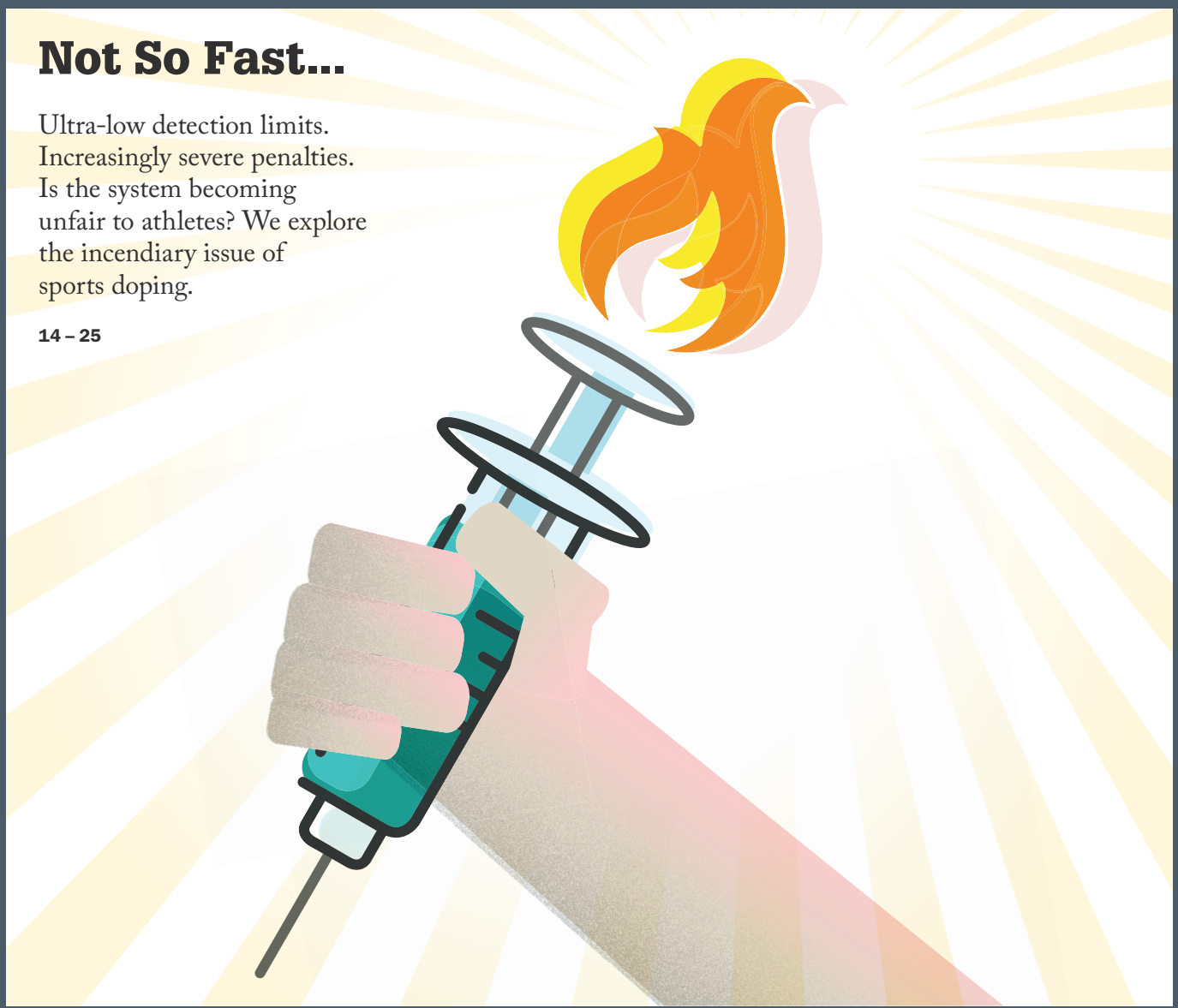
MARCH / APRIL 2022 # 104

the Analytical Scientist®

Not So Fast...

Ultra-low detection limits. Increasingly severe penalties. Is the system becoming unfair to athletes? We explore the incendiary issue of sports doping.

14 – 25



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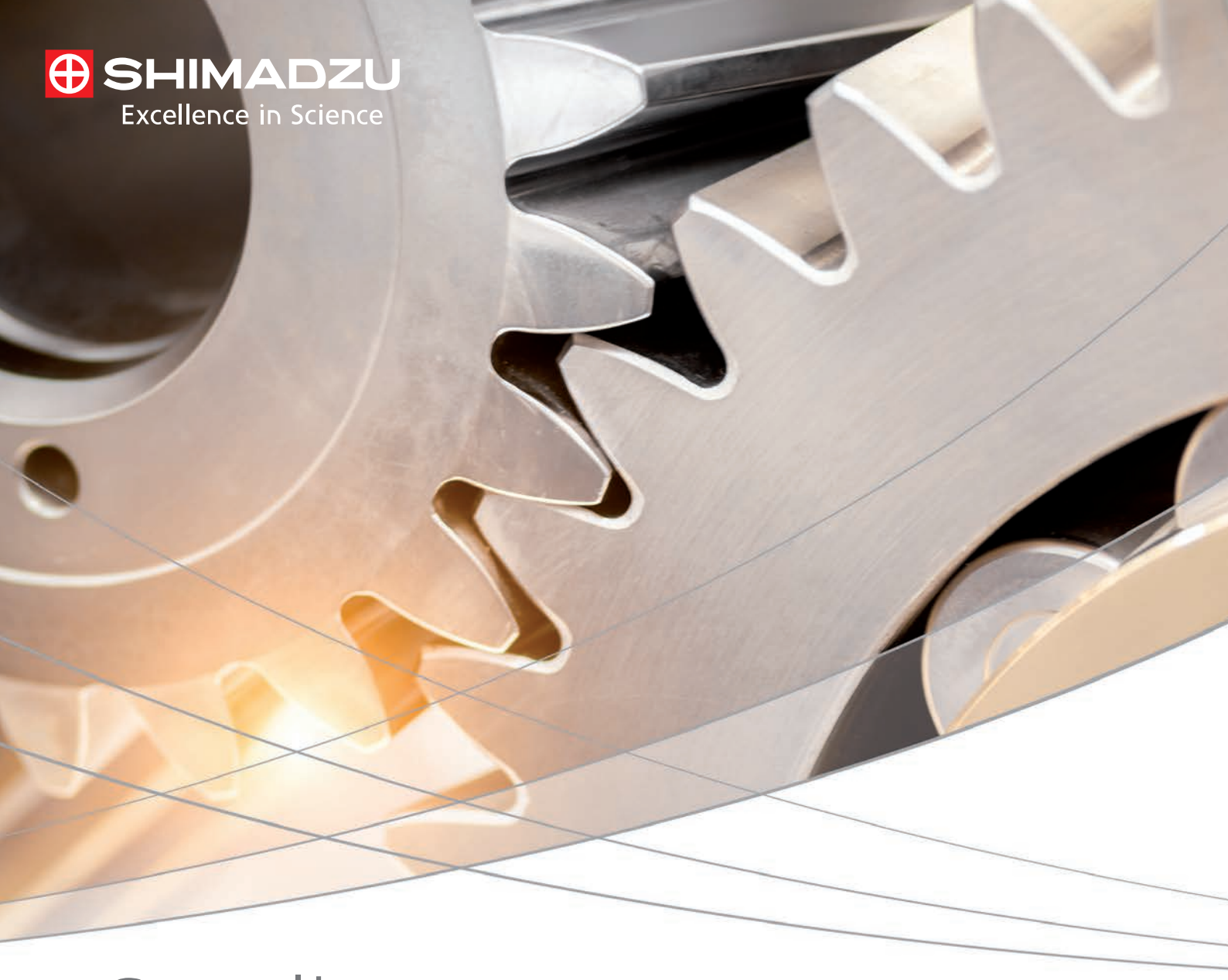

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Straight to the Core

Honing in on the topics at the core of The Analytical Scientist – and revealing some exciting plans ahead…

The eagle-eyed among you may have noticed a slight change to the format of our last issue. Missed it? Well, turn to page 33 – or take a look at the previous digital issue online. That's right – it's my pleasure to retrospectively introduce "Core Topics." From short interviews with key names in the field to practical tips and tricks, each new section – whether mass spec, chromatography, spectroscopy, or (bio)pharma – acts as a hub for content developed for that specific area of analytical science. Moreover, the Core Topics in print reflect a greater emphasis on those areas in the digital sphere…

And that leads me directly to another exciting launch: the Mass Spec newsletter. We all know MS is constantly hitting new strides and the research occurring in this space is frankly exhilarating. Just take a look at our cover feature on sports doping from Douwe de Boer (page 15) – it's clear that MS will play a commanding role in the "war to close Pandora's Box." And, if you head over to the Mass Spec Core Topic (page 33), you'll get a glimpse as to how MS is constantly pushing boundaries – the technology constantly moves in exciting new directions (check out the story behind high-resolution ion mobility MS, as one good example).

With more happening in the space than ever before, we decided it was high time to launch a dedicated newsletter to help better serve the community – a space for #TeamMassSpec to flourish! Mass Spec from The Analytical Scientist will not only keep you up-to-date with the latest advancements in technology and the most exciting research and applications, but also bring together (and amplify) all the different voices in the field.

Since I joined The Analytical Scientist team, mass spec has consistently captured my attention. Was it the many exciting applications passing by my window into the field? Was it the indecipherable new acronyms or the increasingly long chains of advanced technologies? Maybe it was the addictive enthusiasm I've encountered when speaking to some of you. It's likely all of those things. But what I know for sure is that I'm thrilled to have the chance to create and grow a special place for a clearly passionate community.

Lauren Robertson
Deputy Editor

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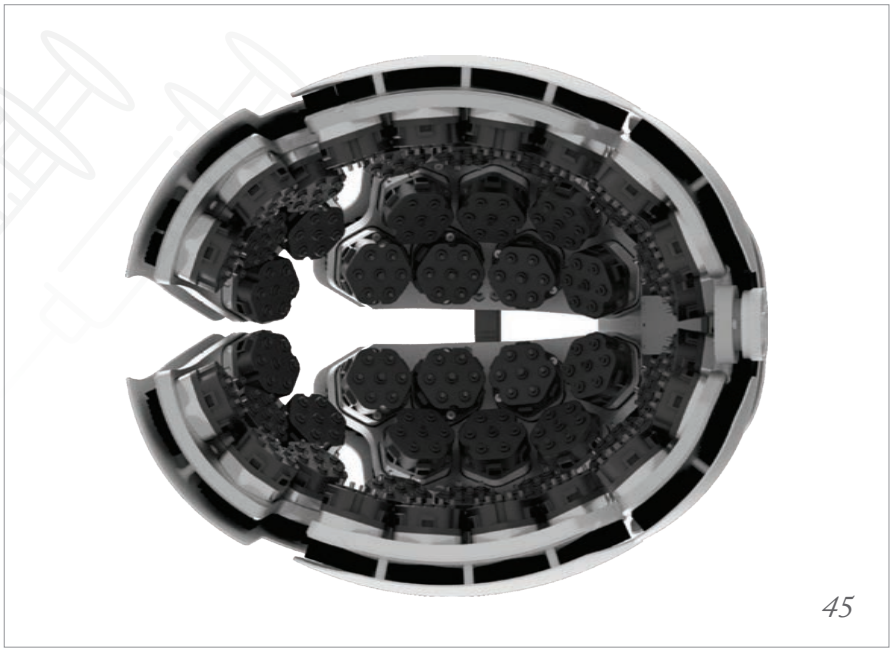
On The Cover

You might think you know who won, but the race isn't over until the analytical scientists have had their say…



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Core Topics

Sitting Down With

the Analytical Scientist

ISSUE 104 - MAR/APR 2022

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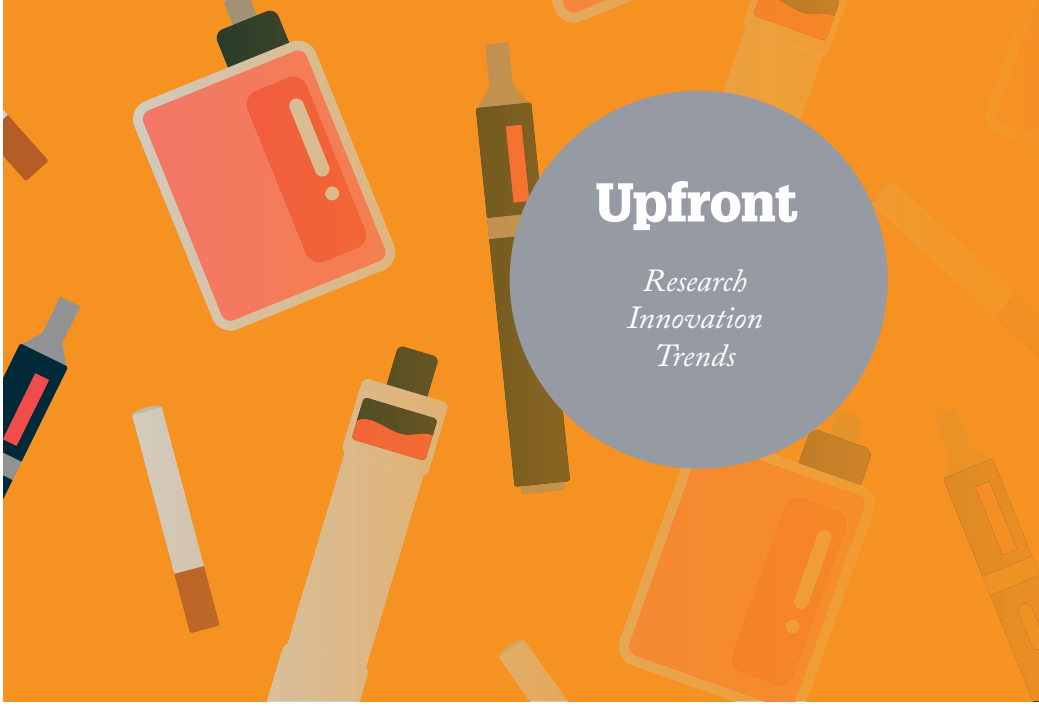
Distribution: The Analytical Scientist (ISSN 2051-4077), is published bi monthly by Texere Publishing Limited, Booths Park 1, Chelford Road, Knutsford, Cheshire, WA16 8GS, UK. Single copy sales $15 (plus postage, cost available on request info@theanalyticalscientist.com). Non-qualified annual subscription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



Fake It Till You Vape It?

Inaccurate labeling is rife among delta-8 THC vaporizers, with many containing unacknowledged adulterants and unintended byproducts

Despite the lack of information around their safety, e-cigarettes and vaporizers containing hemp-derived delta-8 THC continue to rise in popularity. Few would disagree that more data on their health and safety implications are needed – especially in light of the vaping use-associated lung injury (EVALI) outbreak.

To dig into the issue a little deeper, Irfan Rahman and Jiries Meehan-Atrash from the Department of Environmental Medicine at the University of Rochester, New York, analyzed 27 products from 10 brands using a combination of 1H-NMR spectroscopy, GC-MS, and ICP-MS (1). What did they find? "The reported lab test values were all inaccurate – likely due to a lack of optimization in the HPLC-UV methods used by the various labs for testing," says Rahman. On top of this, all of them contained reaction side-products (including Δ9-THC), heavy metals, and a previously undescribed cannabinoid – and 11 had unlabeled cutting agents.

"What was particularly striking was the diverse mix of cannabinoids in the products. Someone consuming traditional cannabis would not be exposed to these, and we simply have no idea what impact they might have on the brain or respiratory system," adds Meehan-Atrash. They suggest people that use delta-8 THC vaporizers should take these findings as a stark warning.

"Our study also highlights the problems with the existing cannabis lab-testing infrastructure; if regulators don't enforce strict certification of testing labs, policies around product potency and composition won't be enforced," says Rahman. As for the future, the duo wants to keep analyzing the chemistry, toxicity, and human health effects of THC isoform products.

Reference

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Cracking Down on Concrete Damage

Photoluminescence spectroscopy sheds light on early signs of concrete damage

Researchers from Rice University and the Kuwait Institute for Scientific Research have discovered by chance that Portland cement emits near-infrared fluorescence, which could lead to new ways of monitoring the integrity of concrete structures (1).

Originally, the researchers intended to test whether nanotube-based strain sensing technology could be applied to monitor concrete structures – but they found an unexpected interfering emission that they traced back to the cement itself. "We deduced that it contains microscopic crystals of silicon emitting near-infrared photoluminescence," says Bruce Weisman.

Armed with this new knowledge, the team applied a layer of opaque paint to a cement block and compressed it to induce microcracks, exposing the substrate's near-infrared emissions and revealing the fracture locations, pattern, and progression.

"This could evolve into a practical method for inspecting critical concrete structures for early signs of damage, thus helping to prevent costly and dangerous failures," says Weisman.

Reference

1. *Weng et al., Sci Rep (2022). DOI: 10.1038/s41598-022-05113-1.*

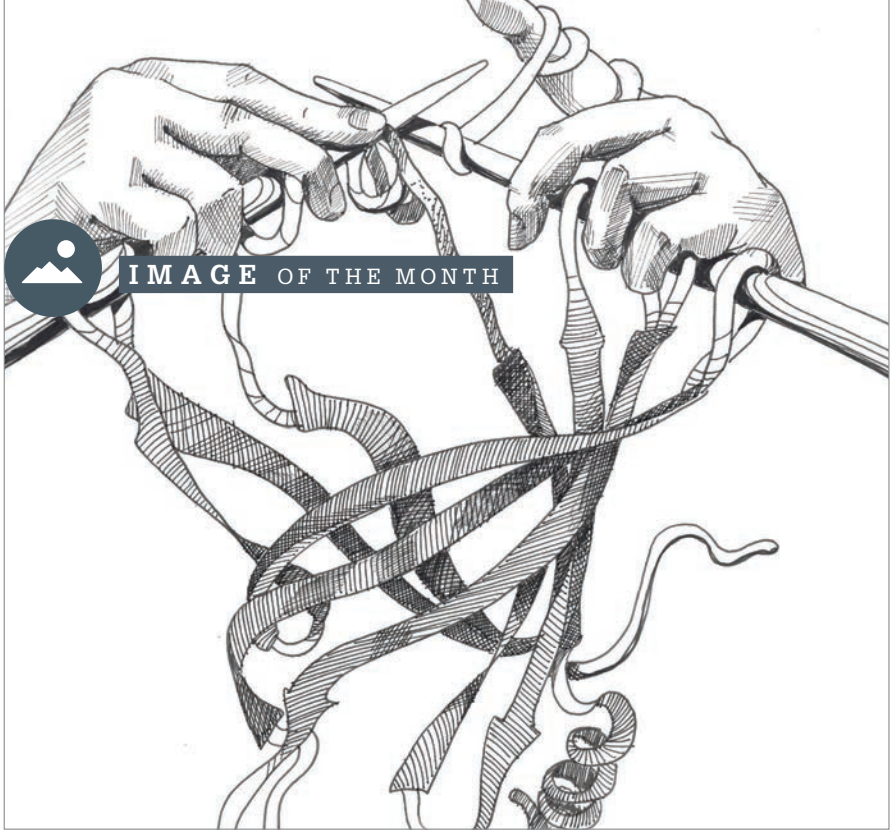

IMAGE OF THE MONTH

The Art of Proteins

Last year, Irina Bezsonova – a structural biologist from the University of Connecticut – created a collection of ink drawings in celebration of 50 years of the Protein Data Bank archive. With some help from the Twitter community, Bezsonova selected one protein every day that aligned with the official "Inktober" prompt – words like "crystal," "knot," and "sour." Above, you can see the drawing created for the "loop" prompt – an NMR spectroscopy structure of the outer membrane enzyme PagP being represented with some knitting needles.

