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# The Buzz About (Prote)Omics

*Is it time for proteomics to take a spotlight in scientific engagement?*



Protein biochemistry tells a tale of diverse protein function. These roles – which range from structural stability to catalysis – are not the only source of variability surrounding these molecules, though. Proteins can also be present in vastly differing amounts, and can be altered in terms of their amino acid sequence and co- and post-translational modifications; the depth of the known proteome covers over 10,000 quantified proteins and 10,000[10] proteoforms.

With the above in mind, it's probably no surprise to hear that capturing protein complexity with accuracy and high throughput is a great challenge. Yet, the technologies behind proteomics are geared to capture this immense landscape of complexity. In fact, the past five years have ushered in many proteomics advances, leading to improved protein coverage, sensitivity, and throughput; we can now cover extremes from single-cell analyses (see page 10) to the processing of hundreds of samples in a single day.

Most excitingly, proteomics, despite its maturity, continues to grow in new ways. As an example: top-down proteomics approaches are expanding to capture the functionally important patterns of protein post-translational modifications. Further examples: the ways in which we can probe three-dimensional protein structure in vivo (important for determining structural changes as a function of disease) are improving; quantification of a protein interaction partners in cells is leading to growing acknowledgement that proteins can choose to stay at home or travel the world; and single-cell proteomics is emerging as a serious tool for studying cell differentiation, evolution, and interactions.

What's more, high-throughput methods with automated and highly QCed workflows are allowing proteomics to boom into a large-scale human population science. In these cases, we can conduct consistent analyses of thousands of samples. Using such methods, we are able to study our natural history, disease progression, and responses to therapies (Amanda Hummon discusses a more personalized approach to the latter application on page 51).

The future is bright, but there's still some space for improvement and innovation in our toolbox. Proteomics promises to answer burning questions about mechanisms in biology, and increased ease of application means that the proteomics buzz continues to grow. Along with other omics approaches (think metabolomics and lipidomics; see "Gurus of Omics" on page 16), previously untold knowledge regarding genetic, metabolomic, and environmental influences lies in wait. Perhaps that's why The Analytical Scientist team was so keen to devote the February issue to the magic of omics… Let's explore!

**Jenny Van Eyk & John Yates**

*Cedars-Sinai Medical Centre, Los Angeles, and The Scripps Research Institute, respectively, both in California, USA*

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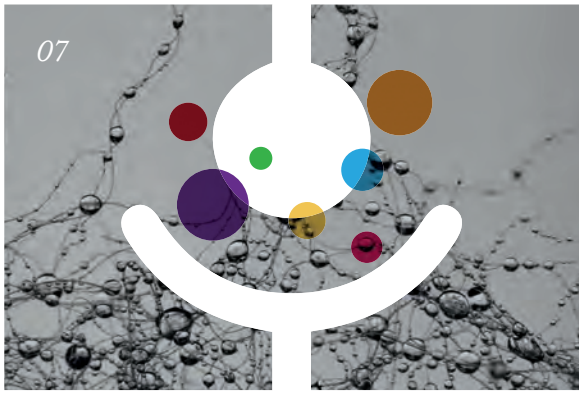




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ISSUE 94 - JAN / FEB 2021





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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@theanalyticalscientist.com). Non-qualified annual subscription cost is available on request.





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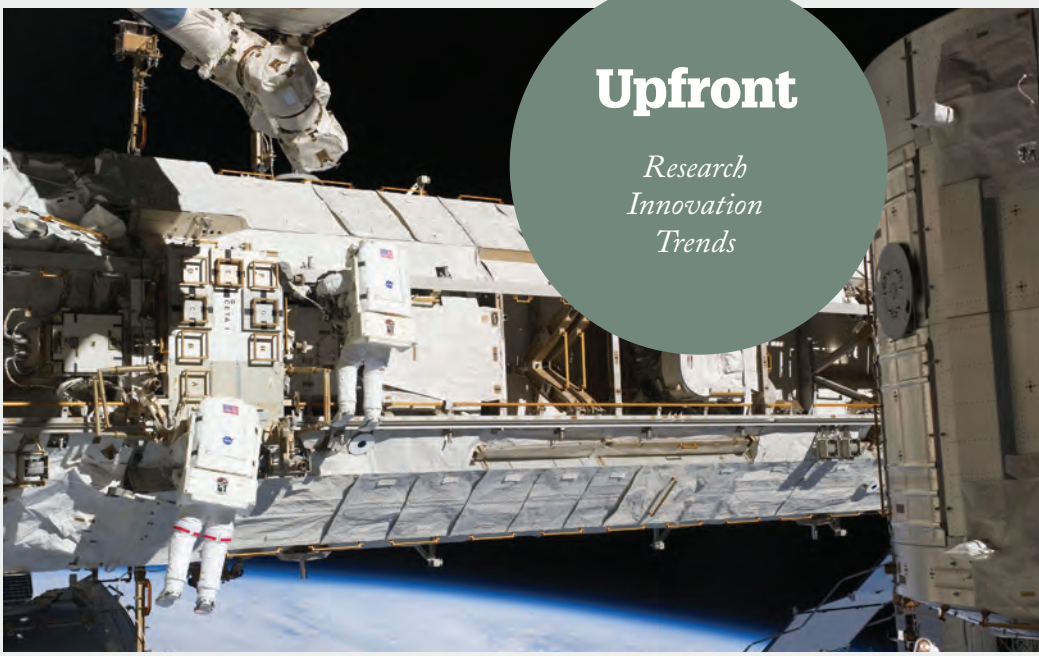


**Upfront**

*Research*
*Innovation*
*Trends*

# The Limits of Life

## New evidence emerges in favor of an extraterrestrial origin for life on Earth

It's a question scientists have been chasing after for years – just how did life begin on Earth? From panspermia to primordial soup, an abundance of theories exists, but evidence is often hard to come by. Now, researchers have added weight to the panspermia hypothesis – the idea that life came to Earth from space – by studying the effects of such harsh conditions on a living entity at the molecular level.

Using a combination of omics and electron microscopy (EM), Tetyana Milojevic and her team investigated the extraordinary mechanisms that allowed the most extreme of the world's known extremophiles, *Deinococcus radiodurans*, to survive one year outside the International Space Station. EM showed that the morphology and cellular integrity of microbial cells was perfectly preserved even after long-term space exposure. Transcriptomics, proteomics, and metabolomics allowed the team to gain further information about the biologically active molecules responsible for the bacterium's recovery from damage induced by outer space.

Their key finding is that the revival of *D. radiodurans* is a multilayered process. The microorganism uses DNA repair systems, specific molecules to neutralize and deactivate reactive oxygen species, elements of a cell envelope, and various metabolic rearrangements to keep cells in "low-energy mode" and help repair damaged molecules.

"Extremophiles such as *D. radiodurans* have outstanding molecular capacities to perform interplanetary journeys," says Milojevic. "Our results emphasize that life in outer space and its transfer across the universe is possible, but these results should also be considered in the context of planetary protection concerns and the development of new sterilization techniques for future space missions."

So what are the team's next steps? "We are currently exposing an ancient microbial form of life, metal-encapsulated *Metallosphaera sedula*, to Mars-like conditions to further study whether mineral encapsulation serves as a natural cellular shield to protect microbes during interplanetary transfer," says Milojevic. This would help scientists evaluate the likelihood of lithopanspermia – the theory that rocks spread biological material from one solar system to another.

*Reference*

1. *E Ott et al., Microbiome, 8, 150 (2020). DOI: 10.1186/s40168-020-00927-5.*

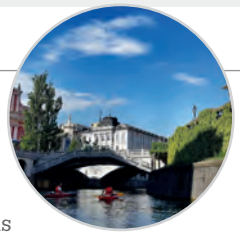
TIMELINE

# The Analytical Calendar 2021

## Not-to-be-missed events (whether virtual or in-person) for the year ahead

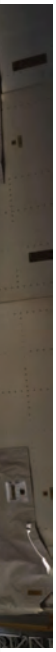
**March 8-12: Pittcon (Online)**

This year's exposition will be held virtually, with booths, short courses, a technical program and networking sessions still available



**June 29 – July 2: ISSS & HPTLC 2021**

The International Symposiums for Separation Sciences and High-performance Thin-layer Chromatography were postponed in 2020 and are currently planned to take place this year in Ljubljana, Slovenia



## BUSINESS IN BRIEF

*A round-up of this month's business news, from the first untargeted analysis of single cells to new guidelines for dioxin testing*

- Bruker and Evosep have teamed up to develop an ultra-low flow method for protein identification using a modified timsTOF Pro MS connected to an Evosep One chromatography system. They have reported the first untargeted, unbiased analysis of single cells – a key step forward in the field of single-cell proteomics (1).
- Intabio, a private company that has developed a system for coupling imaged cIEF charged variant analysis with MS, has been acquired by SCIEX. The hope is that their combined technical and biopharma market knowledge will accelerate biologic drug development through time and cost savings in biopharma analytical labs (2).
- The US Environmental Protection Agency has updated its guidelines on dioxin analysis of environmental samples, confirming the Waters Xevo™ TQ-XS MS is an accepted technology for routine tests (3).
- Oxford Nanopore Technologies

and NVIDIA team up to deliver real-time DNA/RNA analysis at scale. The NVIDIA DGX Station A100 and Oxford Nanopore's PromethION ultra-high throughput sequencing system could help with the analysis of genomes in healthcare, food and environmental analysis (4).
- HORIBA has announced the opening of a new R&D and production site in Loos, France, increasing the company's manufacturing capacity for Raman spectroscopy by 30 percent. The site will also showcase the range of HORIBA's technologies, including the LabRAM Soleil confocal Raman microscope (5).


*References*

1. *Bruker (2021). Available at: https://bit.ly/2Y4Xygk*
2. *Business Wire (2021). Available at: https://bwnews.pr/3iInbgn*
3. *Business Wire (2020). Available at: https://bwnews.pr/3ca89ic*
4. *Nanoporetech (2021). Available at: https://bit.ly/39irMTr*
5. *HORIBA (2021). Available at: https://bit.ly/2Mojyjm*


# Breath of Fresh... Plastic?

**Airborne microplastics have been discovered in the atmosphere surrounding our most remote oceans**

Microplastic particles are widespread in our atmosphere, but the remote ocean was thought to be beyond the reach of such polluting influences – until now. Using micro-Raman spectroscopy, a team of researchers have proven that not even the remotest seas are safe from microplastics.

The team analyzed a collection of ambient aerosol samples taken from the North Atlantic Ocean, taken in 2016. A range of plastic compounds were revealed, including polystyrene, polyethylene, polypropylene, and polysilicone. Shockingly, the team believes their results are actually an underestimation of the particles' true spread because they only measured microplastics larger than 5 µm. Our oceans' plastic problem is already dire – but it now seems that these ubiquitous tiny particles are creating an even worse situation than previously believed.


