AUGUST 2019 # 79

the Analytical Scientist™

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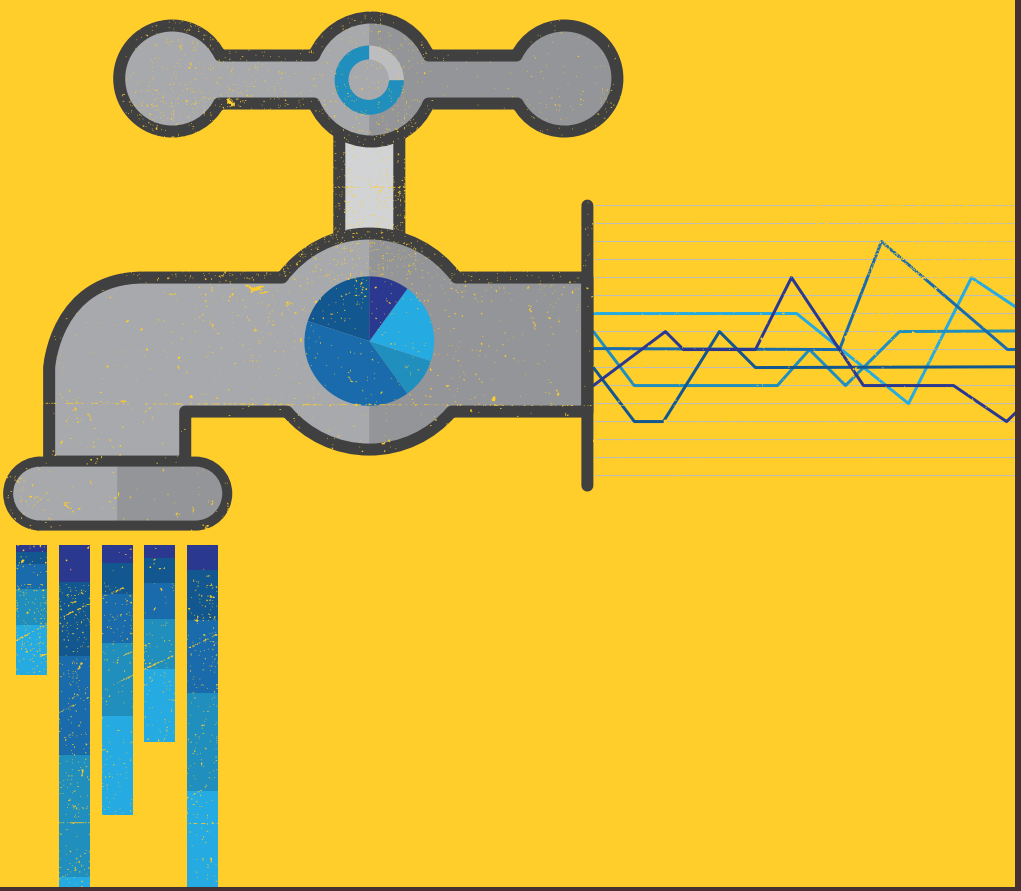
22 – 33



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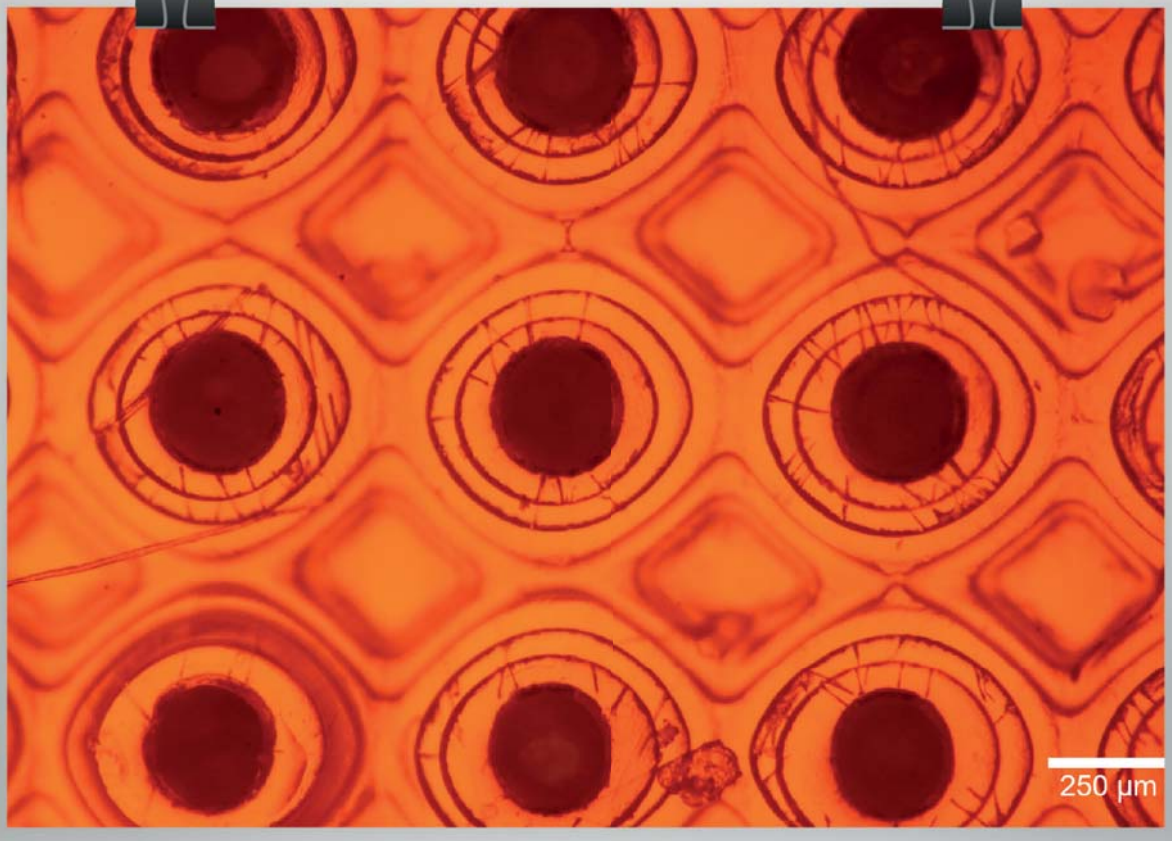

Small Separations

A prototype of a three-dimensional liquid phase spatial separation device, 3D-printed by researchers at the University of Amsterdam using high-resolution projection microstereolithography. Complex mixtures can be spatially separated in the first- and second-dimension channels and eluted in the third dimension. The effluents are stamped out onto a MALDI substrate from the conical tips in the microfluidic device (pictured). Student Leon Niezen produced the device shown here.
Credit: Suhas Nawada, University of Amsterdam, the Netherlands.

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

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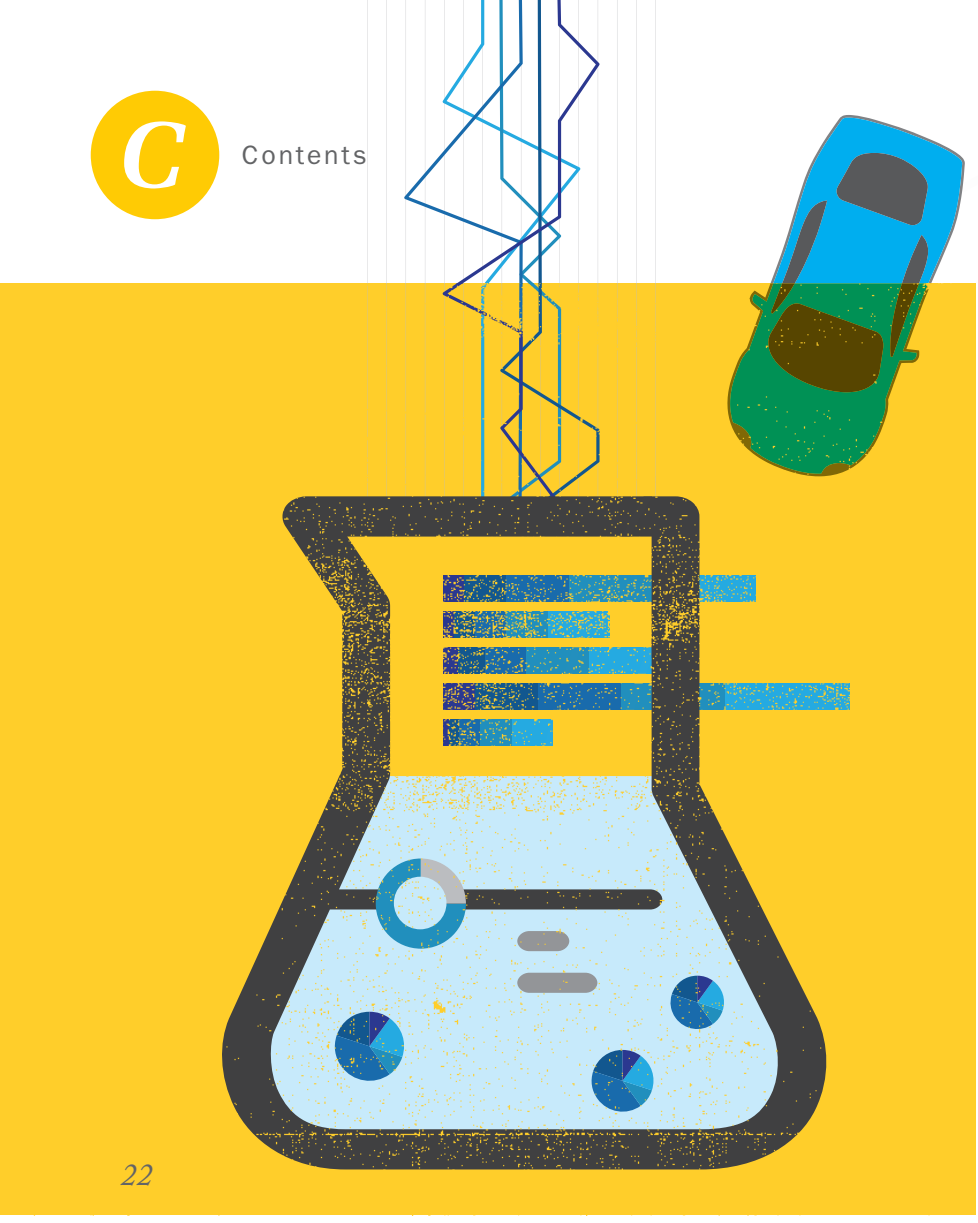
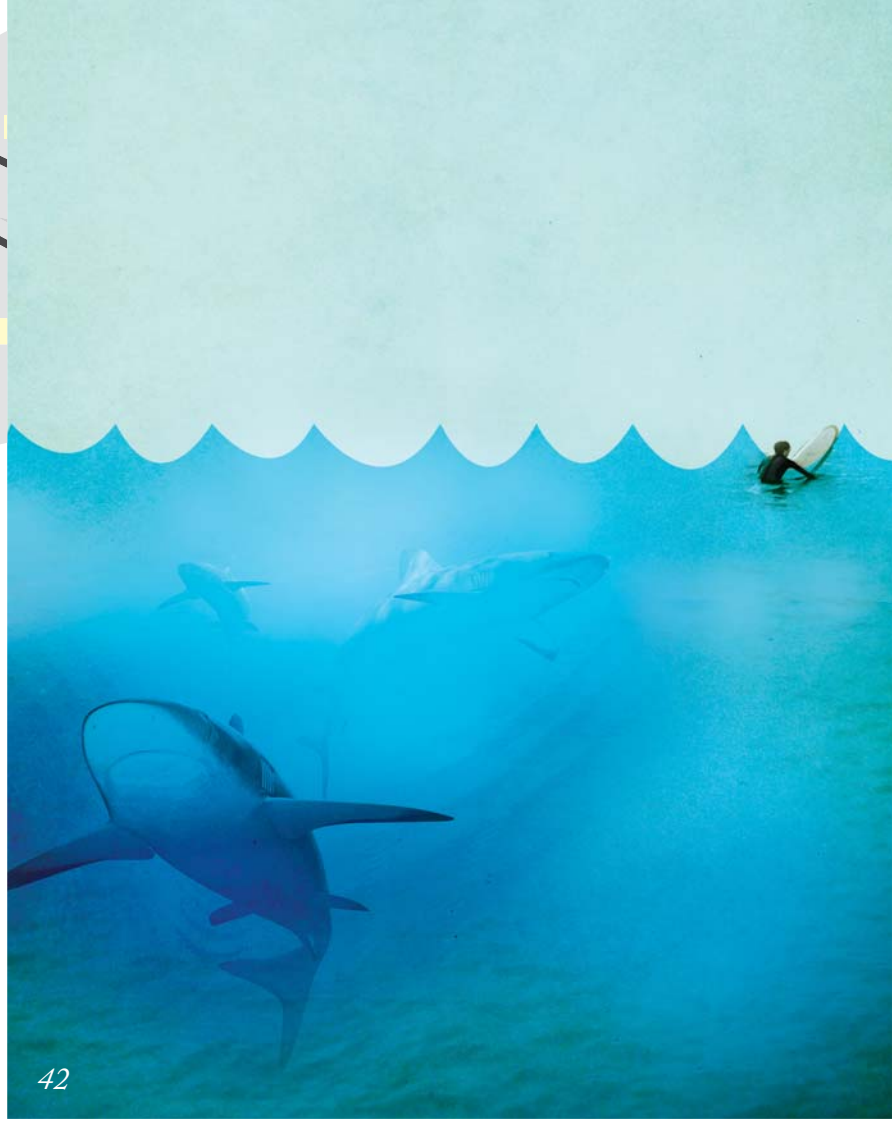


On The Cover

Tapping into a wealth of information from an unlikely source – sewage!



Features

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Report

Sitting Down With

the **Analytical Scientist**

ISSUE 79 - AUGUST 2019

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Chelford Road, Knutsford, Cheshire, WA16 8GS, UK

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www.texerepublishing.com
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Distribution:
The Analytical Scientist (ISSN 2051-4077), is published 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@theanalyticalsientist.com). Non-qualified annual subscription cost is £110 plus postage

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THE INNOVATION AWARDS 2019

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Submit Your Entry for the 2019 TASIAs!

The Analytical Scientist Innovation Awards (TASIAs) are back to showcase the best new technologies, instruments, and software solutions in analytical science.

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To enter, email charlotte.barker@texerepublishing.com with:

Name of innovation

Launch date (must be after 31st October 2018)

Brief description (~10 words)

Detailed description (50-150 words)

Potential impact (50-150 words)

One image (if applicable)

Closing date: November 1, 2019

Pride and Prejudice

Further action is needed to support LGBT+ scientists.

In the sciences, as in society, inequities persist in spite of progress. Inequality adopts different forms in each of our minds, moulded by our own beliefs and experiences – to some, an omnipotent boogieman fat on insecurities; to others, an impenetrable cloud of blissful ignorance.

Here in the UK, reports of hate crimes motivated by race, religion, sexuality and gender have skyrocketed in recent years – 144 percent in five years for those targeting LGBT+ individuals. I had always viewed the scientific community as a safe haven from such issues, in part because my own experiences have been so overwhelmingly positive – but that's not the case for all members of the LGBT+ community.

This reality was made clear to me by the findings of a recent report: "Exploring the Workplace for LGBT+ Physical Scientists" (1). Highlighting rates of workplace discrimination as high as 32 percent, with over a quarter of participants expressing that they had considered leaving the field for related reasons (see "Two Steps Forward, One Step Back" on page 10), it makes for uncomfortable reading. Coincidentally, the Bank of England announced that Alan Turing would feature on the new £50 note just weeks after these findings were published. I needed a stiff drink just to process the sheer dichotomy of these revelations.

Turing's acknowledgement is welcome, but feels bittersweet – a classic case of "too little, too late." The truth is, though much has changed since the wartime days of Turing, the attitudes underlying his persecution remain an adversity faced by many on a daily basis. Recognizing that these barriers exist is a first step - but what comes next?