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

QUOTE OF THE MONTH

"The human immune system can make quintillions of slightly different antibodies – a nearly infinite resource. When one of these antibodies is successful, the body makes more copies. As a result of lifelong training, we only make antibodies that are really needed – only about a couple 100 or so will ever be dominantly present in our blood. What if we could discover which antibody fights which threat the best, and turn them into biotherapeutics?"

Albert Heck during his Pittcon Wallace H. Coulter Lecture on the groundbreaking findings his team uncovered using native-MS – and their potential to transform how we treat disease <https://bit.ly/3qn324z>

Nature Calls for a Mediterranean Diet

Nuclear magnetic resonance (NMR) spectroscopy-based urine profiling has linked the Mediterranean diet with good metabolic health

Metabolic "signatures" in urine can be used to determine diet quality and predict metabolic health, according to researchers from the University of Southern California, USA (1).

The team examined urinary metabolites from 1147 European children and collected information about their dietary habits. Using NMR spectroscopy, they discovered that children who followed a Mediterranean diet had higher levels of hippurate, N-methylnicotinic acid and urea, and lower levels of sucrose. But for those who consumed ultra-processed foods, the opposite was true.

Crucially, the researchers found an association between C-peptide – an accurate marker of insulin resistance and future risk of metabolic disease – and those metabolite profiles; higher adherence to a Mediterranean diet was associated with lower C-peptide levels, while an ultra-processed diet was linked to higher C-peptide levels.

"Our work provides further evidence to support efforts by public health authorities to recommend increased adherence to a Mediterranean diet," said Leda Chatzi, one of the authors of the study, in a press release (2).

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2. *ScienceDaily (2022). Available at: https://bit.ly/3IB8wPY*

In My View

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

Rising to the Analytical Challenge of Advanced Medicine

Cell and gene therapies are incredibly exciting, but without the right predictive analytical methods and tools to ensure safety and efficacy, the field will be held back

By George Buchman, Vice President, Pre-clinical and process development, Catalent Cell & Gene Therapy

Cell and gene therapies have emerged in recent years to stake their claim as one of pharma's "big three" – alongside small molecule drugs and biopharmaceuticals. And, as the cell and gene therapy industry matures, analytical science is playing an increasingly important role – especially when it comes to understanding the safety and potency of therapeutic vector elements ahead of first in human (FIH) studies – and certainly before commercial launch.

Given that cell and gene therapies are relatively immature compared with monoclonal antibodies and other biologics, we are constantly improving our viral vector production methods – and we rely on evolving analytics to accurately characterize efficacy, purity, and potency. The establishment of analytical methods that can demonstrate true comparability is of critical importance – just as important as advancements and improvements in manufacturing methods, which is a major talking point within the field.

An area that is also evolving is the availability of multiple novel viral vector serotypes with specific biologic properties, including improved tissue targeting and reduced immune response. It is necessary that novel serotypes undergo exhaustive physical and biological study. To address these and other testing needs, techniques currently used by analytical scientists in other fields are expanding into use for cell and gene therapies. For novel serotypes, methods may include sequence homology analysis, atomic modeling, cryogenic electron microscopy (cryo-EM), MS, next-generation sequencing (NGS), cell binding studies, packaging efficiency, transduction efficiency, potency and infectivity measures, and of course animal efficacy and toxicology, as well as human clinical safety trials.

Many assays are well established and routine, including compendial testing and viral genome titer by digital droplet PCR (ddPCR). ELISA-based capsid titer assays are readily available for most wild-type, common serotypes. But these immune-based tests are typically not available for novel and proprietary serotypes, so A280 (of purified viral vector) or other means are used to quantify capsid.

More challenging assays measure residual DNA, infectivity, potency, transduction efficiency, and correlation to clinical outcome. For example, residual DNA that is typically measured by quantitative PCR (qPCR) may actually be packaged within the capsid. In addition to qPCR for residual packaged host cell DNA, analytical ultracentrifugation (AUC) provides an effective means to monitor for vector packaging fidelity, including intermediate forms that may not be functional. Small-scale batch processing early in product development in conjunction with key analytics provides valuable insight into vector quality attributes to avoid issues in later development. We are at an early stage in the evolution of cell and gene therapies,

and clinical outcomes will let us know if our current analytics are suitably predictive of safety and efficacy.

Other challenges include the detection of some virus-based contaminants, for example replication-competent adeno-associated viral (rcAAV) vectors. Recombinant AAV vectors (rAAV) are inherently not replication-competent nor are they pathogenic, but there is a risk of the formation of a wild-type form of the vector by recombination during production. These forms are more likely to insert in the patient genome and can increase immune and oncogenic potential. Another testing challenge is looking for contaminating AAV serotypes and other viral vector species (adenovirus, retrovirus, etc.) in a batch, particularly for facilities that produce multiple types. Few laboratories at present perform this testing, though it is gaining regulatory and safety importance.

For both gene and cell therapies, the quality of process inputs is critical. For established serotypes and vectors, including AAV wild types 1–10, master cell banks for viral vector production are tested exhaustively, and particularly around adventitious viral agents. Similarly, plasmid quality is paramount to establish an efficient transfection process (the most typical way to prepare viral vectors) to form gene and gene-enabled cell therapies. Release testing for viral vectors is exhaustive, including standard compendial methods (for example, osmolality and pH), safety testing (adventitious agent and rcAAV detection), residuals testing, titer-based (ddPCR and ELISA), purity and structure (SDS-PAGE and capillary electrophoresis), and aggregation (dynamic light scattering and HPLC), among others.

Viral vector characterization specifically is a significant challenge for the field. The sheer number of vectors (multiple serotypes), vector platforms (AAV, lentivirus, adenovirus, retrovirus), potential candidates for gene of interest, multiple packaging platforms, numerous production methods, and the constant evolution of the field keep viral vector characterization very challenging. As stated for novel vector development and characterization, these analyses may involve many non-standard methods, such as cryo-EM, MS, and in-silico modeling.

In some ways, we are already living in the future. Diseases such as spinal muscular atrophy (SMA) that were previously not treatable, and terminal by age two, now have a commercial cure available. The field will begin to mature as it did for monoclonal antibody therapies, with improvements in production yields that will correlate with reduced levels of impurities per unit dose, access to treatments and cures to more patients, and decreased costs. And that will lead to new treatments and cures entering the clinic for hundreds of diseases. Out of necessity, the analytics will have to keep up – especially with the need to correlate clinical outcomes with our ability to measure critical quality attributes.

It's an incredibly exciting field, but we need vigilance around the analytical methods that are predictive and help to assure safety and efficacy. Novel serotypes will offer better targeting and reduced off target effects, perhaps with reduced dosing. If we all move forward together, the future really does hold amazing promise.

Cite Your Sources: Academic Integrity Revisited

Maintaining standards in citation ethics is a responsibility that lies with each of us

By Victoria Samanidou, The Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, Greece

References form an indispensable part of a manuscript. They ensure integrity, allow the reader to locate existing work, and offer a snapshot of the relevant research.

But, over the years, I have encountered several questionable responses after manuscript submission. For example, editors asking for more citations with references from their journal, potentially altering journal metrics; or reviewers putting put forward additional – largely irrelevant – citations of their own work to the submission. (The latter is comic because it eventually reveals the identity of the reviewer!) And although some suggestions were reasonable due to author oversight and papers being reported inappropriately, more often than not, they were unethical.

I am also well aware of the fact that editors and reviewers should make all possible efforts to improve the quality of a manuscript that will appear in a published journal. What's more, I know that reviewers work on a voluntary basis. But scholars acting as reviewers should not expect anything back – not even in the form of increasing their h-index. And yet what happens when a reviewer realizes that their own work was not given sufficient credit? Should they ask for it? Where do we draw the line between

self-promotion and genuine recognition?

This also raises the question: is it easy for an author to distinguish between a legitimate reviewer suggestion and a manipulation based on self-promotion? In my opinion, the answer is yes. In the first case, the additional suggested papers are always limited to scientifically necessary material and very close to the topic. In the second, irrelevant papers are proposed, sometimes hidden among relevant ones. The author must then decide whether to risk rejection or add all suggested references to ensure the paper is accepted. I hope that most scientists would choose the latter, but there are real incentives to "play the game."

Another ethical question is self-citation. How many self-citations are reasonable? In my view, all recently published papers on the specific topic should be mentioned, regardless of self-citation. Self-citations can help paint a clear picture of the author and their work on the topic. Moreover, self-citations do not increase an author's h-index (though they may skew journals' ratio-based metrics). Personally, I do not see an issue with this, as long as the citations are strictly necessary ones. Authors should not use their research papers to flaunt career achievements, but to provide information that supports the data, the necessity of the research, or its importance to the field.

According to the Committee on Publication Ethics, all references should contribute to the scholarly content of the article (1). There are instances where self-citation and requests for additional citations are legitimate, but all other requests that may violate publication ethics should be avoided. Furthermore, reviewers should refrain from suggesting citations to promote their own work and all suggestions must be based on scientific reasoning. Misconduct should be reported and penalties or respective consequences should be applied (2-4).

Journal guidelines should indicate their policy and potentially ask authors to limit self-citations. But academic integrity and citation ethics should rely on authors' values, not on rules and penalties. Maintaining ethical standards in academia is a responsibility that lies with each of us as individuals and scientists.

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MALDI Imaging's Clinical Research Advantages

Now's the time to realize the many advantages of mass spectrometry imaging in clinical research

By Mike Easterling, Imaging Business Manager, Bruker Daltonics, Billerica, Massachusetts, USA

All pharmaceutical scientists share a common goal – to accelerate drug discovery and development efforts through a deeper understanding of biomarkers and the etiology of a disease. Matrix-assisted laser desorption/ionization (MALDI) imaging is becoming increasingly important in clinical research, particularly when we consider its impact in the study and management of cancer. Over the past couple of decades, as we have uncovered specific molecular markers of disease, we have gradually augmented traditional diagnostic histopathology with the use of molecular labels and probes. If we combine this with the knowledge provided by the Human Genome Project in the 1990s and early 2000s, The Cancer Genome Atlas (TCGA) program that began in 2006, and the development of accessible sequencing technologies, we create a new discipline – clinical molecular diagnostics.

Looking back on these developments, one review stated that "pathologists will become pilots for precision medicine cancer therapy through their unique ability to combine morphological and molecular findings (1)." Put simply, pathologists are pioneers in bringing new cancer drugs to life.

Current tools to analyze the tissue microenvironment of disease and determine the locations and interactions of cellular components that dictate disease outcomes include immunohistochemistry, spatial transcriptomics, and imaging mass cytometry. Although each of these techniques offers useful targeted information about proteins in tissues, they also have challenges – for instance, their inability to capture the variety of post-translational modifications in the proteome.

In contrast, MALDI imaging presents a label-free tool that captures information about the spatial proteome and additional

spatial omic signatures unique to the local cell neighborhood. No prior knowledge of the compounds is required – the technique provides true untargeted molecular analysis in a spatial context. Equally as important, tumor-associated biomolecules that are missed at the gene level can be visualized. Further to this, the MALDI imaging workflow is compatible with standard histological procedures, maintains spatial resolution of around 10 µm, and preserves the tissue section under examination for further study. Its in-depth spatial proteomic, lipidomic, and metabolomic insights complement traditional genomic and transcriptomic methods and can help identify new predictive or prognostic biomarkers and classify heterogeneous tumor subpopulations, yielding important contextual clues to tissue-level communication networks integral to cancer growth and treatment success.

Two recent technological breakthroughs are now being applied to MALDI imaging. First, ion mobility separation has greatly broadened the range of biomolecules that can be analyzed by pre-separation ahead of mass analysis. Second, novel laser-induced post-ionization technology has delivered a quantum leap in MALDI imaging sensitivity – by up to three orders of magnitude.

Clinical research has led the way in using MALDI imaging technology, taking advantage of a label-free analytical tool that can fill in the broad gaps left by spatial transcriptomics and genomics in molecular investigations on tissue samples. It can provide valuable information when it comes to protein modifications after gene expression and visualize additional compounds, such as metabolites, glycans, and lipids – all of which play a role in disease pathology.

In my view, it's not hard to see the potential of this technology in providing a top-down, disease-centric view of tissues that can inform therapeutic strategies, support diagnosis, and improve patient outcomes. Additional developments to the technology will boost measurement speeds, increase sensitivity (without compromising spatial resolution), and even offer deeper molecular content – important factors that may help accelerate the adoption of MALDI imaging in the routine clinical environment.

What I Told Grace

More than 1,300 mentors in the scientific community completed a survey sharing their unique viewpoints and experiences with diversity and inclusion. All responses have been analyzed and the result is Grace – the story of an ordinary girl with the potential to be extraordinary. Here, two of Grace's mentors – Christina Jones and Isabelle Kohler – reveal what they told Grace.

"Extraordinary Grace" could be your daughter, your niece, a student in your lab, even a younger version of yourself. Grace's mentors shape her life and career in science and ultimately guide her to fulfil her potential in spite of challenges she faces. Through this interactive survey, SCIEX aimed to collect experiences that could be used to inspire the next generation as well as shine a light on identity, opportunity, representation and inclusion in science.

When you were younger, did you have a role model who inspired you to pursue science?

Christina Jones: In my middle and high school years, there wasn't one role model in particular who turned me onto science. It was more a case of me enjoying science and doing well in tests, which pushed me in that direction – cultivated by some particularly good teachers. Additionally, when my mom was hospitalized while I was at middle school, I realized I wanted to do something that was going to impact human health.

My first real mentor was Isiah Warner, who was the leader of a scholarship program I was on during my undergraduate studies. He shaped the whole trajectory of my career. As a young student, I was trying to navigate this thing called "science," which was totally unfamiliar – I had no real idea of what a PhD was. And here was this person who had all the knowledge, very senior in his career, renowned for his research, and passionate about increasing diversity in STEM. Not only that, but he was a Black male, with other Black people and people of Color in his scholarship program – a huge confidence booster and counter to much of what I'd seen on TV growing up. He also helped with all the soft skills that are crucial for success, including time management, understanding one's learning style – even the etiquette at a formal banqueting dinner (something a lot of people may take for granted). Overall, his holistic approach made sure everyone had the tools they needed to be successful.

> *"As a young student, I was trying to navigate this thing called 'science,' which was totally unfamiliar – I had no real idea of what a PhD was."*

Women and people of color are more likely to drop out of university STEM courses. Why do you think that is?

Isabelle Kohler: You do tend to find a relatively even split of men and women at the undergraduate level – even in STEM. But most of the professors are male. And when I was a graduate student attending conferences, I got the impression that the few women at the events weren't enjoying themselves as much as the men; they weren't as welcome and didn't feel as free to express their enthusiasm or excitement about their work. I remember thinking, I'm not surprised some women conclude this isn't the path for them. I also saw women dressing like men and imitating the behavior they saw at these male dominated shows. But I think we need to embrace diversity – let people express their unique personalities and perspectives. And that's one thing I say to the younger people who I mentor: Be yourself – you'll get more respect and you'll be happier in the long run.

Jones: The "leaky pipeline" is a complicated problem, but I think confidence plays a big role. Most university students will face some difficulties early on in their studies where they wonder, "Is this really for me?" – especially with the sudden transition to self-directed learning. And when you look around and see few students or teachers, as Isabelle discussed, that look like you or come from similar backgrounds then it can compound the problem – social isolation is a big problem that students from minority backgrounds can struggle with. Diversity at all levels will clearly help here. But effective mentoring can also be instrumental in giving students the confidence they need to keep going. We need to get better at providing – or even just making students aware of – the resources that are available to help them.

Grace talks about being passed over for promotion, while male colleagues with less experience and accolades are being promoted. Have you experienced anything similar or seen it happen to a colleague?

Jones: I've seen it happen – colleagues either not getting the promotion they deserve or being paid less than their male colleagues for the same job. Often it'll lead to the person concluding that the organization doesn't value them – rightly so – and looking for other opportunities. I've advocated for a colleague in the past, warning their boss that they'll end up losing them if they don't invest – to no avail. And sure enough, the employer was looking to hire them back a year later!

Kohler: I think things are getting better in this area, but I do see women being overlooked – often simply because they want a better work-life balance than their older, male colleagues who may not have the same responsibilities at home. Though it is worth pointing out that I have received several opportunities to join the Board of prestigious associations, in part, because of the increased awareness of gender equality.

Grace talks about finishing a paper with a good chance of being published in a high impact journal. But a colleague who previously worked with the editor said they'd have a better chance of getting published if they're named as the first author. How would you advise someone in this situation?

Kohler: I would say, no way – you've got to fight for your rights and scientific integrity! If you've worked hard on a paper, you have to state your case and make sure your name is adequately highlighted in the author list. The alternative is entering into a game that won't help you in the long run while damaging science in the process.

Jones: One thing I've learned is to always agree upfront who will be the lead author based on who is contributing the most to the project. It can be an awkward conversation (and it can be subject to change if roles evolve over the course of a project), but it ensures everyone is on the same page from the outset. But, as Isabelle said, you really have to stand up for yourself in these situations and say, "I contributed X, Y and Z, which means I am the first author." Regardless, any leverage a well-connected person's name brings comes from the fact that they're on the list – not that they're the first author.

How can mentorship be a catalyst for change?

Kohler: Though there are specific mentoring programs for women and people from minority backgrounds – and that's great – I feel we should be aiming towards a situation where everyone, from all backgrounds, have experience working with a mentor and being a mentor themselves. This way, we can make everyone aware of the issues different groups of people face and how their bias can contribute. The issues associated with a lack of diversity is something we should all be aiming to solve – regardless of our background.

Can you give an idea, piece of advice, or solution that could improve science for future generations and, in turn, help create a truer picture of our industry?