*Reference*

1. *M Trainic et al., Commun Earth Environ, 1, 64 (2020). DOI: 10.1038/s43247-020-00061-y.*


**July 11-14: MSB 2021**

The 37th International Symposium on Microscale Separations and Bioanalysis is planned to take place in Boston, MA, USA

**September 26 – October 1: SCIX 2021**

SCIX, hosted by the FACSS, is planned to take place in Rhode Island Convention Center, RI, USA

**October 31 – November 4: ASMS 2021**

The 69th ASMS Conference on Mass Spectrometry and Allied Topics is due to take place at the Pennsylvania Convention Center, Philadelphia, USA

# The Coral Doctors

## Researchers dive into the metabolomics behind bleaching

Our coral reefs are dying. Climate change and local stressors are recognized as the key contributors, causing higher sea surface temperatures that lead to widespread bleaching events. Now, a team of researchers has added to our understanding by uncovering the metabolomic shifts associated with heat stress in our planet's most biodiverse ecosystems (1).

"The goal of our study was to identify metabolite markers that can be used to assess coral health in time to enact conservation efforts before reefs are seriously damaged," says Debashish Bhattacharya, co-author of the paper.

The team used untargeted hydrophilic interaction LC-MS to study the stress-resistant coral species *Montipora capitata* and stress-sensitive *Pocillopora acuta* – both from reefs in Hawaii. "Given the large number of 'dark' metabolites in marine systems, we decided to conduct untargeted analysis with the goal of identifying both known and novel markers in the coral system – broadening our knowledge about these species," says Bhattacharya.

"We focused on the polar metabolites because they have fast turnover and they are more closely related to the central carbon and nitrogen metabolic network," adds Xiaoyang Su, co-author of the paper. "To confidently identify these metabolite markers, we synthesized more than two dozen chemical standards and used these to compare the retention time on the LC and tandem MS spectra."

For both species, the team discovered a variety of co-regulated dipeptides that had the highest differential accumulation under thermal stress. These, along with other metabolites identified by the study, can be used as markers for thermal stress and thus health in wild coral – the diagnostic beginnings of what the team calls the "Coral Hospital."

Furthering its coral health mission, the team has now secured funding to create a microfluidic device that can be deployed by reef managers and members of the public alike to monitor the levels of different stress-related metabolites and proteins.

*Reference*

1. *A Williams et al., Sci Adv, 7, eabd4210 (2020). DOI: 10.1126/sciadv.abd4210*

# A Treacherous Tale

## Analysis of a black smudge casts new light on the final hours of the (in)famous Denmark Expedition

In 1907, three polar explorers set out to survey north-east Greenland as part of the Denmark Expedition. Sadly, none of these men would return home. Now, researchers have cast light on the final hours of Jørgen Brønlund – the last man alive – by analyzing a black spot from his diary.

Synchrotron radiation powder X-ray diffraction uncovered several inorganic compounds associated with 19th century rubber production, and pyrolysis GC-MS determined the organic composition of the sample: petroleum derivatives, oils, and fecal matter.

"The petroleum likely originated from the rubber gasket of a Lux burner, while the oils could originate from Brønlund's various desperate attempts to pre-heat the Lux," suggests Kaare Rasmussen, lead author of the study. The presence of certain markers associated with fecal matter gives a further bleak view of the explorer's final hours.

*Reference*

1. *KL Rasmussen et al., Archaeometry (2020). DOI: 10.1111/arcm.12641*

## IMAGE OF THE MONTH

*Scroll Scrutiny*

Spectroscopic (FTIR and XRF) and hyperspectral imaging techniques have been used to uncover vital details about a severely degraded Jewish ritual parchment (1). The image above shows how different ink components behaved at various wavelengths, enabling researchers to conclude that both iron gall and carbon black inks had been used on the manuscript. As well as revealing the origin and historical context of the document, the multi-analytical approach could prove useful to future investigations of manuscripts whose text is lost due to extensive degradation and could even aid conservation efforts.

*Reference: 1. IM Cortea et al., Front Mater (2020). DOI: 10.3389/fmats.2020.601339.*

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

## QUOTE OF THE MONTH

*"Nobody wants to conduct animal experiments, but nobody in the pharma industry wants to use granny either. Progress is apparent in increasingly powerful analyses that use fewer samples (and fewer animals), and in vitro and organ on-a-chip systems."*

Ian Wilson, Professor, Department of Metabolism, Digestion and Reproduction, Imperial College London, UK

# The Synapse Secret(ome)

### Researchers uncover the hidden proteome of synaptic vesicles

Using a new "ultra-definition" method, a team of researchers has managed to more than treble the number of synaptic proteins identified in the brain (1). Scientists had already discovered a number of proteins associated with synaptic vesicles – the small, sac-like components that deliver neurotransmitters between neurons – but a large proportion of low-abundance proteins were thought to be slipping through the net.

With the goal of uncovering more of this "hidden proteome," the team began by introducing enzymes that target two different cleavage sites, followed by separation of the resulting peptides by electrostatic repulsion-hydrophilic interaction chromatography. Conventional reverse-phase chromatography was also used, along with MS for identification. Using their new approach, the team managed to identify 1,466 proteins – more than three times that of previous studies. The new approach could aid in future investigations into neurological disorders.

*Reference*

1. *Z Taoufiq et al., PNAS, 117, 33586 (2020). DOI: 10.1073/pnas.2011870117*

**In My View**

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

# Down to a Single Cell

**To decipher the functionality of complex biological systems, laboratories should sidestep bulk cell measurements in favor of single-cell proteomics**

*By Erwin Schoof, Associate Professor, Department of Bioengineering, Technical University of Denmark, Lyngby, Denmark*

Biological system functionality is defined by the intricate interplay of its diverse cell types. Yet, cells are often studied in bulk, obscuring intracellular variability and single-cell contributions. This can hinder our ability to study complex diseases like cancer, in which cellular heterogeneity is a significant driver of prognosis. The scientific community needs to step away from averaged protein readouts and towards examining single-cell behavior.

The field of single-cell proteomics is still in its infancy, but is already delivering on its promise to capture transient cellular changes in dynamic systems by capitalizing on key advances in MS/MS-based approaches – particularly those affording improved sensitivity. This is key to make the most of biological samples' limited protein content (because, although present in quantities an order of magnitude greater than mRNA, cellular protein cannot be amplified!). This is particularly tricky when working with samples which are themselves limited, such as patient biopsies.

So how do we boost our proteomic outputs in light of limited sample? There are two main ways: i) by minimizing sample loss with meticulous preparatory steps, and ii) by adopting the most appropriate instrumentation to enhance experimental accuracy.

When it comes to minimizing sample loss, proteins' "sticky" nature is an issue; they cling to the sides of pipette tips and tubes at every stage of sample preparation, making conservation tricky. Robotic liquid handlers with non-contact pipetting can help – as can minimizing sample volumes to an absolute minimum (less than 1 µl). Single cell samples can be isolated through fluorescence-activated cell sorting or laser capture microdissection, which allows for the study of primary tissues in situ.

Experimental accuracy can be improved by including "booster" samples in the multiplexing mix. These increase the number of ions available, allowing us to enhance the lower limit of detection of

*"When it comes to minimizing sample loss, proteins' 'sticky' nature is an issue."*

*"The ability to examine a biological system down to a single cell can pave new paths in research – and clinics."*

MS analyses. In our lab, we use tandem mass tags (TMTs; isobaric labels for the accurate quantification of peptides and proteins in MS/MS-based analysis) for this. We dedicate a single TMT channel to tag a group of up to 200 booster cells, leaving other channels available for single cells. This boosts the protein identification rate by providing optimal ion levels for identifying and quantifying peptides that represent a significant portion of the cellular proteome

New-generation MS systems are also equipped with real-time search features in which MS3 scans are only triggered for peptide precursors identified at the MS2 level. Real-time data acquisition vastly improves proteome coverage, with up to 95 percent of peptides quantified at MS3 level. Matching MS2 fragments with the correct peptide sequence provides improved accuracy. By not wasting runtime on unnecessary MS3-level scans, real-time searching also significantly improves the productivity of single-cell proteomics workflows as a whole. Field asymmetric ion mobility spectrometry (FAIMS) can also filter out background ions and provide deeper sample coverage.

"How effective are these interventions?" I hear you ask. According to a proof-of-concept study we conducted in a heterogeneous acute myeloid leukemia model system (1), very! In fact, we were able to apply TMT multiplexing with a FAIMS-optimized workflow and precision robotics to conduct an entire sample preparation workflow on more than 3,000 cells. Our in-house data processing workflow, SCeptre (single-cell proteomics readout of expression), allowed us to visualize the data and pinpoint protein expression levels within each individual cell type from the sample.

Though it may seem overwhelming to transition from the safe familiarity of bulk protein analyses into the novel and technically demanding world of single-cell proteomics, I would say the time is now. The ability to examine a biological system down to a single cell can pave new paths in research – and clinics. Laboratories can set themselves up for future successes now by venturing into simple proof-of-concept experiments or collaborative efforts that eventually develop into a tailored single-cell workflow.

*Reference*

1. *EM Schoof et al., "Quantitative single-cell proteomics as a tool to characterize cellular hierarchies," bioRxiV [This article is a preprint and has not been certified by peer review] (2020). DOI: 10.1101/745679]*

# A Circulating Solution?

## Liquid biopsy – and appropriate, cell line-derived controls – are essential for improving patient care

*By Prabha Nagarajan, Research Scientist, Diagnostics at Horizon Discovery, Cambridge, UK and Keith Cannon, Director of Commercial Product Management, Diagnostics at Horizon Discovery, San Diego, California, USA.*

Advances in precision medicine are transforming cancer diagnosis and treatment. The potential to detect and monitor both solid tumors and blood cancers has spurred aggressive research programs around the world. Liquid biopsy – the analysis of short nucleic acid fragments (150–500 bp) in blood – provides researchers with a unique opportunity to identify and define signatures for specific tumor types. These circulating free DNA (cfDNA) fragments not only offer the potential for earlier detection and diagnosis, but can also measure therapeutic effectiveness and inform treatment decisions.

Cancers present complex biological pathways that vary across tumor types and patients, and not all tumors respond equally to treatment. For example, therapies targeting pathways in highly proliferative or resistant tumors are more efficient and effective. Personalized genomics has therefore opened a groundbreaking path for molecular diagnostics in oncology.

With increasing numbers of diagnostic assays entering the lab, our need to evaluate these tests' performance is greater than ever – and appropriate controls are essential to calibration, standardization, and routine quality control.

