SEPTEMBER 2018 # 68

the Analytical Scientist™

Life in the Fat Lane

Lipidomics is on the up – can analytical technology rise to the occasion?

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Image of the Month

Bee-ware!

What makes "killer" or "Africanized" bees more aggressive than European bees? Researchers from Brazil used MALDI-MS and mass spec imaging to analyze the brains of 20-day-old Africanized honeybees, discovering that specific neuropeptides were present in those bees that exhibited aggressive behaviour. When young honeybees were injected with these substances, they too became aggressive.

Reference. M Pratavieira et al., "MALDI imaging analysis of neuropeptides in Africanized honeybee brain: Effect of aggressiveness", J Proteome Res, 17, 2358–2369 (2018).

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

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On The Cover

An artist's impression of fat droplets in water graces the cover this month, as we go deep into the lipidome.

Upfront

the Analytical Scientist

ISSUE 68 - SEPTEMBER 2018

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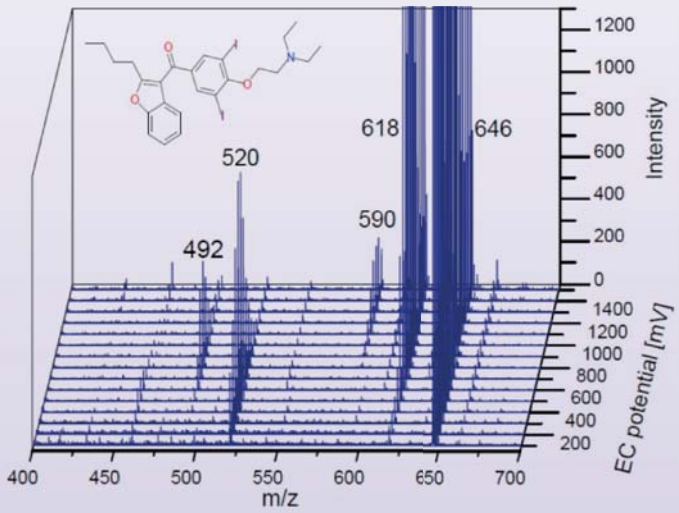

Oxidative "fingerprint" (MS Voltammogram) of Amiodarone (*antiarrhythmic agent*) m/z 646 and its major oxidative metabolites m/z 618, 590, 520 and 492 generated by on-line Electrochemistry-MS using the ROXY EC system equipped with µ-PrepCell2.0 and connected to an MS. Total experimental time 15 min.

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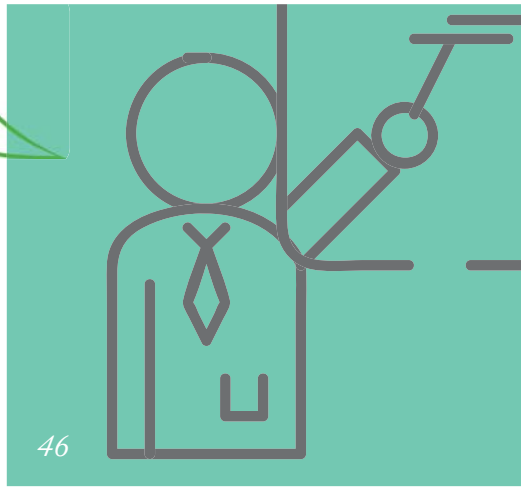

In My View

Features

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Poetic Perspective

"We shall not cease from exploration. And the end of all our exploring will be to arrive where we started and know the place for the first time."

TS Eliot's poem "Little Gidding," from which the lines above were taken, is not easy reading, but the gems hidden in his cryptic language make you want to look for answers.

Analytical scientists are explorers; we love to find new routes – although the road can be long and full of obstructions. We are triggered by questions, and work hard to answer them. And after years of (sometimes tedious) work, it often comes together, crystallizing into simple rules.

It's the story of modern science, perhaps of science throughout the ages, that instrumentation is the springboard for many discoveries. New tools open up new worlds, bring new insights and help us understand the world around us. Modern instrumentation, for example, allows us to delve deeper and discover the complexity of the fascinating world of cell biology. In recent years, we have been getting a closer look into the miraculous world of metabolism, DNA and proteins.

Our cover feature this month is on lipidomics, a rising star in the world of metabolomics. Lipids have long been overlooked, partly because we could not easily measure them. But now, thanks to modern mass spectrometry, we can explore this new world, and it is becoming clear that lipids play a key part in how our cells and organs function – and malfunction. Of course, measuring these complex systems delivers data tsunamis that only slowly release their meaning – standardization will help bring structure to these data so we can interpret them. Michal Holčapek, Markus Wenk, Matej Orešič share their views on page 22.

In our second feature, Albert Robbat from Tufts University serves up some food for thought. His group is focused on "the complex interactions that occur between human and natural systems"; specifically, whether the quality of tea is vulnerable to changing climate conditions (page 34). The findings from Robbat's group, and the software they developed along the way, will allow them to investigate other areas, including developing more palatable meals for cancer patients with compromized sensory abilities, for which they will combine their analytical methods with expertise from culinary chefs and nutritionists.

That brings us to our dessert, an interview with Giovanni Dugo (page 50), a former professor of food chemistry, who laid the foundation for a world-renowned analytical laboratory in Messina, Sicily. He shares his love for Sicilian food and the Sicilian language, which led him – after a lifetime of scientific exploration – to take a new direction: writing recipes in rhyme. Buon appetito!

Frank van Geel
Scientific Director

Upfront

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

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

Choi Yunbyok, PDpics.com

The Early Bird

Who's a pretty (ancient) boy then? Parrots were bred in the USA earlier than previously thought

Archaeologists have uncovered traces of macaw DNA in New Mexico, providing the first evidence that they may have been bred in the American Southwest – more than 1,000 km from their endemic range.

Using a combination of accelerator mass spectrometry (AMS) radiocarbon dating and ancient mitogenomic DNA analysis, the team – comprising researchers based in New York and Virginia – analyzed the remains of 14 birds discovered at archaeological sites in Chaco Canyon and the Mimbres region of New Mexico. Richard George, lead author of the paper, said they were interested in "any evidence for directed breeding or changes in the genetic diversity that could co-occur with different trade networks."

They discovered that the scarlet macaws dated from between 900 and 1200 AD – pre-dating previous known avian colonies. What's more, they were all from the same haplogroup (a population sharing genes from a common ancestor – in the case of mtDNA, the maternal line) and 71 percent of the birds shared one of four unique haplotypes. According to George, this suggests "narrow breeding from a small founder population with little or no introgression or resupply." However, to support their theory, George stated they would need to examine the full genome.

At the moment, you might say they have a bird's eye view of the colony's origin; next, they plan to hone in on a more exact location.

Reference

1. *RJ George et al., "Archaeogenomic evidence from the southwestern US points to a Pre-Hispanic scarlet macaw breeding colony", PNAS, 201805856 (2018).*

Faster Fibrosis Diagnosis

A method for the rapid detection of serum biomarkers in liver disease

"Will Peveler wanted to visit my lab to learn about array-based sensing. In the meantime, he started working in William Rosenberg's lab, where he learned about unmet needs in terms of liver disease diagnostics, in particular the lack of point of care (POC) tools," says Vincent Rotello, Professor of Chemistry at the University of Massachusetts. The unmet need – and the high mortality rate associated with liver diseases – inspired a collaborative effort from the UK and US to develop a rapid diagnostic technique for fibrosis (1).

The new test involves obtaining a small amount of blood (approximately 40 µL) via a fingerstick, adding fluorescent-coated polymers, and then using the enhanced liver fibrosis immunosensing platform – a "chemical nose" – to sniff out serum biomarkers of fibrosis such as albumin, immunoglobulin, transferrin, fibrinogen, and alpha-1-antitrypsin. The simple process takes only 45 minutes and is relatively inexpensive – providing a new diagnostic for the developing world, according to Rotello. "Closer to home, the sensor should enable regular monitoring of liver health using minute quantities of blood," he says. "This straightforward testing would identify issues well before symptoms develop, allowing much more effective preventative and therapeutic strategies to be employed."

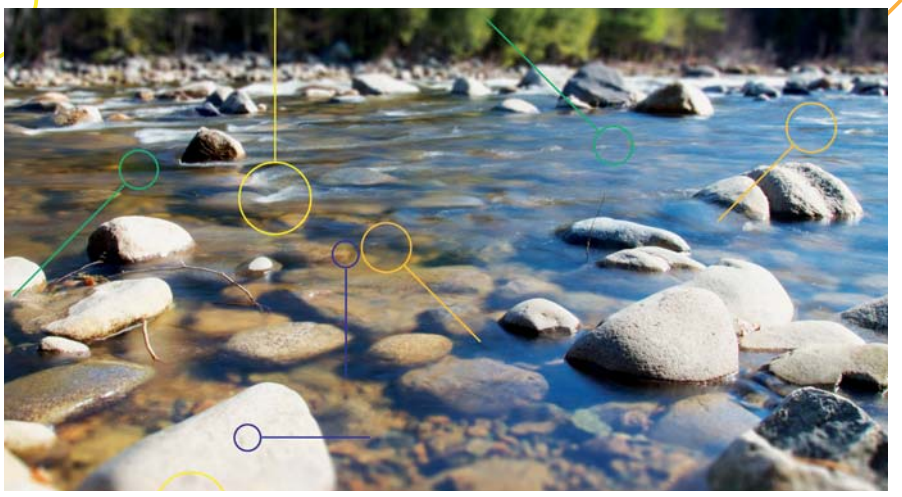
The technique checks the speed and pricing boxes, but how will it fit into existing workflows? Rotello says it isn't far from being pathologist-ready; the researchers are currently streamlining the platform for clinical and POC use, as well as working toward further validating and implementing their technique in various settings. It's not a one-man job, though, and Rotello emphasizes the effort required of everyone on the team: "This project provides an example where a hammer-builder, such as myself, can find an important nail through collaboration."

Reference

1. *WJ Peveler et al., "A rapid and robust diagnostic for liver fibrosis using a multichannel polymer sensor array", Adv Mater, [Epub ahead of print] (2018). doi: 10.1002/adma.201800634.*

There's Something in the Water

Drug emissions in UK rivers raise questions about long-term consequences for the environment



A recent study from the University of York, UK, found traces of 29 different drug compounds within two local rivers (1). The drugs – detected using HPLC-MS/MS – included antidepressants, antibiotics, painkillers, and treatments for diabetes and epilepsy. The levels were in themselves low, but the team are concerned about the long-term impact of the emissions. How can the potential consequences for human (and environmental) health be better understood? And what can be done to help? We spoke to Alistair Boxall, Professor in Environmental Science at the University of York, about his quest to find out more.

Many studies have been done on pharmaceuticals emitted into the environment, but this one looked at emissions over time and in different locations. Why?

We know that pharmaceutical active ingredients occur in the environment, but we have a less developed understanding of how concentrations vary in space and time – something we need to properly assess the risks of these molecules to aquatic organisms. Concentrations of some active ingredients in rivers can be explained based on knowledge of what doctors in an area are prescribing at the time, and of river flows. We have had some surprises; in an earlier study, we detected some compounds that aren't prescribed in the UK, and during periods of heavy rainfall we see elevated concentrations of compounds not usually detected, possibly due to inputs from combined sewer overflows which bypass wastewater treatment.

What sources are these drug traces likely to be coming from?

In York, we think the main source is from patient use, with a small amount arising from inappropriate disposal of medicines. In monitoring in Nigeria, for example, manufacturing inputs appear to be a major contributor.

What can be done to help prevent these emissions?

Some of the measures pharma companies can take include introducing better treatments in their factories, or, if obtaining actives from a supplier, ensuring they only obtain materials from companies with good environmental standards. Longer term, they could move towards developing more environmentally benign medicines to replace the most environmentally risky molecules. Technological developments such as personalized medicine and nanomedicine, which will reduce patient doses, will also help reduce the environmental impact of medicine.

What next steps would you like to see to tackle drug traces in the environment?

In Europe and North America, I suspect that only a small proportion of the 1,500 or so active ingredients we use are causing environmental harm. We need to develop ways in which we can identify these molecules so that mitigation efforts can focus on the compounds that really matter. This will require better sharing of data by industry and academia, and the development of approaches for prioritizing active ingredients in terms of their environmental risk. This is something we are already working on in the Innovative Medicines Initiative's Intelligence-led Assessment of Pharmaceuticals in the Environment (iPiE) project, which involves 13 pharmaceutical companies and ten research and regulatory organizations.

Elsewhere, such as areas of Asia and Africa, the problem of pharmaceutical pollution will be more acute due to things like disease pressures, a lack of connectivity to the wastewater network, and poorer regulation. We need to understand the implications for human health and the environment, and then industry, governments, academics and the NGO community need to work together to solve the problem.