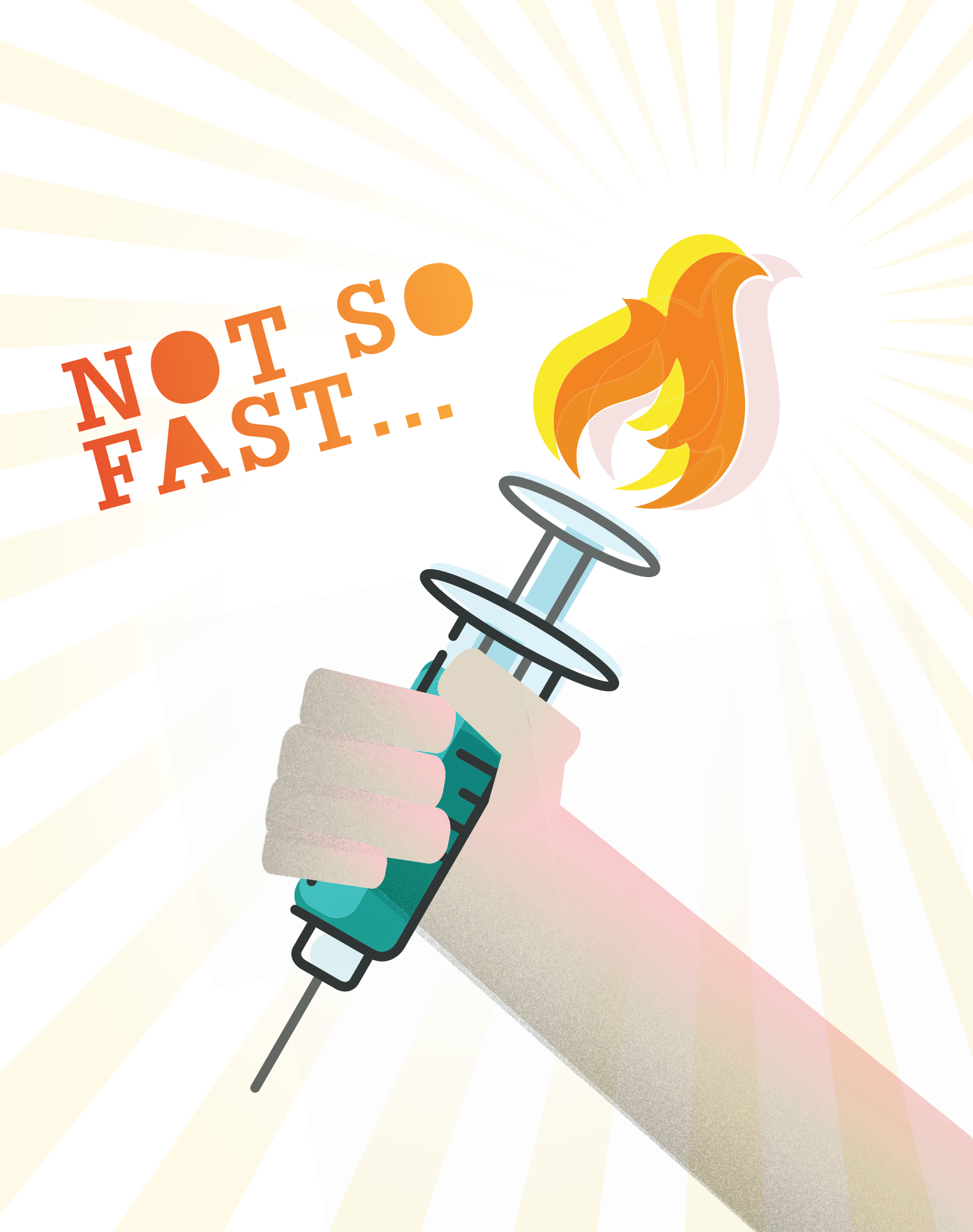
Jones: Innovation in science and technology drives the global economy and will play a key role in solving many of the problems we, as a species, face. And when you think about it from that point of view, it's clear to me that everyone from across the globe with a variety of backgrounds should be represented. Not only will a diverse range of perspectives help us solve problems more quickly, but it will also ensure that we're focusing on the right problems. If you look at the work someone like Renee Robinson is doing as a human health researcher looking at diseases that have a disproportionate impact on people of color – that research wouldn't exist if someone with her perspective wasn't making it happen.

Kohler: I agree. One of my research interests is the differences between male and females at the (patho) physiological level. This is important, because a large proportion of (pre-)clinical studies focus solely on men. How can we be inclusive in academia if we don't follow the same principles in our own research?

Join the science community as they hear from Grace in person. As part of the next chapter in her journey, Grace will give a keynote speech, explaining what she has learnt from her mentors: extraordinarygrace.com. Register to book your seat now!

Christina Jones is a Research Chemist and Advanced Manufacturing Program Officer at the National Institute of Standards and Technology, USA; and Isabelle Kohler is an Assistant Professor, Division of BioAnalytical Chemistry at Amsterdam Institute for Molecular and Life Sciences, Vrije Universiteit Amsterdam, The Netherlands

NOT SO FAST...



You might think you know who won, but the race isn't over until the analytical scientists have had their say. Behind the scenes and headlines, tireless work goes into developing and validating new ways to catch cheaters before the clock runs out. But several big questions are also jostling for the podium. As detection limits continue to push the boundaries – and as penalties become increasingly severe – is the system becoming too unfair to athletes? Are we treating exceptional individuals as guilty until proven innocent? What role should scientists play in ensuring the system is balanced? Douwe de Boer considers how Pandora's Box opened – and whether we'll ever close it.

We also speak with three experts – Mario Thevis, Christopher Chouinard, and Xia Xu – about the cutting edge analytical developments making a difference in the fight against sports doping, including the prospect of gene doping.

CLOSING PANDORA'S BOX

How did Pandora's Box open – and will we ever close it?

By Douwe de Boer

The athletes competing at the 2022 Winter Olympics have had their three-week shot at fame and glory in Beijing. Those who made the podium received their medals. Anthems were played, parties were enjoyed. But we don't yet know for certain who really deserves the accolades. Because analytical chemists have another 10 years to catch athletes cheating with performance enhancing drugs. There may be athletes at this year's games using substances that cannot yet be detected – so the race is on to develop and validate new methods before the decade is out.

The story behind this unexpectedly troubling scenario started to unfold over a century ago, with athletes, classical chemists, and pharmacists all playing their part. Today, Pandora's Box is well and truly open – and analytical chemists have the daunting responsibility of helping the authorities close it again.

The alkaloid arrival

Institutionalized anti-doping analysis started in the 1960s, despite the fact that the use of drugs to enhance sport performance began much earlier. For example, an endurance walker in Britain said in 1807 that he had used laudanum (which contains opiates) to keep him awake during a race (1). Also in the 19th century, pure alkaloids were isolated from plants by classical chemists – morphine (1803), strychnine (1818), caffeine (1819), and cocaine (1859) – piquing the interest of people seeking to apply the effects of alkaloids outside of treating medical problems. One of those non-medical uses was in Coca-Cola, which contained cocaine and caffeine when launched in 1886. It became popular with some athletes, including a group of French cyclists and a champion lacrosse team (2). At the time, the application of alkaloids in human sports was not considered to be a problem, despite deaths surely having occurred as a result (few cases were confirmed). The Box had been cracked open, but nobody was interested in closing it – yet.

In the 1930s, true synthetic compounds like amphetamines were developed (though they were invented much earlier). These psychostimulants were marketed initially as inhalers for congestion or as energy boosters – and, during World War II, as compounds to improve the performance of soldiers. During and after the War, soldiers were thought to have introduced amphetamines into sports, which caused several deaths in

the 1950s and 1960s. This toll was deemed unacceptable to the public and sport authorities had to react. The age of anti-doping analysis had begun in earnest.

Enter anabolics

Pandora's Box also released the phenomenon of anabolic-androgenic steroids (AASs) into sport after World War II. German soldiers had used endogenous AAS testosterone to increase their aggressiveness and to improve their physical strength; and, in the 1950s, Russian athletes were thought to have used testosterone to improve their sports performance. US athletes responded in the 1960s by applying methandienone, an exogenous AAS. Methandienone was developed to treat muscle dystrophy in the elderly, but was withdrawn from the market because of health risks. Athletes, however, had already tasted the forbidden fruit…

Attempts in the 1970s to unambiguously detect the use of exogenous AAS by immunoassays failed, but GC-MS, introduced in the 1980s, made it possible to identify metabolites of exogenous AAS in urine sample of athletes in the range of 100 nmol/L without reasonable doubt. GC was combined with tandem MS (MS/MS) in the 1990s and 2000s to further push the limits of detection down to the lower range of 1 nmol/L. Although GC-MS significantly expanded the identification power by MS, one hurdle was the need for chemical derivatization to increase volatility and stability. The application of liquid chromatography MS/MS in AAS metabolite detection leaped that hurdle in the 2010s, making it possible to detect AAS under non-volatile and less thermo-labile conditions. Nowadays, using LC-Ion Trap MS/MS, detection limits can reach as low as 10 pmol/L. The high sensitivity and the presence of so-called slow excretion metabolites results in a long detection window that makes it very difficult for athletes to continue abusing exogenous AAS.

To detect the abuse of pharmaceutically applied endogenous AAS (testosterone, androstenedione, dehydroepiandrosterone, and so) steroid profiling was initiated based on the ratio between the steroid's testosterone and epitestosterone. However, again due to a lack of analytical specificity, some athletes were falsely accused of doping offenses. GC combined with combustion/isotope ratio MS (C/IRMS) presented a solution in the 2000s and is now the go-to tool for detection of endogenous AAS abuse.

"Not all athletes are innocent; some want to enhance their performance by any means necessary, and so new compounds or methods continue to be found."

So, have we closed Pandora's Box?

I would argue that the Box is open wider than ever before. Though several challenges have been overcome in terms of expanding detection limits for alkaloids, psychostimulants, and AAS metabolites, the question of where the substances came from – and thus the athlete's guilt – isn't always as easy to answer. Banned substances can originate from contaminated supplements, regular medicines, or even in common foods – meat from animals treated with certain agents to increase meat production can be the source of low concentrations of AAS metabolites or other agents.

But not all athletes are innocent; some want to enhance their performance by any means necessary, and so new compounds or methods continue to be found. In the 1980s, athletes became interested in peptide hormones, such as human growth hormone (hGH) and human chorionic gonadotrophin (hCG). The abuse of hGH was initially hampered by limited availability of safe pharmaceutical preparations, but it has become more prevalent with the availability of recombinant hGH. The detection of peptide hormones remained a challenge for decades – clinically adequate immunoassays were able to detect hormones in the 1980s, but identification in the context of anti-doping analysis requires a higher level of legal certainty. Since 2010, identification of hGH abuse has been based on the differential detection of hGH isoforms using specific immunoassays.

In the 1990s, athletes discovered the benefits of using

recombinant human erythropoietins or epoetins (EPOs), often referred to as erythropoiesis-stimulating agents (ESAs). These compounds are highly active protein hormones that stimulate the production of erythrocytes, which improves delivery of oxygen from the lungs to the working muscles.

Today, the direct identification of the abuse of ESAs is based on the analysis of blood or urine using a combination of electrophoresis (for example, isoelectric focusing or sodium dodecyl sulfate- or sarcosysl-polyacrylamide gel electrophoresis) with double Western blotting and immunochemical detection. Indirect identification is tackled by a so-called hematological module of the Athlete Biological Passport (ABP), which, by detecting hematological parameters in blood, applies a Bayesian approach to determine the probability that an athlete's hematological variation might be due to the abuse of ESAs. Also, a growth hormone module of the ABP based on hGH biomarkers has been considered and investigated since the 2000s.

Within the anti-doping community, such approaches to identify protein hormones are considered reliable and robust – though there are doubters (usually outside the analytical chemistry community). A broad scientific discussion may be needed to improve the analytical sensitivity and specificity of the test protocols. As for the classical doping agents, MS-based identification might end this discussion, but continuous development of analytical technology remains essential. In the future, LC-MS/MS and capillary electrophoresis (CE)-MS/MS may be key to unambiguously detecting the abuse of protein hormones.

Who's ahead in the race?

Sports doping is a continuous battle between authorities and athletes. Some athletes – no doubt aided by doping chemists – find new ways to illegally enhance their performance, while analytical chemists work hard to find methods of detection. Who has the advantage in this race? It can take years to adequately validate new analytical tools after the introduction of a doping trend, and that's why anti-doping authorities made it legal to re-analyze biological samples for up to 10 years after collection. But these rules mean that the final winner of a 2022 Olympic medal only will be known in 2032…

Pandora's Box will remain open for the foreseeable future. Moreover, the current anti-doping policy shifts the problem of drugs and methods in sports toward other doping agents and methods instead of closing the Box. For example, it recently became clear that the incidence of the use of thyroid hormones in certain sport populations is significantly higher compared with the prevalence of relevant thyroid diseases in reference

HORSES TO HUMANS

From a purely analytical point of view, anti-doping analysis started relatively early. In the 1910s, analytical methods based on colorimetry were developed to test for alkaloids in race horses. Saliva was chosen as a target biological specimen as urine collection was considered… impracticable (horses were given alcohol to stimulate sufficient amounts of saliva). For extraction, different analytical chemical approaches were available, such as distillation to remove ethanol, and liquid/liquid extraction and precipitation to isolate the alkaloids. Then, using general and specific color reactions, it was possible to screen for alkaloids generally and to confirm certain alkaloids specifically.

In humans, urine is more easily collected, and so it became the sample of choice to detect alkaloids and psychostimulants in the 1960s. At first, thin layer chromatography (TLC) combined with colorimetry was used. In the 1970s, gas chromatography replaced TLC and was combined with nitrogen phosphorus detection to improve analytical sensitivity. However, the lack of analytical specificity resulted in compound misidentification, ultimately meaning that some athletes were falsely accused of doping. These findings forced sports authorities to implement mass spectrometry (MS) for a more adequate identification. Magnetic sector mass spectrometers were used initially, but the introduction of quadrupole MS systems in the 1980s significantly enhanced our ability to identify alkaloids and psychostimulants in urine; for example, ~1 µmol/L. Nowadays, MS is the overall detection method of choice for the direct confirmation of doping agents or metabolites of doping agents. And although urine is still an essential biological specimen in anti-doping analysis, blood has gained more significance for those substances or biomarkers that are insufficiently or not at all excreted in urine.

"Remember that any development in this field can have ethical repercussions; we should carefully weigh the pros and cons in every instance and from multiple angles."

populations. Not currently on the World Anti-Doping Agency's list of prohibited substances and methods, the use of thyroid hormones is raising new medico-ethical questions and of great concern to sport authorities. Manipulation of athletes' genes is another serious issue; in fact, genetic doping has been investigated by official judicial authorities and is an officially forbidden method.

Get in the game

I believe analytical scientists must play a key role in anti-doping discussions. After all, we are the driving force behind the technologies applied in anti-doping analysis. Moreover, the development of sophisticated analytical tools for anti-doping analysis can have an important impact on wider clinical chemistry and thus medicine in general. For example, the ABP module approach could be applied in the pursuit of precision medicine, where individualized approaches are needed to predict and monitor treatments instead of relying on a one-size-fits-all approach.

At the same time, analytical chemists must try to avoid the perils of tunnel vision; for example, only seeing the advantages of an ultra-sensitive method or taking pride in scientific advances without considering the impact on others. Remember that any development in this field can have ethical repercussions; we should carefully weigh the pros and cons in every instance and from multiple angles.

In the context of WADA's list of prohibited substances and methods, my view is that analytical chemists should look beyond the development or continuous improvement of analytical technology, and start asking new questions; for example, what role can analytical science play in determining the origin of a doping agent? And how can we build new ABP biomarker knowledge?

If we continue down the current path of increasingly sophisticated and sensitive technologies that can detect and identify substances beyond current lower detection limits of 10 pmol/L, we may begin detecting pharmaceutical substances that originated from drinking water (residues of certain medicines are already commonly detected in waste water and subject to environmental investigation).

We should not be solely focused on the goal of catching as many doping offenders as possible, but instead making anti-doping activities as robust as possible – and that will almost certainly mean cooperating with other scientific disciplines. If we do not, innocent athletes may be crushed by attempts to close the "lid." With each passing decade, the sanctions faced by potential doping offenders are getting increasingly severe – so we cannot get it wrong.

In criminal law, the presumption of innocence is essential. And any accusing authority must supply a court with as much evidence as possible. Subsequently, any proven violation leads to an appropriate sanction. In sport law, the presumption of innocence seems to function differently. The accusing authority supplies a certain minimal amount of evidence and the accused athlete must prove her or his innocence. Put another way, any accused athlete is guilty unless proven innocent. When it comes to anti-doping analytical procedures, the minimal amount of evidence is well defined in the respective regulations; however, authorities may not always reveal the full details of the analysis – mainly to prevent others using the information to circumvent regulations or develop new methods of doping. But this lack of transparency is arguably unfair to innocent athletes. Without all the details, how can athletes adequately defend themselves? As pressure to reinforce sanctions increases, so too should the pressure to release more analytical details.

As sport organizations and politicians work together to find the right balance, we analytical chemists will continue working behind the scenes, playing our part in the war to close Pandora's Box. But we are not mere foot soldiers – we must also do our utmost to ensure the Box is closed in a fair way.

Douwe de Boer is an analytical biochemist with a PhD in Pharmacy (based on a study of analyzing anabolic androgens in urine samples, conducted at an IOC-accredited Dutch Anti-doping Laboratory). "I specialize in anti-doping analysis and work as an independent anti-doping consultant and expert witness in legal sports cases," says Douwe. He has been active in the field of anti-doping analysis since 1986 and was technical and scientific director of the Portuguese Anti-doping Laboratory in Lisbon from 1998-2004, which was both IOC- and WADA-accredited.

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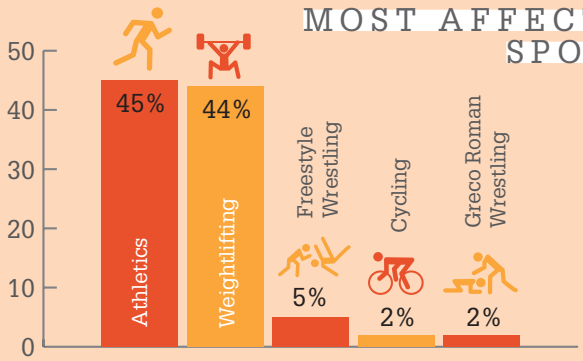
Retrospective Results

Since 1968, 142 summer Olympic Games medal results have been impacted by doping violations, with 74 percent of those identified retrospectively. We break down the key stats.

