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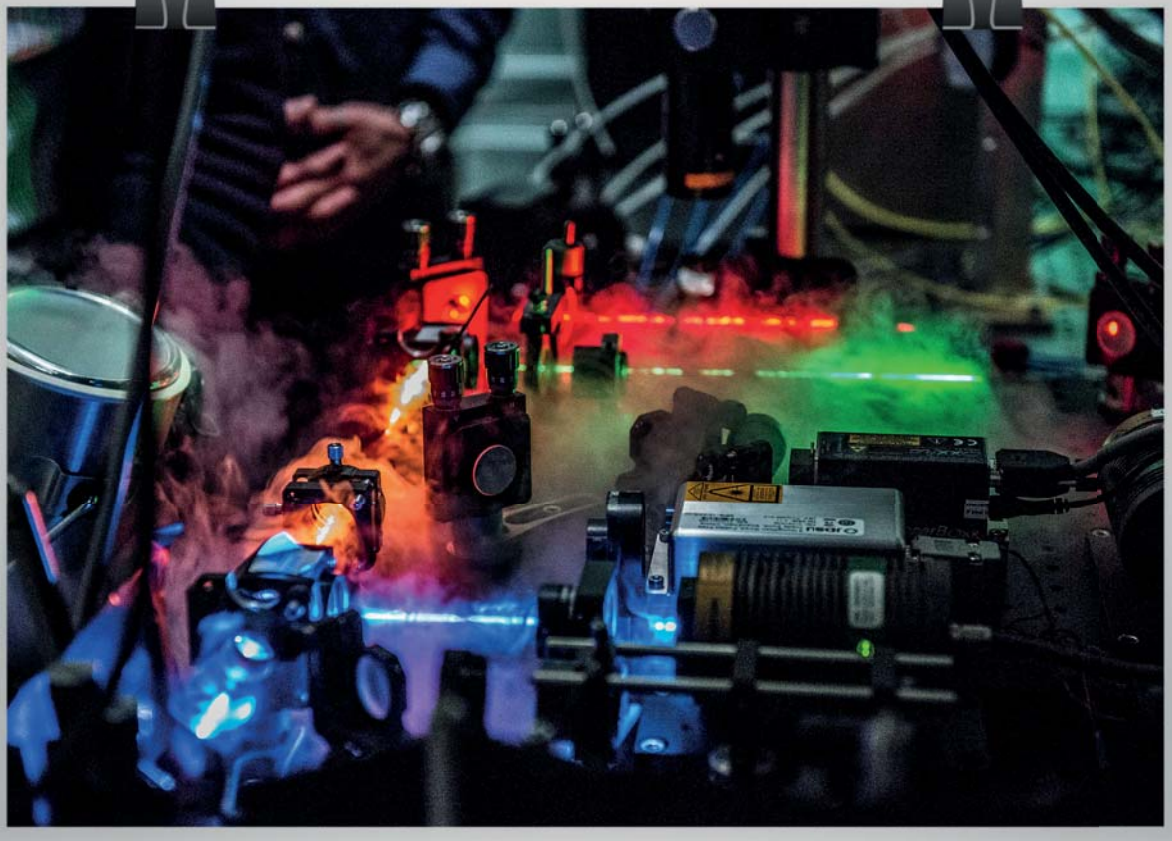

Opposites Attract

Researchers from the University of Zurich got a close encounter of the spectroscopic kind when they looked at the interaction between protein histone H1 and its binding partner, prothymosin α. Using single-molecule fluorescence and nuclear magnetic resonance spectroscopy, the team discovered that there is a strong electrostatic attraction between the two proteins, and that they have extended unstructured protein chains – a finding that could have implications for future drug development.

Credit: Christoph Schumacher, dunkelweiss

Reference: A Borgia et al., "Extreme disorder in an ultrahigh-affinity protein complex", Nature, 555, 61–66 (2018).

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

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Making a wish for the future of HPLC.

Upfront

the Analytical Scientist

ISSUE 65 - JUNE 2018

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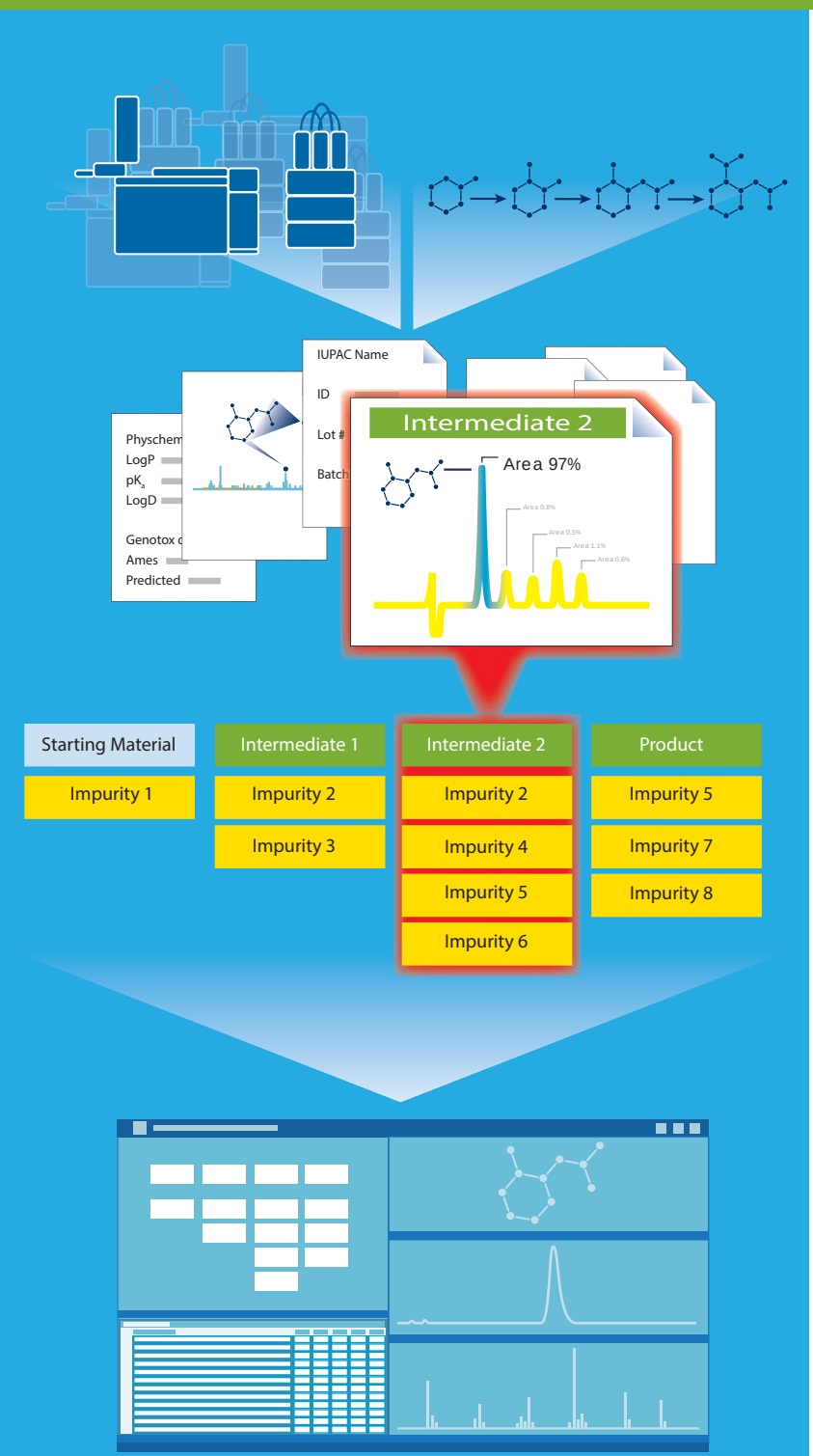

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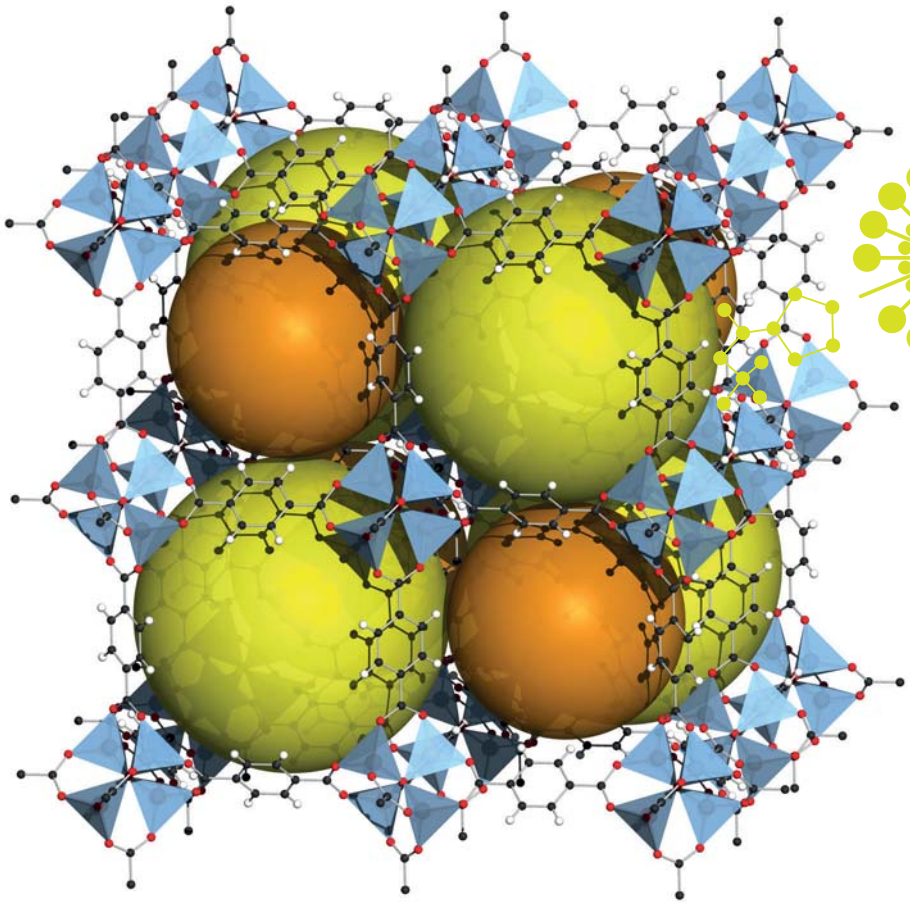

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In My View

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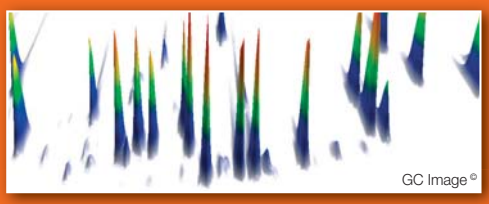

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Time to Think

We can all benefit from taking a moment to consider what could be.

Modern life is fast. Too fast, some would say – and it shows no signs of slowing. Indeed, innovation naturally drives towards getting more done, faster (which can also be called efficiency, but only if quality is not lost along the way). It's easy to get whipped up into the whirlwind, finding ourselves scrambling for a foothold.

But on the gloriously sunny Spring Bank Holiday, life slowed almost to a standstill, as I cruised down a Lincolnshire canal aboard my brother's new home – a narrow boat. The leisurely pace, combined with a lack of technology (my ultrafast smart phone had burned too bright for this world), seemed to grant us access to a secret portion of extra time. Time to catch breath, time to catch up, time to simply think. That's what holidays are for, of course. It feels natural to take time out, when we are supposed to...

So, what can we achieve when we give ourselves a little extra time to think while at work? At a recent "Editorial Retreat," my team and I dedicated two days – enforced thinking time – to find out. And we were all delighted, perhaps even surprised, by our creative output (which will be reflected in future pages of The Analytical Scientist for your benefit!). Our jobs are already creative in nature, and we're proud of the work that we do, so we weren't aiming for faster or better necessarily, but rather for "different" – another direction of innovation.

In this issue, it's great to welcome back Chris Harrison (page 44) – innovator in analytical education and "flipped classroom" advocate. By investing effort in being more creative with technology outside the classroom, Chris has made time more valuable in the classroom for his students – who, in turn, are more able to learn a key analytical skill: creativity. As Chris says, "[Analytical chemists] need to be able to approach problems from unexpected angles, as problems (and their solutions) are not always formulaic."

Slip flow star Mary Wirth is no stranger to tackling challenges from new directions (1), and though her creative work on colloidal crystals in "ultraefficient" chromatography was originally questioned, time, at least, was on her side. On page 38, Mary shares some of her current innovative work on stationary phases for intact glycoprotein analysis, using HILIC-MS. The result of time spent on creative solutions? The characterization of IgG1 glycosylation can be performed not in 1.5 days but in less than 30 minutes.

Innovative (analytical) solutions require creativity; creativity demands thinking time. And though sailing off into the horizon is unlikely to be welcomed by your boss, the fruits of a few hours (or even minutes) of contemplation, as often as you can afford it, almost certainly will be.

