry and academia will discuss practical aspects to choosing the right column for your application.

What else is going on?

We have a record number of posters that will be on display this year, with numerous poster prizes, an engaging vendor exhibition and many opportunities to network through a Texas-style social program.

If you are newer to the field of separation science, short courses on GC×GC, capillary LC, ionic liquids in analytical chemistry, basic HPLC, and sample preparation for capillary chromatography are available. Join us on Sunday and Monday to prime your pump for the whirlwind of great science to follow throughout the week.

I could go on and on about the location in the heart of walkable and historic downtown Fort Worth, the plans for happy hours and conference dinners to facilitate networking and information exchange, the line-up of breakfast and lunch-time vendor seminars where all of the latest commercial developments will be highlighted, the awards and plenary program featuring the brightest minds in separation science… suffice to say I'm very excited and looking forward to seeing you there!

ISCC & GC×GC 2019 will be held May 13–16 in Fort Worth, Texas, USA, www.isccgcxgc.com.

Look out for highlights from the event in forthcoming issues.

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 charlotte.barker @texerepublishing.com

MS and NMR – the Perfect Couple?

Are two technologies better than one in natural product chemistry?

By Ricardo M. Borges, Professor, Walter Mors Institute of Research on Natural Products, Federal University of Rio de Janeiro, Brazil.

From the search for new drugs to a better understanding of biological systems, the use of analytical tools for qualitative and quantitative characterization of samples is essential. Until very recently, groups that focus their efforts in such procedures used mass spectrometry (MS) or nuclear magnetic resonance (NMR) as separate tools. There has been much debate as to whether MS or NMR is most successful for sample characterization; undoubtedly, both have advantages and limitations.

On one hand, MS has high sensitivity and selectivity but more troublesome quantitation. On the other hand, NMR shows quantitative power and unprecedented reproducibility but poor sensitivity and selectivity. When it comes to structural information, both techniques enable the collection of additional orthogonal "dimensions" to help to gather distinctive information for compound identification. In MS, the selection of a certain ion for controlled fragmentation and detection of the product ions enable us to identify pieces of the structural puzzle for a compound. While in NMR, coupling-constant-based 2D experiments enable us to connect characteristic resonances and even link predefined pieces to find the correct structural formula for a compound. The limitation common to both techniques is how comprehensive the available databases are; MS boasts a richer database compared with NMR, but still has far to go. Considerable effort to increase experimental database size is being made by several groups, and others are developing approaches in database-independent compound identification.

In my field of natural products chemistry, dereplication of natural extracts is used as a screening procedure to collect qualitative data for sample selection in high-throughput drug discovery, most commonly using LC-MS. Briefly, the raw extract will be analyzed using a data-dependent analysis LC-MS – a C18 LC column and an electrospray ionization source is a common setup. Once the data have been collected for a whole sample set, a general processing procedure and an extensive database comparison are applied. Bioactive compounds containing sample are then selected for in-depth studies.

In metabolomic studies, the analytical data of a carefully designed study are submitted to multivariate statistical analysis in search of biomarkers that characterize a certain distinction between groups. Once those biomarker features are assigned, the study is largely limited by whether we can establish their identity. Again, there is a preference among researchers to choose a single technique to collect their analytical data. A straightforward, untargeted metabolomics study using only MS data may miss quantitative information, potentially leading to an inconsistent hypothesis. But if only NMR data is used, that same metabolomics study may fail to detect low-concentration biomarkers, even when a targeted sample preparation is used. According to the Metabolomics Standards Initiative (MSI), putative identification using MS or NMR data is level 2 evidence; whereas coanalysis using authentic standards would be level 1.

An obvious solution is to use both MS and NMR, targeted and untargeted, as the source of analytical data for samples (and maybe even fractions) and interpret them all

together as a single multiresponse vector in a multivariate scheme, allowing us to access very high sensitivity and direct quantitative information in the same study. The certainty of an MS-based putative identification is greatly increased once characteristic NMR features are confirmed, and vice versa.

However, every metabolomics study relies strongly upon databases for identification of their biomarkers and, in many cases, this is limited by inconsistent cataloging. I would like to make a plea for the community to routinely catalog identified compounds with all possible spectroscopic data for future access, especially for natural products chemistry, where we can detect dozens of compounds during dereplication procedures. Large-scale cataloging is essential and attention in this area will drive progress in a broad range of areas within life science.

Expect the Unexpected

Jobs for life are a thing of the past – make sure you develop the tools to survive and thrive, wherever your career takes you.

By Yong Guo, Fairleigh Dickinson University, School of Pharmacy and Health Sciences, Florham Park, New Jersey, USA.

Before Forrest Gump's mother died, she told him: "Life is like a box of chocolates. You never know what you're gonna get."

Life certainly is unpredictable, and so are careers. As young professionals eagerly embark on their first forays into the job market, they naturally tend to take an optimistic view of the future, without being fully aware of the unpredictable nature of their chosen path. A case in point – when I started my first job in the pharmaceutical industry, I would never have guessed that 22 years later I would be starting a whole new career as a teacher.

Young scientists starting out today should expect to have not just multiple jobs but multiple careers throughout their professional life. To live under the illusion that you will maintain one job indefinitely would be naïve, but that's not to say you can't find success and fulfillment – perhaps even more so than if you had stayed on a single trajectory. Some of these changes will be by choice to pursue better pay or prospects, but others may be forced by unforeseen circumstances, such as closures or layoffs. I've experienced my fair share of "reorganizations" during my own career in the pharmaceutical industry, and a question I am often asked is whether there is a way to prepare for this unpredictability. While certain situations are beyond our control, I strongly believe that there are steps we can take to be ready for change. My comments will particularly focus on the pharmaceutical sector, but I hope that young scientists in all industries can find something that fits their situation.

First, you need to establish yourself. Young graduates may be surprised to find that work in industry is quite different from their training, even at the PhD level. Whether you are starting your first job or taking up a new position elsewhere, you should aim to establish yourself as a valuable contributor as soon as possible. For example, the pharmaceutical industry is notorious for its use of jargons and abbreviations – take the initiative and learn them. You will not be provided with a course on such things, and this is a very small part of the learning curve ahead.

You will almost certainly be working

in a team. Enthusiasm for the work and a "can do" attitude will help greatly with your success. Another step towards establishing yourself is to find a niche area in which you are recognized as the "go to" person. This is especially important for PhD scientists. A "jack of all trades" is useful, but also dispensable. Recognized expertise in a specific area leads to more opportunities.

Second, work continuously on your communications skills. I find that the young scientists often have excellent training in the technical aspects, but lack sufficient training in communicating information; for example, writing reports and giving presentations. The corporate world is more complicated than the ivory tower. You must communicate well with a range of culturally diverse colleagues, and do so from various functional perspectives and with different agendas in mind. You must communicate effectively across all barriers. Unfortunately, there is no shortcut to acquiring these skills. The only way is to pay attention and practice, practice, practice.

Third, explore new areas and passions. When you are happy in your role, there is a danger of becoming complacent as the everyday becomes routine. It is important to remember that employer requirements often change over time, potentially making your expertise (and maybe even your role) redundant. Many pharma companies offer job rotation programs – a great opportunity to broaden your skills. Another option is to pursue an MBA or business certificates; additional training in business may be an asset when new opportunities knock on your door.

Fourth, build your network. Scientists tend to exist in small social circles within their discipline. While that may be ideal for a certain level of discussion, you may find your address book is rather thin when it comes to pursuing new opportunities. Step outside your comfort zone and expand your network – not randomly, but with your long-term goals in mind. Joining and volunteering your time to scientific and professional organizations is a great start.

Your network should include a mentor and preferably also a sponsor. What's the difference? A mentor advises you, but a sponsor (a senior staff member at your current employer) actively advocates for you and helps you to advance. Sponsors are hard to find but tremendously valuable.

Finally, keep an open mind regarding future opportunities. Unexpected opportunities may come your way, but you should focus on those that are aligned with your long-term goals. When such an opportunity presents itself, you need to be ready; sometimes the perfect opportunity only comes along once and you must be ready to seize it when it does.

Unifying Chromatography on All Fronts

It's time to break through boundaries in the world of separation science.

By Lydia Kisley, Department of Physics, Case Western Reserve University, Cleveland, Ohio, USA.

While reading the January 2019 issue of The Analytical Scientist, the use of the term "unified chromatography" by Caroline West to discuss the combination of supercritical fluid chromatography (SFC) and high-performance liquid chromatography (HPLC) jumped out at me (1). I have myself used "unified chromatography" to describe linking experimental single molecule observations with statistical modeling of ion-exchange chromatography (2). And West and I are not the first – this "unified" terminology can be traced through generations of separation scientists back to the 1965 seminal work "Dynamics of Chromatography" by J. Calvin Giddings (3), which was followed by "Unified Separation Science" by the same author (4).

What does it really mean to "unify"? To me, unification brings together specialties that might seem disparate, but ultimately have a common goal. In the medical field, the "bench-to-bedside" approach connects basic research to disease treatment in the clinic. In physics, linking electromagnetic, weak, and strong forces is pursued in the Grand Unified Theory. Can we similarly take a "Peak to Production" or "Grand Unified Separation" approach?

There are several ways in which we can unify. First, we can link different forms of chromatography. Typically, the physical state of the mobile phase is used to classify instrumentation: gas (GC), liquid (LC), and supercritical fluid chromatography (SFC). As West highlights, these chromatographic methods can be combined (1) – creative instrumentation compatible with all three mobile phase conditions have been demonstrated and are even commercially available. Multimodal and gradient columns unify stationary phases by combining different physical and chemical mechanisms that cause separation, yielding improved selectivity and capacity, along with a reduction in equipment and materials.

Unification can also connect experimental, computational, and theoretical approaches across the molecular, analytical, and industrial scales at which chromatography is studied. Starting from the molecular level, computational molecular dynamics, statistical mechanical modeling, and single molecule experiments seek to understand the fundamental energetics and kinetics that lead

to separations. Moving up a level, we observe the average of many molecules eluting in analytical-scale separations. Experimentally, novel stationary phases with new chemistries and nanomaterials are developed, while ensemble contributions of flow and kinetics are quantified with modeling fluid mechanics and the van Deemter equation. Finally, large amounts of material must be separated robustly and reproducibly in industry. Iterative screening for method development is commonly required, but data scientists can help make informed choices using library-based computational approaches (5).

How can we promote and accelerate unification? I think an easy first step is to stop isolating ourselves in strict classifications, whether that be experimentalist/theorist, academic/industry, physicist/chemist/engineer, or GC/LC/SFC-user. Collaboration, reading the literature, and attending new conferences with a broader perspective can lead to exciting research directions. For example, during my PhD, despite writing my entire thesis on chromatography, I never actually ran a column! I instead relied on my collaborators to do the "real" separations while I hung out with my microscope. My lack of instrumental knowledge became apparent within the first few weeks of my postdoc, when I struggled to use a fast protein liquid chromatography (FPLC) setup. If only I had shadowed my former collaborators, I would have understood some of the challenges and been able to pursue more informed research directions.