MEDAL RESULTS IMPACTED

MOST AFFECTED SPORTS



79%

of detected substances were either dehydrochloromethyltestosterone (CDMT) or stanozolol

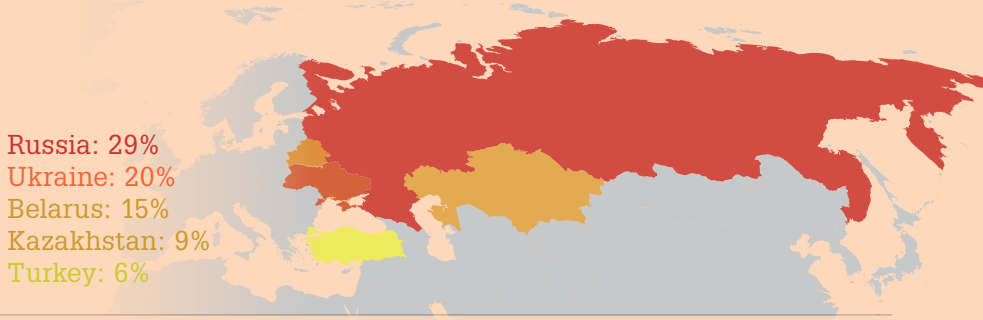
FACT:

CDMT was the key steroid administered to approximately 10,000 East German athletes as part of a secret doping program, known as State Plan Topic 14.25, often without their knowing the nature of the "vitamins" they were forced to take. The program remained in place from about 1968 until the collapse of the German Democratic Republic in 1989.

FACT:

Since 2004, the International Olympic Committee (IOC) has stored all samples collected at summer Olympic Games for retrospective re-analysis with more advanced analytical techniques to catch doping athletes.

MOST AFFECTED NATIONS



74%
of summer Olympic medals impacted by doping violations between 1968–2012 were identified retrospectively

57%
of the total number of impacted medals were International Olympic Committee (IOC) mandated re-testing of the 2004, 2008 and 2012 Olympic Games

6.8 ± 2.0
years was the mean length of time from the medal win to a positive doping test to be announced

90%
of all positive IOC re-tested samples contained metabolites of exogenous anabolic androgenic steroids

Source: A Kolliari-Turner et al., "Analysis of Anti-Doping Rule Violations That Have Impacted Medal Results at the Summer Olympic Games 1968–2012", Sports Med, 51, 2221–2229 (2021). https://doi.org/10.1007/s40279-021-01463-4

REVEALING THE UNKNOWN ENHANCERS

Join Christopher D. Chouinard on a tour of the latest mass spec and ion mobility advances enabling the identification of "novel" – potentially performance enhancing – substances

What is the current gold standard for detecting anabolic steroid doping? And what are its limitations?

The current gold standard for detecting anabolic steroids involve chromatographic separations (either gas or liquid chromatography) coupled to a triple quadrupole tandem mass spectrometer (MS/MS). These methods are highly selective and sensitive, which is why they've been used (and improved upon) over the last several decades. However, these methods are targeted, meaning they are developed to look for specific chemical compounds (such as those listed on WADA's annual Prohibited List). As such, identification of "novel" or unknown substances can be more challenging, especially considering triple quad-based MS is a low-resolution MS technique.

Which emerging technologies might be able to overcome such limitations?

Advances in high-resolution mass spectrometry (time-of-flight, Orbitrap, FTICR, and so on) have allowed for routine molecular composition analysis, especially for small molecules, such as anabolic steroids. These accurate mass methods are capable of reliably measuring m/z to several decimal places and thus providing a confident molecular formula. Coupling these with various MS/MS fragmentation methods can also provide some structural information, but are challenged by stereochemical isomers. And that's where ion mobility (IM) comes in. IM is a rapid gas-phase technique that separates ions based on differences in their size, shape, and charge; our group and others have shown this technique to be capable of resolving numerous isomers in mixtures (where even high-res MS fails). Its timescale allows straightforward coupling with current methods (for example, LC-IM-MS/MS) such that you can get all of the aforementioned data as well as the ion mobility-derived collision cross section (CCS). CCS is a representative property of an ion and can be used to help identify unknown compounds, especially when combined with computational modeling.

What are the main barriers to more widespread adoption?

There are three primary barriers, in my opinion; first, reduced quantitative performance; second, cost of implementation; and third, limited resolving power of most commercial instruments.

How is your work helping to overcome those barriers?

Our work has primarily focused on overcoming the first and third barriers.

Regarding the first barrier, we are implementing several strategies to improve the quantitative performance of LC-IM-MS techniques (1). In collaboration with Agilent Technologies, we are using multiplexed acquisition to increase our duty cycle (or ion utilization efficiency) from <10% to >50%. And that has resulted in improved sensitivity and limits of detection by approximately one order of magnitude. Agilent has also developed a high-resolution demultiplexing software that allows

for post-data acquisition improvements to resolution. Finally, we are using structurally-selective derivatization methods that introduce a fixed charge onto our anabolic steroids, significantly improving the ionization efficiency and thus sensitivity.

To improve resolving power, we are also developing structurally-selective reactions. To date, we have published on alkene-specific reactions, including ozonolysis and the Paternò-Büchi reaction, and have recently submitted a paper focusing on carbonyl- and hydroxyl-specific reactions; the latter (derivatization by 1,1-carbonyldiimidazole) has resulted in significantly improved resolution of stereoisomers.

What other cutting-edge technologies could improve anti-doping analysis if they were more widely adopted?

The ability to definitively identify unknowns and/or long-term metabolites of known performance enhancing drugs is one of the current challenges in anti-doping. Although experimental strategies have considerably improved our capability, computational methods are also crucial to further progress. We currently collaborate with computational chemist Roberto Peverati and his group at Florida Institute of Technology to develop novel computational modeling strategies and machine learning algorithms in an effort to create a predictive database of potentially novel substances.

Should the analytical scientist's responsibility go beyond simply measuring and providing the values?

Computational methods have also come a long way in predicting the biological effects of certain drugs. Our ability to do this for "novel" substances might allow us – in the future – to determine those compounds that will provide the greatest performance enhancing benefits and/or the greatest detriment to athletes' short- and long-term health.

Christopher D. Chouinard is an Assistant Professor in the Department of Biomedical and Chemical Engineering and Sciences, Florida Institute of Technology, USA

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THE NEXT FRONTIER: GENE DOPING

Cheating athletes may be on the cusp of being able to enhance their sports performance through gene modification. Mario Thevis talks us through his team's efforts to answer the crucial question: Could we ever know?

What is the current state of gene doping?

Substantial advances in developing strategies to cure life-threatening diseases have been accomplished over the past decade through gene transfer, gene editing, and gene knockdown studies. But we must consider the potential misuse of these strategies for illicit performance enhancement amongst athletes. Whether or not athletes have been employing gene doping methods to date is difficult to say – but it is vital to continue working on analytical methods that support anti-doping tests to minimize that risk.

What are the major analytical challenges to detecting gene doping?

A major challenge in developing testing strategies for new therapeutic approaches that might be misused for doping is identifying suitable target analytes in the test matrices commonly available in routine doping controls. The available window to detect gene doping is another important factor that we're trying to get to grips with. We're talking about novel approaches, mostly at the experimental stage – so little information concerning the detection of their (mis)use exists. And that's why preventive anti-doping research is vital for future sports drug testing programs.

Tell me about your research into identifying SpCas9 in plasma using HPLC–HRMS/MS

As far as we know, this was the first study to target SpCas9 – a key variable in CRISPR/Cas-based gene editing – for anti-doping purposes (1). Cas9 is of bacterial origin, which makes it xenobiotic to humans; if it's found in a human sample we have strong evidence of deliberate use and injection of the endonuclease. The detection of Cas9 (and its inactivated analog) is based on its extraction from plasma by means of immunoaffinity purification – a highly specific extraction procedure that concentrates the target analyte in a comparably

small volume for subsequent cleavage into peptidic fragments. These fragments consist of defined amino acid sequences that are sensitively analyzed with high specificity by means of high performance liquid chromatography (HPLC) combined with high-resolution mass spectrometry (HRMS), which enables the unequivocal identification of Cas9 in a complex biological matrix, such as human plasma.

Why did you choose HPLC–HRMS/MS? And are there any other techniques that might help detect gene doping?

HPLC-(HR)MS/MS is commonly applied in anti-doping laboratories and, obviously, a typical strategy to detect and identify peptidic analytes in numerous other disciplines. Its proven sensitivity and specificity are extremely valuable for the required performance characteristics of the test methods, especially in terms of reproducibility and robustness. Surely, complementary methods can be employed to help identify gene doping practices, and those are equally relevant and required to comprehensively cover the numerous options of gene manipulation.

Do you expect gene doping to become more prevalent in the coming years?

In a broader context and in all its potential facets, it cannot be excluded that gene doping will receive more attention by cheating individuals than is presumably the case right now. To what extent this will happen is difficult to specify.

Thinking more broadly, should the analytical scientist's responsibility go beyond just measuring and providing the values?

My perception is that the role of the analytical scientist does already go beyond that. For instance, at the Center for Preventive Doping Research and at numerous other centers of expertise in anti-doping, the role of the analytical scientist is interpreted in various ways, including the development and optimization of test methods, as well as providing the required analytical test results for anti-doping organizations and respective case management. In addition, it is a priority also to support the sport community with research data, results, expertise, and communications concerning observations that appear essential for protecting the integrity of sport and, most importantly, the clean athlete.

Mario Thevis is Professor for Preventive Doping Research and Director, Institute of Biochemistry/Center for Preventive Doping Research, German Sport University Cologne, Germany

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SPOTTING THE BAD APPLES

To all honorable athletes out there, be careful where you source your supplements – they could be laced with anabolic steroids! But fear not, because Xia Xu and her team at Zhengzhou University have developed a 2–5 fold more sensitive detection method, which she discusses here.

Why are people adding anabolic steroids to foods!?

Anabolic-androgenic steroids (AAS) are a class of chemical synthesis derivatives, similar to testosterone in structure and activity, and can enhance physical conditioning, including body mass and muscle strength. Banned for the first time at the 1976 Montreal Olympics, they're often abused by athletes to increase muscle quality and improve their overall performance.

Some health foods claim to have strength-enhancing properties, which are attractive to bodybuilders and athletes, but the actual effects are usually not significant. But this interest can tempt some unscrupulous manufactures to add AAS to otherwise healthy foods to enhance their pharmacological effects – and thus increase sales. However, AAS can cause a range of adverse effects, including brain and cognitive abnormalities – not to mention bans from sporting competitions. To ensure food safety and maintain market order, we set out to develop a quantitative analytical method for detecting AAS in health foods.

"Foods that claim to have strength-enhancing properties may be adulterated with anabolic steroids – so dietary sports supplements are certainly on the list."

What sorts of foods are being adulterated with anabolic steroids?

Foods that claim to have strength-enhancing properties may be adulterated with anabolic steroids – so dietary sports supplements are certainly on the list. In China, AAS may also be illegally added to some traditional Chinese medicines. In addition, because AAS can reduce the fat ratio and improve the feed conversion rate, AAS may also be added to animal feed for the purpose of improving the economic benefits of animal breeding.

What methods are usually used for this kind of analysis?

Numerous detection methods for AAS analysis have been developed, including high-performance liquid chromatography with ultraviolet (HPLC-UV), gas chromatography-mass spectrometry (GC-MS), LC-mass spectrometry (LC-MS) and enzyme-linked immunoassay (ELISA). The methods that combine chromatographic separation and mass spectrometry (LC-MS and GC-MS) provide sensitivity and specificity, and for that reason have become the most widely used methods.

What were your most important findings?

We developed a novel stable isotope labeling-flow injection analysis-tandem mass spectrometry (SIL-FIA-MS)-based strategy for detecting AAS in foods, which used 3-nitrophenylhydrazine (3-NPH) to label the AAS prior to mass spectrometry analysis. The 3-NPH labeled AAS showed dual-polarity property, observing chloride adduct ion ([M+Cl]-) in negative ion mode and proton adduct ion ([M+H]+) in positive ion mode. This simultaneous monitoring [M+H]+and [M+Cl]- guaranteed 2–5 fold improvements in detection sensitivity.

Could your research have any implications for anti-doping analysis in sports? Or for athletes?

Our validated method provides very specific and high throughput screening of AAS illegally added to healthy foods, which means anti-doping tests could also be done more quickly. Thus, cheating athletes could be caught on the day of the race – or even before. This would be a huge deterrent to athletes taking AAS.

What are your plans for future research in this area?

In the future, we will explore ways to automate the specific and high-throughput methods we've established here, as well as developing screening methods for other illegal adulterants.


COMPLEXITY
resolved?

Given the fundamental patterns we find in nature, the development of new methods to unravel the most complex mixture of all – petroleum – bears fruit in many other application areas, not least environmental analysis

By Mark Barrow

My research focuses on petroleomics: the characterization of petroleum samples and related mixtures using mass spectrometry (MS), particularly ultrahigh resolution MS. Petroleum has been called nature's most complex mixture: crude oil contains organic components represented by tens or hundreds of thousands of different molecular formulae, spanning different functional groups, polarities, and so on. The complexity of this substance presents a range of challenges for the analytical scientist.

Firstly, we must choose appropriate sampling methods: options include taking a one-off sample at a given time, or using passive sampling over an extended period of time (which loses the time resolution of the one-off approach). Next, we must decide on the best methods of sample storage and preparation; particular extraction and fractionation procedures may demand particular solvent and pH conditions, so we must consider the effect of such conditions with respect to the solubilities of each of the huge number of components in our samples. We must choose ionization methods suitable for the sample components, while also considering the potential for fragmentation and generation of different ion types. Similarly, we must select MS approaches according to factors such as resolving power and mass accuracy, and with regard to likely performance when combined with chromatography or MS/MS systems. Finally, we must have access to effective data storage, processing, and analysis techniques; it is crucial to provide outputs that are easy to visualize, interpret, and understand.

For all these reasons, petroleum analysis – just like the analysis of other very complex samples – demands the employment of multiple tools. And that reminds me of the Asian parable of the blind men examining an elephant (the man at the trunk thinks he is touching a snake, the man at the leg thinks he is touching a tree-trunk, and so on). Each man reaches a different conclusion about the nature of what they are examining. In other words, there are limitations to our perceptions and limits to the information provided by any analysis method. We need multiple, complementary perspectives if we are to see the full picture and thus truly understand complex samples.

This multiple-viewpoint philosophy is key to modern methods of petroleum analysis or petroleomics. And it represents a vast improvement on the original methods used for assessment of petroleum-based environmental contamination. Consider analysis of crude oil extracts using negative-ion electrospray ionization techniques; if you rely on that single technique, you'll primarily observe naphthenic acids but not the many other components of the crude oil. By contrast, the petroleomic approach applies different ionization methods, in both positive- and negative-ion modes, and has thereby identified many problematic compounds other than the naphthenic acids, not least the naphthenic acid fraction compounds (NAFCs).

And so, though I'm convinced of the strengths of Fourier transform ion cyclotron resonance (FTICR) MS, especially with regard to mass accuracy and resolving power, I believe that it is essential to use other complementary analytical methods. Relying on a single technique will reveal only one part of our elephant!

Patterns of success

Petroleomics strategies also assist the analysis of complex samples in fields outside the oil industry. For example, we have been working to improve data analysis and data representation methods for crude oil naphthenic acid analysis – it's essential to have simple ways of presenting complex messages about highly complex mixtures. We've been working with John Headley for many years to apply these same methods to the field of environmental monitoring relating to the Athabasca oil sands in Canada. That's just one example of how developments in petroleomics can contribute to other fields – there are many others (1).

The broad applicability of petroleomics-related methods is in part a reflection of the fundamental patterns they are designed to recognize – simple patterns that we see time and time again. It's analogous to the patterns that often underlie the complex forms we see in nature; remember how the Golden Ratio and the Fibonacci series underpin such forms as the nautilus shell and patterns of plant growth. Interestingly, these patterns also find their way into the world of art – Hokusai's famous print "The Great Wave off Kanagawa" maps onto the Fibonacci series quite closely. I'm often reminded of the phrase "Simplicity is complexity resolved" – which is attributed to Romanian artist Constantin Brâncuși, who attempts to capture the essence of complex forms in his sculptures. Petroleomics has the same philosophy. The analytical tools developed by many researchers within the field essentially help us all to identify common patterns – the petroleomics equivalent of seeing the Fibonacci series – such as differences by H_2 (rings and double bonds) or CH_2 (alkylation). Common patterns are found both in industrially-derived petroleum and many other samples such as naturally-occurring dissolved organic matter. For this reason, the analytical approaches we employ for crude oil analysis, or modified approaches, can also be applied to environmental samples. After all, such complex mixtures are formed by natural processes, such as the decomposition of plant and animal remains. Of course, there are differences between them too, especially with regard to oxygen content, but the key point is that both types of sample present similar fundamental patterns that lend themselves to comparative analysis.

Real-world impact

Our environmental work includes a collaboration with Environment and Climate Change Canada, spanning nearly two decades, to assess environmental impacts of the oil sands industry in Alberta.

These deposits give Canada the third largest oil reserves in the world, but mining the oil sands and refining the oil is very water intensive and environmentally destructive – it demands three barrels of water to produce one barrel of oil, and it has left immense scars on the landscape. Furthermore, under federal regulations, the water used in the extraction process is considered contaminated. It is termed oil sands process-affected water (OSPW) and therefore must not be discharged back into the environment. Instead, the industry stores OSPW in huge man-made lakes called tailings ponds. The water in these ponds is enriched for the molecules that were not retained during the refinement process, such as naphthenic acids. The contamination can actually be visible to the naked eye – you can sometimes see an oily sheen on the water. So advanced methods to analyze OSPW and monitor the environmental impact of the oil sands industry are essential. In one of our papers (2), we compared four different techniques: positive and negative mode atmospheric pressure photoionisation (APPI), which is good for non-polar constituents, and positive- and negative-ion electrospray ionization (ESI). Our results showed that APPI revealed many more peaks than ESI. This further emphasized the point that complex samples contain many components that we won't see, if we don't look for them with the right tools.

Historically, however, monitoring environmental contamination in this region is complicated by the fact that the rivers and lakes are in contact with the oil sands, so when you analyze them, you get a complex signature which looks almost like that of a crude oil. How can you tell if the compounds you see have their origin in industrial processes? We have worked on approaches to address this problem, and developed methods that demonstrate clear differences between environmentally-sourced and industrially-sourced water samples (3). They have unique fingerprints. Furthermore, we also demonstrated that it was possible to differentiate between the origins of samples from different companies according to their signatures, and we summarized this using principal component analysis (PCA). In particular, our analytical approach showed clear ratio differences with regard to sulfur-containing species, depending upon the sample origins. Thus, our petroleomics methods not only reveal differences between environmentally- and industrially-sourced samples, but go further and can also differentiate between one industrial source and another.

Since our earlier work, we have been very pleased to see there has been increased use of ultrahigh resolution MS for environmental monitoring in Canada and we continue to work with Canadian collaborators to develop better analytical methodologies. The aim is to truly understand the nature of these complex samples, and to further explore how sample collection, preparation, and analytical technologies may influence the character of a given sample. The latter insight is important; if we are to compare results from different laboratories, it is essential to understand how changes in analytical parameters can affect results. For example, we recently reported that analysis of the O_2:O_4 compound class ratio (i.e. ratio of $C_cH_hO_2$ and $C_cH_hO_4$ compounds) by different labs does not reliably indicate the source of contamination (4). Why? It can be challenging to compare data because labs that use liquid chromatography may change the sample pH to make it compatible with the column and a change in pH can drastically alter the O_2:O_4 ratio – or even invert it! Our work in this area has emphasized the importance of true, like-for-like comparisons when analyzing and comparing data from different sources.