Reference standards used in molecular tests should be well-characterized, stable, homogenous, and mimic the properties intended for use in analytical measurements. Clinical tests aimed at detecting genetic variations by next-generation sequencing (NGS) can introduce errors at various stages of the workflow, from sample preparation to bioinformatic analysis. To support the use of such comprehensive assays and ensure their accuracy, we must use reference standards that closely represent patient samples at each stage of the process.

That's where cell line-derived controls come in. Cell line-derived materials offer the complexity of the human genome and, when processed into different formats representing a patient sample analyte, serve as a commutable and renewable source of biological controls for assay development and R&D studies. They are comprehensive and, when sufficiently characterized, help establish analytical sensitivity in both quantitative and qualitative measurements.

Every clinical test needs robust controls to ensure reliable results and accurate diagnosis. In lung cancer, for instance, therapeutics tackle at least 10 different disease drivers and elements of the disease pathway – so it's clear that we need new assays to characterize specific cancer types and ensure each patient receives the best possible treatment for their disease. In addition, when studying rare cancers or novel biomarkers, it can be a challenge to obtain reliable, reproducible controls from patient samples. In both cases, cell line-derived reference standards offer a consistent, accurate, affordable way to design these assays.

Tumor DNA shed into blood constitutes a small fraction of the total cfDNA population, but is proving an important noninvasive biomarker in early cancer diagnosis, progression, and remission. However, the variant allele frequencies (VAFs) of tumor-specific mutations can be much lower in the cfDNA population compared to primary tumors – so accurate detection of low-level (1–10 percent) VAFs in cfDNA requires analytical tools with higher sensitivity and specificity. Careful consideration of the preanalytical workflow, which includes sample collection, storage, and nucleic acid isolation, is also critical to cfDNA quality and quantity. Appropriate quality controls for each step of the workflow reduce errors, aid in calibration to achieve higher purity and quality of extracted cfDNA, and facilitate accurate detection of low-frequency alleles.

Cell line-derived DNA, like patient samples, is genomically complex and thus offers an advantage over synthetic reference materials. It's also preferable to non-renewable, non-reproducible patient-derived samples. DNA from cell lines can be processed into smaller fragments corresponding to cfDNA fragment profiles and, when well-characterized for physical properties like quality, purity, quantity, and average size, can be used as controls for preanalytical workflows. Genetic profiling of cell line-derived cfDNA can be useful for developing quality controls to validate mutations and their respective allele frequencies in analytical assays. As the medical community increasingly uses NGS assays to inform diagnosis, prognosis, and treatment, cell line-derived controls will be essential to maximizing their utility and effectiveness in improving patient care.

# Quality Control: The Power of Precision

**A growing number of analytical tools can – and should – be used to assess drug quality and purity. But one technique that is seeing increasing popularity, in part due to its precision and reproducibility, is capillary electrophoresis.**

*By Mark Lies Global Business Manager – Capillary Electrophoresis at SCIEX, California, USA.*

Common methods for the analysis of medicinal products include ligand binding assays (LBAs) and chromatographic methods, such as LC or GC, which are typically used in combination with MS. However, over the past decade, another separation technique – capillary electrophoresis (CE) – is being increasingly used to check and confirm the purity, heterogeneity, and glycan association of biologic drugs (1).

One good example is the application of capillary zone electrophoresis (CZE) for the analysis of synthetic human erythropoietin (EPO), which is used for the treatment of anemia associated with certain clinical conditions, including kidney disease and inflammatory bowel disease. EPO treatments must contain a specific mix of protein isoforms to be fully effective, and so accurate and precise quality control assays are required to check EPO isoform heterogeneity.

Why choose CE? Because it is a simple, quick, and effective technique, with exceptional resolving power and a high degree of precision.

We understand precision as the reproducibility of a method's quantitative and qualitative accuracy relative to data obtained using the same type of instrument and protocol. Industry guidance from the FDA on the validation of analytical methods used for the development and manufacture of biopharmaceuticals specifies that they must be precise, accurate, and of sufficient dynamic range (2). As biologics become increasingly complex, the industry needs – and regulators demand – increasingly precise analytical methods. Precision speaks not only to the analytical validity of a measurement method but, ultimately, to its utility.

In pursuit of CE precision, "home-brew" methods and reagents are progressively being replaced by specialized and standardized reagents and kits optimized for specific CE methods, such as capillary isoelectric focusing (CIEF) and CE-sodium dodecyl sulfate (CE-SDS). The aim is typically to develop a complete workflow solution that is precise but also simple and flexible enough for QC purposes.

Ensuring the precision of commercially available assays is critical for QC during clinical development and commercial manufacture. When addressing robustness, engagement between assay manufacturers and users is key. Critical assay specifications, including precision, of CE analyses can be assessed to meet everyone's requirements. To that end, cross-company collaborations conducted with (bio)pharmaceutical companies and regulatory authorities have demonstrated the precision of CIEF technology for the analysis of monoclonal antibodies, CZE for charge heterogeneity testing of monoclonal antibodies, CE – laser induced fluorescence (CE-LIF) for mapping multi-site N-glycans, and CE-SDS for the analysis of biomolecules (3, 4, 5, 6).

The purity of gene therapy products is also being increasingly monitored using CE and MS technologies. CE-MS data are also used to confirm and corroborate the data obtained using LBAs and other methods. In my view, we will continue to see the rise of CE as an orthogonal tool to address the increasing complexity of QC for new modalities in biologics. After all, precision drives quality.

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# Breath Testing for Early Diagnosis

## Breath testing to advance early disease detection and diagnosis

*By Jonathan Lawson, Head of Content at Owlstone Medical Ltd., Cambridge, UK.*

Invasive, labor-intensive, and potentially risky – a list of characteristics that don't sound desirable in a standard diagnostic tool. Yet tissue biopsy, with all of these characteristics, is the global gold standard in disease detection and diagnosis – and other well-established biopsy methods have their own limitations. It's clear that we have an urgent need for new approaches that are affordable, accessible, reliable, and safe to enable effective early detection of disease and advance precision medicine. Breath biopsy – the detection, identification, and precise quantification of chemicals in breath – has the potential to transform clinical pathology.

Exhaled breath is a valuable source of prospective disease biomarkers, containing over 1,000 volatile organic compounds (VOCs) in addition to respiratory droplets, which carry non-volatile compounds, proteins, lipids, nucleotides, bacteria, and viral particles. Together, these provide a rich source of information on metabolism, environmental factors, and disease processes. Breath collection is completely noninvasive and increasing sampling time allows detection of VOCs that may be present at very low levels in the earliest stages of disease. Such capabilities make breath a unique sampling option.

VOCs in breath can arise from external sources or from within the body itself. As such, they can reflect biochemical and metabolic activity, diet, prescription drugs, and environment. Many VOCs from all parts of the body are readily transported to the lungs via the blood, making breath samples compatible with whole-body disease sampling. Furthermore, because endogenous VOCs link directly to metabolic activity in the body, changes in their levels can be characteristic of specific disease processes from the earliest stages.

Respiratory droplets generated in the deep airways of the lungs have been the center of attention recently because of their role in transmitting respiratory infections. With the right collection approaches, biomarkers relevant to various diseases can be captured from respiratory droplets and analyzed using well-established techniques, such as ELISA and PCR.

Discovering VOC biomarkers in a complex sample like breath requires both highly reproducible tools for collection and advanced chemical analysis to resolve and identify compounds. In the past, technical limitations and a lack of standardized analytical techniques have hindered the development of clinically relevant breath tests. In recent years, though, experts in the field have developed advanced collection technologies that offer consistent breath sampling. At the same time, though many techniques have been applied to breath analysis, GC-MS has emerged as the gold standard for VOC biomarker discovery. The latest high-resolution GC-MS platforms excel in the identification and quantification of biomarkers within the complexity of a breath sample, providing vital biological insight across the full range of exhaled VOCs. Together, these advances enable the development of novel breath tests in areas of high clinical need.

The range of potential applications for breath biomarkers in both research and clinical settings is expansive. Test development programs are under way in areas as diverse as respiratory disease, liver disease, cancer, and environmental exposure.

The early detection of cancer, particularly lung cancer, is a key area of interest in breath research. Despite being the most common cancer worldwide, lung cancer has one of the lowest five-year survival rates. Why? Because early diagnosis is costly and inefficient. Multiple studies have suggested that lung cancer could be diagnosed by the presence of certain carbonyls in a patient's breath (1). The benefits of a low-cost, noninvasive test that can be deployed in screening programs are clear.

Liver disease is rapidly growing as a cause of global morbidity and mortality. Existing liver function tests largely assess liver damage rather than current function and struggle to determine the stage of liver disease. Limonene, however, shows excellent potential as a biomarker of both cirrhosis and broader liver health (2, 3). Originating from diet and detected noninvasively on breath, limonene abundance increases due to metabolic shifts linked to cirrhosis. Work is now ongoing to understand whether limonene and other breath VOCs can also be used to monitor liver disease, which is increasingly widespread due to high-fat diets.

Over half a billion people worldwide suffer from chronic inflammatory airway diseases including asthma, chronic obstructive pulmonary disease, and

*"The range of potential applications for breath biomarkers in both research and clinical settings is expansive."*

idiopathic pulmonary fibrosis. A lack of reliable diagnostic tools means that treatment often depends on trial and error – increasing costs, prolonging periods of poor disease control, and raising the risk of exacerbations. Breath analysis could provide rapid, noninvasive patient stratification to identify steroid responders and enable targeted therapies. Recent research is already exploring the identification and validation of breath biomarkers with the potential to differentiate inflammatory phenotypes in asthma (4), and there is hope for tools that could predict oncoming exacerbations.

Taken together, these three examples highlight the huge untapped potential for breath biomarkers to revolutionize early disease detection and precision medicine. And with the unprecedented attention COVID-19 has brought to breath research, the next few years promise to be an incredibly exciting time of development for the field, with the possibility of disruptive new healthcare technologies just around the corner.