Inspired by this idea of unification, I'm looking into equipping my new lab for HPLC in addition to my microscope so I can study chromatography on multiple scales, and I'm expanding my research from ion-exchange chromatography to chiral separations. These are small steps, but I believe if our field as a whole takes a more interdisciplinary approach, we will be in a better position to tackle the grand challenges – predicting separations and developing routine instrumentation that can handle any separation. I hope others will join me in my quest for unification.

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1. *C West, "Breaking Barriers." In: Landmark Literature 2018: Part 1. The Analytical Scientist, 72, 27 (2019).*
2. *L Kisley et al, "Unified super-resolution experiments and stochastic theory provide mechanistic insight into protein ion-exchange adsorptive separations", Proc Natl Acad Sci USA, 111, 2075–2080 (2014).*
3. *JC Giddings "Dynamics of Chromatography"; Marcel Dekker, Inc., New York, 1965; Vol. 1.*
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Technology with a Real Edge

Since scooping a TASIA in 2017 with their µPAC™ technology, PharmaFluidics has made a real splash. We caught up with CEO Johan Devenyns to find out what's new at "the micro-chip chromatography company."

For any readers unfamiliar with PharmaFluidics, could you give a little background?

PharmaFluidics was formed as a spin-out at Vrije Universiteit Brussel in late 2010 and set up shop as a microfluidics consulting boutique. Since 2014, we've focused exclusively on microfluidic devices for chromatography. In late 2017, we launched our flagship product – the µPAC nano-LC column – onto the market, which immediately attracted a lot of interest, including an innovation award from The Analytical Scientist! In brief, the µPAC is a microfluidic chromatography column with its pillar array stationary phase backbone etched out of silicon wafer; this technology provides exceptional resolution, sensitivity, robustness, reproducibility and retention time stability. Following feedback from our users, we added the µPAC trapping column to our portfolio in 2019.

Proteomics researchers have been keen early adopters of the technology – why?

Having decrypted the genetic heritage of numerous species (including humans), biologists now want to characterize the totality of the proteins in an organism or even a single cell. Technically, this is extremely difficult because of the proteome's complexity and highly dynamic concentration range –and the absence of amplification techniques comparable to PCR; it is not surprising that researchers in this field welcome new technology that can make the task a little easier. The uniform pillar arrays of the µPAC allow for the longer gradient separations needed for complex biological samples, for example by eliminating the need for prefractionation (read more about proteomic analyses with µPAC at tas.txp.to/perfecting.)

What's new for PharmaFluidics?

The company is steadily expanding as µPAC moves into more labs. It's been great to hear from our customers about the difference it is making to their research and to feel that we have played a part in some truly groundbreaking advances (see "µPAC at the Edge").

We have expanded our operations, and we will commission a second dedicated assembly and QC line at our Ghent facility.

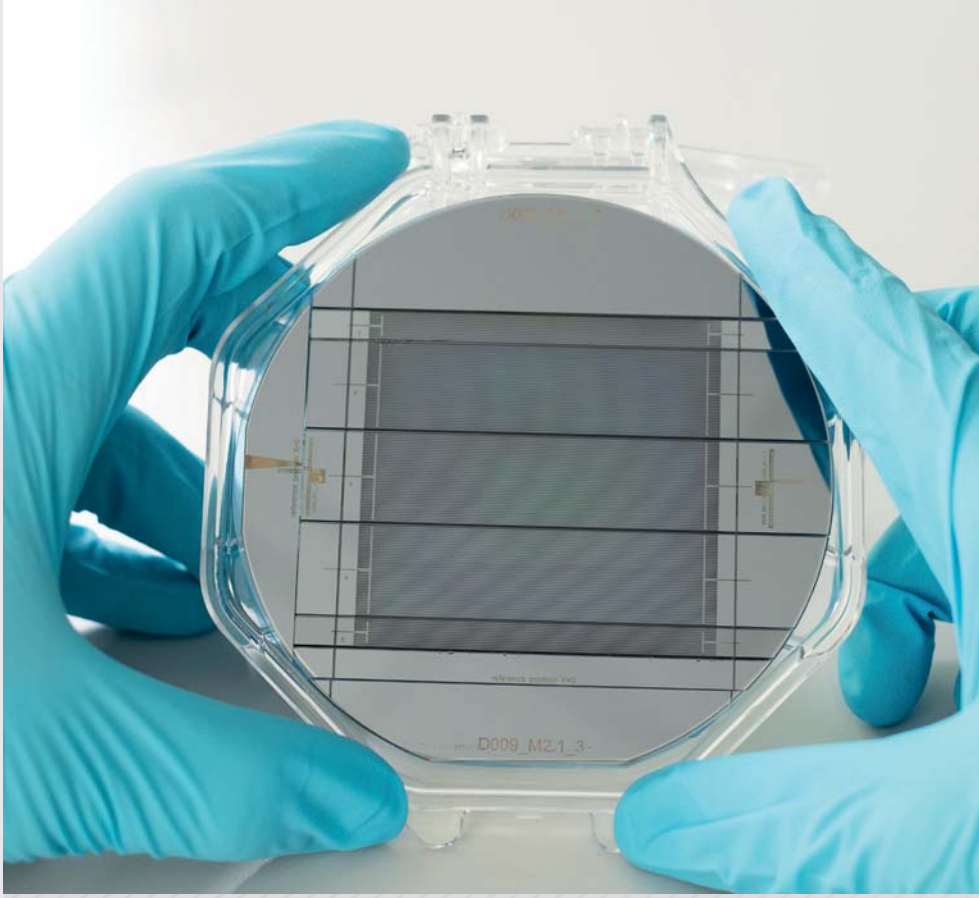
A special highlight of 2019 so far has been receiving the European Proteomics Association (EUPA) Award. We are very grateful to EUPA and the proteomics community for recognizing the contribution of µPAC to improving and standardizing nanoLC/MS proteomics workflows. It is an achievement that belongs primarily to our pioneer users and product champions, who are setting new standards, breaking records and advancing the field of proteomics.

What's next?

Looking to the future, we are focused on two main growth areas: deepening our reach in the proteomics space and broadening access technology in other chromatography segments by accommodating higher-flow-rate regimes.

The company has become well known as a solution provider for deep-dive proteomics, where sensitivity is paramount. As proteomics moves closer to the clinic, other benefits of µPAC – namely, retention time stability and reproducibility – have started to attract more attention. For longitudinal, large-cohort or multiple-site studies, the robustness and reproducibility of all steps in the HPLC-MS-proteomics workflow are of paramount importance, as this is critical to build large data pools with maximum statistical power and minimal noise and bias. Many of the greatest workflow challenges for large-cohort (clinical, diagnostic) proteomics relate to lab-to-lab reproducibility, method standardization, and scalability – all areas where µPAC has a real edge.

Currently, µPAC is mostly used in nanoflow regimes, so the second focus is to



move into areas that are using higher-flow-rate regimes, such as routine biopharma applications. Building on the design and engineering of the popular µPAC trapping columns we launched earlier this year, we will introduce our Capillary LC µPAC Column at ASMS 2019, and start taking pre-orders for deliveries in September.

Has the company faced any "growing pains"?

Early on, it was clear that high-end proteomics was the most suitable entry point into the analytical HPLC market, compatible with PharmaFluidics' capabilities and limitations. Having a clear focus on one high-end, niche market proved to be the right thing to do, allowing PharmaFluidics to get a place on the scene and to grow at a manageable rate. Now that we have further developed our manufacturing and supply chain resources, we are ready to take on broader market segments.

Our customers are now spread over a much wider area, which makes our operations more complex. Although it makes for long days, it's a nice problem to have!

µPAC at the Edge

As uptake of µPAC rises, it's incredibly exciting to see how the technology is contributing to advances in biology and beyond. Here are just three projects that hit the headlines in 2019…

Tree of Life

At the EMBL Wellcome Genome Campus Conference in March 2019, the Matthias Mann Group (Max Planck Institute, Munich, Germany) presented the quantitative proteome atlas of 100 organisms across all three kingdoms, fingerprinted thanks to the high retention time stability and reproducibility of the µPAC. The Tree of Life is the largest open access proteome data set ever reported, with more than 250,000 proteins.

Labs around the world can use the open access database together with µPAC and machine learning to predict a retention time fingerprint for each individual protein in the Tree of Life – the potential for hyper-resolved target data deconvolution is immense.

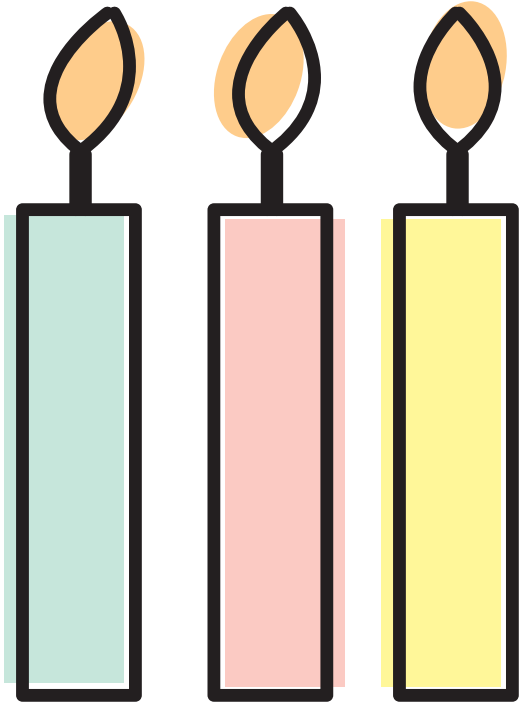
Doubling Up on Single Cells

Single-cell proteomics is poised to revolutionize many fields of biological research, with important implications for therapeutics, discovery, genomics and translational research. In a presentation titled "Double protein IDs in Single Cell protocols", Karl Mechtler (Institute of Molecular Pathology, Vienna) explained how his group have identified 3,500 proteins in a 10 ng HeLa cell sample using the µPAC LUMOS workflow at EUPA Potsdam in March 2019.

Deep-dive DIA

Also at EUPA Potsdam, Lukas Reiter (BIOGNOSYS, Schlieren CH) presented results from 9,000 quantified HeLa proteins, covering five orders of magnitude in a µPAC–HFX–DIA workflow. Single-shot quantitative deep-dive µPACTM-DIA proteomics has great potential for pathway elucidation and endpoint design during clinical trials, and later in body fluid multiplex/panel proteome diagnostics.

MS WISH List



What development in mass spectrometry would you most like to see and why? We posed this question to a diverse group of MS users – here's what they had to say.