The aforementioned report suggests we produce visible support for LGBT+ colleagues, improve policies to address harassment and discrimination, and introduce specialized training where appropriate. Such suggestions are valuable and, alongside movements like 500 Queer Scientists (an initiative to improve LGBT+ visibility in the field) and AZPlus (an empowering resource for LGBT+ AstraZeneca employees), may guide us towards positive change.

All change, however, starts at an individual level – so ask yourself what you can do to support your LGBT+ colleagues. The mountains ahead are daunting, but we cannot quake in their shadows. Together, we must tackle the climb.

Matthew Hallam
Deputy Editor

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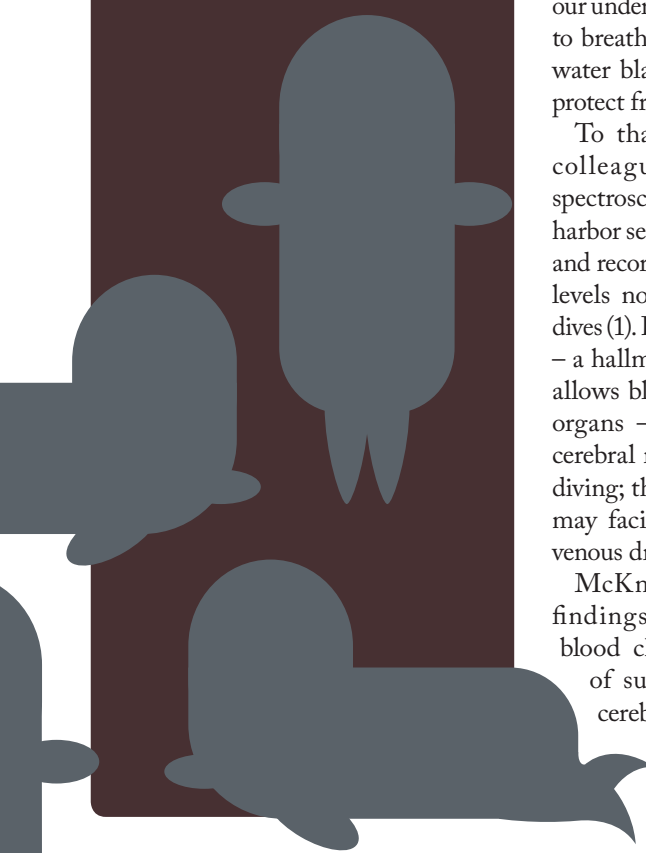
1. *Institute of Physics, Royal Astronomical Society and Royal Society of Chemistry, "Exploring the workplace for LGBT+ physical scientists", June 2019 Report. Available at: https://rsc.li/2Jz9j6Q. Accessed August 5, 2019.*

Upfront

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

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



Into the Deep with Submersible NIRS

Seals are adept ocean divers… Now, near-infrared spectroscopy provides clues as to how they survive extreme depths

Did you know that the deepest recorded seal dive is over 2.3 kilometers? Here, water pressure reaches approximately 240 times the air pressure at the surface, and temperatures plummet to near zero.

Dedicated divers (like the seal) have evolution to thank for their talent – but exactly what physiological changes allow them to thrive rather than just survive? Such knowledge could be used to further our understanding of human issues related to breath-hold diving – such as shallow water blackout – allowing us to better protect free divers.

To that end, Chris McKnight and colleagues attached near-infrared spectroscopy (NIRS) devices to juvenile harbor seals from Moray Firth, Scotland, and recorded oxy- and deoxyhemoglobin levels non-invasively during voluntary dives (1). Peripheral cardiovascular changes – a hallmark of the diving response that allows blood redistribution to essential organs – occurred before diving and cerebral reoxygenation occurred during diving; the team believes that the former may facilitate the latter by increasing venous drainage from the brain.

McKnight was surprised by both findings: "We expected peripheral blood changes to occur at the point of submersion, and thought that cerebral oxygenation would simply decline throughout diving – it appears that seals are more physiologically sophisticated divers than expected." Yet, McKnight also believes that these apparently unique capabilities may be more widespread in the animal kingdom than we currently know.

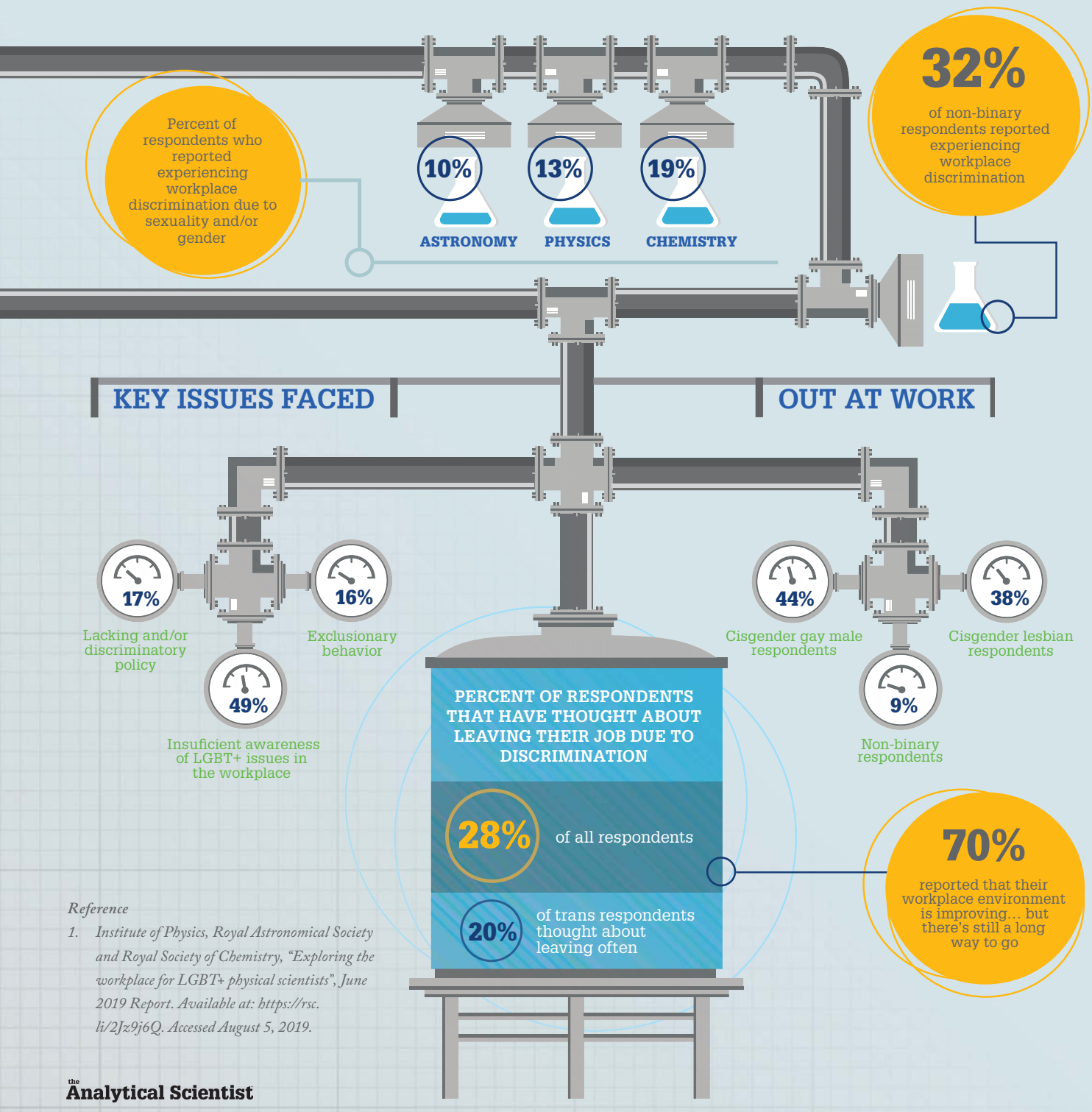
The next steps are to study circulatory and oxygenation changes in other diving animals. Humans – especially competitive free-divers and indigenous diving communities in Asia – are of particular interest to determine how we protect ourselves against cerebral hypoxia. Regarding further work in seals, "Our longer-term goal is to use larger NIRS arrays to highlight which senses seals use to locate and catch prey, and how they balance depleting oxygen stores with the need to maintain brain functions at depths of 2 kilometers," says McKnight.

Reference

1. *JC McKnight et al., "Shining new light on mammalian diving physiology using wearable near-infrared spectroscopy", PLoS Biol, 17, e3000306 (2019). DOI: 10.1371/journal.pbio.3000306*

Two Steps Forward, One Step Back

A survey of 1,025 LGBT+ scientists and their allies reveals that many still face discrimination at work (1). We picked out some of the key findings...



Reference

1. *Institute of Physics, Royal Astronomical Society and Royal Society of Chemistry, "Exploring the workplace for LGBT+ physical scientists", June 2019 Report. Available at: https://rsc.li/2Jz9j6Q. Accessed August 5, 2019.*

Booze, Barbarism and… Beeswax

GC-MS analysis of ancient Celtic drinking vessels paints a colorful picture of life in BCE Europe

So-called "consumption practices" were an integral part of early Celtic life. We know this from writings of the time – some of which describe the abuse of one particular consumable: alcoholic beverages. For example, Diodorus Siculus of ancient Greece apparently documented first-hand experience of this boozey buffoonery in his Bibliotheca historica: "The Gauls (Celts) are exceedingly addicted to the use of wine… and since they partake of this drink without moderation by reason of their craving for it, when they are drunken they fall into a stupor or a state of madness" (1).

Back in modern-day Europe, Maxime Rageot and colleagues set out to learn more about Celtic drinking habits and trading practices by analyzing 99 vessels thought to have been used for preparing and serving alcohol from Vix-Month Lassois – a Celtic site in Burgundy, France (2). The team sampled ceramic objects (16 of which were Mediterranean imports), extracted absorbed lipids, and performed GC-MS analysis.

Not only did the team find scientific evidence that early Celts were indulging in wine, but they also discovered that locally fermented barley and millet beers were also consumed from the vessels. Interestingly, olive oil and beeswax were also identified. Though the presence of olive oil indicates a Mediterranean import, the relevance of the beeswax is less clear. Coinvestigator Cynthianne Spiteri says, "Though we do not know the exact purpose, the presence of beeswax in numerous vessels suggests it was an important product, hinting at local bee management or even domestication." The researchers believe it was most likely used either to seal pots or as a sweetener. Mead, a beverage produced by the fermentation of honey, is another possibility.

Asked how these samples stood the test of time, Spiteri highlighted two key aspects: the hydrophobicity of lipid molecules, and their protection as a residue in the porous ceramic. But not all aspects of the analysis were clear cut: "Some fats require further testing for correct classification, such as ruminant and non-ruminant adipose fats, whose degraded profiles can be very similar," says Spiteri. "We use GC-combustion-isotope ratio MS for this."

Next on the Spiteri's hit list: the Heuneburg in Germany – a prehistoric hillfort by the river Danube. She hopes the time overlap between the locations will result in an insightful comparison, giving us an even tighter grasp on the trading (and consumption) practices of the early Celts.

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Gaining the Upper HAND

Measuring antiretroviral drug accumulation in the brain with MALDI-MSI to inform the best therapeutic avenue for HIV-associated neurocognitive disorder

Thanks to recent advances in antiretroviral (ARV) therapy, the life expectancy of HIV patients is now almost equal to that of the general population – at least in more developed nations. But, as life expectancy continues to increase, long-term HIV-associated complications, such as HIV-associated neurocognitive disorder (HAND), represent a growing health concern (1). The pathology of HAND is underscored by an accumulation of HIV particles in the central nervous system; over time, the disorder prevents sufferers from carrying out basic tasks and eventually results in death. But which ARV drugs are likely to be most effective?

Sooraj Baijanth and colleagues at the University of KwaZulu-Natal in Durban, South Africa, used LC-MS/MS and matrix-assisted laser desorption/ionization MS imaging (MALDI-MSI) to study the spatial distribution of two ARV drugs, elvitegravir and tenofovir, in rat brains (2). The researchers found that elvitegravir demonstrated around 20 times higher blood–brain barrier penetration than tenofovir, reaching a maximum concentration of 976 ng/g.

According to Baijanth, the approach developed by the researchers provides higher spatial resolution than other modalities and should allow them to map numerous analytes in tissue sections. Such capability will prove useful as they complete an evaluation of all FDA-approved ARV drugs before assigning them to a "brain penetration index." The team hopes that the index will eventually help clinicians select the most appropriate treatment regimen for patients presenting with HAND symptoms.

Progress that cannot come soon enough, Baijanth remarks, reflecting on the "constraints and dangers associated with exposure to radioactivity" associated with PET imaging - the technique historically associated with tissue imaging studies. Baijanth is confident that improvements in the speed, accuracy and resolution of MALDI-MSI will continue as MS-based imaging becomes increasingly popular: "Such advances are sure to enhance the ability of researchers to conduct imaging experiments in situ, and will subsequently encourage translation into the clinic."

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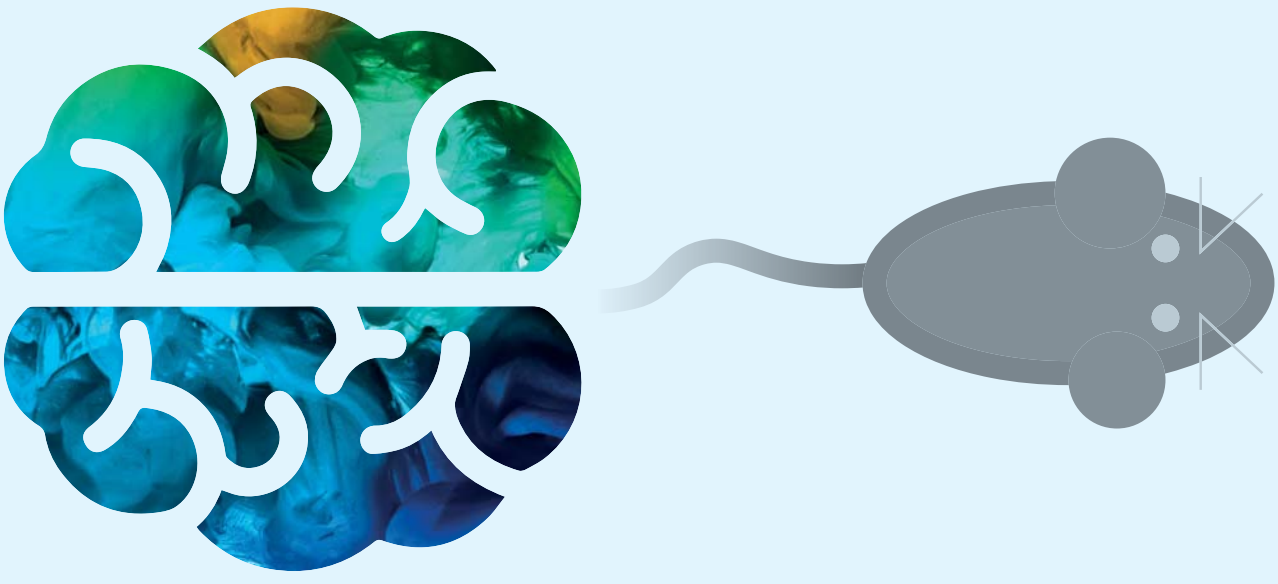

A Golden Ratio for Gut Disorders

Could the permeability ratio be used to diagnose and monitor gut disorders less invasively than traditional colonoscopy?

Inflammatory bowel disease (IBD) is on the rise across the globe – especially in industrialized countries. By 2015, an estimated three million people in the US had received a diagnosis of either Crohn's disease or ulcerative colitis, the two conditions collectively known as IBD (1). Characterized by chronic inflammation of the gastrointestinal tract, symptoms of the disease include stomach pain and swelling, bloody diarrhea, and weight loss. Typically, IBD is diagnosed and monitored through colonoscopy, which assesses structural damage to the intestine's gut-blood barrier. But colonoscopy is invasive and requires anesthesia – and Marcin Ufnal of the Medical University of Warsaw might have found a better alternative.