The methods developed by us and by other researchers help us to better characterize industrially- and environmentally-sourced water samples from the Athabasca region, identify origins, and improve

environmental monitoring. Furthermore, improved analytical methods support development of more sophisticated remediation strategies. Remember, the long-term aim is to decontaminate the tailings ponds, so that the water can be returned to the environment. To do that, we must first know what contaminants are in that water, then develop methods to remove them, and finally have reliable techniques to confirm that decontamination has been effective. Robust analytical methods also allow us to answer questions about nature's involvement. For example, do plants remove contaminants and process them, or do they just store them such that when the plant dies the contaminants are released back into the water? In brief, achieving a better understanding of the range of contaminants assists with both monitoring and future remediation.

We have also compared environmental monitoring methodologies as part of a collaboration with the British Geological Survey (BGS) to analyze soil core samples from Staten Island in the US. As part of the study, we compared two different methods: direct infusion, high field FTICR MS versus lower field FTICR combined with gas chromatography (GC) (5). Interestingly, we also explored how 2-omega detection gives you the option of halving your experiment time for a given resolution or doubling your resolution for a given length of operation. That's useful when you have a GC step; you want the FTICR's scan times to be as fast as possible, as the FTICR is typically much slower than the GC.

As analytical scientists, we must be relentless in our pursuit of new methodologies and in our efforts to improve our data analysis – there is always more information to be extracted from a a given (complex) sample.Once we have developed better tools, we can use them in many different applications and so many fields can benefit from advances made during the course of petroleomics research.

Is there an elephant in the room?

It is clear that ultrahigh resolution MS provides significantly deeper insights into highly complex mixtures. It has helped us establish sample profiles that in turn enable us to better understand both industrial processes. Advanced methods are essential when it comes to understanding the (complex) changes that oil may undergo in the environment, such as through evaporation, exposure to sunlight, and biodegradation. Effective environmental protection strategies need these data to identify and monitor contamination; for tracking contaminants that subtly change over time, we need even more sophisticated methods.

With respect to petroleomics, the ability to analyze very detailed signatures with very high resolution is essential; as I mentioned, each sample contains a huge range of diverse components – polar and non-polar, basic and acidic, large and small. As part of our contribution to advancing analytical methodologies for complex samples, we recently developed a combined experimental and data processing approach, named OCULAR for short, that led to the successful characterization of a particularly challenging non-distillable fraction of heavy petroleum. This resulted in the assignment of a record breaking 244,779 unique molecular formulae – not peaks – within a single sample without the aid of fragmentation or chromatography (multiple peaks may be observed for each molecular formula when using chromatography, due to the presence of isomers) (6). When considering this sort of number of unique molecular formulae and then allowing for the presence of isomers per formula, it is clear that researchers are potentially faced with millions of different structures within petroleum samples! Notably, the existence of many isomers per component of petroleum directly affects the reactivities (and toxicities) of these components and their abilities to interact with catalysts. This latter point is particularly important: the catalyst compatibilities of different isomers determines the efficiency of sulfur removal from petroleum as part of the production of low sulfur fuels.

By developing analytical methods appropriate for complex samples as daunting as petroleum, we contribute to advances in complex sample analysis in many other fields too – and that makes my work even more rewarding.

Although none of us will individually have the full picture, we are collectively getting a better understanding of those elephants!

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GC×GC APPLICATION SPOTLIGHT:

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Core Topic
Mass Spec

Origins of the silk road. Sericulture, or silk farming, has played a pivotal role in modern human history. As a luxurious commodity, silks can tell us a lot about ancient global trade, textile use, and technology developments. Though the mulberry silkworm, *Bombyx mori*, is the most commonly used species for silk production, a range of other region-specific moths appear to have been used throughout time – but classifying silks by species type has proven technically challenging. Now, researchers at The University of Oxford, UK, have developed an improved method for solubilizing and identifying silk fibroins using nano-flow LC-MS/MS. They then applied this to ancient Palmyran silks and found the first evidence of silk production and export using wild silk moths from India.

All in on OrbiSIMS. With the volume of mass spectral data increasing all the time, interpretation of such datasets is proving increasingly difficult. Automated peak assignment based on molecular formula prediction (MFP) is widely used in other areas of MS, but has not yet been applied to secondary ion MS. Enter Max Edney and a team from the University of Nottingham, UK. Using a combination of MFP and double bond equivalence measures, the team was able to chemically filter 3D OrbiSIMS datasets from a series of increasingly complex samples. Though largely a success, the team also highlighted a number of limitations still to be overcome, including the high number of possible protein peak assignments.

PCR or MALDI-TOF? To overcome some of the limitations with RT-PCR diagnosis, a group of researchers from the National Institute of Health Sciences in Japan has developed a MALDI-TOF MS-based method for direct detection of SARS-CoV-2 in nasopharyngeal swabs. Seven peptides derived from the viral nucleocapsid phosphoprotein (NP) were chosen as targets for the clinical specimens, and a purification method for eluting and extracting the NP was developed. Though less sensitive than RT-PCR, the MALDI-TOF method offers advantages in operability, time, and cost – and could help identify contagious patients for isolation.

Where is structural proteomics headed? In a review of the latest developments in structural proteomics, two researchers from Skoltech and McGill University highlighted the increasing availability of high-resolution, high-sensitivity instruments enabling advances in protein structure characterization – especially in cells and tissues. The authors are anticipating further development of MS-based technology, including improvements in top-down analysis, new fragmentation techniques, new reagents for cross-linking and gas-phase reactions, and more user-friendly software programs and servers – perhaps even the integration of machine-learning algorithms.

IN OTHER NEWS

Want to contribute to a space mission and get your hands on $15,000? NASA is looking for someone to build a model to analyze MS data collected for Mars exploration.

A proof-of-concept study shows promise for on-chip MS detection of malaria using a 3D microfluidic paper-based device.

Researchers develop an ultra-high throughput IR-MALDESI sampling approach capable of analyzing 22.7 samples per second.

The 2022 ASMS Biemann Medal has been awarded to Erin Baker for her contribution to the development and application of IMS-MS technologies and her involvement in establishing the Females in Mass Spectrometry group.

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Will the Real SLIM Please Stand Up?

Melissa Sherman, founder and CEO of MOBILion Systems, shares the company's journey over the last five years – from a sketch on a piece of paper to launching at ASMS 2021

Can you tell me a little about your background and the founding of MOBILion Systems?

By training, I am a polymer chemist. But my corporate career, which has spanned the past 25 years, has focused on building businesses. I've always had a passion for the business side of things – and I've worked in a variety of areas, including technology, regenerative medicine, surgical products and the apparel industry (our startup was featured in Wired magazine in 2000 as a disruptive fashion business). I really love growing something from nothing, and this is part of the reason I was so excited to have the opportunity to build MOBILion.

I was introduced to the technology via IP Group, which is an early-stage technology investor that forms long-term partnerships with research institutes to help guide disruptive science in a commercially viable direction – from proof of concept to commercialization. I initially worked with IP Group to build their US business, and once we had the US team in place, we set out to scout disruptive technologies from a handful of universities and federal laboratories. And that's where I first came across structures for lossless ion manipulation (SLIM) – the platform technology behind MOBILion's first high resolution ion mobility (HRIM) product, MOBIE – which was being developed by Richard Smith and his research group at Pacific Northwest National Laboratory. I wrote the investment thesis and told IP Group it was something I thought would have a tremendous impact on the life science tools industry. Our early stage financing milestones included sponsoring research to achieve technical proof of concept and building early prototypes. I was so excited by the possibilities of this technology, I left IP Group in 2017 and became the founding CEO of MOBILion – the rest is history.

What was your "dream" when you set out – and has that been realized?

I saw it as a once in a lifetime opportunity – I truly believed SLIM could have an impact in so many places. Even now, I'd be lying if I said I know exactly where all those places will be or where the greatest impact will be, but that's part of the fun for me. It's almost like being an explorer, discovering uncharted territory. I recognized the potential five years ago and that's why I left IP Group to dedicate all of my time to MOBILion. At the highest level, our mission has not changed at all in five years – we set out to improve how we predict, diagnose, and treat disease. Whether that means better characterizing therapeutics and getting drugs to market faster, better safety and efficacy profiles, discovering the next biomarker, or having a clinical diagnostic instrument that can detect biomarkers to diagnose diseases earlier.

Have there been any major challenges along the way?

The obvious one is COVID, right? But there have been other challenges. When

we set out, I was the only employee and we didn't have an office or lab – it really was like starting with a clean sheet of paper. Our first key milestones were hiring a team, getting a space to work in, and transferring the tech – making sure Richard Smith didn't have to hold everyone's hand to operate the technology. Next was product development – going from a "Frankenunit" primitive prototype to something that was manufacturable and scalable. That was probably the biggest challenge.

Thankfully, we've got a great team who always seem to find a way through whatever roadblock we hit – COVID-19 included. Perhaps our greatest achievement is how we overcame these challenges to launch at ASMS 2021. When we first attended ASMS in 2017, we had nothing but a drawing of our product on a piece of paper. Looking ahead to future ASMS conferences, we saw that 2021 was in Philadelphia (our backyard) – and we set our sights on ASMS 2021 for the launch. Somehow, despite COVID, we did it – which is a testament to the team and their relentless perseverance, dedication, and drive.

How is high resolution ion mobility MS different to other ion mobility systems – and what are the particular benefits of SLIM?

SLIM technology is based on doing separations in the gas phase on printed circuit board technology; one major advantage is that it's one-size-fits-all. So unlike LC systems, for example, you don't need to think about columns and hardware component changeout for the different separations you are performing. In other words, it is an analyte agnostic separation platform with easier, simpler method development that applies to lipids, glycans, peptides, or proteins. Because the separation is in the gas phase, it is achieved in seconds or minutes as compared to minutes or hours. For example, a peptide map fingerprint experiment might take 90 minutes on an LC-MS system, we can achieve that analysis in 5-10 minutes with greater elucidation of key posttranslational modifications. The MOBIE product and the core technology is driven by electronics and the separation is based on physical properties of ionized analytes, resulting in better reproducibility and easier push-button operation. The main advantage is the deeper level of characterization we are able to achieve. We like to say we "reveal what others leave unseen," and it's true – our SLIM technology allows us to separate and identify molecules that other instruments fail to detect. Many times, even if there are other tools able to detect key analytes, we are able to do it faster, easier and with greater reproducibility, eliminating the tradeoffs associated with other approaches.

You seem to be targeting the biopharma space at the moment.

Yes. As I mentioned already, there are a number of application areas for MOBIE – but we have to focus and start somewhere. Initially, we are focusing on the need for better characterization of biologic therapeutics. The current trend toward large molecule therapeutics requires better, more powerful instrumentation to adequately characterize more complex systems and provide that information faster than ever before, to get drugs to market faster. So, for our first product, we are working with our early adopters to enhance monoclonal antibody characterization and peptide mapping – specifically looking at posttranslational modifications (PTMs), among other characterization workflows. Essentially, we are delivering faster, better, deeper characterization of critical quality attributes that a biopharma company identifies and monitors to optimize the safety and efficacy of the drug.

Could you provide more details of the MOBIE system?

Our High-Resolution Ion Mobility product, MOBIE, is integrated with Agilent's 6545, 6545XT and 6546 Q-TOF mass spectrometers to help pharma companies develop safer and more effective biologics, as well as to aid researchers in discovering novel biomarkers. The core SLIM technology inside of MOBIE was designed to have a very long separation path length – 13 meters to be precise – condensed in a small form factor. Other ion mobility approaches max out at a meter. Why do we care about 13 meters versus one meter? Because the longer the path length, the more separation you can achieve. And that translates again to revealing what others leave unseen.

What are you most excited about for the future of MOBILion Systems?

I think I'm most excited about the breadth of potential applications. People often ask, "What's the killer application for MOBIE?" My response is usually along the lines of, "What's the killer application for liquid chromatography?" The fun part is going to be working with our partners, collaborators and early adopters to explore many different applications and uncharted territories with our customers. Gaining customer feedback is also key to our evolution. MOBILion is not a one product and done company – the SLIM form factor is amenable to achieving nearly endless design combinations and permutations. There's a tremendous opportunity for us to succeed in many different areas of life science. In the separation science world, there are many applications and many customers that would benefit from faster, deeper, easier, and more reproducible separations. I am really excited to see how much we are going to learn over the course of the next few years – and to what extent we will help our customers better predict, diagnose and treat disease by putting better characterization tools in their hands.

Let's Make Data FAIR

Embracing accessible, community-supported, interoperable data standards is key to delivering on the promise of Open Science

Dr. Frauke Leitner, Product Manager, Data Suite at Connected Lab, Merck KGaA, Darmstadt, Germany

Collaboration has always been integral to success in science. In the past, collaborators contributed with their knowledge, their instruments, and reports of the results generated. But today, this collaborative spirit also includes the open sharing of the original raw data.

This growing trend towards greater data openness was initially driven by academia, but industry has come to appreciate the immense scientific and commercial potential associated with high quality scientific data accessible to all scientists. Today, there is this wealth of information in databases in the public domain – and it is often the foundation of pharmaceutical R&D.

In this new world of sharing, the pharma industry has typically played the role of data consumer. But that too is beginning to change; many companies now publicly share in-house generated data without the prospect of direct and immediate value – a step forward that was unthinkable not so long ago. However, though companies are increasingly open to sharing data, the community must overcome some technical hurdles – especially, data interoperability – if the Open Science movement is to truly take off. If companies aren't speaking the same language, willingness to share can only go so far. Instead of using countless proprietary file formats, we need to move towards standards that are accepted across the industry, making both sharing as well as reuse of data easier. And that's where FAIR comes into play.

Making data FAIR

FAIR stands for findable, accessible, interoperable, and re-usable:

- Findable – discoverable with machine readable metadata, identifiable, and locatable by means of standard identification mechanisms
- Accessible – available and obtainable to both human and machines
- Interoperable – both syntactically parseable and semantically understandable
- Re-usable – sufficiently described and shared with the least restrictive licenses and cumbersome integration with other data sources

Before I go on, allow me to quash the misunderstanding that FAIR data needs to be completely open to everyone and, therefore, it cannot be applied in certain settings, such as in the healthcare industry (where data sets often comprise sensitive personal data) or in the private sector (where data might be subject to intellectual property rights). In fact, FAIR does not require data to be fully open – it simply requires that access conditions for data sets are open and transparent. In practice, data of a highly sensitive nature can be found via its metadata, with access handled very restrictively following evaluation by an ethics committee.

Despite some misunderstandings, we are seeing increasing adoption of FAIR principles as part of the general embrace of Open Data. Over the past five years, the debate has evolved from a question of whether to implement FAIR to *how* to implement FAIR. The major barrier is a lack of experience – in-house solutions often only address part of the problem and serious discussions are needed to address a couple of questions: Does everything need to be FAIR? What kind of data and metadata provides real added value? FAIRification of data is a journey, and the level of FAIRness that is required depends on the specific use case. Elements that can help to improve FAIRness are identifiers to make data findable, authorization mechanisms to allow accessibility, data standards, and ontologies to make data interoperable.

A different AnIML

The Open Science vision of the future – with companies sharing raw data for the betterment of all – is something we should all be aiming towards. Unfortunately, many companies don't even do a very good job of sharing data *within* their own organization – and this lack of data interoperability can have significant impacts on scientists carrying out their daily work. In many organizations, individual experimental results are shared with colleagues via written reports summarizing the main findings. Usually, this is siloed, where colleagues across the floor don't have access to the original data because it is saved in proprietary files, which only certain people in an organization can open.

The current trend, in line with FAIR principles, is to move towards converting data into open, accessible, and community-supported data standards – an interoperable approach. One such standard is AnIML, the open-source XML standard supported by ASTM International. AnIML provides standardized ways of applying digital signatures to scientific data (which some regulations require), offers the ability to record changes to AnIML documents as

part of a built-in audit trail, and includes Experiment Steps that document how a particular analytical technique has been applied to a sample – a basic building block in any analytical workflow.

AnIML is a standard for analytical and biological data, but since AnIML files can – in principle – capture data from any scientific technology, efforts are ongoing to drive adoption of the AnIML standard across a wide variety of scientific domains.

Sharing prosperity

In the public sector, scientists applying for funding are increasingly expected to provide a data management plan along with their application, detailing how they plan to store their data, what metadata needs to be tracked, where the final data set will be published, and which data standards they will implement. This example shows how people can be encouraged to embrace a "data first" mindset, which might be tedious at first but provides valuable guidance and structure, while also supporting good practice in data management – all of which benefits Open Science.

The same forward-thinking mindset can also be applied to the choice of data format – in both the short and long term. In the short term, increasing interoperability and secondary re-use of data is key. In the long-term, you want to be able to open your files in 30 years from now without the need to maintain outdated software for the sole purpose of opening your files locked in proprietary formats (a problem in many organizations!). Converting your data at the point of creation or after initial processing into an open-source XML standard – like AnIML – provides a solution to both your short- and long-term storage needs.

The movement towards FAIR data is picking up speed as almost everyone agrees that we must keep striving towards high-quality, interoperable, open data standards that are supported by the community and across scientific disciplines. But we're only at the beginning of the journey – companies have a great deal of work to do to define the right level of FAIRness for their data. Regardless of your role within the organization, you can start a discussion on FAIRification of data or participate in already ongoing efforts. Sooner or later, this topic will concern many stakeholders across an enterprise – including lab managers, researchers, data scientists, and QA/QC experts.

For many companies, immediate benefits spring from embracing new ways of collecting and making data accessible to colleagues. And others may have bigger dreams; what questions currently facing humanity will the availability of your data answer in the long-term? In either case, making data FAIR is key.

Core Topic
Chromatography

Cation Catching Combo. The concentration of ammonium cations (NH_4^+) cations in bodies of water is an important environmental indicator, as high levels of ammonium – often caused by industrial pollution or excess fertilizer – can cause toxic algal blooms. The two commonly used methods, ion chromatography and potentiometry, have their advantages and disadvantages – the former is quick and easy, but lacks the sensitivity of the latter. So researchers from Sweden and Portugal combined the two methods, creating a flow cell with space for three ion-selective electrodes coupled to an ion chromatography column. They found that the combination could detect ammonium ions in 10 environmental water samples at micromolar concentrations.

China Strikes Oil. The demand for oil shale is increasing in China as the country aims to move away from coal as part of its commitment to net zero emissions by 2060. In recent years, small amounts of oil shale have been found in the Nyima Basin, Central Tibet, which prompted a team of researchers from China to analyze geological rock samples from the area with GC-MS. The authors were able to demonstrate that the Nyima Basin has good potential for hydrocarbon extraction and production – with potential implications for China's energy strategy going forward.