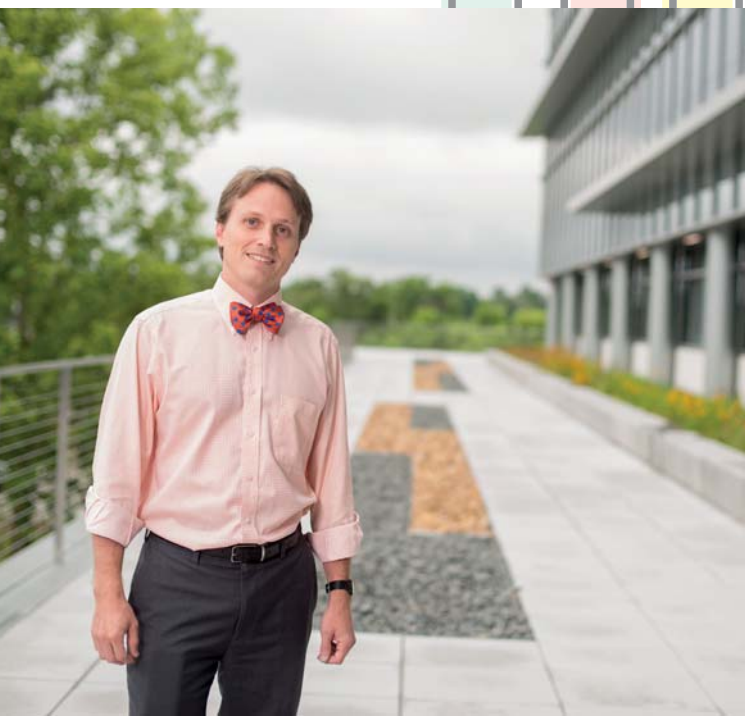
My priorities would be affordability and accessibility. State-of-the-art MS instruments offer substantial advantages for understanding biological diseases and translating advances towards solving human health crises. Discoveries in this realm are made possible by large-scale proteomics, metabolomics, and lipidomics efforts, which rely heavily on MS instrumentation. Though there are many laboratories that have access to high-end MS instrumentation there are many more that do not because of the cost. I wish there were more centralized facilities that researchers could take advantage of to conduct high-throughput MS analyses at substantially reduced costs. Even better, if we can figure out how to manufacture these instruments in a way that reduces purchase costs, I envision many more laboratories worldwide with high-end MS instruments just steps away from their lab doors.

Renã Robinson, Associate Professor of Chemistry and Neurology, Vanderbilt University, Nashville, Tennessee, USA.

From the perspective of using MS in the clinic, there are two developments I'd like to see:

1. With two-minute analysis (no chromatography), flow-injection MS/MS analysis has made population screening possible – with the benefits there for all the world to see in newborn screening. What I would like to see perfected is more rapid chromatographic separation or an "on the fly" separation that doesn't have to be "perfect" like capillary chromatography or micro LC analysis but rather a gas-phase or simpler flow-injection system (perhaps with a little chromatography or separation behavior). Then I can let MS/MS do the rest. Time matters in my business.
2. Much more flexible post-analytical software. I helped develop, at least conceptually, the software needed for two vendors in newborn screening and have used both. Still, they are a challenge to custom design. There seems to me to be a huge opportunity to develop a software application that can be customized to your needs – and yet can work across platforms. Very large enterprise programs exist, but I want something more user friendly – what Microsoft PowerPoint is to presentations or Adobe Photoshop is to photos. Vendors are often slow to meet your needs; software experts are expensive and often either don't finish the job or don't provide support a few years down the road.

Donald Chace, Chief Scientific Officer, Medolac, Swansea, Massachusetts, USA.



One issue with mass spectrometric analysis of small molecules is the source-induced fragmentation that causes confusion in the identification of unknowns for global metabolomics. My ideal instrument would eliminate all source-induced fragmentation, while at the same time reducing ion suppression to create a near-uniform ionization process.

Timothy Garrett, Associate Professor, Department of Pathology, Immunology and Laboratory Medicine, University of Florida College of Medicine, Gainesville, USA.

My wishes for the next generation of mass spectrometers would be:

1. Single molecule detection.
2. Unlimited mass range, and sensitivity in the high mass range that rivals the sensitivity in the low mass range.

Benjamin A. Garcia, Professor, University of Pennsylvania, Philadelphia, USA.

1. For untargeted metabolomics: improvements in sensitivity for low-molecular-weight ions on QTOF systems to make it possible to carry out high-quality, data-dependant acquisition. Plus, improvements in dynamic range, to reduce the loss of lower abundant metabolites and saturation of those at very high concentrations.
2. For imaging, there are lots of areas for improvement, including speed of acquisition, laser resolution, and better software for overlaying multiple image types (MS, histology, and so on).
3. Other "wishes" are to develop approaches to better deconvolve multiplexed MS/MS spectra acquired using MSE, SWATH, AIF, and msxDIA technologies.

Caroline Johnson (Assistant Professor, pictured), Yuping Cai (Postdoc) and Alvaro Santos-Neto (visiting Professor), Yale School of Public Health, New Haven, Connecticut, USA.

If Wishes Were Tweets…

We asked analytical scientists in the Twittersphere what developments they would like to see in MS technology.

@BiswapriyaMisra
Advancements exclusively amenable for absolute quantification.
All data formats open source.
Standardized workflows across vendors for special applications (lipidomics, exposomics).

@ProteinMassSpec
High res ion mobility separation.

@FasciottiMaira
The development of GC-IRMS systems!

@manohar_dange
LC-independent MS… with current resolution and sensitivity.

@ProteinMassSpec
@Shimadzu has a high mass MALDI detector. But everyone needs to start improving intact protein resolution on MALDI. No real advancement in years. Why can't I resolve intact mAb glycoforms... come on!

@Putuma
MS analyzers stripped down to minimize analytical variability in the clinic or point of care. Portable, handheld or adaptable to the operation room.

@TheRealGMW
Faster ETD scan acquisition

@rbharathkumar91
More ions into mass spec and sensitive

@techpeasant
The perennial desire! Also, front optics which never get dirty.

@alan_jarmusch
Open software that is simple to use. Free exchange and deposition of MS data. Improvement and expansion of MS2 databases (e.g. @GNPS_UCSD) for identification of chemicals in samples in untargeted experiments. Portable instrumentation with MS2 capability, e.g. @AstonlabsPurdue.

@SimonaFrancese
A decent PMF software to come with the instrument.

@MohRefai
Pocket size mass spec.

@kiddingyou
Hardware is good enough for me now. I wish there was more intelligent instrument control and definitely downstream data analysis. It's a Wild West world out there in terms of software.

@olgavitek
There is a lot of recent progress in open-source software and algorithms, though.

@ProteinMassSpec
We should all post our go-to open source workflow! #opensource #massspec.

@manohar_dange
Yes, one also learns a lot from using an open source platform... we can actually see the steps of processing in the code. Very useful for beginners. I enjoyed it.

Want to join the conversation? Follow us @tAnaSci

My comments are related to the use of MS in research that involves small- and medium-size molecules. The combination of full automatization, miniaturization and portability would have an important impact on field research, particularly in ecological chemistry and biological studies. For example, studies on pollination and on plant–plant or plant–insect interactions require the detection and identification of trace amounts of substances that are released in a time-dependent manner. Beyond on-the-spot work with individual instruments, a network of autonomous, Wi-Fi-connected mass spectrometers performing real-time monitoring has many applications in biological studies, but also in environmental control and for security purposes.

A field that requires substantial advance is the connection of fully automated monitoring and sample preparation with MS. Highly selective, specific probes capable of scanning, sweeping or somehow covering a surface, while filtering molecules and performing atmospheric pressure ionization, would facilitate many analyses.

Substance identification would be enhanced by computational algorithms that combine the large amount of existing knowledge about the spectra of known molecules, with the physical and chemical principles that govern fragmentation patterns to make reliable predictions about mass spectrum–structure relationships.

Looking further into the future, perhaps a combination of specially designed ion traps may allow us to obtain the proton NMR spectra of molecular ions and fragments. This would greatly facilitate structural identification, especially in the case of different types of isomeric structures.

Elena Stashenko, Director, Research Center for Biomolecules - CIBIMOL Research Center of Excellence, CENIVAM Universidad Industrial de Santander Bucaramanga, Colombia.

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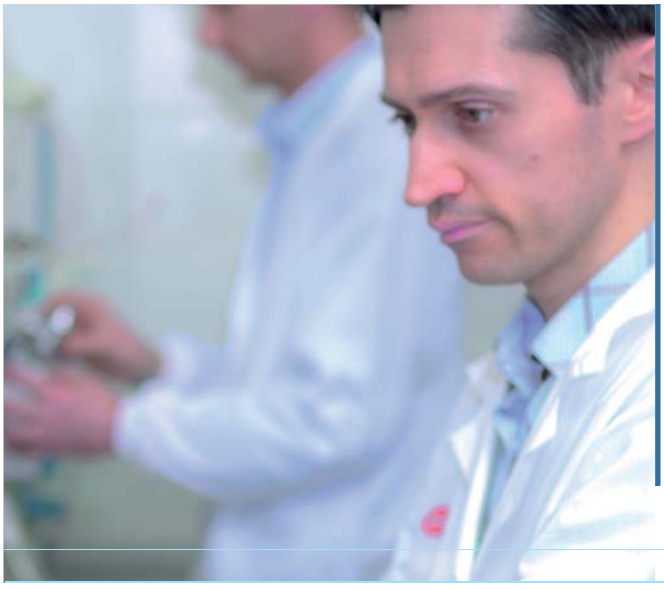

1. A new generation of MS instruments that are miniaturized and portable, totally connected to the web/cloud, and affordable as a field screening tool to solve food quality, authenticity and safety issues.
2. MS instruments that can provide both low- and high-resolution solutions depending on the specific need (or can be integrated with different modules in a sort of "progressive investment") – whether for a targeted quantitation of a single analyte in a simple matrix or a metabolomics approach in a complex one.
3. Significant improvements in post-analytical software for handling and elaboration of big data, in terms of flexibility and easy-of-use, linked to the new frontiers of artificial intelligence and automation.
4. Evolution of reliable software dedicated to ambient ionization MS interfaces, and improved quantitative potentialities of those techniques for direct measurements on food, without sample preparation.

Michele Suman, Food Safety and Authenticity Research Manager, Barilla, Parma, Italy.

In art analysis, we usually only have one chance to analyze a single microsample that can contain mineral pigments, proteins, polysaccharides, lipids and other polymers, such as polyterpenes. Our goal is to identify all of the components but also to characterize degradation caused by age or interactions with other components within the sample, so we need highly sensitive, versatile, robust, and affordable instrumentation.

My "most wanted" advances in mass spectrometry are:

1. Increased sensitivity for trace level analysis.
2. Very high resolution/mass accuracy combined with high scan speed (toward FT-ICR resolution, but faster).
3. High versatility to enable easy shifting between analyses of different kinds of molecules in a single micro-sample.
4. Improved ion mobility for high-resolution conformational analysis plus more robust MS/MS.
5. Indirectly linked to MS: more integrated and robust data processing tools for analysis other than bottom up proteomics.
6. More options for high spatial resolution imaging capabilities for MALDI with high sensitivity.
7. Portable MS that can reliably analyze low and medium molecular weight molecules.
8. Lower purchase, operation and maintenance costs.

Julie Arslanoglu, Research Scientist, The Metropolitan Museum of Art, New York, USA.

What do **YOU** think? Tell us about your mass spectrometry dreams – email charlotte.barker@texerepublishing.com

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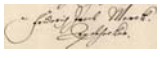
1668
Merck beginning with a pharmacy in Darmstadt, Germany



1827
Transformation into a research-based company

1850
Establishment of partnership EMD

1935
Sigma Chemical Company Founded

SIGMA

1951
Aldrich Chemical Company Founded

ALDRICH

1966
Supelco Founded

SUPELCO, INC.