UK-Wide Web

A new project aims to boost the profile of UK measurement science

What?
The CAMS-UK (Community for Analytical Measurement Science-UK) is an "industry-led membership network" intended to strengthen ties within the UK's analytical community and raise the profile of UK measurement science worldwide. The impetus for the project came from a meeting about the UK's analytical science landscape by the Royal Society of Chemistry Analytical Division, whose members include Melissa Hanna-Brown (Pfizer), Duncan Graham (University of Strathclyde) and Steve Lancaster (pictured; Domino Printing Sciences).

How?
With its strong industry–academia network, CAMS-UK aims to facilitate industry-led research, promote analytical science training, and enhance the skills of up-and-coming scientists.

Why?
"Analytical measurement science cuts across every scientific discipline and touches the lives of every UK citizen," said Melissa Hanna-Brown in a press release. "In the current climate of research and innovation landscape flux in the UK, there is a tangible opportunity for our community to coalesce. We hope… to inspire [scientists] about what we might be able to achieve together as an analytical community if we combine our efforts."

Who?
CAMS-UK is now a coalition of experts from academia, industry and government bodies. Free membership is available to all UK and Ireland-based organizations involved in analytical measurement science, whether companies, universities, institutions or scientific networks.

When?
The project has been in development since May 2016, and was officially launched in early 2018. Collaborative industry-led projects are expected to start in 2019.

Visit the CAMS UK website: www.cams-uk.co.uk
Find out more about Melissa Hanna-Brown, Duncan Graham and Steve Lancaster:

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Sequencing, Spectroscopy, and Standardization

Business in brief: What's going on in analytical science?

Products and launches

- Waters Corporation revealed its Xevo TQ-GC Mass Spectrometer, a tandem quadrupole mass spectrometer that can exceed ppb limits of detection.
- SCIEX announced the launch of their new sample preparation system, the Topaz Prep Station. The automated workstation aims to help speed up testing at diagnostic labs.
- NuGEN Technologies has released a new library quantification method. CEO Nitin Sood said, "as sequencing becomes cheaper, the development of novel technologies to decrease library prep time and cost… is essential."
- The latest version of Agilent's OpenLab CDS ChemStation Edition allows labs to export files in Allotrope Data Format (ADF) – designed to standardize data from multiple sources.
- DataApex launched version 8.0 of its Clarity Chromatography Software.

Collaborations and acquisitions

- Waters Corporation and Restek Corporation have joined forces to provide food safety labs with access to GC-MS instrumentation, application support, and training for pesticide residue analysis.
- Metrohm AG has acquired B&W Tek's Spectroscopy Solution Business, B&W Tek LLC.

Company and people updates

- HORIBA Scientific has cited "continued growth" as the reason for moving to new headquarters in Piscataway, New Jersey. Two years in the design, it is double the size of the previous facility.
- Thermo Fisher Scientific's Chris Pohl (pictured) received the Uwe D. Neue Award for his "continued and significant contribution to the field of separation science." The award was presented at HPLC 2018, where Pohl gave the keynote lecture.
- Over 60 graduates and postdocs attended the ACS 2018 Summer School on Green Chemistry & Sustainable Energy.

For links to original press releases, visit the online version of this article at: tas.txp.to/0918/BUSINESS.

Read our interview with Chris Pohl here: tas.txp.to/0516/Pohl.

THE INNOVATION AWARDS 2018
the Analytical Scientist

The Analytical Scientist Innovation Awards 2018

Nominate now for this year's TASIAs!

The Analytical Scientist Innovation Awards (TASIAs) will return for 2018 to showcase the best new technologies, instruments and software solutions causing a stir in the analytical science community.

The talented teams behind the top five innovations will each have the opportunity to share their development story in a Solutions article in 2019.

To enter, please email joanna.cummings@texerepublishing.com with the following information:

- Name of innovation
- Launch date (must be after 31st October 2017)
- Brief description (~10 words)
- Detailed description (50-150 words)
- Potential impact (50-150 words)
- One image (if applicable)

The deadline is 12th November 2018. All nominations will be put to an expert panel, with the winners announced in our December issue.

Read about last year's winners at tas.txp.to/1217/tasia

In My View

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

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

Contact the editors at charlotte.barker @texerepublishing.com

A Closer Look at the Bigger Picture

How a pixel-based approach can define the unique chemical fingerprint of a complex environmental sample.

By Guilherme L. Alexandrino, Josephine Lübeck and Jan H. Christensen, Analytical Chemistry Group, Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Copenhagen, Denmark.

The latest high-throughput and powerful chromatographs and mass analyzers have found their rightful place in analytical laboratories worldwide. These instruments provide an extraordinary amount of high-quality data, especially comprehensive two-dimensional chromatography (for example, GC×GC or LC×LC) and/or high-resolution mass spectrometers.

One rising star in analytical chemistry is the combination of the pixel-based approach with non-targeted analysis. Here, we aim to look at the whole spectrum of chemical compounds in the samples by using the entire chromatogram in the data analysis, instead of individual peak picking and integrations. The great advantage is that the chemical differences between samples is highlighted in a broader way, particularly useful in petroleum and oil characterization, omics-based studies and the analysis of environmental pollution.

Our team uses a pixel-based approach to carry out oil fingerprinting analysis and to investigate environmental pollution in urban areas. We have two main goals: first, to compare samples to discover the chemical patterns that describe them and second, to simplify the extraction of the information that explains these patterns.

As an example, non-targeted analysis has allowed us to identify a much wider range of potential anthropogenic pollutants released to the environment compared with traditional targeted analysis of conventional persistent organic pollutants (POPs). More specifically, we performed pixel-based analysis, where the main chemical variability of the samples

"Our team uses a pixel-based approach to carry out oil fingerprinting analysis and to investigate environmental pollution in urban areas."

"There is no peak picking or peak integration steps in this approach – but the data analysis is nevertheless more complex because of the high number of chromatographic pixels."

is assessed pixel-by-pixel from entire chromatograms. There is no peak picking or peak integration steps in this approach – but the data analysis is nevertheless more complex because of the high number of chromatographic pixels.

We also use pixel-based analysis in forensic investigations of oil spills. The aim is to find the source(s) of the spill (1,2), with positive matches of spill–source pairs obtained after comparing their respective chemical fingerprints. We use GC-MS and GC×GC-MS to analyze hydrocarbons in oil spills, and pixel-based analysis to match spills to their source (3,4).

Pixel-based analysis is usually divided into two parts: i) preprocessing, and ii) modeling. In the first step, it is recommended that the sample chromatograms are "corrected" to minimize the influence of experimental noise. A key preprocessing step to make the pixels adequately comparable is the correction of retention time shifts that can occur because of multiple injections and column aging. The modeling step focuses the analysis on the most relevant chemical information from the chromatographic signals.

In our research, principal component analysis (PCA) has been the preferred solution when using models for pixel-based analysis, because it efficiently extracts the systematic patterns in the pixels that allow us to group the samples according to their chemical similarity. Similar samples should have similar pixel-by-pixel patterns, and will therefore belong to the same group in the principal components score plot. As an example, we investigated the entire chemical fingerprint of sediment samples analyzed by GC×GC–(HR) MS from two polluted sites (Utterslev Mose and Fortress channel) in Copenhagen, Denmark. The chemical similarity of the samples was assessed through the score plot of the PCA model. The samples were clearly grouped according to their origins in the PC1 versus PC2 subspace, which means the chemical fingerprints were different in each place.

We therefore encourage the use of pixel-based analysis for a deeper understanding of the complex chemical fingerprints analyzed by today's high-end instruments, so that we get a closer look at the bigger "chemical" picture we have in our hands in non-targeted analysis.

References

1. *JH Christensen et al, Environ Sci Technol, 38, 2912–2918 (2004).*
2. *FDC Christensen, JH Christensen, J Chromatogr A, 1235, 149–158 (2012).*
3. *JH Christensen, G Tomasi, J Chromatogr A, 1169, 1–22 (2007).*
4. *S Furbo et al., Anal Chem, 86, 7160–7170 (2014).*

Coming Soon: Third-Wave Diagnostics

The need for continuous and contextual biochemical data is clearer than ever – and enabling technology may be just around the corner.

By Jason Heikenfeld, Professor at the University of Cincinnati and Chief Science Officer at Eccrine Systems Inc., Cincinnati, USA.

When is it appropriate to attach the moniker "stone age" to a previous era of science and medicine? You could easily argue that pathology was in just such a stone age before biofluid- and tissue-based diagnostics came of age. So when will our present-day capabilities be similarly relegated?

The last century produced the first wave of modern diagnostics based on collected biosamples that had to be sent to a laboratory for analysis. More recently, we have seen a second technological wave of point of care diagnostics that put the lab right in the hands of the doctor. This second wave brings added convenience and can even allow the doctor to validate a diagnosis while in the presence of the patient. Despite these advances, the remaining gaps in patient care are so significant that – one day not too far in the future – we may agree that pathologists in 2018 were in the "stone age" of medical diagnostics. To visualize the gaps, it may help to start thinking about what might soon be possible…

Imagine personalized therapeutics, where the dosing is adjusted in real time based on each individual's unique rates of absorption and metabolism and their treatment responsiveness. Or something even simpler: knowing for certain that the patient is actually taking the drug at all. Imagine a complete, continuous biochemical view of lifestyle choices for a cardiac patient, measuring potassium and brain natriuretic peptide continuously on both good and bad days. Imagine mental or stress disorders without the need for biased self-reporting, with treatment based instead on quantitative cortisol responses to daily stressors. Or imagine a workforce safety system in which chemical toxin exposure is reliably recorded as internal exposure and organ loading, not just in terms of what volume of toxin may have breached protective clothing.

Imagination may soon become reality with the third wave of diagnostics – one that allows patients to take the laboratory with them in the form of wearable biochemical monitoring systems. That's what prompted our research group (in partnership with Air Force Research Labs) to seek not a technological solution, but rather to first uncover the fundamental questions and challenges that would face such diagnostics. It led us to a biofluid that was at the time underused, but arguably had the highest upside potential: eccrine sweat. Seven years after that first inspiration, we have now demonstrated a wearable device that can locally stimulate sweat for multiple days, wick a tiny sweat sample up off the skin surface, and transport it within minutes to a Bluetooth-connected array of sensors that can continuously report analyte concentrations. In essence, we are extracting blood-level information continuously and noninvasively, with less than five-minute time stamps. And it works exceptionally well for small, hydrophobic analytes that partition readily through the tissue layers between blood and the sweat glands (such as steroid hormones or small-molecule drugs). Proteins and antibodies are larger and therefore more challenging because they are highly diluted in sweat, but we can now pre-concentrate such analytes by several orders of magnitude – also continuously and within minutes.

"Imagination may soon become reality with the third wave of diagnostics"

With this device, we hope to ride the crest of that third wave. Our goal is something even more powerful than continuous biochemical data for a patient; we want that data to be contextual. As doctors, you know just how limited a single data point can be – and you know that, in many cases, you would find it more powerful to trade absolute concentration accuracy for the ability to closely monitor relative changes in chemical analytes. Measuring such changes can be particularly powerful when they are placed into context. Coming back to the cardiac patient from earlier – was the spike in blood pressure due to eating a cheesesteak sandwich or because of a daily stress event? Or did the patient simply stop taking their statins? For many diseases, the coming wave will make current diagnostics look like interpreting a connect-the-dots picture before the connecting lines have been drawn.

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Intelligent Innovation

From the very beginning, KNAUER's success has hinged upon developing cutting-edge measurement tools. We caught up with Christian Benkhäuser, Head of Hardware Development, to uncover the company's approach to innovation in the "on demand" era.

What drew you to KNAUER?

I used KNAUER products during my PhD, and the company was on my radar as a manufacturer for high-quality lab devices. When a job opportunity came up – straight after my PhD – I jumped at the chance! It was a big risk to move more than 600 km with a young family, but I have no regrets. I've always loved the idea of moving technology forward, so working for an instrument developer is perfect for me.

I enjoyed my academic studies, but I wanted to find work with a more tangible impact – something that really makes a difference to scientists in all sorts of fields. It really motivates me to think that my work here could ultimately play a part in curing disease or protecting the environment.

What are your top priorities as Head of Hardware Development?

The obvious answer is to build the best devices! But of course there are many different aspects to "best." My main priority is to make sure we know what our customers really need and want. Honest feedback is absolutely crucial for us here at KNAUER. I want to know how the customer works with our product, what problems they face, and what they would improve.

As a relatively small company (around 135 people), our devices can't always be offered at the lowest prices on the market, so it's important that we distinguish ourselves with the quality of our technology. Our reputation is built on great performance and a long lifetime – and that must continue in future products.

KNAUER seems to have a real passion for innovation – where does it stem from?