Novasep Acquisition Confirmed. Sartorius has closed the acquisition of Novasep's chromatography division following approval by the US Federal Trade Commission. Novasep's portfolio comprises chromatography systems primarily suited for smaller biomolecules, such as oligonucleotides, peptides, and insulin, as well as systems for the continuous manufacturing of biologics; and employs approximately 100 people, the majority of whom work at its Pompey site in northern France but with some in the USA, China, and India.

From Ashes to Masses. Most of the smoke from wildfires consists of well-studied carbon particles. But much less is known about the other sulfate, potassium, and fine ash particles. In 2019, a NASA aircraft flew through smoke plumes produced by wildfires, which researchers from the US and Japan analyzed using ion chromatography and transmission electron microscopy. They found that the fine ash-bearing particles were mostly collections of calcium- and magnesium-containing compounds, and made up eight percent of smoke particles by number and five percent by mass. Experiments have shown that these chemicals can affect the climate by promoting the formation of both clouds and ice. Their effect on health is currently unknown – though they are small enough to enter the lungs.

IN OTHER NEWS

Researchers from the Punjab Forensic Science Agency, Pakistan, uncover heroin hidden in a fabric coating with a modified GC-MS protocol.

Chromatography column provider Phenomenex celebrates the 40th year anniversary of its founding – beginning as a "small idea in a garage" in 1982.

China-based researchers combine "bubbling extraction" – a new sample pretreatment method – with GC-MS to successfully analyze volatile compounds in beer.

France-based researchers use UPLC-MS/MS to evaluate matrix effects in water samples from lakes and rivers, and describe strategies for minimizing them, in a bid to quantify steroid hormone pollution in surface water.

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Popular Reflections: Is Chromatography Still Losing the War?

"Chromatography: Winning Every Battle, Losing the War?" In 2015, Hans-Gerd Janssen argued that other techniques are beating chromatography and mass spectrometry to the hero's reward all too often. Has best-in-class triumphed over fit-for-purpose?

Credit: Pixabay

Has progress been made in generating "faster first results" since you wrote the article in 2015?

To be honest, I don't think that massive fundamental improvements have been made. But we have seen a decent number of small incremental improvements. Higher resolution GC-MS and LC-MS allow us to cover more than 1000 target compounds in one run. So clearly the number of methods needed has gone down. Libraries of LC-MS spectra are now available, improving black-box automated identification and operation. Universal sample preparation methods and data integration routines have been implemented, reducing the time to first results; faster analyses allowing a higher time resolution are possible using fast GC-MS methods; and continued miniaturization of sample preparation has further reduced the use of chemicals. Unfortunately, there are also areas where not that much progress has been made. Localized compositional analysis and "molecules in context – neighbors and interactions" are examples of such fields. I wouldn't say we have focused on the low hanging fruit only, but I would argue some of the most difficult fields remain.

Are you still concerned that chromatography might go the way of the "typing department, the calculation department, the organic chemistry department..."?

Chromatography will never disappear. Mankind is facing massive challenges in the environmental field, energy, health and mental wellbeing, food supply, and so on. Chemical analysis of problems and solutions will remain needed, and this will very likely become even more important. Other techniques – in particular, spectroscopic methods like NMR – may be very powerful, but they cannot provide the molecular resolution and sensitivity of chromatography. It is also interesting to think about why the typing department and the mail/telex room disappeared. It wasn't because the work was no longer needed; on the contrary! It was because the equipment needed became much smaller, simpler, cheaper, and easier to use. At this moment, it seems fundamentally impossible to miniaturize the high-end spectroscopic and microscopy methods to a size that would allow operation by anyone anywhere.

Thinking broadly, what has been the most exciting development in analytical science since 2015?

Improvements in the field of mass spectrometric detection have initiated a cascade of events, making chromatography (both LC and GC) faster and better than it has ever been. Because the detector has become more sensitive, injected amounts can be reduced. And that makes the system less vulnerable to dirty samples and reduces fluctuations in retention times and sensitivity – data becomes more stable and less prone to fluctuations. In turn, the results of automated identification and quantification software are improved, allowing automated interpretation where in the past manual adjustment and interpretation was needed. Instruments can now be operated with less operator interference, increasing the sample throughput, reducing the time-to-first results, and reducing analytical errors and failed analyses.

Digitizing GC×GC

How can we take GC×GC to the next level? Katelynn A. Perrault, Associate Professor of Forensic Sciences and Chemistry at Chaminade University of Honolulu in Hawaii, believes data science is the key…

Can you give me an overview of your background and research interests?

I am an Associate Professor of Forensic Sciences and Chemistry at Chaminade University of Honolulu in Hawaii. I teach courses in analytical chemistry, instrumentation, forensic chemistry, and more.

I got involved in GC×GC when I began analyzing odors from decomposing remains to see which chemicals could prompt cadaver-detection canines to alert. We wanted to know more about cadavers' complex odor and multidimensional GC was the best tool to tackle this challenge. Since then, I have developed my career as a faculty member focusing on odor analysis using flow-modulated GC×GC systems. Our applications of interest include method translation, forensic odor analysis, food analysis, traditional medicines, and more.

Outside research, I am interested in undergraduate pedagogy surrounding instrumentation in analytical chemistry (incorporating research experiences into the classroom environment) and implementing culturally sustaining pedagogy. I am also passionate about promoting the adoption of GC×GC for end users who can significantly benefit from transitioning from conventional 1D GC to a multidimensional approach.

What is the state of GC×GC today?

GC×GC has come a long way since its inception. Our workshops and conferences once revolved around fundamental instrument design and optimization – important topics to develop a foundation for an emerging technique. Now, we see conferences dominated by exciting applications across a range of topics – metabolomics, biomedical diagnostics, forensic science, environmental monitoring, drug analysis, agriculture, and forestry, to name just a few.

We've also seen a major shift toward using GC×GC for large batch data. Now, the challenge lies in how to handle that data when conducting research in complex applications. We are starting to see the emergence of data science as the next critical tool in our multidimensional chromatography toolbox. It will be exciting to see where this data revolution takes the field in the next five years.

What do you see in your crystal ball for GC×GC?

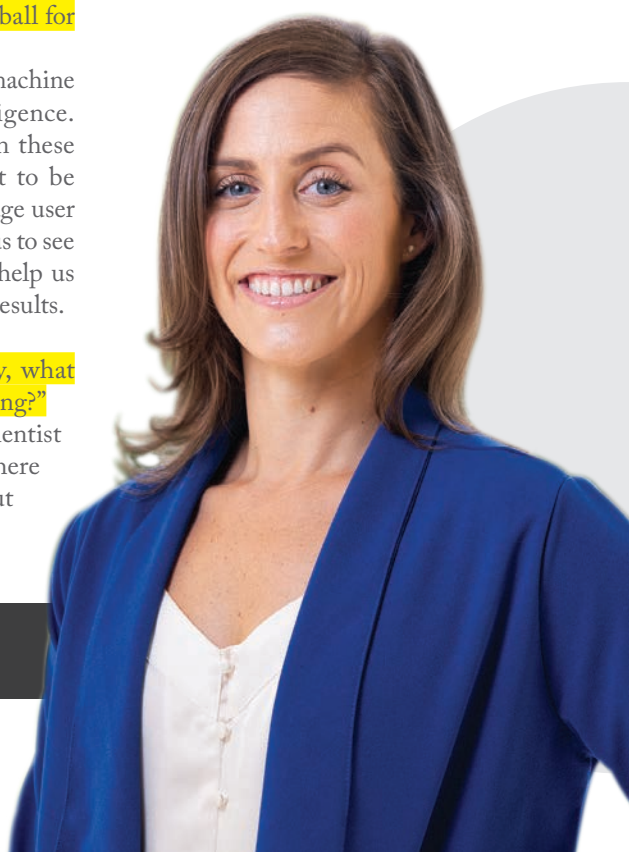
I anticipate a huge emergence of machine learning and artificial intelligence. We are recognizing the value in these technologies, but they have yet to be presented in a way that the average user can easily implement. I am curious to see what tools become available to help us get the most meaning from our results.

In terms of your research today, what "gets you out of bed in the morning?"

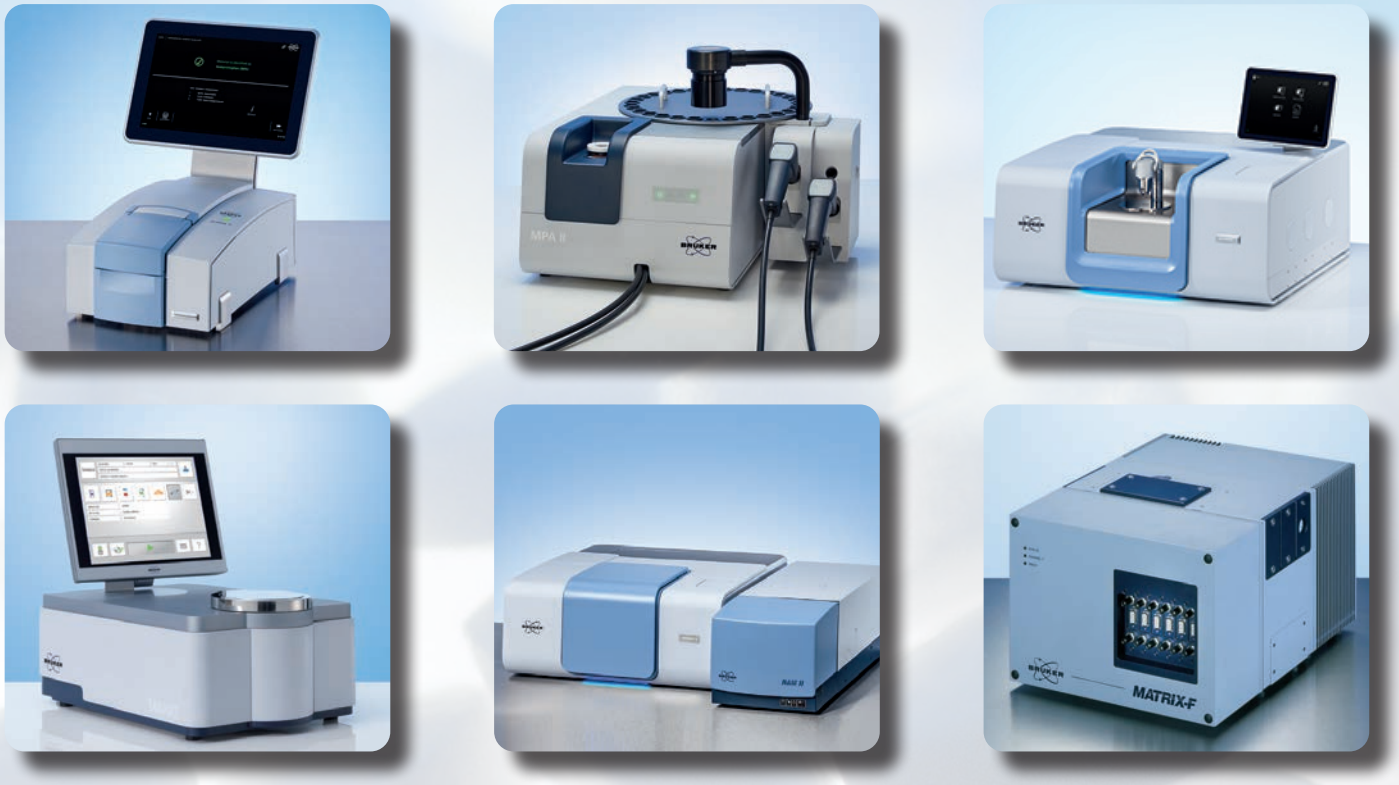
One of my greatest joys as a scientist is the pursuit of knowledge. There is something exhilarating about creating knowledge that did not previously exist. The thrill of a new discovery, a new tool, a new approach, or a new way of looking at something – that is what gets me out of bed in the morning. There is plenty of space for failed experiments, troubleshooting, and other challenges; however, the moment you stumble across something exciting, it is all worth the trouble.

Do you have any "top tips" for getting the most out of an instrument or technique?

My best piece of advice is to network with individuals in the relevant scientific community using the technique or instrument. In the case of GC×GC, conferences and other networking events were critical for me as I learned the technique, began using it, and eventually developed a research program centered on it. The GC×GC community is filled with individuals who want to share their expertise. Developing relationships with instrument vendors is also a valuable resource, especially when it comes to day-to-day operations.



Spectroscopic Solutions for the Validated Pharmaceutical Industry



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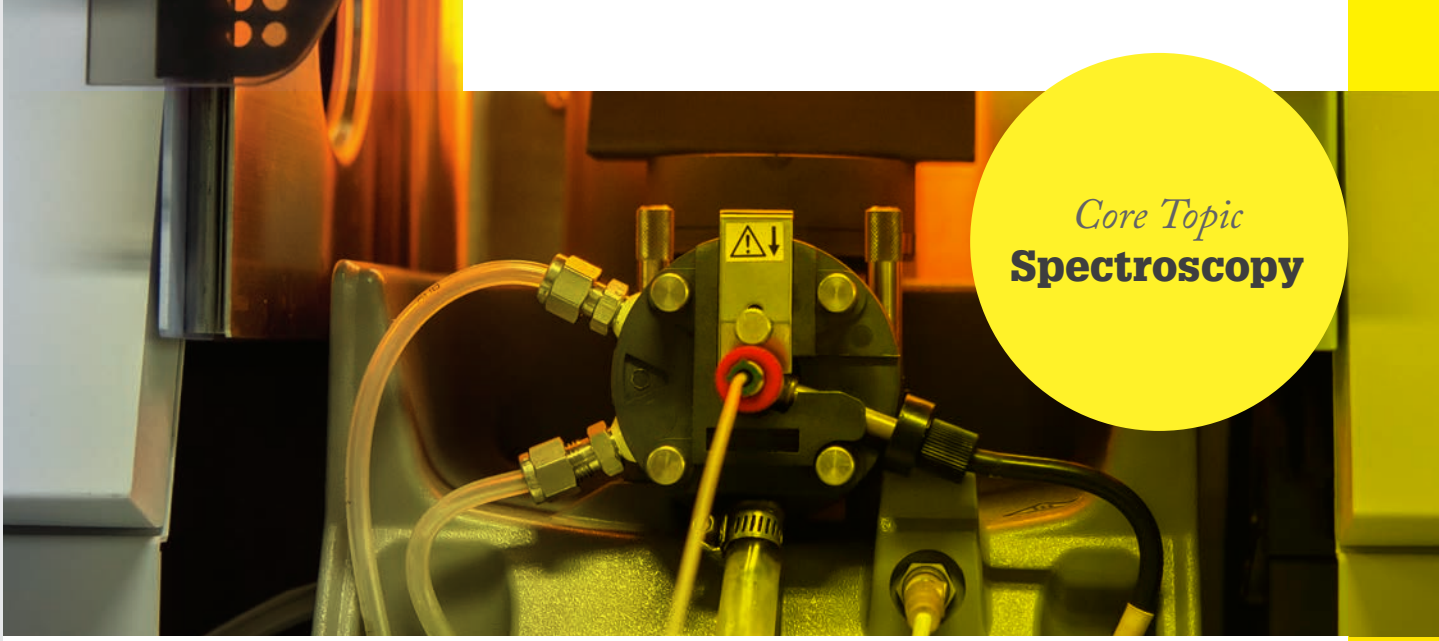

Core Topic
Spectroscopy

ID, please. A new separation-free bacterial identification technique has shown promise in reducing the time it takes to diagnose infectious diseases. Current techniques, such as surface-enhanced Raman spectroscopy (SERS), are limited in terms of their ability to obtain a clear spectra of bacteria because of numerous overlapping sources, such as proteins in cell walls. But researchers from the Korea Advanced Institute of Science and Technology decided to pair SERS with a new deep-learning model called the dual-branch wide-kernel network (DualWKNet) to avoid time-consuming bacterial separation steps, while detecting bacteria with 98 percent accuracy. The authors hope to apply their platform to additional bacterial species in both food and clinical analyses.

Award-winning research (literally). Rohit Bhargava, Founder Professor of Engineering and Chemistry, and Director of the Cancer Center, at the University of Illinois (and one of our 2021 Powerlisters), was presented with the 2022 Pittsburgh Spectroscopy Award – demonstrating his outstanding achievements in the field of spectroscopy. Bhargava was recognized for his contributions to new instrumentation and technologies in the field of digital molecular pathology. His most recent work is focused on understanding and using the native molecular content of tumors and their microenvironments for improved cancer pathology.

Probing COVID-19 immune response. Researchers from the Institute of Infectology in Sao Paulo, Brazil, have used Fourier-transform infrared reflectance spectroscopy (micro-FTIR) to explore blood serum samples of healthy and COVID-19 positive individuals, finding that the 1702–1785 cm–1 spectral window (carbonyl C=O vibration) is a spectral marker of the degree of IgG glycosylation, which was in turn linked to the degree of COVID-19 symptom severity. "Considering the minimal and reagent-free sample preparation procedures combined to fast (few minutes) outcome of FTIR, we can state that this technology is suitable for fast screening of immune response of individuals with COVID-19," say the authors.

Revolutionary ribosome research. Two researchers from the University of Houston, Yuhong Wang and Shoujun Xu, have been granted US$1.2 million from the National Institute of General Medical Sciences for new research into ribosomes. They will be developing a new type of super-resolution spectroscopy to observe exactly how ribosomes make proteins within cells – new knowledge that could potentially lead to new drug design to treat cancers and viral infections.

IN OTHER NEWS

Thermo Fisher Scientific acquires Max Analytical Technologies, a producer of FTIR-based gas analysis solutions for process monitoring.

Single-cell Raman microspectroscopy reveals the combination of disinfectants most effective at preventing re-infection, which researchers hope will prevent overuse of antibiotics.

Using atomic-scale vibrational spectroscopy, researchers demonstrate isotopic imaging of 12C carbon atoms embedded in 13C graphene, noting the technique's utility in nanoisotope engineering and monitoring.

Researchers use quantum-memory-based time-frequency processor to bypass constraints of the Rayleigh limit and achieve optical-domain spectral super-resolution.

References available online

Live, Laugh, LIBS

Richard Hark, conservation scientist at Yale's Institute for the Preservation of Cultural Heritage, argues that chemometric tools are what's needed to take LIBS to the next level

Are you using laser-induced breakdown spectroscopy (LIBS) in any of your projects?

We've used it for a few projects. We utilized LIBS to find out whether a piece of Chinese jewelry – a gigantic necklace in a series of interconnected little silver cones – was silver plated or solid silver. XRF can detect silver but cannot penetrate deeply into the metal. We decided to apply LIBS in a very inconspicuous spot, slowly making a tiny crater the width of a human hair down through the silver to explore the stratigraphy of the metal. We were able to determine that it was silvered and not solid silver. Admittedly, there may be other methods to achieve the same goal, but LIBS was also a really convenient way of gaining an approximate thickness of the silver coating at the same time.