Rich Whitworth
Content Director

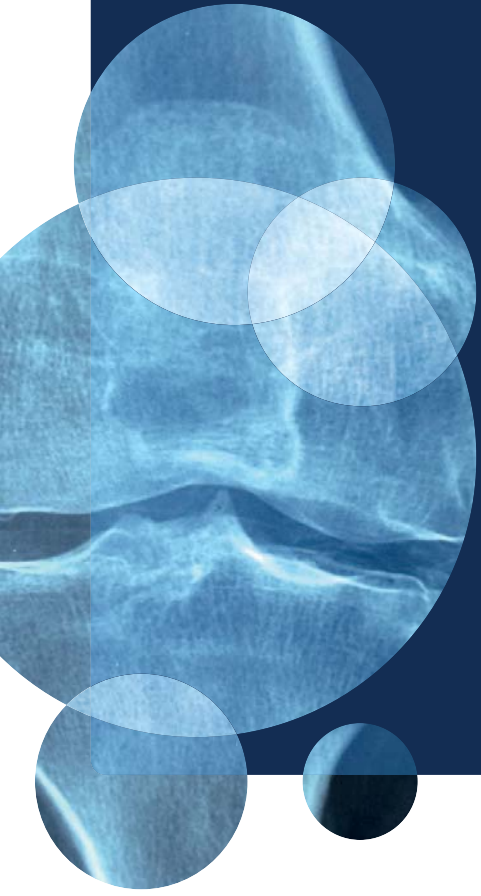
Reference

1. *www.theanalyticalscientist.com/issues/0713/slip-flow-star).*

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



A Joint Venture

Nanopore sensors boost the sensitivity of an osteoarthritis biomarker

Hyaluronan (hyaluronic acid) plays an essential role in the physiological functions of joints, giving rise to its use as a biomarker for osteoarthritis. However, in analysis, results are only semiquantitative because of a lack of sensitivity and insufficient dynamic range. Now, researchers from Wake Forest School of Medicine, Cornell University, and the University of Oklahoma Health Sciences Center are looking to boost sensitivity to improve quantitation using a solid-state nanopore sensor (1). We spoke with Adam Hall, lead researcher and Assistant Professor of Biomedical Engineering, to find out more.

How did the investigation come about?

My lab has a strong interest in applying nanopore technology to biomarkers. Ellie Rahbar, a colleague who has prior experience working with hyaluronan as a biomarker of trauma and knows the mechanism of our technology, recognized that nanopores might be able to measure its concentration. With the help of Paul DeAngelis of the University of Oklahoma College of Medicine, we determined that we could identify the size of hyaluronan polymers very accurately on a molecule-by-molecule basis. The final piece of the puzzle came when I met Heidi Reesink, a veterinary scientist who was using conventional technology to study hyaluronan in the knee joints of osteoarthritic horses. The fit was too perfect to ignore, so we initiated a collaboration that allowed us to apply our technology to an ideal in vivo system.

How easily will this translate to the clinic?

The technology itself is well-positioned for translation. We and others have developed advanced technology to increase affordability, make the results relatively easy to collect, and keep the measurement system compact. In fact, we believe the entire apparatus could be attached to, and powered by, a smartphone at some point.

There is clear evidence that osteoarthritis strongly affects hyaluronan, but definitive linkages between the disease's molecular characteristics and its grading and progression remain to be determined. This is mostly because of limitations in the technologies available for studying it; we believe our technology will fill that gap. A key advantage of our platform is its sensitivity: we may be able to test blood or urine (instead of synovial fluid drawn directly from the knee joint) – allowing us to make analysis less invasive.

How does your approach compare with current methods?

It rivals the precision and resolution of existing techniques, which tend to be much more expensive and time-consuming (and also require significant expertise and infrastructure). As with any new technology, it will take time for people to accept, but as our system becomes more accessible, and as we continue to show how our results compare with – or even exceed – those of conventional techniques, we think its advantages will become clear.

What's next?

We are pushing hyaluronan analysis further with more physiological testing as well as expanding into other diseases in which it may be important. We are also extending to other related molecules to diversify the utility of the platform, including our continued development of nucleic acid biomarker analysis.

Reference

1. *F Rivas et al., "Label-free analysis of physiological hyaluronan size distribution with a solid-state nanopore sensor", Nat Commun, 9, 1037 (2018). PMID: 29531292.*

What a Waste!

Better manure management could help fight antimicrobial resistance

Antibiotics are widely used in livestock, and when manure is re-purposed as fertilizer or bedding, traces of the drugs can leach into the environment, potentially contributing to the global antibiotic resistance crisis. Yet studies of antimicrobial residues in the environment have mainly focused on municipal wastewater effluents.

Farmers typically use waste management systems to treat solid and liquid manure before re-use – reducing offensive odors and making it suitable for use as fertilizer. A team from University at Buffalo, New York, wanted to know how effective these farmyard systems are in removing antibiotic residues. Their ultimate goal? "We are looking for strategies to minimize the environmental dissemination of antimicrobial compounds and antimicrobial resistance genes to reduce the agricultural contribution to the emergence and spread of antimicrobial resistance," explains Professor of Chemistry Diana Aga, lead researcher.

The team primarily used LC-MS/MS for the identification and quantification of antimicrobial residues in treated cattle manure. "The main analytical challenge we faced was developing sufficient extraction and clean-up procedures for achieving trace level detections, while preventing significant matrix suppression during analysis," Aga says. They overcame this by performing extensive sample cleanup using solid phase extraction (SPE).

The results indicate that standard waste management is not enough to remove antibiotic residues. The team found that tetracyclines were not completely removed during anaerobic digestion, and water recovered from the Livestock Water Recycling system (a reverse osmosis technique) was found to contain trace levels of ionophore antibiotics. Overall, solid waste contained higher levels of antibiotics than the liquid part of the manure.

"We were surprised to see that even high temperature conditions (such as pasteurization or composting) did not degrade the antimicrobials," says Aga. "But it's worth remembering that manure management strategies (including anaerobic digestion, composting, and so on) are designed to reduce odors, organic carbon and nitrogen levels – they are not specifically designed to remove antimicrobials."

The researchers would like to study transformation products of antimicrobials in manure using high-resolution mass spectrometry (HRMS), as well as digging into the effects that other contaminants have on antimicrobial resistance. The research can't come soon enough, according to Aga. "We are running out of options for effective drugs to fight pathogenic antimicrobial-resistant bacteria – it is one of the grand challenges of our time."

Credit: TU/e, Bart van Overbeeke

Pure and Simple(r)

Making cancer drugs cheaper and more effective – with a paper "coffee" filter

The action of breast cancer drug Tamoxifen is mediated not by the drug itself but by its metabolite, Z-endoxifen. The body's ability to convert the drug varies between patients because of genetic differences in enzyme production. Administering Z-endoxifen directly would remove this variability, but, until now, synthesis of the drug has been prohibitively expensive. Now, researchers from Eindhoven University of Technology, Syncom BV and the Antoni van Leeuwenhoek hospital in the Netherlands have found an inexpensive way to produce Z-endoxifen directly – using only a simple paper filter similar to those used for making coffee to isolate the pure drug.

The development has been some years in the making. "In 2011, Jos Beijnen (Netherlands Cancer Institute) asked us if we would be willing to synthesize a gram of Z-endoxifen for his research group, which is looking into the pharmacology of different tamoxifen metabolites (among them Z-endoxifen). At the time, Z-endoxifen was hypothesized to be the active form of tamoxifen and its efficacy in clinical trials had not yet been shown," says British-born Lech Milroy, Assistant Professor at Eindhoven University. "As part of her Bachelor's project, Daphne van Scheppingen and I produced milligram quantities of the Z-endoxifen as a 95/5 mixture of Z/E isomers using an optimized route." Beijnen then contracted out the synthesis to coauthors Syncom (1), where further optimization work was performed by Bartjan Koning to increase the safety and scalability of the synthesis, ultimately enabling production of the drug on a multi-gram scale, in a single batch and with higher purity.

So how did they filter this special "brew"? By carefully controlling the work-up and purification conditions (including changing the pH of the separating medium) and replacing expensive preparative HPLC with trituration and paper filtration (see picture) at the last step, the Eindhoven team managed to simplify the separation of pure Z-endoxifen from the undesired E-alkene isomer.

They also increased the stereoselectivity and further improved the reaction conditions and safety of the synthesis, allowing them to raise the overall yield of Z-endoxifen to 37 g in a single batch – a significant improvement on the 200 mg delivered after preparative reverse-phased HPLC reported in previous literature. "Our synthesis relieved a significant bottleneck in the process, enabling straightforward access to multi-gram – in other words, scalable – quantities," says Milroy.

TU/Eindhoven and Syncom's work has made Z-endoxifen more synthetically accessible to pharmacology groups. Says Milroy, "The mission now is to replace tamoxifen with Z-endoxifen in the clinic for the treatment of breast cancer."

Reference

1. *L-G Milroy et al., "A multi-gram-scale stereoselective synthesis of Z-endoxifen", Bioorganic Med Chem Lett, 28, 1352–1356 (2018).*

Materials Science and Small Molecule Detection

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

Products and launches

- B&W Tek has announced the launch of the QTRam, its latest portable Raman spectrometer.
- RedShiftBio has launched the AQS3pro – an automated protein characterization platform that uses integrated bioanalytics software.
- Rigaku will release its latest X-ray fluorescence (XRF) benchtop instrumentation at the American Association of Petroleum Geologists 2018.
- SCIEX has released BioPharmaView Software 3.0, which aims to streamline LC-MS-based workflows in biopharma and research labs.

Collaborations and acquisitions

- Camena Bioscience and TTP are partnering to develop a desktop analytical instrument to monitor cell culture health.
- Eurofins Scientific will acquire Covance Food Solutions in the next few months. Eurofins CEO Gilles Martin said, "[It will] strengthen Eurofins' global offering in the very competitive food testing market."
- Microsaic Systems will be working in partnership with Knauer to provide a compact integrated MS for Knauer's LC platform.
- The Materials Science and Engineering Research Facility (MSERF) has been established at the University of North Florida, thanks to a grant awarded by Shimadzu Scientific Instruments. The facility will provide state-of-the-art research facilities for students, as well as analytical services for the R&D sector.
- Agilent has announced that it will acquire software-developer Genohm. According to the VP of Agilent's Software and Informatics Division, "The modern architecture of [Genohm's digital platform] SLIMS is perfectly aligned with the values of Agilent's OpenLab products."

Companies and people

- Nightingale Health, known for its blood biomarker testing services, is expanding to include metabolomic analysis of urine, cerebrospinal fluid and umbilical cord blood samples.
- Genomic services provider Novogene will be establishing The Novogene UK Genomic Sequencing Center at the Babraham Research Campus in Cambridge, UK.

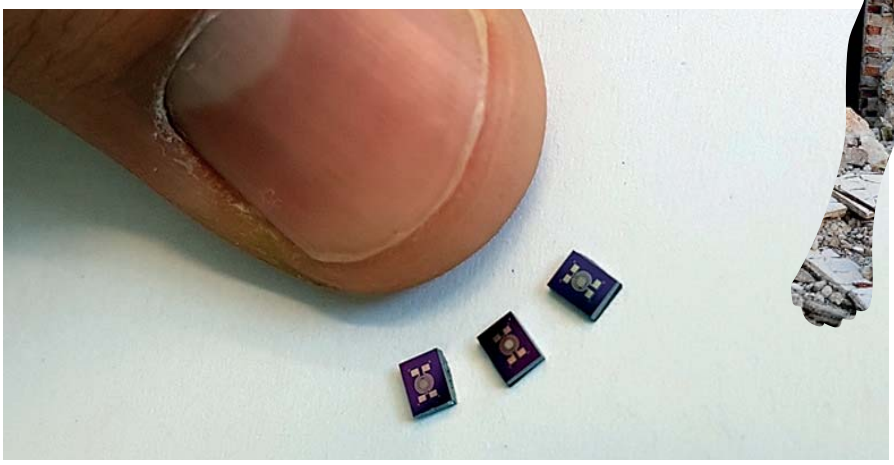
For links to original press releases, visit the online version of this article at: tas.txp.to/0618/BUSINESS.

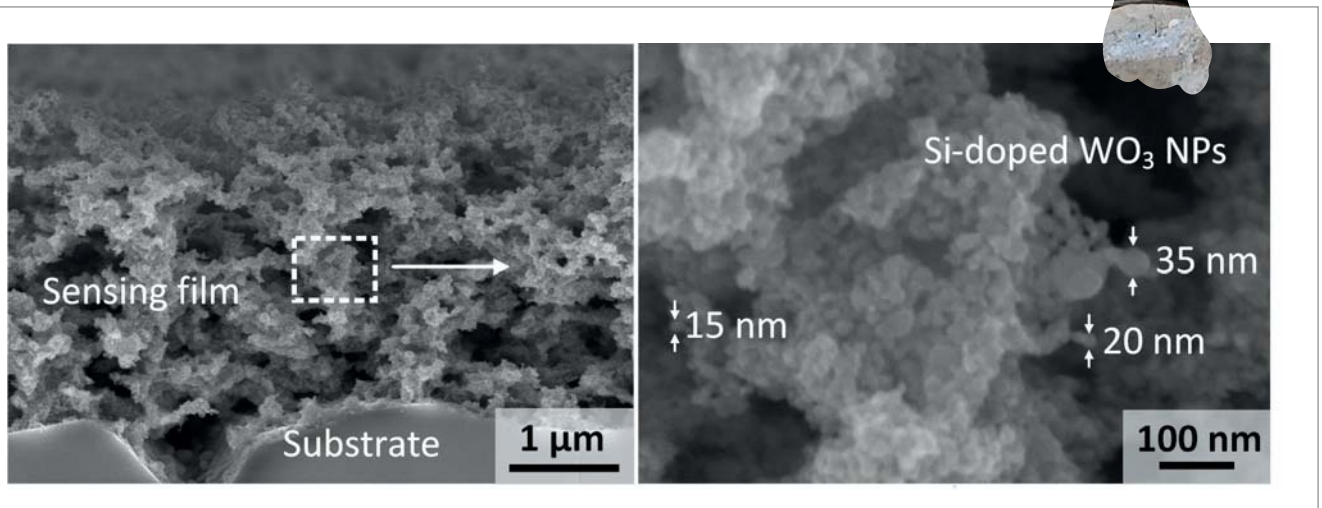
Working Like a Dog

A new portable sensor could sniff out trapped humans in search and rescue missions

An earthquake strikes, buildings crumble, survivors are buried beneath the rubble. When it comes to search and rescue in the aftermath, every minute counts. Rescue dogs are a valuable resource – but they are far from perfect.

"I was surprised when I spoke to first responders about search and rescue missions – I was not aware how limited the operational capabilities of rescue dogs are," says Andreas Güntner, research associate and team leader in the Particle Technology Laboratory, ETH Zurich. "Sniffing is tremendously exhausting for them, and so they may only operate for ten minutes to half an hour before they need hours of rest." This revelation kicked off the development of a device designed to sense human breath- and skin-





The sensor consists of three nanostructured metal-oxide sensors, including Si-doped WO3 to monitor acetone (shown here using top-view scanning electron microscopy).

emitted chemicals to assist in search and rescues.