Patients with IBD suffer from an impaired gut-blood barrier, which Ufnal and his team have harnessed to develop a novel test that compares the ratio of bacterial products in the patient's blood and stool (2). "We initially wanted to use the concentration of gut bacterial products in the blood, but this didn't work because there were significant inter-individual differences in bacterial composition, including geographic, dietary, and drug-related factors," says Ufnal.

In contrast, the blood-to-stool ratio of bacterial products isn't affected by differences in the composition and metabolic activity of bacteria. "The permeability ratio (Pr) assesses the extent to which bacterial products have passed through the gut-blood barrier," Ufnal explains. "A healthy individual will have a low Pr, whereas the ratio for an IBD patient will be higher." Specifically, the Pr analyzes short-chain fatty acids in just 1 mL of blood and stool, measuring their concentration via liquid chromatography coupled with triple-quadrupole mass spectrometry.

Ufnal believes that the technique could also be used to diagnose other disorders that affect the function of the intestinal wall, such as celiac disease. In addition, it offers promise for the detection of heart failure, high blood pressure, and liver ailments, because they may all result in a leaky gut that affects the concentration of bacterial products in the blood.

Future efforts will be directed toward assessing which bacterial metabolites are most useful in terms of calculating Pr. "We are doing a lot of basic research to look for bacterial products that aren't metabolized by the liver, because that can affect their concentration in systemic blood," says Ufnal. Given that gut disorders can develop before any structural changes can be seen with traditional colonoscopy, this method of diagnosing and monitoring IBD offers hope that symptoms can be controlled at the earliest stage.

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IEM Identification

Combining metabolomics and DNA sequencing could be the key to discovering new inborn errors of metabolism in children

Although individually exceedingly rare, together inborn errors of metabolism (IEM) make up a sizeable portion of the broader spectrum of genetic disorders. Nevertheless, they remain underdiagnosed and undertreated (1). A multidisciplinary group based at the University of Texas Southwestern Medical Center in Dallas are working to improve our understanding of these diverse conditions. In a recent study, they combined genomic and metabolomic data to diagnose lipoyltransferase-1 deficiency (LIPT1D), an IEM characterized by abnormal brain development, seizures, and lactic acidosis (2). The team are optimistic that the new approach could provide the basis for more routine identification and treatment of IEMs.

"We've long known you can treat many IEMs if you pick up the underlying metabolic disturbance quickly," says Ralph DeBerardinis, Professor of Pediatric Genetics and Metabolism at UT Southwestern and a co-author of the paper. Phenylketonuria (PKU), a well-known IEM, is characterized by a failure to metabolize phenylalanine, resulting in the accumulation of phenylalanine and related metabolites in blood and urine – abnormalities easily detected by laboratory testing (3). But many other diseases remain poorly characterized and much more difficult to pinpoint – something that DeBerardinis hopes to address with advanced techniques. "It has become apparent that applying broad profiling technology will allow us to understand metabolic disturbances at a more granular level, helping us to uncover these conditions and ultimately to develop new therapies," he says (4).

Part of the problem is that current diagnostic approaches are narrow in scope. DeBerardinis certainly believes so; after all, even the most sophisticated clinical tests can only pick up a small fraction of potential markers. "You might be able to detect 50 biomarkers or so in a high-end laboratory," says DeBerardinis. "But there are potentially thousands of detectable metabolites in the blood – each of which could be associated with a novel IEM."

Part of the problem is that current diagnostic approaches are narrow in scope. DeBerardinis certainly believes so; after all, even the most sophisticated clinical tests can only pick up a small fraction of potential markers. "You might be able to detect 50 biomarkers or so in a high-end laboratory," says DeBerardinis. "But there are potentially thousands of detectable metabolites in the blood – each of which could be associated with a novel IEM."

There certainly seems to be plenty of reason for optimism, but DeBerardinis is keen to stress caution, at least for now. "We really need to know more about metabolic variability in the normal population first," he says. To get that data, the team are looking further afield. "We have established collaborations with medical geneticists in Pakistan, where the frequency of undiagnosed IEMs is high. Because that population has remained relatively understudied, there's opportunity for discoveries that will help us better understand and treat IEMs," says DeBerardinis. And although that project only began a few months ago, progress is already being made. "We have around 150 samples so far – we're very excited to see where the work takes us."

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In My View

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

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

Contact the editors at charlotte.barker @texerepublishing.com

Chip Off the Old Block

Mini-organ technologies are on the rise, but could analytical approaches hold the key to supercharging their potential?

By Steven Wilson, Department of Chemistry, University of Oslo, Norway.

Organoids and organ-on-a-chip technologies represent two potential game-changers in the study of human biology. The former are three-dimensional tissue models derived from primary tissues, embryonic stem cells or induced pluripotent stem cells, while the latter are miniature organs that are grown in or introduced into a microfluidic device, allowing for the real-time tracking of responses to a given stimulus.

Not only are these developments conceptually exciting, but they're also emerging as important tools in drug development. Animal models are often not accurate predictors of human physiology, and so preclinical success is not necessarily indicative of subsequent success in humans, which results in anticlimactic results, lost time and wasted money. Here, organoids and organ-on-a-chip technologies serve as important complementary techniques.

If we have access to a tiny clone of a patient's own organs (a kind of "mini-me"), we can overcome the physiological disconnect of animal studies – and find ourselves with an invaluable asset to progress personalized medicine. In fact, mini-livers, mini-hearts, mini-kidneys, and even models resembling the embryonic human brain are already available to us. Efforts are also being made to monitor how these mini-organs can interact with one another, pointing towards the eventual possibility of "human-on-a-chip" systems. It may sound crazy, but these applications are very much within the reach of our capabilities.

Though organoids and organ-on-a-chip systems remain a focus in developmental biology and drug development, they are yet to be partnered with key approaches in

"Efforts are also being made to monitor how these mini-organs can interact with one another, pointing towards the eventual possibility of 'human-on-a-chip' systems."

"I believe it's only a matter of time before we witness a union between the mini-me and the mini-LC!"

analytical chemistry; for example, with separation science and MS. One reason for this is that mini-organ systems are still in their infancy, but it's my opinion that many mini-organ researchers simply do not know enough about the power of these approaches – and may harbor some misconceptions.

One example is that the mini-organ scientists often see LC-MS as a technique reserved for larger samples, and thus believe it would not be compatible with sub-millimeter-sized organoids. Perhaps this belief stems from the fact that some mini-organ scientists interact solely with small-molecule LC-MS researchers, who are used to running samples with conventionally sized LC columns that would admittedly struggle with the small samples warranted for mini-organ studies.

I think that scientists who work with miniaturized LC systems (capillary and nano, for example) would find excellent partners in mini-organ scientists. Miniaturized LC is known for being highly compatible with limited samples… yet, with the exception of proteomics, the "mini-LC" system is avoided by many due to the inherent difficulties of working with nanoliter to microliter per minute flow rates while also trying to minimize "dead volumes" or leakages. But, considering both the increasing evidence behind mini-organ systems as tools in biomedicine and the unquestionable power of LC-MS and related techniques, I believe it's only a matter of time before we witness a union between the mini-me and the mini-LC!

I would also wager that miniaturized, online sample preparation approaches will find homes in mini-organ research, too. Take the immobilized enzyme reactor, for instance; in-tube reactors have been developed with the promise of automated analysis in proteomics for decades, but these devices have never really caught on because off-line alternatives are considered more suitable. If online proteomics of organ-on-a-chip systems were to be performed, maybe this would create space for the immobilized enzyme reactor, and see it adopted to a loving home.

It's a fantastic area of research to work in; many exciting partnerships and developments lie in wait. To facilitate this, biologists in the field of organoids and organ-on-a-chip must be shown the applicability of mini-LC for challenging samples with the potential for high-throughput and reproducibility. As in any area of science, communication and collaboration between these groups is essential for progress.

Helping Undergrads Bloom

From science to social experiences, grant-funded STEM programs provide undergraduates with a valuable foundation for their careers.

By Mark T. Stauffer, Associate Professor, Department of Chemistry, University of Pittsburgh, Pennsylvania, USA.

There is an old saying: "I hear, and I forget. I see, and I remember. I do, and I understand."

Experiential learning has been shown to help students learn concepts much more effectively than simply reading about a subject. Coupling experience with reading then makes what we read more easily understood and meaningful, as many of us appreciate from our own learning experiences. After all, if you do something often enough, that "something" will eventually become second nature.

Most of the learning strategies being developed for today's undergraduates in the STEM fields, which includes analytical science, are being geared toward a generation of students whose learning abilities are visually based, compared with the reading-based learning of baby boomers (such as I) and previous generations. The technology-oriented society in which we live is partly to thank for this. STEM undergrads today have greater opportunities than their predecessors to engage in experiential learning activities. From journal clubs to undergraduate research experiences, scientific seminars and the formation of scientific learning communities, opportunities abound.

All of these activities are associated with their own benefits for the students. Journal clubs, for instance, allow the students to develop skills for the critical appraisal of published research, while participation in research experiences allows students to gain exposure to scientific practice. What's more, each of these program elements facilitates social interaction. The only issue is that money is needed to make these helpful undergraduate programs happen.

So, how do colleges and universities go about obtaining the money to fund all of this? They apply for funding, the source of which is public (governmental), private, or a mix of the two; colleges and universities have access to lists of potential funding sources for STEM projects that fall into all of these categories. Of course, these grants do come with stipulations on how the funding may be used, and those stipulations vary from donor to donor (whatever these stipulations may be, donors must make the grant applicant aware). A well-prepared grant application, based on a well-thought-out idea for a project to enhance undergraduate performance in a STEM discipline, can lead to the acquisition of funding and a successful program – and well-prepared future members of the STEM workforce.

"Coupling experience with reading then makes what we read more easily understood and meaningful, as many of us appreciate from our own learning experiences."

Presentations from the Pittcon 2019 workshop on grant-funded STEM programs for undergraduates highlighted innovative project ideas that build the intellectual foundations of STEM undergrads and also explored the financial support needed to make these programs a reality. My campus' S-STEM (Scholarships in STEM) grant from the US National Science Foundation not only made it possible for its principal investigators to examine the effectiveness of science learning communities on academic performance among STEM undergrads and their retention in their majors, but it also provided scholarship funds for the cohort of students who met the requirements of our NSF-funded grant program.

Thinking on this, I'm reminded of another well-known saying: "Experience is the best teacher." With this in mind, please consider the following: how will you and your institution contribute to building tomorrow's STEM workforce?

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Sugar Rush

The addition of polysaccharide chains to therapeutic monoclonal antibodies can have a dramatic impact on their safety and efficacy – and that's why glycosylation is a critical quality attribute. Here, we speak with Tomasz Walski, Project Group Leader in the R&D department at Mabion S.A., to find out how the biosimilar developer is keeping glycan analysis short and sweet.

What exactly does your team do – and what analytical technology do you rely on?

In short, my team develops chromatographic methods to analyze the quality attributes of the therapeutic antibodies we develop. Our workhorse analytical tools are U/HPLC and LC-MS/MS systems.

What keeps you motivated?

As a company, our priority is creating safe, effective and accessible drugs for the patients who need them. The safety of a biological drug relies on the quality attributes of the antibody, so having sensitive and accurate methods to analyze them is of outmost importance during the drug development phase. It is incredibly rewarding to feel that our work is contributing directly to patient safety.

My favorite part of the job is when we are able to improve a method to generate less waste, be more analyst-friendly and less time-consuming – all while still generating high-quality results. Ultimately, that enables us to develop projects faster and deliver safe drugs to patients at an affordable price. We are constantly looking for technological innovations that help us to achieve that.

Why is N-glycosylation so important in biopharma analysis?

N-linked glycosylation describes the attachment of oligosaccharides (sugars) to a protein, via an asparagine residue. We pay very close attention to N-glycosylation of biologics – the composition of the sugar chains attached to the drug molecule is one the key quality attributes for therapeutic antibodies.

N-glycosylation strongly affects the way the drug interacts with its target (for example, cancer cells). It determines how efficient it is in its therapeutic action, how long it remains active and how long it circulates in the bloodstream. In addition, making sure that the N-glycosylation stays the same during development, through clinical trials and onto the market helps to minimize the possibility of any adverse reaction. It is crucial to carefully monitor the N-glycosylation throughout project development and then in the production process to ensure that patients always receive safe, high-quality medicine.

What challenges does glycan variant analysis typically present?

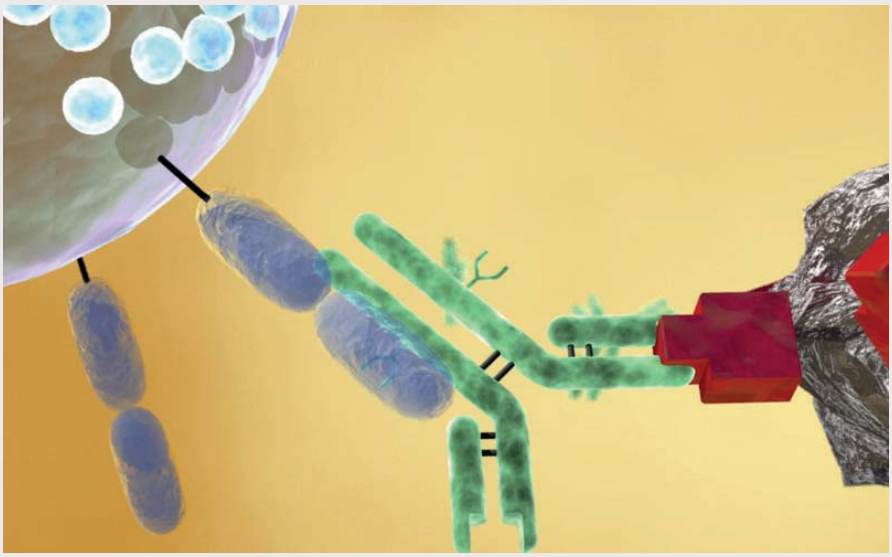
There are a number of powerful, gold-standard methods for the accurate analysis of antibody N-glycosylation. These methods typically require sample purification followed by multi-step preparation of some sort; for instance, proteolytic digestion or glycan separation and labeling, several clean-up steps, and finally UPLC or LC-MS analysis. All of these steps can be automated, of course, but it still takes significant time. The ideal approach would keep the reliability and high quality of results while simplifying and accelerating the workflow.

You've used a number of different LC columns over the years. How is the FcR column different?

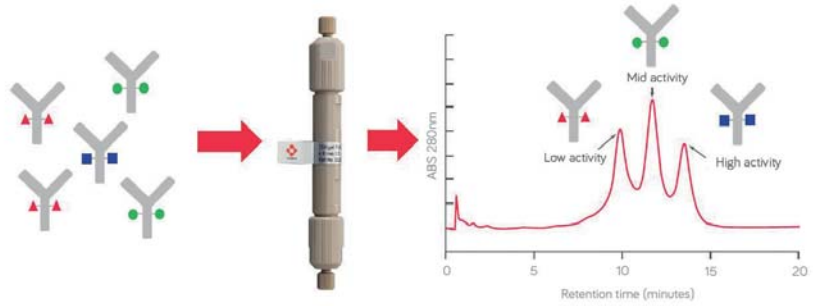
By using an FcR HPLC column, we can make the whole process much simpler. For a significant number of therapeutic antibodies, interaction with the Fcγ receptor is crucial for their activity. This interaction is strongly dependent on the

"The accuracy of the data really surprised us. We could predict the content of galactosylated glycans with less than five percent error in most cases."

TOSOH



"I am certain that the FcR column will be extensively used in our current and prospective biosimilar development projects."



Separation of mAb glycoforms according to their affinity to the FcγIIIa receptor.

type of N-glycosylation. By making good use of the unique characteristics of the TSKgel FcR-IIIA-NPR column, which uses the Fcγ receptor as the ligand, we are able to get information on the N-glycosylation of the antibody and its biological activity. The resulting pattern of chromatographic peaks strongly correlates with the N-glycosylation composition.

