JULY 2016 # 42

# the Analytical Scientist



## A Breath of Fresh Air

Giuseppe Petrucci tackles the complex chemistry of organic aerosols to better understand atmospheric processes.

26 – 35

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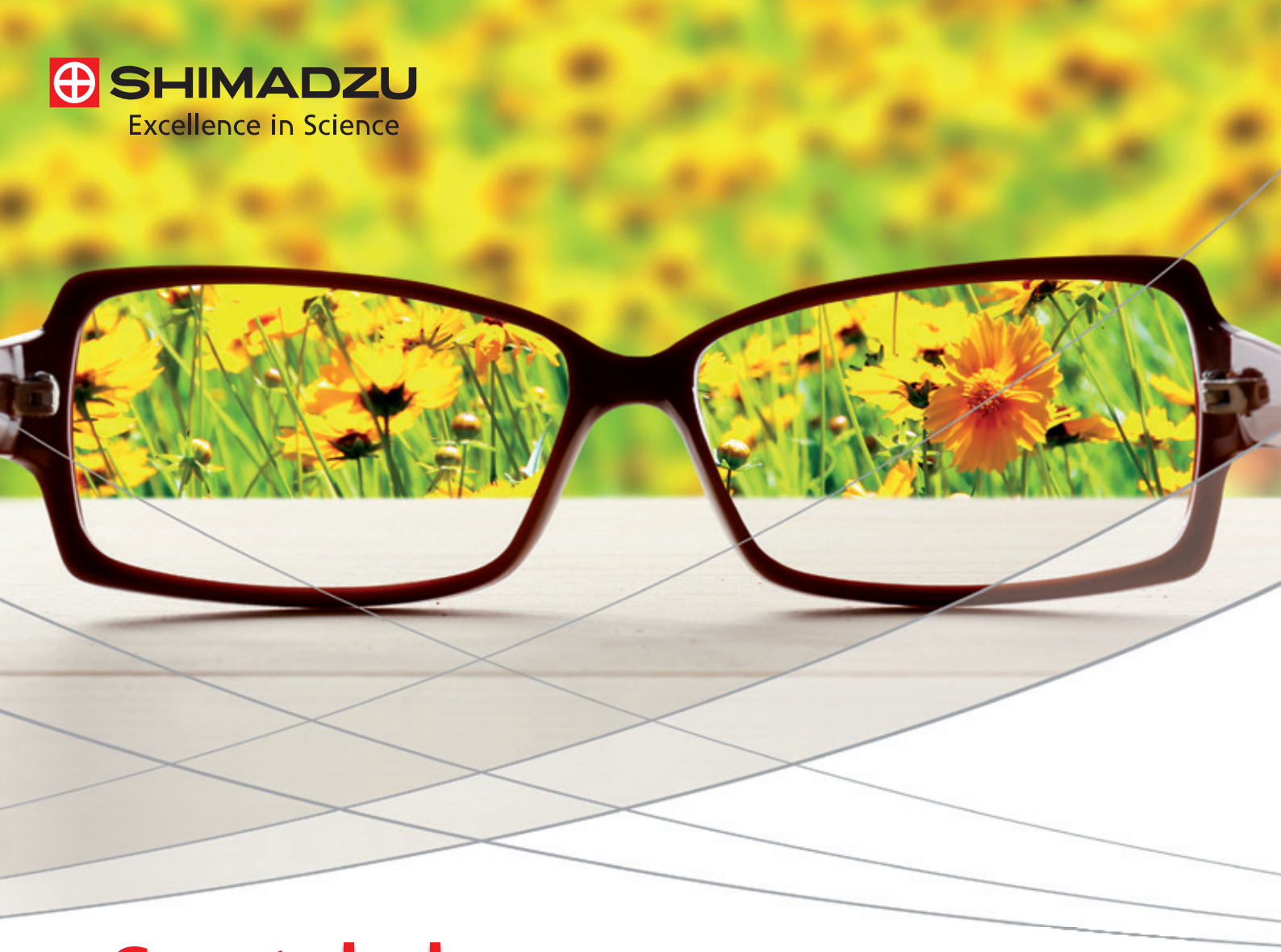

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# Image of the Month

*An Exciting Pro[spec]t*

NASA engineers inspect the array simulator installed on the Near-InfraRed Spectrograph (NIRSpec) engineering test unit. The array – featuring microshutter technology developed specifically for the NIRSpec – controls how light enters the spectrograph via thousands of adjustable microscopic windows. It is one of four instruments set to be aboard the James Webb Space Telescope, launching in 2018 – and will be the first spectrograph in space with the capacity to simultaneously capture data for multiple objects (http://jwst.nasa.gov/nirspec.html). Credit: NASA

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

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## On The Cover

*The Earth's beautiful atmosphere, courtesy of NASA.*

## Upfront






the Analytical Scientist

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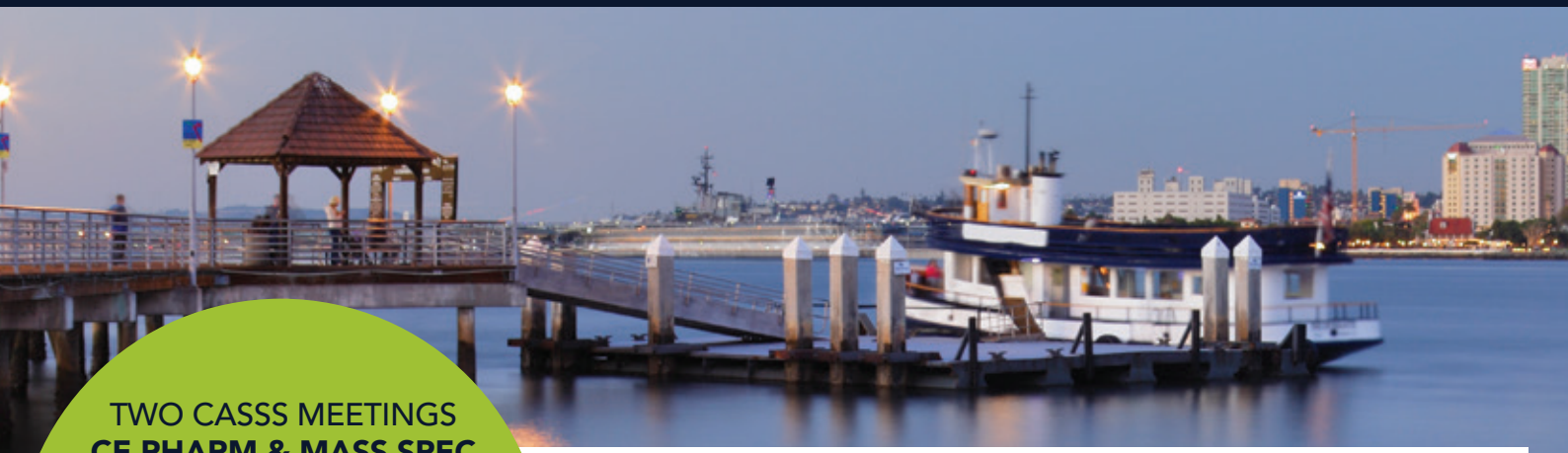

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## ABSTRACT SUBMISSION DEADLINE:

July 1, 2016 oral presentation | August 26, 2016 poster presentation

## SYMPOSIUM CO-CHAIRS:

Christopher Yu, *Genentech, a Member of the Roche Group*
Eef Dirksen, *Synthon Biopharmaceuticals B.V.*

## PLENARY SPEAKERS:

Jennifer Brodbelt, *The University of Texas at Austin*
J. Michael Ramsey, *University of North Carolina*

## TOPICS

Product Characterization
- Recombinant proteins
- mAbs
- Fusion proteins
- Protein conjugates

Process characterization
- Impurities
- Aggregation
- Protein formulation and stability
- Host cell protein

Innovative MS Technologies
- High throughput and automation
- Hyphenated and 2D methods
- Novel instrumentation
- Software advances

Higher-order Structure
- Protein folding
- H/D exchange
- Ion mobility applications

Regulatory Aspects
- Comparability
- Quality-by-design (QbD)
- Biosimilars
- CMC

R&D Applications
- Protein design
- Ligand-binding studies
- Proteomics/metabolomics
- Pharmacokinetics
- Quantitation
- Biomarkers

Identification and Characterization of Unknowns
- Biosimilars
- de novo sequencing
- Post-translational modifications

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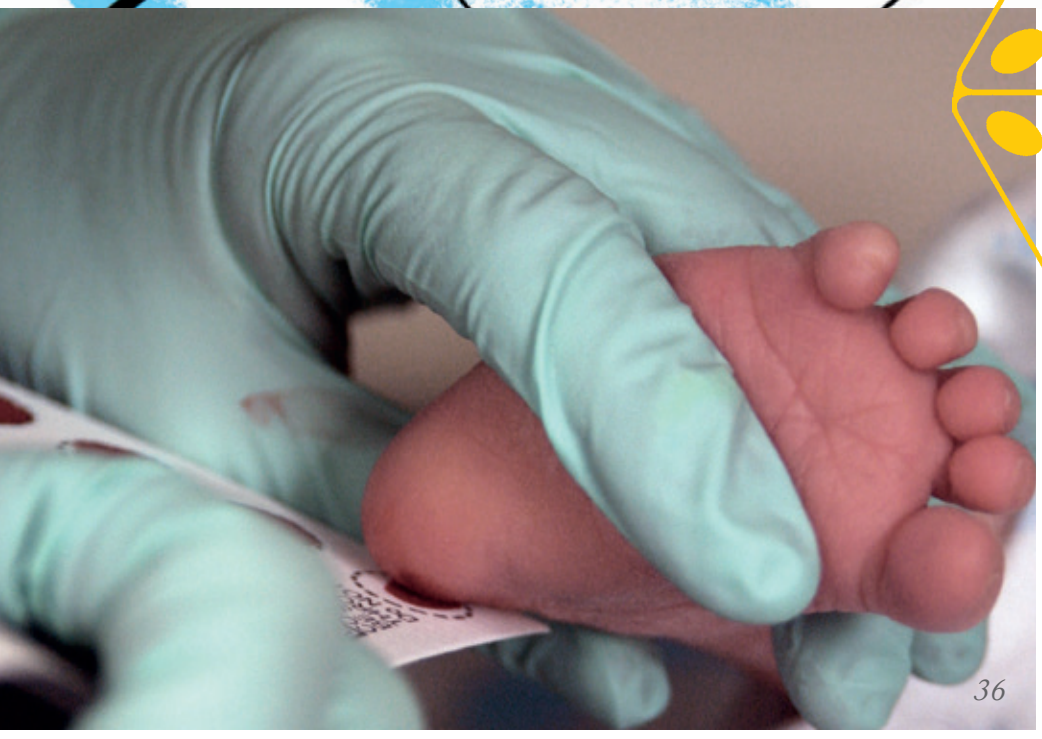



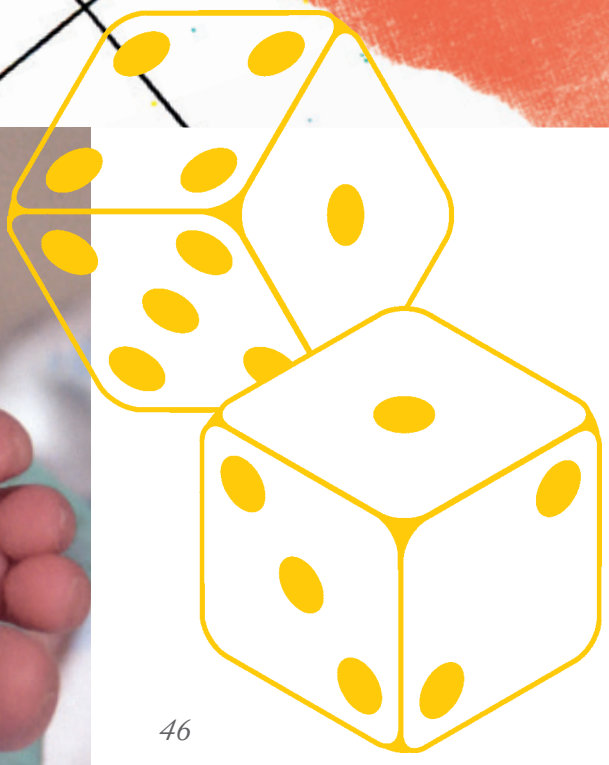



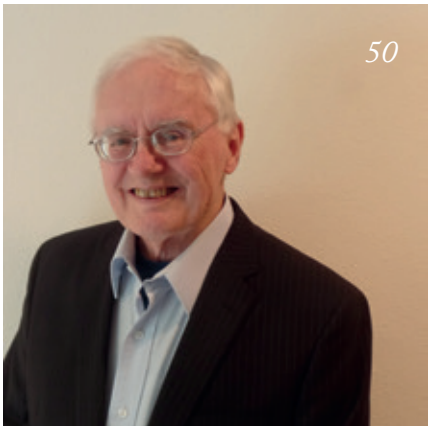



## In My View



## Features



## Reports



## Departments



## Sitting Down With

**September 25-28, 2016** | The Westin San Diego Hotel, San Diego, CA

## Symposium Co-chairs:

Henry Luo, *Regeneron Pharmaceuticals*
Steffen Kiessig, *F. Hoffmann - La Roche Ltd.*

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# Tacky Factor

*What should "impact" really mean? And what is the future of the over-popular and sometimes-abused metric that is often used to describe it?*



"We want to make [Impact Factor] so tacky that people will be embarrassed just to mention it," stated Stefano Bertuz (chief executive of the American Society for Microbiology) in a recent Nature news article (1).

Last month, I focused on publication quality and the potential for fraud in the open access model (2) – clearly unwelcome. But the use and misuse of Impact Factors arguably has a greater negative... erm... impact on science.

Impact Factor – the origin of which dates back to 1955 (3, 4) – essentially takes the number of citations to a journal in a given year (say 2015) and divides it by the number of articles published by the journal in the previous two years (in this case, 2013 and 2014). For 2015, Analytical Chemistry is 5.886 and Trends in Analytical Chemistry is 7.487. But does it make sense to assign a simple number, such as Impact Factor, to something as multifaceted as"quality"? And, if so, how should it be used?

Some have been clear on the answer to the latter question: not at all. Three days after Bertuz was quoted in the Nature article (itself a response to a preprint on bioRxiv [5]), ASM announced that it would stop supporting (and promoting) Impact Factor (6) – "to avoid contributing to a distorted value system that inappropriately emphasizes high IFs." Apparently, ASM hope that other high-profile journals will follow suit.

There are a couple of problems with (Journal) Impact Factors, including the fact that the number is very often skewed by a small number of very highly cited papers (the main argument of the bioRxiv paper). But perhaps people's (mis)perception of the metric is the more damaging aspect; an author's ability to publish in a "high-Impact Factor" journal can positively influence promotion and funding decisions (in cases where hirers and funders are too lazy to delve into specific metrics). Using Journal Impact Factor as a surrogate for individual research (or researcher) quality is clearly flawed.

The bioRxiv paper – "A simple proposal for the publication of journal citation distributions" – could be a new catalyst for change, or at least discussion, and should not be taken lightly; its authors represent Nature Research, Science (AAAS), and PLOS amongst others. Together, they suggest that greater transparency is needed.

Given an inherent focus on representative sampling, quantitative data, and robust statistics, shouldn't analytical scientists be leading the charge for change?

**Rich Whitworth**
*Editor*


*References*

1. *http://www.nature.com/news/beat-it-impact-factor-publishing-elite-turns-against-controversial-metric-1.20224#/b1*
2. *E Garfield, "Citation indexes to science: a new dimension in documentation through association of ideas", Science. 122:108-111 (1955). http://garfield.library.upenn.edu/essays/v6p468y1983.pdf*
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4. *https://theanalyticalscientist.com/issues/0616/open-access-fraud/*
5. *http://biorxiv.org/content/early/2016/07/05/062109.article-info*
6. *https://www.asm.org/index.php/asm-newsroom2/press-releases/94299-asm-media-advisory-asm-no-longer-supports-impact-factors-for-its-journals*

# 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:*
*rich.whitworth@texerepublishing.com*

## Killer Smile

### Potentially dangerous levels of lead discovered in cheap lipsticks

After reports of high lead levels in Chinese lip products, a research group at the State Key Laboratory of Pollution Control and Resource Reuse, Nanjing University (Hongbo Li, Lena Ma, and PhD candidate Di Zhao) carried out a study into the concentrations of lead in popular cosmetics – and whether they pose a threat to lovers of lippy.

"To accurately assess the health risks of lead in lip products, understanding lead bioavailability – the fraction of lead absorbed into the bloodstream following oral ingestion – is essential," says Hongbo Li. "Until recently, there were no systematic studies available, with previous studies (1–3) focusing only on total lead concentrations in lip products without considering the bioavailability." The research group had been focusing primarily on the bioavailability of contaminants in various matrices – including soil, dust, and food – following oral ingestion by humans, so lip products were an obvious progression.

The group purchased 93 lip products (75 lipsticks and 18 lip glosses) in different colors and at varying price points from retail stores and online. The total concentrations of seven metals (cobalt, cadmium, arsenic, nickel, chromium, zinc, and lead) in all lip products were determined using inductively coupled plasma mass spectrometry (ICP-MS, NexION 300X, Perkin Elmer) following digestion of lip products with repeated additions of concentrated $HNO_3$ and $H_2O_2$.

"To determine the relative bioavailability of lead, we selected 15 samples with relatively high lead (87–10185 mg/kg), and fed these to mice via their diet for an exposure period of 10 days, using lead accumulation in the femur as the biomarker of exposure," Li says. The femurs were then freeze-dried and analyzed for lead concentration using ICP-MS (USEPA Method 3050B). "Based on this information, we calculated the lead intake via lip products for women and assessed its contribution to overall daily lead exposure, taking into account lead intake from other possible pathways." Ingestion of the 15 lip products contributed 5.4–68 percent of the aggregate lead exposure for women depending on lead concentration; lip products with lead at concentrations over 1800 mg/kg contributed more than 30 percent, while ingestion of samples with lead concentrations under 500 mg/kg contributed less than 10 percent.