*References*


1. *Y Saalberg, M Wolff, "VOC breath biomarkers in lung cancer," Clin Chim Acta, 459, 5 (2016). DOI: 10.1016/j.cca.2016.05.013*
2. *R Fernández del Río et al., "Volatile biomarkers in breath associated with liver cirrhosis – comparisons of pre- and post-liver transplant breath samples," EBioMedicine, 2, 1243 (2015). DOI: 10.1016/j.ebiom.2015.07.027*
3. *ME O'Hara et al., "Limonene in exhaled breath is elevated in hepatic encephalopathy," J Breath Res, 10, 046010 (2016). DOI; 10.1088/1752-7155/10/4/046010*
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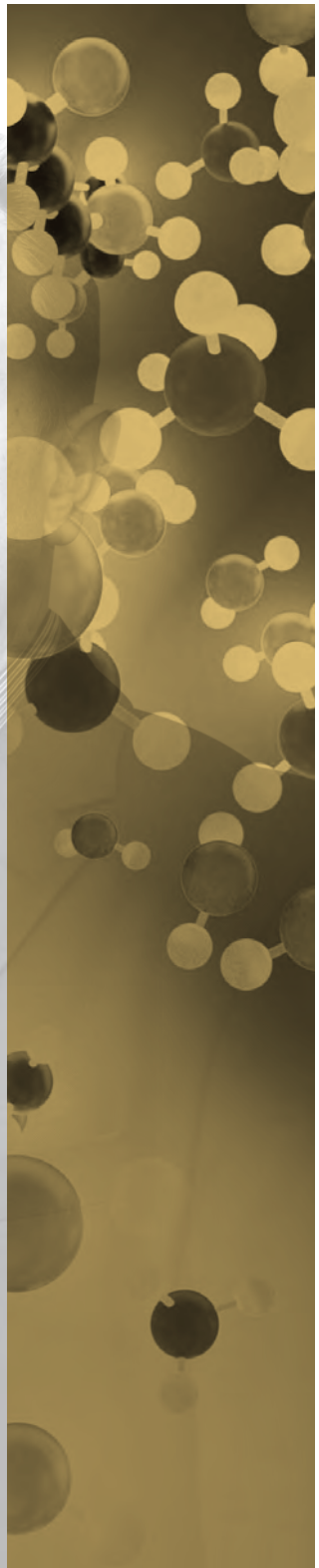
# GURUS *of Omics*

LEADING MINDS FROM CORE OMICS DISCIPLINES WEIGH IN WITH THEIR THOUGHTS ON WHERE WE ARE NOW – AND WHAT WE CAN EXPECT FROM TOMORROW



# QUICK INTRODUCTIONS

**Alejandro Cifuentes,**
representing foodomics
*Professor, Laboratory of Foodomics, Spanish National Research Council, Madrid, Spain*

My research has a core objective: to demonstrate that food can benefit our health in countless ways. A great aim in this is the investigation of revalorizing food by-products, including algae, plants and food waste. My main line of research for some years now has honed in on how these bioactive compounds could be applied to the treatment of colon cancer, and my group was the first to combine transcriptomics, proteomics and metabolomics to investigate bioactive food compounds in this context.

**Claire Eyers,**
representing proteomics
*Professor, Institute of Systems, Molecular & Integrative Biology, University of Liverpool, UK*

I develop proteomics strategies to enhance our understanding of protein post-translational modifications, largely phosphorylation, in cellular regulation. We think that we understand a great deal about the roles of phosphorylation, but studies in vertebrates have focused almost exclusively on the phosphorylation of serine, threonine, and tyrosine. We recently found that at least six more of the 20 common amino acids are also phosphorylated in humans. Considering this, it's clear that our job defining the functions of protein phosphprylation is far from over. There really isn't an area of biological and clinical science that won't benefit from proteomics, and I feel privileged to play a role in its progress.

**Gary Siuzdak,**
representing metabolomics
*Professor & Director of The Scripps Center for Metabolomics, The Scripps Research Institute, San Diego, California, USA*

We aim to create technologies that facilitate identification of metabolites that can alter biological systems (hydrocortisone's immunosuppressive function is a great example) – I like to call such efforts "activity metabolomics." From a technical perspective, we have achieved this through our development of XCMS and METLIN. XCMS was the first chromatographic MS platform that allowed correction of the nonlinear alignment of chromatographic and MS data, while METLIN was the first database of tandem MS data – it now hosts over 850,000 molecular standards.

**Michal Holčapek,**
representing lipidomics
*Professor, Faculty of Chemical Technology, University of Pardubice, Czech Republic*

I always seek the best analytical performance possible. In our laboratory, we don't like to use "features" or "tentatively identified molecules." We strongly prefer to report lipid species confidently – identified based on the retention times, accurately determined masses, and fragmentation behavior. The result: false identification rates close to 0 percent. Our approach goes against the mainstream in omics, but is a powerful force in my team's application of lipidomics to solving problems in human disease and beyond.

## *We've come a long way since the Human Genome Project…*

The mapping of our genetic self was an incredible milestone in scientific history. And, for omics, it was the Big Bang that gave birth to an ever-expanding universe of holistic molecular analyses. Today, that universe is almost unrecognizable from the one we inhabited at the turn of the millennium – and its associated applications are booming, too.

As for the use of the suffix -omics, there has been an equally impressive explosion – epigenomics, microbiomics, lipidomics, foodomics, interactomics, even CRAPomics – as countless scientists find a niche and dedicate their labs and their lives to digging ever deeper into nature's molecular mysteries. We invited four such scientists (each a leading mind in their own omics discipline) to talk about the state of their field today.

How did we get here? What can we achieve with the tools available to us? And are we nearing a Big Yawn in the expansion we've witnessed thus far? Let's see what our gurus had to say…

### WHAT DREW YOU TO OMICS?

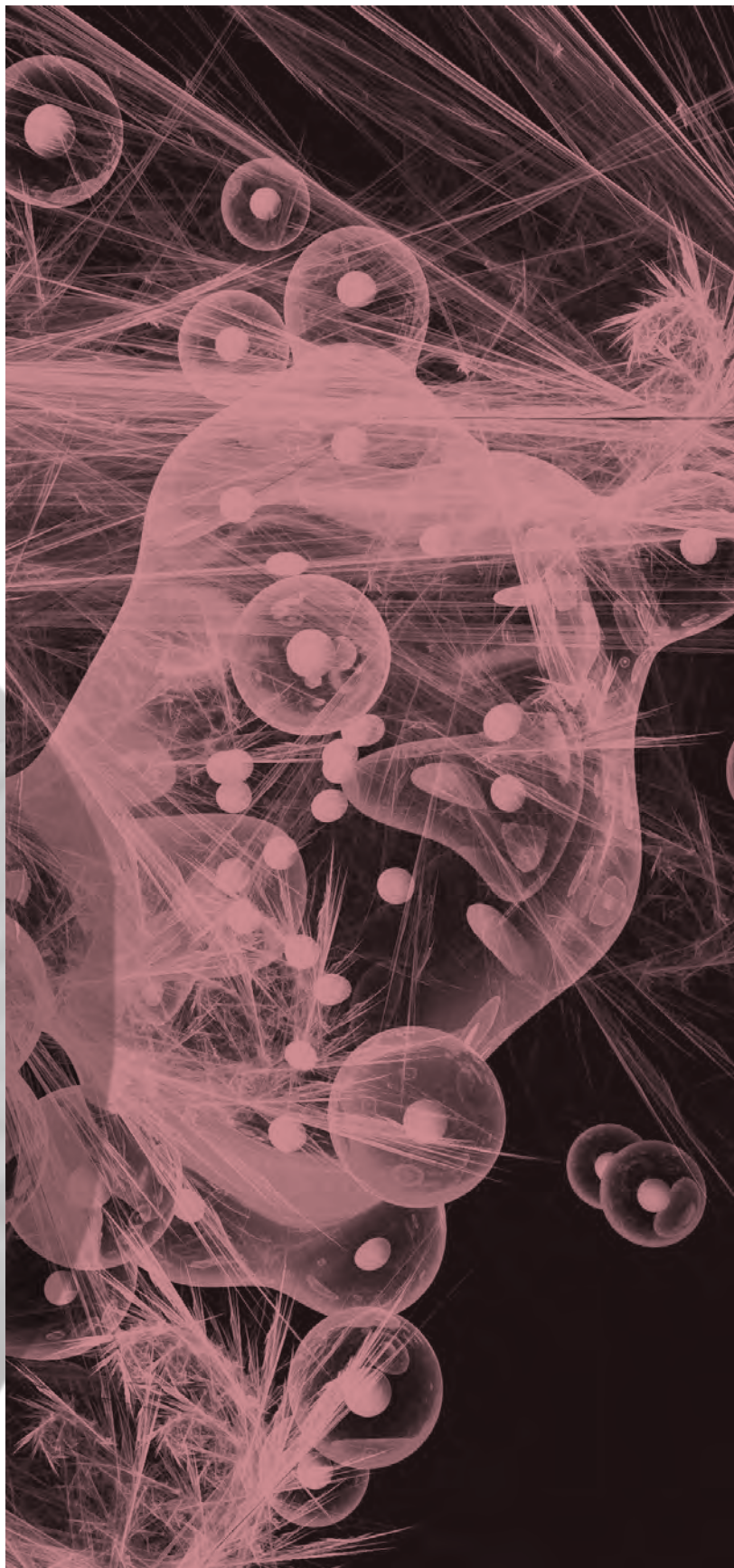
*Claire:* My interest in proteomics started as a PhD student working on phosphorylation-mediated cell signaling in Dundee. I believed (and still do) that proteomics can help us to better delineate and understand complex biological systems, and the ways in which proteins act – both individually and in concert – to regulate biological function.

*Michal:* Like Claire, it all started with my PhD. I joined Pavel Jandera's lab in 1995, and we were fortunate to have the first benchtop LC-MS system in the Czech Republic. It was equipped with a single quadrupole mass analyzer, which is almost laughable today. One of my first tasks was to analyze reaction mixtures from biodiesel production (these complex samples contain tri-, di- and monoacylglycerols). It was then that I realized the beauty of lipid analysis. These species have regular increments per their individual classes, resulting in regular retention patterns and predictable m/z values. This is part of the reason that I decided to focus on lipids in biological systems, and – 25 years and a new name (lipidomics!) later – I consider this the best choice I ever made.

*Alejandro:* Other areas, such as biotech and pharma, have typically been the first to benefit from omics – that's where the big money is, after all. Food science was always left behind. I found this hard to stomach (if you'll pardon the pun), especially considering the gigantic impact food has on our lives. I introduced the concept of foodomics in 2009, with the aim of applying omics technologies to studies of food and nutrition. The ultimate aim is to improve food quality and safety, and to explore the relationship between food bioactivity and human health.

*Gary:* Unlike my friends above, I was never drawn to "omics" per se, but instead focused on the lack of information available regarding the role that metabolites play in so many areas of biology. Metabolomics, in my view, has turned out to be an ideal technology to address this problem.

*"In 20 years, we will still be trying to comprehend the huge complexity of interactions between food ingredients and our body!"*



## WHAT'S THE STATE OF YOUR FIELD TODAY, AND HOW HAS THIS CHANGED DURING YOUR CAREER?

*Alejandro:* I would say foodomics is still in its infancy. As I said before, it was only defined 12 years ago, so there's much room for growth. Though foodomics itself hasn't changed much throughout my career, the methods that we rely on certainly have. For example, early proteomics investigations relied on two-dimensional electrophoresis followed by image comparison, protein cutting from gels, trypsin hydrolysis, and sequencing by MALDI-TOF-TOF tandem MS. Now we can analyze the proteome of a biological system with a single injection by combining nano-LC and high-resolution MS with isotopic probes bound to hydrolyzed proteins.