The accuracy of the data really surprised us. We could predict the content of galactosylated glycans with less than five percent error in most cases, compared with the orthogonal method – HILIC-UPLC using released and labeled N-glycans.

The method was sensitive enough to use low microgram amounts of sample and, on top of that, we got virtually the same results regardless of whether we used highly purified sample or the antibody still in the cell medium. And that means we can perform high-throughput and highly informative screening of clones, cell culture conditions and lead molecule selection with minimal sample handling. Not to mention that the lack of sample preparation makes this approach cost-efficient and environmentally friendly.

Given these characteristics, I am certain that the FcR column will be extensively used in our current and prospective biosimilar development projects.

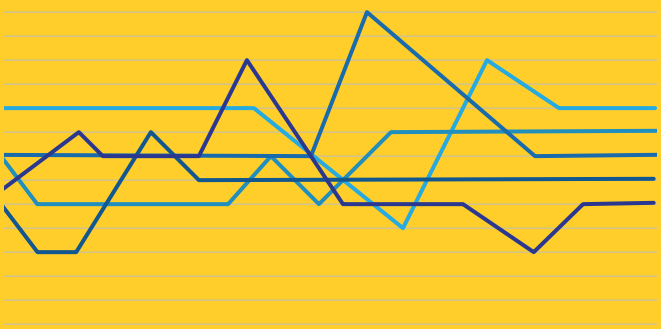
How else could the column be used?

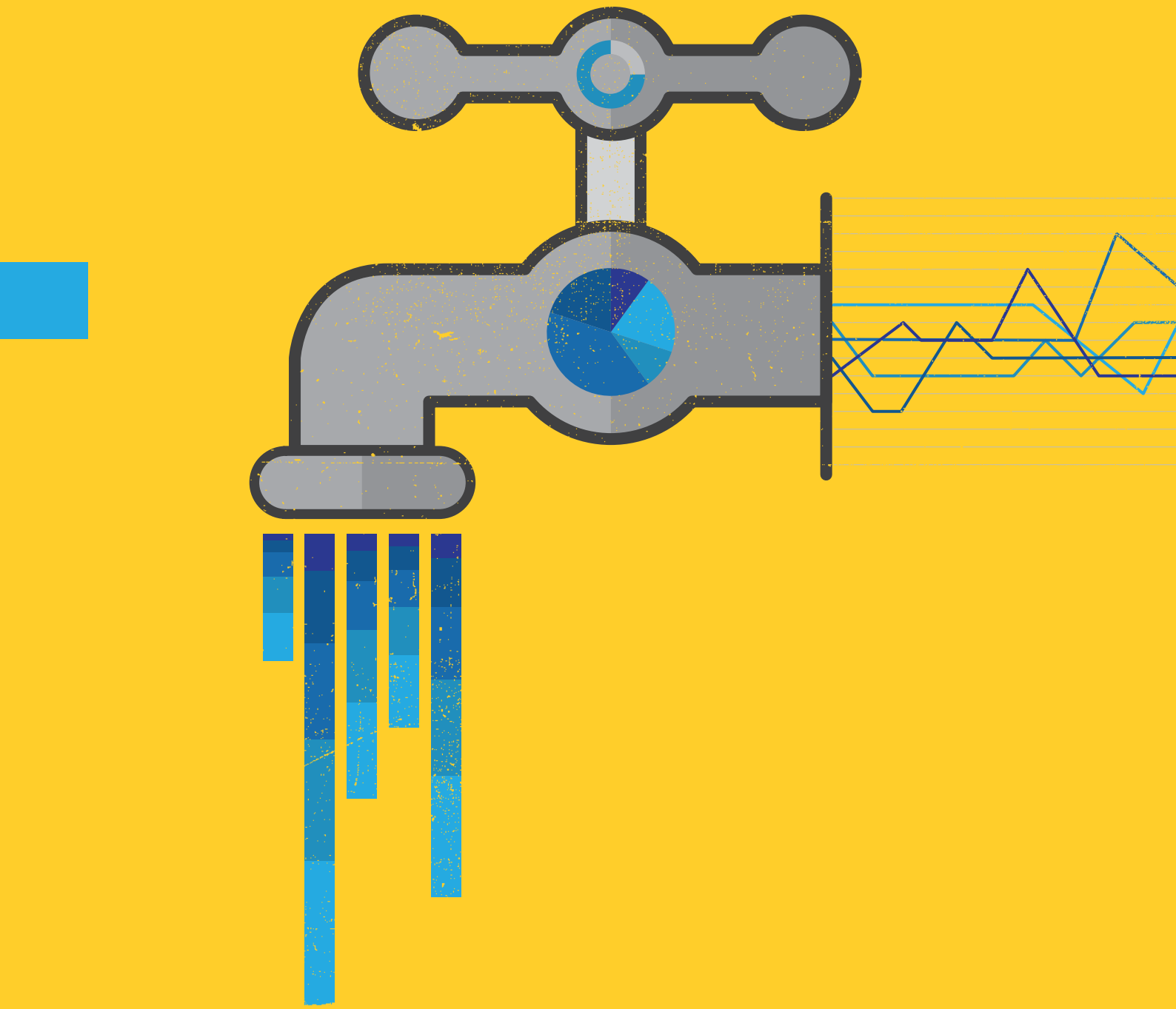
As well as targeted screening for activity and glycosylation, I believe that the column has potential in a number of areas. Estimation of binding affinity on non-purified samples is one thing that comes to mind. The fact that the Fc region of the antibody interacts with FcR can be used to detect antibody fragmentation or changes in the quaternary structure. FcR could make a great ligand for antibody purification, so a preparative-scale FcR-IIIA column is something I'd be interested in exploring. I imagine that our colleagues in the Quality Control department might find some new uses for the column too; for example, in one of the batch-release methods.

The Great Sewage Census

From revulsion to revolution: wastewater analysis not only informs us of human health and habits, but could even help transform our approach to healthcare.

By Rolf Halden





The first thing people ask when they hear that we analyze wastewater: "Why on earth would you pick that as your subject?" And, while the question is understandable, the answer is simple: to monitor health and protect the general public.

I was not the first to hit on the idea of studying sewage; people have monitored microbes in wastewater to study epidemics as far back as the 1950s, and European researchers have measured levels of illicit drugs across multiple cities since the early 2000s. Our primary contribution to this area has been expanding the study of wastewater into a platform for evaluating matters of public health. My story, however, did not start with sewage. It started with something even less pleasant…

PROTECTING THE PUBLIC

My first job after completing my doctorate was as an environmental engineer at Lawrence Livermore National Laboratory – a weapons lab – where I dealt with hazardous chemicals, such as organochlorines, dioxins and polychlorinated biphenyls. It was five years later, in 2001, that I moved to Johns Hopkins University to start up the "Center for Water and Health" program as an assistant professor and found myself surrounded by public health experts – it is the biggest school of public health in the world, after all. I hoped that my knowledge of organochlorines (persistent molecules incompatible with natural degradation systems) and dioxins (polychlorinated carcinogens consisting of two aromatic rings) would be of use in this new environment. It was, and I quickly identified not just one but two chemicals to study in wastewater – triclosan and triclocarban, both antimicrobials.

The significance of triclosan (a trichlorinated sanitary agent) in the public health sphere was obvious. Not only was it recognized as a pre-dioxin by the US Environmental Protection Agency (EPA), meaning it can serve as a precursor to more persistent and increasingly chlorinated dioxins during incineration, but it could also contribute to the development of microbial drug resistance. Coupled with the fact that it was present in many personal care products at the time, triclosan represented a promising research target and many teams had already set out to study its relevance. I saw an opportunity to bring a new dimension to our understanding using wastewater analysis.

The second antimicrobial that piqued my interest, triclocarban, also has a persistent structure, comprising two aromatic rings and three chlorines, and was in widespread use at the time. And yet, it hadn't been studied at anywhere near the level of triclosan. Why not? Because triclosan could be studied by GC-MS while triclocarban could not – it reacted with the column and wouldn't make it to the detector. To make matters worse, sample derivatization to improve the analysis was also difficult. Luckily, I like a challenge!

Given the lack of information available, I chose to focus on triclocarban first, with a view to answering two fundamental questions: i) what methods could we use to study this compound, and ii) where does triclocarban end up after its use in hand soap?

To address the first question, we adopted LC-MS and LC-MS/MS. LC-MS was fine for measurements in simple environmental samples (ground water and drinking water, for example) (1). Initially, the triclocarban eluted with another compound of the same molecular weight, but the addition of acetic acid led to the formation of a triclocarban adduct whose heavier reference ion moved it away from the interference, providing a much clearer result. In time, as we moved on to more complex samples, like sewage sludge or human materials, our team also started to use a tandem MS method we developed (2).

When it came to finding triclocarban, I knew that it was formulated into soap – and what happens to soap? It is used and washed down the drains into wastewater. From our lab in Baltimore, we followed wastewater pipes to the nearest treatment plant and found triclocarban not only in the water entering the plant, but also in urban streams near leaky sewer pipes and in the process flows exiting the plant – both the reclaimed "cleaned" water and in the "sewage sludge" produced as a byproduct of water reclamation. Next, we studied additional urban streams passing through Baltimore (and representing potential drinking water sources) as well as brackish waters of nearby Chesapeake Bay and Jamaica Bay near New York; we found triclocarban at all of these sites, including in the sediments of Chesapeake and Jamaica Bay. At that point, we decided to look at triclosan, and found that it was also present in all of the samples.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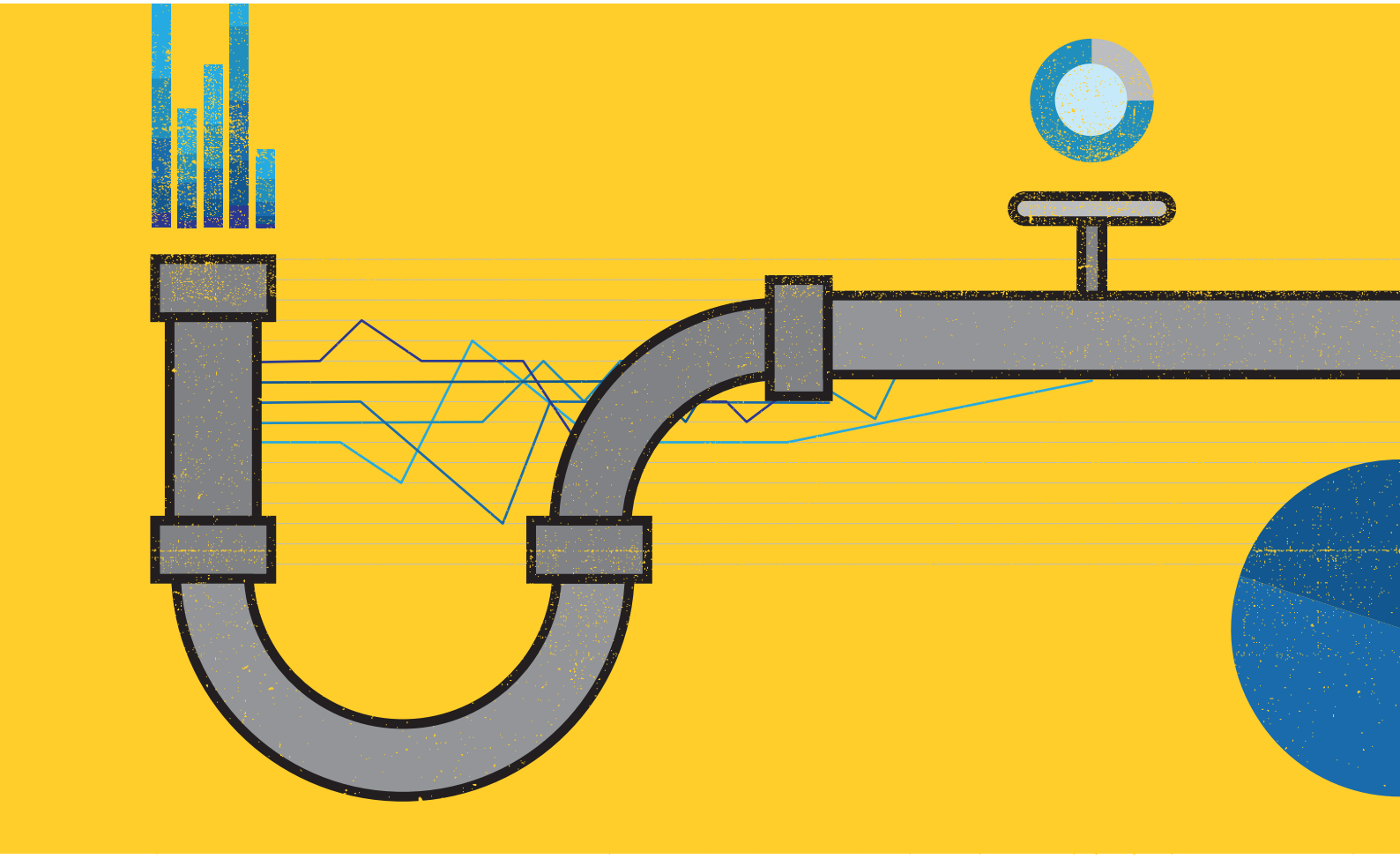
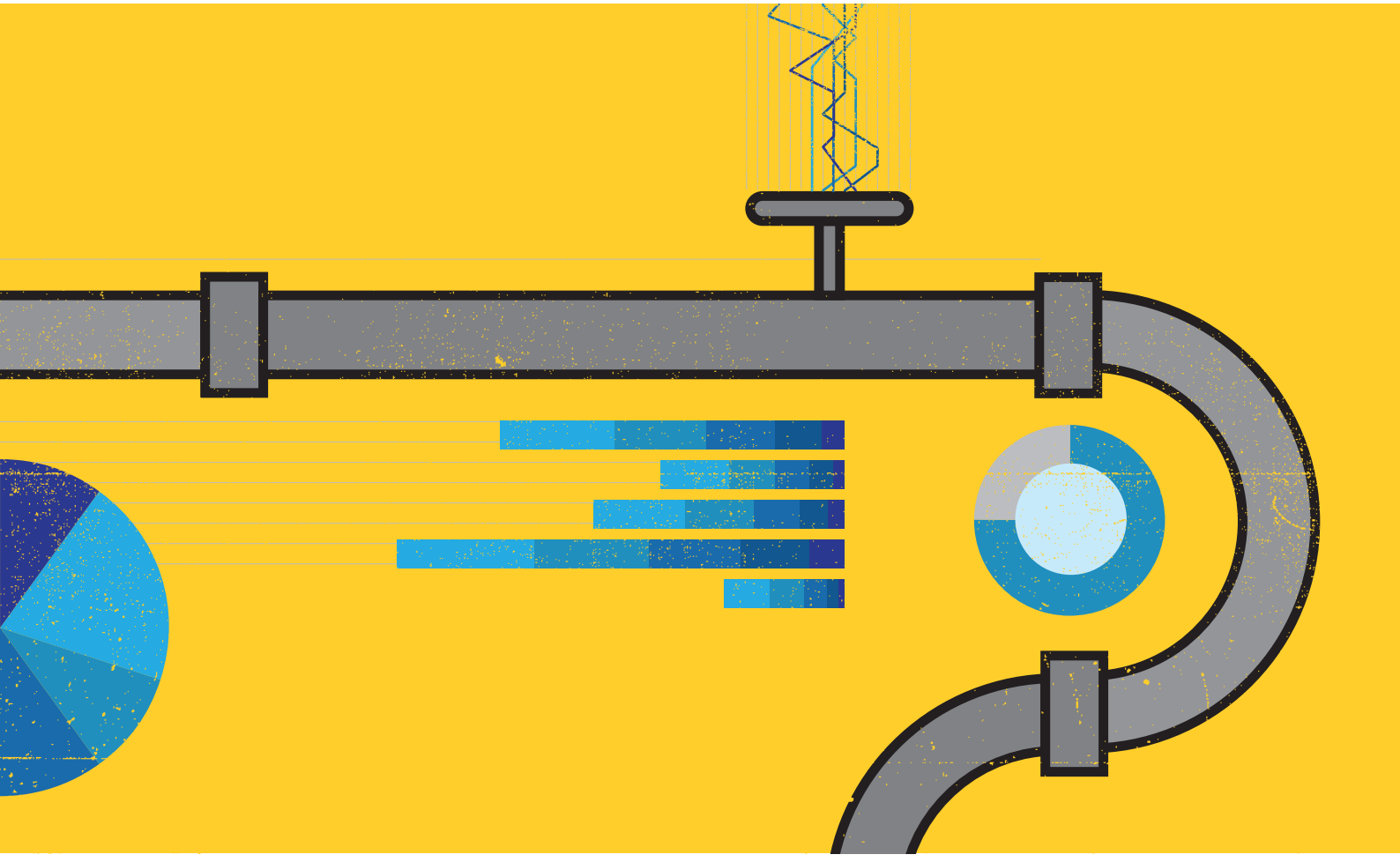


DOUBLE TROUBLE

From a public health perspective, the co-occurrence of the two drugs spelled trouble but it provided a benefit in terms of predictive power. We developed a simple model forecasting the concentrations of triclocarban based on triclosan levels detected across the nation – our model predicted that triclocarban was likely a top 10 contaminant in drinking water across the US (3). The prediction was largely based on data from the EPA and the US Geological Survey, who had measured triclosan in numerous locations, including in sewage sludge and freshwater streams. Of course, when we published our paper, we came under pressure to prove our estimates… And so that's what we did.