1975
Merger to form Sigma-Aldrich Corporation

SIGMA-ALDRICH

1993
Supelco Acquired by Sigma-Aldrich

SUPELCO

1995
Establishment of Merck KGaA and public listing

2010
Acquisition of Millipore

MILLIPORE

SigmaAldrich.com / Supelco

Lit. No. MK_AD2917EN 03/2019

Founders of Supelco

Dr. Walter Supina
Born in Hartford, Connecticut, Walt obtained his doctorate in chemical engineering in 1960

Mr. Nicholas Pelick
Born in Scranton, Pennsylvania, Nick obtained his master's degree in biochemistry in 1964

Footprint of Supelco

1966: Enters GC business with adsorbents and packed GC columns
1971: Enters carbon adsorbent business with Carbosieve®
1979: Enters HPLC with SUPELCOSIL™ line of stable 5 µm spherical particles with true monolayer bonding
1983: Enters the air sampling market by introducing a line of solvent desorption tubes for industrial hygienists to help protect workers from being exposed to toxic chemicals
1985: Enters sample preparation business with Supelclean™ SPE tubes
1993: Launches SPME fibers
1994: ISO 9001 – Quality Management System
2006: Sigma-Aldrich acquires Astec®: leader in chiral chromatography
2007: First to market globally Fused-Core® particles jointly with Advanced Materials Technology, introduced Ascentis® Express
2018: Supelco® expanded to include all analytical products from Merck KGaA for a comprehensive range of analytical techniques

TODAY

2015
Acquisition of Sigma-Aldrich

Comprehensive analytical portfolio

Sample Preparation,
Chromatography,
Titration,
Spectroscopy,
Photometry

Reference materials for accurate results

Cerilliant®, Certipur®, TraceCERT®

Chemical Standards and
Certified Reference Materials

Analysis with mobility and ease-of-use

Analytical test kits
and test strips

HYPER-FAST GC

– and a New World Perspective

In the past two years, we've moved on from beginner's luck to results obtained through expertise. Our work is being received with increasing enthusiasm, and we've even claimed the top spot on The Analytical Scientist Innovation Awards 2018. However, the story of field flow thermal gradient gas chromatography is far from over.

By Peter Boeker

It is a shame that I cannot tell a truly heroic story about the development of our technology. Sometimes, start-up stories seem almost mythical: the inventors are smarter than everyone around them and fight their way through all the difficulties and bitter setbacks into a bright future! Maybe these stories really exist, but the development of HyperChrom is more of a long-lasting effort – and with less drama!

When I told the first part of the flow field thermal gradient gas chromatography (FF-TG-GC) story – "Beginner's Luck and Hyper-fast GC" – in The Analytical Scientist after Riva 2016, I chose luck and ignorance as leitmotifs (1). Luck has continued to play a big role; fortunately, ignorance is in slow decline! For this article, I was asked to write a retrospective of the last couple of years. Certainly, a lot has happened, but much more is still unclear and the future remains uncertain... So how can I draw any real conclusions before the end of our story?

Today, we have five years of technology development and a lot of experience behind us. And, although this is a short time compared with many colleagues in the field, we now better understand many of the complex processes involved in GC.

To really test a new idea, it takes a great deal of stamina. When I look back at the chromatograms produced by our first experimental setups, I seriously wonder why we didn't give up! The resolution was far from the published results of other researchers and commercial companies. Ultimately, however, curiosity got the better of us; the incentive to finally see the expected benefit of a temperature gradient was simply too strong. What's more, our developmental journey has been consistently positive, with a constant flow of new improvements, ideas and technology – a wonderful way to work. Thus, we finally reached the preliminary peak in developing temperature gradient GC... and towards the end of 2018, we were awarded the top spot in The Analytical Scientist Innovation Awards!

During my doctorate, I studied the philosophy of science and epistemology, which has allowed me to observe my own modus operandi – and though that may sound a little strange, it teaches patience. It takes a great deal of time and experience to gain a real understanding of complex processes (and high-level GC is really complicated), but when one has reached this state – you might call it inner representation – interrelations and new solutions emerge intuitively and creativity can unfold much more effectively. Even scientists and engineers need philosophy!

Technical development

I don't know exactly how technical development is organized in larger companies. I imagine that groups work on very structured projects that are well planned and well managed. The small HyperChrom team can't match that. What does our approach look like? My working day at the Institute for Agricultural Engineering at the University of Bonn almost always begins with a visit to the mechanical workshop, not the laboratory. There, I sit down with our precision mechanic and self-taught designer Wilfried Berchtold. He doesn't always have as much time as I would like, as he also has to design and build large agricultural machines for scientific experiments. When he has time, however, a very creative phase always begins. Most of the time I have hatched new ideas, which we then discuss with a view to realization (and reject some crazy ideas). Wilfried is a guru when it comes to manufacturing processes and can design in such a way that even delicate parts can be produced. Furthermore, he makes sure that anything he designs also looks beautiful!

To exploit the full potential of fast GC, great design is required. Even small errors (for example, cold spots, dead volumes, leakages) lead to dramatic deviations from the ideal behavior, masking possible progress. That's why an excellent scientific workshop – and someone like Wilfried – are so extremely important!

A large part of the technical development of HyperChrom GC is not as obvious as the mechanical components would lead you to believe. Behind the gray aluminum plates, electronics, microcontrollers and firmware are hiding. Here, luck played a big role again. In my e-nose days (around 2003), I met a physicist and technology developer called Martin Theisen. He develops and builds very complicated devices, from medical to superconductivity. Three years ago, we met again and he started to develop a commercial device from the concept of the FF-TG-GC. The result was brilliant - and much better than I had dreamed! When I was already satisfied with something, Martin always found an even better solution – often leading to new measurement methods and new possibilities in chromatography.

Martin has coupled multiple multicore microcontrollers with a dedicated bus to synchronize all controls in real time. And that allows each sub-process to run on its own processor core without latency. The temperature control of the column temperature is incredibly fast and precise. The pressure controls also come from our own development efforts, as the control speed requirements are much higher than with existing GCs. When we were unable to obtain a fan with a speed that could be precisely controlled, Martin developed it himself, with its own processor and a brushless precision motor.

Of course, even the best hardware in GC is only one side of the coin. After my visit to the workshop in the morning, I go to the laboratory to spend time with my colleagues – Jan Leppert, Peter Müller, Miriam Schäfers and Marco Aust. The working group is now larger: four scientists and a technician! Sometimes we see an unexpected tailing in the chromatograms, sometimes

there are persistent carry-overs. Or a mass spectrometer shows strange artifacts in the signals. Just the daily problems of analysts everywhere. We must always focus on drawing the right conclusions so that we can improve our designs. And that means you first need to understand the problems – very challenging with a highly complex processes like GC.

Theory and modeling

It is already a running gag at conferences: Leonid Blumberg, the great theoretician of chromatography, denies the benefits of temperature gradients. This was the case at Riva 2016 and also explicitly at Riva 2018, during his lecture for the Marcel Golay Award. But Leonid would not be the excellent scientist he is if he were not open to a possible revision of this view. And so, Leonid is now helping my colleague and physicist Jan Leppert to settle the debate on the basis of theory and a simulation model. Jan has already created a complete simulation model that precisely reproduces the results of established models, but also takes the temperature gradient into account during the simulation (2).

I sometimes think that numerical models are only necessary for scientists who are too stupid to develop analytical solutions. Because of the enormous possibilities of fast computers, highly differentiated numerical models are now possible. But what about the efforts of our scientific predecessors of the pre-computer era – where the development of analytical solutions was associated with intelligent simplification? Such simplification can get lost in the computer-driven complexity. On the other hand, simplification can also lead to the loss of essential elements... In this area of conflict, we will re-evaluate the many open questions in the field of thermal gradient GC.

Virtual experimentation with a simulation model is in fact an independent area of research. The model must be very well validated and able to reproduce the physical–chemical processes of GC with the required level of detail. Yet, the computing time for the simulation of hyper-fast GC is even longer than a measurement would take!

A new world of analytics

Science philosopher Thomas S. Kuhn has described how innovations in science can create a new world perspective. Thermal gradient GC gives us an inkling of what that might feel like.

The possibilities of temperature gradient GC originally led us to start developing a HyperChrom, but at the same time, we wanted to build a system that was as easy to use as a conventional GC, could use any separation column, and did not require complicated and expensive modules or consumables. As university scientists with a small and ever-decreasing budget for consumables, we also had our colleagues in mind. All of these demands led us to the flow field principle.

The development, however, has gathered momentum of its own, which has led to many further improvements. In the meantime, HyperChrom FF-TG-GC has become a separate field of GC.

To enable exchange of the separation columns, we had to integrate connectors into the analytical pathway, which automatically provides us with a guard column between the injector and the separation column and a restriction to the detector. But we have found that no commercial GC connector is good enough for very narrow peaks. The minimal dead volumes always lead to peak tailing. Also, these connectors are often very delicate to assemble, with tight tolerances between missing leak tightness and column breakage due to excessive connection tightening. Therefore, we had to find our own solution: purged connectors.

Purged connectors posed a new challenge. To generate purging flows in the correct order of magnitude, an exact calculation of the pressures and flows at all junctions is necessary. It is good that we had already solved this problem for the Deans' Switch (3). The electronic pressure controls (EPCs) also had to be designed with special manifolds and additional restrictions. These proprietary developments are all precisely adapted to the conditions and requirements of fast GC – and integrated into the new HyperChrom GC.

The transformation from conventional – slow – GC to very fast GC also requires new thinking in method development. Do the old methods really only have to be translated (4)? Does a one-to-one translation to drastically shortened times make sense at all? Measurement cycles that are shortened by a factor of 20–30 (including cooling) open up new opportunities for method development. Even a double measurement of the same sample requires only 2 min. Different sample quantities or concentrations can be injected to compensate for the lower sample capacity of microbore columns. Alternatively, two different temperature and gradient programs can be used to separate different critical pairs.

Since 10 conventional GC-MS instruments can easily be replaced at the same sample throughput, a particularly powerful mass spectrometer on a high-throughput GC is much more worthwhile. And that allows residual deficits in chromatographic resolution to be compensated by deconvolution or by measuring exact masses.