We've also analyzed over 100 Chinese ceramics for a book being written by one of the curators at the Yale University Art Gallery. For this project, we've been using a combination of LIBS and XRF because they are so complementary. LIBS allows us to detect some elements that XRF cannot, but XRF is actually better at seeing other elements that fall below the detection limit of LIBS. We also use chemometric tools to see if there are patterns in elemental composition.

What important advances have been made in portable LIBS?

I remember using a backpack unit that was developed by Ocean Optics. It looked (and felt) like an army backpack – and it came with a wand that looked a little like a metal detector. You could zap things on the ground, which was satisfying. It actually worked quite well for some applications, but it wasn't always practical for some geological or archeological applications I was interested in. Since then, we've seen great developments in handheld units driven primarily by industrial applications – using LIBS for scrap metal sorting, or for positive material identification, to make sure you're using the right component in a manufacturing process, or even looking at the carbon content of steel. Now there are five or six companies that make handheld LIBS systems that work much the same way as a handheld XRF device – and that's remarkable. These devices have only been made commercially available over the past five years or so and represent a significant improvement on previous portable approaches.

What advances would you still like to see in LIBS?

When we use LIBS, we're often looking for clues that tell us whether an object is from a particular region – our Chinese ceramic project is a good example of this need. But NIST standards do not exist for such work, so you have to use chemometrics and machine learning tools to group similar things together. Right now, you need to become a competent data scientist or collaborate with one to access the power of LIBS. I would love to see more development of chemometric tools for non-expert users, to increase accessibility. It would be great if a scientist could start using LIBS without in-depth data science knowledge and instead simply input data into a program that tells them whether there's any clustering. I believe there are some companies working on such data analysis tools – and they will really complement handheld LIBS.

Tackling TD-fNIRS Head On

Kernel Flow: How a new wearable brain scanner plans to make brain imaging mainstream

Although time-domain functional near-infrared spectroscopy (TD-fNIRS) is currently considered the pinnacle of non-invasive optical brain imaging techniques, it can be complex, cumbersome, and costly, and, therefore, is not yet widely employed in the field. Sensing a gap in the market, engineers from Kernel, a US neurotechnology company, designed a wearable brain-imaging device called Kernel Flow (1). Weighing in at 2.05 kg, the TD-fNIRS headset contains 52 modules arranged in eight plates that fit on either side of the head. Using picosecond laser pulses and detectors to estimate photon scattering and absorption in tissues, Kernel Flow measures changes in blood oxygenation that correlate with groups of neurons firing.

When developing Kernel Flow, the engineers evaluated other non-invasive brain imaging techniques, including EEG, ultrasound, fMRI and MEG, but ultimately homed in on TD-fNIRS - "the perfect combination of scalability, temporal resolution, spatial resolution, and wearability to build a mainstream brain interface," according to Ryan Field, Kernel's chief technology officer. Importantly, the team wanted to overcome the limitations of traditional TD-fNIRS, while maintaining the performance of a research grade system. "Our goal was to build a scalable brain interface that could someday be used by anyone and everyone, and to create the infrastructure to enable the mainstream adoption of brain measurement," says Field.

The team used standardized methods of assessment for brain imaging instruments and a commonly used validation task to assess the system's performance; the results showed that the Kernel Flow headset demonstrated performance comparable to existing TD-fNIRS benchtop systems. Although these results are very promising, Kernel highlights the need for future work, including the collection of additional human neuroscience data and the evaluation of system performance with different hair and skin types. Both are things the team at Kernel is currently working on.

Kernel has expressed interest in applying their system to novel drug discovery, pain measurement and management, healthy brain aging, cognitive changes, and elite performance. "We believe that a large amount of high-quality data collected in a standardized way will be the key driver of innovation in applying brain measurements to personal insights," explains Field. "We also hope that, one day, using a brain interface will become as common as picking up your phone."

References

1. *Ban et al., Journal of Biomedical Optics (2022). DOI: 10.1117/1.JBO.27.7.074710*

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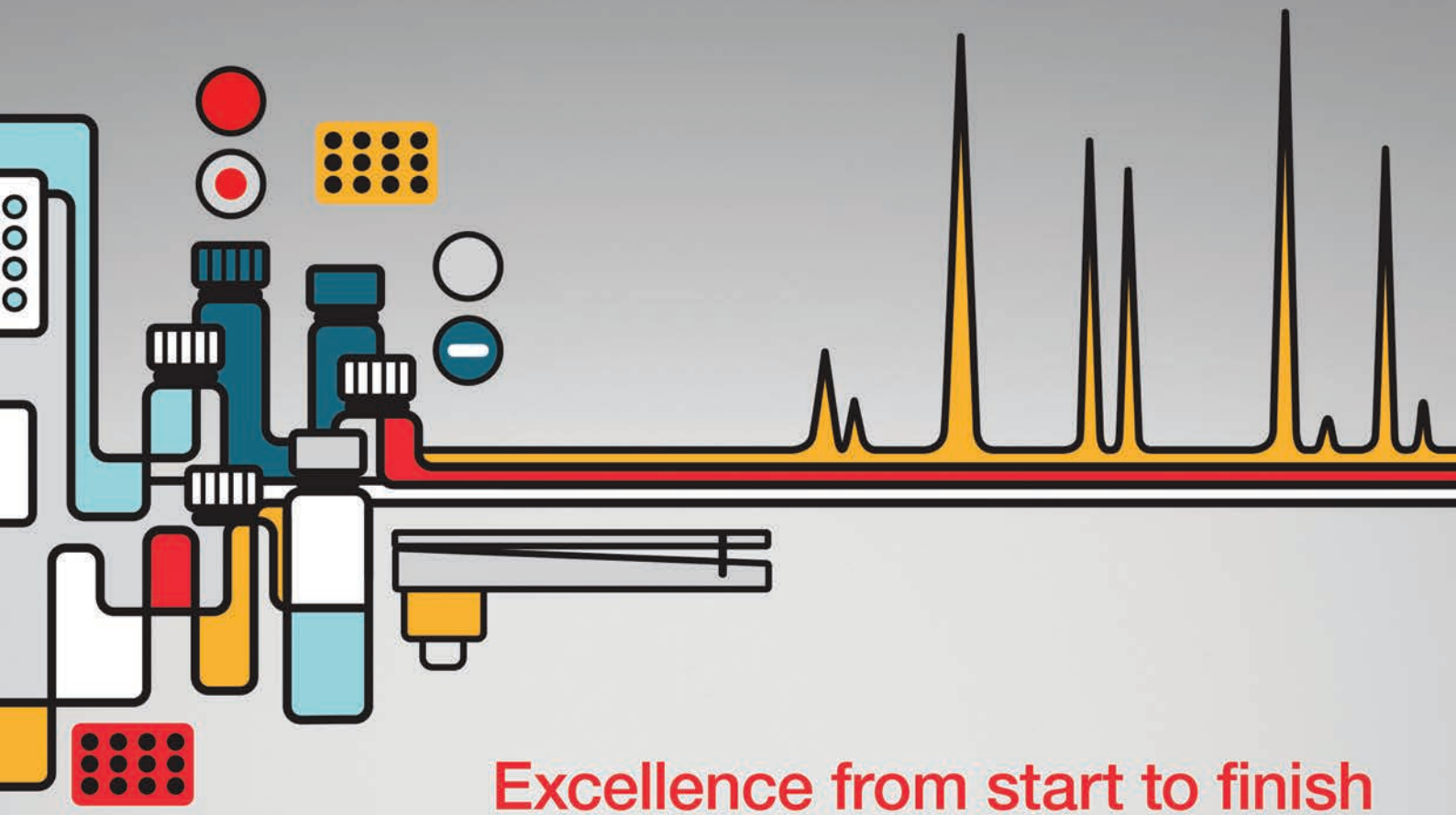

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Core Topic
(Bio)Pharma

Super-speed cell separation. Researchers from Hiroshima University have created a microfluidic chip that uses dielectrophoresis to sort living cells in just 30 minutes – eliminating the need for sample pretreatment and chemical tagging. The lab-on-a-chip device could come in handy for stem cell developers, where cell separation techniques are crucial, and for cancer diagnosis, which often involves circulating tumor cell separation. "Future research may examine refinements, allowing us to use dielectrophoresis to target certain cell types with greater specificity," said Fumito Maruyama, who led the research.

Remembering Anthony Franklyn Fell. Anthony Fell, founder and former editor of Journal of Pharmaceutical and Biomedical Analysis, Professor Emeritus at the University of Bradford, UK, and "Tony" to his friends, passed away on 13 January, 2022, at the age of 80 years. Tony was a leader in pharmaceutical and biomedical analysis, publishing on separation methods (GC, HPLC, CE), spectroscopic methods (UV–VIS, FTIR, NMR, MS, AAS) and hyphenated technologies (DAD-LC, LC-MS). His research focused on analyzing bulk drugs, formulations and clinical samples for research, drug development, and quality assurance.

Why multiple myeloma returns. An interdisciplinary team of researchers in Berlin were able to show that the production of CDK6, a cell division-promoting cell cycle regulator, is particularly high once multiple myeloma has become resistant to treatment. They used integrated global quantitative tandem mass tag (TMT)-based proteomic and phosphoproteomic analyses along with RNA sequencing, which Evelyn Ramberger – one of the authors – believes could "unveil further treatment targets and biomarkers for use in personalized cancer medicine."

The battle to improve AAV characterization continues. The latest weapon in the arsenal? Online two-dimensional liquid chromatography-mass spectrometry (2DLC-MS), developed by Regeneron researchers. The technique uses high-resolution anion-exchange chromatography (AEX) in the first dimension to separate and measure empty and full capsids in AAV samples, followed by reversed-phase liquid chromatography coupled with mass spectrometry (RPLC-MS) to separate and characterize viral proteins. The method "allows for high-throughput and multi-attribute AAV characterization in a single run, with minimal sample handling required for different AAV serotypes", concluded the researchers.

IN OTHER NEWS

Bruker launches new timsTOF-based MALDI platform for label-free high-throughput screening for drug discovery.

Waters acquires Megadalton Solutions, an early-stage developer of charge detection mass spectrometry – a useful tool for cell and gene therapy characterization.

Thermo Fisher and Symphogen extend their collaboration with the aim of developing new analytical tools for the characterization of complex biotherapeutics.

Biognosys expands its suite of MS-based proteomics platforms aimed at supporting drug discovery and clinical development.

Liquid chromatography–tandem mass spectrometry used to monitor alcohol-use-disorder medication compliance in Korea.

References available online

From Analytical Chemistry to Computers

Can games be used to predict human behavior – such as unethical decision making in a pharmaceutical production environment?

By Robert Lodder

I received my PhD in analytical chemistry from Indiana University Bloomington, but I really enjoyed combining computers and chemistry. Over time, I became good at using computers, and I even won first prize in an international IBM supercomputing competition in the life science division. I've spun out a few drug and medical device companies from academia, and I've also worked on projects with the likes of DARPA and the Department of Homeland Security. For example, Homeland Security had an analytical method they wanted to test to look for chemical weapons being placed in the US food supply. We came up with a gaming approach that used simulated attacks to test their method (in some cases proving the analytical method wouldn't work).

Such gaming approaches can be used in many different industries – and I've been working with Heather Campbell to show how gaming can be used in drug quality.

The University of Kentucky Hospital is the only hospital in the US that has a regular, full-time program analyzing incoming injectable drugs before they are used. The program was launched in late 2019 and testing began during the pandemic. The program has found a number of potential problems that have already been reported to the FDA through MedWatch and Citizen's Petitions as well as published (see Drugs With Reported Problems below). Pharma companies must adhere to GMP and GLP – and there are regulatory inspections to enforce this. In her research, however, Heather read that Valisure (a company in Connecticut that tests oral dosage forms) found around 10 percent of drugs have something wrong with them.

Drug companies often buy their ingredients from China or India. Companies will look at the label, the chain of custody, and then test the ingredient, too, before using it. On the other hand, when a pharmacy receives a drug, they look at the label and the chain of custody only – and then use the drug. If we want to eliminate the last 5 to 10 percent of problems, pharmacies need to test drugs on-site before they are used. And if the drug isn't right, they need to send it back to the manufacturer and tell them to try again! We should not assume that everything is equivalent. Generics, for example, are all assumed to be the same as the brand product. During the pandemic, the FDA halted foreign and even many domestic inspections. Even in normal times, there are still things an FDA inspection won't always catch (like the employee running down the hallway with bags of shredded documents as described in Catherine Eban's book, Bottle of Lies). Many inspections are announced beforehand so companies will be able to prepare. In other words, there is always a danger of low quality drugs entering pharmacy supply chains. Counterfeit products are also an issue.

Even at our hospital, we don't have the manpower, space, or equipment to test everything when it arrives so we prioritize drugs for testing. Some of the considerations include the manufacturer's Form 483s from the FDA, whether the company is the sole source of a drug, how much a drug costs and how profitable it is to counterfeit, among other things. Typically, however, that information can be months or even years old. Current prediction models tend not to fare well when faced with unexpected events – like the COVID-19 pandemic. They are also not always good at predicting human behavior.

Heather's work looked at how a serious gaming approach could simulate how humans react in a pharmaceutical environment. If we can understand how humans behave – and the quality shortcuts they may take in the pursuit of profit – it may help us to understand where the biggest risks lie and help to improve current risk prediction models.

Robert Lodder is a Professor in the Pharmaceutical Sciences Department of the University of Kentucky College of Pharmacy, USA

Cell Therapy's Live Analytical Challenges

Analytical science can take cell therapy manufacturing to the next level: in-line measurement of critical quality attributes

By Dalip Sethi

Cell therapies are a new, exciting, and complex branch of medicine that uses living cells as drugs. And with that complexity comes multiple analytical challenges – depending on the initial cell source and the nature of the manufacturing process.

One main analytical challenge is establishing in vitro potency assays that represent the therapy's mechanism of action in vivo, which is either undefined or far more complex than one would encounter with small molecules or monoclonal antibodies. Plus, cellular products cannot be terminally sterilized. The regulatory agencies understand this challenge and have issued specific guidance (1) to address the raw material (donor cells, master, and working cell banks) and in-process and release testing parameters.

At release, the product should be tested for sterility (ruling out the presence of microbiological and adventitious agents), identity (product characterization), purity (testing for acceptable limits of contaminants), and potency. Autologous therapy products are manufactured as one batch per patient and are infused back to the patient as soon as possible – a 14-day sterility test for release testing isn't ideal here. Aseptic techniques are maintained throughout product manufacturing, and sterility may be tested 48 to 72-hour prior to final cell harvest/formulation or after the last re-feeding of the cell culture. The product may be released at risk with a STAT Gram stain on the final formulated product, with a 14-day culture in progress.

Developing rapid, reliable, and validated analytical assays for microbiological agents is another major challenge for cell therapy. And it's equally important to control critical quality attributes (CQAs) – defined as a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution (2). As the industry matures, analytical sciences will play an increasingly significant role – providing the tools to enable cell and gene therapy developers to define the ranges and limits and test it at various steps of the manufacturing process.

The primary analytical techniques used for cellular product characterization are cell counting, cell size, and viability analysis, using dyes and image-based analysis. Other CQAs, such as chimeric antigen receptor (CAR) expression, are assessed by phenotypic characterization using flow cytometry. Enzyme-linked immunosorbent assays (ELISA) are used to quantify the residual cytokines, with various cell activation agents used in the process. If the activation is bead-based, the residual bead amount in the final drug should be quantified – microscopy-based methods quantify the bead agents. Finally, PCR-based methods are used to quantify the vector copy number in gene-modified cell therapies.

Analytical science has a plethora of tools that can potentially be employed to the cell and gene therapy space. For example, Fourier-transform infrared spectroscopy (FT-IR) could potentially enable in-line measurement of metabolic data, such as glucose and lactate values, which are currently measured using off-line or at-line enzyme-based sensors.

To develop these kinds of in-line process control tools, industry players are collaborating. For example, the UK's Cell and Gene Therapy (CGT) Catapult has formed a consortium of over 20 organizations to assess the application and combination of multiple technologies for process analytics within the cell and gene therapy industry (3). I also think we'll see a move towards AI-based process control tools in the next 5–10 years. In-line monitoring of manufacturing processes, off-line measurements of cell and gene products, and in vivo data on cell product potency and persistence should allow AI tools to make better decisions in manufacturing processes.

Dalip Sethi is Director of Scientific Affairs at Terumo BCT

Mass (Photometry) Effect

How mass photometry could become a new go-to tool for AAV characterization

Gene therapies rely on vectors, such as recombinant adeno-associated viruses (AAVs), to deliver genes. From early research to commercialization, characterization of those AAVs is essential to ensure purity and safety – but the task can present analytical challenges, especially in less well-equipped research labs. In search of an alternative analytical solution, researchers from the National Heart, Lung, and Blood Institute, (NHLBI) USA, applied a newly developed tool, called mass photometry (MP) – a single-molecule technique that measures mass distributions of biomolecules. And they found that MP can measure multiple AAV attributes (including heterogeneity, relative species content, and packing efficiency) accurately, reproducibly, quickly, and with minimal sample preparation (1).

We spoke with Grzegorz Piszczek, director of the Biophysics Core Facility at the NHLBI and one of the co-authors of the study to find out more.

What is the current state of AAV characterization?

It used to be that precise characterization of AAV preparations was primarily done within the pharmaceutical industry. Recently, because of the increased availability of new AAV characterization technologies, more extensive AAV

"We were surprised to find that some samples we analyzed were more complex than expected."

testing procedures are becoming standard in research laboratories, such as those working with animal models.

Established techniques like qPCR, ELISA, and spectrophotometry are still routinely used in many labs, but they can't assess the purity of AAV samples. Recently, the SEC-MALS (size exclusion chromatography coupled to multi-angle light scattering) applications have become more widespread. However, the full-to-empty capsid ratio calculations based on the SEC-MALS data can be affected by the presence of differently loaded capsid species. Additionally, SEC-MALS requires relatively large samples. And all the aforementioned methods are also relatively slow.

Why did you decide to assess mass photometry (MP) to AAV characterization?

MP is a revolutionary new technology – it's fast and requires small amounts of material. The technique can also be used to characterize a wide range of molecules of different sizes. We had used it to characterize antibodies and protein complexes and were looking at additional samples to analyze – AAVs were an obvious material to try.

How does the technique work when applied to AAVs?

The mass photometer is a highly specialized microscope. A few microliters of diluted sample solution is applied on the microscope cover glass. The MP camera observes individual AAVs "landing on" the surface of the cover glass, which are visible as dark spots appearing in the image. The intensity of this signal – the darkness of the spots – is proportional to the molecular mass of the particles. Since AAVs containing encapsidated genome are heavier than empty capsids, spots representing full particles appear darker than spots representing empty capsids, allowing for the quantification of different species. The whole MP measurement takes only a few minutes and does not require any special sample preparation.