To identify the sources of lead in lip products, the two samples with the highest lead concentration were analyzed by X-ray absorption near-edge structure (XANES) spectroscopy at the lead LIII-edge. There was a close agreement in spectra between lead in the lip products and lead chromate, suggesting the source of lead from the addition of lead chromate.

The researchers found that most lip products contained lead levels below the US Food and Drug Administration (FDA) threshold of 20 mg/kg (see Figure 1) – but that with some cheap lip products, you get what you pay for (see Figure 2A). "In general, most lip products are safe to use," says Li. "However, cheap lipsticks (under 5 USD) tended to have high lead levels – with some containing hexavalent chromium, another carcinogen." Finally, orange and pink products contained higher lead concentrations than brown, red, or purple products (see Figure 2B). *JC*

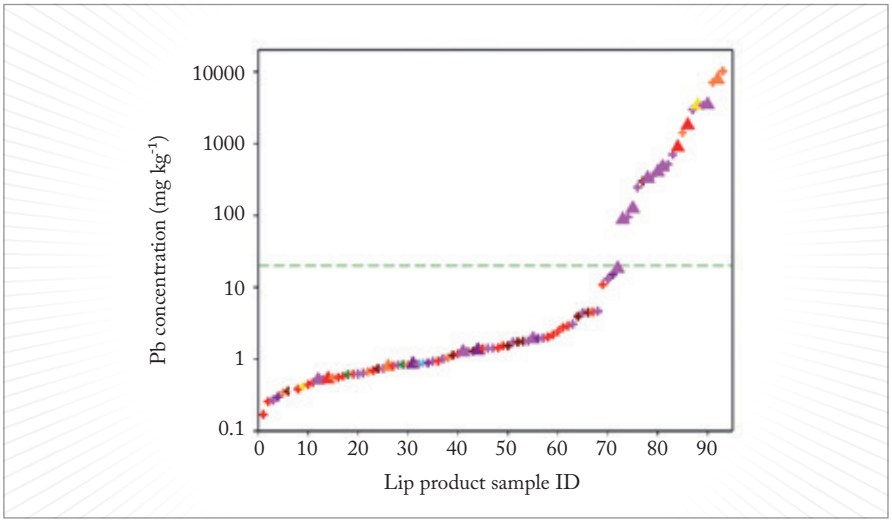
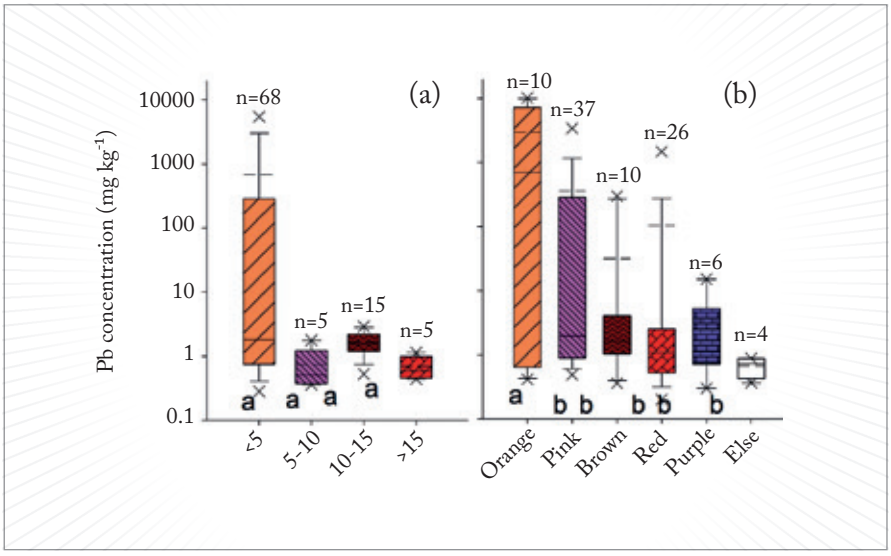



Figure 1. Lead concentrations in 75 lipstick (+) and 18 lip gloss (▲) samples from retail stores and the Internet in China. The color of each point in the figure represents the color of the lip products. The green dash line indicates the US Food and Drug Administration limit of 20 mg/kg for cosmetics.




Figures 2a and 2b. Variation in Pb concentration by price (A) and color (B). Boxes represent the 25th to 75th percentiles, while solid and dashed lines in boxes denote the median and mean values, respectively. Error bars represent the 5th and 95th percentiles, and multiplication signs represent the 1st and 99th percentiles, respectively.

*References*


1. *NM Hepp et al, "Determination of total lead in lipstick: development and validation of a microwave-assisted digestion, inductively coupled plasma-mass spectrometric method", J Cosmet Sci, 60, 405–414 (2009).*
2. *NM Hepp, "Determination of total lead in 400 lipsticks on the U.S. market using a validated microwave-assisted digestion, inductively coupled plasma-mass spectrometric method", J Cosmet Sci, 63, 159–176 (2012).*
3. *S Lui et al, "Concentrations and potential health risks of metals in lip products", Environ Health Perspect, 121, 705–710 (2013).*

# Maple Mayday

## Assessing the effect of climate change on maple quality – using LC-MS

Is the future of high-grade maple syrup at risk? A multidisciplinary team (Acer Climate and Socio-Ecological Research Network - ACERnet) aims to find out by investigating the impact of climate change on this beloved breakfast product. Here, Joshua Rapp, a forest ecologist, and Selena Ahmed, Assistant Professor of Sustainable Food Systems at Montana State University, tell us more about their project.

What prompted your research?

*Selena:* During a trip to Vermont in 2012, I visited a couple who had been farming and producing maple for many decades. They led me through a tasting of several samples of maple syrup with differing quality, saying that climate variability over their lifetime was changing the taste of maple, and that they were getting lower amounts of the highest grade of maple syrup than in previous years. At that time, I was studying the impact of climate change on tea quality via changes in secondary metabolite profiles, antioxidant activity, and sensory discernment. But after a few spoonfuls of maple syrup, I was intrigued by how climate change impacted maple quality and how producers can mitigate risk in their maple systems.

*Joshua:* I study masting in trees (the episodic and synchronous production of seeds), and I became interested in maple sap as a way to measure the resource status of trees (1). I've shown that syrup production is lower after mast years, presumably because making lots of seeds uses a lot of energy, with less left over for sugar in sap. Last year we recruited several colleagues, and got grant funding through the Northeast Climate Science Center.

Tell us about your methods – traditional and analytical.

*Joshua:* We collect sap from trees using traditional tapping methods used by syrup makers for centuries (although our equipment is a bit newer!). At the beginning of the tapping season, we drill a hole into the tree and insert a spile (small metal peg) through which the sap runs, dripping into a plastic bag. Each time we collect the sap, we weigh it and measure the sugar content using a refractometer. We also collect a small amount of sap in a plastic vial, which gets stored in a freezer until the end of the season. All of the vials then get sent to Selena for further processing. We also have access to daily weather data, which we use to compare sap flow, sugar content, and chemistry, against the local conditions.

*Selena:* We assess the quality of maple sap via reagent-based spectrophotometry and LC-MS in order to quantify overall and individual phenolic constituents in maple sap that contribute to its quality – including flavor and nutrient attributes. The maple samples are lyophilized, re-dissolved in methanol, filtered, then analyzed. We also use LC-MS to measure individual phenolics, such as vanillin and coumarin. Essentially, we measure how specific compounds responsible for crop quality vary with changes in environmental, management, and processing factors.

What do you hope to get out of the project?

*Joshua:* We want to understand how sap yield and quality is related to climate conditions. Past studies have focused mostly on yield, so we are focused more on quality – both for sugar content and chemistry of the sap. Our results should help producers understand what conditions make for the highest quality sap, and also how sap quality may change as the climate changes.

*Selena:* In addition to climate, sap flow, and sap chemistry data, we are collecting social science data – making this a truly interdisciplinary project. Specifically, we are interviewing maple producers to understand their perceptions of climate effects on maple resources and the ability of management to mitigate climate risks.

*Follow the project here: http://blogs.umass.edu/acernet/*

*Reference*

1. *M Rapp & EE Crone, "Maple syrup production declines following masting", For Ecol Manage, 335, 249–254 (2015).*

# From Precision Medicine to Proteomic Maps

**What's new in business this month?**

In our regular column, we partner with www.mass-spec-capital.com to let you know what's going on in the business world of analytical science. There's a real focus on collaboration this month, with academics and vendors uniting to tackle key issues in diagnostics and biopharmaceuticals.

For more information plus links to other launches and deals, please visit the online version of this article: tas.txp.to/0716/BUSINESS

## Products

As the latest addition to the Thermo Scientific Vanquish UHPLC platform, the new Thermo Scientific Vanquish Flex Binary UHPLC system adds a binary solvent delivery option in the 1000 bar (15,000 psi) performance range.

## Collaborations

1. Thermo Fisher Scientific and Dublin-based NIBRT announced a scientific collaboration for biopharmaceutical characterization.
2. The MultiModal Molecular Imaging Institute (M4I) at Maastricht University has joined the Waters Centers of Innovation Program.
3. Waters collaborates with Singapore's Bioprocessing Technology Institute to develop new strategies for identifying cancer markers and probing cancer biology.
4. Bruker announced the results of a successful collaboration with the Special Bacteriology Reference Laboratory at the US Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia to create an expanded microorganism reference library for the Bruker MALDI Biotyper.
5. Thermo Fisher Scientific announced a partnership with West China Hospital of Sichuan University, to develop a joint platform for researching precision medicine.
6. Sciex will work with the Francis Crick Institute and the University of Cambridge to build a comprehensive metabolism-centric proteomic map (focusing on enzymes involved in the control of metabolism) using Sciex micro-flow chromatography and data-independent SWATH acquisition.

## Financings & Acquisitions

- Eurofins announced a EUR 200 million private placement to La Caisse de dépôt et placement du Québec to secure its growth options. Eurofins also strengthened its footprint in food and water testing in The Netherlands with the acquisition of Bureau de Wit.
- 908 Devices has been awarded $165,000 in tax incentives through the Massachusetts Life Sciences Center's Tax Incentive Program.
- Agilent Technologies is set to acquire assets of iLab Solutions, a leader in cloud-based laboratory management software.

# Great SciX-pectations

## The chairs of SciX 2016 offer a sneak preview of this year's conference

*by Alexandra Ros and Mary Kate Donais*

The Federation of Analytical Chemistry and Spectroscopy Societies (FACSS) is hard at work putting the finishing touches to SciX 2016, which takes place in Minneapolis in September. With diverse programming, a wide variety of exhibitors, major award presentations, and numerous networking opportunities, attendees will experience everything they've come to expect from FACSS/SciX. Here are a few highlights of the strong and diverse conference planned by the SciX 2016 team.

Two new hands-on workshops will be presented this year, both focusing on STEM education and partially funded through a grant from the Kerith Foundation. On Sunday September 18 Celeste Morris from Northern Kentucky University will be teaching the Introduction to Arduinos workshop followed by the Advanced Uses of Arduinos on Monday September 19. Also designed with educators in mind is the Flipping the Analytical Classroom workshop on Tuesday September 20, taught by Chris Harrison, San Diego State University. Attendees will learn how to use various low-cost hardware and software technologies to produce instructional videos for use in teaching. Both workshops have low fees to promote enrollment by students and educators, and other low-cost workshops will be available.

The SciX conference has expanded its separations programming in recent years, and we are co-meeting with the 23rd International Symposium of Electro- and Liquid Phase-Separation Techniques (ITP) in 2016. ITP programming will be across the first four days of the conference, and all SciX attendees have the opportunity to attend any of the 17 sessions organized by ITP co-chairs Ziad El Rassi and Blanca Lapizco-Encinas. There will also be two workshops organized by ITP on Sunday plus various social events specific to ITP attendees.

Lastly, the SciX section chairs have worked hard to organize strong section programs for the 2016 conference overarching most aspects of analytical chemistry. There will be cutting-edge symposia in hot areas such as atomic spectroscopy, pharmaceutical analysis, process analytical technology, security and forensics, surface plasmon resonance – and a Surface Science and Nanotechnology program. All sessions will include aspects of fundamental science as well as applications.

A special focus this year will be on Easing World Poverty, with a closing session on Science Beyond Borders also planned. Other special sessions at SciX 2016 focus on Women and Diversity in Analytical Sciences, and Art and Archeology.

We look forward to welcoming you to Minneapolis!

*Alexandra Ros is Program Chair and Mary Kate Donais is General Chair of the SciX conference.*

*SciX 2016 will be held September 18 – 23, 2016 at the Hyatt Regency Hotel in Minneapolis. www.scixconference.org*

*Deadline for poster abstracts: 31 July, 2016. Submit via the SciX website.*

# King Tut's Space Blade

## X-ray fluorescence spectrometry uncovers the meteoritic origin of a dagger in Tutankhamun's tomb

Tutankhamun's tomb and its contents have been the subject of fascination since its discovery by archaeologist Howard Carter in 1922. Results of previous analyses of Tutankhamun's iron funerary objects have proved controversial, with scientists arguing over whether the high nickel content is suggestive of meteoritic origin.

Most recently, a bilateral project between Italy and Egypt – focusing on the non-destructive analysis of objects from the ancient Egyptian culture – has allowed a multidisciplinary team to analyze one of two daggers found in the mummy's wrapping. They have concluded that the dagger blade is in fact made of meteoritic iron.

The team analyzed the bulk composition of the blade using non-destructive x-ray fluorescence (XRF) spectrometry. "The chemical compositions of iron meteorites are typically determined by means of sensitive (but destructive) analytical methods, including instrumental neutron activation analysis and inductively coupled plasma mass spectrometry," says Daniela Comelli, Researcher at the Polytechnic University of Milan, and an expert on the development and optimization of scientific non-destructive methods on cultural heritage objects. "By contrast, XRF spectrometry allows you to get information on the composition of an object (in terms of chemical elements) in a quick, easy and non-destructive way."

The composition of the metal of the dagger blade (iron, plus 10 wt% nickel and 0.58 wt% cobalt), is characteristic of nickel-rich iron meteorites, according to two of Comelli's colleagues, planetary scientists Massimo D'Orazio and Luigi Folco (University of Pisa). In particular, the nickel-cobalt ratio is close to the cosmic ratio typically observed in meteorites.

The result has important implications. "This confirms that before the advent of The Iron Age, ancient Egyptians considered the rare pieces of meteoric metal a valuable source for the production of precious ornamental objects," Comelli says. It also provides insight into the iron working capabilities at that time. "The high manufacturing quality of the dagger suggests that ancient people of the eastern Mediterranean area had acquired significant mastery in iron smithing already close to the end of the Bronze Age."

The idea for the research was originally conceived in 2010 by iron meteorite experts from Pisa University, who proposed analysis of Tutankhamun's dagger but were unable to gain access. Though it is still too early to judge the response of the archaeological communities to the research, the team now hope the discovery will allow them to analyze the other iron objects in Tutankhamun's tomb. Comelli is tentatively optimistic: "I hope our discovery reinvigorates interest in this kind of scientific research!" *JC*

*Reference*

1. *D Comelli et al, "The meteoric origin of Tutankhamun's iron dagger blade", Meteorit Planet Sci [online] (2016). DOI: 10.1111/maps.12664*

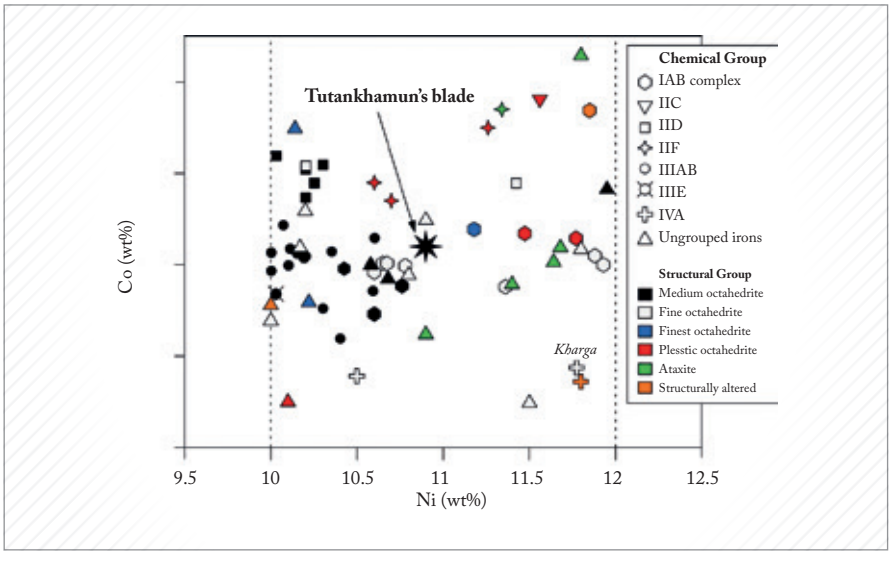



Figure 1. Co versus Ni diagram for Tutankhamun's iron dagger blade (black star) and for iron meteorites with a moderately high Ni content (10–12 wt%), i.e., with composition similar to the Tutankhamun blade, sorted by chemical and structural groups. Published by: Wiley © The Meteoritical Society, 2016

# What We learnt: Riva 2016

## Four experts share highlights from "the forum on microcolumn separations"

Beautiful Riva del Garda (Italy) played host to the 40th International Symposium on Capillary Chromatography and the 13th GC×GC Symposium in late May. Here, a select group of attendees tell us what stood out from an impressive crowd.