*Claire:* Proteomics has changed considerably since I started in this field 20 years ago. Advances in chromatography instrumentation and column chemistry have dramatically improved sensitivity, partly because chromatographic resolution has focused MS instrument time. Combined with vast improvements in the speed and sensitivity of mass spectrometers, we are now able to identify and quantify changes in protein abundance in very small sample amounts – even to the single-cell level in some systems. Routine implementation of different forms of peptide fragmentation during tandem MS, ion mobility separation to complement (or replace!) chromatographic peptide separation, and better understanding of the chemical mechanisms driving peptide fragmentation have all improved the automated analysis of peptides and their post-translational modifications.

*Gary:* Metabolomics is on a tremendous growth streak. The trajectory of this growth today is higher than ever before. This is largely due to many different biological and therapeutic fields realizing the wealth of information that can be gleaned from metabolomic analyses. Whether it is fundamental biology, the microbiome, the exposome, therapeutics, or any of myriad other areas, metabolomics can add both mechanistic and functional information – or even identify metabolites that can alter phenotype. Lots has changed since I first got involved in LC-MS-based metabolomics 27 years ago!

*Michal:* Scientific knowledge evolves constantly and dramatically. Lipids were initially known for their roles as energy stores and building blocks of cellular and intracellular membranes. Today, however, we know that they also play roles in countless other biological processes, including cell signaling.

The lipidomics toolbox has needed to expand rapidly to study the crucial roles of lipids. We have, for example, benefited from enormous improvements in analytical instruments and bioinformatics. I was happy with HPLC coupled to a single quad spectrometer 25 years ago. Today, ultra-high, ultra-fast performance is ubiquitous; examples include qTOF mass spectrometers with ultra-fast scan rates, the ultra-high-resolution Orbitrap, and UHPLC and UHPSFC. These powerful configurations quickly generate tremendous amounts of data, putting pressure on the bioinformatics processing and statistical evaluation. Indeed, bottlenecks tend to present themselves on either side of such impressive systems, with both sample preparation and data analysis demanding further attention.

## WHAT'S THE GREATEST FEAT ACHIEVED IN YOUR FIELD THUS FAR?

*Gary:* The sheer number of tools that have been created to support metabolomics is likely the most impressive collective feat. A new technological (typically informatic) tool comes out almost every week, and these developments are helping the field to accelerate at an incredible rate. I am personally fond of my own team's success in developing the XCMS/METLIN platform, which facilitates metabolite and chemical identification. METLIN now boasts over 850,000 molecular standards, each with experimental tandem MS data. This is (I hope) helping move the field forward, especially combined with other technological achievements.

*Claire:* Scientists outside of omics may think of proteomics as a "plug and play" technology: put the sample into a (black) box and out comes a list of identified and quantified proteins. Though this isn't really the case (much expertise is required for good sample preparation, instrument setup and maintenance, experimental design, data handling, and so on…), the fact that many people perceive proteomics to be routine indicates that this technology has come of age!

As for specific points of excitement, I don't think I could

highlight a single one, but single-cell proteomics is up there! Our ability to quantify proteins at this level allows us to understand heterogeneity – a crucial factor in studying, for example, signal transmission across cell populations and disease. We can also explore proteins from species that we don't have a genome for and examine combinations of post-translational modifications via top-down proteomics approaches. "Native" MS (to understand protein complexes and the conformational dynamics of proteins subject to ligand binding or protein modification) can be used to conduct particularly powerful protein analyses.

*Alejandro:* The greatest achievement in foodomics is a tricky question, as we are still building our relatively new discipline. We have a long way to go to catch up with more established omics fields like genomics and proteomics. So, for now I would say: let's wait and see! But some great examples of the potential of our field can be found in foodomics studies looking at the antiproliferative activity of some food ingredients against colon cancer both in vitro and in vivo; for example, my group found that the green extraction and concentration of specific compounds (and the elimination of other not so positive natural compounds) from rosemary creates a product with such effects. Foodomics also helps corroborate the work that goes into ensuring the safety and quality of food commodities.

## AND WHAT ABOUT THE PROUDEST MOMENT IN YOUR OWN CAREER?

*Michal:* I am proud of my group's achievements in oncolipidomics. We found that the lipid profiles (in blood) of subjects with various types of cancer are distinct – and multivariate analyses could differentiate cases from controls with over 90 percent accuracy. Our most convincing results concerned a large set of pancreatic cancer samples (we were recently granted a European patent for our pancreatic cancer diagnostic method), but similar dysregulation is also apparent in many other cancers. We are now working on the translation of our diagnostic method into clinical practice. If successful, it would be a real breakthrough in pancreatic cancer screening and, hopefully, other cancer types as well!

*Alejandro:* Coining the term foodomics was certainly a proud moment. And yet, nothing would have come of it without the positive reception among colleagues – and the incredible work they conduct in this area. It also makes me very happy to think about how many (mostly young) researchers have been prepared for life as capable professionals following time in our lab!

*Gary:* I feel the same way, Alejandro. My answer is simply the number of individuals who came to my lab and left for exciting metabolomic careers across academia and industry!

*Claire:* Demonstrating the true extent of "non-canonical" phosphorylation in human cells (my team used a novel analytical pipeline to show that protein phosphorylation in human cells is much more diverse that anybody previously thought) and the excitement that this has created within the cell signaling community is one of my proudest proteomics moments. It took a number of years to get a working method, given how unstable atypical phosphorylation events are under standard proteomics conditions, and I am incredibly proud of the students and postdocs that contributed to this project.

Also, working with other members of the COVID-19 MS coalition, we have developed robust MS assays for the simultaneous screening of both COVID-19 and winter flu in saliva samples. These are currently being evaluated in clinical hospital labs. Exploiting the sensitivity and specificity of targeted MS assays that can be multiplexed for rapid screening of diverse markers of infection or disease in this way will have significant societal benefit.

## WHERE DO YOU SEE THE FIELD IN 20 YEARS' TIME? WHAT CHALLENGES WILL NEED TO BE OVERCOME?

*Claire:* We have this discussion quite often in our lab, and I personally think that proteomics in 20 years will probably not be conducted using today's classical LC-MS workflows. Proteomics (or rather bottom-up proteomics) has an issue that stems from the inference of protein identities from peptide identification using tandem MS.

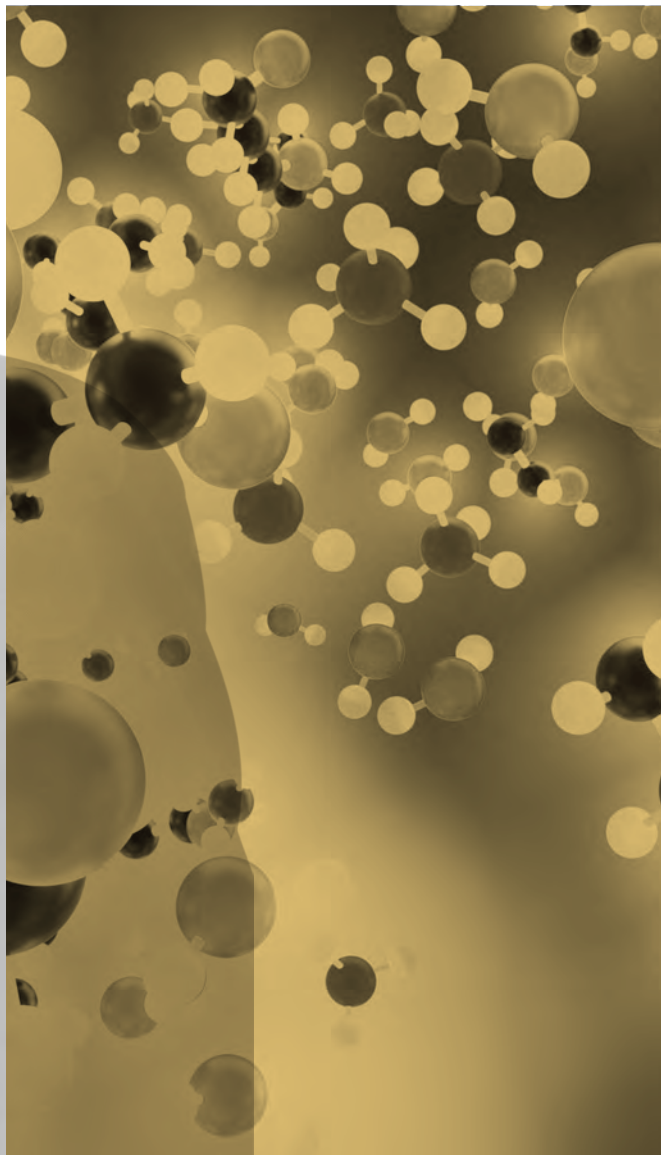
Problems with "missing peptides" and our inability to fully characterize sites of covalent modifications mean that we don't currently define the large numbers of proteoforms present in systems. Current instrumentation also struggles

*"Proteomics has changed considerably since I started in this field 20 years ago."*

*"I am proud of my group's achievements in oncolipidomics."*

with the robust separation of large (50 kDa or bigger) proteins that may differ only by the addition of a number of small chemical entities (methyl, phosphate, and sulfate groups, for example), and we are still unable to completely fragment these proteins inside the mass spectrometer and interpret these data. The sequential way in which we typically analyze samples means that high-throughput "complete" protein characterization remains outside of our current capabilities. Overcoming this limitation will be a great focus for the next 20 years, and will likely require radical new ways of thinking about our technology and data analysis tools.

*Gary:* If the question is "Where would I like to see the field in 20 years' time?" then the answer is pretty straightforward… I'd like to see metabolomic tools with a dynamic range sufficient to comprehensively quantify and identify all metabolites – from



the most ubiquitous to the least common.

The instrumental challenges are rather daunting, though. A dynamic range improvement from the current state of the art (six orders of magnitude) to ~12 orders of magnitude would be needed. And maybe 20 years is not long enough!

The identification challenge is also daunting. PubChem lists 93 million molecules in its existing chemical space; identifying these rapidly and with stereoisomer precision is almost impossible. Major leaps will be needed regarding the size of our own METLIN database and technologies for high sensitivity stereoisomer assessment. Again, 20 years may not be enough…

*Alejandro:* In 20 years, we will still be trying to comprehend the huge complexity of interactions between food ingredients and our body! I anticipate a deeper understanding of the microbiome by then, but so-called "personalized nutrition" will take longer still. I would say that overcoming current limitations in data treatment is the main challenge ahead, including the integration of the huge amounts of data generated at different levels of expression (genomics, transcriptomics, proteomics, metabolomics by existing analytical techniques) and subsequent transformation of this data into useful biological information.