The sensor was inspired by the team's work in the clinical field. "Our research at ETH focuses on the development of gas sensors for medical breath diagnostics. We had developed portable sensors to detect metabolic tracers, such as acetone, ammonia and isoprene," says Güntner. "So we asked ourselves: can our sensors sniff out trapped humans as well?"

After years of research, the team delivered a sensor array that incorporates highly porous films based on metal-oxide nanoparticles. "The particles are chemoresistive, changing their resistance upon surface interaction with the analytes, which can be detected as a signal," says Güntner. "The chip has three such films all based on different materials (Si-doped MoO3, Ti-doped ZnO and Si-doped WO3) designed to sense human breath- and skin-emitted ammonia, isoprene and acetone, respectively." They applied statistical response evaluation to the array to optimize the sensing performance further, and added commercial humidity and CO_2 sensors.

Urban search and rescue requires part-per-billion level detection of ammonia, isoprene and acetone, but the state-of-the-art technology needed to detect such low concentrations is not only expensive but also lacks the mobility necessary in such missions. The ETH team's sensor (pictured) addresses both problems. "We carried out parallel measurements with a bench-top selective reagent ionization time-of-flight mass spectrometer. Our sensor array performed well, with high accuracy of of 19.3 and 21 ppb and outstanding precision for the target compounds acetone, isoprene and ammonia."

The sensors may be referred to as 'electronic rescue dogs', but they're unlikely to replace our faithful friends any time soon. "We are aiming for field tests with first responders, ideally done in parallel with rescue dogs on their training sites," says Güntner. The team have also used the acetone sensor to monitor fat burn through breath during exercise and fasting and say the ammonia sensor is also promising in the non-invasive detection of kidney dysfunction.

References

1. *AT Güntner et al., "Sniffing entrapped humans with sensor arrays", Anal Chem, 17, 4940–4945 (2018).*
2. *2. AT Güntner et al., "Noninvasive body fat burn monitoring from exhaled acetone with si-doped Wo3-sensing nanoparticles", Anal Chem, 89, 10578-10584 (2017).*

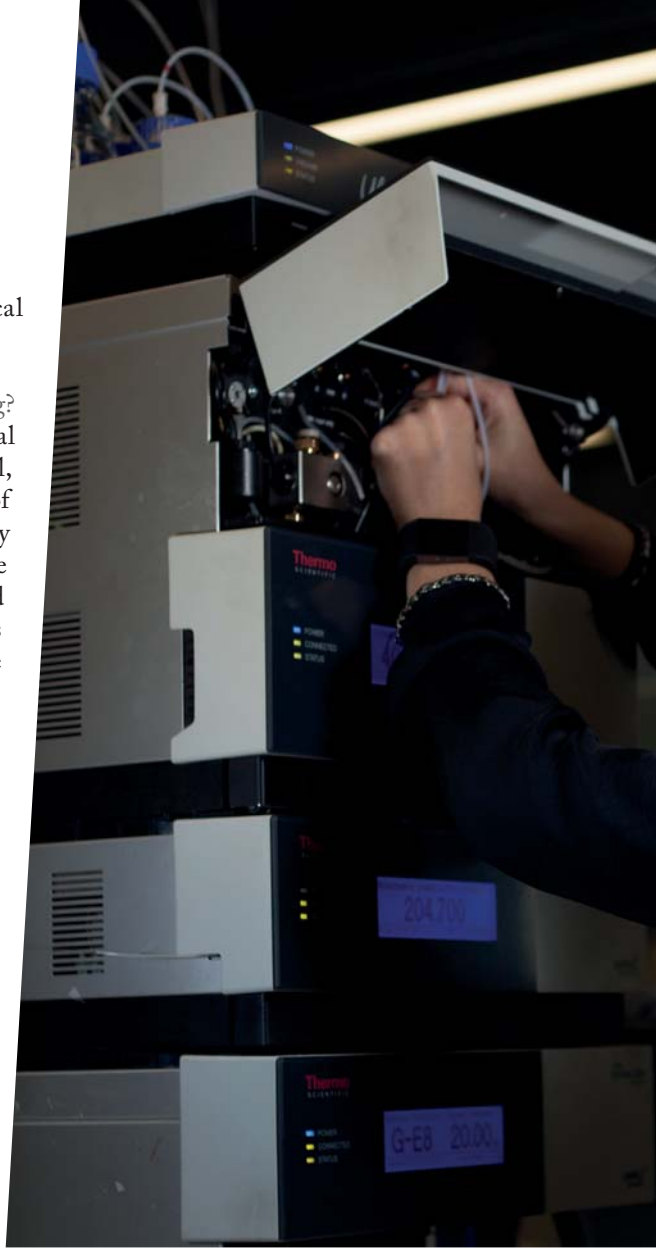
Data With Destiny

A chromatography consortium has been awarded four million euros as part of the Belgian government's Excellence of Science (EOS) program. We spoke to two of the awardees, Gert Desmet (Free University of Brussels) and Deirdre Cabooter (KULeuven) to find out more about their collaborative project focusing on the development of multidimensional methods.

What can you tell us about the application process?
The grant is part of a new funding program that aims to establish collaborative networks in the areas of science that Belgium is strongest in. Given the amount – and duration (four years) – of funding you can imagine there was fierce competition, with contributions ranging from astrophysics to cell oncology and from clinical psychology to archaeology.

Why focus on chemical data mining?
We want to bring multidimensional chromatography to the next level, partly by increasing the number of compatible modes, but especially by supporting analysts in making the best possible choices for method selection and optimization. In this process, the main focus will be on the end result of the analysis; in other words, on the quality and reliability of the measured data, rather than on the "beauty" or ingenuity of the chromatographic methods.

Could you describe the project?
The "Chemical Data Mining in a Complex World" (CHIMIC; www.chimic.be) project aims to revolutionize the way complex samples are analyzed with multidimensional methods. By developing a combination of clever decision algorithms and innovative hardware solutions, we can better explore and expand the available separation space for complex



samples. The first steps will involve pulling together all the consortium's knowhow on method selection in multidimensional analysis and compiling it into a rational workflow.

What do you hope to achieve?

By the end of the four-year period, we hope that we will have established a renowned Center of Excellence on chromatography and chemical analysis that will shape new generations of separation science specialists, as well as helping industry professionals solve their toughest problems. In the longer term, we would like to end up with a functional expert system that can autonomously select and combine the most suitable separation methods for complex samples.

You can find out more about Desmet and Cabooter's work by reading our Sitting Down With... interviews:

Gert Desmet: tas.txp.to/1117/Desmet
Deirdre Cabooter: tas.txp.to/1215/Cabooter

In My View

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

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

Contact the editors at edit@texerepublishing.com

Bioanalysis 2.0

How can pharmaceutical companies and CROs work together to drive innovation?

By Scott Summerfield, Head of Bioanalysis, GlaxoSmithKline, Ware, UK and Eric Yang, Vice President, Bioanalysis Immunogenicity and Biomarkers, GlaxoSmithKline, Philadelphia, USA.

Twenty years ago, mass spectrometry swept into the field of bioanalysis and rapidly replaced existing detection methods, such as UV. As a result, pharma was able to scale-up pharmacokinetic (PK) and toxicokinetic measurements in drug discovery and development like never before, allowing portfolio decisions to be based on circulating in vivo concentrations rather than merely the dose administered. Nowadays, bioanalytical measurements extend far beyond drug concentrations in plasma, and support a more translational medicine-centric mind set within the pharmaceutical industry. Increasingly, data-rich preclinical and clinical trials demand much more from limited R&D budgets, and to keep costs manageable, the pharma industry often looks to develop partnerships with contract research organizations (CROs). Indeed, the majority of regulated bioanalytical work is conducted at CROs.

The pharma-CRO partnership should be mutually beneficial but, in reality, innovation in bioanalysis has faltered – caused in no small part by the evolutionary course of the arrangement. Given the current high level of outsourcing, CROs have become the majority shareholder, while pharma has seen a concomitant contraction of internal capital expenditure and bioanalytical staff. In parallel, today's marketplace for novel medicines means that pharma procurement groups must maximize return on investment (ROI) for any externalized activities, with the goal of getting medicines to market at a price tolerable to payers, shareholders and healthcare providers alike. In turn, CROs are under pressure to keep costs low.

Oftentimes, significant investment is required to adopt a new technology. Justifying the original capital outlay will almost certainly need recurring revenue, and then there is a significant training burden, regulatory uncertainty and a need to "pull" pharma companies to agree to using innovative workflows on fledgling drug products. Whereas pharma looks at the long game of new medicine development, bioanalytical CROs are inherently more focused on short-term results; business is booming for CROs, so why change? Larger CROs can afford to be rapid followers, skipping the risk of trailblazing. Smaller CROs simply can't afford to invest more capital into newer technologies and remain competitive.

CROs may be reluctant to update their technology but it's clear that advances like high resolution mass spectrometry (HRMS) and lab automation will win out in the end; the results speak for themselves. These technologies will not only drive better data quality but will also enable lab scientists to focus on higher-level tasks, rather than holding pipettes or moving 96-well plates around.

Momentum is already building; major bioanalytical conferences have more sessions than ever on lab automation and HRMS. However, to speed up adoption, we need a collaborative endeavor involving vendors, CROs and pharma that looks at how we can reduce cost and risk. Pharma

must take its fair share of the innovation burden. In our own laboratories, the philosophy is now to embed new technologies more assertively into internal support and bring them into the early regulated environment. The resulting efficiency gains allow us to retain more bioanalytical activities on strategically important assets, or divert skilled scientists to more challenging tasks.

The next step is to push these technologies into receptive CRO partners – we believe CROs are much better placed to drive industrialization and scalability. The value that we can bring to internal support should and must translate to even greater value in our outsourced activities, with improved efficiency in CROs leading to lower costs or increased capacity for pharma.

There are a number of exciting CRO start-ups that are aiming to disrupt the bioanalytical sector, including highly automated platforms that would slash the costs of delivering regulated bioanalytical support by providing i) reduced outsourcing costs for pharma, and ii) 24/7 scalability for CROs based on instrument cells rather than recruitment. The technology is ready and waiting, so the question now is: who will be the key players in bioanalysis 2.0 – and who will be caught napping?

Mineral Oil Analysis: A Slippery Problem

What are the analytical challenges – and dangers – posed by MOAH and MOSH – and how can we tackle them?

By Maurus Biedermann, Chemical Analyst, Official Food Control Authority of the Canton of Zurich, Switzerland.

In food analysis, people are increasingly talking about MOAH and MOSH – but what are they, and why are they in the spotlight?

The terms mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) were first used in 2008, when our lab started to distinguish between these two fractions of mineral oil contamination by HPLC separation. In fact, we began studying them two decades before this; it was 1988 when we were using on-line LC-GC coupling to perform a different analysis entirely, but by coincidence, the mineral oil contamination showed up in the chromatograms.

In these initial experiments, we discovered that jute bags – used to transport cocoa beans, rice, hazelnuts, and more – were the source of the mineral oil contamination. The oil is used to soften the fibers in the jute bags, and during transportation or storage there is migration of the volatile parts of these mineral oils. Since then, many other potential sources, such as recycled paper and board, lubricants and the environment, have been found to contaminate our food supply with mineral oil hydrocarbons.

Why is this important? It has since been discovered that MOSH accumulate in the human body, and concern has therefore been growing about their possible adverse effects – though quite how much harm they pose to humans is still unclear. It is suspected – though has not been adequately proven yet – that the toxicity of saturated hydrocarbons is different to aromatic hydrocarbons. In the class of three and more ring polycyclic aromatic hydrocarbons, there are certainly species which are carcinogenic; for example, benzo-a-pyrene.

MOSH/MOAH are a complex mixture of thousands of isomers; they don't produce single signals in the chromatogram, but rather "humps". Interpreting such chromatograms can be difficult, and needs experience and training.

On-line LC/GC-FID is already used in routine analysis, and there are auxiliary methods that are able to remove interfering long-chain n-paraffins or olefins from natural origin. For example, epoxidation renders olefins more polar and they are therefore removed from the MOAH fraction, and long-chain n-paraffins are removed by chromatographic pre-separation on activated aluminium oxide. If there is a need for further characterization, comprehensive 2D-GC is a very powerful tool that allows further separation of the MOSH or MOAH humps. GC-MS and GC×GC-FID/MS add further tools to the search for mineral oil markers.

There are no general regulatory limits for MOAH and MOSH... yet. But there is an ongoing discussion. In Germany, for example, there is a draft regulation regarding the use of recycled paper and board for food packaging. Given the inherent challenges of MOAH and MOSH analysis, it is critical that we prepare analytical methods that are able to adequately support upcoming regulatory decisions.

THE NEXT BIG (OR SMALL) THING

The huge diversity of applications – old, new, and emerging – that must be addressed by modern liquid chromatography presents challenges and opportunities. In this double-barreled feature, we attempt to predict LC's way forward in our leaders' "wish list" – and profile advances in one important direction: portability.

THE WISH LIST: LIQUID CHROMATOGRAPHY

In this issue, we're exploring a host of exciting advances in separation science – from miniaturized HPLC systems (page 26) to the evolution of sample preparation (page 30). But what's the next big priority for LC development? We asked leading chromatographers what advances they would most like to see and why. Here's what they told us…

"I'd like to see the development of columns covering all LC modes with internal diameters (ID) of 1 mm, packed with particles (porous or superficially porous) and offering, with high reproducibility, the same efficiency as columns of 3 to 4.6 mm ID. To make this wish possible, we need instrumentation that provides dead volumes able to cope with such small IDs. Not only do we need optimal mobile phase flows in the order of 50 µL/min (20 times lower compared with the 1 mL/min for 4.6 mm ID columns) for R&D purposes, but there is also no fundamental reason not to implement such columns in QA/QC (green chemistry!)."