"Unexpected insights are always the most impressive – all the more so if they also offer a glimpse of a new horizon. The lecture from Restek's Roy Lautamo on 3D printing of capillary columns was such a highlight. Printing of capillaries is not such an obvious idea, and the presentation offered both historical roots and a visionary approach. Roy's very lively story began with solutions, included modeling results, and finally described a convincing path to alternative and potentially improved separation columns of the future." – *Peter Boeker, University of Bonn, Germany.*

"The presentation of the Golay Award to Rob Synovec was most eye-catching for me. It meant utterly deserved recognition for Rob, but also for the field of chemometrics. Smart data handling and data treatment is increasingly important in analytical science, and Rob Synovec has been a leader in this field for many years. There were other interesting contributions with a chemometric flavor as well, such as that of the ferocious (looking) Martin Lopatka, who is using incredibly smart statistical methods to obtain forensic evidence from GC×GC data." – *Peter Schoenmakers, University of Amsterdam, the Netherlands.*

"What struck me as very interesting were the opening remarks from Pat Sandra's plenary lecture. He stated that LC×LC will become more important than GC×GC in the future, because it can handle more complex samples. Further, a key to the development of LC×LC (and GC×GC for that matter) has been the availability of commercial systems. While not a seasoned practitioner, I have recently had the opportunity to contemplate the power of LC×LC while on sabbatical with Luigi Mondello and Paola Dugo in Messina. Given the solutions from major instrument manufacturers and the range of separation modes available, the application space of LC×LC seems quite limitless. Certainly, as Sandra stated and demonstrated, an area where it can be immediately extremely powerful is in the protein analysis space." – *Kevin Schug, University of Texas at Arlington, USA.*

"Pat Sandra's lecture on the latest developments of 2D-LC for the enhanced analysis of macromolecules was of great interest to me, because it signified an endorsement of multidimensional chromatographic techniques. A similar evolution occurred in conventional 1D chromatography: GC evolved first (in the 1950s), and LC then followed (about a decade later). Today, a large share of the market belongs to LC, but GC is still relevant. Pat's lecture to me was an announcement that multidimensional chromatographic techniques are now here to stay, which is quite exciting indeed.

I was also very impressed with the high quality content of the multi-dimensional GC lectures and posters that I attended. While some people lamented the fact that they were not able to attend every GC×GC talk because of the split sessions, I actually take that as a positive sign of the growth of the technique. After all, we do not expect to be able to hear every lecture on conventional GC when we go to Pittcon or ACS, do we? We need to get used to the fact that, just like at those conferences, we need to pick and choose which talks we go to, or plan on bringing more people for full coverage of all sessions. I shared many wonderful conversations with young scientists over the week, and I look forward to seeing more of this at future ISCC/GC×GC conferences." – *Jean-Marie (John) Dimandja, Georgia Institute of Technology, USA.*

# The Power... Then the Glory

## Nominations are open for an all-women Power List

In our May issue, we announced the 2016 Power List – we felt it was high time to showcase the work of female analytical scientists, some of whom may not be getting the recognition they deserve. Our readers obviously think the issue is as important as we do, because the nominations have been pouring in... But we'd like to keep the momentum going. Visit http://tas.txp.to/power/2016 to nominate up to three women in the field – the top 50 will feature in our first all-women Power List, announced in October.

# In My View

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

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

*Contact the editors at edit@texerepublishing.com*

## Only Gene Deep

**Advances in genomics are certainly thrilling, but let's not forget that a tumor is more than a bundle of genetic information.**

*By Han van Krieken, Chair of Pathology, Radboud University Medical Center, Nijmegen, Netherlands.*

As histopathologists, we try to understand disease by looking at tissues. We see a snapshot of cells in their tissue environment. We can see whether they are normal or abnormal, whether there are too many or too few cells, how they are organized and how they interact. We can localize enzymes and proteins, measure expression levels, determine DNA alterations, and so on – and by bringing all this knowledge together, we can form a fairly complete picture of the disease that manifests itself in the tissue. These efforts provide the patient and the treating physician with information that can be used to choose the best possible treatment (or no treatment).

In this era of genetics, we are increasingly able to sequence the DNA of individuals and tumors, which allows us to quickly diagnose many different diseases that are caused by changes in genes, such as cystic fibrosis or Noonan syndrome. For cancers, we get information on the gene alterations that drive the tumor; for example, c-Erb2 amplification or ALK-fusions. Increasingly, it is suggested that whole genome sequencing will replace traditional forms of diagnosis. Indeed, if a child with an intellectual disability comes for a diagnosis, physical examination is already replaced by DNA analysis. And I was informed that in Hong Kong, where the incidence of EGFr mutated lung cancer is quite high compared with western countries, lung cancer is already diagnosed using genetic tests on blood samples in patients with inaccessible pulmonary lesions; if an EGFr mutation is found, it is regarded as sufficient evidence that the patient should be treated using an anti-EGFr approach. But in my view, although sequencing is an important diagnostic tool with much potential, it will never give the complete picture.

An example: it was recently shown that the cells within a tumor the size of a ping-pong ball will carry a total of 100 million mutations, with only a few of those mutations present in the majority of cells (1). Not only does this finding indicate that tumor heterogeneity on the cellular level is enormous, but also that complete sequencing of tumors provides us with so much data that it becomes useless. Quite interesting, of course, but not surprising for pathologists. In fact, that the nuclei

*"Although sequencing is an important diagnostic tool with much potential, it will never give the complete picture."*

in cancer cells are extremely variable compared to normal cells has been one of the most important criteria a pathologist uses when making a diagnosis of cancer for more than a century...

Furthermore, a tumor consists of not only neoplastic cells but also stromal cells, such as fibroblasts, inflammatory cells, endothelial cells and others. There is enormous variation in the ratios of these cell types between tumors – variation that has been shown to relate to treatment response and survival of the patient. Such variation cannot be found by sequencing the tumor or even the germline DNA.

Genes act through proteins, but proteins are not only modified by genetic mechanisms. Indeed, proteomic approaches are likely to give even more information, but replacing genomics with proteomics (which will take quite some time) will also not tell the whole story. Cells and tissues are so complex that we cannot fully understand what is going on by extracting only the genes and proteins. Spatial orientation, communication between cells, composition of tissues are all critical.

To that end, analyzing tissues with the microscope will remain an extremely cheap and fast way of providing useful information. But I am also convinced that we can benefit from new approaches in this field to extract even more information; for instance, deep-learning approaches – where standard tissue image analysis is supplemented with new information based on automated quantification of structures and protein levels – have great potential.

Of course, sequencing of tumors has given us a lot of valuable information – and will continue to do so – but we must remember that many other factors are equally important. As we all know, we are more than our genes – and a tumor is more than its genetic make-up.

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# Splendid Isolation

**It's time to embrace a new way of obtaining pure components from complex natural samples.**

*By Sebastiano Pantò, Application Chemist at LECO EATC, Berlin, Germany.*

Over the past few years, several efforts have been made to obtain bioactive molecules from natural sources, but the most widespread technique for obtaining pure chemicals is organic synthesis, which plays a major role in many fields such as pharmaceutical, food, flavour and fragrances. The practice is often inefficient; in fact, sometimes kilograms, even tons of raw material are required to obtain enough pure product. And the approach certainly cannot be considered "green" because of the huge production of waste, including solvents and hazardous by-products.

Over the last century, both liquid and gas preparative chromatography appeared in analytical chemistry as a possible substitute for organic synthesis for the isolation of pure molecules. However, neither technique – and in particular, preparative gas chromatography – was seriously considered from a commercial point of view, because of the low quantities collected per run, as well as the degree of purity attained. Preparative chromatography also had other limitations: difficulty in separation of very complex samples and the low relative concentration of the components to be isolated.

Many scientific papers have been published on the subject, reporting the isolation of pure components, such as PAHs and volatiles, components from distilled spirits and small molecules from complex samples (1-4). Almost all these applications exploited a huge number of repeated injections (100-500) to obtain only micrograms of pure components. The reason lies in the low injected amounts, often mandatory to preserve separation and resolution of the components of interest prior to collection.

With this in mind, during my post-doc experience in the group headed by Luigi Mondello, some colleagues and I decided to develop an all-in-one lab-made instrument – the first on-line LC-GC-GC-GC prep system capable of collecting milligrams of pure components in a very short time (5, 6). The system represented a step forward in the field of preparative chromatography because of its ability to meet the demands of private companies who, until that moment, had not considered the technique a valid tool for their needs.

The instrument consists of a HPLC system equipped with a normal phase 25 cm x 4.6 mm ID LC-Silica column, connected through a special syringe-

*"The entire instrumentation may appear very complex to handle, but the results attained surely justify its use."*

type interface to a large-volume injector (LVI) and three GC ovens. The ovens are equipped with three wide-bore columns (0.53 mm i.d.) of different selectivity and three Deans Switch devices that allow the heart cut of single fractions from each dimension to the next. Finally, the collection of the pure components is performed using two different collection devices: i) a low-cost, lab-made one, positioned in the second oven, which allows the collection of the already resolved peaks and ii) an automated commercial system positioned at the end of the third column that is capable of collecting up to 10 components in one run.

The four-dimensional preparative system described above has been exploited for the isolation of two non-commercially available sesquiterpene components from vetiver essential oil – namely alpha-amorphene and beta-vetivone – as well as the collection of seven of the most important oxygenated components belonging to sandalwood essential oil. In both cases, thanks to this system, we were able to collect milligrams of pure component in under a day.

Although the entire instrumentation may appear very complex to handle, the results attained surely justify its use in many specific applications where other techniques – such as distillation and organic synthesis – fail.

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# The Dark Side of Scientific Publishing?

**Third-party review is a viable – and faster – alternative to "journal shopping".**

*By Robert Kraus, Assistant Professor, University of Konstanz, and Research Scientist, Max Planck Institute for Ornithology, Radolfzell, Germany.*

Nature News recently highlighted the fact that scientific publishing is getting slower – even though in the digital age everything about publishing and communication should be faster (1). A closer look, however, shows that the process of individual review is fast enough. Email communication, online manuscript management tools, outsourced and digital design, plus the now common procedure of making PDFs of accepted articles available way ahead of print are all modern advances of the Internet age. The problem lies in the trend of "journal shopping".

Hiring committees and grant agencies have placed so much weight on the journal impact factor (IF) that CVs are screened for publications in those journals with the highest standing (with reference to this indicator). Much has been written about the fallacies associated with the IF; however, the pressure is still on the individual scientist. Publishing in shiny, high-impact journals is a

mighty weapon in the hands of a scholar who tries to snatch that grant or get that all-important permanent contract at a university of their choice. Obviously, high-impact journals receive an overload of submissions by eager scientists and can only publish a fraction. Scientists take their chances and submit anyway: one journal at a time, down the IF ladder from high to low. Many will be rejected, and fairly often this only happens after external review – something that consumes the time of the reviewers as well as the authors. Chances are, a set of new reviewers will hand the submission to the next journal on the authors' wish list, while time is lost in a serial fashion.

*"Publishing in high-impact journals is a mighty weapon in the hands of a scholar trying to get that grant."*

One solution to delays caused by serial events is parallelization. In computing, parallel processors are used to permit faster calculation (or more calculations at one time); parallel DNA sequencing has dramatically increased the supply of genetic data. While the Ingelfinger rule prevents simultaneous submission to more than one journal, third-party review organizations (such as Axios Review, Peerage of Science, and Rubriq) can assess the 'fit' of a paper to multiple journals simultaneously and pass the paper to the outlet that is thought to be most suitable. Early evidence suggests that the parallelization approach significantly shortens the review process.

I am an academic editor at Axios Review (https://axiosreview.org) and so know firsthand that this trial project has run quite successfully since its official launch only two years ago. Our open letter in a follow-up issue of Nature covers the essence of this idea (2). For instance, 85 percent of papers reviewed by Axios Review get accepted at the first journal to which they are sent, and more than half of the accepted papers are not peer reviewed again by the journal. These are impressive numbers and support the general idea that independent, external review can be de-coupled from processes at the individual journals.

We scientists know that other solutions to the problems associated with journals and impact factors are currently tested and discussed, too. These include ideas to either get rid of the impact factor altogether, or to get rid of pre-publication peer review in favor of a system where manuscripts may be openly discussed by the community and improved in an iterative forum fashion on preprint servers. These measures appear equally suited to doing away with journal shopping. However, there are many reasons to keep a system in which journals rely on invited expert opinion and the associated review process. Peer review is a tool that acts like a filter for high quality presentation of experiments and analyses, as well for balanced and neutral conclusions. Within this setting I urge colleagues to try out the new system of third-party review. As a reviewer you will be grateful for potentially receiving fewer submissions, and as an author you will appreciate taking much fewer steps when writing and revising your manuscript.

# The Analytical Triumvirate

**The reawakened interest in SFC shows that, all too often, academics are followers and not leaders.**

*By Caroline West, Associate Professor at the Institute of Organic and Analytical Chemistry, University of Orléans, Orléans, France.*

The development of analytical techniques is dependent on the interest and investment of three possible contributors: i) manufacturers who build innovative systems and promote them; ii) academic researchers who stimulate innovation, improve understanding of the fundamentals, demonstrate feasibility of applications and educate future users; and iii) industry users who demand technology improvements in setting high requirements on system quality, reliability, robustness and cost-effectiveness.

When one of these three elements of the triumvirate is missing, analytical methods do not progress well. For an example of such impaired development we need only look at the history of supercritical fluid chromatography (SFC).

First described in 1962, SFC initially attracted the attention of academics for allowing the elution of thermolabile analytes with the help of a denser fluid than the hot gases employed in gaseous phase chromatography. Note that HPLC did not exist at the time. Carbon dioxide became a favorite eluent, because the supercritical conditions could be reached at low pressure and temperature values, and because of the added benefits of a cheap, abundant, non-toxic, non-flammable, non-corrosive solvent. However, the limited elution strength of neat $CO_2$ prompted the use of co-solvents in the 1980s (most often an alcohol, such as methanol).

Technical development demanded real innovation – pumping a compressible fluid and mixing it with a liquid co-solvent in well-controlled proportions; injecting liquid samples into this compressible mixture; controlling back-pressure to obtain the desired fluid density – after all, such requirements surpassed those of typical GC systems and then-developing HPLC systems. No surprise then that only a few manufacturers settled on designing dedicated SFC systems – most users employed homemade systems.

In the 1990s, although much research showed the potential of SFC in many application areas, SFC never really saw the light of day. The limited involvement of manufacturers in the SFC space was compounded by HPLC coming of age, deterring industry users from investing time and money in SFC.

In 2000, SFC had essentially survived as a preparative-scale technique for the resolution of enantiomers, where the economic advantages of $CO_2$ were widely recognized. At this point, academics had essentially abandoned the technique, which was when I, as a new chromatographer, entered the field and learnt about SFC (and other types of chromatography) with someone who had practiced the technique for over 10 years. When attending chromatography conferences during this period, we were rather lonely. There was barely a talk to go to and only a couple of posters mentioning SFC – usually the ones we had brought ourselves. You could count on the fingers of one hand the academic teams still putting effort into SFC. However, in user meetings, where only industry users attended, we could feel a strong demand for fundamental work to help improve their understanding, as well as improved systems to perform more reliable and effective analyses.

It took the efforts of two major manufacturers, who finally introduced analytical systems that met the high-level requirements of the industry at the beginning of 2010, to reawaken the attention of academics. Today, chiral separations are still of interest, but achiral applications have particularly increased. The publication rate of SFC papers is higher than ever – about twice the number produced ten years before. A close examination of the contributors to SFC literature shows that industry contributions have not varied much in the past 20 years, possibly indicating that the level of interest has remained constant. It is only the contributions of academic researchers that have drastically reduced – but that number is now increasing again.

As an academic researcher, I feel really concerned by my observations, which make me wonder about the responsibility of academics in the development of a particular technique – not just in terms of fundamental understanding, but also in education. Even though I have worked on SFC for over a decade, I have only recently introduced SFC lectures to my students. I guess I also felt that it was not worth spending time on a technique that they were so unlikely to need in their future careers... Most importantly, I am concerned about our role as stimulators of innovation. Though I am happy about the current interest in a technique I have supported for several years, I wonder about our tendency to follow trends (possibly because it is easier to publish on a trendy topic) when we should, on the contrary, inspire new directions. In other words, academic researchers should be thought-leaders, not followers.

# Addressing Diagnostic Error

## How can we harmonize testing to prevent laboratory-related diagnostic errors?

*By W. Greg Miller, Professor of Pathology, Virginia Commonwealth University Medical Center Richmond, Virginia, USA.*

Harmonization of laboratory test results is one of the most pressing issues in laboratory medicine – around 40 percent of medical encounters are informed by laboratory tests and pathology consultations. The landmark 1999 report from the US Institute of Medicine "To Err Is Human: Building a Safer Health System" emphasized the importance of clinical practice guidelines to standardize decisions and treatments. Using guidelines was not new in 1999 but has assumed increasing importance in the practice of medicine. A 2015 follow up report from the Institute "Improving Diagnosis in Health Care" again emphasized the importance of guidelines and stressed that cooperation among the health care team, including laboratory professionals is essential to reduce diagnostic errors.

Neither of these reports recognized that laboratory test results frequently vary, depending on the measurement procedure or laboratory performing the test. Consequently, diagnostic errors are possible when non-harmonized laboratory test results are interpreted using fixed decision values in clinical practice guidelines. For example, parathyroid hormone (PTH) results varied four-fold across different laboratory methods, yet a guideline recommended the drug Cinacalcet for treating calcium and phosphate imbalance in chronic kidney disease when the PTH exceeded a fixed value (1). Urine albumin to creatinine ratios of 30 mg/g (3.4 mg/mmol) and 300 mg/g (34 mg/mmol) are almost universally used in guidelines to identify micro- and macro-albuminuria in diabetes or hypertension, despite a 45 percent difference in median results among different laboratory measurement procedures for urine albumin (2). Steroid hormone measurements such as testosterone and estradiol have 100 percent or more variability among different measurement procedures making clinical guidelines difficult to develop or apply (3).