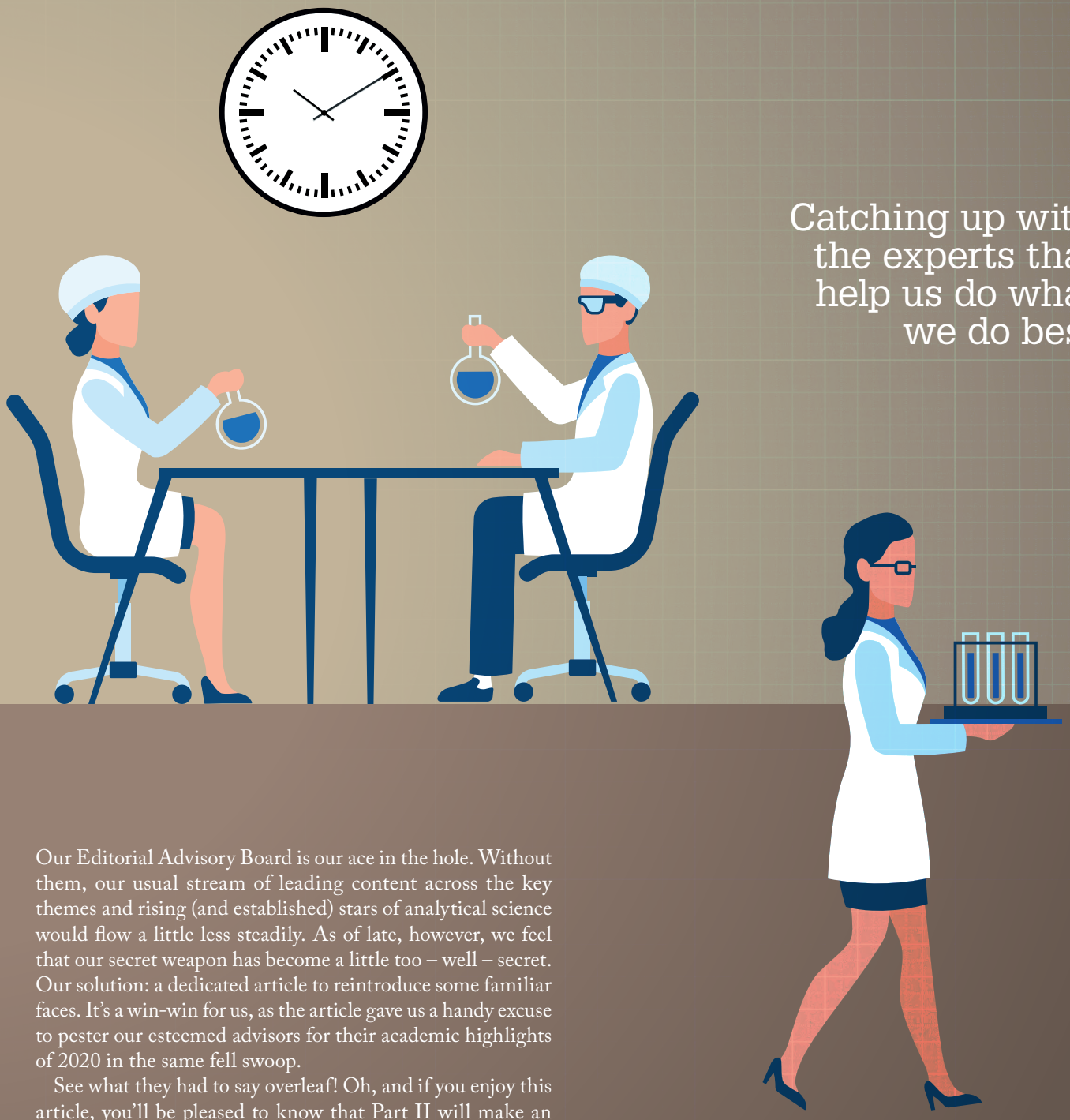
*Michal:* We have made significant progress in lipidomic quantitation, but we aren't perfect. In the words of my coworker, Denise Wolrab: "It seems that all labs have troubles and, even for the so- called leading groups in lipidomics, it is far from perfect. Improvements are necessary." Harmonizing our protocols so that different labs can report comparable results with high confidence and structural detail represents a crucial step. Other hurdles we must overcome are the identification of specific lipids' roles in metabolic pathways and ability to merge information from separate omics disciplines in a systems biology approach – a huge challenge for bioinformaticians.

In addition, the alterations in lipid metabolism occurring across numerous diseases (cardiovascular and liver diseases, cancer, Alzheimer's, and so on) remain poorly understood. There is much work to be done by the lipidomic community on this front! As methodological and instrumental improvements materialize, we will also be able to dig deeper into structural details with MS and ion mobility approaches.

# *Meet our*

# Editorial Advisory Board

*(PART I)*



# Catching up with the experts that help us do what we do best

Our Editorial Advisory Board is our ace in the hole. Without them, our usual stream of leading content across the key themes and rising (and established) stars of analytical science would flow a little less steadily. As of late, however, we feel that our secret weapon has become a little too – well – secret. Our solution: a dedicated article to reintroduce some familiar faces. It's a win-win for us, as the article gave us a handy excuse to pester our esteemed advisors for their academic highlights of 2020 in the same fell swoop.

See what they had to say overleaf! Oh, and if you enjoy this article, you'll be pleased to know that Part II will make an appearance later this year. Watch this space!

# Ian Wilson

*Professor, Department of Metabolism, Digestion and Reproduction, Imperial College London, UK*

## Tell us about yourself and your research!

That must be the most embarrassing thing you can ask an Englishman, but here goes... I'm a visiting professor at Imperial College London, having (semi-)retired from an academic position there, and I also work with Waters, Evotech, and Liverpool University – among others. I like to think that I've fallen into the James Lovelock paradigm of being an "independent scientist." This has a nicer ring to it than a freelancer, which once described medieval mercenaries for hire. My main research interests are drug metabolism, omics, instrumentation, separations, and toxicology.

## What makes you tick?

My main driver is a desire to know more, and to improve the efficiency of drug discovery and evaluation. An example is movement away from animal models. Nobody wants to conduct animal experiments, but nobody in the pharma industry wants to use granny either. Progress is apparent in increasingly powerful analyses that use fewer samples (and fewer animals), and in vitro and organ on-a-chip systems. Yet, the latter could one day raise ethical questions. An example: at what point does a human-on-a-chip constitute a human?

## What keeps you up at night?

Despite working from home, I'm exceptionally busy. And, with many of us spending half of our time on Zoom calls, we end up with less time to do our real work. The worry then becomes: who am I letting down today? Another concern – one I think all professional scientists share – is making an appalling mistake in a publication. You hear horror stories of comments like, "Should we cite the crappy Gabor paper here?" making it to print (that example from Ethology is now sadly corrected in the online version!).

## Do you have any personal (or general) milestones from 2020 you'd like to share?

Well I finally saw the last of my PhD students successfully launched! If you're ever in a position to take on PhD students, my advice is to always select students who are brighter than you are – it saves you a great deal of thinking!

## Top paper of 2020

"Crystalline Sponges as a Sensitive and Fast Method for Metabolite Identification: Application to Gemfibrozil and its Phase I and II Metabolites" (1). The technique described, which uses crystalline sponges to enable X-ray crystallography of micrograms of metabolites isolated following HPLC separation, gives more defined structures than NMR.

*Reference*

1. *L Rosenberger et al., "Crystalline Sponges as a Sensitive and Fast Method for Metabolite Identification: Application to Gemfibrozil and its Phase I and II Metabolites," Drug Metabolism and Deposition, 48, 587 (2020). DOI: 10.1124/dmd.120.091140*

# Gary Hieftje

*Retired – Distinguished Professor and Robert & Marjorie Mann Chair, Indiana University Bloomington, Indiana, USA*

## Tell us about yourself and your research!

I spent 50 years investigating atomic, molecular, and biomolecular emission, absorption, fluorescence, and MS on the faculty at Indiana University Bloomington and have been retired for a year now. But that doesn't mean I'm not still scientifically active. I contribute to ongoing research projects with former students, serve on a number of editorial boards (including The Analytical Scientist's!), and serve on review committees for several different government agencies, including the Department of Energy.

## What makes you tick?

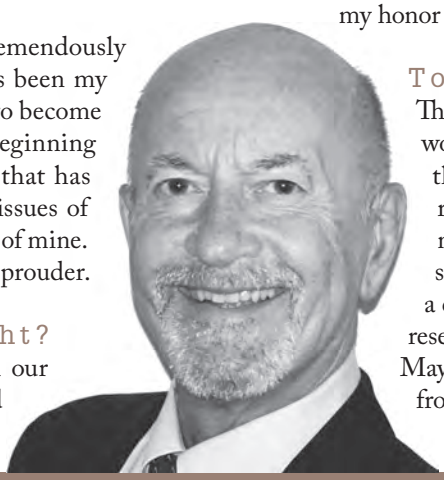
Research is, of course, enjoyable and tremendously useful, but educating students has always been my focus. Some of my students have gone on to become award-winning scientists – or are even beginning their own retirements! Another activity that has kept me busy is pulling together special issues of journals dedicated to these former students of mine. It's incredibly gratifying and I couldn't be prouder.

## What keeps you up at night?

The long-term impact of COVID-19 on our world is a big worry – especially with regard to science and education. Schools and universities are not built to operate in vacuums like they are right now – our remote ways of working are a poor substitute for in-person teaching and research. The impact of politics on science is also of concern (an impact now visible in many countries), as is the tendency for analytical chemistry to lean excessively toward bioscience. This is an important field, but it's a mistake to forget all other fields in its pursuit. I fear our focus has become too narrow.

## Do you have any personal (or general) milestones from 2020 you'd like to share?

I'd say retiring is a pretty big milestone! I've also moved to a considerably smaller dwelling, albeit in a much nicer climate, and become a great-grandfather for the third time (I have 13 grandkids!). Closer to my scientific career, a special issue of the Journal of Analytical Atomic Spectrometry was published in my honor to commemorate my retirement.



## Top paper of 2020

There are countless good papers out there and I wouldn't want to make any seem less important than the others! I have, however, started reading a lot of trashy novels and renewed my love for science fiction. I've always enjoyed sci-fi and, looking back to the books I read as a child, it's strange to see that a lot of my best research ideas have come from science fiction. Maybe my best idea in 20 years' time will come from the reading I'm doing now!

## WHERE YOU MAY HAVE SEEN HIM BEFORE

*Reflecting on Gary's prior moments of glory with The Analytical Scientist*

**The Three Sides of Spectroscopic Investigation**

This one's a flashback to an insightful editorial that Gary penned as the Guest Editor for The Spectroscopist. He took the opportunity to guide us through the nature of the symbiotic relationship between theory, application and instrumentation in analytical science. This relationship, he says, is too often overlooked in research.