I think it's a combination of our history and our small company size. Our founder, Herbert Knauer, started the company in 1962 after coming up with a revolutionary temperature measurement device. With this instrument he built up the company, and went on to invent and launch a series of innovative products. In other words, innovation has been built into KNAUER from day one, and we continue to invest a lot in development – not only for our products but also for our internal processes and training for our employees in many different areas.

What are your favourite projects?

They are all exciting! Launching a product in a new area is particularly fun because we are building up a new technology base. Improving existing products, on the other hand, can be very challenging but no less exciting. All in all, I have to say that it is very rewarding to find the root cause of a technical problem: once this is done, the solution is developed much more easily. I really enjoy this as an team effort - working with very smart and dedicated people in a team together.

Above all, we are lucky that our CEO Alexandra Knauer is so willing to invest in innovation, whether we are developing products for customers or in-house systems (for example, 3D-printing for faster prototyping), and state-of-the-artsoftware systems.

What is the key to innovation?

From Galileo to Hawking, curiosity is the key to problem solving. Without curiosity, you cannot improve things and ultimately your work cannot progress.

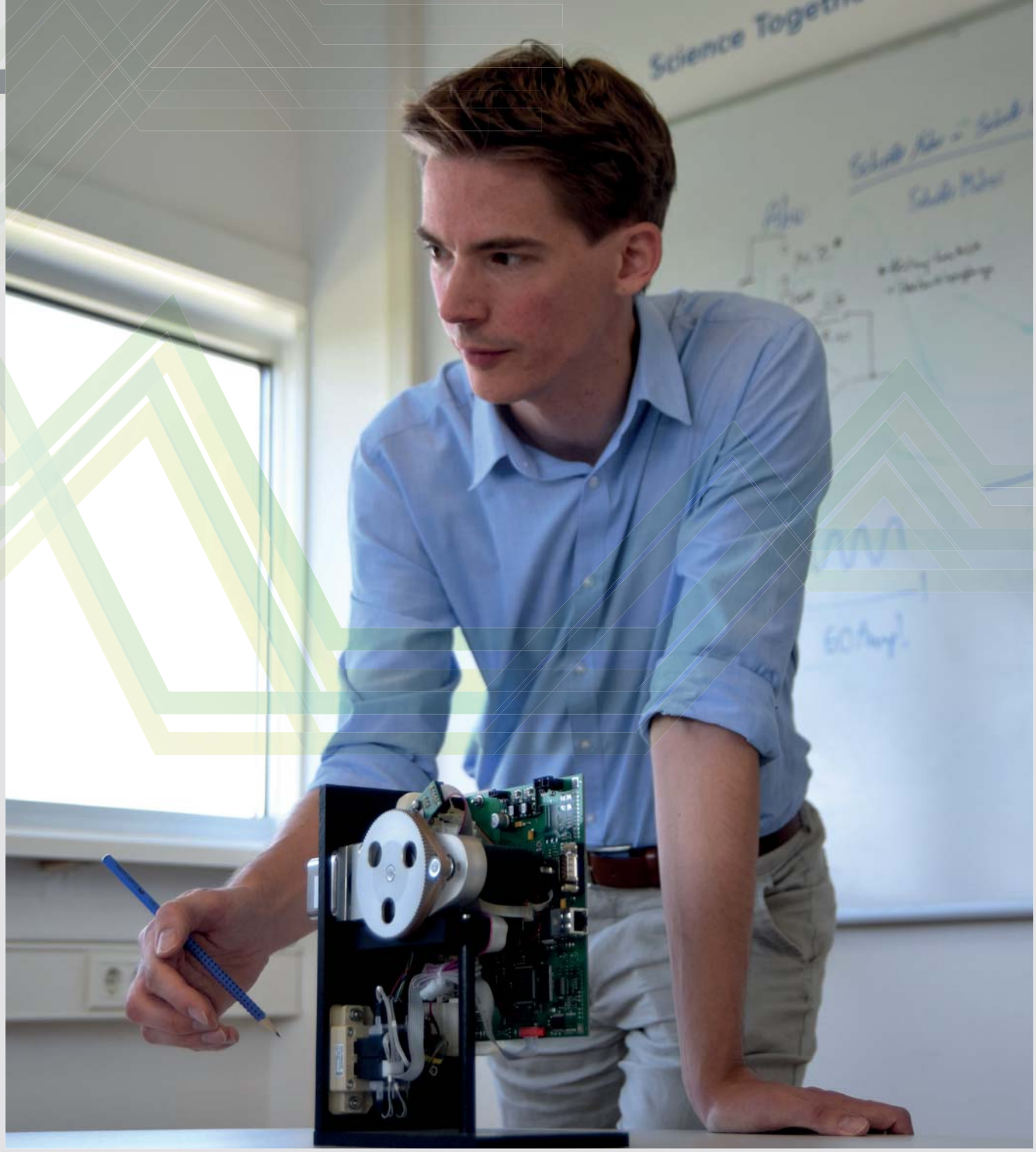
I also believe that you cannot be innovative without accepting failure. Every time you make a mistake or hit a dead end it's important to learn from it. You have to be critical of your own work and identify what went wrong, so that you can start afresh with new knowledge. Every failure is a step closer to success. That's why it's important to have complete honesty in the team. If you spot a problem at an early stage, you have to be able to discuss it there and then, not after the project fails! All members of the team need to feel that they can be honest, even with those in senior roles.

"I've always loved the idea of moving technology forward, so working for an instrument developer is perfect for me."

How can a company make sure that it is future-proof?

I don't think it's possible to be 100 percent future-proof. Sometimes you may put a lot of time, effort and money into developing a new product, but before launch the science moves on and it's no longer innovative. It is the nature of this business. But if you know your market and your customers really well, you can avoid costly missteps and introduce advances that help your customers achieve their goals.

For example, digitization and laboratory automation is a big topic for the whole market, and something our customers ask about, so we have developed a mobile app to monitor and control a complete system from anywhere, using a tablet. We are also developing augmented reality tools for marketing and customer support. Digitization

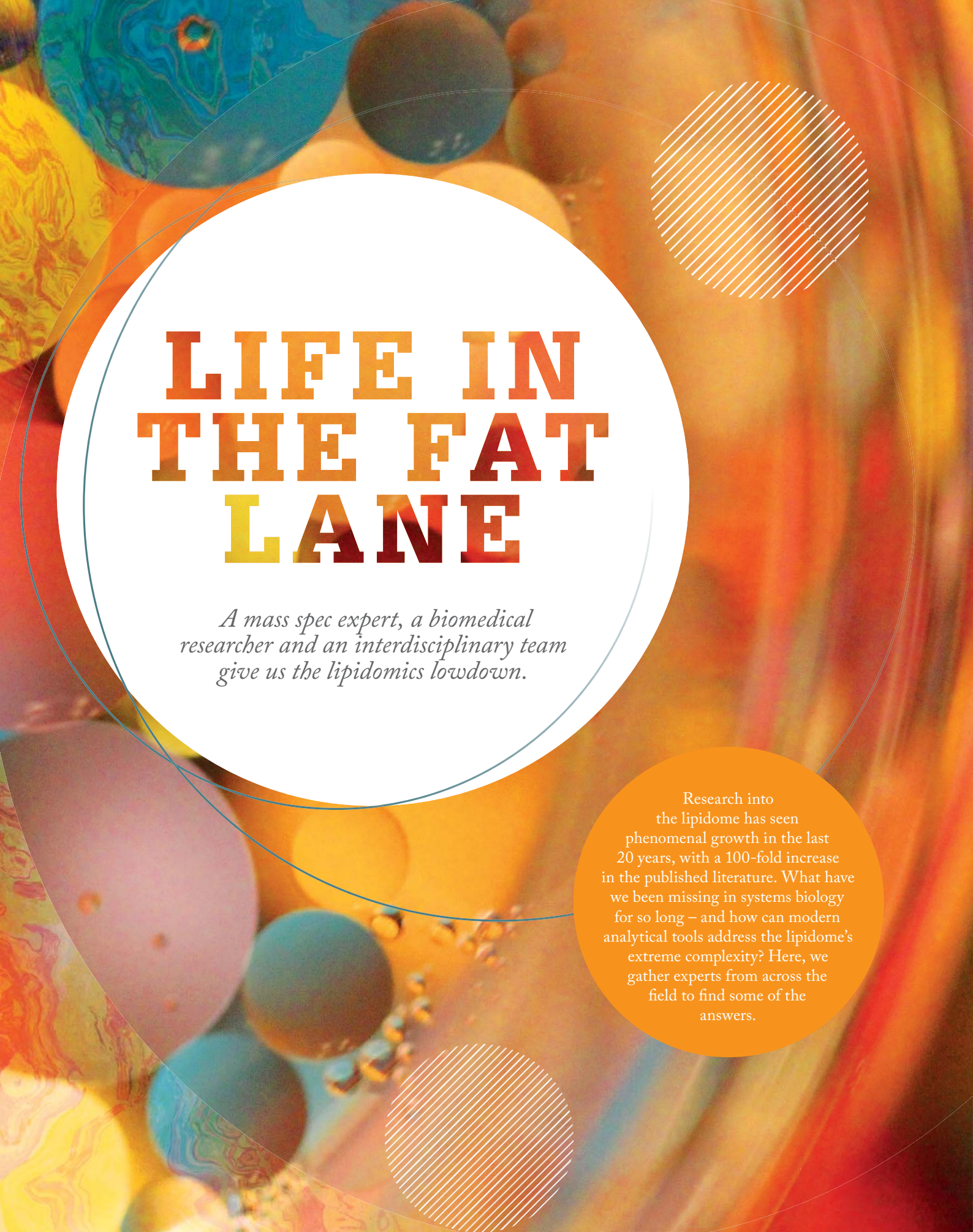


speeds up innovation, because now we all have instant access to information.

And you are extending that approach to training too…

Yes, we are working on a project in collaboration with several academic and industry partners to develop an online training platform. Our goal is to have a tool with which new employees and customers can learn how to use our products and services.

As a globally acting company, we face the challenge to support a wide-spread net of distributors and service technicians every day. With the development of such an elaborate online training platform we are able to train our partners and customers all the time and everywhere. Training gets faster, more effective and resource-saving while improving the quality of the service we provide. With this platform we want to make this knowledge available "on demand." In today's culture, we expect instant delivery of goods, services and information – and it's the same with training; we want it when and where we need it.



LIFE IN THE FAT LANE

A mass spec expert, a biomedical researcher and an interdisciplinary team give us the lipidomics lowdown.

Research into the lipidome has seen phenomenal growth in the last 20 years, with a 100-fold increase in the published literature. What have we been missing in systems biology for so long – and how can modern analytical tools address the lipidome's extreme complexity? Here, we gather experts from across the field to find some of the answers.

MEETING THE CHALLENGE

Lipidomics has come a long way – but there are still many analytical challenges ahead.

WITH MICHAL HOLČAPEK

The word "lipidomics" did not even exist 20 years ago. Back then, only a limited number of lipid classes and species were analyzed, and rarely in connection with other biomolecules and biological backgrounds. Since then, the field has changed dramatically; nowadays, the application of lipidomic analysis in various fields is increasingly prevalent.

Why have lipids and lipidomics taken on greater prominence? First, because they play crucial roles in human biology. Numerous lipids are present in every cell in our body and have a huge influence on health and disease: they form the lipid bilayer that surrounds the cell and intracellular compartments, they are involved in energy storage and cell signaling. Consequently, there has been a serious drive to develop methods for lipid analysis, which – together with rapid innovation in the area of mass spectrometry – has led to the rapid growth of the lipidomic field.

I first began analyzing lipids in 1995, with an initial focus on monitoring biodiesel production – specifically, the transesterification of triacylglycerols to methyl esters of fatty acids. We had recently had an LC-MS system installed (the first in the Czech Republic – a single quadrupole from Waters!). Starting with the analysis of triacylglycerols, we developed analytical methods for all existing types of isomers up to triacylglycerol enantiomers, extending our interest to phospholipids, sphingolipids, and other lipid categories to build a more comprehensive picture of the lipidome. From there, we decided to look at the application of lipidomic analysis for cancer biomarker research, which remains our main focus today.

Lipidomic analysis is very complex. There are now dedicated proteomic databases and standardized approaches for structural elucidation, whereas such tools are not yet available in lipidomics. In lipidomic analyses, there are too many sources of variability – different lipid categories, classes, subclasses, and many types of isomerisms inside classes – including fatty acyl chain lengths, the double bond number, positions, geometry, positional isomers, and enantiomers. The theoretical complexity is extremely high and we are still not sure why the body needs such a huge diversity of lipids.

METHODS IN THE MADNESS

There are three major approaches in lipidomic analysis (1-3). The first involves direct infusion (often called shotgun): the lipidomic extract is infused directly to the mass spectrometer at a low flow rate, so all required scans can be performed using either a low-resolution (precursor ion and neutral loss scans with triple quadrupole or Q-LIT instruments) or high-resolution (QTOF, Orbitrap or, more rarely, ICR) approach.