*"Diagnostic errors are possible when non-harmonized laboratory test results are interpreted using fixed decision values in clinical practice guidelines."*

A substantial infrastructure has been developed to provide tools and procedures for harmonization of laboratory test results (4). The International Standards Organization (ISO), for example, has standards for reference materials, reference measurement procedures, and reference laboratory services. The Joint Committee for Traceability in Laboratory Medicine (JCTLM) reviews specific components of reference systems that conform to one of the ISO standards and lists those that meet the criteria. Measurement procedure producers use these approved reference systems to establish calibration traceability for the measurement procedures used in medical laboratories. At present, the JCTLM lists reference methods for 79 analytes and reference materials for 162 analytes. However, no reference system exists for most of the 1,000-plus medical laboratory tests. Clearly, our profession has a challenge to fill this gap so that more test results can be harmonized.

In principle, calibration traceability to reference systems should produce harmonized results among different measurement procedures. Unfortunately, some analytes with reference system components remain non-harmonized. One of the main reasons for ineffective harmonization is lack of commutability of reference materials with authentic clinical samples (4). Commutable reference materials are those that have the same relationship for results between different measurement procedures, as do clinical samples. Calibration traceability to commutable reference materials effectively harmonizes results for clinical samples. Unfortunately, a number of older JCTLM-listed and other international reference materials are not commutable, so when they are used for calibration traceability the results for clinical samples do not agree among different measurement procedures (4). JCTLM now requires commutability validation for reference materials intended to be used as calibrators for medical laboratory tests. Therefore, all providers of reference materials should ensure commutability for new reference materials.

*"Our profession needs to collaborate with regulatory agencies to streamline and lower the cost for approval of harmonized measurement procedures."*

Another challenge for harmonization is the large number of analytes for which there are no reference system components available. This problem was addressed at a conference in 2010 (5) and mechanisms are now being developed by the International Federation of Clinical Chemistry and Laboratory Medicine, the International Consortium for Harmonization of Clinical Laboratory Results and ISO to use international consensus harmonization protocols to achieve agreement for clinical sample results among different measurement procedures.

An interesting challenge for implementing new calibration schemes to achieve harmonized test results is conformance to regulatory requirements. Many countries have regulations that require measurement procedure manufacturers to resubmit for approval when a test has been recalibrated to conform to international harmonization recommendations. Our profession needs to collaborate with regulatory agencies to streamline and lower the cost for approval of harmonized measurement procedures – such realignment of calibration is clearly in the best interest of good medical care. New measurement procedures should be required to demonstrate calibration traceability to approved reference systems, when they exist, rather than simply demonstrating agreement with another measurement procedure already on the market.

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# Breaking New Ground with IC-MS

## Then & Now, with Stuart Adams, Higher Analytical Chemist at Fera Science Ltd, York, UK.

Then: a bright but chilly day in 2007...

About nine years ago, we were having problems with the analysis of glyphosate and glufosinate. We'd been using derivatization prior to GC-MS/MS, but too many repeats of samples told us we needed a new solution. We got in touch with Dionex to assess whether ion chromatography (IC)-MS could help reduce the amount of time spent in the laboratory and produce more reliable results. That first conversation kicked off a collaboration with Dionex, who supplied an ICS-3000; we provided a mid-range mass spectrometer with a few upgrades. Sensitivity was always going to be an issue, so we sought inventive ways to get the most out of the system. We came up with the idea of using inline concentrators for sample cleanup. But if you inject as much as 4700 µL of extract onto a system, you add an awful lot of background matrix, so we needed to flush the concentrator with water to remove the non-ionic components of the matrix before bringing it inline with the rest of the system.

For years, we worked with this solution. But it wasn't without its own challenges. We had to use two sets of control software (and therefore two PCs), so errors occasionally but inevitably crept into the sequences, meaning that the IC and MS systems were not always synchronized. We fudged around those problems by running the two control systems on the same PC, but we were still a little uneasy.

We spent the first couple of years – the "honeymoon period" – understanding what preventative maintenance was necessary to keep the IC-MS system running as smoothly as was possible. In fact, all of our systems go through weekly preventative maintenance – something that we've found to be a real time-saver in the long run. For our IC-MS system, the most important task was re-conditioning the columns each week.

There was always a certain "home-built" feel to the system – after all, we were one of the first labs working at this particular frontier. Nevertheless, the benefits were also clear; the number of repeated runs dropped dramatically. Essentially, we'd moved on from analysis that was very difficult using any other technique to much improved analysis on an albeit slightly cranky system. It also allowed us to expand our analytical services; the scope of IC-MS was not limited to glyphosate and glufosinate. A third compound – ethephon – appeared; I remember running the first batch of grapes for the Pesticide Residue Committee Survey and finding an MRL exceeding sample. We'd not done such analysis before, so we weren't sure what to expect – but from that point on, we regularly found ethephon in grapes...

Now: June 8, 2016

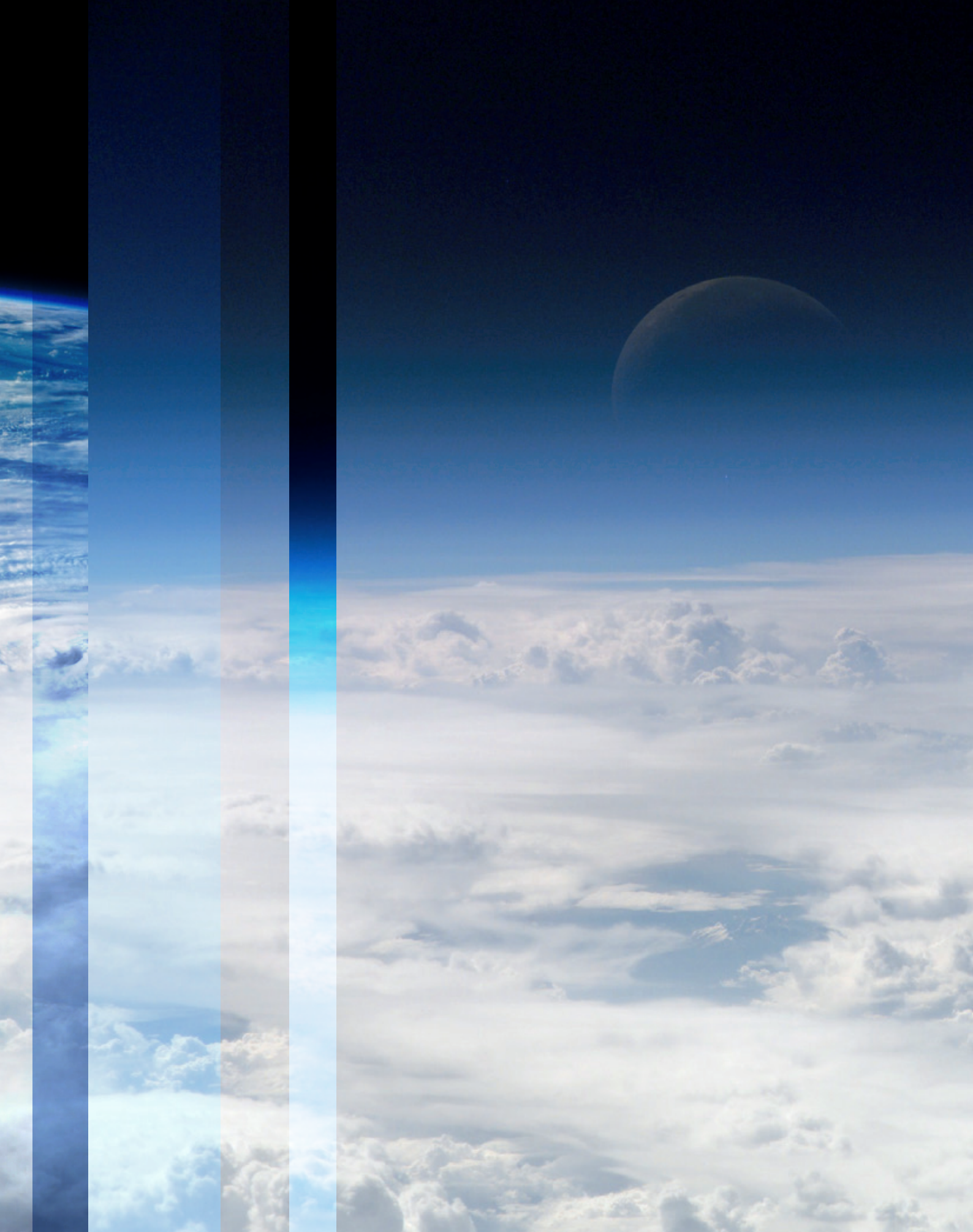
Where we are today is very different. Sample injection volumes have dropped from 4700 µL of extract to 100 µL of 10-fold-diluted extract (so 10µl in reality) – less is more! A stark and pertinent difference between "then and now" is how much the technology has advanced. Our Thermo Scientific™ Dionex™ ICS-5000™ is paired with a TSQ Quantiva™ MS system, both of which are controlled with a single software platform, TraceFinder™. Not only is the system easier to use but it is also much more reliable. In other words, we've progressed from the initial excitement of getting our first system to (mostly) work to the excitement of using a system that works the way we want it to out of the box. Columns have also become much more efficient in the intervening years, which allows us to get better peak shapes. And the TSQ Quantiva has got a special low-mass tuning solution – perfectly suited to our compounds of interest in IC-MS.

The scope of IC-MS analysis has also increased with chlorate, perchlorate, and phosphonic acid, all of which have become very topical. Rather than using an LC-MS system with uncertainty about the retention mechanism, we've got a tool that's designed specifically for anionic compounds. It's another robust tool in our toolbox that allows us to step away from the constraints of other techniques when we need to.

Nine years ago, we were certainly an early adopter of IC-MS for pesticide residue analysis. Today, I get the sense that IC-MS is being embraced by an increasing number of organizations in our field and beyond. And now that we've got a reliable system – and experienced staff – we certainly sell the technique internally.

When I started at Fera, there were a lot of single-residue methods. Over the years, such methods are diminishing as compounds are getting slotted into multi-residue methods. IC-MS fits into that evolution with its ability to target a suite of 40-50 analytes. We have developed and validated methods for anionic pesticides and going forward we hope to work with Thermo Fisher Scientific to evaluate cationic pesticides.

It's clear that we all want to test for more compounds with less effort – and in 5–10 years' time, I suspect we'll be working on unknown screening, which will complement our targeted analyte approach. We are also evaluating the Q Exactive™ Focus Hybrid Quadrupole-Orbitrap™ Mass Spectrometer for other analyses, and hopefully IC-Orbitrap MS, especially given that, as Amadeo Fernandez-Alba noted last month, high-resolution accurate-mass MS systems are likely to become more dominant in the future. As analytical chemists, we don't want to be tied to a list, waiting for a problem – we want to be able to identify upcoming problems and trends. And for that, we need the right tools for the job.



# UNDERSTANDING OUR ATMOSPHERE

How do we untangle the complex molecular level chemistry of organic aerosols? And is such knowledge even necessary when studying atmospheric processes? Here, I share my sometimes lonely – but always fascinating – journey into the unknown.

*By Giuseppe Petrucci*

Aerosols play an undeniably important role in atmospheric processes. And our understanding of that role has become increasingly clear over several decades. Initial research focused on inorganic aerosols, in large part because they posed questions that we thought could be answered with established methods. Sure enough, we began to answer some important questions – but, as is typical in research, some answers inspired new questions that pushed the limits of what was then current analytical science.

It soon became clear that bulk chemical measurements with time scales of days to weeks would not yield the information necessary to advance the state of knowledge with respect to aerosols. We had several important yet unanswered questions; for example, how important is the chemistry of single particles? What is the role, if any, of organics? Aerosol mass spectrometry was developed with these new questions in mind and has been revolutionary in providing tantalizing glimpses into the complex life cycles of aerosols. However, it has fallen short when it comes to organics. Questions still remain: How do we untangle the molecular level chemistry of organic aerosols? Do we even need to? What methods exist that could give us information to this end? Unfortunately, the answer to the last question is "none."

I would say that, for the scientific community in this field, complacency is a major enemy. Because we are able to produce some data with aerosol MS – data that can easily be handled by models and used to gain some predictive model outputs – many of us simply stopped looking for better data. And indeed, the general thinking has become: "why bother getting more detailed data when the models can't handle it anyway? Let's just work with what we have." I wasn't – and am still not – satisfied with that conclusion...

## More lasers, more fun

My fascination with aerosols actually started at the same time as my career in academia, some 20 years ago. I was trained as an analytical chemist at the University of Toronto before entering a PhD program at the University of Florida. I was under the mentorship of the brilliant James Winefordner where I learned to appreciate the power of lasers (pun intended). In fact, my motto has since been "more lasers, more fun." Shortly before completing my postdoc, I received an NSF Fellowship that allowed me to continue my work at the Joint Research Center (JRC) of the European Commission in Ispra, Italy. And in late 1993, I had the good fortune to work with another great mentor – Nicolo Omenetto, who I first met in Florida during one of his annual summer pilgrimages to the Winefordner group.

The fellowship proposed a continuation of my PhD work on the use of two-step resonance-enhanced laser ionization as an ultrasensitive, monochromatic photon detector capable of operating effectively in bright light conditions. And what a project that was – two lasers, a low-pressure lamp and a voltage-to-frequency converter that whistled when the laser wavelength was tuned!

It was toward the end of my fellowship that I met an energetic and overly-enthusiastic German postdoc (Ulrich Panne, now President of the Federal Institute for Materials Research and Testing) who had a contagious excitement for all things aerosol. In fact, he had joined our lab to initiate an aerosol laboratory – in keeping with one of the focuses of the Fourth Framework Programme of the European Commission. We were tasked with developing a new instrument for the chemical analysis of atmospheric particles. The analysis needed to be accomplished on a single particle basis, in real time and in situ – a daunting task. There were several groups worldwide working toward the same goal and, without exception, all approaches used mass spectral detection of ions generated by one of several different means. Most of the approaches used high-power lasers to intercept the particles in the ionization region of the mass spectrometer. During this laser-particle interaction, copious numbers of ions were generated for analysis. At the time, all the groups were focused on the analysis of inorganic particles.

Of the groups working on this task, two produced commercial instruments – one of which has been revolutionary in this field (the Aerodyne aerosol mass spectrometer). The innovative, plug-and-play approach presented a paradigm shift in the analysis of atmospheric aerosols. It was accessible to a large number of research groups and provided, for the first time, a glimpse into the chemical makeup of organic aerosols (OA) primarily in the form of oxygen-to-carbon ratios (O:C). The approach quickly gained a strong following among the atmospheric chemistry and physics communities, leading to an expansive user network across the globe. Its impact in the field cannot be overstated.

And yet, despite the advances made possible by this instrument, something inside me still continued to ask: "Can something as simple as O:C really paint a meaningful picture of the role of OA in global change?" As chemists, should it not be "obvious" that distinct molecules are important? And that chemistry fundamentally governs the behavior of OA in the atmosphere? It was my belief that to advance our understanding of the governing principles of OA chemistry and atmospheric impact, we must push aside the naivety or complacency of our field.

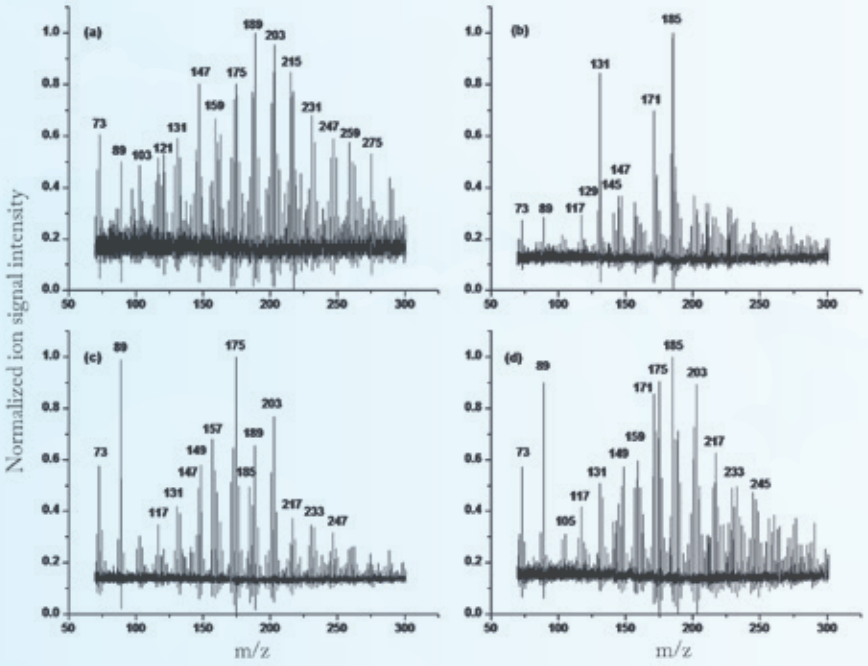



Figure 1. NIR-LDI-AMS mass spectra for four different chemical systems. Inherent simplicity of the soft ionization mass spectra makes direct, semi-quantitative comparison possible. Mass spectra are for SOA derived from (a) grass clippings at SOA mass loading (COA)= 16.3 μg/m$^3$ sampled for 2 minutes (3.9 ng) (b) cis-3-hexenylacetate (CHA) at COA = 14.7 μg/m$^3$ sampled for 6 minutes (10.6 ng) (c) cis-3-hexen-1-ol (HXL) at COA = 10.3 μg/m$^3$ sampled for 10 minutes (12.4 ng) and (d) CHA and HXL mixture at COA = 3.9 μg/m$^3$ sampled for 5 minutes (2.3ng).