**Three Gurus of Spectroscopy**

Another three (a coincidence, most likely). We enlisted Gary's help to explore the past, present, and light-filled future of spectroscopic analysis back in 2014. In his words: "Over the last 50 years we've been moving towards extremes: faster, smaller, higher resolution, higher power, lower noise." And regarding the future? "We need to encourage new small instrument firms that employ breakthrough technology."

**Free Up Funding**

"Following trends and buzzwords is delaying progress. We need more support for diverse, curiosity-driven research projects." This was the message Gary conveyed in this opinion piece from the archives. "We need a rethink," he continued. "To paraphrase Richard Feynman, if a person publishes in a less crowded field, he would not only seem better and bigger, he would be better and bigger."

# Emily Hilder

*Chief Maritime Division, Defence Science and Technology Group (DSTG), Department of Defence, Australia*

## Tell us about yourself and your research!

Previously the Director of the Future Industries Institute of the University of South Australia, I've recently moved to a new role within DSTG, the Australian government's lead agency responsible for applying science and technology to safeguard Australia and its national interests. I lead ~300 scientists and engineers across Australia, covering research in acoustic and non-acoustic signature management, materials science, sensors, systems integration, human performance, corrosion and more – all supporting Australia's maritime defence capability. Though there is plenty I can't discuss, I can say that I love the role so far.

## What makes you tick?

I'm definitely driven by the clarity of purpose in my role and this is something that has driven me throughout my career. I'm most happy when I can support providing solutions to real-world problems. An example: how do we provide and sustain the best technology to enhance the safety of the people who need to use it, especially in a contested environment? Providing clarity for my team in such uncertain times also keeps me ticking.

## What keeps you up at night?

In honesty, I usually sleep perfectly well. One concern I do have, however, is how early-career scientists are coping right now. COVID aside, many careers – for example, those in academia – are arguably more difficult than they used to be. Luck can play a big role (despite the extraordinary work people are doing) in success and progression, and that's stressful. In a year that's challenged many peoples' mental health, this could have a very negative effect.

## Do you have any personal (or general) milestones from 2020 you'd like to share?

The chaos of last year made me re-evaluate what's really important in life. I'd say that's a pretty big milestone! More generally, the speed with which our field has focused on COVID-19 is unbelievable. An example: the rapid development and application of methods for COVID monitoring in wastewater, which have been used to identify and manage outbreaks (especially in Australia). We have developed technology at a rate we previously thought impossible!

## Top paper of 2020

I'd say "Rapid High-Resolution Visible Light 3D Printing" (1). It addresses a really exciting "what if?" and allows many new possibilities for 3D printing. The potential for visible light photocuring changes the rules for what materials can be used and should lead to new applications both in 3D printing and beyond (for example: coatings, adhesives, lithography).

*Reference*

1. *D Ahn et al., "Rapid High-Resolution Visible Light 3D Printing," ACS Cent Sci, 6, 1555 (2020). DOI: 10.1021/acscentsci.0c00929*

## WHERE YOU MAY HAVE SEEN HER BEFORE

*Reflecting on some of Emily's prior moments of glory with The Analytical Scientist*

**The Art of Writing**

Emily lent us her wisdom (and words) in this 2018 piece about preparing top-quality manuscripts and posters that are sure to make waves. Along with Paul Haddad and Frantisek Svec, Emily covered some of the key questions we must ask ourselves in manuscript development (what journal will I submit to, and are my results really suitable for publication? ), as well as common mistakes to be avoided!

**Gurus of the Monolith**

One of our classic "gurus" articles, Emily took prime position in our 2015 discussion of monolithic columns. As she says: "Monoliths were the first practical alternative to particle-based stationary phases in chromatography. Both silica and polymer monoliths were able to demonstrate fast separations without significant loss in separation efficiency. Irrespective of whether or not monoliths outperformed other column types, the development of this new column format triggered many other innovations in separation science."

**Tea with Emily Hilder**

At ISC 2014, our beloved Content Director Rich Whitworth had the pleasure of enjoying a cup of tea with Emily - as well as discussing an exciting and ambitious collaborative project focused on portable analysis: the ARC Training Centre for Portable Analytical Separation Technologies (ASTech). Watch the video online!

# Peter Schoenmakers

*Van't Hoff Institute for Molecular Sciences, University of Amsterdam, Netherlands*

## Tell us about yourself and your research!

I lead a very international lab group, with members from all over the world (Iran, France, Portugal, Greece, Italy, and beyond). And that's less of a surprise when you consider that we take on roughly 80 master's students each year. We also run a number of PhDs – five of which are taken by extremely tall Dutch men right now! Luckily, we remain somewhat diverse, as the sixth spot is taken by a very talented woman. Together, we focus on separations (largely HPLC and multidimensional LC) for all sorts of applications.

## What makes you tick?

At the moment, the prospect of in-person interaction is a big factor. I think this will have to wait until summer at least; teaching could then potentially return to normal much later. Of course, we should wait until this is totally safe. The last time I went back into the university was on Sep 2, and I caught COVID-19 immediately. Luckily it was a mild case. It looks like it might be a while before we can do nice things like celebrate birthdays together again. For now, our lab has agreed that we will begin celebrating birthdays again after the middle of March 2021 (presuming things improve by then)!

## What keeps you up at night?

The impact of COVID-19 on colleagues and peers. I know groups and companies where people have ended up in intensive care; other departments have lost many. Another (much less serious) thing that keeps me up are the logistical issues of cybersecurity concerned with working remotely. I often find myself at war with cybersecurity processes...

## Do you have any personal (or general) milestones from 2020 you'd like to share?

The way our communication has changed is a huge milestone. We connect much more logically now – it's easier, and distances are no longer an obstacle. A great example is a PhD defence. Usually at least one or two people won't make it, but that's not an issue right now. Another positive: we've had much more time to write without lab access – a lot of people are writing literature reviews! As a result, there's also more to read! At least that can keep us busy.

## Top paper of 2020

Definitely: "Multiple heart-cutting two-dimensional chip-HPLC combined with deep-UV fluorescence and mass spectrometric detection" from Belder and colleagues (1).

*Reference*

1. *SK Piendl et al., Anal Chem, 92, 3795 (2020). DOI: 10.1021/acs.analchem.9b05206*

# Chris Harrison

*Associate Professor, San Diego State University, California, USA*

## Tell us about yourself and your research!

I've been teaching analytical chemistry at San Diego State University for over 10 years now, and during this time I've really been focused on using technology and other digital tools to aid the teaching process. The "flipped classroom" (see Flipping The Analytical Chemistry Classroom on The Analytical Scientist website!) is something I have implemented in my classes, where lectures are pre-recorded and class time is instead used for group assignments. In terms of research, our work in the Harrison lab is mostly focused around capillary electrophoresis and its application to bioanalytical problems – such as blood doping identification..

## What makes you tick?

What drives me most is securing opportunities for students and pushing them to realize their full potential. I've had students who didn't know of a scholarship opportunity or didn't realize there was a chance to do research as an undergrad or go to grad school – it's greatly satisfying to see one of your students succeed and go beyond what they thought was possible. And that's what keeps me going.

## What keeps you up at night?

What's been keeping me awake at night recently has been how to best prepare my students for practical lab work while we are all working remotely. We've been able to do some in-person teaching but it's been infrequent, so I'm constantly thinking about the best way to get those lab skills across – because it's so important! In past years, I've had students who have been top of the class in written work, but with little practical aptitude. I think the effects of this pandemic are going to plague our field for years to come, because we are going to need to do some sort of remedial teaching to get those students back up to speed when it comes to the practicalities of analytical science.

## Do you have any personal (or general) milestones from 2020 you'd like to share?

I think one of the biggest milestones for me this year was that one of my graduate students (who started as one of my undergraduate students) secured a four year NASA fellowship for a research project that she developed to detect traces of past life on rocks; it's incredible to see her succeed. In terms of personal milestones, I think I am just happy that I've managed to keep things moving along and make it through 2020!

## Top paper of 2020

In one really interesting paper relating to electrophoresis, the authors identified a secondary electrokinetic force that is involved in electrophoresis but that we've essentially overlooked in chemistry (1). I think it's really going to revolutionize dielectrophoresis research and make it more predictable – and it's got me thinking about things we can look at in our own lab...

*Reference:*

1. *Direct current electrokinetic particle trapping in insulator-based microfluidics: Theory and experiments, Anal Chem, 92, 12871 (2020). DOI: 10.1021/acs.analchem.0c01303]*

## WHERE YOU MAY HAVE SEEN HIM BEFORE

*Reflecting on some of Chris' prior moments of glory with The Analytical Scientist*

**Those who can, Teach**

Turning those oft-recited (and irritating, at least to teachers) words on their head, we asked Chris to tell us about his approach to teaching analytical science, and why it is arguably the greatest legacy a scientist can leave behind. His advice? "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."

**COVID-19: Improvise, Adapt, Overcome**

Back in May 2020, Chris helped us out with a special feature exploring the effect of the pandemic (so far) on scientists – from conference organizers to professors. Chris was kind enough to offer us a day-by-day account of the dramatic changes he witnessed across campus over two weeks as the pandemic took hold in California.

# Jenny Van Eyk

*Director of the Advanced Clinical Biosystems Research Institute and the Precision Biomarker Laboratories at Cedars-Sinai Medical Centre, Los Angeles, California, USA*

## Tell us about yourself and your research!

Our lab applies proteomics to two main aspects of personalized medicine: diagnostics and prognostic indicators. These approaches represent great promise but mean little without individualized therapies. To meet this need, we have also developed high-throughput methods to quantify the proteome in body fluids, animals and induced pluripotent stem cell-derived organoids to elucidate mechanisms of cardiovascular and neurological disease. Overall, it's going really well!

## What makes you tick?

I feel a huge debt to society, which I intend to pay by changing the way we practice medicine. Luckily, my group shares this mission. At the moment, this team comprises almost 45 individuals over multiple labs, including the Advanced Clinical Biosystems Research Institute (which is a mouthful, I know!) and the new Precision Biomarkers Labs. These latter laboratories are focused on bringing the expertise of targeted MS to biomarker discovery – and the development of targeted assays for translation into clinical environments.

## What keeps you up at night?

COVID-19 and the urgency of associated science. Another concern is that we aren't giving early-career scientists the entrepreneurial skills they need. We should be reaching out to young scientists with specific training that allows them to seek business opportunities – and this is especially important for fields like omics, in which equipment costs are high. I believe that we can form a cooperative for equipment, bioinformatics and knowledge sharing to overcome some of the hurdles involved in proteomics. At least, that is one business model.