"Though not everyone is concerned for the environment, most do take great interest in their own health and the health of their families."

I spoke to the EPA in the hope of expanding our wastewater data and, luckily, they had just completed their nationwide sewage sludge survey. They had recovered samples of sewage sludge from all over the US, stored them, and were preparing to dispose of them (they had reached the end of their four-week shelf life). I rushed over there with a bunch of coolers to take as many as I could off their



hands; I figured that any organic chemicals remaining in the sludge at this point must be highly resistant to degradation and would likely be of anthropogenic origin – and therefore important to my study. Using the EPA samples, we were able to produce national inventories demonstrating the total mass of triclosan and triclocarban in wastewater across the US, quickly expanding to over 200 wastewater treatment sites across the nation.

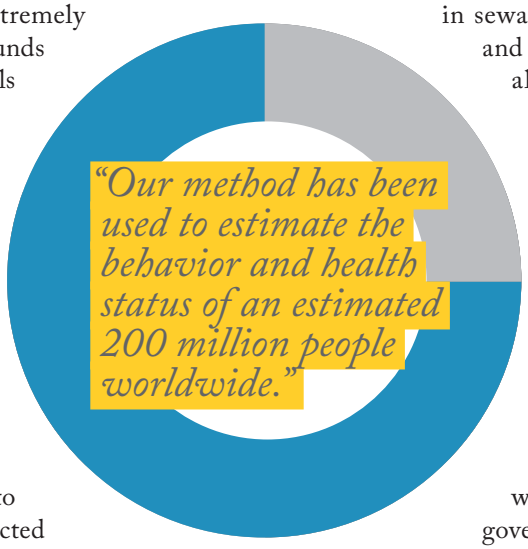
Armed with data on of the widespread co-occurrence of these compounds and their persistence after wastewater treatment – a process that should destroy anything degradable – our next priority was to investigate the potential for people being exposed to these chemicals, which could result from drinking contaminated water or the consumption of agricultural products from land that has been treated with sewage sludge – over 14 billion pounds of sludge are produced each year in the US, and approximately half of this is spread on land. Perhaps unsurprisingly, we found the two antimicrobial compounds pretty much everywhere we looked for them – in breast milk, in umbilical cord blood and in the urine of young children, to name a few examples.

Though not everyone is concerned for the environment, most do take great interest in their own health and the health of their families. Accordingly, our findings in humans triggered a cascade of new research. The link between chlorinated aromatic compounds and endocrine disruption was already known, but as more and more researchers began to look at the health effects of these chemicals, the list of their harmful effects mushroomed. When you consider the entire lifecycle of these compounds, the list grows even more damning (4); for instance, triclosan manufacture generates a carcinogenic dioxin byproduct and triclocarban degradation gives rise to further carcinogens. It became very clear that these chemicals posed a risk to public health.


Avalanche

THE HUMAN HEALTH OBSERVATORY

We soon began using the wastewater as a diagnostic matrix to study much more than exposure to just one or two select contaminants. First, we added antibiotics to our hit list, because of concerns surrounding the rise of antibiotic resistance, and found extremely high concentrations of these compounds in sewage sludge. Soon, other chemicals relevant to public health were added – illicit drugs, prescription drugs, stress hormones, antidepressants, and nicotine. Before long, our panel of analytes had expanded from two chemicals and their transformation products – maybe a dozen in all – to several hundred different compounds that we investigated in raw and treated wastewater as well as in sewage sludge.



"Our method has been used to estimate the behavior and health status of an estimated 200 million people worldwide."

Our work was no longer limited to the US, either. To date, we have conducted measurements at over 100 locations in China alone and probably well over 350 locations around the world in total, meaning that our method has been used to estimate the behavior and health status of an estimated 200 million people worldwide. This data and the corresponding global sample archive, dubbed the "Human Health Observatory", is collected at relatively low cost and gives us the power to manage health in a preventative manner, rather than reacting to observed outcomes. In other words, we can measure threats before they make people sick – not only chemicals but also biological agents, such as viruses and antibiotic resistance genes. Communities can then take the appropriate precautions against the identified threat; for example, equipping emergency responders with an appropriate drug or antidote.

Such proactive healthcare management is an important goal. The US alone spends three trillion dollars a year on healthcare, yet we still focus most of our efforts on curing sick people rather than on preventing the healthy from becoming sick in the first place – it's like cleaning up your flooded bathroom without first shutting off the valve to the broken pipe. We have already demonstrated that wastewater analysis can flag the arrival of a new dangerous drug or disease to a neighborhood or area (5,6), and we have outlined how this approach can be used to avoid epidemics and pandemics in the future (7).

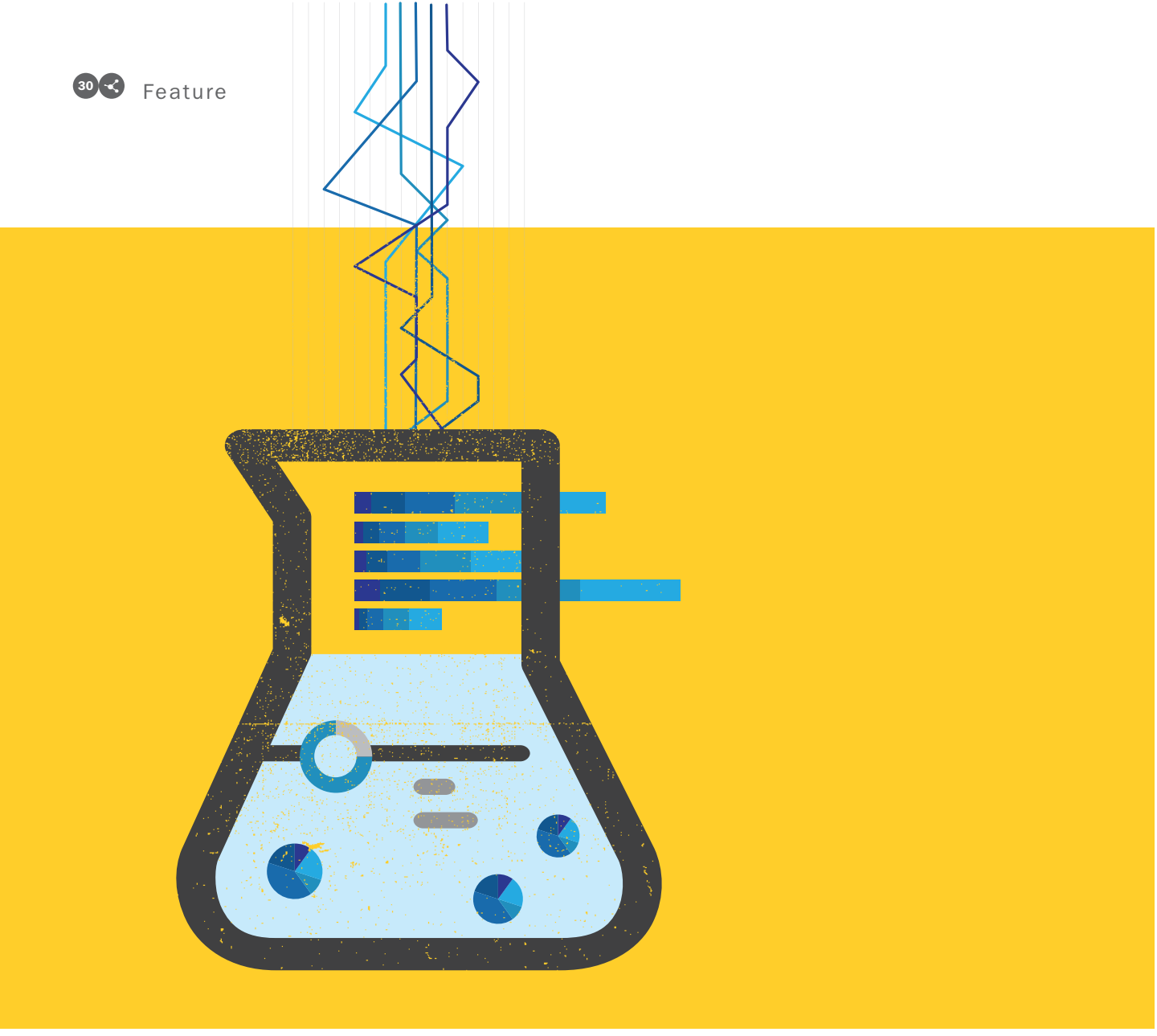
In the case of persistent molecules like dioxins and polychlorinated biphenyls – which are lipophilic and accumulate in adipose tissue – another bonus is the ability to avoid invasive biopsy procedures. In fact, we published a paper in Nature Scientific Reports showing a tight correlation between the levels of these chemicals in sewage sludge persisting after treatment and in human adipose tissue, essentially allowing the estimation of the types and magnitude of toxic burdens in populations based on measurements made at the wastewater treatment plant serving these exposed populations (8).

At a more local level, we contribute to proactive healthcare efforts in our home city (Tempe, Arizona) by talking to the mayor's office on a monthly basis about threats we identify in the wastewater of our community (see page 30), and we also work closely with the federal government. I have presented to the US EPA on multiple occasions regarding the inventories and trajectories of persistent chemicals and these sessions opened up discussion with the CDC and FDA, with the latter agency eventually making the decision to ban triclosan, triclocarban and numerous other antimicrobials from products under their supervision. That marked the beginning of the end of overuse of risky antimicrobials in personal care products, and it is a rewarding feeling that our team played a part in it.

Ultimately, when I think about the journey my research has taken me on, I'm reminded of a quote from the philosopher Arthur Schopenhauer:

"All truth passes through three stages. First, it is ridiculed. Second, it is violently opposed. Third, it is accepted as being self-evident."

We have certainly seen ridicule ("Why would you search for old samples that are past their shelf life?") and have heard plenty of criticism ("You'll never know which individuals are represented in the samples!"). In fact, I left Johns Hopkins back in 2008 to join the Biodesign Institute at Arizona State University because federal funding – on which Hopkins relied – was so hard to obtain for this unconventional work. But our results speak for themselves, many critics now recognize the benefits of the approaches we use, and we now have the satisfaction of seeing our work make a real difference in the world.



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An Opioid Observatory

In the Arizona city of Tempe, researchers are tracking drug use in real-time.

By Matthew Hallam

The opioid crisis has become a prevailing and pervasive issue underscoring America's transition into the 21st century. An estimated 47,600 deaths were caused by drug overdoses in the US in 2017 (1) – and more recent data is expected to demonstrate an even bigger impact, as the spread of illegally manufactured fentanyl and the rise of synthetic opioids continue to drive the epidemic forward.

Within this feature, Rolf Halden discusses how his analytical techniques have been used to compile the so-called "human health observatory" – a massive database that tracks community behaviors and predicts imminent threats based on chemical measurements in sewage samples. So, what can one see through their telescope? Now, with the Public Health Dashboard developed in an ongoing collaborative effort between Arizona State University and the city of Tempe, Arizona (accessible at https://bit.ly/2NruWey), you can square up to the eyepiece and watch the skies shift with your own eyes.

This first-of-its-kind dashboard shares data on opioid consumption in the city with Tempe's citizens – and anybody else who might be interested – in near-real time (see Figure 1). The intuitive interface lets users filter through the data presented to show detected levels of parent opioids (fentanyl, heroin, oxycodone and codeine) or their primary metabolites (norfentanyl, 6-acetylmorphine and noroxycodone) across distinct areas of the city, which can then be viewed in context with individual neighborhoods – and even streets – by navigating the central map. But how are these numbers calculated, and how are they used?

Following the detection of a drug in wastewater, the amount present can be used to estimate the equivalent number of regular users in the area, by calculating the "population normalized mass load" for each sample. For every gram of heroin found in wastewater, it is reasonable to estimate that there are 20 regular users in that area based on an average consumption of 50 milligrams of heroin a day; as each sample represents approximately 1,000 people, the number of suspected users can then be estimated as a proportion of the local population. This novel technique has been successfully implemented to provide a fast stream of anonymous health data for US cities (2), but has also been used to track narcotic use within smaller areas such as a US university campus (3).

Given the scale of the opioid crisis in the US, powerful methods to track opioid prevalence have never been more important. Armed with knowledge of how opioid levels change over time in Tempe, Halden's data allows policymakers to monitor the impact of interventions (for example, education efforts or drug amnesty points) and modify next moves accordingly (see Figure 2).

The observatory is certainly a powerful tool, but a huge number of points must be mapped to give us a national view of the opioid crisis. What constellations will reveal themselves – and how will they inform new or modified intervention approaches?

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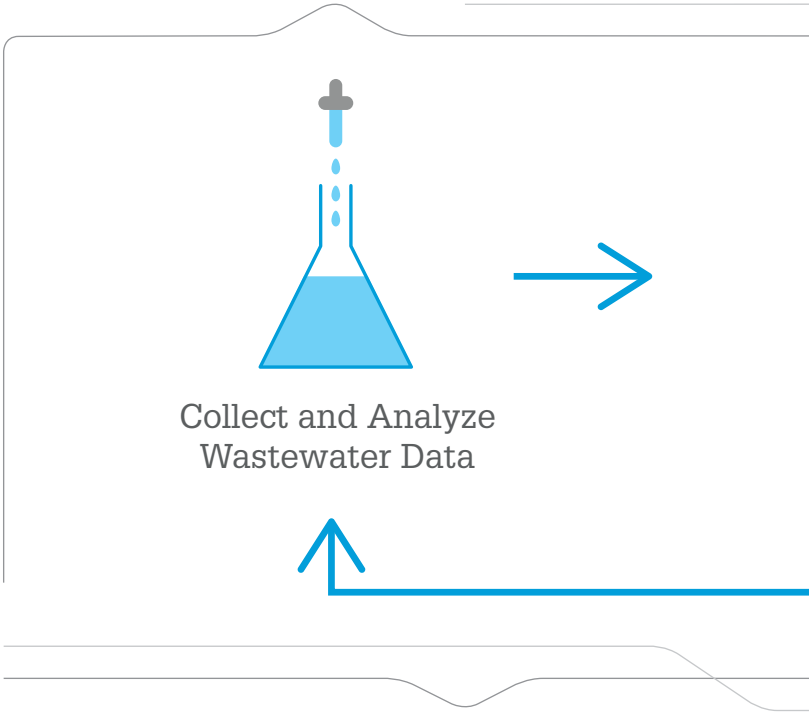


Figure 1. The Tempe opioid tracker interface, showing the tracking of fentanyl and heroin across distinct areas of the city. The options (what data is displayed and for what city area) can be modified by the user online.