*"I would say that, for the scientific community in this field, complacency is a major enemy."*

But how could we approach the task of analyzing femtogram quantities of molecules on/in OA without destroying the molecular identities of the compounds? And how could we accomplish this goal while working at atmospherically relevant mass loadings and time scales?

At the JRC, we also developed an aerosol mass spectrometer based on a relatively high-power pulsed laser that was capable of measuring positive and negative ions simultaneously. Although we anticipated observing predominantly positive ions, we were surprised to measure negative ions with greater resolution and even better sensitivity. Unfortunately, our negative ion spectra were highly fragmented and much of the molecular information was lost. Nonetheless, we were excited at the prospect of making nascent OA measurements.

## The birth of NIR-LDI-AMS

In mid-2000, my stay at the JRC ended and I took up my current faculty position at the University of Vermont. I started to think about ways we could be gentler in our vaporization and ionization approaches, so that we could reduce – or ideally eliminate – molecular fragmentation. Was there a way of keeping molecules intact, while still maintaining the sensitivity needed for measurements at ambient levels?

My original approach was to use infrared laser vaporization with resonance-enhanced multiphoton ionization, harking back to my PhD studies ("more lasers, more fun"!). Unfortunately, the funding-powers-that-be made it clear that they thought my approach was quite limited, while others expressed skepticism of the need to measure organic aerosols at all. "After all," they wrote, "how much organic mass could there really be in PM2.5?"

At this point in my life, I started to question my career choice!

Fortunately, while describing the photoelectric effect to my general chemistry class at UVM, I remembered an observation from my JRC work about the measurement of negative ions. Could it be that the particles were acting as little sources of

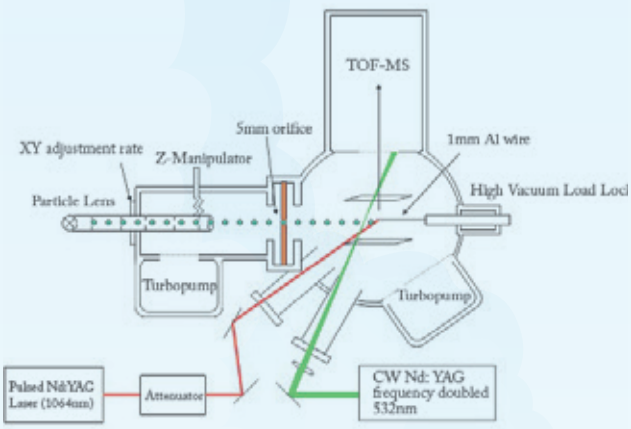



Figure 2. General instrumental layout for NIR-LDI-AMS instrument

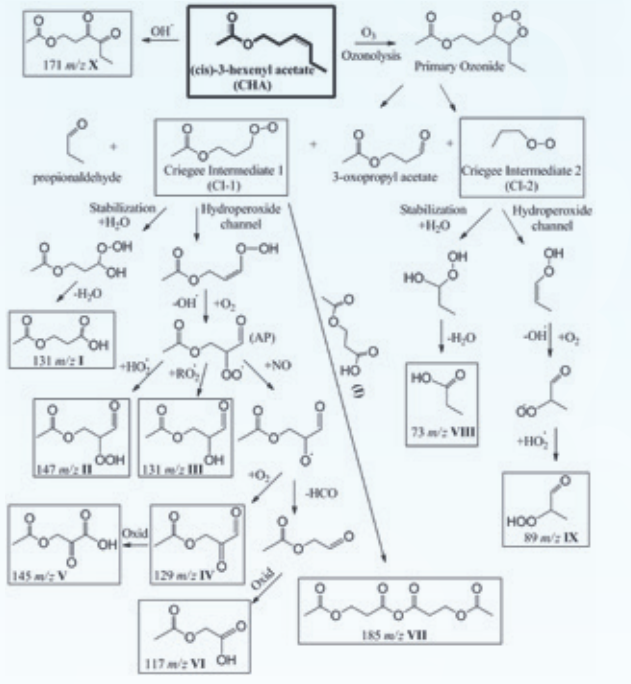



Figure 3. Scheme 1 CHA mechanism. Abbreviated reaction mechanism for the ozonolysis of Cigs-3-hexenyl acetate, one of the predominant SOA precursor emissions from turfgrasses. This is a chemical mechanism we were able to derive solely as a result of eliminating molecular fragmentation by way of using the soft ionization capabilities of our NIR LDI AMS.

photoelectrons, which then attach to vaporized molecules? I rushed back to my lab right after class and discussed this idea with my graduate student (Brian LaFranchi) and within a day or so, we had modified our aerosol mass spectrometer to use a home-built thermal vaporizer and a photoelectron source for testing. It took more than three weeks of constant fiddling to record our first spectrum of pure particles of oleic acid that showed absolutely no measureable fragmentation; and we were off to the races... But after several years of studying the ionization method of photoelectron capture, we accepted that it would never provide us with the necessary analytical figures of merit to work at ambient levels.

Now what? My first thought was to couple the photoelectron source to laser vaporization of the particles. In this way, we could optimize the temporal overlap between the vaporization laser and the UV laser generating the photoelectrons. A simple, back-of-the-envelope calculation suggested that, if we vaporized from a small collection probe in the vicinity of the photoelectron source, we could get signal enhancements on the order of 1,000 – 10,000 fold. Once we had incorporated laser vaporization from a small

1-mm diameter pure magnesium probe, we were rewarded with an approximately 3000-fold increase in ion signal, still with no molecular fragmentation! For once, a "rationally thought-out mechanism for improvement" worked as expected – that is, until we realized that the UV laser was not firing, which meant that the near-IR pulse was solely responsible for the signal enhancement!

Of course, I was elated that this meant that the complexity of the instrumentation would be reduced greatly. But I will confess that I was disappointed to have been wrong once again. In fact, the addition of the UV pulse in this instrumental configuration provided no substantial benefit to using the near-IR pulse alone. And thus, near infrared laser desorption/ionization (NIR-LDI) aerosol mass spectrometry (AMS) was born.

*"It took more than three weeks of constant fiddling to record our first spectrum of pure particles of oleic acid that showed absolutely no measureable fragmentation; and we were off to the races..."*

## Simplifying the puzzle

One could liken the difference between hard- and soft-ionization mass spectrometry to the difference between the frustration of assembling a 1000-piece puzzle after several glasses of wine (and without a copy of the final picture), and the simple enjoyment of helping a toddler put together a 50-piece floor puzzle while enjoying your first glass of wine...

In our instrument, aerosols are sampled via an aerodynamic

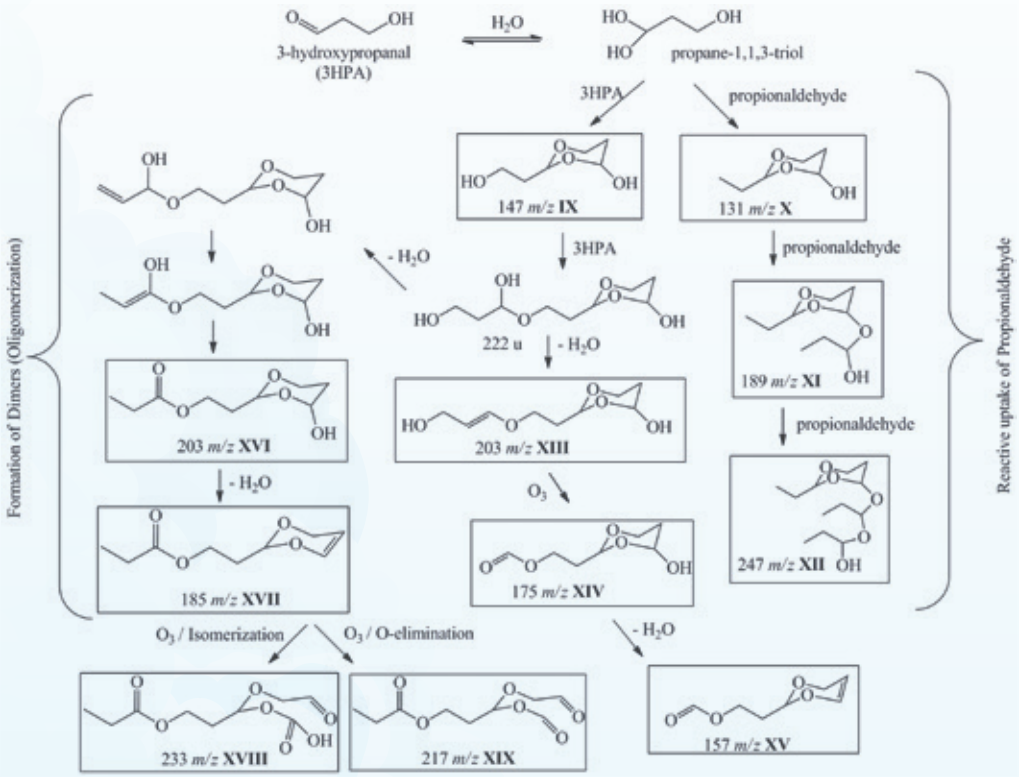



Figure 4. Scheme 2 HXL trimer mechanism. Proposed reaction mechanism for the formation of higher molecular weight products resulting from oxidation of HXL and reactive uptake of propionaldehyde.

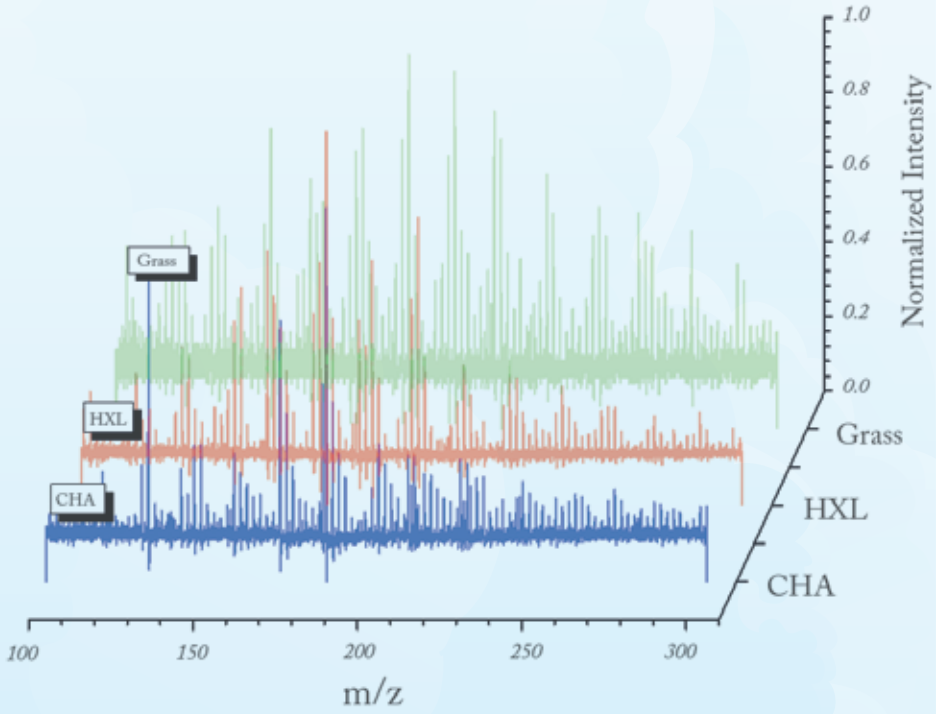



Figure 5. TOC abstract art. A different representation of NIR-LDI-AMS mass spectra from turf grass and the two predominant SOA precursors emitted (CHA=cis-3-hexenyl acetate and HXL= cis-3-hexenol).

lens to produce a tightly collimated particle beam that is directed onto a metal probe in the ionization region of the mass spectrometer. After some suitable collection time of seconds to minutes (depending on the organic aerosol mass loading), the NIR laser is fired, generating a 4 ns burst of ions that are chemically analyzed by time-of-flight mass spectrometry. The power density of the laser at the probe surface (<10 MW cm-2) is below the plasma formation limit and most organic molecules are transparent to the wavelength we use (1064 nm). Furthermore, even for those few photons that may be absorbed, the photon energy (~1 eV) is not sufficient to photoionize organic molecules directly. The net result is that all molecules are measured as their intact, pseudo-molecular ions ([M-H]-), greatly simplifying interpretation of the chemical state of the particles.

Of course, our original experiments were conducted with pure organic particles, such as oleic acid, to characterize the analytical figures of merit of our approach. We then reacted these oleic acid particles with ozone in a flow reactor, to demonstrate the exceptional capability of our method in deconvoluting the heterogeneous oxidation that takes place as ozone molecules are reactively used by the oleic acid particles. It should have been a simple undertaking, with only four chemical products predicted from homogeneous chemistry in the bulk (actually, a beaker). Basically, ozone will add electrophilically across the double bond of oleic acid, cleaving it to produce four oxidization products of lower molecular weight. Imagine our surprise when we measured not only tens of oxidation products of lower molecular weight, but also products of much greater molecular weight than the parent oleic acid molecules. What was going on? We spent the next several years trying to understand this heterogeneous chemistry, and it turns out that the reaction of even a simple, one component organic particle was full of surprises: stabilized Criegee intermediates participating in multigenerational chemistry, leading to oligomers, formation of extensive hydroperoxide networks and high concentrations of volatile products measured in the particle phase, to name but a few. Clearly, our picture of heterogeneous aerosol chemistry was far from complete.

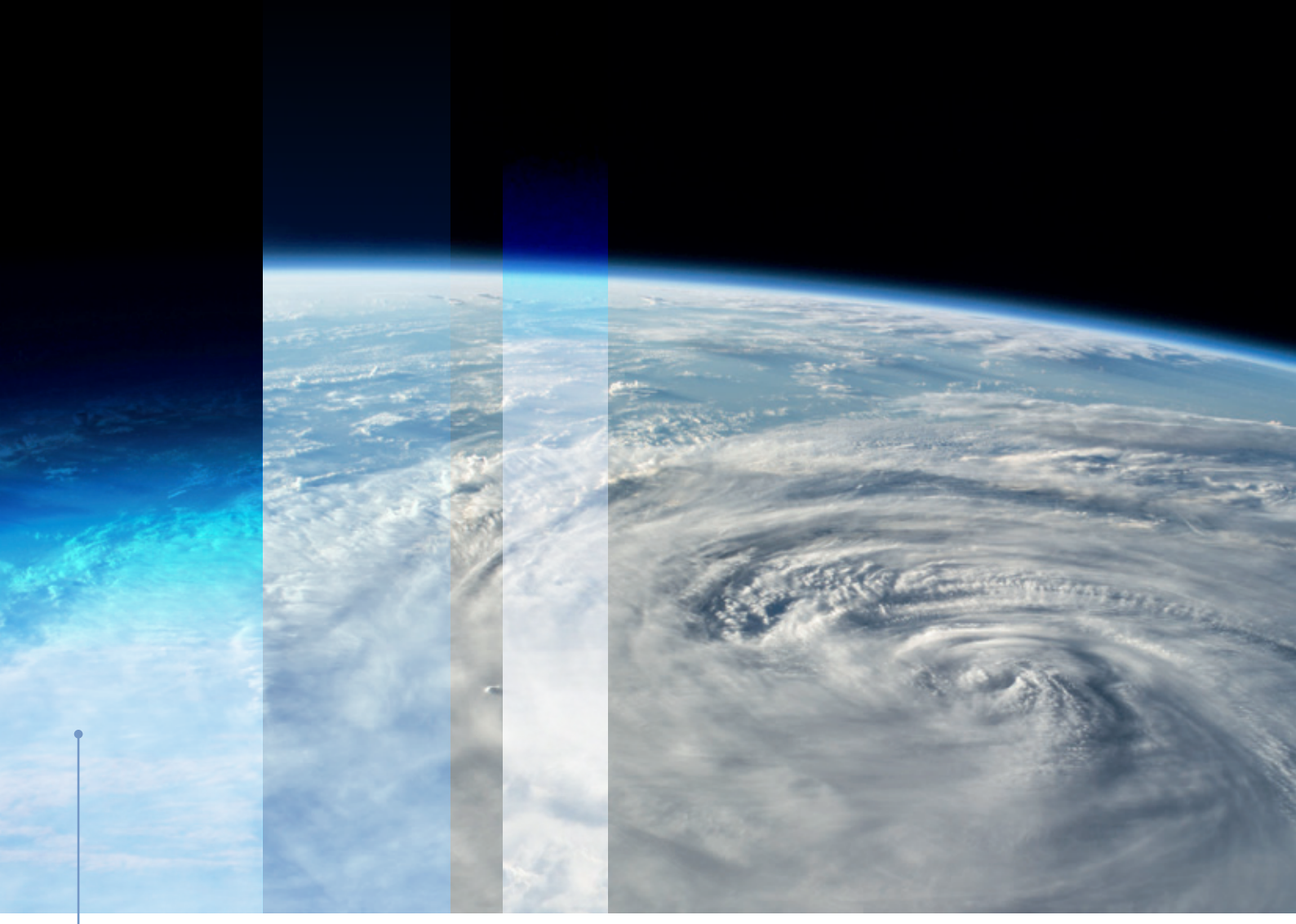
Our next step was to measure secondary organic aerosol (SOAs) formed by oxidation of common and extensively studied terpenoid compounds emitted primarily by tree foliage. The experiment that has become a "rite of passage" for anyone working in this field is the ozonolysis of α-pinene. During these first experiments, we noted that, after 20-30 minutes of aging in our environmental chamber, the SOAs no longer produced any measurable ion signals in our NIR-LDI-AMS. We conducted a similar experiment with limonene as the SOA precursor and saw the same behavior, albeit after more than 45-60 minutes.