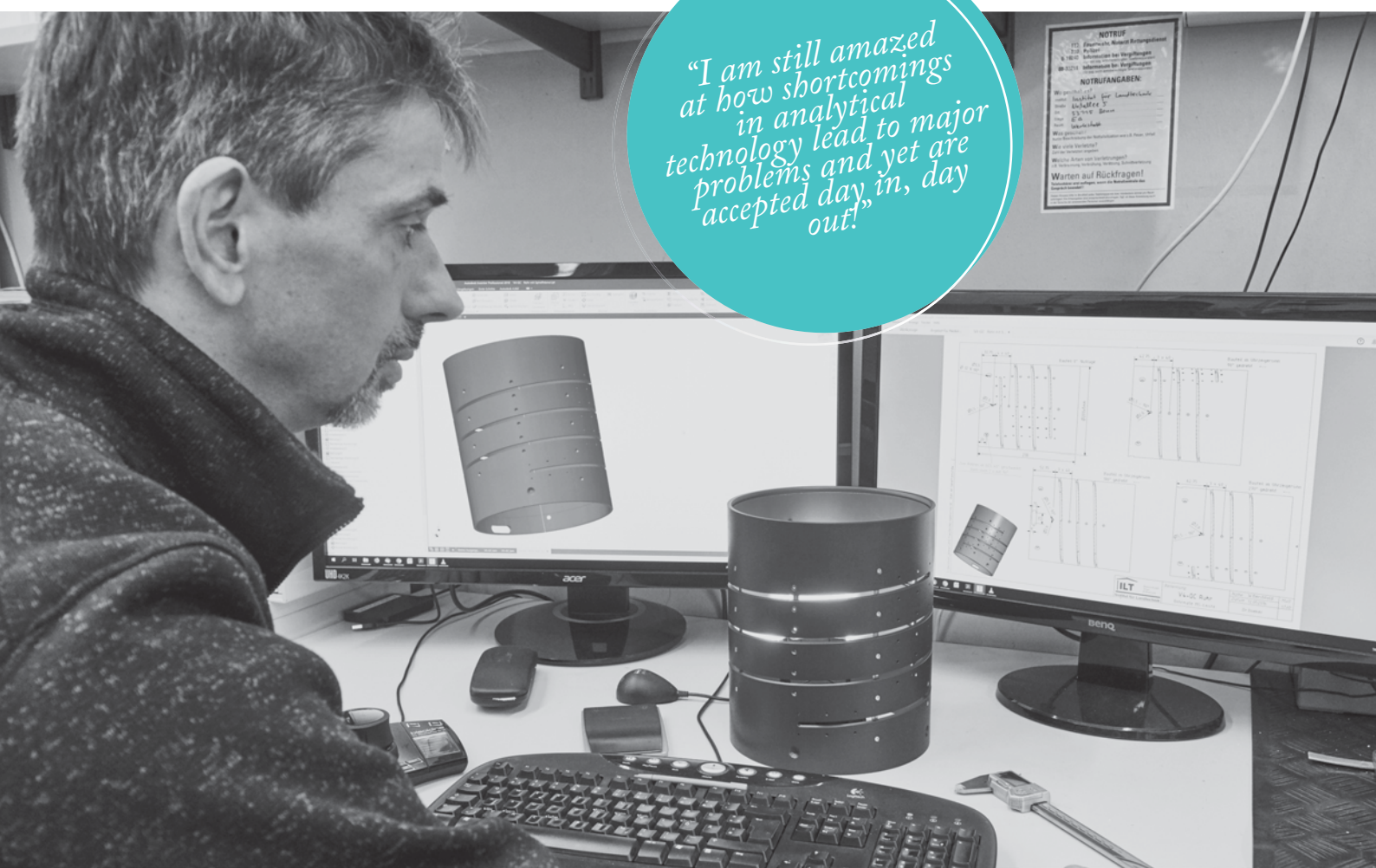
Key customers

How can technical developments be made without future customers? Again, we were lucky. Our first customer was a global petrochemical group, our second customer one of the world's largest laboratory companies.

It's not the big names that are important, though – it's the people behind them. Everyone in the scientific community knows

"I sometimes think that numerical models are only necessary for scientists who are too stupid to develop analytical solutions."

"I am still amazed at how shortcomings in analytical technology lead to major problems and yet are accepted day in, day out!"



Jan Blomberg from his many contributions to chromatography and GC×GC, and Jan did much to encourage us at an early stage. Of course, we all know what challenges lie ahead of the HyperChrom-GC before it can be used in chemical production processes – but we believe we have made a strong start and the positive feedback from customers is highly motivating.

Beyond the scientific community, we found an extremely experienced analyst at our laboratory company customer. Manfred Lux was the first to open our eyes to the world of high-throughput laboratory analysis. The challenge of delivering high analytical quality with thousands of samples is huge. It's a miracle how this is even possible, given the ever-changing samples and unavoidable technical malfunctions!

Manfred's experience was invaluable in showing us how non-discriminatory sample injections are possible, and which components and materials deliver the best results. GC is also an empirical science!

The benefit of perspective

I came to GC relatively recently and one of the advantages of being an "outsider" is a different perspective – the ability to see things that are unfiltered by experience.

I am still amazed at how shortcomings in analytical technology lead to major problems and yet are accepted day in, day out! For example, with the injector: why do we have to be constantly annoyed by septum particles that fall into the liner, especially during solid-phase microextraction (SPME) measurements? Why do we have to endure septa that start to leak? Do outgassings of a new liner O-ring really have to appear in the chromatogram?

I have been collecting designs for septumless techniques for quite some time, and I am trying to understand their advantages and disadvantages. Additionally, I am open to dialogue with colleagues who have experience with septumless techniques. Even if the problem will be (very) difficult to solve to perfection, a novel septumless injector is worth the effort!

Another big focus of our future efforts will be in multidimensional technologies – with the modulation outside the GC oven and with our fast GC as the second dimension. The gas pulse modulation is especially interesting, which the highly innovative Robert Synovec presented to us in Riva in 2018 (5). It would be fantastic to combine this with fast GC...

We will also continue to work intensively in the field of simulation, not so much to compete with the excellent web-based simulation "Pro EZGC" from Restek, but to better understand the possibilities of gradient GC and to develop new measurement methods.

I am not daunted by the fact that GC is considered by many to be a "mature" technology – we will continue to work on further improvements for a long time to come!

Peter Boeker is a research associate at the Institute of Agricultural Engineering, University of Bonn, Germany.

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Detecting Change

Agilent Technologies and The Analytical Scientist recently conducted a survey to collect your thoughts on trends in liquid chromatography. Here, we speak with Lester Taylor, Agilent's Pharma Marketing Manager, to discover what the results mean in the pharmaceutical market – and beyond.

How has the use of LC-MS systems grown over the years?

Focusing on the life science and pharmaceutical arena, the biggest advance has been the ability to do routine analysis of biologically relevant compounds, such as drugs and metabolites. Typically polar and labile, these molecules are not amenable to direct analysis with GC-MS techniques, so there was a big gap in the market for LC-MS. The introduction of techniques such as electrospray ionization in the 1980s and 1990s, made LC-MS much more reliable and robust, and its use for routine analyses has grown steadily ever since. We've seen that reflected in the growth of conferences like ASMS, which has gone from under 300 attendees to over 6,000 – many of whom work in the biological sciences.

The continuing evolution of software systems able to deal with high-speed data acquisition and processing has also played a crucial role in promoting adoption of MS detection.

Where is LC-MS most commonly used in pharma, and where does UV-Vis detection still hold sway?

Mass spectrometry is widely applied in R&D – used by synthetic or medicinal chemists looking for a quick verification of compound synthesis. Drug metabolism groups use it to help understand the nature and extent of metabolic products, and it's the gold standard analytical technique for DMPK and bioanalysis studies.

However, it is less commonly used in method development, where companies tend to adopt the "fit-for-purpose" approach of using tried and tested instrumentation rather than technology that may have more analytical capability than is required; for example, a routine method for screening a target drug once it has moved into development.

In pharma QC, UV-Vis detection is predominant. By the time drugs make their way through to this stage, the analytical characterization of the drug is very well understood, so companies want to apply the simplest, most robust methodology that meets their needs. For many purposes, UV-Vis may well be sufficient. In cases where unexpected peaks arise in the LC-UV chromatogram more analytical information is required to identify these compounds. Mass spectrometry can help identification in such cases. However, as MS technology evolves and becomes much more automated, I think analysts will gain confidence and start to apply it in more areas where greater specificity and sensitivity is required to identify eluting LC components.

The Analytical Scientist reader survey represents a useful insight into the barriers and pain points that limit the use of MS.

Survey: Trends in Liquid Chromatography

Survey respondents in pharma, chemical and academic settings identified their analytical challenges (Figure 1). Here are the top three:

Certain samples/methods push us beyond the capabilities of our analytical setup (58 percent)

Lester says: Some samples are much easier to deal with than others; for example, looking at drugs in matrix may be particularly challenging.

We are concerned about hidden impurities/compounds of interest (49 percent)

Lester says: Because of the exquisite sensitivity and selectivity of mass spectrometry as a detector, you can find very low levels of compounds that you may not have expected.

We regularly experience downtime due to hardware maintenance/failure (28 percent)

Lester says: Instrument downtime is always a huge area of frustration because (unlike the other two challenges) it is often outside your control...

The majority (56 percent) of users in pharma, chemical and academia used UV-Vis detection on a routine basis, but 57 percent turned to MS detection for more challenging samples (either in their own lab or a core facility).

Lester says: One reason for that, of course, is because users often have to go to either another lab or another instrument to use MS. If you had an MS detector on the same system as the UV-Vis detector then you would be able to utilize that information without having to switch instruments. The other factor is the cost – MS detection has traditionally been more expensive than UV-Vis. However, costs have come down dramatically over the past few years and I think we will see MS being used more as a first-line detector.

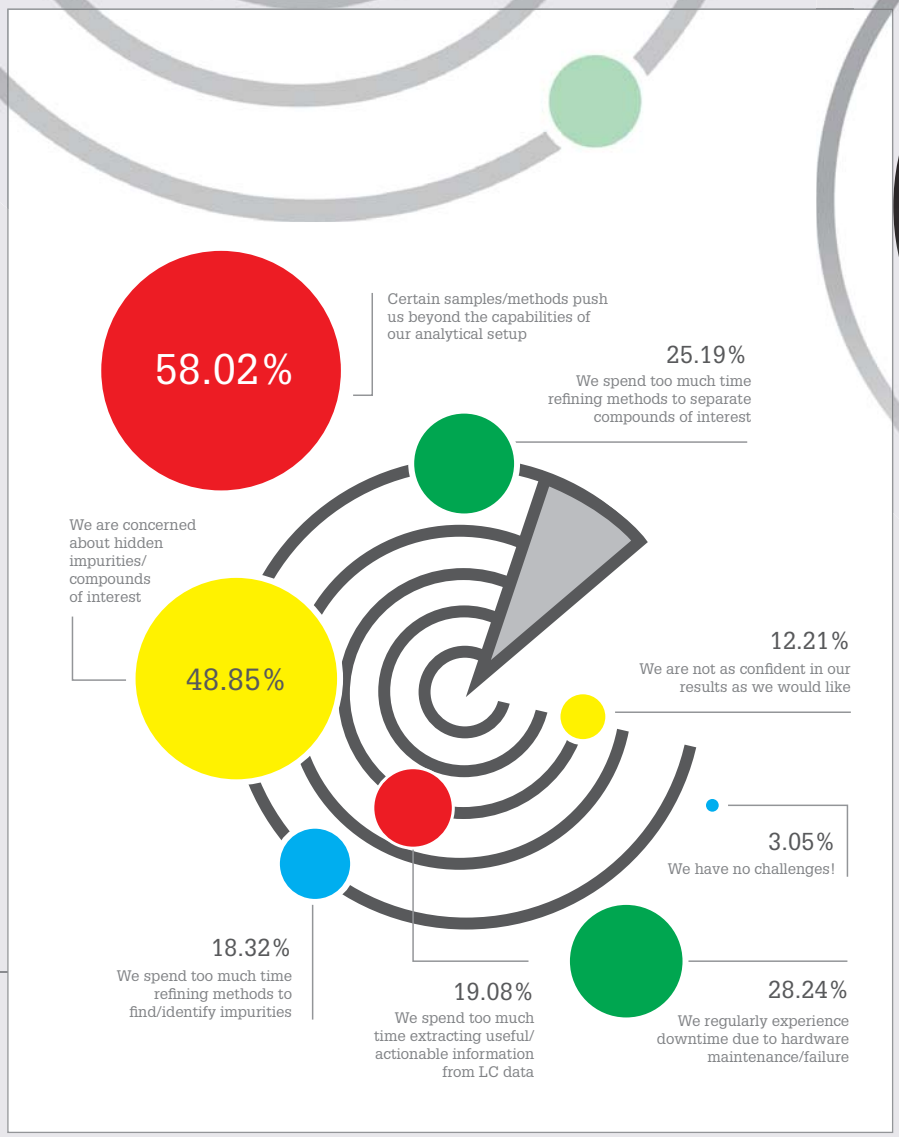

Figure 1. Which of the following statements do you identify with?