The second area is MS coupled to chromatographic techniques, which brings obvious advantages, including the chance to separate various types of isomeric lipids. Chromatography offers a wide variety of different separation mechanisms, but by far the most common mode is reversed-phase LC, which provides an excellent separation selectivity for isomers differing in the hydrophobic part of the molecule. Silver-ion chromatography is a special mode that uses embedded silver ions in the stationary phase, which interacts with double bonds in lipids or other molecules. Increasing numbers of double bonds mean stronger interactions, making it possible to separate lipids differing only in the double bond position or cis/trans configuration. These methods provide the selectivity for species separation.

"One challenge we have to be particularly aware of is the biological variability among individuals."

Alternatively, lipidomic class separation can be used, such as hydrophilic interaction chromatography (HILIC) and normal-phase chromatography. Lipid species within one class coelute with the lipid class internal standard, which makes the quantitation more robust. In fact, there is a small partial separation even inside classes, but this only affects quantitation to a very limited extent. Reversed-phase LC can also be used for quantitation, but careful attention should be paid to the selection of an adequate number of internal standards to cover the whole retention window, and to full method validation, including the determination of the matrix effect. In principle, any MS method can be used for quantitation, provided that all requirements of quantitative analysis are followed; method reliability is clearly demonstrated using validation parameters, quality control samples and cross-validation; and the quantitative data obtained are in agreement with other laboratories.

Desorption ionization MS and imaging MS is the third area. Matrix-assisted laser desorption/ionization (MALDI) is the most common ionization technique, but desorption electrospray ionization (DESI) and other desorption ambient techniques are also now available. A typical application is MS imaging of tissues to show the distribution of lipids and other biomolecules within tissues and organs. There are some limitations in terms of quantitation (the signal in desorption ionization MS is not as stable as atmospheric pressure ionization techniques) but semi-quantitation can also be achieved (4).

In future, I anticipate further implementation of ultra-high-resolution mass spectrometry (UHRMS), ultra-high-performance liquid chromatography (UHPLC) and ultrahigh-performance supercritical fluid chromatography (UHPSFC) in common practice. In my group, we are fans of UHPSFC-MS coupling for high-throughput lipidomic quantitation (5); it is robust enough for high-throughput lipidomic analysis, and the sensitivity for less polar lipid classes is much higher than any other available techniques. We expect to see more groups implement this relatively new approach in the near future.

FROM ANALYTICAL METHOD TO CLINICAL PRACTICE

The next step for the field of lipidomics is to gain a better understanding of the biology behind the changes observed in analytical experiments. For this, we urgently need experienced

biologists to collaborate with analytical chemists. Many biologists consider the lipid class as a whole, but it's important to note that – even in the same lipid class – some lipids are upregulated while others are downregulated. Therefore, we need biologists who are able to interpret observed changes lipid-by-lipid.

Our own group's focus for the next few years is quite clear. We have obtained really exciting results in the area of lipid cancer biomarkers, and we are working hard to get the methodology fit for implementation in real clinical practice for early cancer screening and monitoring of treatment progress.

One challenge we have to be particularly aware of is the biological variability among individuals. To achieve acceptable accuracy for lipidomic bodily fluid analysis, full optimization of the whole methodology is required, starting with sample collection, storage, transport, sample preparation, analysis, and data processing up to statistical evaluation. With so many steps, there is a risk of introducing artefacts and errors in addition to the biological variability – so we have to be as rigid as possible in terms of method optimization, validation, and quality control. Currently, it is possible to differentiate cancer patients from healthy volunteers – but the analytical methodology must be as robust as possible.

Our ultimate goal is to convince a strategic partner to move the methodology from the academic lab into real clinical practice. To do that, we need experts from various fields (clinicians, analytical chemists, statisticians, and biologists) – and finding a common language among these experts is another challenge ahead of us!

Michal Holčapek is Head of the Lipidomics group at University of Pardubice, Czech Republic.

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A PICTURE OF HEALTH?

Lipids are a crucial and complex component of the 'omics' puzzle.

WITH MATEJ OREŠIČ

A diverse group of metabolites, lipids are under tight homeostatic control and exhibit spatial and dynamic complexity at multiple levels. It is thus not surprising that altered lipid metabolism plays an important role in the pathogenesis of many common diseases (1).

My main research area is systems medicine. In particular, I explore metabolomics applications in biomedical research and related integrative bioinformatics in a range of conditions, including non-alcoholic fatty liver disease (NAFLD), metabolic co-morbidities in psychotic disorders, and immune-mediated inflammatory disorders, such as type 1 diabetes and celiac disease. We have found that lipid-related disturbances underlie many complex diseases and their co-morbidities.

For example, NAFLD is defined by accumulation of storage lipids in droplets in the liver. In studies of NAFLD, lipidomics not only uncovered biomarker candidates for diagnosing and monitoring the disease, but also revealed key underlying processes and disease heterogeneity. Specifically, lipidomics studies have shown that NAFLD, typically associated with insulin resistance and diabetes development, is also characterized by accumulation of triglycerides with low carbon number and double bond count, as well as increase of hepatic ceramides, which are known to cause insulin resistance (2). On the other hand and because of a common genetic variant in *PNPLA3* gene, NAFLD associates only with storage of harmless dietary triglycerides, and not with insulin resistance. The NAFLD lipid signature found in liver can also be identified in the circulation, thus offering promise for new diagnostic tools for NAFLD (3, 4). Interestingly, studies by us and others have shown that the triglycerides associated with NAFLD are also associated with the risk of type 2 diabetes (5, 6).

ANALYSIS IN THE BALANCE

Lipids have high structural and functional diversity, so there are an enormous number of combinatorial possibilities. Specific specialized targeted methods are required, as these lipids may need specific types of sampling, extraction and analysis. Another level of complexity is introduced by the fact that even minor changes in lipid concentrations may have a large effect on cellular physiology. Therefore, accurate, quantitative measurement of lipids is important – which is, after all, the ultimate goal of lipidomic analysis.

Comprehensive "lipidomics" approaches usually refer to methods that cover the major lipid classes, including phospholipids (including major membrane lipids such as phosphatidylcholines and -ethanolamines), sphingolipids (sphingomyelins and ceramides) and neutral lipids (mono-, di- and triacylglycerols, cholesterol esters). Two main approaches have been adopted for these lipidomic analyses, based on direct infusion (shotgun approach) and on LC-MS (mainly reverse-phase LC, which is the most commonly applied approach for lipidomics). Each has its own advantages and disadvantages (7).

In the shotgun approach, because sample infusion to the mass spectrometer is at a constant concentration and the inevitable matrix effects are relatively constant, internal standard quantification may work well if the lipid profiles between the study groups do not vary too considerably. However, it comes at a cost: considerable and matrix-dependent ion suppression, as well as lower sensitivity compared to LC-MS. In LC-MS, lipids are separated prior to the introduction to the mass spectrometer, thus reducing the matrix effects and improving the sensitivity. However, because the internal standards do not elute at the same time as most of the lipids covered, the quantification may only work well for some of the compounds.

The aim of both approaches is to quantitate lipids as accurately as possible, which is aided by the increasing number of pure lipid standards becoming available. Nevertheless, strict quality control is essential (7). In our studies, about 20–25 percent of all samples analyzed are QC samples.

Over the years, much attention in lipid analytics has been devoted to somewhat contentious discussions about the superiority of one lipidomics technique over others. Confusing terminology such as "absolute quantification" has been introduced for some (particularly shotgun) approaches, despite not being truly quantitative. As the field matures, and as we gain a better understanding of the advantages and limitations of different techniques, I hope we will see more attention paid to issues that matter most, such as having proper QC, lipid standards and harmonized ways of reporting lipidomic analysis.

MARCHING ONWARDS

There has been a rapid increase in the volume of lipidomics publications over the last 15 years, partly reflecting better understanding of the importance of lipids in life sciences and medicine, and partly because of improvements in analytical tools for lipidomic analysis. Technological advances look set

to continue in the near future, with better MS instrumentation, and new technical solutions for more detailed lipid identifications. LIPID MAPS (www.lipidmaps.org) has become an important resource for lipid analysis and has also contributed to the development of new pure lipid standards in collaboration with reagent companies. Lipid imaging, including in vivo imagine, is also developing fast.

Our group is currently engaged in studies of NAFLD, not only to develop better diagnostic and monitoring tools, such as in the LITMUS project, but also to study the role of fatty liver in the development of metabolic co-morbidities. Specifically, we are interested in how metabolic co-morbidities develop in patients with psychotic disorders, a topic of considerable public health interest. Our findings indicated that fatty liver plays a crucial role (8), though this line of research will require further study of the lipidome across the gut–liver–brain axis.

Matej Orešič is Visiting Senior Lecturer in the School of Medical Sciences, Örebro University, group leader in systems medicine at the Turku Centre for Biotechnology, University of Turku, Finland, and guest professor at the Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences (Wuhan, Hubei, P. R. China). He also initiated the open source MZmine project, and is one of several experts working on LITMUS (Liver Investigation: Testing Marker Utility in Steatohepatitis).

MZmine – http://mzmine.github.io/
LITMUS – https://litmus-project.eu/

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SINGAPORE'S "SLING"

The Singapore Lipidomics Incubator, established by Markus Wenk and based at the National University of Singapore, is an interdisciplinary program dedicated to innovation, education and partnership in lipidomics research. Here, four of the team members – Markus Wenk, Anne K Bendt, Federico Torta and Amaury Cazenave Gassiot – discuss the growing importance and impact of lipidomics.

Why focus on lipids?

Anne K Bendt: Coming from a proteomics and transcriptomics background, I used to consider lipids "the slime" you tried to get rid of! I was amazed to discover that lipids constitute 50 percent of the brain, and that popping a painkiller directly influences lipid signaling.

Amaury Cazenave Gassiot: I came to the lipidomics field almost accidentally after my PhD in analytical chemistry, and never left. The analytical challenges brought up by lipids, their chemical diversity, and the potential they offer – for instance in clinical research – are of tremendous interest to me.

Federico Torta: My interest in lipidomics grew from my interest in mass spectrometry, and proteomics in particular. A few years ago I was looking for new and exciting applications of MS, and the lipidomics field seemed an obvious choice. As I learnt more about lipids, I realized how ubiquitous these molecules are and how many important roles they play in biology.

Markus Wenk: I was motivated by a single question: "Why are there so many different lipids in nature?"

Why are lipid metabolites deserving of their own "omics"?

FT: Once seen as enemies of a healthy life, lipids are essential players in any biological process. Hence, if we want to understand the inner workings of an organism, we need to measure not just proteins but lipids too.

ACG: As Federico says, lipids have a bad press for a long time, but it is becoming increasingly clear that the picture is more complex. Current clinical tests measure cholesterol and total triglycerides, but there are thousands of individual molecular species or lipids in a drop of blood… We need to understand what they are and what they do.

AKB: The size of the lipidome is still not well understood. Why are there so many different lipid molecules? What is the degree of sheer randomness versus functionality, especially given that subtle differences in molecular fine structures have been shown to have effects on their immunomodulatory properties? Differences in the lipid repertoires of pathogens and their human hosts have already been exploited; for example, as targets for more selective antibiotics and as pathogen-specific diagnostic biomarkers.

What makes lipidomic analysis so complex?

ACG: The chemical diversity of lipids is staggering. Under a single mass spectrometric peak, even measured in a high-resolution instrument, many lipids can be hidden, in the form of isomers. Many techniques, therefore, including relatively new ones (such as ion mobility and on-line chemical reactions – ozonolysis, for example), are needed to obtain more detailed information. The use of shotgun, LC, GC, SFC, high-resolution MS or low-resolution MS/MS should be determined by what kind of lipids you are looking at, what level of information you require, and what your application is.

FT: The extreme heterogeneity of their chemistry means the number of lipid species has so far been quantified in the hundreds of thousands. These different molecules have different properties and characteristics and it is impossible to measure all of them at once. The complexity is reflected by the proliferation of methods and techniques used to characterize different classes separately, compared with proteomics, where the spectrum of analytical methods might be smaller. Many different lipids can be generated by the same enzyme, making the biological investigation more complex.

AKB: Proteins consist of known amino acids and a number of post-translational modifications, whereas lipids have vastly diverse chemistries, with a large number of isobaric compounds complicating their characterization. They also cover an extensive dynamic range, from low-abundance signaling molecules to bulk lipids, further complicating their

comprehensive extraction. Plus, we still don't have good databases.