*"Our next step was to measure secondary organic aerosol (SOAs) formed by oxidation of common and extensively studied terpenoid compounds emitted primarily by tree foliage."*

## (Un)phased

The physical state of particles can affect particulate phase chemical reactions, and thus the growth rates of newly formed atmospheric particles. The phase state may also markedly increase the lifetime of organic aerosols, as the oxidation caused by atmospheric ozone and other oxidants is confined to the surface. Recent results suggest that biogenic SOA particles can adopt a solid, and most likely glassy state. Furthermore, it has been shown that for mixed compositions of isoprene and alpha-pinene derived SOA, a significant number of particles bounce off (rather than adhere to) solid surfaces, suggesting a fraction of the particles are not in the liquid state.

After much effort trying to determine the loss of signals, we thought perhaps that the SOA particles were not liquid (as has always been assumed), but rather were turning "solid" and bouncing off the probe surface upon impact, rather than being captured. Of course, it is a difficult to make the case that "the particles must be changing phase because we lose our signal," so an independent measure of the particle phase was required. Once again, no method existed for the on-line, near real-time measurement of the particle phase in a polydisperse aerosol and we found ourselves in the "exciting situation" of having to develop a method to make measurements that were previously not possible.



*"We've developed instruments and made measurements that have allowed us to greatly advance our understanding of the life cycles of organics in the atmosphere."*

Building on the work of Annele Virtanen (now at the University of Eastern Finland) and her group on the use of particle bounce to infer particle phase (1), we modified an electrostatic low pressure impactor (ELPI+) to use, in turn, impaction plates that either promoted or eliminated bounce (2). Using this approach, it was readily apparent that the phase-state of SOA changes rather dramatically over short time periods, supporting our earlier observations of the loss of ion signals from the NIR-LDI-AMS.

## Understanding SOAs

Ultimately, the question arises whether the ability to measure particle phase and particle chemistry at the molecular level is truly needed to better understand the formation, aging and role of SOA in our atmosphere. For example, is our original hypothesis that distinct molecular products are important in defining the optical properties of SOA supported by these new data?

Having taken advantage of the analytical capabilities of NIR-LDI-AMS to measure the chemical composition of SOA at the molecular level, we were able to identify new SOA precursors emitted from turfgrass. These compounds, termed green leaf volatiles, or GLVs, were dominated by two reactive compounds: cis-3-hexenol (HXL) and cis-3-hexenyl acetate (CHA). Interestingly, we were able to

distinguish that ozonolysis of each compound proceeded via different chemical pathways. HXL proceeded via an oligomerization pathway that leads to greater concentrations of higher molecular weight products than was the case with CHA, which proceeded through a hydroperoxide channel. The difference in reactive pathways bore itself out in the measured bounce behavior, with the HXL-SOA exhibiting much greater bounce (which is to say, higher viscosity). It is interesting to note that the bounce behavior of the SOAs were amplified more than ammonium sulphate, which is used as a reference solid aerosol. What is one to make of that? Such time dependent phase behavior makes treatment in models difficult at best.

On the question of optical properties, it has become common to relate particle optical absorption and scatter with the average O:C ratio of the aerosol, with higher O:C indicative of greater optical absorbance. However, this approach can be misleading in some cases. For example, in collaboration with Scot Martin and his group at Harvard University, we generated GLV SOA in a continuous flow environmental chamber, all the while monitoring O:C ratio with an Aerodyne AMS, chemical composition with our NIR-LDI-AMS and optical scatter with a nephelometer. For the two SOA precursors under low relative humidity (~10 percent), while the CHA SOA has a lower O:C, it exhibits a stronger absorbance than HXL SOA. Similarly, when comparing the absorbance of HXL SOA at 10 percent and 70 percent relative humidity, the SOA formed under humid conditions has a significantly greater absorbance, despite indistinguishable O:C for the two systems. In the former case, comparison of the NIR-LDI-AMS particle mass spectra for SOA formed from each precursor clearly show very different chemical fingerprints. In fact, if we calculate the carbonyl content (that is, the area of all ion signals known to originate from carbonyl-containing compounds normalized to the summed area of all ion peaks), we see that CHA SOA has a carbonyl content of 3.5 (±0.8) percent, as compared to 2.1 (±0.5) percent for the HXL SOA, indicating a higher predicted absorbance for CHA SOA.

## The punchline

Our molecular level analyses have given us insights into the life cycles of organic aerosols that were entirely new and, in some cases, completely unexpected. We've developed instruments and made measurements that have allowed us to greatly advance our understanding of the life cycles of organics in the atmosphere. Our new question: "can this knowledge be used to help understand the roles and quantify the impact of organic aerosols in our atmosphere?"

Box models have made great strides in their ability to make these predictions. However, owing to still-limited computing power, we must make substantial assumptions and simplifications. With the availability of molecular-level data, the challenge we now face lies in incorporating these fundamental data to generate models that, although more complete and accurate, are computationally unfeasible. Given the current computational constraints, what are we willing to sacrifice within these models to include this new chemical understanding? Time will tell.

*Giuseppe Petrucci is Professor in the Department of Chemistry at the University of Vermont, Burlington, USA.*

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# Forensic Science for the Living

In clinical diagnosis and treatment, the ultimate goal of analytical scientists is to develop robust tests that will be used by physicians. Could the convergence of forensic toxicology and metabolomic analysis provide the right tools?

*By Donald Chace*

I testified at a trial perhaps a decade ago and was asked to explain "metabolism" to a jury. They all looked at me with what I think was dread – all except one juror who really seemed to want to know. They certainly didn't want a complex science lecture, so I put this to them: "You eat all kinds of food for energy. That food is mostly made of carbon and hydrogen, just like coal. Of course we don't eat coal, but we do burn coal for energy in the form of heat. We burn the food we eat to generate the energy (and heat) we need. When you burn coal you produce carbon dioxide – $CO_2$ – the greenhouse gas (lots of head nods) and we exhale $CO_2$ as well. Coal needs oxygen to burn – and we need it to live.

"Metabolism is that process – a process that, in all its complexity, ultimately takes oxygen and carbon and converts them to $CO_2$ and water while producing the energy we need. Anything that interferes with that process is a metabolic disorder."

## *From postmortem to newborn*

During the four years of my Bachelor of Science in chemistry (Boston College), I realized that my interest lay in applied analytical chemistry – particularly with a connection to health or the environmental. I chose forensic toxicology and received my MSFS (Master of Science in Forensic Science) from the George Washington University. As it turned out, those were the two years that most influenced my life and set its direction. I roomed in the home of one of the first forensic toxicologists – Leo Goldbaum – in Washington DC. We debated science almost every night and I met many experts in the fields of both toxicology and forensic science. My research focused on the analysis of carbon monoxide (CO) in blood, and I decided I would like to pursue the laboratory side of forensic toxicology. I went for a PhD in pharmacology at the same institution, at which point my path diverged from toxicology with an emphasis on drug metabolism.

My dissertation research introduced me to mass spectrometry (MS), and I developed a new MS method that essentially converted the mass spectrometer into a stable isotope detector. Essentially, you could take a labeled drug and find peaks that contained the label amongst the hundreds of metabolites that are present in urine. Once you knew where those peaks were in terms of retention time, you could reanalyze by MS to get the mass spectra and identify those compounds. My colleague (who had developed a similar method based on sulfur) and I ultimately found a new metabolite of a drug in dogs that was not known previously.

A one-year post doc at University of Maryland in Baltimore (School of Pharmacy) doing MS was followed by my first job as a medical research assistant professor at Duke University Medical Center, Department of Pediatrics. While there, I did much of the research on what is now the "newborn screening by MS method" used around the world.

My first task was to develop an MS-based newborn screening test for medium chain acyl CoA dehydrogenase (MCAD) deficiency, a disorder than could result in sudden death. The Duke laboratory was a clinical lab with a specialty in metabolic disorders, my colleagues having pioneered much of the work on clinical diagnostics of fatty acid oxidation disorders (of which MCAD deficiency is the most common). In brief, I had to adapt the tandem MS method that detected MCAD deficiency in liquid plasma to a method that could detect the disease from a blood sample collected from newborns. Such blood samples come in the form of a dried spot on filter paper – my first exposure to dried blood spot (DBS) analysis. The history of much of the research I performed is part of a paper I recently submitted for publication focusing on phenylketonuria (PKU), but the analysis is much broader (1).

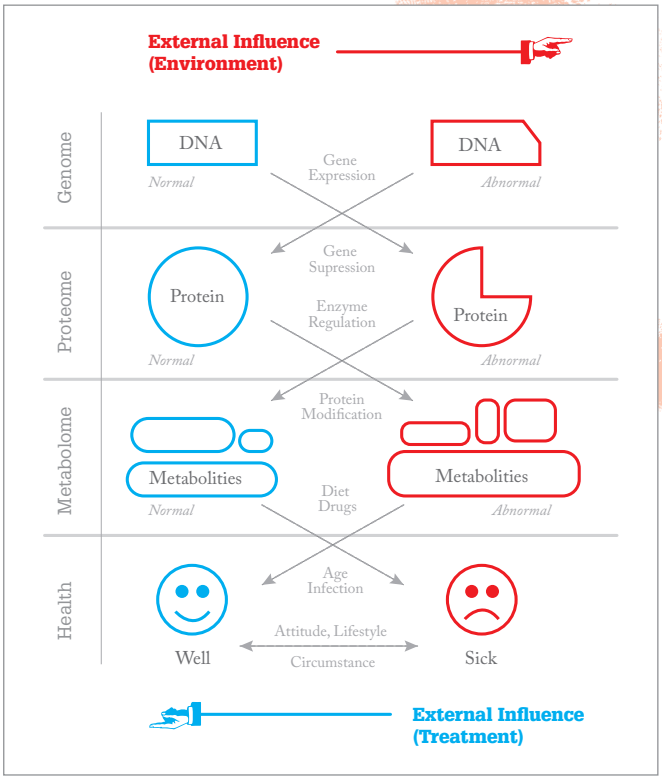
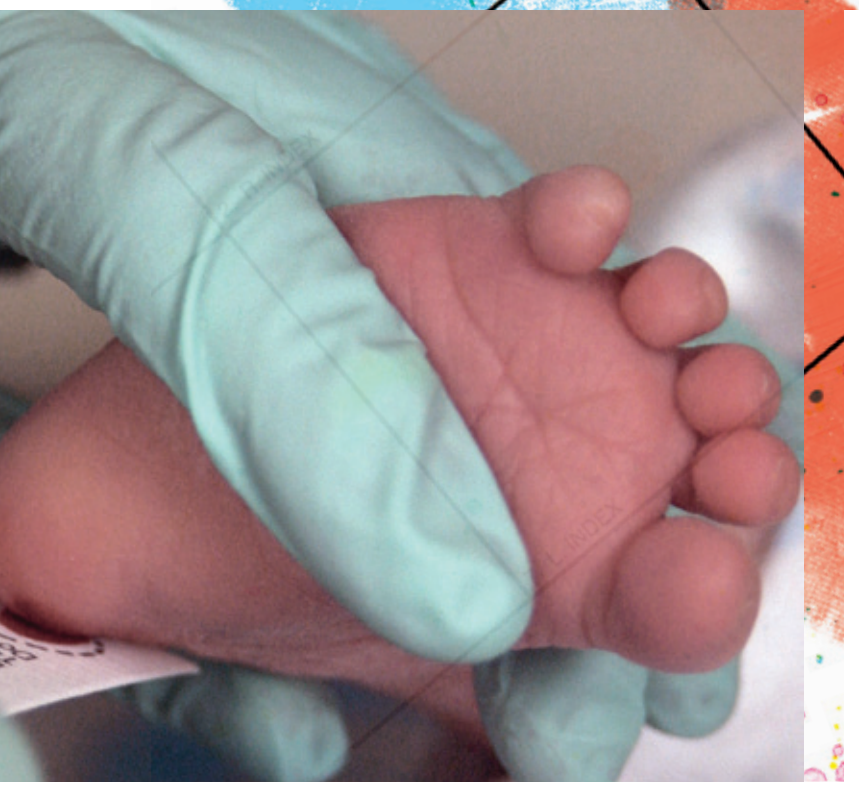



Figure 1. Omics and Health. Wellness and Disease states are a complex interplay between our genetics and the environment. The illustration is designed to show that our DNA blueprint (genome) provides instructions as to how we are made and how we function, via its expression in the proteome (proteins) and the metabolome (metabolites). A mutation in a gene may be expressed as an abnormal protein, which is then expressed as abnormal concentrations of metabolites, producing an unwell state (right column) compared to a normal gene (left column). The external influences of our environment can have adverse effects on our "omics", pushing us from a normal state to an abnormal one (diagonal arrows from left to right) or in the case of a medical treatment, push us from an abnormal state to a more normal one.

It was a challenge getting labs to accept our new test. But one group was particularly interested: a lab in Pittsburgh called Neo Gen Screening (at the time), which was run by Edwin Naylor. Naylor offered supplemental screening for a variety of conditions, some of which were amino acid disorders. Supplemental screening was offered to birthing centers in addition to the public health (state mandated) screening, but none of the tests used MS at the time. Naylor purchased the required instrumentation (tandem MS and sample preparation equipment) and training so that he could begin the first commercial application of newborn screening with tandem MS offered in Western Pennsylvania. I joined the lab to ensure the success of a method that I'd had a big role in developing and to develop and refine automated interpretation and analysis.

Much of the work over the next few years was to optimize the method for large numbers of samples (500-1000 per day), to introduce new tandem MS technology and, most importantly, to develop an interpretation system that could detect multiple metabolic disorders from the MS profiles obtained for each newborn. (I am the "inventor" of five patents related to the interpretation of metabolic profiles in DBS samples from newborns.) In addition to the supplemental screening, we conducted a pilot study for the State of North Carolina to determine incidence of the 35-plus disorders we could screen for using tandem MS.

Pediatrix Medical Group (now MEDNAX Health Solutions Partner) purchased the lab in 2002 and renamed it Pediatrix Screening. Pediatrix was solely focused on neonatology at the time, and newborn screening was complimentary to their goals of screening for metabolic disorders affecting preterm infants. During that time, when the analysis was robust, automated and routine, I was able to focus more on research. I shifted to the screening of a subpopulation of newborn – the premature (preterm) infant – a group that gives the highest number of presumptive positives (false positive results) as a result of their prematurity, low birth weight, immature metabolism and nutrition.



## Advice to analytical scientists

1. Get out of the lab and listen to other scientists. Attend seminars in biology, biochemistry, medicine, pharmacology, forensic science, agriculture, environment.
2. Find out what problems people in the field are encountering; what solutions are needed? My experience is you will hear those magic words: *I wish I could measure X, Y and Z*. You can then consider the problem and think about whether you can use your expertise and specialization to reach a solution.
3. Don't live in a laboratory bubble (probably good advice for any career). Technology is all around us and we can learn from other areas.
4. Create a solution that is uniquely your own, whether working independently or as part of a team. Improving an existing assay with a unique solution is as important as developing a new test.

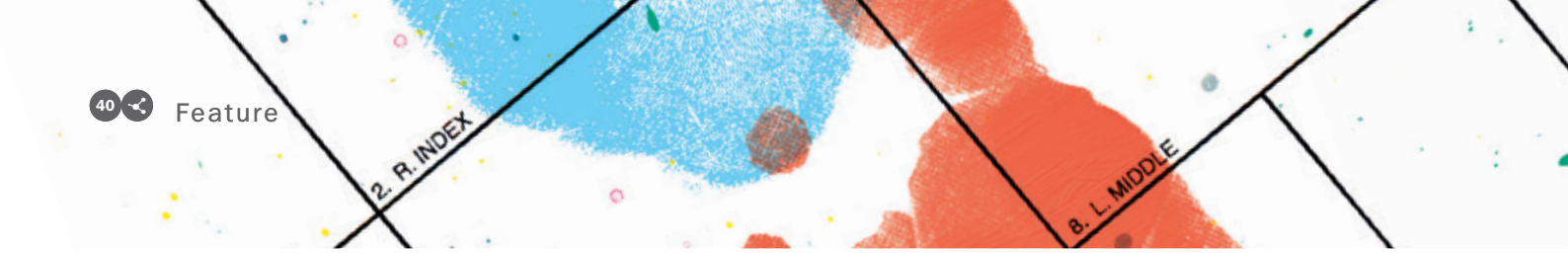
> "Looking back, my earlier experience in forensics and toxicology has been invaluable in the field of newborn screening."

More recently at MEDNAX, we have done two clinical trials on the effect of total parenteral nutrition in infants on metabolism as observed in their metabolic profile. The goal is to develop a better newborn screen for preterm infants and the possible monitoring of their metabolism while in the NICU (neonatal intensive care unit). I have focused on the interpretation of these profiles using newer bioinformatics software, and we continue to publish in this area.

Looking back, my earlier experience in forensics and toxicology has been invaluable in the field of newborn screening. Specifically, I learnt the importance of screening in the "two test rule", where screening is performed with either a simple test or a broader profiling test that – by design – aims to pick up potential positive cases. All positive cases are then reanalyzed by a more specific confirmatory or diagnostic test. In fact, forensic and clinical screening are very similar – apart from the patient population (postmortem versus newborn). Indeed, during the screening laboratory days, we used the same metabolic profile as on sudden unexplained deaths. We found that about one percent of all infant deaths from unknown causes actually did have a cause – a metabolic disorder. We called this test the "Metabolic Autopsy" and I used to go a step further to say that newborn screening is forensic science for the living. Of course, in forensic science, the metabolites are often illicit drug metabolites or poisons, whereas in newborn screening they are endogenous biochemical metabolites (but note that a metabolite that is endogenous in nature can be a poison when at very high concentrations, as in an inherited metabolic disease).