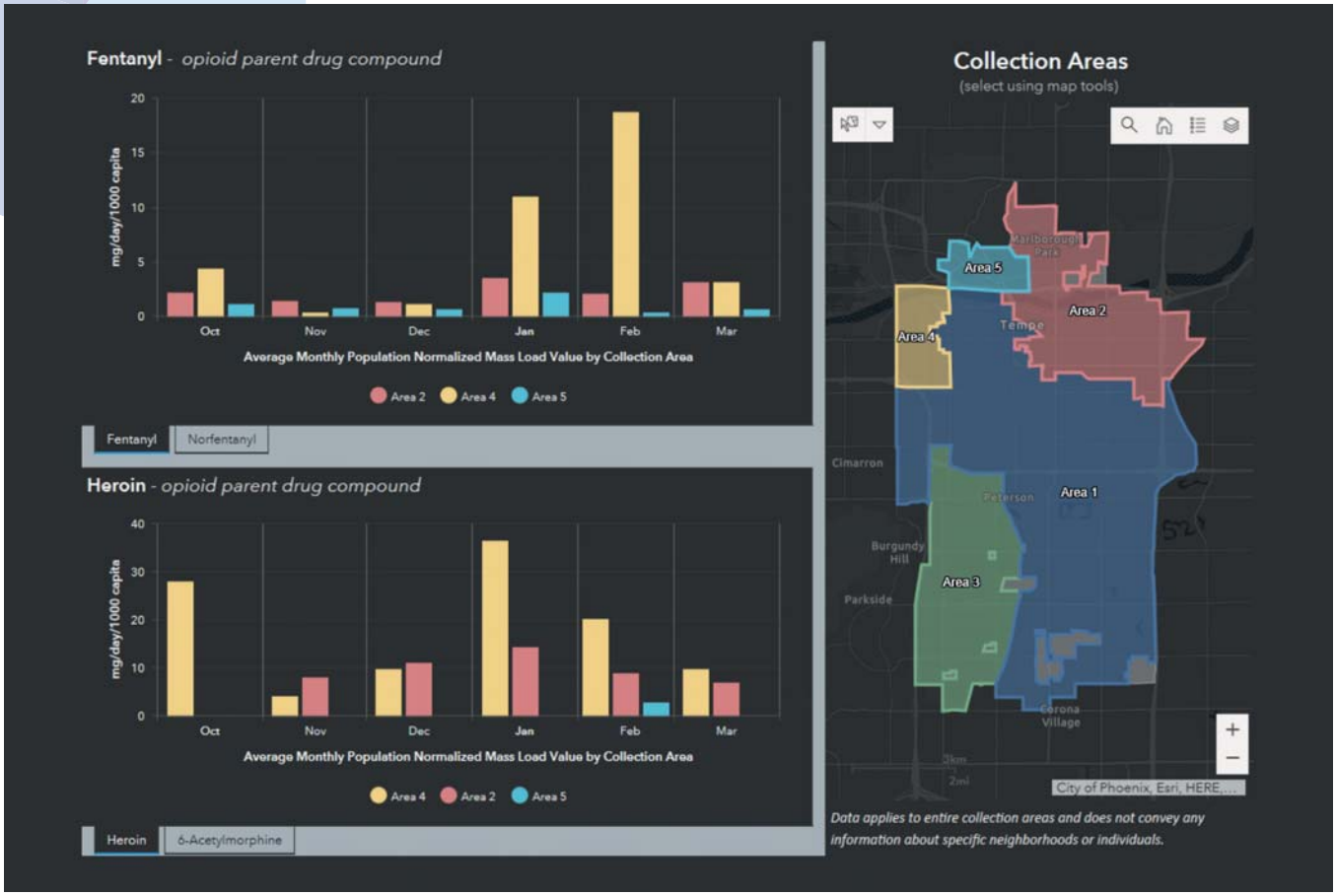
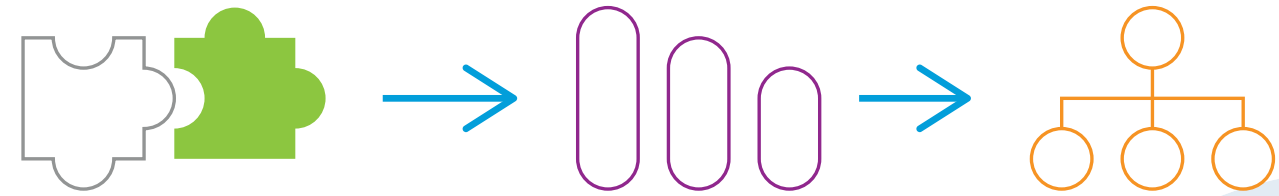


Figure 2. A schematic illustration of how the observatory is guiding interventions to tackle the opioid crisis in Tempe.



Gurus of IMS-MS

Ion mobility spectrometry (IMS)-MS has been around since the 1960s, but it was the launch of the first commercial systems in the 2000s that brought the technique into the mainstream. With an increasing role in applications from national security to omics, what's next for this versatile technique? David Clemmer, Erin Baker, and Kevin Giles delve into the past, present, and future of IMS-MS.

What's so special about ion mobility?

Erin Baker: IMS provides rapid structural separations based on the balance of two forces that impact the movement of an ion: the pull from the electric field and the drag force from collisions with buffer gas molecules. Variations in the electric field and stationary state of the buffer gas have given rise to multiple IMS techniques, including drift tube IMS (DTIMS), traveling wave IMS (TWIMS), trapped IMS (TIMS), field asymmetric IMS (FAIMS) and differential mobility analyzers (DMA). The different properties of each technique allow IMS devices to be constructed from low to high pressure values and have sizes ranging from just a few inches to many meters. Furthermore, each of these techniques can be interfaced with mass spectrometers to permit simultaneous acquisition of IMS structural and MS mass information on a rapid millisecond to second timescale. These multidimensional measurements are proving essential in assessing isomers and structural changes that cannot be evaluated with MS analyses alone.

David Clemmer: The timescale – millisecond measurements are slow enough that many types of experiment can be carried out, and fast enough that the observer doesn't need to schedule more instrument time to make the measurements. Essentially, the mobility measurement is obtained for free (from a laboratory time perspective).

Kevin Giles: I would only add that the speed at which separation takes place (the peak capacity per unit time) allows effective sampling of temporally variant upstream processes such as chromatography or imaging.

IMS-MS was first developed in the 1960s – how has it evolved since then?

Erin: IMS measurements have evolved throughout the last 60 years and become an increasingly important component of analytical analyses. One important development was the introduction of ion funnels to couple low-pressure IMS systems with MS in the late 1990s. This greatly increased the sensitivity of IMS-MS platforms by avoiding the losses associated with transferring ions between different regions, and thus enabled the rapid analysis of low-concentration compounds. Commercialization of these more sensitive IMS-MS instruments in the early 2000s gave a lot more groups access to the technology. Many groups found that the additional IMS dimension uncovered molecules missing or inseparable with MS-only analyses. These results have shown the value of the IMS separation and driven the commercialization of even more IMS-MS platforms.

David: I entered the field in the 1990s, when I joined Martin Jarrold's group at Northwestern University. At that time, the structure of C60 was still unknown and our group was in a race with Mike Bowers' group to understand the structures of small carbon clusters. Great theoretical work and increased computing speed made it possible to calculate mobilities, and Jarrold and I were the first to couple ESI to IMS with the aim of resolving protein conformations. It would be hard to overstate how bad our initial measurements were! We would begin recording the spectrum early in the morning and not stop until early the next morning… And yet we only counted a few hundred ions in the largest peaks. But compared with the calculated theoretical cross sections for simulated structures it became clear that we were onto something exciting.

I set up my own group at Indiana University, focusing on IMS but a lot people thought we were wasting our time, asking "Who cares about the structures of proteins in the gas phase?" We responded by improving the measurement. Steve Valentine, Cherokee Hoaglund, Anne Counterman and I coupled ion traps to drift tubes to accumulate ions and then added a time-of-flight (TOF) mass spectrometer to make the first nested-IMS-TOF measurements. Jim Reilly and Ray Sporleder knew how to make TOFMS measurements and they helped us greatly. Milos Novotny told us to forget the model systems and work on a real sample, and his group taught us how to make tryptic digests. Before long we had 2D-IMS-TOF measurements of complex systems, including mixtures.

Soon, we had it all coupled to an LC and were able to run proteomics experiments by LC-IMS-MS and LC-IMS-CID-MS. None of this worked very well! But we could resolve isomers and charge states and that was enough to get Kevin Giles and Tim Riley from Waters interested in developing it. IMS-IMS and circular instruments came along a little later and made sure that IMS was no longer seen as the poor relation of MS. We know now that there is a lot to learn from the ions before sending them into the MS.

Kevin: The availability of a commercial GC-IMS-MS system in the 1970s (made by PCP, West Palm Beach, FL) had a role in bringing the technology to more laboratories, but the real resurgence in interest began in the 1990s as key groups (Michael Bowers, Martin Jarrold, Herbert Hill, David Clemmer) began to study more biologically relevant species. Some of the biggest breakthroughs came from the Clemmer group. As David describes above, they showed nested LC-IMS-MS acquisitions on complex mixtures and improved sensitivity through ion trapping in sub-ambient pressure IMS and sequential IMS experiments followed by MS. Central to this advance was the utilization of TOF-MS rather than quadrupoles. Another major contributor to the expansion of IMS-MS came in 2006 with the commercial availability of a quadrupole/TWIMS/TOF-MS instrument, the Synapt HDMS from Waters.

> "IMS-MS has proven its worth in solving a wide range of analytical problems."

In recent years, Dick Smith's group developed their structures for lossless ion manipulation (SLIM) approach towards extreme IMS resolving power (>1800) over separation path lengths of many hundreds of meters. Since 2013, two other companies have produced mainstream commercial IMS/quadrupole/ToF-MS systems; the DTIMS-based 6560 from Agilent and the TIMS-ToF from Bruker. These instruments, combined with the Waters Vion (TWIMS/quadrupole/ToF-MS) and Synapt systems, make IMS-MS more readily available than ever. The most recent addition to the commercial landscape is the Select Series Cyclic IMS instrument from Waters, released in 2019.

Where are we today?

David: We have only scratched the surface of this technique. Buried in the broad peaks we generate today is a new type of vibrational spectroscopy that promises to tell us not only how many heavy isotopes are present, but also where they are located in each molecule. Dick Smith's SLIM measurements and Waters' cyclic instruments are beginning to reveal this information. Large ions remain mostly untouched. For example, IMS is enabling Vicki Wysocki's IMS-SID and SID-IMS work on protein complexes. Exploiting fast gas-phase ion-molecules for all kinds of mixture analysis is another promising avenue – Steve Valentine leads the way here. The marriage of IMS with traditional chromatography and MS is ideal for addressing the emerging problems in the post-genome era. Fast computing and information management approaches present many new ways to interpret data and gain predictive power.

Erin: Over the last decade the use of IMS in analytical measurements has rapidly increased, with applications in national security-related analyses, patient screening, environmental monitoring and numerous other areas. DTIMS, TWIMS, TIMS, DMA and FAIMS have all proved excellent tools for separating molecular classes and interfering ions, especially when coupled with MS analyses for the assessment of complex samples.

Kevin: IMS-MS has proven its worth in solving a wide range of analytical problems. Firstly, for analysis of complex samples, where the added peak capacity provided by IMS allows detection of lower-abundance species. Secondly, in screening applications, where collision cross section (CCS) libraries (as well as the added peak capacity) can aid confirmation of the identity of target species – a particular area of focus has been in pesticide screening of foodstuffs. Thirdly, where measurement of CCS values in conjunction with calculated values allows structural elucidation. Compounds from small molecules to large protein assemblies have been studied, providing insight into areas such as isomeric heterogeneity of samples and structural biology.

In what applications will IMS-MS have the biggest impact in the future?

Erin: While IMS-MS was once characterized as an emerging technique in the omics field, it is now well-integrated into

Meet the Gurus

David Clemmer

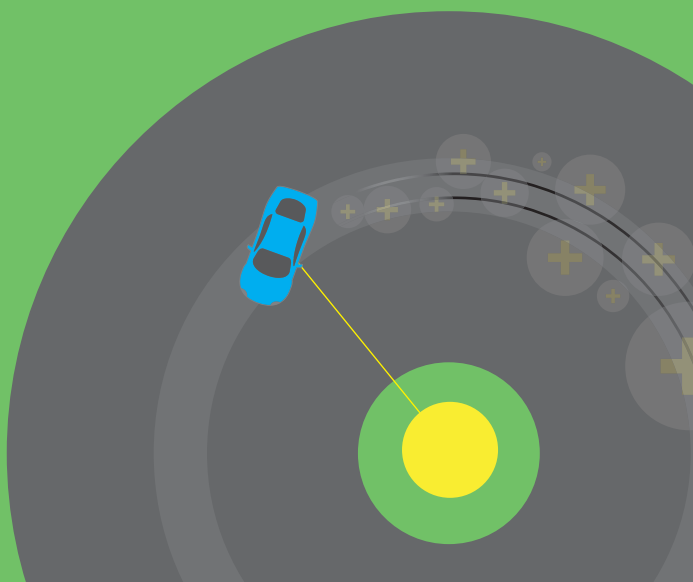
A chemistry Professor at Indiana University, Bloomington, David is a well-known figure in the world of analytical science. His work developing methods for the study of structures of complex low-symmetry in the gas phase has seen him recognized with several awards; the Fresenius Chemistry award and the John B. Fenn Distinguishing Contribution Award in 2018, to name just two.

Erin Baker

Erin completed her PhD in Chemistry at the University of California, Santa Barbara in 2005, before specializing in multi-dimensional separation techniques including LC, IMS and MS. Erin's work focuses on evaluating molecular changes in biological and environmental systems.

Kevin Giles

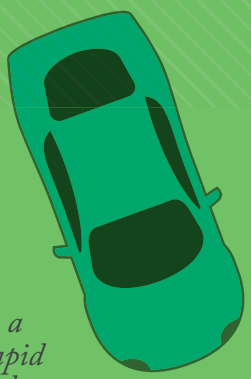
A Scientific Fellow at Waters in Wilmslow, UK, Kevin has been with the company for over 20 years. He has a PhD in Interstellar Chemistry. His work at Waters has primarily focused on the development of ion mobility – MS instrumentation, for which he was recognized in 2014 with the Industrial Analytical Science Award from the Royal Society of Chemistry.



modern omic measurements. Its utility in separating peptides and proteins with only minor structural differences has proven extremely powerful in bottom-up, middle-down and top-down studies. IMS-IS has enabled the evaluation of isomers, which were previously inseparable or required long chromatography assays. Plus, the ability of IMS-MS to distinguish contaminant ions and molecular classes is proving essential across all omic analyses of complex environmental and biological samples. By measuring CCS values for available standards and compiling databases, rapid targeted analyses and identification of small molecules, lipids, glycans and peptides is possible. These CCS values are also being incorporated into computational methods, such as theoretical modeling of structures and machine-based approaches, enabling molecular insight when standards are not available.

Kevin: IMS is particularly powerful in conjunction with ambient ionization approaches, where the simplicity of sample introduction is augmented by the additional peak capacity provided prior to MS analysis. This is of particular benefit when real-time decisions need to be made, for example in food safety testing or histopathology. Another high-impact application is in screening, where the combination of peak capacity and CCS libraries can be used to increase sample throughput whilst reducing false positives and negatives, saving time and money.

David: I believe the most important future work will relate back to some of the early criticisms of gas-phase protein work. Because we all emerged from physics backgrounds, when we made the early measurements, we gave our critics more respect than they probably deserved. Gas-phase proteins are often solution structures that become trapped upon removal of solvent in configurations that do not reflect native structures. In the early days, everyone was focused on visualizing native structures, so this was seen as undesirable. But what we are seeing is dozens of new non-native structures for proteins that cannot be observed with any other approach. A "cooperative, two-state" transition is really a composite of many structures. IMS-MS measurements appear to provide more information about the non-native states than any other method. If we are really going to understand protein



Introduction to IMS

New to the field? Need a refresher? Read on for a rapid recap of IMS–MS technology and the factors behind its rising popularity.