Pat Sandra, Emeritus Professor, Organic Chemistry, Ghent University; Founder and President, Research Institute for Chromatography, Kortrijk, Belgium.

"A great deal of research is focused on improving efficiency of separation. The other important practical aspect of SPME application would be to improve background and carry-over issues, which would require understanding the sources of column contamination, as well as improvements in the design of LC components to minimize carry-over. Longer term, I'd like to see improved fundamentals and instrumentation to facilitate on-line multi-dimensional separations, including heart cutting. The miniaturization of LC systems and use of alternative pumping systems, such as electro-osmotic pumping, are also important future directions."

Janusz Pawliszyn, Professor, Department of Chemistry, University of Waterloo, Ontario, Canada.

"I would like to have a single, robust, high-resolution (UHPLC or better) universal stationary phase capable of resolving the whole spectrum of low molecular mass metabolites/small peptides (from polar ionic, polar-neutral, through mid-polar, all the way up to non-polar lipids) in a single LC-MS compatible separation, to be able to rapidly and reproducibly metabolically phenotype biological samples for metabolomics/metabonomics applications."

Ian Wilson, Professor, Chair in Drug Metabolism and Molecular Toxicology, Department of Surgery & Cancer, Faculty of Medicine, Imperial College London, UK.

"Stationary phases allowing for an even better retention and separation of compounds, exhibiting an extensive spectrum of polarities, would be particularly desirable. Especially in light of the growing relevance of multi-analyte test methods, employing targeted as well as non-targeted approaches, we need the highest performance in LC systems."

Anonymous (working in forensics)

"During my 30 years in chromatography, I have been amazed by the technical improvements in (U)HPLC, but I get sticker shock at the costs and miss the ability to use modular LC components with any detector from any vendor. So my wish is for better modularity and interchangeability between vendor LC and detection systems."

Steven Lehotay, Lead Scientist, USDA Agricultural Research Service, Eastern Regional Research Center, Pennsylvania, USA.

"I would like:
1. An expert system that suggests the right column and mobile phase once you enter the structures you want to separate,
2. Routine LC in less than 10s,
3. Lipid isomer columns."

Bob Kennedy, Hobart H Willard Distinguished University Professor of Chemistry; Professor of Chemistry, Chair-Chemistry, College of LS&A; Professor of Pharmacology, Medical School, University of Michigan, Ann Arbor, USA.

"A transfer interface/strategy that makes fully uncoupled operation between the two separation processes in LC×LC possible, while still allowing complete and focused transfer of the eluent from the first dimension to the second, providing a flexible, universal and easy to optimize analytical platform."

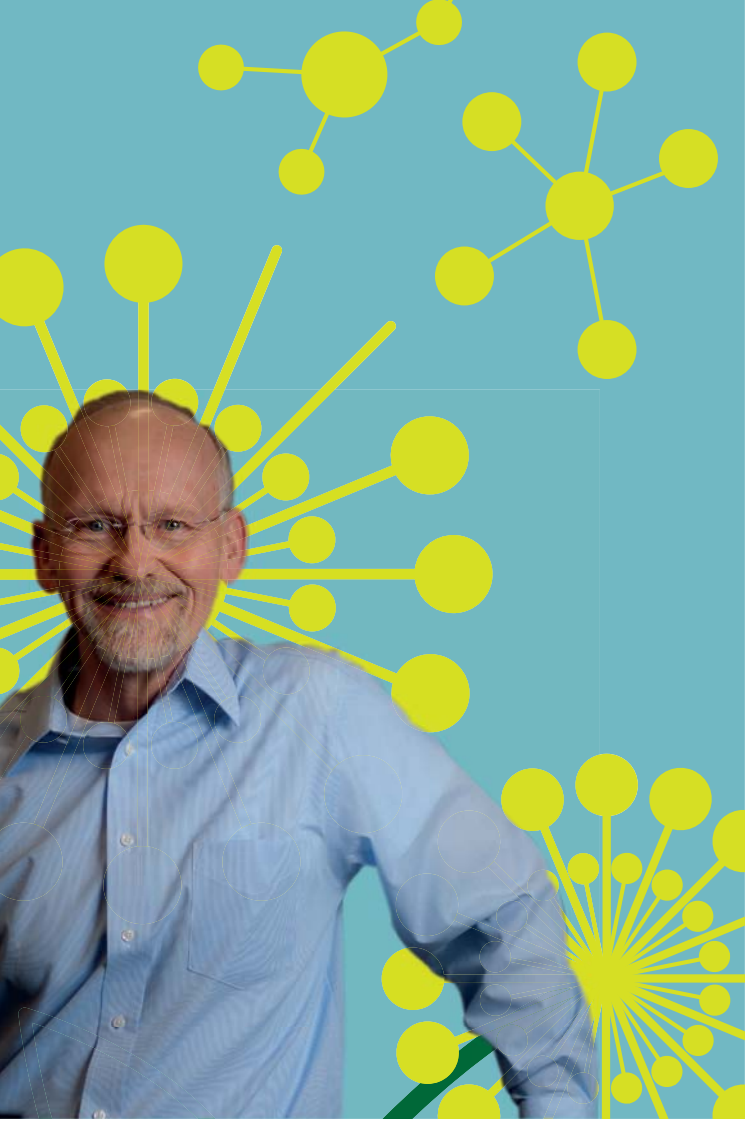
Lourdes Ramos, Research Scientist, Department of Instrumental Analysis and Environmental Chemistry, Institute of Organic Chemistry, Scientific Research Council (CSIC), Madrid, Spain.

"This Christmas, I wish Santa would bring me a really sensitive on-column UV absorbance detector with a physical diametric path length of 25 microns or smaller."

Sandy Dasgupta, Hamish Small Chair in Ion Analysis, Department of Chemistry and Biochemistry, University of Texas at Arlington, Texas, USA.

"Separations providing increased peak capacities and peak generation rates (essentially more resolution, and faster!), so as to enable analyses that provide increased dynamic range and speed for applications involving highly complex samples in conjunction with mass spectrometry, such as those in proteomics and metabolomics."

Dick Smith, Battelle Fellow and Chief Scientist, Biological Sciences Division, Pacific Northwest National Laboratory (PNNL), Washington, USA.



"LC separation of a wide range of polar and non-polar compounds in water would be nice."

Xing-Fang Li, Professor, Division of Analytical and Environmental Toxicology, University of Alberta, Alberta, Canada.

"I would most like to see highly efficient 3D printed columns. These computer-designed columns need to be identical, so we need suitable materials to create both the column and the filling at the same time, and high-speed high-resolution printers. By default, the filling must be a monolith."

Frantisek Svec, Facility Director, Organic and Macromolecular Synthesis, Lawrence Berkeley National Laboratory, Berkeley, USA.

"A universal LC-MS interface that allows the ionization of all compounds irrespective of their polarity, size, volatility and so on; plus, gives a more or less constant response for all species – so that universal calibration factors can be employed and compounds for which no standards are available can be quantified."

Hans-Gerd Janssen, Science Leader Analytical Chemistry, Unilever Research Vlaardingen, and Professor of Biomacromolecular Separations, van't Hoff Institute for Molecular Sciences, University of Amsterdam, the Netherlands.

"The desire for intact protein analysis has grown tremendously. We need more and new liquid chromatography stationary phase/support combinations and concepts to provide a wider range of selectivity for intact protein separations. Ideal products would be able to work over a wider pH range (especially above pH 8), have potential to recognize variable and changing protein conformations, and be extremely robust."

Kevin Schug, Shimadzu Distinguished Professor of Analytical Chemistry, University of Texas at Arlington, Texas, USA.

"My coworkers and I would appreciate having 1 mm columns with various chemistries and robust long-term performance at ultrahigh pressures, providing ultrafast separations easily interfaced with mass spectrometry. Such columns would use optimal flow rates for maximum sensitivity with electrospray, but would still be robust enough for high-throughput LC-MS quantitation."

Michal Holčapek, Professor, Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Czech Republic.

"My long-term wish is the development of comprehensive spatial 3D-LC chip technology, overcoming classical sequential analysis of fractions that incorporate novel flow control mechanisms between different developments. This technology has the potential to achieve truly high peak

capacities in the minimum amount of time (compared to classical 2D-LC technology)."

Sebastiaan Eeltink, Department of Chemical Engineering, Vrije Universiteit Brussel, Brussels, Belgium.

"My wish list would include:

1. 2D and 3D HPLC separation methods with a total peak capacity that can reproducibly separate thousands of compounds.
2. Preconcentration methods that can concentrate compounds based on compound class.
3. Column technology that is even more efficient than existing sub 2-micron particle technology.
4. Columns and preconcentration devices that can improve the dynamic range of analyses."

Susan Olesik, Dow Professor and Chair, Department of Chemistry and Biochemistry, The Ohio State University, USA.

"My big LC wish is for hardware that allows us to achieve the full potential of fast separations and miniaturization. For example, can we re-engineer how we introduce the sample (the injector) and the detector to take advantage of these performance gains?"

Emily Hilder, Director: Future Industries Institute, University of South Australia, Australia.

DREAM SMALL

A wish come true for analytical scientists in the field? We profile the new breed of portable HPLC instruments.

FOCUS LC – AXCEND

Serial inventor and CSO Milton Lee tells us about his patented nanoflow LC technology.

Vital statistics

Size: 30×20×20 cm
Weight: 6.8 kg
Release date: Fall 2018

Why is size important?

The bulk and weight of existing LCs mean that they are almost always fixed, lab-based systems, which leads to obvious analytical delays, given that samples are gathered outside the lab. But it also means scientists often jump through logistical hoops just to get samples from one end of a lab to the other.

Talk us through the tech

Given my focus on capillary separation techniques, I had often wondered what the boundaries might be if I was to start with a truly 'blank slate' for a new HPLC. The result of this tabula rasa approach was the Axcend Focus LC, an HPLC that measures just 11.8×7.9×7.9 inches (~30×20×20 cm), weighs under 15 lbs (6.8 kg), and operates on either battery or electrical outlet power.

To create an HPLC the size of a toaster we used 150µm nano-flow internal diameter capillary columns packed with 1.7 µm–3.0 µm fused silica particles. As we experimented with smaller capillary columns, we realized we needed a significantly flatter angle for detection purposes. But 150µm ID capillaries also meant volumes dropped to almost nothing – for analytes and water – while also producing virtually zero waste. Additionally, we found we could support up to 20,000 psi (1,379 bar) and generate 100 times greater sensitivity than traditional LCs.

Visual/statistical output from the Axcend Focus LC will be delivered securely and wirelessly to any Web-connected smartphone, tablet or personal computer capable of HTML5 display, with the additional ability to connect via a USB cable to any PC.

What are the applications?

The instrument was designed specifically for applications involving analysis in the field. Among the applications we're targeting are environmental contamination, crop testing, and criminal investigations, to name just a few. A pleasant surprise has been how many traditional LC users have expressed interest in a smaller instrument.

What's next?

Initial availability for the Axcend Focus LC is slated for Summer 2018, with general availability beginning in the Fall.

Sum up your product in a tweet

Under 15lbs, with 150µm nanoflow ID capillary columns and up to 20Kpsi, it uses 1/500th the H_2O and analytes of conventional LC and has 100× greater sensitivity, but produces just half a teaspoon of waste per week.

"I had often wondered what the boundaries might be if I was to start with a truly 'blank slate' for a new HPLC..."


Axcend
Focus


Axcend
Focus LC

SMART HPLC – POLYLC

CEO Andrew Alpert gives us the lowdown on the latest product from PolyLC – a small company with a big impact.

Vital statistics

Size: 41 × 32 × 17 cm
Weight: 11.3 kg
Release date: January 2018

Why is size important?

A portable HPLC system permits real-time analyses in the field, facilitating point-of-care clinical diagnostic testing and environmental analyses, to name just two.

Talk us through the tech

Advances in several technologies made the Smart HPLC (and application-specific instruments such as Smart LifeLC and Smart CannaLC) possible, including:

- Tablets that can replace computers as the controllers
- LED-based compact detectors
- Compact high-pressure pumps

Together, these factors permitted the design of a rugged system that fits inside a plastic instrument case and weighs just 25 lbs.

The Smart HPLC system works like any other HPLC and operates with conventional columns. It uses Clarity software for instrument control, data collection and generating reports. Though the system is compact, all components are readily accessible.

Repairs involve simply shutting the case and sending the unit overnight to a central repair facility, while a loaner unit heads the other way – a new paradigm for customer support.

What are the applications?

We envisage applications in public health campaigns and environmental analysis, and for student use in college labs.

What's next?

We are planning a demonstration tour in sub-Saharan Africa to screen for hemoglobinopathies in remote towns, in conjunction with public health authorities.

Sum up your product in a tweet

The Smart HPLC is a fully featured portable HPLC instrument, designed for assays that can benefit from mobility as well as functionality. This includes diagnostic testing in remote locations and testing food and water in the field.

"The Smart HPLC system works like any other HPLC and operates with conventional columns."

CROMITE CHEMICAL ANALYZER – SIELC TECHNOLOGIES, INC.

Company president Yury Zelechonok tells us how Soviet-era technology has been reinvented for cutting-edge analysis.

Vital statistics

Size: 18×9×9 cm
Weight: 2 kg
Release date: Fall 2018

Why is size important?

First, in most cases it is more economical to produce and operate a smaller instrument. Second, as instruments get smaller, new opportunities are opening up for mobile applications. Third, if the price and cost of ownership are low enough, new types of consumers can begin to enter analytical chemistry; for example, cannabis growers could carry out their own tests.