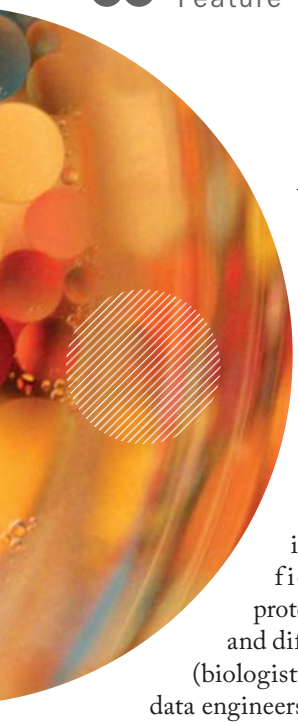
Interest in lipidomics has risen steeply in the last 20 years – why?

AKB: With the increasing focus on personalized health and preventive monitoring, there is a growing interest in lipids as indicators for the status of health and disease, prompting investments by manufacturers (instruments, medical devices), big data and service providers. Though this increasing attention is great for the overall awareness and growth of the field, we must be aware of immature solutions (such as lipid biomarker panels) that are marketed only to fulfill business interests.

FT: I believe the development of analytical technology used to measure lipids, especially mass spectrometry, has played a major role. Once researchers could accurately measure lipids, they began to realize how important they are in biology. The fact that many lipids are also bioactive molecules that can be used as disease treatments/biomarkers or as active components in nutritional interventions has also fueled business growth around lipidomics – which is positive for the field as it brings more possibilities, new ideas and new players. Perhaps our task as scientists in the field is to watch this growth closely, to avoid the false claims and promises that can arise when business takes over science…

ACG: I would concur with Federico here. We have been told for decades to avoid certain kinds of fats and favor others, but were these recommendations based on good science?

MW: As Federico says, lipidomics is an area of growth driven by technological advances. Better analytics inevitably leads



to new opportunities, be they in scientific discovery or applications. The latter part of this process – the translational aspects beyond descriptive biochemistry – will require considerable additional efforts and time to mature successfully.

What's next for your group?

FT: Higher throughput and more quantitative approaches, with integration of different fields (lipidomics, proteomics, genomics) and different expertise (biologists, physicians, data engineers).

AKB: I will drive our efforts towards clinical mass spec, through engagement of key stakeholders from lipidomics R&D, data integration and clinicians. We aim to establish clinical utility for select lipid markers, and work closely with the intended "end users," toward needs-driven assays for routine applications.

ACG: As Federico mentioned, our future lies in teaming up with other specialties. Whether it be a MS-based lab test for a specific disease, or a better understanding of how lipid metabolism affects health outcomes, we are definitely moving toward more pre-clinical and clinical work.

What analytical techniques will have the greatest impact over the next 10 years?

AKB: The currently available technologies are great tools for R&D applications because of their sensitivity, specificity and versatility. However, in clinical diagnostics, where reliability, turnaround time and of course costs are crucial, further development towards robustness, ease of use and automation are crucial.

FT: Instruments have reached an amazing level of resolution and sensitivity, but more time will have to be invested in separations (ion mobility is a very exciting application), robustness and data analytics.

ACG: Current analytical workflows are robust enough and identify lipids of interest with enough precision that we can hope to usefully roll them out to clinical application. However, new techniques that will enable us to discriminate between lipid isomers could potentially shed new light on specific diseases.

How do you expect to see our understanding of lipids evolve?

FT: I expect us to gain a little more understanding of the molecular aspects of life – this would be a giant step in scientific knowledge.

MW: Hopefully, we will gain better insights into how the oceans of different lipid molecules interact at the molecular as well as organismal level. The former will contribute to our fundamental understanding of membrane biology, the latter will give us a more fine-grained picture of lipid transport and metabolism relevant to medicine.

AKB: I would like to see the perception of lipids in health to shift from foe to friend, with an increasing awareness of how we can take specific actions to improve health and disease outcomes.

ACG: A better understanding of lipids will bring us better diagnostics and better health outcomes. But a better understanding of the chemistry of life in its own right is a worthy pursuit!

> *"I expect us to gain a little more understanding of the molecular aspects of life."*

Anne K Bendt is Associate Director of SLING and Principal Investigator at the Life Sciences Institute at the National University of Singapore.

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Federico Torta is Research Assistant Professor, SLING, Department of Biochemistry, YLL School of Medicine, National University of Singapore.

Markus Wenk is Director of SLING and Head, Department of Biochemistry at the National University of Singapore.

LIPIDOMIC HARMONY?

The Lipidomics Standards Initiative was initiated in early 2018 by Kim Ekroos and Gerhard Liebisch. What are its aims, and what will it bring to the table? Ekroos explains the grand plan.

We launched the Lipidomics Standards Initiative (LSI) because we saw that the number of studies reporting low-quality lipid data was increasing and felt there was a need to develop guidelines.

The aim of the initiative is to create a common standard for minimum acceptable data quality and reporting for lipidomics. We want to centralize lipidomics standards with the goal of guiding and stimulating users, reviewers, editors, and funders toward harmonized lipidomic outputs. The initiative covers the major lipidomic workflows, including sample collection, storage, as well as data deconvolution and reporting. It is a community-wide effort covering methodological progress based on the input of researchers in the field.

Why is finding a "common language" for lipid species reporting important? First of all, it provides a foundation for data comparability and exchange in both basic and clinical research, as well as for development of bioinformatics tools. Together with quality standards, this common language will improve our understanding of the functional role of lipid species. Moreover, common standards will set the cornerstones for the regulatory environment, which is necessary for applying lipidomic methods in diagnostics.

Already, we have 20 key opinion leaders as members, and we look forward to seeing our number grow. We are working together with the LIPID MAPS Lipidomics Gateway and both the Metabolomics and Proteomics Standards Initiatives – but we welcome interactions from other disciplines and initiatives. We believe the LSI will help the ultimate goal of discovering the true potential of lipidomics.

Kim Ekroos is Founder and CEO of Lipidomics Consulting Ltd, Esbo, Finland.

Lipidomics Standards Initiative (LSI): https://lipidomics-standards-initiative.org

Coordinators: Gerhard Liebisch – gerhard.liebisch@ukr.de, Kim Ekroos – kim@lipidomicsconsulting.com

SETTING THE STANDARD

Why does the field need standardization?

Michal Holčapek Despite the rapid growth in recent years, the field of lipidomics is still not "mature," and we are still missing generic guidelines. Lipidomic quantitation can be used for clinical cohorts, as illustrated by the literature; unfortunately, many papers do not follow basic requirements for analytical quantitation, such as the use of internal standards, method validation, quality control, cross-validation, and so on. The result? A chaotic situation whereby published results cannot be reproduced by other groups.

Federico Torta As with all new fields, issues around standardization have manifested themselves as the area has become more established, generated by a demand from the scientific and clinical community to be able to compare different studies confidently and to increase reproducibility.

Anne K Bendt Standardization – or at least harmonization – of analytics is crucial to cost efficiency. Multiple international organizations are active in standardization efforts, including the Centers for Disease Control (CDC), offering schemes for standardization of LC-MS-based analysis of cholesterols and triacylglycerides. Various working groups within the European Federation for Laboratory Medicine (EFLM) are leading international harmonization efforts for clinically relevant molecules. Standardization of data curation, interpretation and reporting as well as linking results to electronic medical records (EMR), international biobanks and making use of artificial intelligence for lipidomic datasets are also critical endeavors.

Amaury Cazenace Gassiot With so many variations between and within lipidomics workflows (one lab = one method) and specific issues, such as the lack of adequate internal standards, we are faced with a challenge: can we, as a community, deliver consistent and useful data based on agreed guidelines?

Matej Orešič Harmonization of reporting lipidomics experiments and developing guidelines for good practices is an important goal, and recent consensus publication (1) was an important step in this direction. In regards to standardization, the question is what is meant by this term. There is already the Metabolomics Standards Initiative (MSI), which also, in principle, includes lipids. Lipidomics data deposition to public databases such as MetaboLights, which is supported by many journals, has to follow MSI. Nevertheless, there is room for improving MSI from the lipids perspective, and the lipidomics community would have the greatest impact if it joined forces with MSI. This would also be the best way to develop practical tools needed to implement standardization in the field.

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Perfecting Proteomic Separations

An Innovation Award-winning micro-chip chromatography "column," µPAC™ promises high-resolution separations of complex biological samples. Kris Gevaert and Francis Impens, from Ghent University and the VIB Proteomics Core, embedded in the VIB-UGent Center for Medical Biotechnology, have been trialing the new column. We caught up with them to find out more about their work, and how µPAC can expedite routine analysis.

How did you get involved in proteomics research?

Kris: I did my Master's thesis in a proteomics lab in the mid-1990s, and have been in the field ever since. I became a professor at Ghent University in 2004 and a group leader at VIB in 2005. Proteomic technologies developed in our lab are made available to other researchers via our VIB Proteomics Core.

Francis: I'm a biomedical scientist by training. It was Kris who introduced me to proteomics – I did my PhD thesis in his lab. After a stint at the Pasteur Institute in Paris, working on bacterial pathogens, I returned to Ghent University and VIB in 2015. I manage the proteomics core facility – a spin-out from Kris' lab. I also have a small research group "on the side," which focuses on host–bacteria interactions using proteomics technologies.

What motivates you?

Francis: For me, it's the strong belief that this technology is enabling real discoveries, as we witness on a weekly basis. There's still so much to discover – novel interaction partners, novel protein modifications, novel biology!

Kris: I also enjoy the opportunity to work with scientists in a variety of disciplines, from plant biologists to clinical scientists.

What recent trends have you noticed in the daily routine of the proteomics center?

Francis: Proteomics technology as a whole has matured, with a very rapid evolution of mass spectrometry instruments, separation instruments, and supporting technologies. It's a very different landscape compared with just a decade ago. As a consequence, some proteomic analyses (such as shotgun analysis for biological samples and mapping protein interaction after affinity purification) have become routine. Of course, there are still many novel kinds of analyses, which are still very much dependent on the expertise of single labs or even single people.

Kris: There are certainly some exciting new techniques that allow us to focus on aspects of a protein that we could not study some years ago. In my lab, we invent or trial such techniques to master them and then transfer them back to our Proteomics Core as a new tool for the wider user community.

What are the biggest limitations in proteomics?

Francis: With shotgun analysis, we can only routinely measure about half of the proteins expressed in the average mammalian cell. And that is not taking into account the millions of different proteoforms that could also exist. The question is: do they all have meaning? To me though, it's not a limitation – it's a challenge.

Will the resolving power of mass spectrometers eventually eliminate the need for separation technology?

Kris: The short and simple answer is no. We will always need resolving power, be it LC or other types of chromatography. It is simple mathematics: there could be hundreds of thousands of different peptides in a sample – and it's impossible to identify that number of molecules without separations.

Can improvements in separation technology help overcome the challenges facing proteomics?

Francis: In my view, improving separations is key. Mass spectrometry has seen huge advances over the past 10 years, for example, Orbitrap technology and hybrid machines. To help mass spectrometry reach its full potential, we need to see advances in fractionation too – smaller peaks, more resolution, and higher peak capacities can only increase the overall performance of the analysis. To that end, we have been excited to trial the new micro-pillar array chromatography (µPAC) columns from Pharmafluidics.

How can µPAC technology improve proteomic analyses?

Francis: Personally, I think it has the potential to revolutionize the field of mass spectrometry-based proteomics. It is the type of technology that speaks for itself – you can look inside the column, see the perfectly ordered structure, and understand exactly how it increases separation power.

Kris: In comparison with traditional columns, µPAC columns result in more sensitive analysis, so you need less material to identify the same number of proteins. Another positive of the µPAC columns is the

"We're looking forward to finding out what else we can do that we haven't been able to do before!"

ease of use – it's very quick and efficient to put a µPAC column in front of your mass spectrometer. The back pressure is very low, meaning that you can work with long columns that allow greater sensitivity.

The µPAC columns have been designed by scientists who have been in the field of chromatography for decades, so we felt confident in agreeing to collaborate with them to implement the technology.

How have you applied µPAC technology?

Kris: So far, we have been evaluating them for routine shotgun analysis, where we try to gain a total protein profile. Compared with traditional columns, we see an overall increase in identifications, both on the peptide and the protein level, which results from the improved LC parameters that you can readily see just by looking at the chromatograms.

Beyond routine analyses, we are excited to try out µPAC technology as part of a wide range of proteomic methods. These columns have already made it possible to do incredibly long runs of eight or ten hours – something that was previous unheard of. We're looking forward to finding out what else we can do that we haven't been able to do before!

Will you be implementing µPAC into daily operation?

Kris: Yes, that is on the horizon. We are just waiting for some final aspects, such as a compatible trapping column, but we hope it won't take longer than a few weeks.

In the longer term, where do you expect µPAC technology to have the greatest impact?

Francis: One of the biggest gains the company reports is in analyzing minute amounts of sample – µPAC boosts the performance of the mass spectrometer by keeping peptide concentrations undiluted and high prior to ionization. This particular aspect could be a game changer for analysis of small samples; for example, in biomarker discovery.