Survey respondents without access to MS in their lab identified a number of barriers to adopting MS detection. The top four challenges were:

1. Limited budget – 52 percent
2. Insufficient time to train staff on new detection system/software – 18 percent
3. MS would be too complicated for analysts – 16 percent
4. Management don't see the value – 11 percent

Lester says: I believe MS will be of significant value for many analyses currently carried out with UV-Vis, but there may be an opinion among non-mass spectrometrists that MS is not routine or is challenging for non-experts to use. To overcome these barriers, I think we need to focus on ease of use. Software is key, as we've seen with our Open Access LC/MS, which only requires use of a simple sample entry process.

Profiling Lester Taylor

During my undergraduate degree in organic chemistry at The University of Manchester Institute of Science and Technology, I was intrigued by my professor's research into instrumentation and its application to the analysis of chemical compounds. I went on to choose a PhD focused on the design of mass spectrometers and later worked with Professor Dudley Williams at Cambridge University on the characterization of N-terminally blocked peptides by mass spectrometry, which were not amenable to standard Edman sequencing used at the time. At that time, LC-MS was not yet a routine technique, so it was a significant challenge to apply it to biological molecules, which include many polar or labile compounds. It was a very exciting time for the field of mass spectrometry!

Later, I was hired by Burrough Wellcome Co. in North Carolina, where LC-MS was being applied to the analysis of drugs in various phases of drug R&D. After many years in the pharmaceutical industry, I eventually found my way back to the analytical instrument side of the field, and have been with Agilent for the past 10 years.

Profession

Leadership
Talent Development
Career Planning

Everything You Wanted to Know About a Career in Chemistry* – Part II (*But Were Afraid to Ask)

Peter Kissinger is a highly experienced university professor and entrepreneur. Last year, he shared his responses to ten frequently asked questions from the hundreds of students and postdocs he has mentored. His advice proved very popular with students and teachers alike; now he's back to answer another batch of killer career conundrums.

What constitutes a good scientific paper? The reader will decide. I, for one, don't like sloppy work seasoned with hype, excess superlatives, and even more excessive insignificant figures. I know these tendencies well, having practiced them more than once myself!

Some view citations as a mark of quality but I'm not convinced. A more important point for me is whether the work can be replicated – science advances through repetition and confirmation and if critical details are left out, you are off to a poor start. Consider the example of a paper published in Nature, which received a lot of attention in the popular press. I looked at the supplementary material referring to LC/MSMS of a potential drug in mouse blood. There was no mention of how the mice were sampled or the volume sampled and the LC column, mobile phase and flow rate were not fully specified. This aspect was not central to the paper, but it suggests that anyone studying the pharmacokinetics of the drug in future would have to start over.

Clear, concise writing is also crucial. Some say that people who write are driven to careers in law and those who don't are driven to careers in science or engineering. Like many generalizations, this one should not apply to you. Don't wait till the end of your studies to become an effective writer. There are a lot of great resources available to help develop writing skills in Purdue's Online Writing Lab program (https://owl.english.purdue.edu/owl). In the end, investing in your communication skills will pay off. Without it, you will find obtaining employment in science very difficult and you will not advance in the profession.

The very worst thing you can do when preparing a paper is to fabricate, falsify or plagiarize – academic careers and whole companies have been destroyed by research misconduct of this type. One of the most disturbing aspects of scientific work is the tendency to commit to presentations and publications in advance of having carried out the work to be described, creating huge pressure between students and faculty. If the data don't fit the plan, go with the data, back out of the commitment or change it. No matter how much pressure you get from a Professor, it is you who would be held accountable in an investigation. It is

"The very worst thing you can do when preparing a paper is to fabricate, falsify or plagiarize."

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My advisor covers my drafts with red ink. What should I do?

Write more! Read more! Think about the structure of what you are reading. Do you like it? Why? Why not? Writers write, rewrite, edit themselves, and seek help from others. Perhaps before you hand anything to your advisor, it would be smart to share it with another willing student or postdoc. Remember that novelists, biographers and journalists all have editors.

There are many cases where it is better to let a file sit for a day or three before hitting "SEND". You will make the text better and save yourself from embarrassment or worse.

A common writing problem with young scientists is the tendency toward hyperbole – I'd certainly like to make many of my early publications disappear for that reason. It is not necessary to glorify your own accomplishments – the reader can decide if your accomplishment is faster, better, cheaper, more accurate, more sensitive, and truly amazing.

Aren't patents more important than papers?

Not necessarily. Graduate students are typically very confused about patents. They will say "I've filed a patent", when what they mean is an invention disclosure was filed with the University's Technology Transfer Office, who will decide whether or not to pursue filing for a patent or trademark protection. At other times a bona fide patent application is confused with an issued patent. For example, there is much confusion about a US-published patent application versus a US-issued patent. The former is the publication of a previously confidential patent application and the latter is the result of patent application approval by the United States Patent and Trademark Office (USPTO). Getting to the former typically occurs 18 months after the first patent application filing; the latter can take longer than achieving a PhD.

There is much more to say here, but I'll leave you with the fact that a very tiny number of patents bring any economic return to the inventors, with an estimated 95 to 99.9 percent of patents leading to no financial gain whatsoever. Likewise, very few scientific publications are widely cited, with many not being cited at all. Whether you collect patents or publications or art, a few truly impactful examples are better than many "rabbit pellets".

"It is not necessary to glorify your own accomplishments – the reader can decide if your accomplishment is faster, better, cheaper, more accurate, more sensitive, and truly amazing."

Is it better to be a specialist or generalist?

I often refer to "T-shaped" people. This is a convenient metaphor for a good scientist, meaning that they are deep experts in a field (vertical), but also have a broad perspective on its significance (horizontal). A T-shaped professional can interact across disciplines and see synergies with other fields and endeavors. I've met a number of top scientists with global reputations and inevitably find them interested in music, visual arts, theater, poetry, history and so forth. While breadth doesn't guarantee depth and vice versa, it does seem that the most productive people avoid being very narrow. Seeing how your research fits your subfield is one thing, but thinking beyond that to other disciplines in science and beyond is both smart and fun. Your T will be N = 1 out of >7 x 10^9.

"Try to learn something about everything and everything about something." – Thomas Henry Huxley

My lab has too many meetings and they are too long OR We don't have research group meetings at all

Many articles and books have been written on the topic of professional meetings, because so many find the subject annoying. The recommendations are quite consistent:

- Have an agenda and a purpose, with no surprises
- Be on time to start, and finish on time
- After 90 minutes productivity drops exponentially as exhaustion sets in; shorter is better
- Invite people who need to be there and who will contribute
- Wrap up with a conclusion and directive for next steps
- Prepare brief minutes to update the larger team

Common meeting mistakes:

- Dominating the conversation yourself or letting others do so (listening is the harder skill)
- Running late
- Inviting people because you think they will be offended if not invited, but have no need to attend (You can't

win here. If you don't invite them, they will be offended. If you do invite them, they will say the exercise was a waste of their precious time; so don't invite them.)
- Tolerating fiddling with smartphones and the like, or worse, doing it yourself as the organizer
- Drifting off topic and losing control of the agenda (the organizer must manage the meeting, not just book it)
- Slipping into an emotional, accusatory tone. War must not break out.

Like most people, I am not good at this; I doubt you will be either. I try to improve my listening skills, but like most people I still prefer to listen to myself! One principle that I strongly recommend as an alternative to meetings in business and academia is MBWA – management by wandering around. This means getting out and talking to people in their own work setting, getting to know them one-on-one or in very small groups, listening to their concerns and sharing yours. If you do this once a day, many meetings can be avoided. In summary, some research teams will have no meetings, some will have too many. If you object to either, you are normal. Hang in there.

How can I get good letters of recommendation?

I'm asked this question by undergraduates every year when it is already too late; sophomore year is not too early to start. Simply getting a good grade is not enough, you need to engage with your professors. I recommend that you:

- Sign up for independent study with an undergraduate research component.
- Meet with faculty outside of classes and discuss career options and your objectives.
- Speak up in classes. Debate. Participate in extracurricular activities and muster up some enthusiasm.
- Join organizations in science and/or engineering related to your interests. You can learn from and develop conversations with other professionals.
- Take part in competitions or programs offered by the University or national organizations e.g., student business plan competitions.

Too often, all I can honestly write about a student is: "Ms Smith took my

"Classroom learning is overrated by both faculty and students; in reality, it simply can't compete with real-world experience."

introductory organic class and received a B+." Worthless! Consider the next version and how many conversations with Ms Smith were required to obtain it. "Ms Smith is one of the most enthusiastic undergraduate research students I've worked with in the last five years or so. She takes initiative, is very dedicated and has contributed to three papers my group has submitted for publication. I recommended her for an internship at XYZ Company in the summer of her junior year and they reported that she was very productive. She will be able to hit the ground running in graduate school and be an asset to any group she joins. Unlike many chemistry students, she can also write."

How long should it take to get a PhD?

I'd rather use words like 'achieve', 'attain' or 'earn'. It is now half a century since I entered graduate school at UNC Chapel Hill. In that time I've seen it take anywhere between 3 and 7 years, and a median of five years feels about right for the chemical sciences. However, research is by nature a great unknown – think of breaking the sound barrier in the late 1940s or landing on the moon a generation later. If the time spent in graduate school is critical to you, let me suggest dental school. A life of looking at teeth seems less stressful and pays rather well.

How can we narrow the gap between the PhD program curriculum and the needs of hiring companies?

Classroom learning is overrated by both faculty and students; in reality, it simply can't compete with real-world experience. Lectures are great for teaching, but much less so for learning. PhD students have been fabulously successful in industry for decades – how is this possible if there were no courses to orient them with industry or government labs? The answer is that the basic ingredients for success are largely the same in every occupation – curiosity, self-motivation, teamwork and communication. Respect for others and a sound ethical foundation matter a lot everywhere.

That said, you should take opportunities to interact with alumni from commercial settings, take short courses with business schools, and (especially) read business-oriented publications. Resources for learning are all about us and waiting for the learner to access them. If you are interested, just do it! One place is ScienceCareers.org/booklets sponsored by AAAS and another is nature.com/careers.

I find I no longer enjoy lab work. What should I do?

How often is your principal investigator in the lab running experiments? Are they even safe in a lab? A PhD does not assign you to a life of preparing solutions or executing mice. The opportunities are broader than many graduate students and postdocs realize; few scientists are at the bench more than five years after their PhD. Some are in technical sales or service, working as patent agents, selling real estate or writing for trade magazines (as I do from time to time). Many manage science, lead it or teach it.