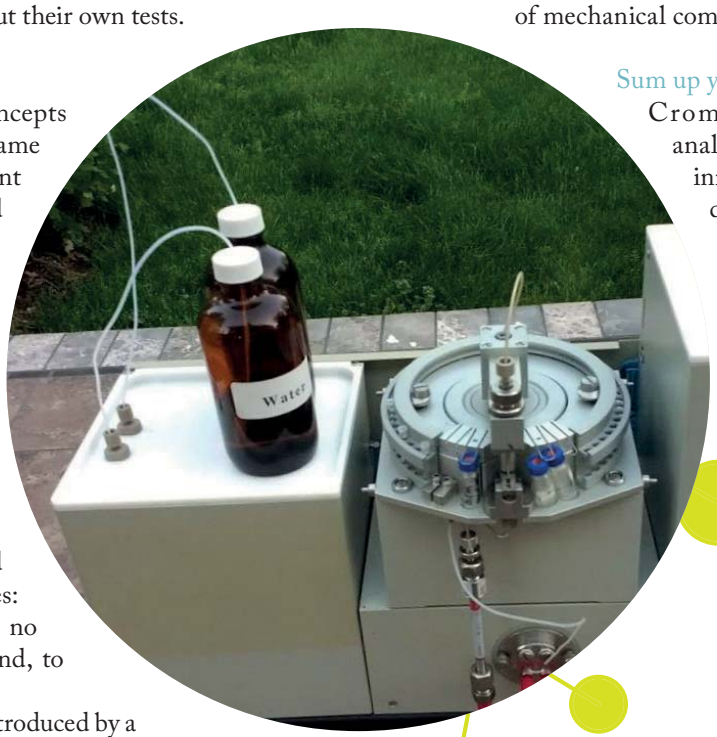
Talk us through the tech

Several of the important concepts behind the miniature HPLC came from a Soviet-era instrument called the Milichrom, developed in the 1990s by G. Baram and colleagues in Novosibirsk (Russia). Milichrom failed to penetrate the international market, but it was a pioneering development.

Our miniature instrument works just like any other HPLC, but every major component of the device has been re-evaluated and redesigned with two principles: first, to make it smaller, with no loss of performance, and second, to make it easier to use.

For example, the sample is introduced by a special disposable probe, eliminating the need for a syringe, a high pressure valve, and sample loop.

Compared with conventional HPLC systems, Cromite is 10 times cheaper, about 10 times smaller, and does not require any special skills to operate – and can still perform most methods currently carried out in conventional HPLC.



Who is it for?

The main users of the miniature HPLC are non-analytical lab customers, for example:

- Chemical/food/cosmetics manufacturing
- Synthetic labs
- Environmental field tests
- Schools/universities as an educational tool
- Consumer health and drug abuse monitoring (urine test)
- Counterfeit detection (drugs, food, chemicals).

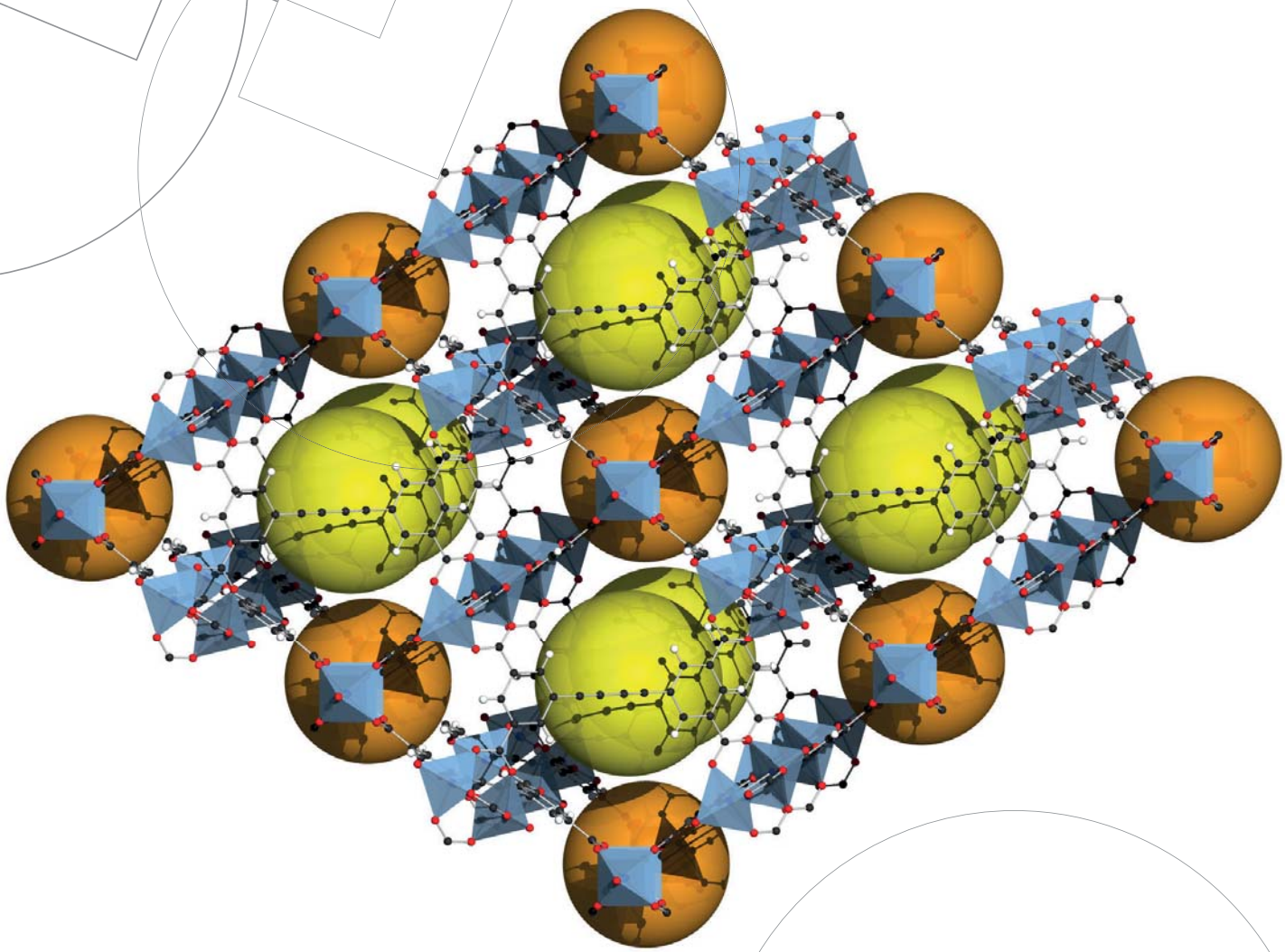
What's next?

We are busy finalizing the design and streamlining production. We are also making the operation of the instrument as simple and reliable as possible through smart, user-friendly software.

It is hard to charge a premium price for smaller devices and I think the instrumentation industry will resist the change unless real competition starts to emerge. But change is inevitable due to miniaturization, integration, and cost reduction of electronic elements, which leads to simplification and miniaturization of mechanical components.

Sum up your product in a tweet

Cromite is a small HPLC analytical instrument with an innovative approach to solvent delivery, column geometry, sample introduction, and detection, controlled by smartphone software with cloud-based data storage and interpretation.



Structure of BAF-4, with yellow and orange spheres showing the pores. *Credit: Tony Boehle.*

AN UNDERUSED **FRAMEWORK** FOR SIMPLER **SAMPLE PREP?**

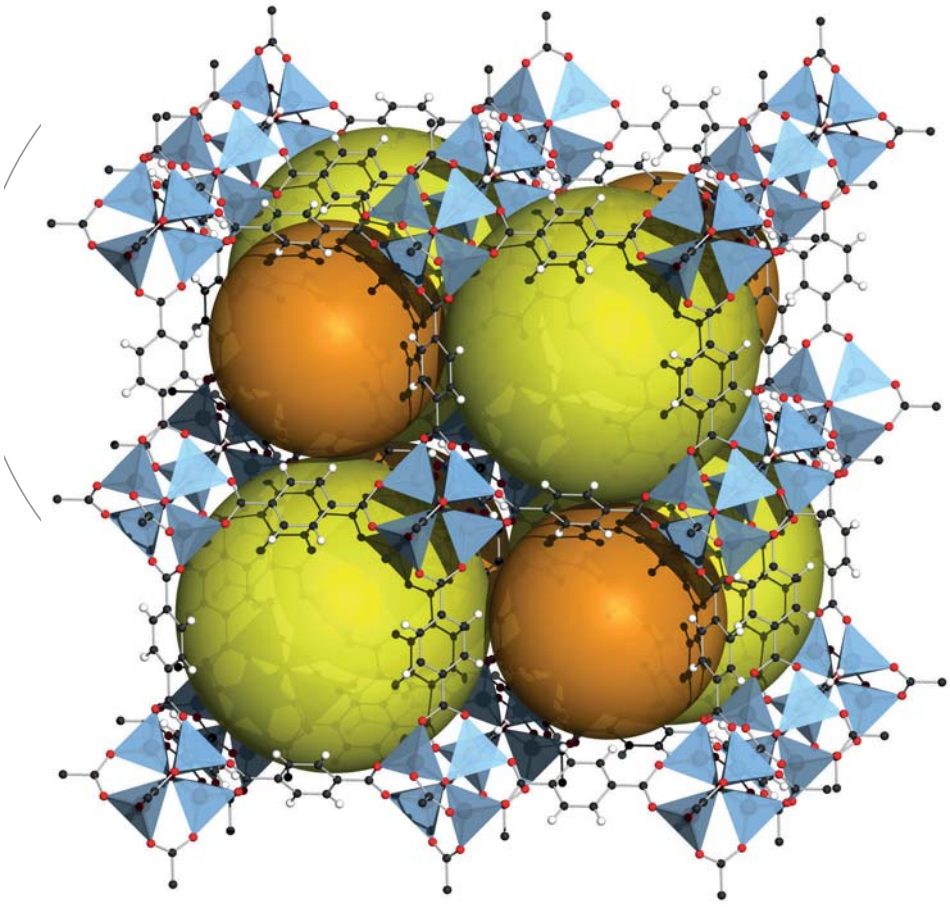
Metal–organic frameworks (MOFs) are some of the most promising innovative materials of the moment. Here, I present my "MOF 101" and highlight how almost limitless configurations open up a wealth of opportunities to improve sample preparation.

By Victoria Samanidou

Sample preparation is typically seen as a necessary evil – and a somewhat neglected field of research. Sorptive extraction techniques, such as solid-phase extraction (SPE) and solid-phase microextraction (SPME), dominate. The evolution of these approaches is mainly driven by the design and synthesis of new materials bearing extraordinary properties – such as metal–organic frameworks (MOFs).

MOFs are crystalline materials with coordination bonds between metal clusters (for example, metal-carboxylate clusters and metal-azolate clusters), metal atoms, or rod-shaped clusters, and multidentate organic linkers with oxygen or nitrogen donors (such as carboxylates, azoles, nitriles, and so on). Within a given material, the length of the organic linker that is used in the synthesis process determines the number of available adsorption sites. Additionally, the characteristic properties of both metal ions and linkers determine the physical properties of MOF networks with regards to their porosity, pore size, and pore surface. The structural properties of the produced frameworks can be controlled by the solvent system, pH, metal-ligand ratio, and temperature (1, 2).

Structure of MOF-5 (also known as IRMOF-1), with yellow and orange spheres showing the pores. *Credit: Tony Boeble.*



Exceptional **properties**

MOFs were first introduced in 1990 and their initial uses included catalysis, gas separation, membranes, and electrochemical sensors. In 2006, MOFs were introduced as SPE sorbents for polycyclic aromatic hydrocarbons (PAHs) in environmental water samples. Hydrophobic properties and π–π interaction give them a stronger affinity for aromatic pollutants than conventional sorbents (2, 3).

Since then, their use has expanded further into the field of analytical chemistry, both in chromatographic separation and sample preparation. Analytical chemists have taken advantage of the unique structural characteristics and properties of MOFs in several sample pretreatment approaches, including SPE, dispersive SPE, magnetic solid phase extraction, SPME and stir bar sorptive extraction – with many others likely to be included in the future. The number of analytical applications implementing MOFs as sorbents in sorptive sample preparation approaches continues to increase steadily – mainly owing to the tuneability afforded by the near infinite number of structures that can be designed and synthesized. In the meantime, they have been designed in various formats so that they can meet more challenges with improved analytical features.

"Analytical chemists have taken advantage of the unique structural characteristics and properties of MOFs in several sample pretreatment approaches"

MOFs exhibit many interesting characteristics: mechanical resistance, thermal stability up to 300–600°C, cavities of structures with specific pore size, ultralow densities, simple synthetic tuneability, and the highest surface areas (ranging from 200 to 7000 m^2/g). And it is these properties that allow them to be used as excellent sorbents in different kinds of analyte extraction (non-polar compounds, polar compounds, and metal ions), such as with pesticides, hormones, antibiotics and phenolics from complex matrices like food, pharmaceuticals, biosamples or environmental samples.

Recently, MOFs have also been applied to the recognition and separation of chiral molecules by incorporating enantiopure building blocks into the porous framework. Enantioseparation by MOF materials is achieved by interactions between the framework and individual racemic agents, such as hydrogen bonding and the homochiral environment in the pores (4–6).

In **fine** form

MOFs are typically classified according to their hydrostability: either moisture-sensitive or water-stable. The instability of moisture-sensitive MOFs in aqueous solution is due to their open metal sites; occupation of those sites by molecular water results in the damage of their structure with a subsequent loss of extraction efficiency. Combining MOFs with other materials can strengthen the stability and dispersibility of moisture-sensitive MOFs in aqueous samples (for example, carbonization, and post-synthetic modification). In this way, their advantages – high surface area, microporosity, and lower density – can still be exploited.

The stability of water-stable MOFs in aqueous matrix makes them compatible for use in SPE, SPME and so on.

Know Your MOFs

ZIFs

Constructed by metal atoms (e.g. Zn, Co) linked with imidazolate or functionalized imidazolate ligands through nitrogen atom. They are characterized by high porosity, large surface area, and exceptional thermal and chemical stability either in water or organic solvents (7).

MILs

Built by trivalent metal centers (Cr, Fe) and carboxylic acid bridging ligands. They are characterized by nanoscale porosity, good stability, outstanding surface areas, and availability of in-pore functionality and outer surface modification which make them promising for adsorption and separation (8).