ALL THE TEA IN CHINA

How we applied advanced software to unravel the impact of climate changes on the complex chemistry of an ancient crop.

By Albert Robbat, Jr., Tufts University Sensory and Science Center, Chemistry Department, Medford, MA, USA.

The primary motivation of our group is to explore the complex interactions that occur between human and natural systems – including environmental contamination, pesticides and petroleum products. Recently, we have used tea as a model system to learn how small and extreme changes in climate affect plant chemistry. Studies to date have mainly focused on yield, with very limited attention given to impact of climate variability on flavor, aroma and functional quality. Tea is an ideal crop system for climate or variability studies since it's harvested several times throughout the year. Tea is also an economically significant crop, with 6 million tons produced in 2017 – worth $39 billion.

How and why may tea quality become vulnerable to changing climate conditions? And what would be the impact on consumer purchasing decisions, markets, farmer livelihoods, ecological knowledge and management practices? The objective of our research team – which consists of graduate students, postdocs, and faculty from Tufts University, Montana State University and University of Florida – is to provide quantitative data to answer these questions.

Teatime tech

Tea is a complex sample containing thousands of compounds. To investigate natural system interactions, we knew we needed to analyze hundreds of samples both in the field and in the lab. We also knew we needed to track the changes in plant metabolites over several years. Our challenge was to find analytical tools that were up to the task.

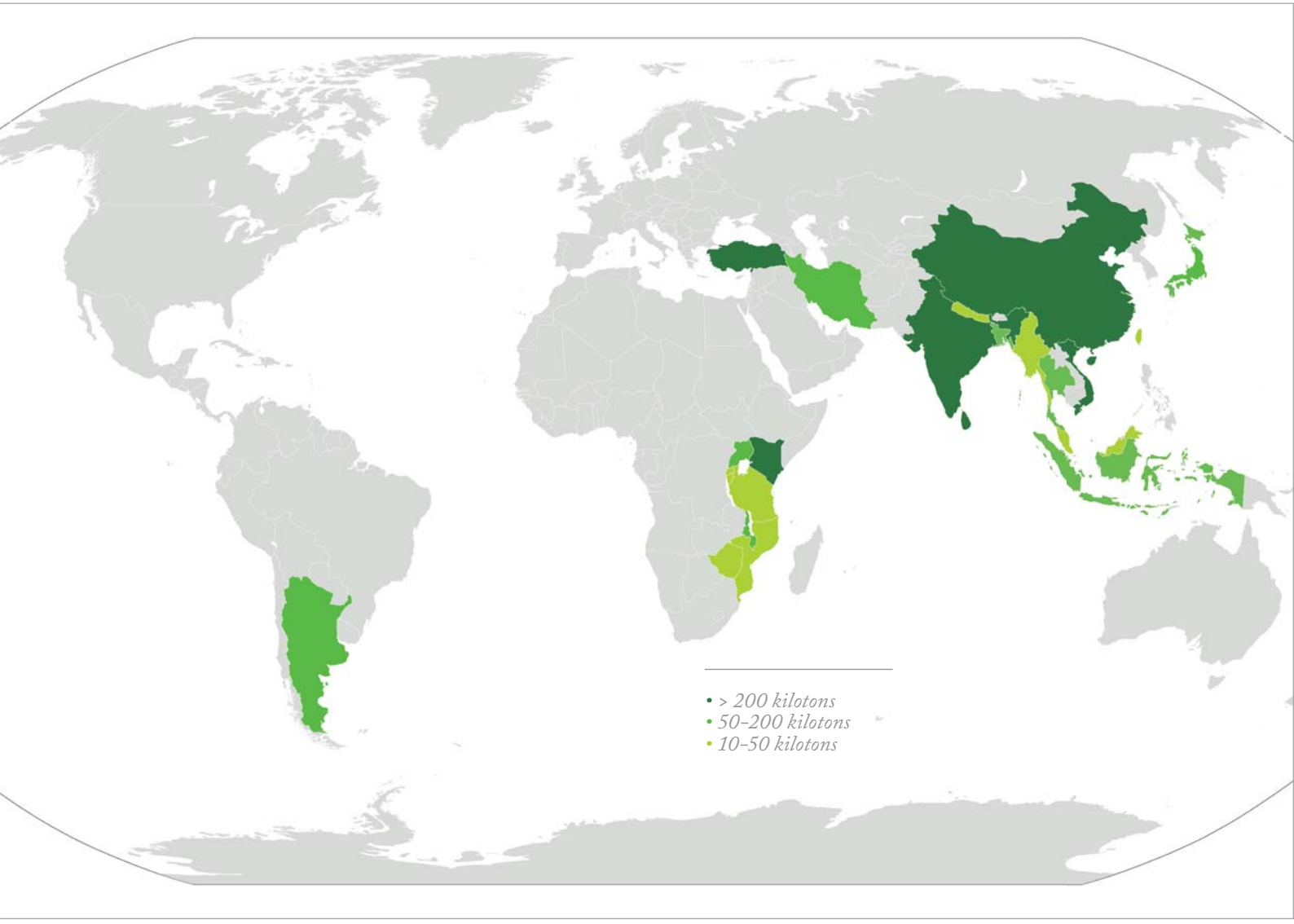
First we looked at sampling, and investigated static and dynamic headspace, SPME, and others, before settling on stir bar sorptive extraction. By placing a small, inexpensive magnet on one side of the leaf and a Twister on the other, we can collect dozens of samples from many locations in the same day to investigate plant–climate and/or plant–herbivore interactions. By using the same sampling technology to sample brewed tea, we can compare direct-sampling leaf, unprocessed "environmental" and "processed" green tea infusions.

GC/MS is universally used to analyze the volatile fraction of tea. Most investigators focus on just 50–100 compounds; in contrast, we study the total metabolome using advanced automated, sequential 2D GC/MS. In the past, GC-GC/MS has been used to identify specific compounds of interest by transferring small fractions of the sample from the first to the second column, but because of long runtimes and data analysis times, GC-GC/MS is not routinely used to identify all components in a complex sample. For example, if one-minute sample portions are analyzed and the separation time on each column is one hour, it will take four days to analyze one sample. Analysis of the fortieth sample portion requires 41 minutes on the first column and 60 minutes on the second column, as well as equilibration time before the 41st sample portion can be analyzed. Despite the long analysis time, we have shown that a wax (first column) peak that appears to be the product of a single high concentration analyte can actually mask (due to coelution) as many as 25 compounds on a DB-5 (second column) stationary phase. The same is true when the columns are reversed. Such detailed metabolomic profiling necessitated the development of new data analysis software to automate the library-building process.

> "TO INVESTIGATE NATURAL SYSTEM INTERACTIONS, WE KNEW WE NEEDED TO ANALYZE HUNDREDS OF SAMPLES BOTH IN THE FIELD AND IN THE LAB."

Data day

Our Ion Analytics software (sold by Gerstel, Germany) automatically inspects each peak to ensure that the mass spectrum at each peak scan is constant. If so, the software annotates the database by importing the compound's retention time, mass spectrum, 3–5 ions and relative abundances, identity and CAS number, if available. Sample-specific information such as sample identifiers, sensory or nutraceutical properties can also be added to the database. If not, the software identifies 3–5 constant peak spectra, averages them, and then subtracts the spectrum from the total ion current signal at each peak scan. If two compounds coelute, the software inputs the information above into the



Major tea-producing countries 2012-2013. Source: Paju, Creative Commons.

database. If compound information cannot be assigned, a numerical identifier is applied. If more than two compounds coelute, the process continues until the residual signal approximates background noise.

Initially, we developed the software to do target analysis of pollutants in the field. Because the sample matrix was typically crude oil, petroleum or coal tar, I developed spectral deconvolution software to quantify target compounds in the presence of concentrated matrixes.

Ion Analytics software differs from other spectral deconvolution software because it eliminates additive ion effects from coeluting compounds, which makes concentration measurements accurate. The deconvolution software is used in the metabolomic library-building process, along with MS subtraction algorithms, to process the data files produced by GC-GC/MS analyses.

We have already demonstrated the accuracy of the software when analyzing EPA-targeted pollutants by GC×GC/MS, and work is now in progress to assess if the software offers advantages when GC×GC/MS is used to produce detailed metabolomic profiles. Current software is inefficient, so most investigators use non-targeted analysis to discern differences in samples, but these

"CLEARLY, CLIMATE CONDITIONS HAVE A DRAMATIC IMPACT ON THE CHEMISTRY OF TEA."

software platforms bin ions, which often result in thousands of molecular features. It's common for researchers presenting at conferences to begin by stating the number of features, then quickly move on to discuss the 20–30 compounds they use to present results. In our work we use compounds (based on their clean spectra) to discern differences.

High tea?

It is well known that the quality of tea is affected by climate. For example, tea from Yunnan Province, China, experiences extreme rainfall effects due to the onset of the East Asian monsoon rains between spring (a few mm) and summer (hundreds of mm). Consumers know that tea harvested from Yunnan Province in the summer is poor in quality, with buyers paying $0.10 on the $ compared to spring tea. What we didn't know, however, was how much the tea metabolome changed. That was a question we were able to answer – our research revealed that more than half of the ~400 volatile tea metabolites changed in concentration with heavy rainfall; some concentrations are 100 to 1000-times higher in spring tea, others much higher in summer tea. These striking differences in concentration include compounds that impact on the taste and/or health properties of the tea.

Yunnan also served as an ideal location to test temperature effects – plants grown in the same farm on the same mountain can differ in elevation by up to 1000 m – we produced the first quantitative data documenting how volatile plant metabolite concentrations differ with temperature. We showed that a 5 °C difference in temperature between plants grown at 600 and 1500 m yielded striking differences in metabolites, with concentrations of some compounds increasing or decreasing by hundreds of percent. High elevation tea has a sweeter, more floral taste compared to low elevation tea, which tastes grassy and barnyard-like. We discovered that low

"OUR HIGHLY TRAINED SENSORY PANEL WILL WORK WITH CANCER PATIENTS, CULINARY CHEFS AND NUTRITIONISTS TO IDENTIFY PLANT-BASED FOODS THEY BOTH LIKE AND WILL EAT."

elevation tea contained most of the health beneficial compounds found in high elevation tea, but high elevation tea contained nutraceuticals not found in low elevation tea.

Finally, we studied the effects of monsoon season on total phenolics and antioxidant potential. We learned that catechin (antioxidants and polyphenolics) concentrations decreased significantly within the first ten days of the heavy rains, but that total phenolics and antioxidant potential increased. Once the summer rains fell heavily, however, the plant seems to adjust to this stress factor, since catechin concentrations differed little between seasons.

Clearly, climate conditions have a dramatic impact on the chemistry of tea. Soon we will submit our findings from Yunnan and Fujian in China for publication. But previous studies on tea grown in South Carolina suggest that location has most impact, followed by rainfall and then temperature.

Left: Nonvolatile metabolites provide taste (e.g. bitter) and color. Right: Volatile metabolites provide aroma (sweet, floral, green, etc).

In Yunnan, the Tea Research Institute is already harvesting tea from plants that consumers dislike and extracting health beneficial compounds for commercial application. This offers the farmer an opportunity to obtain a higher price and/or sell leaves for medicinal purposes.

Beyond tea

The software we developed can be applied to any GC/MS (including GC-GC/MS and GC×GC/MS) analysis. Outside of the realms of tea, we at TUSSC are interested in helping cancer patients who have experienced changes in their ability to smell and taste, which often results in patients losing interest in eating and suffering from malnutrition. According to the Academy of Nutrition and Dietetics, current recommendations for patients suffering from altered sense of smell and taste is insufficient. Using a sensory-directed chemical approach, we plan on investigating what cancer patients with compromised sensory abilities can and cannot smell, and what they like and dislike. Our highly trained sensory panel will work with cancer patients, culinary chefs and nutritionists to identify plant-based foods they both like and will eat. We will then analyze the foods using the techniques described above, and olfactometry, to identify the relevant flavors and odors.

The same approach could be used to assess authenticity and purity of raw ingredients and final products, especially if sensory-active compounds lead to brand quality and equity. The emerging cannabis market would certainly benefit from this type of analysis, especially as it relates to product purity and potency.

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Your Efficiency Challenge – Part II

Since late 2017, we have been collecting your responses to a survey on laboratory efficiency and liquid separations – kicking off a special project: "Your Efficiency Challenge." Here, we present select results, consult with our efficiency experts – and introduce "The Road to Improved Efficiency" – an unmissable video webinar master class. It's time to challenge yourself!

Only 36 percent of respondents adopt the latest methodology and exploit state-of-the-art performance in LC. Lack of consideration or lack of foresight?