### *Applied omics*

Genomics is a key component of generating a biochemical or clinical profile, and is used to assess whether an abnormality is genetics-based in origin as oppose to acquired (such as an infection, or when a child is born too early or has been exposed to poisons). DNA analysis is also important in the identification of microbes/




## MS Tech in metabolic profiling

Before the introduction of tandem MS there was no profiling in newborn screening. Essentially, you had to do eight separate single analyte tests. For a more comprehensive view of the technology evolution, look out for my upcoming paper (1).

In terms of MS technology, most metabolic experts use gas chromatography-mass spectrometry (GC-MS). When used to analyze metabolites extracted from urine, hundreds of compounds can be detected in a single analysis. It is considered a diagnostic test and is quite complex. Each test takes approximately 45 minutes to run (as well as a couple hours of sample preparation). It is the quintessential metabolic profile and is still very important in clinical labs – notably, interpretation requires a great deal of expertise. Tandem MS analysis of amino acids and acylcarnitines is similar in terms of profiling, but doesn't require chromatography and takes about two minutes per sample; diseases have different sets of metabolites. Today a positive newborn screen by tandem MS is often followed by confirmation and diagnosis using GC-MS.

Modern technology in metabolomics emphasizes automated analysis from sample preparation using robotics to the analysis by MS – usually multiple tests with multiple profiles. It is also heavily dependent on software tools to process all of the data. Anything is possible with modern technology. You could obtain a blood sample (even as a blood spot) and have 3-5 profiles from 2-3 sample preparations (our current screen has two profiles plus one screening marker for Tyrosinemia type 1) all analyzed on the same tandem MS instrument. The only limits are time, sample size – and cost. And if cost is no issue, you can throw in molecular analysis as well.

Beware the pitfalls. With new technology in mass spectrometry and automated analytical chemistry, the sky's the limit when it comes to analytical. In one sense, the term TMI (too much information) should be used here. It is the old balance of "quality versus quantity". You have to ask yourself: what are you analyzing? Do you know why? Do you know what the metabolites are? Do you know how much?

> "Genomics helps confirm what we observe in metabolism – and vice versa when we can confirm the expression of a mutation"

viruses. Genomics helps confirm what we observe in metabolism – and vice versa when we can confirm the expression of a mutation. In general, cost is an issue with molecular technology, because in a multiplex assay (similar to a multiple metabolite assay) each mutation needs a probe – and that can get expensive.

I always like to say that the gene is what we are thinking (what we want to do) but what we actually do is dependent on how we express that desire. Molecules are the instructions, metabolism is the result – and sometimes, actions speak louder than words! Notably, not all health disorders are metabolic; some are the result of defective proteins (for example, sickle cell hemoglobin) or chromosomal errors, i.e. Down's Syndrome.

Metabolomics is a broad term. For example, lipidomics analyzes a vast number of lipids to target aberrant metabolism in cells based on their lipid metabolic signature. Other studies may focus on finding drug metabolites during the screening of thousands of samples. Most metabolomics efforts are still in the research and discovery phase, whereas newborn screening or metabolic diagnostics is an actual application in metabolism – quantitative tests that use internal standards and target specific diseases that have a known signature or metabolic profile. Clinical analysis of small molecules (also called metabolites) is the same type of application. Both are subject to regulation, quality assurance programs, inspection by government or state agencies, and so on. Simply put, newborn screening for inherited metabolic disease is a subset of the broad field of metabolomics (or metabolism), no matter how you define it.

The approach ultimately depends on your goals. Are you trying to understand a disease and its markers or are you developing a clinical test to detect the disease? Certainly, you must understand the disease process before you can detect the marker (see Figure 1). It really is that simple I think. What's the best or fastest way to find and characterize disease? Well, that's a challenge, and there are many factors involved, with cost and effectiveness at the very center. Here are a few of my thoughts on...

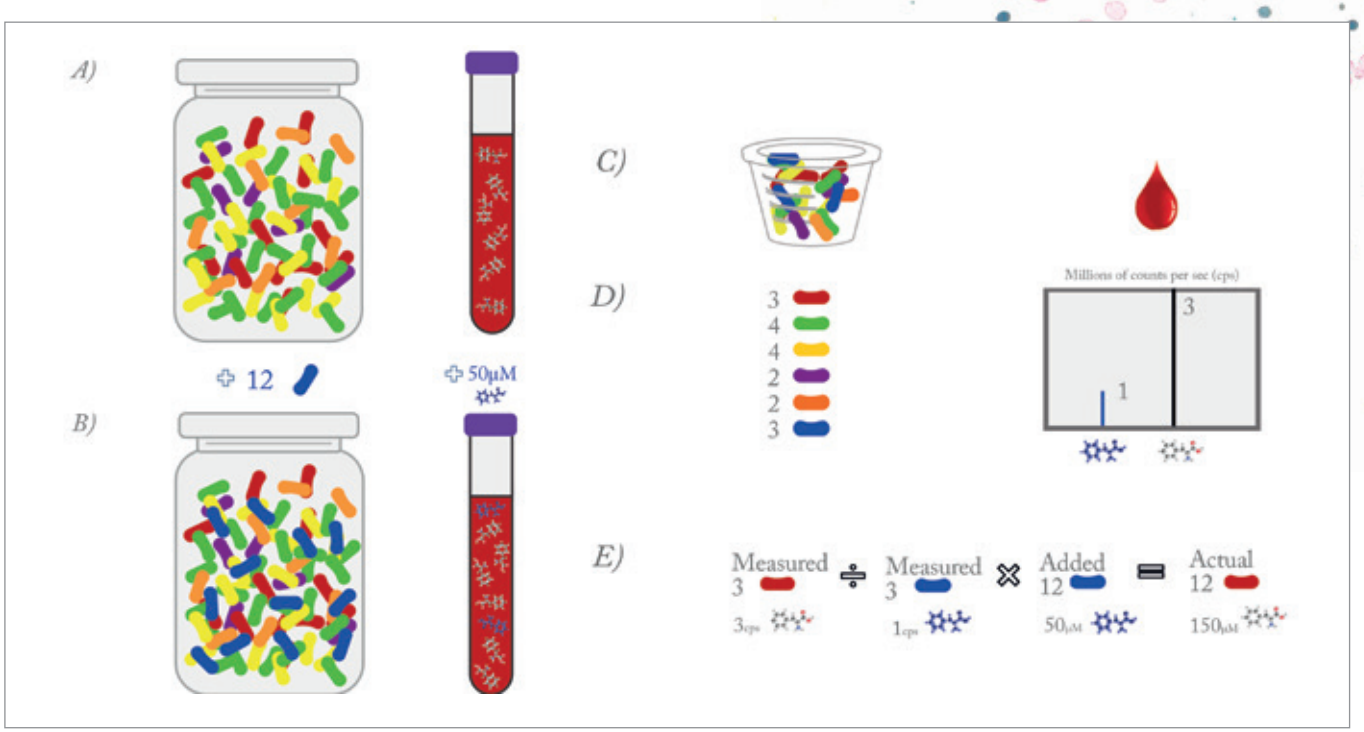



Figure 2. Jelly bean-omics and blood metabolite measurement. Consider the "guessing" game we've all played: how many jelly beans are in the jar? We wouldn't want a "guessing" game on how much of a particular compound, say phenylalanine (an amino acid) is in our blood. In fact, we don't guess as to how much of a particular metabolite is in our blood; rather we take a sample, add a standard for reference, detect both standard and the metabolite using a chemistry based analysis and calculate an amount. Panel A shows a jar of jelly beans, or a tube of blood containing phenylalanine. How many cherry red jelly beans are in the jar? Or, how much phenylalanine is in the blood? Using the rules of a clinical lab we can add a reference standard to blood (stable isotope labeled phenylalanine, or blue molecules). We can add 12 blue berry jelly beans to the jar as a standard (B). After mixing, we now take our samples and then make our measurements. In the clinical lab, we can take a small aliquot (a drop of blood – or in the case of the jelly beans a small cupful) as illustrated in Panel C. Note we don't attempt to analyze the entire blood sample collected nor count every molecule in our blood as we cannot count every jelly bean (as per the rules of the contest). The next step is to analyze our metabolite and standard – or sort our jelly beans – and count how many in the sample we obtained (Panel D). In the case of phenylalanine and its isotope standard, we can sort these compounds by their mass using a mass spectrometer. For jelly beans, it is as simple as sorting by color. In both cases, we calculate how many of each group (color or mass). Finally, as illustrated in Panel E, we have to translate our results from Panel D into our analytical answer: how many cherry red jelly beans are in the jar or how much phenylalanine? In the case of jelly beans, we know we added 12 blue jelly beans and measured three red and three blue jelly beans. A simple ratio of red to blue multiplied by how many we added originally will produce the amount of cherry red jelly beans in the jar, which is in fact 12. For blood measurement, we added 50 µM (micromoles per liter) of the isotope standard. We measured a ratio of three times as much phenylalanine relative to standard, a ratio of three to one in terms of molecules (ions) counted. We simply multiply this ratio by the isotope internal standard and obtain the actual concentration of phenylalanine in blood. It's certainly much simpler than the impossible task of counting every molecule in a blood sample.

*...complexity of samples*

All biological samples (blood, plasma, urine, sweat) are complex, with hundreds of compounds. Obtaining a metabolic profile does not require measurement of each metabolite, of which there are hundreds; rather, developing an assay to detect families of compounds makes the most sense, for example: amino acids, acylcarnitines or a lipid profile.

Spending time developing the proper sample collection and sample preparation system is key to success and that is much of what analytical scientists do: collecting, separating, detecting, quantifying and reporting!

*...quantifying markers*

As noted above, I think the markers in a profile should be related and analyzed by family (amino acids or acylcarnitines, steroids or bile acids). You quantify those markers that are the most important with internal standards and semi-quantify the rest. You have software that can sort out all the normal samples, which is key –

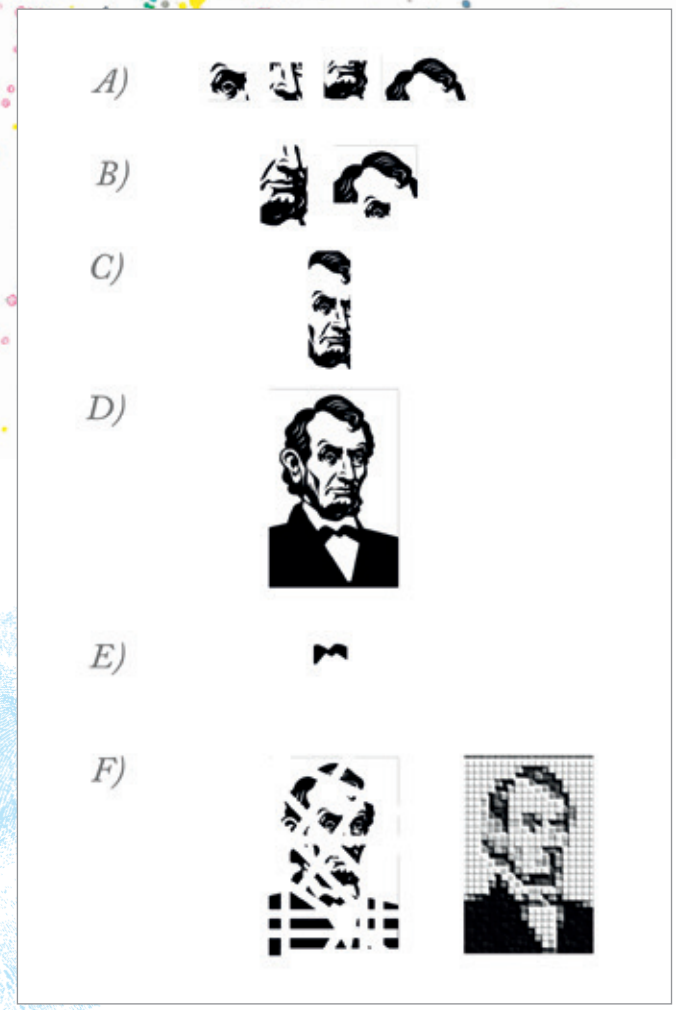



Figure 3. Profiles in metabolism. Classical clinical analysis of small molecules – metabolites – that are biomarkers for disease is based on single marker, single analyte analysis. Let's use a character portrait of Abraham Lincoln as the goal of identification. Single markers (partial illustrations) do not readily identify Abraham Lincoln (Panel A). However, if the target answer is highly specific, i.e. is the question if a beard is present, then a single biomarker assay will suffice. Of course, you can perform many single assay biomarkers and ultimately assemble a profile. Metabolism is primarily characterized by the conversion of one substance (a substrate) to a chemical modified compound (a product, sometimes called a metabolite) of an enzyme. If the goal is to detect a disease characterized by the deficiency of an enzyme, measuring its product and substrate together may be a better profile of that disease. This is the case in phenylketonuria, where the substrate phenylalanine is not metabolized to tyrosine because of a deficiency in the phenylalanine hydroxylase enzyme. The result is an accumulation of phenylalanine and a decreased production in tyrosine. Two linked metabolites are like linking two profiles in close proximity, as shown in Panel B, where the picture of Abraham Lincoln is more closely approximated than with any single metabolite. The addition of three or more metabolites may clarify the disease even more, so that there is almost no difference between Panel C (four metabolites) and the complete picture in Panel D. Metabolomics is often characterized by detecting a variety of biomarkers, many unknowns that add to a picture but do not provide clinical information. Panel E describes that. The bowtie does not uniquely identify Abraham Lincoln and would be a poor biomarker in any screening panel, for example. Finally, Panel F attempts to illustrate that metabolomics analysis does not have to analyze every single biomarker nor always be perfectly precise. You could remove portions of the portrait or make the image less precise by pixilation and still identify Abraham Lincoln. Metabolomics research into biomarkers with clinical validation ultimately helps the clinical chemist working with a clinician to determine the best way to obtain a profile of a disease they are evaluating with the lowest cost, high enough accuracy and sufficient selectivity to reduce errors or false identification.

now, you don't have to spend time focusing on those. I have always liked visual profiles for the abnormal. It is easy to see a disease that matches a quantitative result or an artifact (where the analysis was all noise or a spike in the electronics). The software can also calculate ratios of compounds or any mathematical relationship you can consider, like sums of metabolites. Some labs opt for software that provides a score in terms of certainty, but I never liked the idea that you have a 98 or 86 percent probability. Borderline results are the worst (but metabolic profiles actually help reduce these).

*…pinpointing disease*

Any picture or profile is a signature and still requires a confirmation. Suppose you had ten artists paint their versions of the Mona Lisa and one of those was Da Vinci's. You could only verify by a different kind of examination – using an expert who may analyze the age of the paint, for example. In terms of having the best confirmation, that is where molecular confirmation or cell culture or separate tests come into play.

*…visualizing results*

Observing ten markers is certainly simpler than 100 or a 1000. Software is needed to assist in this matter and is critical to metabolomics. I like viewing profiles when searching for unknowns or clear abnormalities. My recent work in nutrition doesn't simply look at very abnormal profiles that suggest toxicity or a metabolic disease (though it does do that), it also uses those quantifiable profiles to determine if nutrition is adequate or if toxic markers are accumulating. Software is critical in this case for providing a report.

*…quantitation*

Regardless of the analytical test, classical clinical chemistry still dictates the quantification of compounds using standards,




statistical validation, reference materials and quality controls. This is a cornerstone and I think the only way to a clinical test. With metabolic profiling of urine, even by GC-MS (organic acids analysis), a lab has a reference standard to estimate quantification. Some labs doing GC-MS are now using multiple standards for true quantification. Newborn screening using MS/MS has 20-23 standards, most of which are stable isotope labeled – the gold standard in quantification using mass spectrometry.

Ratios, by which I mean concentration ratios or molar ratios, require two compounds. The advantages of the ratio parameter are i) it is already based on good quantification (both compounds in a ratio have a standard reference). A ratio helps normalize a variation in blood volume in the case of dried blood spots; and ii) it better characterizes a disease like PKU, where two metabolites are connected to the same metabolic pathway. A block in that pathway should increase one metabolite while causing a decrease in another. Ratios are endogenous references and, I believe, very important in metabolic profiling. In fact, I would argue that they are essential.

### *…false positives/negatives*

False results are a problem in any clinical or forensic lab. Much more often than not, lab error is not the issue (and is reduced by good laboratory practice and QA/QC). Missing a disease is also quite rare because we interpret the results with a great deal of caution. Normal variations in metabolism between individuals/patients are quite large. To ensure you don't miss a disease characterized by an elevation of a metabolite, you set your criteria for a positive result lower (into the upper normal range). The resulting problem is, of course, false positive results – but we tolerate false positive results in newborn screening because we want to reduce false negative results (or a missed case) down to zero. In forensic science, on the other hand, the issue of false results is reversed, as a false positive implicates a result that is not true. The goal in forensic science is to find the truth, hence detecting a drug that is not present is obviously not desirable.

False results create issues such as increased costs, due to repeat testing and follow-up, hence a technology that can reduce these results is important. That technology is tandem MS and multiple metabolite analysis. In any profiling, screening or diagnostic test, there has to be a continuous effort, no matter how good the test, to push these false alarm rates toward zero on both sides.

### *The analytical balancing act*

From analytical scientists, what we need most are methods of sample preparation that can optimize the extraction (as close to 100 percent as possible) while also making samples cleaner. We also need to improve the chemistry to gain more sensitivity and selectivity in the mass spectrometer (or any other instrument for that matter). With a better instrument, the efforts in sample preparation always pay off, since the instrument will stay cleaner and be at its optimum for longer. The better the extraction and any improvements to the derivatization, the smaller the sample needed. Then the less sample needed, the more is left for other metabolic profiles or confirmation tests. With DBS, an advantage – and one that is often only realized after you begin to use it – is a cleaner extracted sample (it's also safer from an infectious disease point of view). An organic solvent extraction usually leaves salts and other highly polar or ionic compounds behind, compounds that reduce sensitivity (and ionization efficiency).