UiO

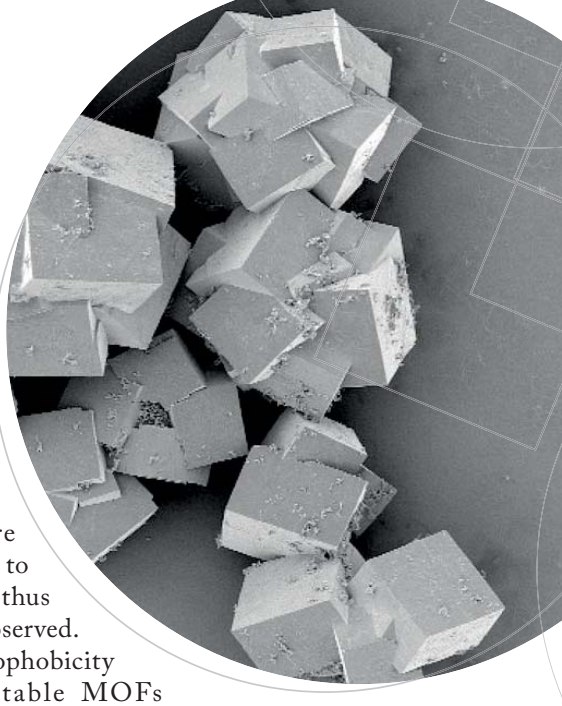
Built from Zr-based blocks (for example, $Zr_6O_4(OH)_4$). They are characterized by a high surface area and unexpected chemical and thermal stability (9).

IRMOF

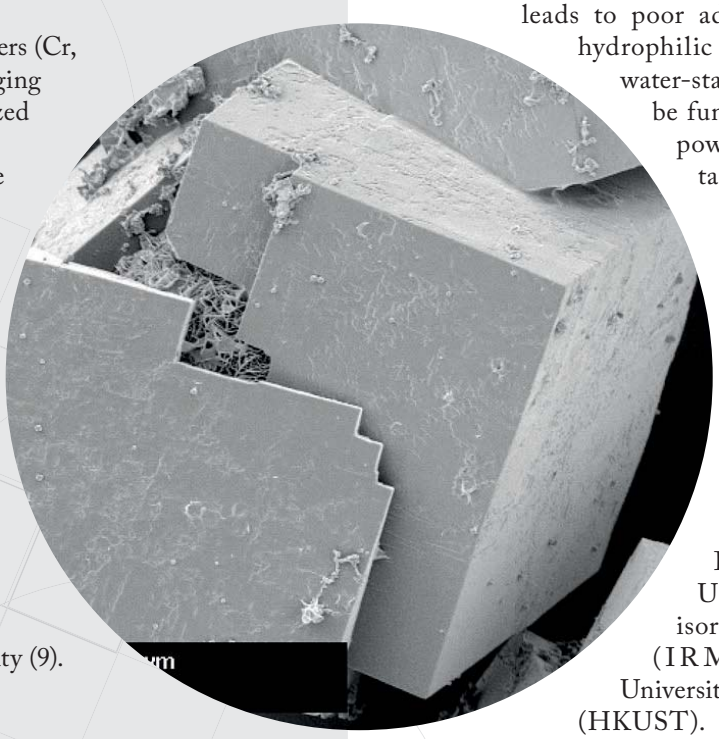
Characterized by a cubic construction with a high surface area (2833 m^2/g), great porosity, and easy preparation. They are mainly used in gas adsorption, and are not suitable for aqueous samples because of instability in humid environments.

HKUST-1

3D Cu-based MOF with a big pore size and unique structure. They are especially effective for adsorption of heavy metal ions.



Right and below: Scanning electron microscope images of MOF crystals. *Credit: P Falcaro and D Buso, CSIRO*



However, their pore sizes are too small to trap target analytes, thus poor recoveries are observed. Moreover, the hydrophobicity of some water-stable MOFs leads to poor adsorption to hydrophilic compounds. In this case, water-stable MOFs may also need to be functionalized to become more powerful and selective toward target analytes.

To simplify the sample preparation procedure, magnetic composites based on MOFs have also been introduced in magnetic microextraction. These composites, often named after the institution where they were developed, include zeolite imidazolate frameworks (ZIFs), Materials Institute Lavoisier (MIL), University of Oslo (UiO), isoreticular metal frameworks (IRMOF) and Hong Kong University of Science and Technology (HKUST). For more information, see "Know Your MOFs."

Magnetic MOFs are designed using a composite material with a magnetic center (typically Fe_3O_4) and a MOF bonded to the outside layer of the magnetic center nanoparticles by means of physical or covalent bonds; for example, via bonding to a polydopamine (PDA) linker (10).

The synthesis of bimetallic MOFs is another approach for synthesizing magnetic MOF materials using a self-magnetic zinc-cobalt MOF with tuned performance by optimizing the ratio between the two metal ions. Other magnetic centers include permanent magnetic materials, such as MFe_2O_4 (M = Mn, Zn, Ni), which have been recently introduced with various crystal structures and particle sizes (2, 11, 12).

"Chemical approaches are comparatively simple and can produce large-scale MOFs, and therefore are preferred in sample preparation applications."

Making a **MOF**

More "traditional" approaches to MOF preparation include crystallization by slow evaporation (the metal salt and the ligand are mixed in a solution, and crystallization follows solvent evaporation) and diffusion in liquids or gels (the metal and the ligand are dissolved in two separate phases, and the complex is formed when they react after diffusion of one into another). These approaches lead to the formation of large crystals and small amounts of MOFs, and take time – from as little as one day to as long as several weeks.

Modern fabrication of MOFs is based on chemical and electrochemical methods. Chemical production involves solvothermal or hydrothermal processes (depending on the solvent media; the most frequently used organic solvents are dialkyl formamides, alcohols, and pyridine) that synthesize MOFs by means of a cooperative ionic interaction between organic linkers and metal salt in mixed solution. Chemical approaches are comparatively simple and can produce large-scale MOFs, and therefore are preferred in sample preparation applications. The use of microwaves, ultrasounds, or mechano-chemical synthesis speeds up production, and can reduce or eliminate solvents, making the process more environmentally friendly.

In the electrochemical method, MOFs are directly deposited onto a conductive electrode under cathodic potential. In this fast and mild approach, there is no need to add metal salts into the sample solution because they are produced from electrochemical reactions in a solution containing organic ligands and electrolytes. MOFs obtained by electrochemical methods are mainly used in gas separation, membranes, and electrochemical sensors. The use of ionic liquids as either solvent or template has

MOF imaged at ×20,000 using a focused ion beam scanning electron microscope. Image from the US Department of Energy.

also been applied to the preparation of MOFs and is known as the ionothermal method (1, 13–17).

A promising **future**

The future applications of MOFs in sample preparation will be determined by the synthesis of new MOFs with improved chemical stability, selectivity, adsorption capacity, and reusability. I expect that a large number of applications using MOF-based sorbents will arise in the near future, because of the almost endless possibilities for synthesizing MOFs and further post-synthetic modifications. When it comes to improving analytical performance, the formation of multifunctional composites – MOF/nanoparticles, MOF/graphene, MOF/silica, and MOF/organic polymers – looks very promising.

Victoria Samanidou is a Professor at the Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, Greece.

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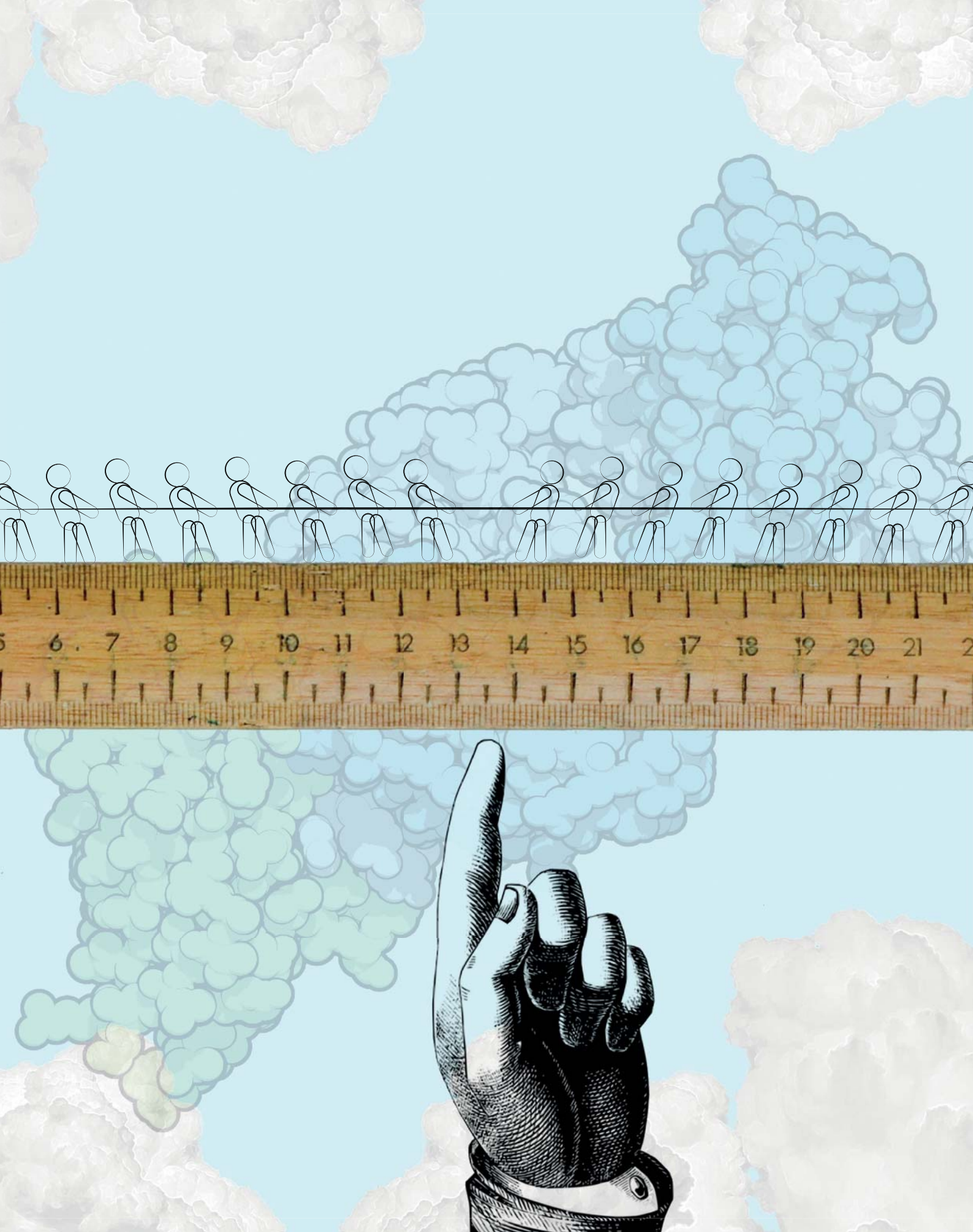
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Tricks of the Trade-Off

The detailed characterization of post-translational protein modifications relies on performance improvements in both HPLC and MS. But how do we strike a balance between LC resolution and MS sensitivity?

By Mary J. Wirth, Rachel E. Jacobson and Edwin Jhovany Alzate Rodriguez

It's an unfortunate fact that the best conditions for chromatographic resolution are those least conducive to high sensitivity in mass spectrometry. Specifically, ion-pairing agents or high salt concentrations are the basis for improved resolution in chromatography – but they are the bane of mass spectrometry. Reversed-phase liquid chromatography with mass spectrometry has arguably been the most successful marriage between HPLC and MS, because acidic modifiers that avoid ion-pairing have been a tolerable compromise. Other separation modes fare worse, including hydrophilic interaction liquid chromatography (HILIC) and hydrophobic interaction chromatography (HIC). Here, we present new strategies that make HPLC-MS work with less compromise in HILIC and HIC modes.

HILIC into shape

HILIC (for the uninitiated, a type of normal-phase chromatography based on hydrophilicity, where the mobile phase is acetonitrile/water and the stationary phase is a hydrophilic layer [1]) has attracted increased interest in recent years due to its ability to characterize protein glycosylation (sugars are very hydrophilic so retention is longer when the glycan has more sugar groups). Why is glycosylation of such interest to bioanalytical chemists? Two reasons: first, many biological therapeutics are glycoproteins and their effectiveness relies on having the same glycan sequence as the native human protein (2); second, most of the human proteome is comprised of glycoproteins, and aberrant glycosylation is a hallmark of cancer (3). Thus, improvements in speed and sensitivity in characterizing protein glycosylation could have a broad impact in medicine.

HILIC has high efficiency and compatibility with MS for glycans but lower resolution and less MS compatibility for glycoproteins. The current workaround for characterizing protein glycosylation is to enzymatically release the glycans from the glycoproteins, label the glycans, and perform HILIC-MS. However, this process is slow and labor-intensive, and doesn't tell us where the glycan is attached.

We wanted to improve HILIC resolution for intact glycoproteins, and this led us to make a bonded phase that allows us to use HILIC-MS for the characterization of intact glycoproteins (Figure 1). In Figure 1a, conventional small-molecule bonded phases allow for electrostatic interactions between the positively charged protein and the negatively charged silanols on the silica surface. Our idea? We use a thick polymer layer to create a greater distance between the protein and the silica surface (Figure 1b). Since the electrostatic potential decays exponentially from the surface, distances longer than a few nanometers will screen the charge well. We have shown that this approach gives better resolution of an intact model glycoprotein, ribonuclease B, compared to commercial columns (4).

In making the bonded phase, the polymer layers are grown from the silica by atom-transfer radical polymerization. This technology was first demonstrated in 1997, where polymer chains were grown from surface-bound initiators on silica in a controlled way, largely avoiding polymer formation in solution (6).