By Robin H.J. Kemperman, Department of Chemistry, University of Florida, Gainesville, USA.

Ion mobility spectrometry (IMS) is an analytical technique that separates ions based on differences in:

1. Their mobility whilst under the influence of an electric field;
2. Their interactions with a buffer gas.

While the use of IMS for analytical measurements and structural characterization is not novel, publications and conference proceedings over the past decade highlight a tremendous increase in IMS applications (1,2). IMS has been successfully applied in a number of areas, including isomer separation, reducing analysis time in LC-MS, kinetic studies, structure elucidation, molecular modeling, and the compilation of collision cross section (CCS) databases.

Acronym overload?

There are a variety of different IMS platforms available commercially, with some important differences (and a wealth of acronyms).

Drift tube IMS (DTIMS) is the most traditional, in which ions are propelled through a series of stacked ring electrodes by an applied uniform electric field, undergoing interactions with a neutral buffer gas. Separation of the analytes is based on the size, shape, and charge of the ions. Larger or more extended ions have longer travel times compared with smaller and more compact gas phase ion structures due to larger drag forces. Using DTIMS, CCS values can be directly calculated from measured drift times, which is useful for compound identification and structure elucidation (3,4).

Traveling wave IMS (TWIMS) moves ions forward

in a buffer gas-filled cell in the direction of the detector. In contrast to DTIMS, a traveling or pulsed direct current (DC) voltage is applied, generating voltage waves that drive ions forward. In addition, a radio frequency (RF) voltage is used to confine the ions radially for higher transmission (5).

Trapped ion IMS (TIMS) separates ions based on a non-uniform electric field that increases towards the detector. Ions are pushed forward by a gas flow and, depending on the size-to-charge ratio, ions are trapped at different axial positions in the TIMS funnel while being radially confined by a RF voltage. When the electric field is decreased, ions are released towards the detector from high to low size-to-charge ratio (6).

Field asymmetric IMS (FAIMS) separates ions at much higher electric fields based on differences in mobility between high and low electrical fields. Ions with different mobilities have different trajectories and might strike one of the electrodes. Therefore, a DC compensation voltage is applied to shift the ion trajectories closer to the opposite electrode to detect specific ions of interest (7). FAIMS is a continuous IMS system without ion gates and is therefore well suited for use as an ion filter.

Keeping it clean

Despite the rich information that IMS-MS provides, most complex samples, such as biological mixtures, need some form of front-end separation prior to IMS-MS to remove interference.

LC, GC, and online extraction devices have been reported to be helpful in sample clean-up and reduction of ion suppression (8). Direct sampling techniques, such as matrix assisted laser desorption/ionization (MALDI), desorption electrospray ionization (DESI), liquid micro junction-surface sampling probe (LMJ-SSP), liquid extraction surface analysis (LESA), and laser ablation electrospray ionization (LAESI) have also been shown to benefit from a fast millisecond-based IMS separation prior to mass detection (9).

A valuable tool

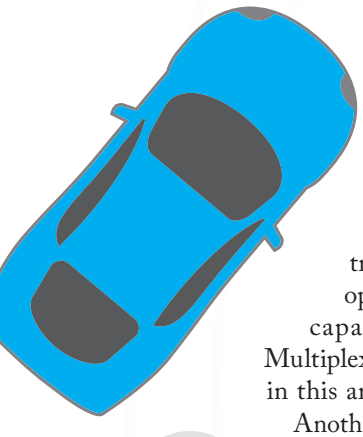
IMS, in combination with direct sampling or font-end separation techniques, provides fast analysis whilst increasing the signal-to-noise ratio through background reduction. In addition, IMS provides isomer and isobar separation and can assign CCS values in some cases. These CCS values are reproducible for the same gas-phase structure on any IMS platform, in comparison to LC retention times, which can shift over time or change drastically when comparing different systems.

A loss in transmission occurs when an extra stage of separation is added to a workflow. This problem has been addressed in IMS-MS through the use of better ion guides and ion funnels, and improving the IMS duty cycle using multiplexing with DTIMS (8). For FAIMS, which often suffers from significant transmission losses, different geometries have been investigated (planar, cylindrical, hemispherical) to balance separation power and transmission (10). The resolving power of IMS has also been a limitation in some applications. To overcome this, multiple IMS cells have been coupled back to back, such as a FAIMS-DTIMS and TIMS-TIMS, to increase the peak capacity and take an IMS zoom of a previously isolated IMS peak. Structures for lossless ion manipulation (SLIM), which uses extremely long TWIMS path lengths on print plates, and cyclic TWIMS have been developed to increase the IMS resolving power dramatically (11,12).

structure – perhaps the most important problem in biology – we will need to understand the role of non-native structures. How stable are they? What are their lifetimes? Do they aggregate?

What are your predictions for the future?

David: MS methods didn't come about overnight. There was nearly a century of work before we saw Alan Marshall's high-resolution measurements. After that came Alex Makarov's Orbitrap and suddenly everyone could make MS measurements easily and without cryogens. High-resolution IMS-MS has only been around for decade or so. In 50 years, it will have found its place and we won't think so much about the difficulties. The new cyclic instruments allow us to carry out IMS^n experiments – enabling an exciting new approach for state-to-state-(to-state)n experiments.



Kevin: There have been significant advances in transmission and resolving power in the past two decades. The challenge is that higher resolution requires longer separation times and to maintain performance (both transmission and resolution), the ion optics need to have increased charge capacity, mandating larger devices. Multiplexing approaches have proven useful in this area but are not without limitations. Another key challenge is to further improve the accuracy of CCS determination, both through instrumental measurement and theoretical calculation. These two requirements go hand-in-hand if more subtle structural differences are to be unraveled between species and if cross-platform CCS databases are to be produced. Instrument precision and reproducibility is, however, generally good, facilitating platform-dependent screening applications. One of the biggest issues has been a lack of software support for mobility data analysis. Significant inroads have been made in this area but there is still plenty of scope to ensure IMS-MS is accessible and embed it into routine workflows.

> "There have been huge strides made in hardware capability."

Erin: Undoubtedly, the utility of IMS-MS hinges on increasing its resolving power and successfully coupling it with other pre-separation, fragmentation and software tools. Fortunately, developments in all these areas are taking place to enhance the capabilities of IMS-MS measurements. Higher-resolution IMS and MS instrument platforms, such as the SLIM approach Kevin and David mentioned earlier, are becoming available and provide greater confidence in global measurements of complex biological samples. New fragmentation approaches such as electron-capture dissociation are being added to various instrumental IMS platforms, and software tools enabling rapid analyses are also being developed to push the technology further. The analysis of the multi-dimensional LC-IMS-MS data has proven difficult, due to the many measurement dimensions (LC, IMS, MS and data-independent or data-dependent acquisition MS/MS). Despite this, the future seems promising. The incorporation of numerous software packages including the open source Skyline software means that the capabilities of IMS-MS are only expected to increase in the future, making it an essential tool for high-quality sample analyses.

Kevin: I think the next few years are going to be extremely interesting for IMS, particularly in application development. There have been huge strides made in hardware capability and the application of this added horsepower to more challenging or complex studies is providing more detail than previously possible. Simply increasing IMS resolution will not provide all the answers.

The added flexibility and specificity of IMS^n, in the form of either sequential IMS devices or multipass cyclic IMS instruments, will be particularly enabling. I feel the broader acceptance of IMS-MS will come from its increasing use as a routine tool to provide better/quicker answers to analysts' questions. This will require ongoing development of software and integrated hardware.

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Profession

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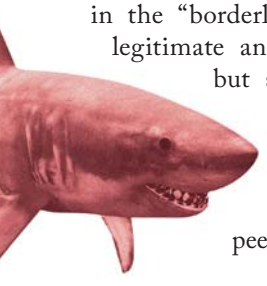
When Progress Becomes Predation

Predatory publishing can lead to academic ruin, especially for developing countries

By Yaman AlAhmad, Ibrahim Abdelhafez, Faruk Skenderi, Semir Vranić

"Pseudojournals" – predatory journals or publishers – abuse the academic system and community solely to gain profit. They do so by charging for the publication of questionable scientific content, devoid of standard editorial procedures like peer review. These journals operate globally, but their deleterious effects on the academic environment are felt most keenly in developing countries, where local researchers who publish in such journals build careers and gain tenure based on their publications, but fail to advance their scientific and research skills. On the other hand, these journals may also attract honest but inexperienced researchers who find their article "hijacked" after submission – no longer permitted to withdraw it, but subject to pressure to pay the fee for publication.

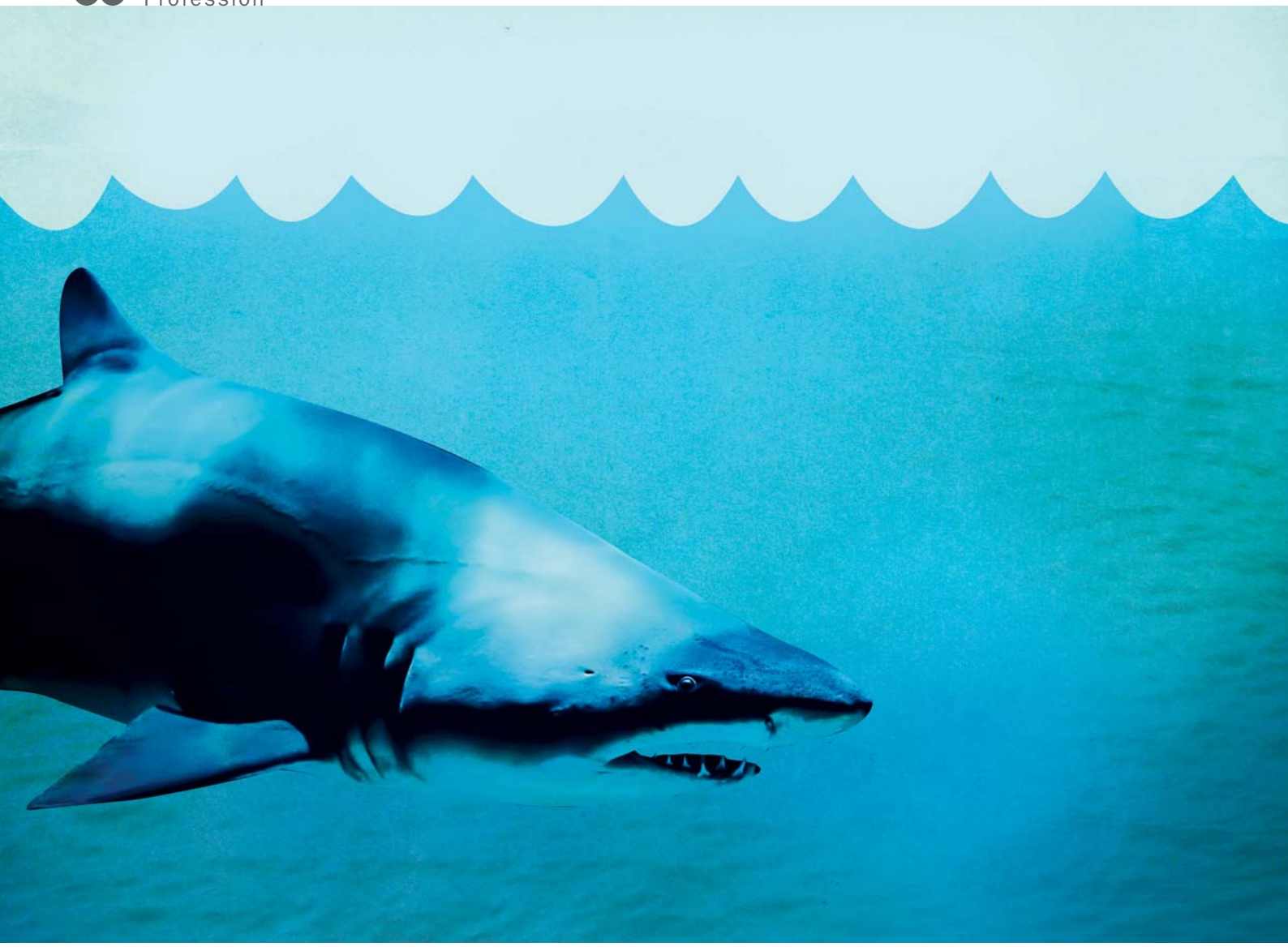
Predatory journals disguise themselves in different ways. Some of them operate in the "borderline" zone, balancing legitimate and for-profit practice, but still consistently and notoriously publish low-quality or even fabricated articles without conducting any peer review. Unfortunately, there are no firmly established criteria to help distinguish a predatory journal, particularly a "borderline" example, from a low-quality but legitimate one.



In more pronounced cases, it is easy to tell. At first sight, these journals use titles similar to legitimate journals, or label themselves as American, British, or originating from another scientifically sound nation despite being based in a completely different location. At the same time, they display false, misleading, or irrelevant scientific metric indices. Usually, information on the pseudojournals' editorial policies is vague, limited, incomplete, or altogether missing. The contents are especially revelatory; such a journal publishes everything it gets, regardless of its aims and scope, and sometimes even crossing the boundaries between branches of science, as with the Journal of Medicine, Radiology, Pathology and Surgery. Published articles lack basic editing and the layout is usually poorly done. These journals are almost never covered by PubMed/MEDLINE, Scopus, Web of Science, or the Directory of Open Access Journals (DOAJ).

"Predatory journals affect not only individual authors, but can have a negative or even ruinous impact on the academic environments of entire countries."

In more discreet cases, it is not easy to recognize predatory journals. They look deceptively like legitimate ones, with fitting titles, appropriate articles, and professional-looking layouts. Some of these journals even manage to become members of authoritative associations dealing with editorial and publishing



best practices – a fact they then misuse to improve their reputations. In addition, some of them have their contents covered by PubMed or Scopus – which not only makes it more difficult to spot the fakes, but also creates "noise" in the literature and spreads irrelevant, trivial, or wrong information while making it more difficult to locate and identify true science. Of note, our research revealed that only one predatory journal was listed in a reputable database (1). However, we do not know how many of them have applied and may be accepted for inclusion in reputable databases.

Another common feature that should raise suspicion of a predatory journal is its focus on authors – for instance, advertising or overemphasizing rapid peer review, database coverage, journal metrics, and emails with non-selective calls for papers. In contrast, legitimate journals put emphasis on scientific content to attract readers. Nevertheless, sometimes a more detailed analysis of the journal is necessary to rule out its potentially predatory nature, including looking at the reputation of the editor-in-chief and the journal's validation by databases or authoritative bodies. Sometimes, even a simple Google search for the journal can reveal other people's experiences that may assist determining its legitimacy.

The impact of predatory journals is astonishing – far more severe than it appears at first glance. They affect not only individual authors, but can have a negative or even ruinous impact on the academic environments of entire countries. Developing countries have limited scientific infrastructure and human resources. Unfortunately, without equivalent scientific resources, many researchers in these countries cannot reach the level of research quality and importance required to publish extensively in reputable

"For those whose academic work has a direct impact on patients, illegitimate journals might even lead to a negative effect on patients' health."

journals, so these countries usually have loose criteria for academic tenure. Nevertheless, they have adopted the academic organizational structures of developed countries, including the "publish or perish" mindset that makes authorship essential for an academic career. This is where predatory journals come in to fill the gap.

This setting creates a vicious cycle leading to extreme detriment of under-resourced academic communities. Inexperienced or incompetent researchers publish in pseudojournals, use those publications to rise through the ranks of academia, and train the next generation of researchers in the same methods. Eventually, the scientific potential of such a community could be devastated.

These journals also pose a significant challenge to individual scientists. In particular, young and inexperienced researchers might be tricked into contributing to predatory publications. This can have a deleterious impact on their academic and professional careers. Consider that the misfortune of publishing in a predatory instead of a legitimate journal could result in a failure to fulfil the requirements for a doctoral thesis – or that relying on results from an inadequately peer-reviewed publication could mean a waste of months or even years of scientific work. For those whose academic work has a direct impact on patients, illegitimate journals might even lead to a negative effect on patients' health.