The other very important area is bioinformatics. Any commercial metabolomics platform needs to have a heavy focus on data processing and result interpretation. Although it is not necessarily obvious to an analytical scientist, often "the result" requires more than a concentration value. In medicine, interpretation is extremely important and the subsequent communication to a clinician even more so.

A test with a low false alarm rate that is selective, sensitive and reasonably priced relative to its impact – and that helps the clinician make a diagnosis or improve therapy for his patients – is more likely to be ordered. And the ultimate goal of any analytical scientist should be to develop a test that is used! A good test targeted toward early diagnosis will have a positive impact on disease, by facilitating earlier treatment, prevention, or better monitoring of therapy. And ultimately, the test will reduce healthcare costs. But the reality is, unless you get the test into the hands of the physician, you're unlikely to have any impact at all.

Finally, in today's healthcare environment, cost (or cost–benefit) is critical. A metabolic profile gives you more bang for your buck, but you also have to make sure you aren't performing more tests than necessary or providing information that is not desired or needed. Scientists really do have to perform a unique balancing act when it comes to analytical research and development.

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*Donald Chace is Director, Pediatrix Analytical, Mednax Centers for Research, Education and Quality; and a guest researcher at the Newborn Screening and Molecular Biology Branch, Centers for Disease Control and Prevention (CDC), based in Massachusetts, USA.*

# Wanted: Winning Project for 2017

## Nominations for the 2017 Humanity in Science Award are now open

Analytical science has the power to change human lives for the better, but rarely receives the same fanfare as other scientific disciplines. The Humanity in Science Award was launched to recognize and reward a recent breakthrough in analytical science that has the potential to make the world a better place.

Do you know of a project that is worthy of the prize?

Nominate an analytical science project with a humanitarian impact now at:
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# Cheaper HIV Diagnostics

Waseem Asghar, Assistant Professor at the Departments of Computer Engineering & Electrical Engineering, Computer Science, and Biological Sciences, Florida Atlantic University, USA, was given the 2016 Humanity in Science Award for "development of a new paper and flexible material-based diagnostic biosensing platform that could be used to remotely detect and determine treatment options for HIV, *E. coli*, *Staphylococcus aureus* and other pathogens."

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# Time to Roll the Dice

Have you considered that there may be hidden factors preventing you from progressing in science? In my experience, there is a lot to be gained from taking a leap of faith.

*By Sarah Maurer*

Analytical scientists have a reputation for being accurate, for understanding probability, and for using caution when drawing conclusions about data. One personality trait typically associated with our field is looking before we leap!

Being overly risk averse can hold you back, however. And women have it even harder; from a young age, girls are often discouraged from taking risks like climbing trees and asking someone on a date, which leads to inexperience at risk-taking for female scientists.

A father recently asked me, in front of his teenage daughter, if I had experienced any sexism in my career. I haven't, but it was clear that he was concerned about his little girl being treated unfairly because of her gender. Why? Well, because of a lack of role models, people assume there must be a reason why women don't want to become engineers and computer scientists. It's a way of thinking that leads to fewer women entering these fields – and research careers may seem too risky to pursue.

Fortunately, we can all take heart. There are certain steps we can take to help ourselves and the people we know – and some of my advice isn't limited to women.

## Ask and you shall receive

One risk that we should all take is asking for what we want; it means overcoming the fear of possible rejection. It starts from the moment we apply for college, carefully considering which major to choose, but doesn't end when we get diplomas. For example, the average start-up funds given to professors at biomedical schools vary significantly by gender (1); men were awarded $889,000, while women received $350,000. And though the article referenced does not make conclusions about the reason for this disparity, one of the possibilities is that women are asking for less.

*"One risk that we should all take is asking for what we want; it means overcoming the fear of possible rejection."*

Perhaps women are not provided with the same insider information about the upper limits of these requests, or women are more conservative in their estimated costs because they want to increase their chances of acceptance. The inequality could be mitigated easily by increasing the transparency of start-up proposals, and by providing more mentorship opportunities for applicants. Mentorship can also minimize the amount of risk a decision holds.

I had a hard time learning to ask for what I wanted. When I finished my BS in biochemistry, I had some time before graduate school started. I wanted a job as a chemist, and was interested in a group from Los Alamos National Laboratory (New Mexico, USA). I was scared to email or call the principal investigator, so I took the easy way out; I submitted my resume to the general student applicant pool, hoping the group would see it and hire me on the spot.

It worked out, just not the way I imagined. I was hired by a wonderful group and moved to New Mexico to work with honeybees. While there, I made contact with the

principal investigator I originally wanted to work for and established the basis for my PhD thesis. However, the first thing he said to me was, "Why didn't you contact me sooner?" And he was right. The only repercussion of sending him an email was rejection, and yet I couldn't do it.

The problem of "asking" is addressable at many levels, beginning with ourselves. When it is time to ask for additional funds to cover travel, meeting a senior scientist, or getting the last donut at the group meeting, it is important to be assertive. Secondly, many people do not know what is available or have been taught that asking is inappropriate. To counteract the fear of asking, mentors can guide and reassure the timid scientist. It is important that we mentor young scientists to be self-assured, and calm their fears of rejection. Because rejection happens throughout our scientific endeavors – to be afraid of it can cripple an otherwise successful career.

## Not all who wander are lost

Another risk that occurs frequently in scientific careers is relocating for a better position. From starting a bachelor's degree to achieving a permanent position, a scientist will typically relocate three or four times for education and/or employment. Acceptance letters and job offers tend to be the reason rather than personal preference. During this time, it is difficult to maintain friendships and romantic engagements, in addition to the stress of settling in a new location and having new job expectations. Knowing these risks, many scientist choose to "play it safe", finding less optimal local opportunities to sustain them.

My experience is an exaggeration of the nomadic scientist. While working on my PhD, my advisor was awarded a large grant to start a "Center of Excellence" in his home country of Denmark. Moving away from friends, significant others, and family is always scary, and moving to a foreign country is doubly so. I was reluctant, but when it was made clear that my research stipend in America was running out, I boarded the plane.

*"Don't just visit foreign countries – live in them, immerse yourself in the culture. And when you return home, embrace your experience."*

Living in Denmark was very rewarding. For one, living abroad has been shown to make people more creative (2), which is great for a scientist who is looking for research inspiration. I learned to communicate without a common language, navigate public transport, enroll in and visit my government appointed healthcare provider, and thrive in an international community of scientists. Working in a European lab was a very different experience to the American academic environment, and shaped the way that I run my current research group.

There were penalties for taking this risk. Living abroad can be lonely, and even though I had friends, I felt linguistically and culturally isolated most of the time. It was also difficult to transition from Europe back to America. Because most of my contacts were in Europe, available postdoctoral positions were limited to me. I do not regret living abroad, but I am also glad to be "home".

I encourage everyone, of any background, to travel for extended periods abroad. Consider taking a sabbatical in Europe, or taking a position within the company in their Beijing office. Don't just visit foreign countries – live in them, immerse yourself in the culture. And when you return home, embrace your experience. Encourage your children, your students, your employees, and yourself to get out and see the world. It will make you a better manager, mentor, student, and researcher.

## Creativity takes courage

One of the most rewarding parts of our jobs is sharing our research with others, whether personally at conferences or remotely through journal articles and patents. It inspires intellectual discussion about our research, helps us get over hurdles, or takes us in a completely new direction. However, it is also needs to be highly scrutinized for accuracy, significance, and integrity. Science has formalized this process to make it less intimidating and more emotionally detached, using tactics like anonymous review and technical language.

There are many other formats for creatively communicating scientific results. From the "Vizzies" (US National Science Foundation) to "Dance your PhD" (American Association for the Advancement of Science), scientific exchange is hardly limited to technical presentations. However, there is a difference between presenting data that

represent cold, hard, facts and a work of art that represents a personalized vision. It is much riskier to put yourself into your work artistically, but the results can be inspiring, wide-reaching, and professionally enriching.

During my PhD, I was directed to a TED talk on knitting and hyperbolic spaces and how art can help understand science (3). This was the inspiration for "Knitting a Protocell", the poster I presented at Artificial Life 12, in Odense Denmark, March 2011 (4). My use of embroidery, crochet, knitting, and quilting allowed me to present my PhD thesis in an engaging and understandable way. The attention gained from this "poster" continues to attract people to my work much better than a traditional poster could.

To pass this experience on to my students, every semester, I ask them to make a creative version of molecules. General chemistry lab students make molecular models from electron dot structures, while biochemistry students reproduce glycolysis and the citric acid cycle. These activities allow them to be creative and earn bonus points for thinking outside the box. Each year, a student surprises me and designs something unbelievable. This semester I received all of my intermediates modeled within decorative soaps (usable) and a 12-minute beat poem, plus the ultimate Frisbee team modeling of xenon tetrafluoride.

Conversely, many students are paralyzed by these projects. "I don't know how to be creative" or "I'm not good at anything" are regular assertions. Part of this hesitation is a fear of not being good enough; fear that their creativity will not meet my standards. My job is to encourage, give suggestions, and help them feel comfortable showing me a little bit of themselves. And reward them for their efforts. These students are the greatest successes. They learn an important lesson: hard work and passion at the very least will make you proud of yourself, but could also help you be better than you thought.

## Risks, rewards, responsibilities

We may not like taking risks, but it is a necessary part of our lives. And remember: putting yourself out there and taking a risk can send you places you never thought you would go – and help you do things you never thought you could do.

Ultimately, when making decisions, we practice loss aversion, because we feel the pain of loss more strongly than the pleasure of gain. This mentality is not easy to break, but can get easier with practice. Being mindful of our behavior is the first step to changing it.

Likewise, it is important for us to calm the fears of others and advise them when risks are being calculated. We have the ability to turn a frightening choice into a comfortable one through effective mentorship. And instead of being hesitant, we need to approach these challenges with all of our energy – regardless of gender.

*Sarah Maurer is an assistant professor in the Department of Chemistry and Biochemistry at Central Connecticut State University, New Britain, Connecticut, USA.*

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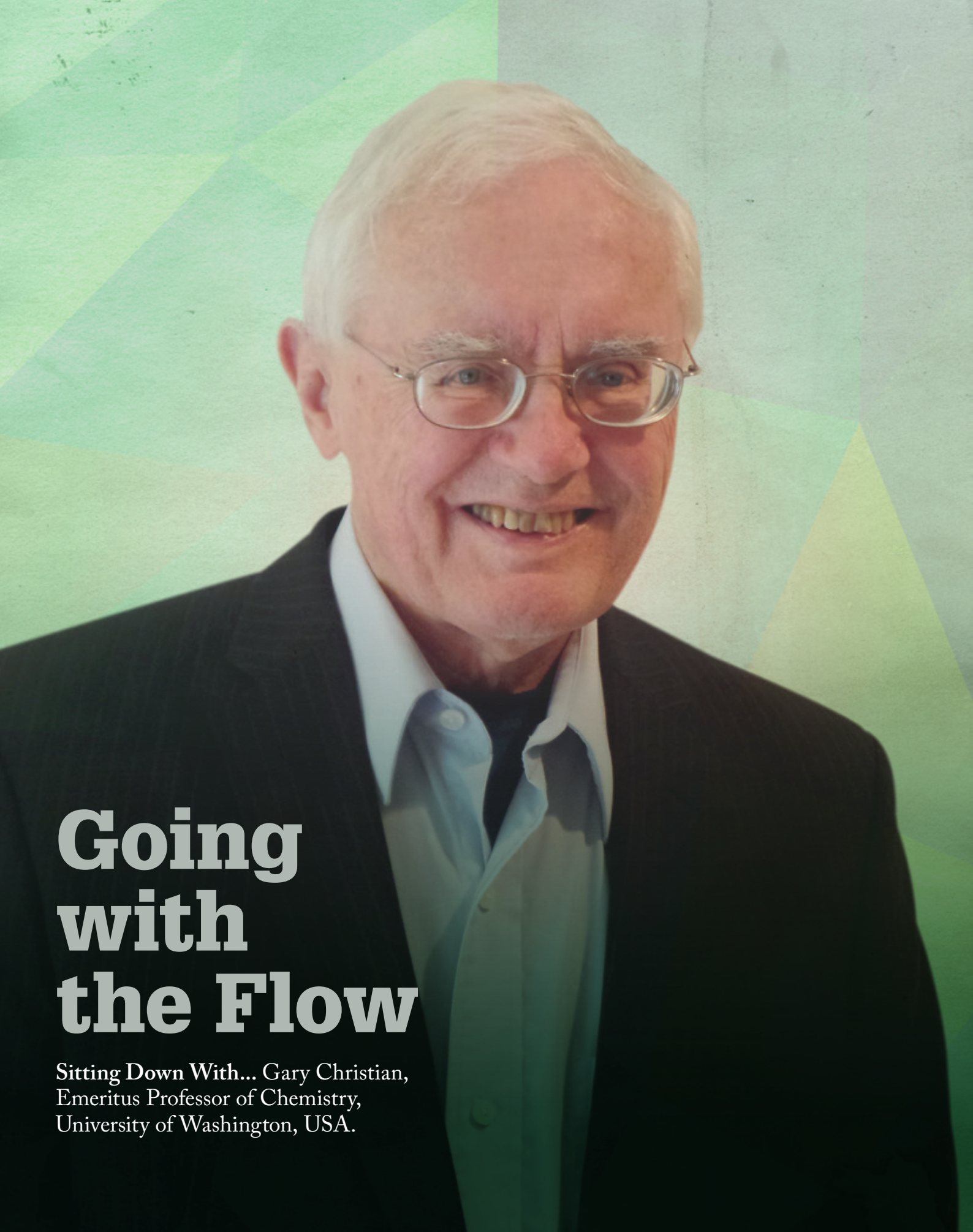

# Going with the Flow

**Sitting Down With...** Gary Christian, Emeritus Professor of Chemistry, University of Washington, USA.

Who sparked your interest in science?
Teachers make a big difference in your life. I had a tremendous science teacher, who taught me general science, biology, physics, and chemistry. He was also my track coach and a real character. We remained friends for years, but sadly he died a couple of years ago (he was in his nineties). Initially, I wanted to be a high school teacher to emulate him. I knew that I'd need a Master's degree and decided I wanted to get it in chemistry, so I applied to the University of Maryland. It felt a long way from home – especially as I'd never been out of my state!

But you never made it back to high school teaching?
Again, my instructor, Bill Purdy, had a great influence on me. I wound up doing research for him and he talked me into continuing on. He was the kind of guy who just let you loose. I wrote my own papers and worked in the lab on my own "creations" (initially, in electrochemistry). He was also a consultant at Walter Reed Army Institute of Research in Washington DC and found me a job there. After a couple of years, I decided to take the academic path – I didn't know what real chemists actually did(!) – and I ended up at the University of Kentucky, where I stayed for five years. But when you're young and impatient (and they're not promoting you fast enough) you start to look around...

So you moved again?
Right. I have a twin brother who graduated from the University of Washington, and his professor hired me as a full professor in 1972. I got into atomic absorption spectroscopy and, with a friend of mine (Fred Feldman), wrote one of the first books on the subject for biological and agricultural applications. Eventually, I moved into flow injection analysis and recruited Jaromir (Jarda) Ruzicka. I unexpectedly wound up in administration when I was appointed Divisional Dean of Sciences with 15 departments to look after, including chemistry. At first, I wondered what I'd got myself into, but I enjoyed it – for nine years.

Did you miss research or teaching?
I kept the research going for a while, but it had to stop. And I really missed teaching. I actually came back to teaching for a couple of years before retiring. I was a little bit nervous because I hadn't taught for almost a decade, but I got the best ratings I'd ever had. A student from the very last class I taught invited me to a luncheon for "favorite professors" – that was a nice way to end my career.

Could you share some highlights of your career?
A couple of things happened that kind of prodded me on. One year, I was elected chairman of the analytical division of the ACS and I got the award for excellence in teaching. I eventually got the Fisher Award in Analytical Chemistry as well. Such achievements make you feel like you're doing something worthwhile. It's not the most important part of your career, of course, but it's nice to have the recognition– and to know that you've made an impact. I also made an interesting discovery or two...

How did you get involved with the Talanta journal?
Another unexpected turn! Jim Winefordner had been the chair of the editorial board for years and wanted me appointed. Back in those days, we used paper manuscripts, so things moved a little more slowly. Today, although it's faster in some ways, it's also easier to cut and paste, self-plagiarize, even plagiarize... I actually have a presentation on ethics of scientific writing (http://tas.txp.to/0716/garyonethics).

*"I've learnt that students are the ones who 'make' your career, more than anything else."*

What makes a good teacher?
Being a friend, being accessible, and trying to teach students to be creative and independent. I've had students tell me the best thing they learnt from me was how to communicate and write – which is almost more important than the science itself in some ways. No matter what job you get, you need to communicate. I've remained friends with many of my students. And some have gone on to fantastic things; take Isiah Warner, an African-American who went to Southern University as an undergraduate in Baton Rouge. At the time, he wasn't allowed to go to Louisiana State University because he was black – but now he's Vice President there. Over the years, I've learnt that students are the ones who "make" your career, more than anything else.

What's your best advice?
Something along the lines of: "Life will take you where you do not expect. Take advantage of what you have when you have it." I can give three examples in my case: i) I never thought I'd become a college professor – that happened by accident, as I wanted to be a high school teacher; ii) I never thought I'd become Dean; and iii) I never thought I'd become a father again at the age of 55 – my wife, Sue, and I ended up raising two wonderful granddaughters from the ages of 2 and 5. In short, life goes where you don't anticipate – you have to enjoy what it brings.


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