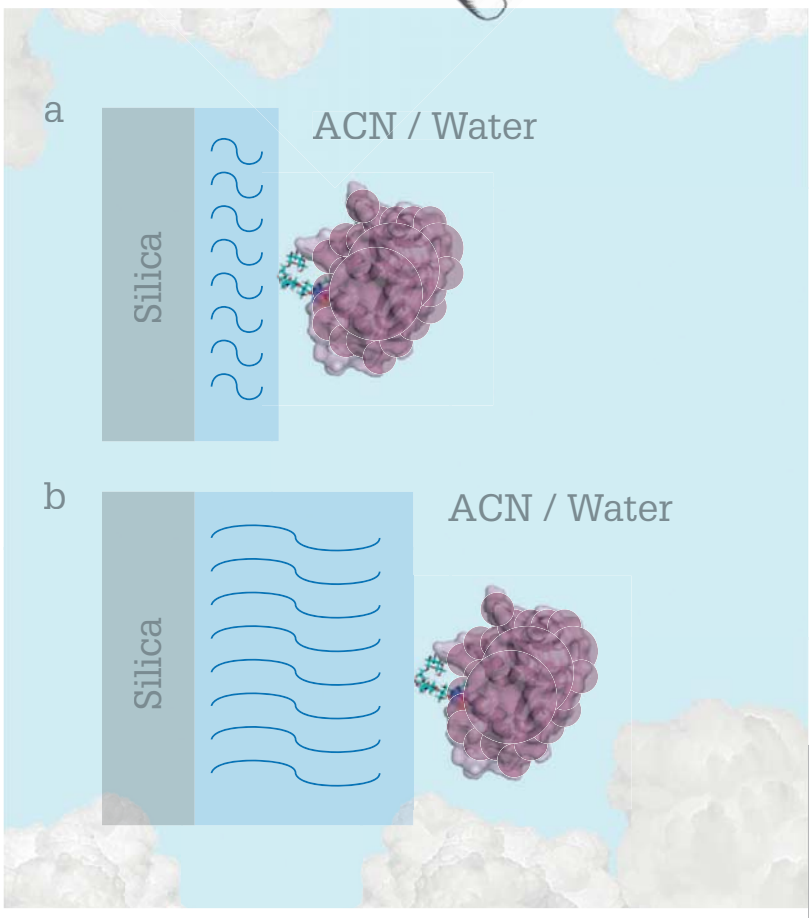
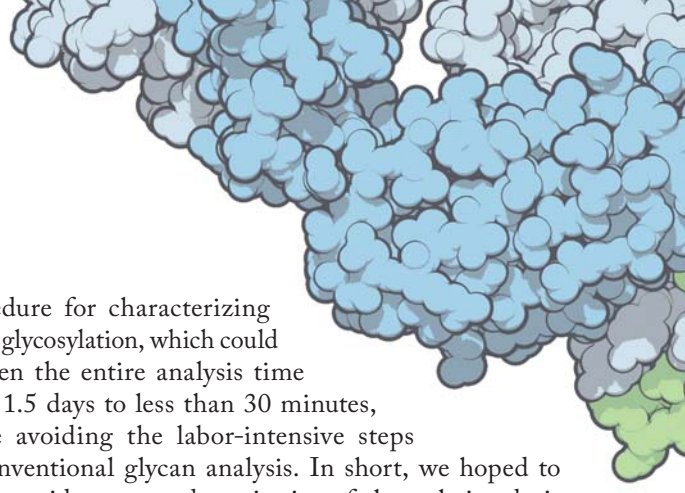


Figure 1. a) The conventional HILIC bonded phase is made of short, covalently bonded functional groups, and b) the co-polymeric bonded phase is thick enough to screen charges effectively. The glycan preferentially interacts with the hydrophilic bonded phase, as depicted. The structure of the glycoprotein, ribonuclease B, was adapted from Jayaprakash et al (7).

A middle-down middle ground

The approach of using a thicker bonded phase allows the use of MS-compatible mobile phase modifiers. Trifluoroacetic acid (TFA) is used as a mobile phase modifier for HILIC of glycoproteins because it is a strong enough acid to neutralize the most acidic silanols on the silica, but it greatly reduces sensitivity in MS because its anion forms adducts with proteins. Formic acid is compatible with mass spectrometry, but resolution in HILIC is lost because the pKa of formic acid is much higher than that of TFA, which makes the silica surface more charged. With a much thicker bonded phase, formic acid can be used with very little TFA because the surface charge is screened by the polymer.

To illustrate how much a polymeric bonded phase improves HILIC-MS, take a look at the chromatograms in Figure 2 for ribonuclease B. The change from 0.1 percent TFA to the MS-



"The **middle-down approach** is performed in one vial, with **fast reactions**, and the contents can be directly injected into the column **without further sample preparation**"

compatible mobile phase of 0.1 percent formic acid plus 0.025 percent TFA results in a negligible change in resolution, but means that we can now consider using HILIC-MS on intact glycoproteins instead of the released glycans.

We collaborated with Genentech to test a middle-down procedure for characterizing IgG1 glycosylation, which could shorten the entire analysis time from 1.5 days to less than 30 minutes, while avoiding the labor-intensive steps of conventional glycan analysis. In short, we hoped to enable rapid, automated monitoring of glycosylation during manufacturing, allowing for real-time adjustments (the two procedures are compared side-by-side in Figure 3). The middle-down approach is performed in one vial, with fast reactions, and the contents can be directly injected into the column without further sample preparation. Such an approach has been studied previously, but the HILIC separation did not provide sufficient resolution even with the use of TFA (5). Our HILIC columns provide the resolution needed to make this approach work (publication is pending so we can't share the data here) – and the resolution is maintained for 0.1 percent formic acid + 0.025 percent TFA.

In another application, we collaborated with scientists at Abbvie on HIC-MS of a model antibody–drug conjugate. This project might seem like an impossible dream, given that HIC

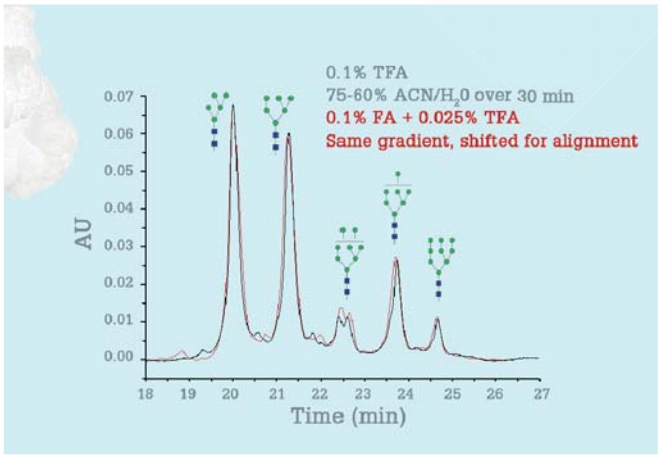


Figure 2. HILIC separations using 0.1 percent TFA (black) and the MS-compatible modifier of 0.1 percent formic acid + 0.025 percent TFA (red).

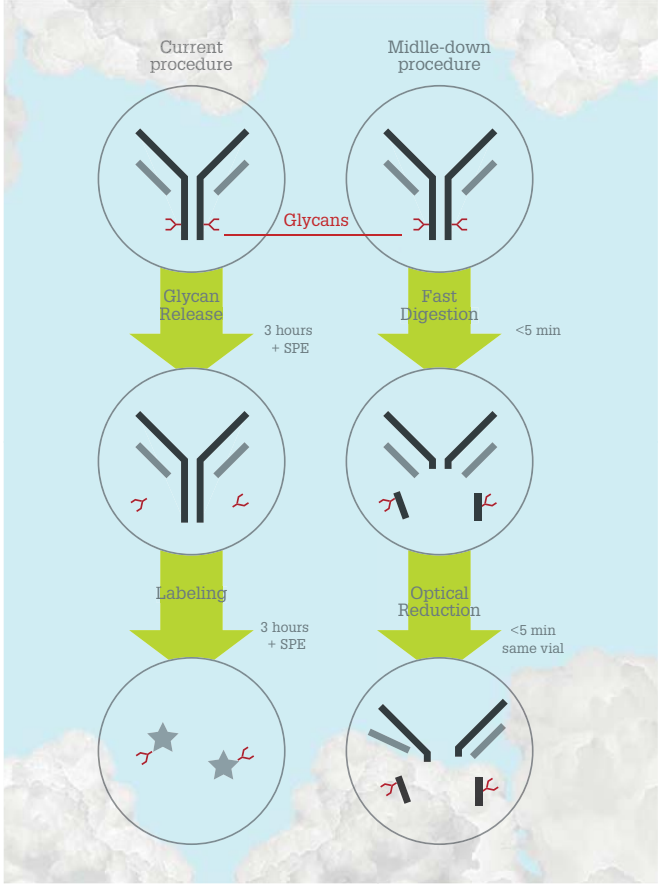


Figure 3. Comparing the current procedure for glycan analysis, where HILIC is performed on the released glycans, and the faster middle-down procedure where the Fc/2 fragment bearing glycans are generated simply by addition of reagents and separated by HILIC.

> "The most **essential factor** in commercialization is **collaborating with** potential customers."

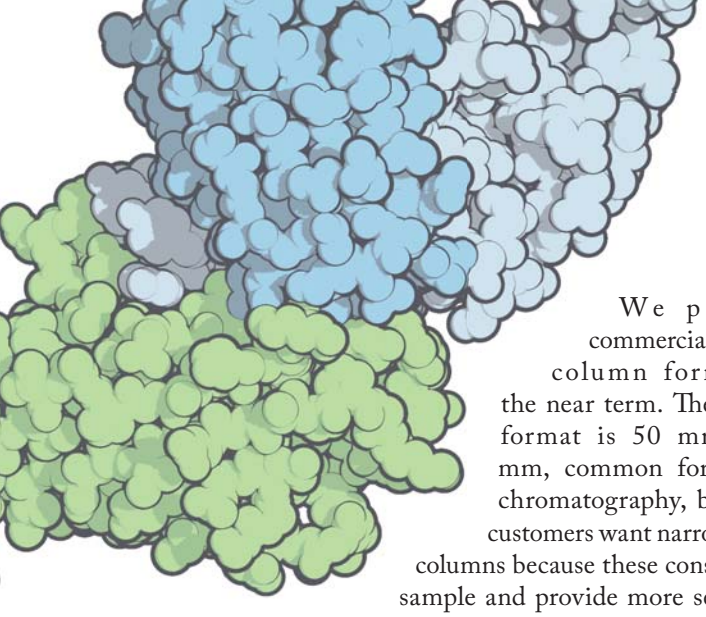
for such drugs currently requires a salt gradient containing a high concentration of sodium ions, but with the lessened electrostatic interactions, high resolution was maintained using low concentration ammonium formate for HIC-MS. We expect to publish on this soon, as well.

From collaboration to commercialization

We have commercialized this technology through bioVidria (www.biovidria.com), located in the Purdue Research Park. The HILIC and HIC columns, as well as RPLC column, are stainless steel, 50 mm × 2.1 mm, packed with 1.2 µm silica particles with the appropriate covalently bonded copolymer for the separation mode. The intellectual property is protected under an issued patent, US Patent 9,758,542, 2017, with international rights pending.

Commercializing the technology has been an adventure for us. Why? Academic research is directed toward the future, whereas commercialization has to survive in the present. To illustrate the dichotomy: the chromatographic resolution is better with submicron particles, but the variability of available frits prevents cost-effective production right now. Another illustration of the difference between academic research and commercial practicality? Polyacrylamide works great for HILIC-MS of intact proteins, but the acrylamide monomer is a neurotoxin that would have to be carefully weighed out in large quantities for reproducible and cost-effective bonded phases. Polyacrylamide swells, which gives high back-pressure and makes the particles fight back during high-pressure packing. It also degrades too fast for the customers' satisfaction. All of these problems matter little in basic research, but weigh heavily when bringing the technology to practice. The learning process has enabled us to develop a safe, cost-effective, high-performing copolymer for HILIC-MS.

The most essential factor in commercialization is collaborating with potential customers. Our collaborators at Genentech, Abbvie and Pfizer were valuable in developing our HILIC, HIC and RPLC columns, respectively.



We plan to commercialize more column formats in the near term. The current format is 50 mm x 2.1 mm, common for protein chromatography, but many customers want narrower bore columns because these consume less sample and provide more sensitivity in mass spectrometry. We continue to explore new polymers and, on the basic research side, we are using capillaries and fluorescence imaging to understand the gradient elution process better, identifying where the broadening occurs so that we can design columns for even better separations in the future.

Mary J. Wirth is W. Brooks Fortune Distinguished Professor — Analytical Chemistry, and Rachel E. Jacobson and Edwin Alzate are doctoral candidates in the Department of Chemistry, Purdue University, Indiana, USA.

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Profession

Leadership
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Career Planning

Those Who Can, Teach: Christopher Harrison

In a new series, we ask analytical scientists about what is arguably their greatest legacy – enlightening the next generation.

Chris Harrison is an associate professor at San Diego State University, where he has been teaching analytical chemistry for 10 years. Over that time, he has incorporated progressively more technology into the classroom to help his students better engage with the material. In recent years, Chris has implemented a "flipped classroom" – the lecture material is pre-recorded and class time is used for group problem solving (read more at tas.txp.tp/0614/Harrison). We caught up with Chris to find out more about the good, the bad and the ugly of teaching analytical science.

How do you motivate your students?

A few are truly passionate about uncovering the details of chemistry and clearly plan on pursuing careers in the field. For this group of students, there is an inherent curiosity about chemistry, and they need no other motivation. Other students are taking chemistry courses to fulfill requirements for their degrees, and ultimately want to progress towards programs in medical or pharmacy schools. And then there are students who are not at all motivated to be there – many appear to be at university merely because they see it as the next prescribed step in becoming an adult.

"One factor that can motivate a student is being shown exactly how the chemistry that they are learning is applicable in everyday life."