Our research in our own field has shown that the number of predatory journals in pathology is approaching the number of legitimate pathology journals listed in Science Citation Index Expanded and the Web of Science. If this trend continues, it is expected that pseudojournals may outnumber the legitimate ones at some point in the future. Our next step is to look into the prevalence of predatory journals in laboratory medicine more generally to determine whether or not the threat is equally great – and to help raise awareness and build reliable tools for identifying these journals.

Like any other revolutionary development, open-access publication can be – and has been – misused. However, we do not believe that open access itself is to blame for the rise of predatory journals. In fact, open access is definitely the way of the future, especially in areas where researchers may not be able to afford multiple expensive subscriptions. The availability of legitimate, peer-reviewed information facilitates research dynamics and the overall progress of science and medicine. The academic community widely recognizes the importance of open access, and many institutions support their researchers in publishing with open-access journals. And the benefits extend to authors as well as to readers; many prefer open access because it means their papers are immediately available for reading and citation, thus increasing the author's reputation and disseminating knowledge faster. No wonder, then, that an increasing number of reputable publishers are offering open-access options to their contributors. Nevertheless, we believe that predatory journals are damaging the open-access model – once again, with a more pronounced effect in developing countries.

As the issue of predatory publishers is

"As the issue of predatory publishers is brought to the forefront, the academic community will need to adopt quality control criteria."

brought to the forefront, the academic community will need to adopt quality control criteria that can clearly differentiate between predatory and legitimate journals – particularly in the countries that are most severely affected. Eventually, such precautions will improve the publishing landscape for both academia and legitimate publishers.

Many researchers may find that they are pursued by predatory publishers, who ask them to submit manuscripts. And many more will likely come across these publishers online while seeking appropriate venues for future articles. How can legitimate academics protect themselves from danger? In addition to the criteria mentioned earlier, they should request the advice of senior colleagues and fellows – a task made much easier by social media; science and medical professionals all over the world can share information about pseudojournals almost instantly.

It's also important to question what you find, just as you would any scientific result. Have I ever read an article from this journal? Have I seen it cited in other legitimate publications? Have any of my colleagues or collaborators published articles in this journal? If all of the answers are negative, or if any of the journal's characteristics raise suspicion of a potential predatory nature, you should definitely apply the criteria used in our study (1) to ensure that your article is not being submitted to a pseudojournal.

A plethora of excellent and legitimate open access journals enable the free and unrestricted sharing of scientific and medical knowledge. Before deciding whether or not to submit your research to any of them, you should carefully evaluate the journal's impact factor (as provided by Journal Citation Reports, Clarivate Analytics) and indexing status – especially given that even legitimate journals, if recently launched, may lack an impact factor and may not be indexed in the major bibliographic databases. Notably, important information may be obtained from sources such as the World Association of Medical Editors (WAME) (2) and the Committee on Publication Ethics (COPE) (3), which are dedicated to promoting best principles and practices in publication ethics – including those related to research and publication misconduct. A coalition of scholars has also developed an online tool known as "Think. Check. Submit." to help scientists identify trusted journals in which to publish their research. When contributing to our collective knowledge, researchers would be well served to familiarize themselves with the basic principles of academic publishing and indexing using the above sources. Such knowledge is your best defense against academic predation!

This article was first published in our sister publication, The Pathologist. Read more at www.thepathologist.com.

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Weather-Induced Degradation Study of Polystyrene Using the Photoprobe

High Intensity UV Irradiator at 800 mW/mm²

By Karen Sam

Environmental conditions play a critical role in polymer lifetime, and traditional degradation studies take time ranging from hours to days due to limited light intensity. CDS's Photoprobe uses free-space focusing technology and improves the light intensity to 800 mW/mm² from 260 nm to 400 nm wavelength, which reduces the time on weather-induced degradation studies down to minutes.

A CDS 6200 Pyroprobe equipped with Drop-In-Sample Chamber (DISC) and Photoprobe was used, and an autosampler module was installed to automate the weathering-pyrolysis sequence. 11 µg of polystyrene was irradiated in the DISC with the presence of air as reactant gas at a flow rate of 10 ml/min. The volatiles generated from the photoreaction were trapped on the analytical trap, and then desorbed to the GC-MS after the photoreaction is completed. The remaining polymer was pyrolyzed at 600°C as the last step. A DISC quartz tube was used as the sample vessel.

CDS Pyoprobe Setting:

Method 1 - Weathering

DISC:	60°C
Photoprobe:	60% Intensity
UV irradiation:	5 min
Reactant Gas:	Air 10mL/min
Trap Rest:	40°C
Trap Final:	300°C 3 min
Trap Sorbent:	Tenax

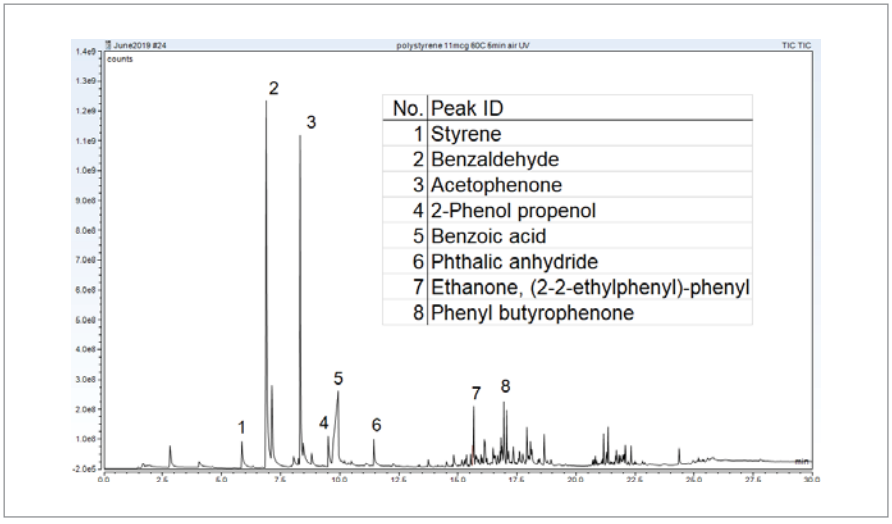

Figure 1. Photo thermal oxidative degradation products of Polystyrene

	BA:S	*AP:S*	*BA:AP*
Rep 1	8.23	5.24	1.57
Rep 2	8.88	5.98	1.49
Rep 3	7.90	5.75	1.37
Rep 4	8.52	5.91	1.44
Rep 5	8.14	5.48	1.49
Rep 6	8.21	5.96	1.38
Average	8.31	5.72	1.46
RSD%	4.09	5.26	5.16

Table 1. Peak area ratio reproducibility of degradation products: BA-Benzaldehyde (Peak 2), S-Styrene (Peak 1), AP-Acetophenone (Peak 3)

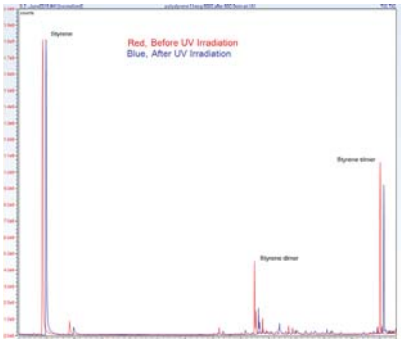

Figure 2. Polystyrene Pyrograms (600°C) before and after UV irradiation.

Method 2- Pyrolysis

DISC:	600°C	30seconds
Interface:	300°C	
Valve Oven:	300°C	
Transfer Line:	300°C	

Weathering studies of polymers with the Photoprobe took minimal time – on the scale of minutes compared to hours with traditional techniques. Polystyrene under UV irradiation and an air atmosphere at a 60°C set-point produces many UV thermal oxidative degradation products, ranging from volatile to non-volatile components. The resulting chromatogram is shown in Figure 1.

Six replicates of the volatiles from weathering polystyrene provided area ratio RSDs ≤5.25 percent for Benzaldehyde, Acetophenone and Styrene (Table 1). After this degradation analysis, the remaining degraded polymer was automatically pyrolyzed and studied with a second GC-MS run (Figure 2). Six replicates of the pyrolysis of weathered polystyrene produced an area ratio RSD of 3 percent.

In addition to analytical pyrolysis, the Photoprobe, the newest member of the CDS Pyroprobe family, can perform quantitative online weathering studies within minutes.

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WITec's ParticleScout is an advanced analytical tool for the alpha300 Raman microscope series that surveys, classifies and identifies particles over even large sample areas. Automated routines sort particles, acquire their Raman spectra and generate detailed reports. Microparticles are of ever-increasing interest in environmental science, cosmetics, packaging and many other fields.
www.witec.de/products/accessories/particlescout

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Learn more at https://www.cdsanalytical.com/thermal-desorption-7550s

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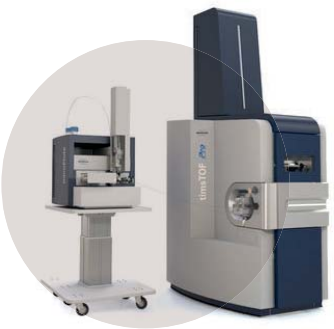
Learn more at http://chem.markes.com/Pro



Bruker's timsTOF PRO with PASEF

MS-based proteomics has become a powerful technology for the identification and quantification of thousands of proteins. Bruker's proprietary timsTOF Pro system uses Parallel Accumulation Serial Fragmentation (PASEF) acquisition method to provide extremely high speed and sensitivity to reach new depths in shotgun proteomics and phosphoproteomics, using low sample amounts.
Learn more at https://bit.ly/2uJ41ys

An Eye on Mobility

Sitting Down With… Erin Baker, Associate Professor, Department of Chemistry, North Carolina State University, Raleigh, California, USA.

Where does ion mobility fit into your research?

We like to combine multiple analytical techniques to gain answers to complex questions. Ion mobility is great for separating molecules of the same molecular weight and studying their structures – this structural information can then be used in combination with other separations to improve molecular identifications. We work across omics fields – proteomics, lipidomics, metabolomics, exposomics, glycomics – and ion mobility is invaluable when it comes to separating isomers in these studies.

For example, in lipidomics, we're trying to determine if different double bond positions are related to disease states by comparing samples from healthy patients with those suffering from a given disease. Our main focus, however, is the combination of different omic analyses – or multiomics. Here, we try to gain a more systems-level (holistic) view of the protein, lipid and metabolic changes to paint a more complete picture of the impact of a disease. When studying toxins, for instance, we can use exposomics to quantify toxin levels in a patient, and then proteomics to study potential changes in protein levels, which gives a more rounded view of how that toxin makes a person sick.

How did you become attracted to ion mobility?

My fourth-year undergrad project at Montana State University focused on ion mobility spectrometry, and I enjoyed it so much that it encouraged me to continue with this technique. From there, I joined Mick Bowers' group at UC Santa Barbara for my doctorate and then moved onto a postdoc position with Richard Smith; both focused on ion mobility. I will admit that the transfer to Smith was largely by chance… I needed a job and they needed the position filled as soon as possible – the fact that they were working on ion mobility was just a bonus really.

Tell us about your role at Pacific Northwest National Laboratory (PNNL).

I worked as a postdoc there for two and a half years, after which I was hired as a research scientist. My job was to bridge ion mobility technology to specific applications, and so I worked on all kinds of assays from soil to those based in human health. In these studies, we found that ion mobility was capable of identifying more molecules than MS alone. At PNNL, some groups were funded by the US Department of Energy, but we relied almost completely on external donors like the NIH. Now I'm using the grant-writing skills I developed at PNNL to apply for a number of further grants to support my research.

As an associate professor, what's most challenging?

The biggest challenge is probably the acquisition of funding, which is time consuming – especially trying to figure out which programs you fit into best; we've looked into environmental exposure for toxin analyses, and health analyses for molecular pathway elucidation. Plus, technological funding is really hard to come by in the US, which complicates matters further. At North Carolina State University, I was lucky enough to start a solid program, and was able to use my previous 13 years of research experience and a grant I had obtained to negotiate an amazing postdoc. We started on the same day and I have been acquiring graduate students over the last year. It is so important to build a strong team – nobody thrives in this field alone! And I've been blessed with a great group.

"For me, it's about staying motivated and, when rejection slaps you in the face, you just need to shake it off and keep going!"

How important is a positive attitude?

Generally, I would say it's important… but especially so in the sciences! In our field, rejection and failure are around every corner – from having papers turned down by journals to refused proposals to bad days in the lab and broken instruments. It's important to look at the positives to survive. For me, it's about staying motivated and, when rejection slaps you in the face, you just need to shake it off and keep going! That's definitely the attitude I encourage in my group.

And what keeps you motivated?

Our overarching motivation is to help the scientific community, hence our focus on developing tools that everybody can use. We've released databases for different molecules and we're also working to incorporate ion mobility functions into commonly used software, which can then be passed onto further teams; we recently accomplished this with the Skyline informatics group. We're hoping to achieve a sort of symbiosis: we produce the tools to help other groups and in return we can use the outcomes of other groups' research to challenge the big issues. After all, nobody will cure cancer alone… We need to centralize our collective knowledge and push forward together.

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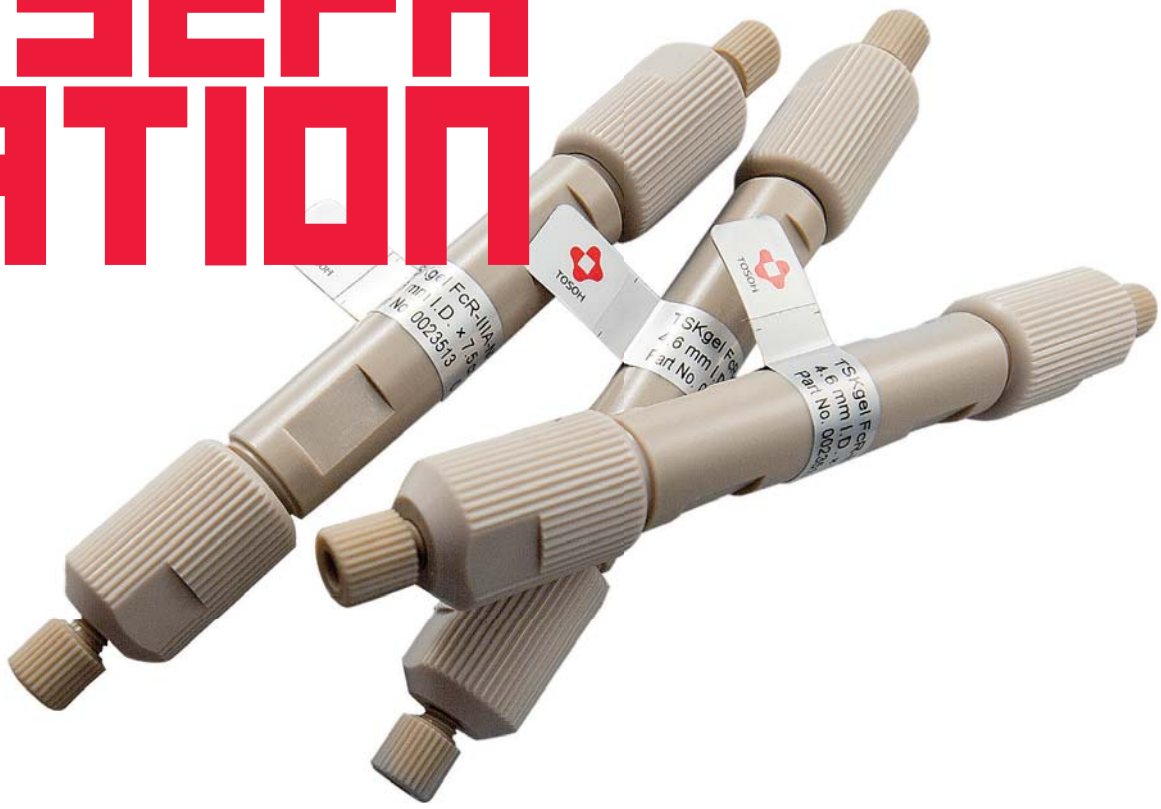

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