Udo Huber, Director LC Application Solutions, Agilent Technologies…

On analytical efficiency:
People started to think about analytical efficiency when Agilent introduced the first sub-2-micron columns in 2003 – kick-starting the UHPLC age. Suddenly, columns and instruments were available that allowed the same results with much shorter run times. However, although UHPLC can reduce run times to a few minutes, for many labs a more important benefit was the increased plate number or peak capacity. High peak capacity, especially in combination with orthogonal separation techniques like CE or SFC, gives scientists confidence that they are seeing all compounds in their samples, which is important for customers in all industries. Missing an unknown impurity in an API in the pharmaceutical industry, for example, could have a major impact on patient safety. Of course, detector sensitivity and dynamic range are also important aspects of analytical efficiency, but I think increased peak capacity is not only the biggest improvement so far, but also the area where major gains can still be made. The next step in this direction is 2D-LC, which gives peak capacities that can never be achieved by UHPLC.

On the survey result:
Does it surprise me that around a third of respondents use state-of-the-art methods? Yes and no. On one hand, increasing analytical efficiency should be beneficial for almost every analysis, whether it is increased peak capacity, better sensitivity, more dynamic detector range or lower carryover, to name a few. On the other hand, scientists cannot always make use of these possibilities because the method is validated and cannot be changed without significant effort for re-validation. Even for unregulated methods, scientists are reluctant to change a proven method. So, if there is no significant thread, like for example the acetonitrile shortage several years ago, scientists do not take the risk of changing methods or workflows.

Kelly Zhang, Principal Scientist, Genentech…

On analytical efficiency:
To me and my team, analytical efficiency means the amount of useful information we can obtain per time unit, per mg of sample, per manpower, and per dollar (or put other way: efficiency = useful information/second/mg/person/$).

Efficiency is very important to us, as our goal is to deliver first-in-class and best-in-class medicines to patients in the shortest possible time.

In the case of liquid phase separations, there are many ways to gain analytical efficiency, with faster and universal methods, automation, and intelligent data analysis. When it comes to method speed, it's not only how fast a method run time is, but also how fast one can develop that method. Method development can take significant time. Furthermore, to characterize one sample, many analytical instruments and methods may be needed, which significantly reduces analytical efficiency. One way we approach boosting analytical efficiency is by using multi-dimensional UHPLC with hyphenated universal detectors – with one sample injection, we can obtain information for multiple critical attributes.

On the survey result:
If you're involved in research, you're more likely to be in the quoted 36 percent – using the latest technology. But when it comes to the regulated environment of clinical drug development and the commercial stage, there is an inevitable delay in implementing the latest technology. For example, if you want to apply a new technology to drug analysis and file it in an IND or NDA dossier, there may be questions about whether the FDA will accept it, which could be considered a significant risk.

76 percent of laboratory analysts already work in a challenging multi-user, multi-method environment. Are your current processes and systems enabling smooth workflows?

Stéphane Dubant, Product Specialist Liquid Chromatography, Agilent Technologies…

On instrument/software efficiency:
Many analytical laboratories using LC are "service-providers" for internal or external customers. In this environment, it is critical to have the most efficient lab possible – and instrumentation and software efficiencies are an important contributor. Hardware features that give increased robustness or flexibility (broader use-range) to an instrument can have a significant difference in your day-to-day work. For example, dedicating a simple system to one application is ideal if you have a

high volume of a single analysis, but if you keep changing methods all the time then column/solvent selection becomes an asset. Often, instrument downtime could be avoided by good laboratory practices.

Another thing we often hear about from customers is software inefficiency; in particular, the manual inputs required (for example, sample list parameters). There are tools, such as barcodes or LIMS interfaces that can speed up the process, but there is room for improvement.

On the survey result:
Companies are minimizing their footprints by having the same lab cover as many analytical aspects as possible (and outsource what is not possible or cost-effective to do internally). I expect that workload will continue to increase and staff numbers to decrease as these cost pressures intensify.

In the multi-user, multi-method environment, hiring the right people is paramount. Robust and simple instruments/software are important but the results are only as good as what you ask the instrument to do. I believe proper training on instrumentation, software and good laboratory practices can have a significant impact.

Laboratory management is considered a 'service function' by 50 percent of respondents. One in three laboratory managers already feel the threat of the competition – what about you?!

Wolfgang Kreiss, Independent Consultant, Germany…

On laboratory efficiency:
When we think about laboratory efficiency, we tend to focus on speed (for example, number of analytical results per time) and costs (for example, number of analytical results per cost). For analytical projects, costs are closely related to the time taken, so the speed of analysis is a good indicator of the efficiency of an analytical lab. Even when increasing speed incurs greater expense, in my experience, the majority of customers will go for the faster analysis. Thus, for many laboratories, speed is the most important factor governing laboratory competitiveness.

Some laboratories are focused on improving quality criteria, such as sensitivity, precision or validity of the results; for example, in research environments. When it comes to assessing efficiency in such cases, laboratory managers can modify efficiency metrics by relating the numerical results for the quality criteria to their operational costs or the analysis time.

On the survey result:
For day-to-day analyses that follow well defined workflows, lab management is a service function, but beyond such daily routine, laboratory management includes long-term planning and the shaping of a lab's strategy. Successful lab strategies will depend on an appropriate understanding of the future development of technology and analytical instrumentation, as well as on the correct assessment of future customers' requirements. In my experience, most lab managers today are feeling the pressure of competition, so developing adequate strategies will be crucial for the long-term success of their laboratories.

Oliver Rodewyk, Account Manager Strategic Accounts, Agilent Technologies...

On laboratory efficiency:
Cost-saving initiatives are the typical starting points for lab efficiency projects, but there is never a one-size-fits-all solution, taking into account equipment usage, know-how, transition plan and flexibility to new techniques as well as budgetary plan. Rather than cost-saving, the more forward-looking approach is to concentrate on cost-effectiveness. A lab manager can only boost efficiency if they understand four key issues: the workflow dependencies in the lab, utilization of equipment, target service level and lifecycle management.

On the survey result:
"Service" is linked to cost, systems are linked to investments. Lab management includes the whole lifecycle, not just of equipment and services but of the whole lab.

Utilization and flexibility of adapting new requirements or techniques must be combined with a clear view for the next 3–5 years. Lab managers must regularly ask themselves:

- Is this technique or equipment an opportunity or a burden?
- Is my sample throughput fixed for upcoming years?
- How will my lab handle changes?
- Should any aspect be outsourced?

The Road to Improved Efficiency

Are you a scientist, laboratory analyst or lab manager – or all of the above? Are you willing to challenge your perception of efficiency or do you already know you need to make efficiency gains?

Join our experts – Kelly Zhang, Roman Szucs, Udo Huber, Stéphane Dubant, Wolfgang Kreiss and Oliver Rodewyk – for an exclusive series of video webinar masterclasses:

Webinar 1 – Analytical efficiency: How to push your results to the next level by selecting suitable technology

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Webinar 3 – Laboratory efficiency: How to plan for success and secure your future

For more details, visit: http://tas.txp.to/0918/YEC

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Those Who Can, Teach: Janina Kneipp and Katharina Schultens

In part three of our series on analytical science education, we talk to one of the professors and the managing director of the School of Analytical Sciences Adlershof (SALSA), about their multidisciplinary teaching program – which combines analytical sciences with data analysis, statistics, risk assessment, and science communication.

SALSA began in November 2012 as a graduate school funded as part of the German Excellence Initiative, and currently has 52 doctoral fellows working on co-supervised research projects. So far, 22 students have graduated from the program and 20 more are about to graduate; plus, there are several postdoctoral fellows completing short- and longer-term research projects within the SALSA framework. The school's international partners include ETH Zurich in Switzerland, Hebrew University of Jerusalem in Rehovot, Israel, and Universidad de Oviedo, Spain.

Janina Kneipp is a co-founder and one of the two co-speakers of SALSA and a professor of Physical Chemistry at Humboldt Universität, Berlin. Katharina Schultens is managing director of SALSA.

What is the aim of the SALSA project?

As part of the SALSA project, we focus strongly on collaboration and development of a common "language" at the interface between chemistry, physics, and the life sciences. Our main motivation is to educate excellent, highly specialized young researchers who think outside the box. We want to instil a sense of the many different problems that analytical scientists are required to solve – today and in the future.

SALSA's administrative structure and its communication culture have shaped graduate education in the natural sciences at our university and with our German university and non-university partners. Our university has committed to fund the project structure further until at least 2022. Of course, the project can only be kept alive if interesting research is carried out. Therefore, we recently started an initiative called "Make & Measure," which will bring together scientists on the campus and SALSA's old and new academic partners to secure funding for new collaborative research for analytical science graduate students.

Why do students choose SALSA?

Along with the funding of a collaborative research project, SALSA provides both a curricular framework and a social environment. Compared with other graduate schools or similar structured doctoral programs, SALSA probably stands out because it does not focus on a single research area. Instead, we focus on analytical problems that can be found virtually everywhere in science – and anybody who is willing to think about their research from a problem-solving perspective is welcome. We have therefore attracted extremely diverse cohorts of graduate students over the years. In an extremely competitive application process, only genuinely motivated students can succeed – and curiosity is an important driving force for them. Several students – particularly female fellows – also explicitly state that they want to achieve a degree in the analytical sciences because they want the chance to shape policy and decision making in their home countries, so that they can have a different life to that of previous generations.

Have you noticed a difference in the motivation of students today?

"Our main motivation is to educate excellent, highly specialized young researchers who think outside the box."

Not in terms of curiosity or interest in fundamental scientific questions, but perhaps more due to differences in (scientific) cultures. Culturally, SALSA could be considered a melting pot! In addition to different parts of the world, SALSA students also come from different natural science subjects. As an example, the motivations of chemists going for a doctoral degree have – on average – always deviated a bit from those of physicists or biologists, at least in the German system.

What is the best approach to teaching analytical chemistry?

Analytical sciences require problem-oriented thinking, in addition to substantial knowledge of instrumentation, data analysis, and, of course, basic chemistry. To drive progress in analytical science, we need input from those in other disciplines – and the same can be said for teaching analytical chemistry.

We have established several unique but successful teaching and learning formats. For example, we host sessions prepared by the more advanced doctoral fellows, who share the approaches and methods they are most familiar with and explain them to their fellow students. Such sessions, in particular, see the "teachers" and students working in groups to solve analytical problems. They also invite experts to give short lectures on specific aspects of the topic. We find that our fellows really benefit from working in groups and taking on an active role in mutual teaching.

How has technology changed the teaching of analytical chemistry?

We still teach face-to-face a lot, and student-to-student teaching is invaluable in graduate education. Of course, we try to gain as much use from online material and learning as we can. For example, organization and scientific preparation of SALSA's annual "Make & Measure" event is managed by groups of fellows, some of them located at different universities – including our international partners. For this, the online exchange of data, video teaching, and discussions are crucial.

What skills do your students have when starting your course?

The students entering SALSA come from extremely diverse scientific backgrounds. Some students come with an excellent ability to tackle the most diverse kinds of questions, some have gone through an extremely specialized education and still need to develop those skills – but SALSA provides ample opportunity for development with a structured program emphasizing and developing problem-solving abilities in a case-based curriculum.

Left to right: Katharina Schultens, Janina Kneipp and co-speaker Ulrich Panne.

Esther Santel

What advantages does the current generation have over those who have gone before?

The current generation is extremely skilled in gathering information from very diverse sources and then quickly, even informally, distributing that information. Such working habits could change the way we present our data, moving towards a real "open source" culture. The Internet has made gathering information much easier than it was for previous generations. On the other hand, taking a broader perspective and checking whether new ideas have been published in "really old" papers from the 1960s and 1970s takes a lot of persistence, sometimes courage. It is our responsibility as teachers to help them set their filters and develop the courage to assess the novelty, originality, and potential impact of their own scientific result.

What is your biggest challenge in teaching analytical chemistry?

To not teach it in a traditional way, but to convey that all kinds of chemistry and many aspects of physics and data analysis are needed to push the limits of current analytical chemistry. At the graduate level, students must look beyond their particular research project. But as teachers we need to convey much earlier, at the undergraduate level, how essential it is to gain deep understanding of concepts from different fields, if one wants to become a "real" analytical scientist.

What do you spend most of your teaching time focusing on?

We find our students have different ideas about "measuring" things. We spend a lot of time discussing the fact that analytical science starts with a sample, and fighting the misconception that they are just an application of existing "techniques" to mostly known samples. We believe we do a great job in preparing our fellows by having them work in projects supervised by two different but complementary supervisors, and by arranging meetings with renowned scientists and remarkable personalities from the international analytical sciences community.

What instrumentation do you use in your teaching labs?