That being said, a student's motivation can change over time. For many students (and I was one such student), the lower-level, general courses generate little engagement. But as the courses progress, and become more focused and challenging, they develop a greater interest. Another factor that can motivate a student is being shown exactly how the chemistry that they are learning is applicable in everyday life. Discussions of equilibrium constants may be rather dry and abstract, but showing how they are employed with lateral flow assays and antibodies to create a pregnancy test can make a world of difference for a student's enthusiasm.

What is the best approach to teaching analytical chemistry?

I believe that getting students to experience chemistry is the ideal. We need to move students away from trying to memorize facts and formulas, and towards understanding the processes. I do not believe that lectures are the best approach; instead, getting the students involved in active learning – and asking important questions about the chemistry – is the best way to stimulate their interest and foster their understanding of the material.

What is your biggest teaching challenge?

Mathematics, and relating the math to chemistry, has been an ongoing struggle. Presently, I find that students lack the number-sense to understand that the mathematical equations we use in analytical chemistry (particularly quantitative analysis) represent the chemistry that is taking place. There is a tendency for students to want to memorize how the equation is applied, without any understanding of the process, resulting in significant difficulties when problems are modified slightly.

Consequently, much of my time is spent translating the mathematical equations into a picture of chemistry that the students can understand. Surprisingly, the concept that I need to re-explain the most is the idea that reagents are consumed in reactions, and how that is accounted for in the math. Without explicit prompting, many students seem to ignore the concepts of limiting and excess reagents.

How has classroom technology changed how you teach?

I have moved all of my lecturing out

"Instead of merely using the class time to present the material to the students, I now use it to challenge the students' understanding of that material."

of the classroom and on-line – made possible because all of my students can readily access these course materials through their personal devices at any time. Some lecturers worry that by making material available online, their role in the class will be diminished. I have found quite the opposite – the time I spend in the class is more valuable now. Instead of merely using the class time to present the material to the students, I now use it to challenge the students' understanding of that material. More importantly, I am there while they are working on the problems, and can help address their struggles right away. Moving course material on-line frees up my time with the students, so that I can help them refine or clarify their understanding of those concepts on a one-to-one basis.

How does the instrumentation your students use compare with that found in a contemporary analytical chemistry lab?

Much of what we have is a decade or more old, lacking many of the modern bells and whistles. On one hand this is a disadvantage, as students are not seeing the best that analytical chemistry can offer but, on the other hand, older instruments can be more accessible and hands-on, providing the students greater insight into how the system functions.

For example, we have a GC that uses a manual syringe for injection and a manually triggered integrator. Though this is not comparable with an Internet-connected GC with an auto-sampler, it does provide students with key insights into how the GC works. They can manually change the volume of sample injected, and immediately see how that impacts peak height, and potentially peak shape and resolution. We can also discuss why the peaks appear to come out at different times, based on when the integrator is started, and how we can account for that variability with a deadtime marker.

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"I feel we may be reaching the limit of what technology can achieve in education."

Can new technology improve education?

I feel we may be reaching the limit of what technology can achieve in education. However, virtual reality is one appealing avenue – being able to immerse oneself directly in an instrument to experience the inner workings of techniques such as chromatography and mass spectrometry from the perspective of a molecule could be great.

An artificial intelligence (AI) that is capable of teaching chemistry concepts may be more revolutionary, though likely very difficult to achieve. An AI that could understand and answer students' questions and misconceptions would be a very powerful tool. Some publishers are trying to get there with adaptive learning/teaching modules associated with their textbook problems, but it is far from ideal.

Are students graduating with the skills required to be effective analytical chemists?

Overall, I believe that most of the students graduating from our programs will be effective analytical chemists. The skills that I see as being most useful in this field include attention to detail, creativity/flexibility, and communication. A successful analytical chemist needs to be detail oriented, but must also be flexible and creative when it comes to problem solving. They need to be able to approach problems from unexpected angles, as problems (and their solutions), are not always formulaic. Finally, they must be able to clearly communicate their results to colleagues in different divisions of chemistry, and in other fields entirely. This last skill requires both clarity and some fundamental understanding of fields beyond analytical chemistry.

The two greatest weaknesses I see in my students are the struggles with mathematics I already alluded to – and the fear of failure. The weakness in mathematics results in a lack of comprehension of how to translate chemistry into calculations, which is a clear problem in analytical chemistry. The fear of failure is more insidious, as it often results in students being unwilling to try to solve problems alone. These students rely on solution guides and other students to help, and struggle to even start to solve a problem on their own.

On the flipside, the current generation of students may be the most naturally collaborative yet. Though in some instances it may seem that they are overly co-dependent, they are for the most part inherently drawn to, and skilled in, working in groups. This collaborative nature is likely to be very useful in their careers beyond the classroom.

How could your education program be improved?

A few of my students move onto graduate school programs after they complete their degree, but the majority go out in search of jobs. All of our graduates are required to complete at least a semester of research so they all have hands-on lab experience and are well equipped to succeed. However, there is clearly a disparity in the way that academic research labs are run as compared with commercial labs, notably the absence of formal protocols. Most students will graduate our program without having seen an SOP or completing a formal report. We are still more focused on the development of pure research scientists, and not as cognizant of what will make a good laboratory scientist in a commercial laboratory.

Analysis of Polyethylene by Pyrolysis-GC×GC-MS

A new dimension added to analytical pyrolysis

By Daniela Peroni

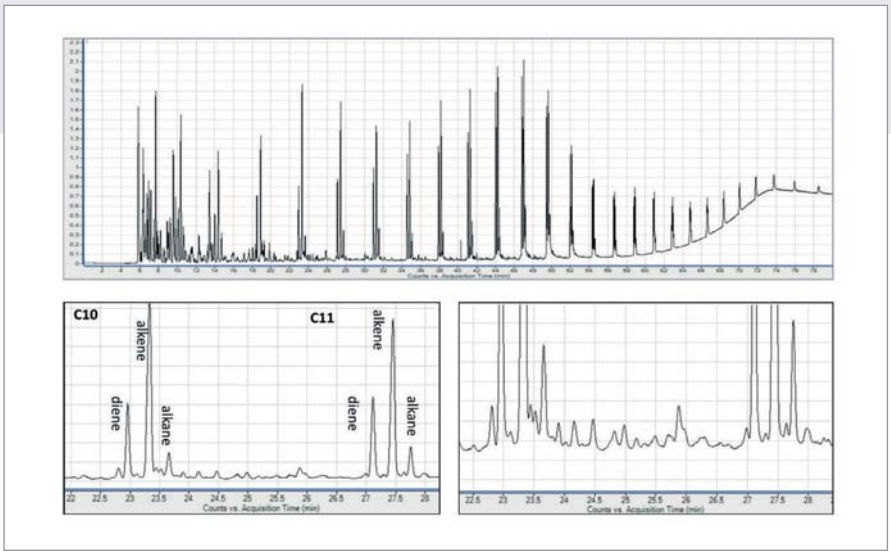

Figure 1. Py-GC-MS chromatogram of PE. The zoom-ins show a detail of the profile and an example of a complex area.

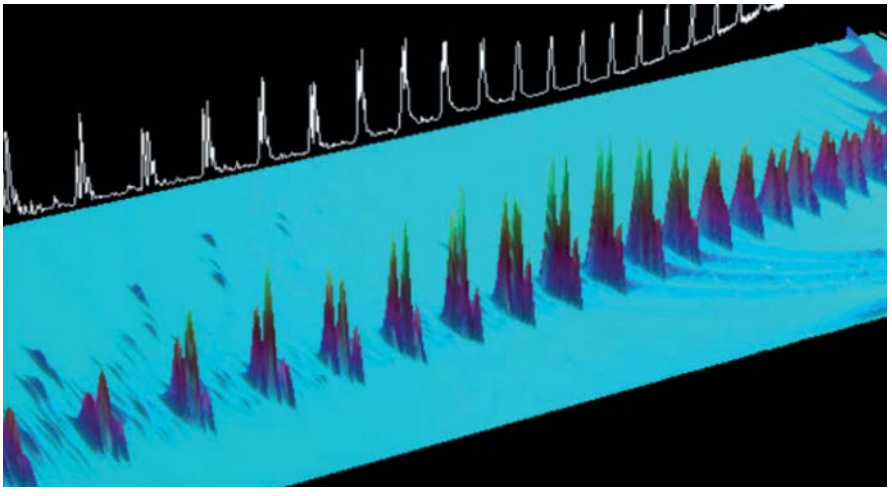

Figure 2. 3D view of the py-GC×GC-MS chromatogram of PE.

Pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) is widely used for polymer characterization. The precisely controlled pyrolysis heating step provides informative and specific products that can be separated and identified by GC-MS to estimate the polymeric composition and structure. However, these profiles are sometimes too complex to be characterized properly by conventional GC, leading to potential loss of information.

Comprehensive two-dimensional gas chromatography (GC×GC) provides enhanced resolving power and peak capacity by combining two different separation mechanisms in one analysis. This allows for more detailed separations and more complete characterization of complex matrices. Here we demonstrated the Py-GC-MS and Py-GC×GC-MS profiles of polyethylene (PE) to prove the advantages arising from coupling pyrolysis and GC×GC.

Fig. 1 shows the GC-MS chromatogram of a PE sample subject to pyrolysis at 750°C through a CDS Pyroprobe and separated on a non-polar column. The characteristic PE pyrolysis profile shows a repeating unit of triplets of paraffins (diene-alkene-alkane) for every carbon number. Between these major peaks, there are a number of smaller, unresolved peaks commonly identified as branched paraffins.

The two-dimensional separation of the same pyrolysis profile is shown in Fig. 2. The second dimension separation based on polarity grants additional composition information. Several peaks are visible between the triplets, providing a better idea of the number of branched paraffins present. Additionally, there are a number of polar compounds more retained on the second dimension (Fig. 3). These analytes are not visible at all in the GC-MS analysis due to their small abundance and the co-elution with the aliphatic components, like in the case of naphthalene and C12 triplet. In the 2D plot they are fully separated and show clean MS spectra that allow for easy identification.

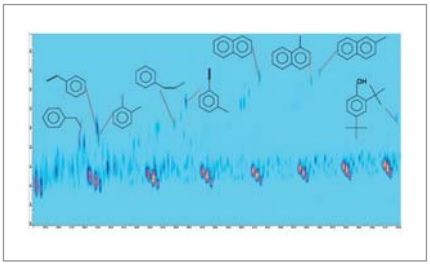

Figure 3. Py-GC×GC-MS chromatogram of PE.

In conclusion, Py-GC-MS analysis of polyethylene shows a characteristic pattern of paraffin triplets for every carbon number. However, separation is not sufficient to unravel the sample complexity. Characterization obtained by coupling pyrolysis with GC×GC provides more information. Several aromatic compounds are identified in the 2D pyrogram of polyethylene which are not detected in the mono-dimensional separation.

The Accidental Scientist

Sitting Down With...
Monika Dittmann,
Principal Scientist,
R&D Agilent Technologies,
Germany.

What was your route into science?
I went to an all-girls school, where scientific subjects were not prioritized, so I never had a firm plan to go into science. Eventually, I excluded everything I didn't want to do – and only science remained... Physics was too mathematical; biology was too vague, so chemistry seemed a good compromise.

And did you grow to love it?
Not right away! At the beginning, studying chemistry involves a great deal of learning things 'by heart,' which I found very boring. I persevered, and when the work became more applied, it started to get interesting. I went into physical chemistry, because that struck me as the most logical and most explainable; once you understand the concepts it is easy to go from there.

When did you become interested in analytical chemistry, specifically?
During my PhD on thermodynamics, I worked with Hewlett Packard (HP) GCs, and when we received a HP 1090 HPLC instrument in 1983 – the first LC equipped with a diode array detector – I was very impressed by the breakthrough in performance. That inspired me to move into analytical chemistry, particularly instrument development. After my post-doc (again in thermodynamics) I successfully applied for a job with HP and that was my entry into analytical chemistry.

How was the transition from academia to industry?
Initially it was a culture shock! At university, you select a topic with your supervisor, and as long as they are happy and you have grant money, you can continue to pursue your ideas. When you join a company, your interests are mainly driven by those of that company. Luckily, in my career at Agilent I have had the opportunity to combine scientific research and product development.

What do you enjoy most about your job?
I really enjoy collaboration with people from different fields. At Agilent, we run large projects involving people in hardware, software, firmware, physics, optics, chemistry, and so on. You have to work together to find compromises, and ultimately end up with a product that is suitable for a specific application. I like that exchange – and it gives us all a broader understanding. The best moment is when you release a product; if you've worked with a group of people who are really engaged, it's almost like your baby.

"In my view, multidimensional chromatography is the natural progression. It's not mainstream yet, but long term I think it's the way to go."

Where do you think the field is heading?
In my view, multidimensional chromatography is the natural progression. People struggle generating peak capacity in one dimension, maybe getting to 700 or 800 with very long separation times – but with the second dimension, you not only boost peak capacity, but the different separation modes give you a different angle on your separations. It's not mainstream yet – it's still too expensive and complex – but long-term I think it's the way to go.

Do you anticipate any challenges with increasing dimensions?
We generate so much data that it gets increasingly difficult to make use of it all. We are currently collaborating with a professor in Germany who uses 2D-LC IMS QTOF, which generates data in five dimensions – but if you can't analyze that data efficiently, there is no point. This is likely to be the bottleneck now and in the future.

What about miniaturization?
I worked for ten years developing miniaturized instruments, and they do have a lot of potential in certain application areas. However, I wouldn't say that miniaturization is a benefit per se; you need a very compelling reason to go down that route.

Has science changed much for women during your career?
It's not quite so male dominated. When I started, I was the only woman in the R&D department, except for the administration team. Today, we have many more women, specifically in the fields of chemistry and biology. It is still hard, however, to find female software or hardware engineers.

Balancing family and work is still difficult for women. In industry, it's easier to pull back for a year or two, but in academia, if you stay out of the field for too long, you risk being forgotten. The whole environment needs to change. I was lucky – when I came back part time from maternity leave, I was still able to work on interesting projects. Agilent do encourage a good work–life balance – and it's so important for a positive environment. If people are happy, they do a better job!