A key point here is to find your strengths and interests and not be burdened by some vision impressed on you by others. Break the mold. Find your own way. N = 1 = you. For example, I was never that good at deep and narrow science, and was more interested in the application of science and engineering to solving medical problems. I would not have been happy as a "pure" academic, so I chose to start companies instead. To this day, I remain very "impure" and find this fun and rewarding, but I likewise very much respect those who have taken different forks in the career path.

Someone else is getting all the credit for work we did together

Are you sure? Why? Are you shy? Did you not write up your work professionally in a progress report? Are you obsessing over getting a chapter in your dissertation? In my lab, teamwork is encouraged and it is possible to include the same work as a part of several dissertations.

Remember that jealousy, fear and greed are in our DNA source code. It seems to start at about age 2; for some it settles down around 40, for others it never ends. If you want to learn more about the many historic arguments over "who gets the credit", look at the invention of the laser, MRI imaging, electromagnetic induction, and many others. Even Nobel Prize winners have been subject to claims that they took credit for another's work. The more important the topic, the more likely war will break out.

Faculty can easily forget the relative roles of student A versus student B, or at least the students can imagine it is so. In fact, the mentor and mentee are likely both missing something that can be settled with a respectful chat.

Peter Kissinger is a Professor at Brown Laboratory of Chemistry, Purdue University, and a founder of Bioanalytical Systems, Inc. (BASi), Prosolia, Inc., and Phlebotics, Inc. Indiana, USA.

Have a career question you'd like Peter to answer? Email the Editor: charlotte.barker@texerepublishing.com

Evaluation of mAb ADCC Activity through Fc Receptor Affinity Chromatography

The new HPLC column TSKgel FCR-IIIA-NPR is based on recombinant FcγIIIa receptor - a key player in antibody-dependent cellular cytotoxicity (ADCC). A thirty-minute analysis gives valuable first information on the distribution of glycoforms and expected ADCC activity of therapeutic antibodies.

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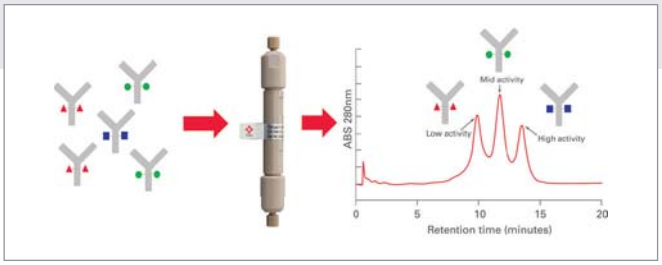

FcR-IIIA affinity chromatography separates mAb glycoforms according to their ADCC activity

N-glycans are known to play an important role in Fc-mediated effector functions. Accordingly, peak patterns of therapeutic antibodies on TSKgel FcRIIIA- NPR can be correlated to mAb glycoforms. Early eluting peaks (low affinity to Fc receptor) represent mAb glycoforms with low ADCC activity while late eluting peaks represent glycoforms with high ADCC activity.

Chromatographic conditions:
Column: TSKgel FcR-IIIA-NPR (5 µm 4.6 x 75 mm); Mobile Phase: A: 50 mM Citrate, pH 6.5 ; B: 50mM Citrate, pH 4.5; Flow rate: 1 mL/min; Detection: UV@280 nm; Sample: Rituximab

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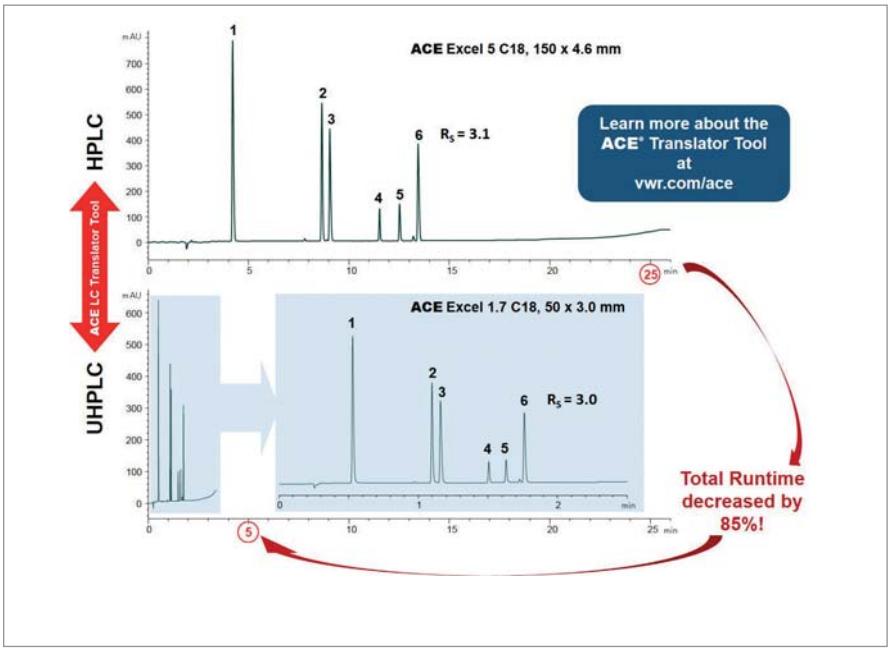

Top: Separation of 6 peptides on an ACE Excel 5 C18 HPLC column installed on an HPLC instrument. Bottom: The same separation translated to an ACE Excel 1.7 C18 UHPLC column installed on a VWR Hitachi Chromaster UltraRs UHPLC system. The method translation was achieved using the ACE LC Translator Tool. Mobile phase: A=0.05% TFAin H_2O, B=0.05% TFA in MeCN. Temperature: 60°C. Detection: UV, 220nm. Sample: 1.Gly-Tyr,2. Tyr-Tyr-Tyr, 3.Val-Tyr-Val, 4.Oxytocin, 5.AngiotensinII, 6.Leu-enkephalin.

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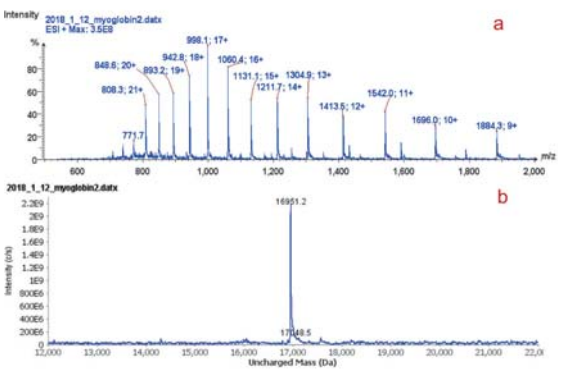
Direct Analysis of Intact Proteins Using the Advion Touch Express™ Open Port Sampling Interface (OPSI) with the expression Compact Mass Spectrometer (CMS)

Touch Express OPSI coupled to the expression CMS provides fast benchtop analysis of intact proteins. Here, 2 µL of myoglobin (horse) at a concentration of 1 mg/mL in 10 mM ammonium acetate was used and deposited directly at the open port by pipette tip.

Following sample analysis, a charge deconvolution feature within Advion Data Express software automatically calculated the uncharged protein mass from the characteristic multiply-charged spectrum envelope obtained from ESI of biomolecules. The full mass spectra and the deconvolved, uncharged mass for myoglobin is shown in Figure 1.

A total of 14 multiply charged ions of myoglobin were detected with charge state ranging from +9 to +22 (Figure 1A). The uncharged mass of myoglobin is calculated to be 16,951.2 (Figure 1B), within 1 Da of the theoretical mass, 16,950.5.

Figure 1



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Certain fungi that can grow on agricultural products produce toxic metabolites known as mycotoxins. Modern food processing procedures cannot completely remove these compounds if they are present, so strict monitoring protocols have been established. Although a universal method for the analysis of mycotoxins would allow highly efficient screening, it is very challenging to develop such a method due to differences in physiochemical properties of mycotoxins, extraction efficiencies, and matrix effects. Zhang et al. published a multi-lab study [1] aimed at providing labs with an analytical procedure that could be broadly applied to the analysis of a variety of mycotoxins in many different matrices. Using that work as inspiration, we developed the following LC-MS/MS method that resolves 12 FDA regulated mycotoxins within the pressure limits of traditional HPLC instruments.

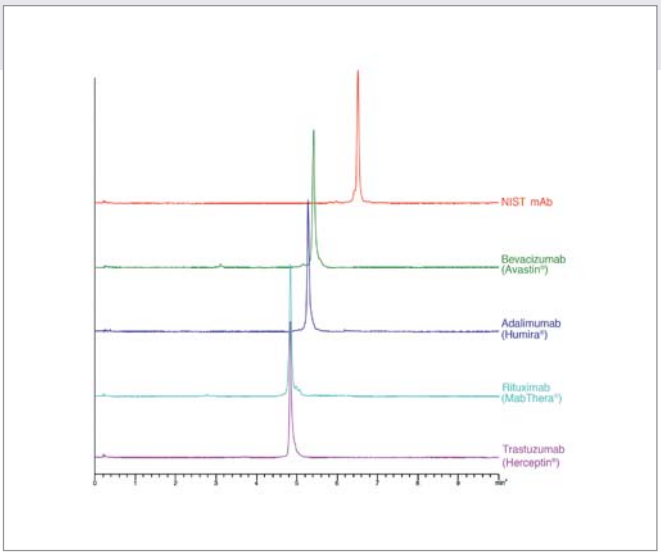
Read more at https://www.restek.com/Technical-Resources/Technical-Library/Foods-Flavors-Fragrances/fff_FFSS2971-UNV

Generic Analyses of Different Monoclonal Antibodies with RP-(U)HPLC

Due to their molecular weight of about 150 kDa, intact antibodies are usually analysed by IEX, SEC or HIC. In addition, RP methods have become an easy tool which is compatible with mass spectrometry (MS). However, lack of sensitivity and resolution has been a hurdle in the past.

Successful analysis in RP mode for MAbs is enhanced by employing a modern, widepore and temperature-stable stationary phase, such as YMC-Triart Bio C4. As a result of its extended stability, it is possible to use temperatures up to 90 °C.

This application note shows how to achieve robust chromatographic results for 5 commercially available MAbs: Trastuzumab (Herceptin®), Rituximab (MabThera®), Adalimumab (Humira®), Bevacizumab (Avastin®), and



Nist mAb. This generic analysis is carried out in less than 7 min using 1.9 µm particles for UHPLC and an elevated temperature of 80 °C resulting in higher sensitivity and sharper peaks for all the MAbs analysed.

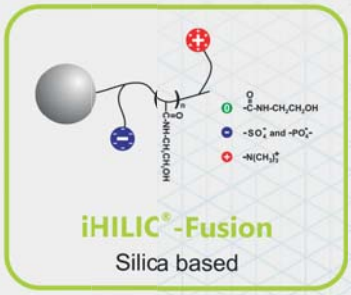
Download the application note with the full method details here (www.ymc.de/files/Generic Analyses.pdf).