It is certainly not your typical chemistry lab equipment – progress in analytical sciences means progress in markers, probes, and materials, as well as advances in measurement capabilities. We use our labs for teaching both basic and advanced courses in mass

spectrometry, optical spectroscopy, or characterization of specific materials, to name some examples. The funding we received enabled us to set up two so-called application labs run by SALSA, one in a brand new lab building provided by Humboldt-Universität zu Berlin. The Application Lab Photonics, established by our junior research group leader Zsuzsanna Heiner, has recently become home to novel instrumentation for vibrational sum-frequency generation spectroscopy that can be considered unique worldwide. Over the years, we have acquired equipment for different imaging methods, ranging from Raman microscopy to mass spectrometry imaging.

Furthermore, collaboration with university and non-university partners enables us to incorporate unique measurement capabilities; for example, the synchrotron HZB-BESSY, which includes brilliant X-ray sources for applications ranging from molecular structure analysis to X-ray tomography.

How can technology further support (analytical science) education?

Technology provides unique possibilities in education: for several semesters, we have made extensive use of an excellent class taught via video by some of SALSA's PIs (located at ETH Zurich). However, I still think that personal interactions surpass any other teaching format – so our aim should be to make these interactions more efficient. Of course, in some cases, new technologies will help us achieve this.

What are your students' prospects?

Naturally, the students are very much focused on life after the classroom! So far, our graduates have pursued a diverse range of careers after completing their studies. Many of them have gone for postdocs in Germany and abroad, some working in industry, and others in science management and administration.

Toward Better Biotherapeutics

When digging into the inherent complexity of antibody-drug conjugates, choosing the right chromatography column is key. We spoke with the University of Geneva's Alexandre Goyon about the evolution of UHPLC, and how to streamline separations for complex biotherapeutics.

How did you get into analytical chemistry?

My first experience with analytical chemistry instrumentation was during my Master's degree in Philip Jessop's lab at Queen's University, Canada. I knew straight away that it was an area I'd like to explore more. I joined the Nestlé Research Centre and spent two years developing and validating LC-MS/MS methods for determination of chemical contaminants in food materials. Next, I joined the pharmaceutical analysis lab in Geneva for my PhD, working with three well-known scientists – Jean-Luc Veuthey, Davy Guillarme and Szabolcs Fekete. While there, I used different liquid chromatography and capillary electrophoresis approaches for biopharmaceutical analysis.

What keeps you moving forwards?

It's easy to be motivated about improving therapies for cancer patients! Of course, my research is a long way from the patient, which is one reason why I wanted to spend time in biopharmaceutical companies. There, I can see first-hand the impact of analytical chemistry on drug discovery, development and manufacture.

Your work has focused on antibody–drug conjugates (ADCs). Why is analysis of these biotherapeutics particularly challenging?

Antibody–drug conjugates (ADCs) combine a lipophilic drug with a monoclonal antibody, meaning that the hydrophobicity of an ADC is much more pronounced than that of an unconjugated antibody. In theory, SEC separates different species based on their size, but in practice the picture is complicated by a range of non-specific interactions, including hydrophobic interactions.

To reduce hydrophobic interactions, biopharma scientists typically include isopropanol in the mobile phase, but this introduces two drawbacks: i) you may no longer be working under native conditions and ii) isopropanol has been found to have deleterious effects on some antibodies.

How have recent innovations in SEC columns helped tackle the complexities of ADC analysis?

The ability of the previous generation of ultrahigh pressure SEC columns to limit non-specific hydrophobic interactions with little or no isopropanol has helped to establish the validity of these analyses.

In addition, most ultrahigh pressure columns are packed with sub-micron particles, which has allowed for smaller columns and faster separations. You can now do in a 4.6 mm ID, 150 mm length column what used to require 7.8 mm ID and 300 mm length, and a separation that used to take 45 mins can be completed in 10 mins. Such improvements start to look attractive in other areas of biopharma development, such as process control.

I would go as far to say that, when it comes to ADC analysis, the biggest advances over the last five years have been seen in SEC columns.

You published an article in the Journal of Chromatography A this year on "Extending the limits of SEC" (1) – what was your aim?

A critical quality attribute of ADC products is the amount of free payload in solution. Typically, the payload molecule is cytotoxic, so you don't want it being released before the ADC reaches its target. Establishing the free payload content of a candidate drug in solution by SEC alone can be challenging. During SEC of an ADC, the high molecular weight species (whole ADC, monoclonal antibody, etc) will emerge first, followed by smaller molecules (payload, linker, etc). The smallest molecules all elute as a single band, making it impossible to accurately quantify the payload. Previously, researchers had tried a 2D-LC method – coupling SEC to reverse-phase chromatography to separate the smaller molecules – but we were able to find a faster, more streamlined method.

How did you develop the new method?

With colleagues at the University of Geneva and a major pharmaceutical company located in Basel, I was conducting experiments using an ultrahigh pressure SEC column (Tosoh). We found that the payload species were highly hydrophobic, and were therefore absorbed onto the stationary phase. It gave us an idea: why not try a two-part separation using the same SEC column? We allowed the largest proteins to elute, then applied an acetonitrile gradient to elute the smaller molecules. We were pleasantly surprised to find that, thanks to

secondary hydrophobic interactions, the acetonitrile gradient allowed separation of the smaller molecules. For some ADCs, this meant we were able to quantify both the high molecular weight species and the free payload in a single run – in under 10 mins. By taking advantage of (usually undesirable) hydrophobic interactions, the streamlined method could allow R&D scientists to quickly rule out ADC candidates that release payload in solution. It's a great example of how analytical chemistry can help in the development of new biotherapeutics.

What technology is likely to have the biggest impact in biopharma – now and in the future?

Right now, the most important trend is towards multidimensional LC, as demonstrated by the large number of presentations on the topic at HPLC 2018 in Washington. Most vendors now sell at least some 2D-LC-specific solutions, although software development for these systems has lagged behind somewhat. I believe that over the next five years, most companies will be using multi-dimensional LC approaches, coupled to high-resolution MS instruments.

Looking ahead 10 years, I think we will see separations going beyond two dimensions and into three, four or five dimensions. In parallel, I hope that suppliers will be able to improve column chemistry even further, in particular to reduce non-specific interactions in SEC and allow it to be coupled directly to MS.

Pharma is (rightly) a cautious industry and, before any new technology is adopted, companies and regulators must be sure it won't cause unexpected issues. Nevertheless, despite the technical and regulatory challenges, all the company scientists I have worked with have been very open to new technology.

Reference

1. *A Goyon et al., J Chromatogr A, 1539, 19-29 (2018).*

Keeping Apace with Biopharma Trends

Regina Roemling, Senior Marketing Manager, Separations at Tosoh Bioscience GmbH, Germany fills us in on the company's latest innovations for the biopharma market.

What challenges do vendors face in developing products for biopharmaceutical applications?

The biopharmaceutical industry is highly regulated and established methods are not easily replaced. Consequently, products such as chromatographic resins used in manufacturing or columns applied in QC of an approved biologic have to maintain a consistent quality over a very long period. On the other hand, the development time for new analytical tools needs to be reduced to cope with the increasingly rapid development of new, complex biopharmaceuticals. Looking just at therapeutic antibodies, there is a huge range, from antibody–drug conjugates (ADCs) to small, single-chain variable fragments (scFv). Analytics need to keep pace with this variety.

What do biopharma customers want?

R&D labs dream of multidimensional analytical platforms allowing the thorough characterization of tiny amounts of candidate molecules overnight. In production, throughput is of higher importance, but robustness and reproducibility are essential too.

What trends in biopharma analysis have you seen in recent years?

As Alexandre describes, hyphenation and multidimensional chromatography have been amongst the hottest topics at recent HPLC meetings. Another trend is the increasing use of affinity-based separations, not only in purification but also in HPLC analysis. A good example is Protein A affinity, which can be applied for fast analysis of cell titers or as a kind of sample pretreatment of a crude feedstock in process analytics when it's coupled with techniques like size exclusion chromatography (SEC) for monitoring aggregate contents. In fact, we will soon launch a new affinity column, which is designed to analyze the antibody-dependent cell mediated cytotoxicity (ADCC) activity of antibodies with high selectivity and reproducibility.

Why develop a column for measuring ADCC activity?

ADCC plays an important part in the mechanism of action of therapeutic antibodies, particularly cancer-targeting mAbs. When developing an antibody for cancer therapies it is extremely important to select a clone delivering the required ADCC activity, but current methods (in vitro cell-based assays or surface plasmon resonance techniques) are less reproducible than chromatography. In addition, there are many more potential applications for a fast and highly reproducible HPLC method; for example, monitoring lot-to-lot differences for antibody products or comparing the ADCC activity of an antibody biosimilar to the innovator. We are keen to hear further ideas from academia and industry for potential applications and are looking forward to releasing the new affinity column by the end of 2018, which we are confident will be a real asset to biopharma scientists (1).

Reference

1. *M Kiyoshi et al., Nature Scientific Reports, 8, 3955 (2018).*

La Dolce Vita

Sitting Down With… Giovanni Dugo,
Professor Emeritus, University of Messina, Sicily, Italy.

The 2018 ISCC meeting in Riva del Garda saw the presentation of the inaugural "Giovanni Dugo Award" – how did you feel?

It is always rewarding to be recognized by your peers. But what makes me proudest is to feel that I have made a contribution to the successes of students and colleagues who now occupy high-level positions within the scientific community. Carlo Bicchi, recipient of the Award this year, perfectly embodies the qualities of a good researcher and good professor, and, in addition, he is a true friend.

And what makes a good researcher?

Curiosity, passion, willingness to make sacrifices, and intellectual honesty. A good researcher should be able to study and work hard, should not fear comparison, and should not be self-righteous or protective of their own skills. Their personality needs to combine modesty, nerve and courage. They should also be happy knowing that their students are successful in their careers, and be ready to discover that they have been surpassed.

What piqued your interest in food chemistry?

My career in this field started by chance, when a professor I knew predicted that gas chromatography would eventually be applied to food analysis and suggested I work in this newly developing field. I'm happy I took his advice; it was the beginning of a long, successful and satisfying career. I've always considered myself lucky to be working with GC instruments, because I feel that scientists using GC have a close link with the instrument – a more intense, intimate interaction than with spectroscopy, for example. My research has been focused on essential oils, olive oil, wines, and other local foodstuffs, which are important to the Sicilian economy.

The people of southern Italy, including Sicilians, suffered after Italian unification in 1861. Sicilians were seen as people to be dominated, rather than being understood and integrated into the newly born Italian nation. As a result, the wealth and skills of the south were transferred northwards, and while we now live in a more democratic nation, the economic legacy of those times still persists. So it is important for me to be able to support the local economy.

How has food analysis changed over the decades?

In Italy in the 1960s and 1970s, food chemistry was mainly studied in numerous non-academic public labs devoted to research and routine analysis. Limited attention was dedicated to research and teaching in this discipline within the universities. It was regarded as the "Cinderella" of chemistry, neglected by the chemical academic community. At that time, I was a pioneer – I felt brave and enterprising enough to commit myself, and ultimately I contributed to the foundation of the Italian group for Food Chemistry, an inter-division of the Italian Chemical Society. I also chaired the first two conferences on food chemistry. Nowadays, there are several outstanding academic research groups active in this field.

What are the highlights of your academic career?

My career has been very intense and varied, and I have fulfilled many different objectives, but my commitment to everyday life in the lab never faltered. I went into the lab every day and spent most of the day there, exchanging ideas with researchers and students. Most of all, I am proud of the development of a research group that has grown over time and now occupies a prominent position within the scientific community. When the research group was first formed, students from Messina went abroad to be trained or to pursue doctoral research. It is now the opposite – we have more requests to study here than we are able to satisfy. Students, professors and PhD/postdoc students come from all over the world. In recent years, I have been gratified to see that the two people who now coordinate the laboratory and the research – Luigi Mondello and Paola Dugo – are among the most influential people in The Analytical Scientist Power List.

"Lucky are those who never stop 'doing' – once you give up your dreams, your spirit slowly falls asleep."

What drives you?

Playing, dreaming and desiring. Lucky are those who never stop "doing" – once you give up your dreams, your spirit slowly falls asleep. In 2014, I decided to stop doing research and commit myself to a new dream – writing. Since then, I have published eight or nine books on Sicilian food and other topics, which I write in verse.

What appeals to you about writing?

I like spending time with friends and family, but I'm a very private person and find it hard to share my feelings – my deepest emotions, my disappointments or delights. Writing poetry has become cathartic, permitting me to put into rhyme what I cannot describe in normal words. After writing, I feel a sense of "release" and contentment. Poetry says what I cannot express, and cooking is the same.

What is your greatest passion?

Everything related to Sicily – the language, the customs, the cuisine, the history, the cigars, the wine – and my family.

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MPS robotic Performance

SPE / Evaporation / Filtration


GC Liner Exchange (QuEChERS)


quickMIX Extraction / Liquid Handling

Solvent Free

Headspace / SPME

Solvent Free

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