Were any of your results unexpected?

We were surprised to find that some samples we analyzed were more complex than expected. We used analytical ultracentrifugation to confirm our findings and to validate the MP results. We were also surprised that we could not only differentiate full and empty capsids, but also estimate the size of the encapsidated genome from the MP data. This result really showcases the capability of the MP technique.

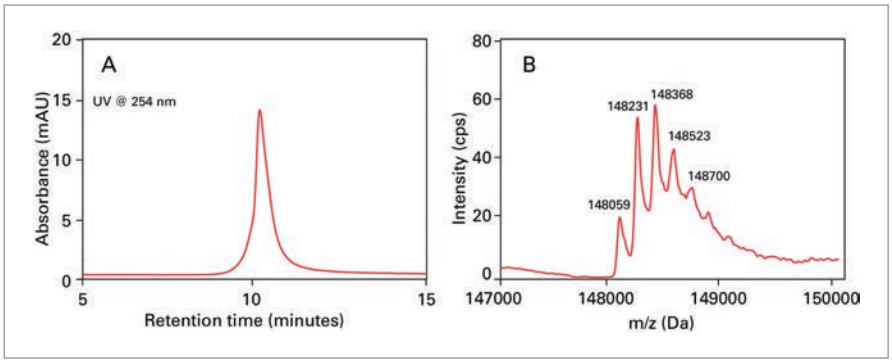
On the downside, we quickly realized that higher concentrations of some impurities, particularly capsid fragments, can impede MP measurements. Repeated sample freeze-thaw cycles tend to degrade the AAVs and create those fragments.

Where will your research take you next?

We are developing a protocol that combines SEC-MALS and MP to fully use the strengths of both technologies; SEC-MALS can isolate impurities affecting the MP measurements and provide titer information and MP can precisely characterize different AAV species eluting as a single peak on the SEC column.

Considering how fast the MP measurements are, and how little sample they require, I expect that this technique will become one of the standard methods for AAV characterization.

Characterization of Monoclonal Antibodies Using Native SEC-MS



Native intact SEC-MS analysis of NIST mAb. Elution profile (A) and deconvoluted spectrum (B)

Size exclusion chromatography (SEC) remains the gold standard for determining the molecular weight (MW) distribution of mAbs expressed in mammalian cell culture. However, obtaining structural information beyond the physical size (hydrodynamic volume) typically requires the combination of SEC with MS.

Native ESI has proven particularly useful for generating multiply charged ions of intact proteins with lowered charge states, providing increased spectral resolution at higher m/z values. Still, the analysis remains challenging and involves biomolecule-specific optimization on both the chromatography and mass spectrometry side.

A new application note illustrates an intact mAb analysis workflow solution integrating U/HPLC technologies and high-resolution mass spectrometry. It permits rapid and accurate mass characterization of mAbs leading to excellent mass accuracy for glycoform distribution. Detailed information was obtained about the heterogeneous composition of mAb proteins, with minimal sample preparation involved.

Download the application note here: https://bit.ly/SEC-MS

Enhanced LC Sample Preparation with J.T.Baker® High Performance Syringe Filters

Many samples analyzed by liquid chromatography (LC) contain particulates that can potentially damage both the LC instrumentation and the analytical column. Insufficient sample filtration can result in system blockages, increases in back pressure, reduced column lifetime and distortion of the analyte peak. It is therefore important to remove particulates from the sample prior to analysis. J.T.Baker® syringe filters are premium filters that have been specifically designed for the filtration of chromatography samples.

The extraction efficiencies of J.T.Baker® 0.22 µm syringe filters were experimentally determined by filtering a 0.01 percent latex of 0.3 µm polystyrene beads using the various membranes, followed by determination of extraction efficiency against a set of prepared calibration standards by spectrophotometry (UV, 272 nm) (Figure 1). Triplicate analyses were performed for multiple batches. Samples were prepared in water for hydrophilic membranes and in methanol for hydrophobic membranes.

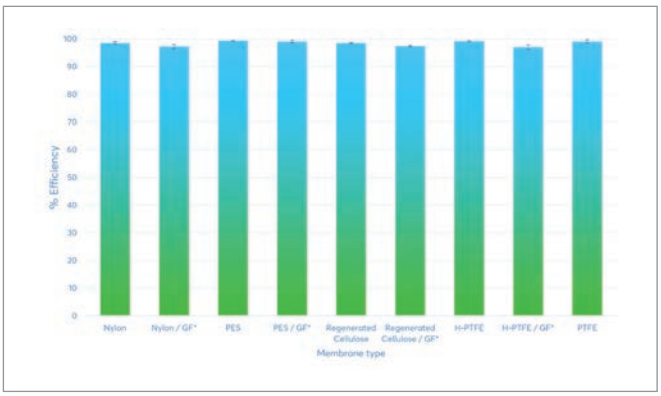


Figure 1: Extraction efficiencies of J.T.Baker® Syringe filters according to membrane type. * Refers to filters with built-in glass fiber pre-filters

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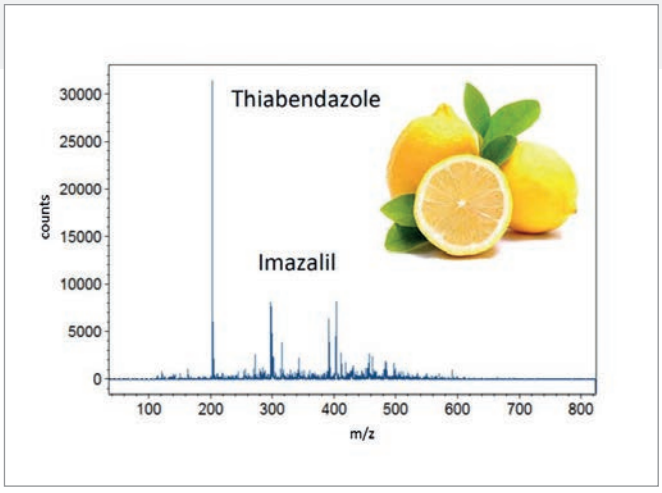
Detection of Residual Pesticides on Fruits and Vegetables Using Portability™ Miniature Mass Spectrometer



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

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

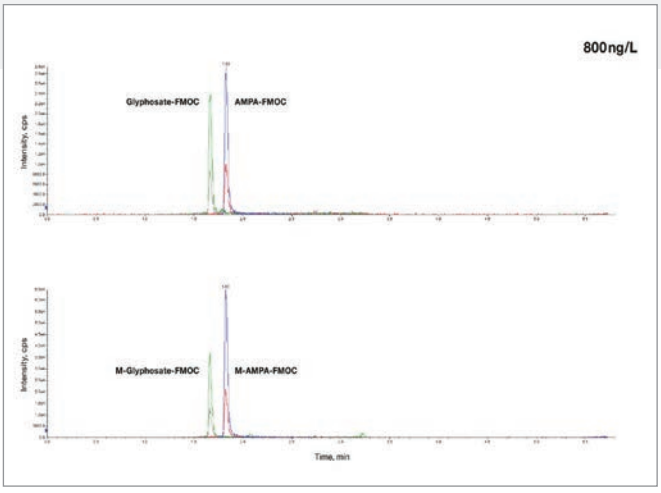
Read the full app note at http://tas.txp.to/1018/ANBaySpec

UHPLC/MS Analysis of Glyphosate and AMPA According to DIN ISO 16308

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

Glyphosate and AMPA are both highly polar compounds which make them difficult to retain on C18 columns. The derivatization with FMOC-Cl (fluorenmethyloxycarbonyl chloride) according to DIN ISO 16308 is used to lower the analytes' polarity and therefore increase their retention. DIN ISO 16308 is recommended for the analysis of drinking, ground and surface water, whereas for salt and sea water the applicability has to be checked.

The analysis of FMOC-derivatized glyphosate and AMPA is performed using a YMC-Triart C18 UHPLC column. Due to the highly robust hybrid silica base particle of YMC-Triart, the challenging pH value of 9.5 can easily be used. Since the detection capability of the MS was sufficient, no enrichment was needed. Analyses were performed for very low concentrations of 30 ng/L up to 800 ng/L. Stable isotope M-AMPA-FMOC and M-glyphosate-FMOC were used as internal standards.



Download the application note with the full method details here (https://ymc.eu/d/brDno).
Application data by courtesy of: Dr. Dirk Skutlarek, Universitätsklinikum Bonn, Institut für Hygiene und Öffentliche Gesundheit, Bonn, Germany.

Electrochemical Investigation in Research With Advancing Technology and Increase in Energy Consumption

Studies of electrolyte systems and surfaces are of real importance for further development

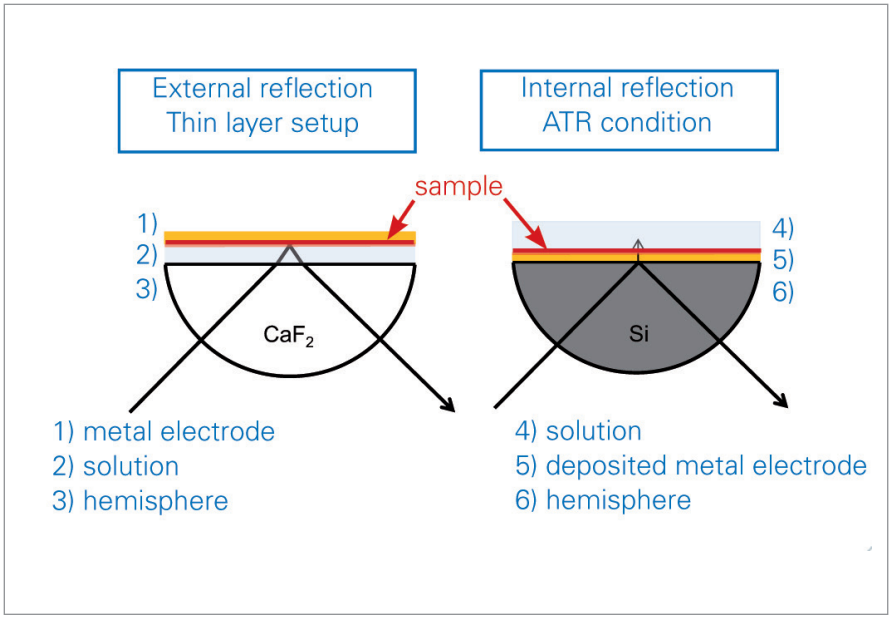
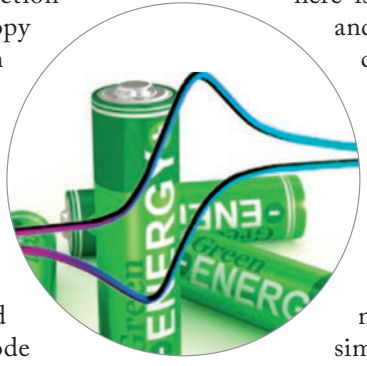


Figure 1: Different measurement modes of the AF30/x reflection unit. On the left side external reflection (materials: CaF_2, BaF_2), on the right side internal reflection (materials: Ge, Si).

Electrochemical investigations are a very current topic in research. In recent times, advancement in technology and industry has resulted in a worldwide increase in energy consumption. A future requirement to face this trend is the development of high capacity and low weight rechargeable batteries for energy storage. Therefore, studies of electrolyte systems or electrode surfaces are of great importance for possible further improvements.

Moreover, in other fields such as biochemistry or catalysis, electrochemistry is greatly beneficial, enabling access to molecular information – depending on an applied electrochemical potential. For example, the redox-active center in biomolecules (1), the reaction behavior of catalysts, or the formation of carbon oxides during alcohol oxidation.

A combination of FTIR spectroscopy with electrochemistry offers insights into the molecular change and reaction processes of studied molecules, in addition to the electrochemical response of the experiment. This valuable method can be applied to investigations of electrolytes or of electrode surfaces. With the BRUKER A530/x reflection unit, prepared for electrochemical cells, both may be studied (2). For an external IR reflection-absorbance spectroscopy (IRRAS) set-up, a thin layer configuration is used, which allows for the study of the electrolyte and the electrode surface. Alternatively, an internal reflection ATR set-up can be used to analyze the electrode surface directly with limited influence of the electrolyte (see figure 1).



Overall, the study considered the electrochemical oxidation of a metallic complex, ferrocyanide [Fe(CN)6]4-, with FTIR spectroelectrochemistry. This combined analysis offers the possibility to investigate the molecular change of this compound, depending on the applied electrochemical potential. The oxidation to ferricyanide [Fe(CN)6]3- was illustrated in the form of a SNIFTIRS spectrum and a 3D-plot with the OPUS software. One main advantage of the set-up reported here is that the potentiostat and the spectrometer are connected by a trigger functionality for an all-over communication. After setting the experimental parameters and the desired potential procedure, the measurement can be simply started in OPUS and will run automatically. The resulting spectra will be well sorted and assigned to their corresponding potential for subsequent evaluation. In the end, the combination of FTIR spectroscopy and electrochemical investigations makes in situ monitoring of electrochemical processes easy and reliable.

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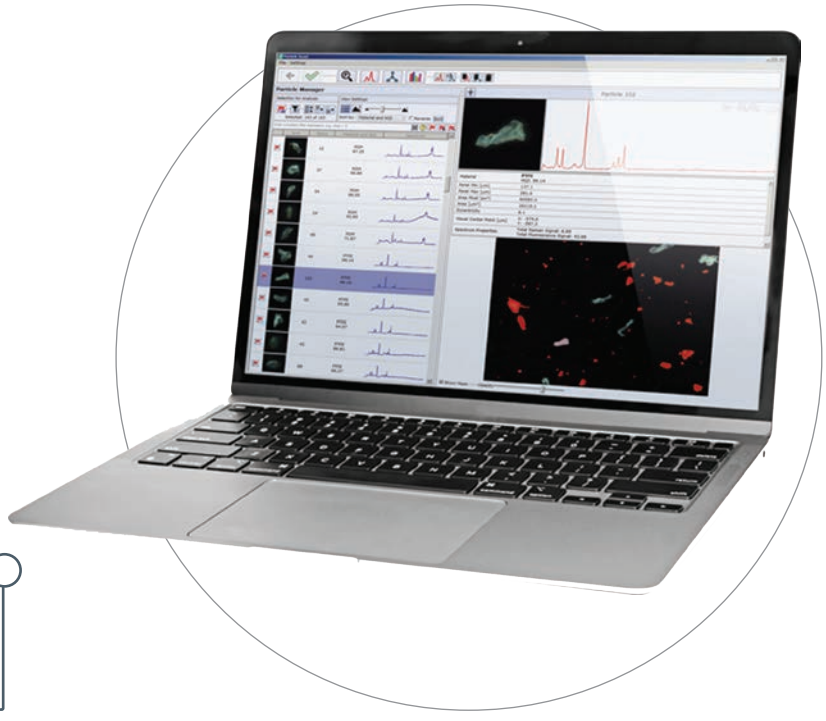

FPOP Star

Sitting Down With… Lisa Jones, Associate Professor of Pharmaceutical Sciences, University of Maryland, Baltimore, USA

Did you always want to be a scientist?
No. In middle school, I thought I would be a lawyer. And in high school, I thought I'd go into medicine. At University, I majored in biochemistry with the ultimate intention of applying to medical school. But I had to do some research as part of that major, and found it much more interesting than medicine! So that's how I ended up doing a PhD in science – there was no real "aha" moment, I just did what interested me.

What attracted you to the area of research that you work in now?
It's been a long journey. I took the first steps at college, where I fell in love with proteins. Protein structure, protein folding – it all fascinated me, so when I began looking for graduate positions, I focused on protein structure laboratories. I ended up working for Jenny Yang at Georgia State University, where we employed a variety of techniques from biochemistry, analytical chemistry and biophysics; for example, fluorescence, circular dichroism, and NMR. We were working on small model proteins, but my interests increasingly gravitated towards large protein complexes. To pursue that interest, I joined the lab of Peter Prevelige (Department of Microbiology, University of Alabama, Birmingham), who was working on virus capsids using advanced techniques, including hydrogen-deuterium exchange MS. And that was a revelation for me; I didn't know you could do structural studies with MS, until then. After working with Peter, I joined Michael Gross's laboratory – and that opened up a whole new world! Not only had Mike been using MS for decades – which allowed me to benefit from his wealth of experience – but he had also begun using a new technique called fast photochemical oxidation of proteins (FPOP). It seemed to me that it would address my interests better than conventional MS could, so I started using FPOP for the analysis of large proteins; for example, we were the first to report epitope mapping using FPOP. Then I began thinking about using FPOP for the analysis of very complex systems – and that is how I got into the analytical optimization and method development work that I pursue today. As I said before, I didn't plan to end up where I am – I just followed what I found interesting!

Do you think your career path has given you a different perspective on analytical method development?
Definitely. Being able to draw on both biochemistry and analytical chemistry helps me to align method development with the unique challenges of biology. For example, cellular processes occur on different time scales, some of which – like signaling cascades and protein folding – are very fast. Conventional flow systems are too slow to capture these processes, so I started developing more high-throughput systems in well-plate formats. Having a foot in both camps has been very helpful – I can cover a range of technologies without necessarily having to seek collaborators.

Your work analyzing protein complexes in cells has been described as "groundbreaking" – is your unusual perspective necessary for this type of endeavor?
Well, it doesn't seem unusual to me – I just like to try things that nobody has done. It can be a little scary at times, but for the most part it's fun – pushing boundaries is exciting. I always tell graduate students who want to join my lab that they won't find any protocols to follow – they have to make the protocols. Embracing this philosophy requires a degree of fearlessness – there's no real safety net when you're trying things for the first time. The graduate students who succeed in my laboratory enjoy the challenge of innovation; they like trying something new, and they do it fearlessly. On the other side of the coin, I've had some people leave the lab because they cannot operate that way, and I understand that.

Do you anticipate a greater role for automation in the coming years?
In my research, definitely. In fact, we're about to publish a paper on automation of the FPOP method. In general, automated systems speed up our analysis, which help us study cellular processes. Also, we've learnt from Jenny van Eyk's approach to automating sample processing by means of a Beckman liquid handling device; we are now trying to couple this with the Thermo Scientific 96-well plate system. The idea is to automate sample processing from cell lysis all the way through to sample digestion for MS, and we are working with Thermo Scientific to achieve this. Automated sample processing should improve reproducibility of MS outputs; we want our samples to be as good as possible so that we can get the most out of our MS methods. In brief, anybody engaged in proteomic analysis of biological samples will benefit from process automation, as it improves throughput and, by removing manual errors, offers better reproducibility. In short, I think automation will be hugely important in the continued development of the MS field.

Do you have a particular mission for the next five or ten years?
Two things. First, I want FPOP to become accepted as a standard structural biology tool. Second, I want to facilitate training of underrepresented minorities in science. Those are my two key ambitions for the future.

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