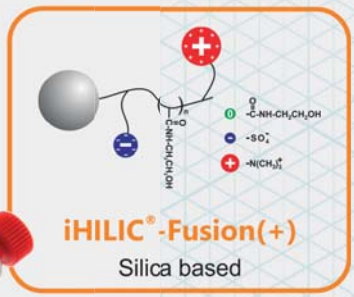
Spotlight on...
Technology

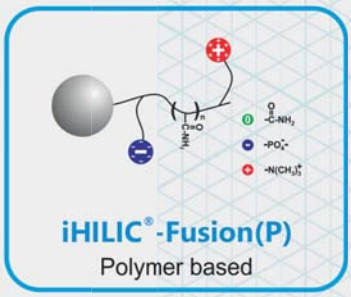
iHILIC®-Fusion Advances HILIC separations in UHPLC and HPLC

iHILIC®-Fusion, iHILIC®-Fusion(+), and iHILIC®-Fusion(P) are three lines of UHPLC and HPLC columns that are designed for the separation of polar compounds in HILIC (hydrophilic interaction liquid chromatography). They provide customized and complementary selectivity, ultimate separation efficiency, and ultra-low column bleeding. The columns are particularly suitable for the LC-MS based applications.

More information, please visit www.hilicon.com or contact info@hilicon.com







CDS Analytical Acquired the Rights to 3M™ Empore™ Products

CDS Analytical, the global leader of gas chromatography (GC) sample introduction instrument, announced that it has acquired the rights to Empore™ products, including production line, patents and trademarks, from 3M™ Purification Inc, who manufactures the world-recognized Empore™ brand solid phase extraction (SPE) media which is used in numerous analytical applications.

Learn more at https://www.cdsanalytical.com/empore

Obtain Direct Mass Analysis from TLC Plates with the Advion Plate Express™

The Advion Plate Express provides a simple, automated means of obtaining mass spectra directly from TLC plates. Paired with the expression Compact Mass Spectrometer (CMS) this creates the TLC/CMS technique. From this, users can quickly and confidently identify products, even in complex mixtures, without additional sample preparation.

Learn more at www.advion.com

IonSense DART-OS with OpenSpot – Simple, Fast Identification

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CALYPSO: The most advanced LCMS N2 generator on the market (Four models)

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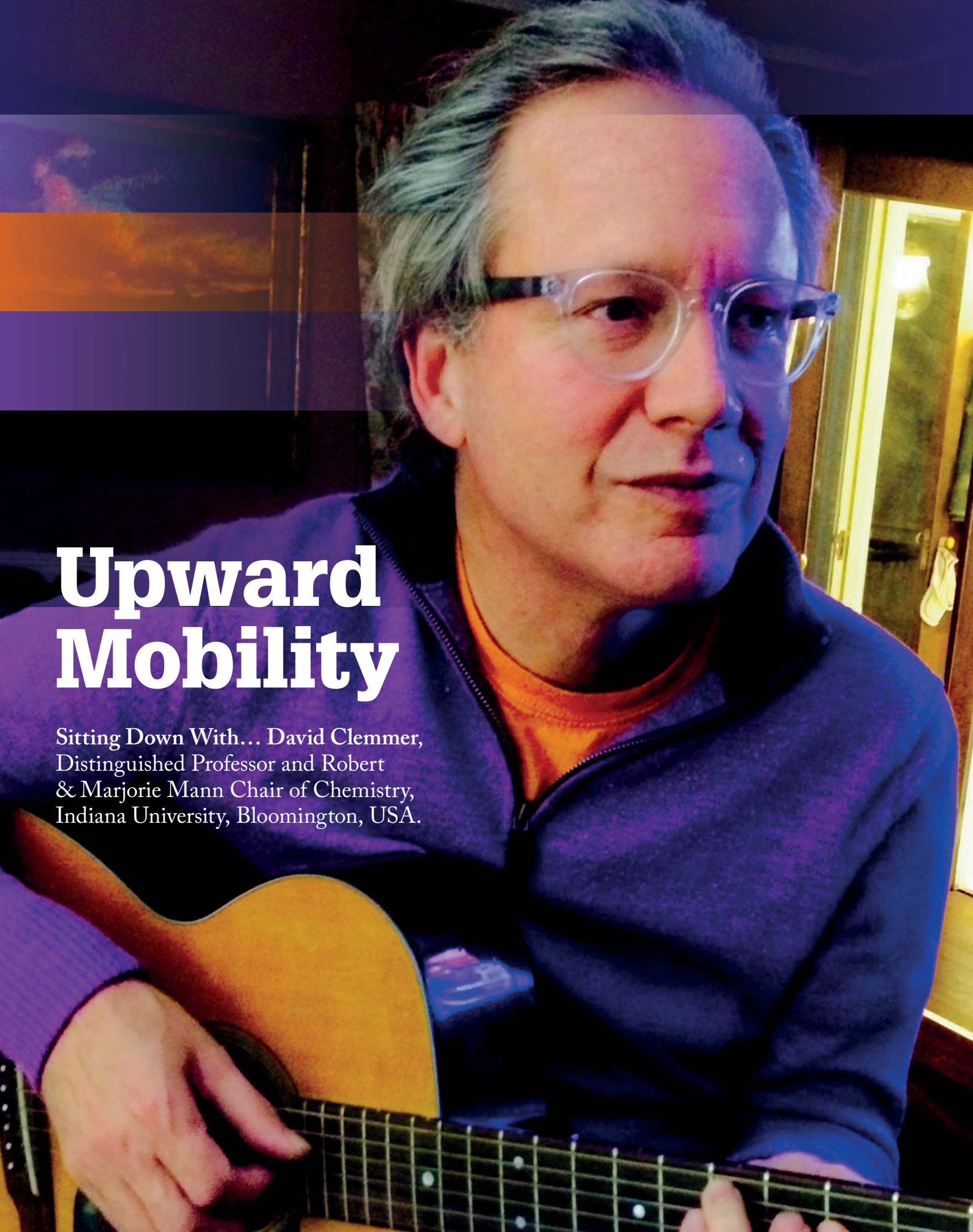
Ocean HDX is anchored by High Definition Optics for high throughput, low stray light and great thermal stability. Its X-Platform Electronics include powerful onboard processing and communications including USB, Gigabit Ethernet, Wi-Fi, AP Wi-Fi and RS-232. The Ocean HDX is compact, robust and ideal for integrated, industrial, biomedical and research applications.

More information available at https://oceanoptics.com/product/ocean-hdx/

Bruker's timsTOF PRO with PASEF

Mass spectrometry (MS)-based proteomics has become a powerful technology for the identification and quantification of thousands of proteins. Bruker's proprietary timsTOF Pro system uses Parallel Accumulation Serial Fragmentation (PASEF) acquisition method to provide extremely high speed and sensitivity to reach new depths in shotgun proteomics and phosphoproteomics, using low sample amounts.

Learn more at https://bit.ly/2uJ41ys



Upward Mobility

Sitting Down With… David Clemmer, Distinguished Professor and Robert & Marjorie Mann Chair of Chemistry, Indiana University, Bloomington, USA.

What sparked your interest in analytical chemistry?

My dad taught sculpture and art history, so it was natural that my first interests were in the humanities. I started college as a music major, but took some classes in science and math – and I was fascinated. When I learned that you could go to graduate school for free if you studied chemistry, my fate was sealed!

I joined Peter Armentrout's lab for my PhD to study transition metal ions in gaseous reactions. Coming to science rather late, I lacked much of the background knowledge of my graduate student peers, but Peter and his students soon brought me up to speed. Peter takes real joy in getting things right and he soon instilled that in me.

How was the shift from music to chemistry?!

Through the years, I've met many people with overlapping interests in math, chemistry, and music, so it's not as unusual as you might think. I was lucky enough to play guitar in working bands as a college student and was able to pay for some of my tuition this way. Growing up in a small community, the style of music depended upon what instruments the members of each group could play, and making the most of the abilities of others is something I have carried through into my science.

How did you come to focus on ion-mobility spectrometry-mass spectrometry (IM-MS)?

I went to Japan as a Japan Society for the Promotion of Science postdoc and studied laser-induced fluorescence in flow tubes. When I returned, I joined Martin Jarrold's lab at Northwestern and used drift tubes to get structures of carbon clusters, but at that stage I was using MS purely as a physical technique. I really started thinking about the combination of ion-mobility spectrometry and MS as an analytical technique when I started my assistant professor post at Indiana University in 1995.

Around Christmas 1997, we made the first nested ion-mobility time-of-flight (TOF)-MS measurement, which gave us cross sections and masses in a single experiment for all of the ionizable components of a mixture. My colleague, Jim Reilly, encouraged me to patent our technique, which ultimately led to the invention of parallel dissociation, and became a part of the MS^E technology now sold as "high-definition mass spectrometry" by Waters Corporation. The large capacity and high speed of our technology was clearly useful (at least in our minds) for analyzing complex mixtures. This made the emerging omics revolution a target for applications of our measurements.

How much of your success was luck versus skill?

Luck is a big component, but it is also the environment at Indiana – there are so many talented students. Students really drove the development of the technology. Parallel disassociation is a great example – Stephen Valentine (then a student of mine) saw the potential long before I did. After I dismissed the idea for the third or fourth time, he pasted a spectrum on the door of the laboratory that I went into several times each day. It wasn't until months later when Ryan Julian, then interviewing for a post-doctoral position with Martin and me, walked through the lab and pointed out that the fragmentation pattern was entirely different from typical CID, that I finally understood what Stephen had done. It's a little embarrassing to think about the number of times my students have led me to a breakthrough.

Are we any closer to solving the big questions in biology?

In some ways, we've advanced quickly, but in others, we've just begun; we have a blueprint but we don't know what all of the different components do or why they are valuable.

The problem I have been looking at recently is how exosomes transmit information between cells through the blood. Martin Jarrold has invented the first type of MS that can measure exact charges (charge-detection mass spectrometry; CDMS). Once you have the exact charge of a molecule it extends the mass range into the mega-Dalton or giga-Dalton region, allowing us to measure the masses of exosomes. Meanwhile, Milos Novotny has figured out how to pull urinary exosomes out of solution, purify them and concentrate them. There's a lot to learn about these vesicles – back in the 1960s, they were thought to be junk carriers, and now it is looking more and more like they may be another way of controlling function within organisms. They carry genetic information between different organs and perhaps organisms – nature's gene therapies.

What's next for ion mobility?

I think we will increasingly be able to look at large ions. Martin Jarrold and I have a grant to put a drift tube on the front of the CDMS instrument I mentioned earlier so that we can gather information about the shape, exact charge and mass-to-charge of very large molecules – viruses, extracellular vesicles, lipoproteins, nanoparticles, and environmentally relevant molecules such as soot and small plastic particles. That last idea – small plastic particles in the 10 to 100 nm size range is going to be a huge problem as big pieces of plastics decompose into smaller particles. Anyway, I think the combination of ion mobility with CDMS is likely to have a future in these areas.

There's also a lot to do with small ions. There are a couple of new technologies – instruments from Waters and Bruker, and Dick Smith's SLIMS technology – that will go to very high resolution. Here, ion mobility gives you a quick way to look at subtle differences in molecules that otherwise have the same mass.

I think IM-MS is in a good place and I imagine there will be a drift option included with every mass spectrometer in the future – the applications are limitless.

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