## Do you have any personal (or general) milestones from 2020 you'd like to share?

Several! For one, the COVID-19 pandemic has accelerated the development of self-sampling blood collection devices, which can be deployed for population and healthcare proteome assessment (and will be of great use in telehealth). The use of remote sampling devices has afforded us valuable knowledge about many diseases, including COVID-19, and responses to vaccines. This knowledge, we hope, will inevitably help patients.

## Top paper of 2020

My favorite is actually from our own lab: "Lysine and arginine protein post-translational modifications by enhanced DIA libraries: quantification in murine liver disease" (1). This work allowed us to quantify site-specific data on 7 different protein modifications while simultaneously quantifying the total protein by DIA-MS. That's a lot of data content.

*Reference*

1. *AE Robinson et al., "Lysine and arginine protein post-translational modifications by enhanced DIA libraries: quantification in murine liver disease," J Proteome Res, 19, 4163 (2020). DOI: 10.1021/acs.jproteome.0c00685*

## WHERE YOU MAY HAVE SEEN HER BEFORE

*Reflecting on Jenny's prior moments of glory with The Analytical Scientist*

**Diagnostics Everywhere**

Jenny wrote this In My View article to share her expertise on precision diagnostics alongside student Kimia Sobhani. Here's a particularly striking quote from the piece: "Healthcare affordability and disease prevention remain at the forefront of the minds of our medical community, government and public in the US. And yet, approaches to improve the situation have not centered on the most obvious tools at our disposal: well validated laboratory diagnostics."

**Lessons I've Learned, With Jenny Van Eyk**

Having walked a scientific path many might describe as unconventional, we jumped at the chance to explore some of the most important lessons Jenny has learned throughout her career. Among them: there is much we don't know, collaboration is crucial, and - perhaps most importantly - that science changes lives (usually for the better).

# Sam Kounaves

*Professor of Chemistry at Tufts University in Massachusetts, USA, and a visiting Professor in Earth Science and Engineering at Imperial College, London, UK*

## Tell us about yourself and your research!

I'm an analytical chemist by training, and for the first 10 years of my career I was an environmental chemist. Somewhere along the way my group started writing proposals to NASA because we wanted to do analytical chemistry on Mars. People thought we were crazy! They kept telling me I was going to ruin my tenure and things like that. Luckily, I didn't listen, because in 2003 we eventually had one of our proposals funded, and my group performed the first wet chemical analysis of Martian soil with the Phoenix Mars Lander. Currently, we have a mission funded to analyze water, geochemistry, and habitability on the moons of Saturn – Enceladus and Europa.

## What makes you tick?

My drive to explore the universe comes from the fact that when I was growing up in the 1960s (I'm giving away my age here) I was a massive Star Trek fan. To add to this, my Dad was a mining engineer and he was always bringing home funny little instruments that I could tinker away at. So that grounding really set me in this direction. I've always loved science and just exploring new things, but now that desire has matured into a question that I believe is similar for a lot of scientists – are we alone in the universe? The only way to answer that is to find a sign of of past or present life elsewhere in our solar system and beyond, and that's what I want to do. I wake up every day trying to figure out ways I can contribute to that answer.

## What keeps you up at night?

The problems that keep me awake are probably quite similar to those that keep most scientists awake – how do we get funding and how do we keep our labs running? But quite literally, I'm also usually thinking about the next day's research, what people are going to be working on in my lab. At this point in my career, I'm not really looking to establish myself anymore; I'm mostly focused on creating those opportunities for my students and postdocs to allow them to progress in their careers.

## Do you have any personal (or general) milestones from 2020 you'd like to share?

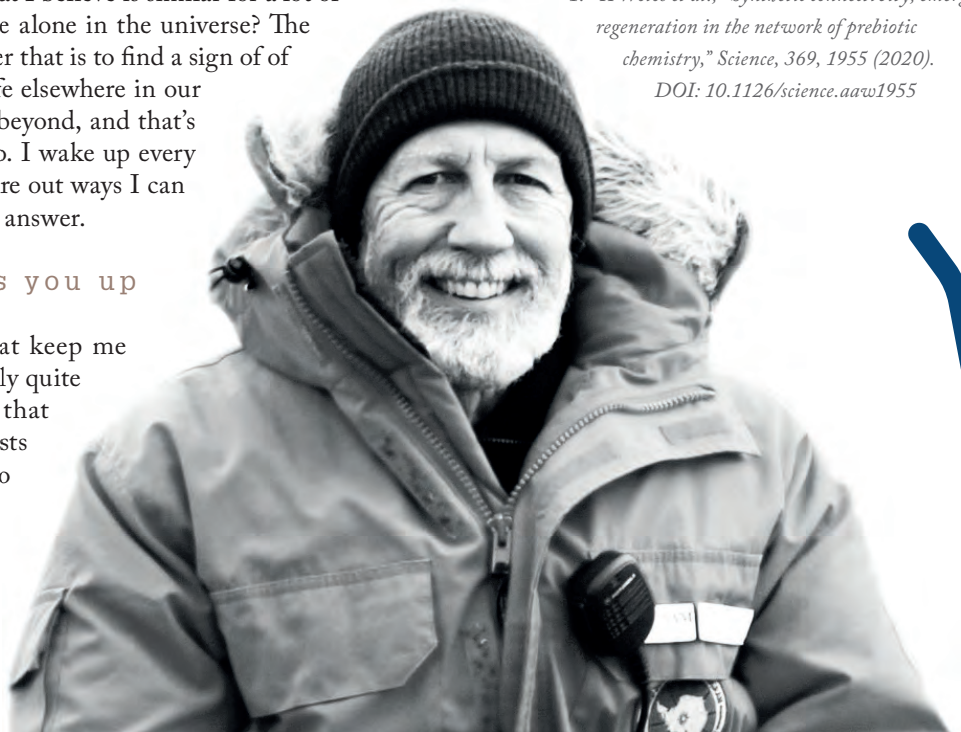
It's been a tough year! March through June our labs were shut down (as for everyone else), and you can only write so many papers without actually doing more research! I was also trying to hire a postdoc this year who would be based both here with me in the US, and at Imperial in London. Unfortunately that didn't happen because of the pandemic. I think the biggest milestone would just be adapting to this new situation, especially in terms of learning how to give on-line lectures and run analytical labs.

## Top paper of 2020

I've saved a couple on my desktop that have been particularly interesting, but if I had to choose just one: "Synthetic connectivity, emergence, and self-regeneration in the network of prebiotic chemistry" (1). They basically modeled and showed the different reactions that could result in biotic synthesis – essentially the emergence of life on Earth – some of which had never been considered before!

*Reference*

1. *A Wolos et al., "Synthetic connectivity, emergence, and self-regeneration in the network of prebiotic chemistry," Science, 369, 1955 (2020). DOI: 10.1126/science.aaw1955*



# Frantisek Svec

*Professor, Faculty of Pharmacy in Hradec Kralove, Charles University, Prague, Czech Republic*

## Tell us about yourself and your research!

Well, I started out in polymer chemistry; I received my PhD from the Institute of Chemical Technology in Prague, Czech Republic. I then worked at the Institute of Macromolecular Chemistry, during which time I began doing some work in chromatography – and I've never looked back! In 1992, I moved to the US and joined Cornell University; in 1997, I joined UC Berkeley and became Director of a Facility in the Molecular Foundry at the Lawrence Berkeley National Laboratory. I also spent a few years as a professor at the Beijing University of Chemical Technology. And since 2017, I have been a member of the faculty here in the Czech Republic in the Faculty of Pharmacy at Charles University. But the thing I'm probably best known for is the development of monolithic materials!

## What makes you tick?

I like to search for new things – and that's why we started working on monolithic columns in the first place. When I came up with the idea, many of my colleagues told me: i) it would never work and ii) even if it did, no one would be interested anyway. But if you never try, you will never know what is possible! It's that drive to make the impossible possible that has kept me going throughout the years. My other motivation is simply to share knowledge – I would never like to stake a claim to a particular area, and I've always shared new results and research on monolithic columns because it simply extends the range of knowledge available in the field. And this philosophy also applies to working with younger people in the field. I find it incredibly interesting to watch them coming up with their own ideas. Some of them might be crazy, but, as I like to say: if even one percent turns out okay then that's a pretty good yield!

## What keeps you up at night?

The worry I have is that the heavy weights in chromatography are getting older – and I'm not seeing the younger generation getting as highly engaged in the field. I'm not sure if it's such a big issue in Europe, but it certainly is in the US. Chemistry is not an area you enter if you want to get rich! Chromatography in general is not particularly attractive in that sense, and it makes me very uneasy to see the numbers dropping off…

## Do you have any personal (or general) milestones from 2020 you'd like to share?

It's always difficult to single out one highlight from any particular year, especially last year! But something I'm still proud of is the fact that my work on monoliths remains relevant. There is no better feeling than knowing that your research is still being put to use in so many different applications. In January, a large review paper was published on column technologies, and a significant part of that concerned monoliths. It's great seeing that the technology is still alive!

## Top paper of 2020

I wouldn't like to pull out a certain paper actually, but something I've been really interested in and watching closely in 2020 is the rapidly booming application of metal organic frameworks in chemistry. They are getting a lot of interest from the separation community for being specifically designed for sample preparation – or even as stationary phases. I also think 3D printing of columns is on the horizon, and I'm excited to see where that technology will go in the future!

## WHERE YOU MAY HAVE SEEN HIM BEFORE

*Reflecting on some of Frank's prior moments of glory with The Analytical Scientist*

**Gurus of the Monolith**

Making an appearance once more, this classic "gurus" feature saw great minds from across the separations field discussing the impact of monolithic columns – a list that would not be complete without Frank. In his words: "I believe the future for monoliths is bright. They are likely to be used extensively in sample preparation, a rapidly developing area. I also expect a renewed interest in TLC using ultrathin monolithic layers."

**Solutions**

*Real analytical problems*
*Collaborative expertise*
*Novel applications*

# GC×GC: The Road Not Taken?

Comprehensive two-dimensional GC has arguably established itself as a superior solution for detailed characterization of complex samples – so why has it not been more widely adopted? And what can we do to help new users access a tool with such great potential?

*By Tadeusz Górecki, Department of Chemistry, University of Waterloo, Ontario, Canada*

Some time ago, I received an email from a company producing renewable hydrocarbon oils. They were looking for a laboratory offering routine GC×GC analysis, so that they could convince potential customers that their oils are suitable for cosmetic and personal care products. They used GC-MS for characterization of the oils, but were looking to GC×GC for a much more complete picture. Unfortunately, I could not refer them to any routine laboratory that offered such services because – to the best of my knowledge – no such laboratories exist.

## The antinomy of analysis

The exchange got me thinking about the paradoxical situation GC×GC finds itself in at the moment. On the one hand, most chemists now realize the true power of the technique in the analysis of complex samples. As the number of GC×GC papers continues to grow, so does the awareness of the benefits of the technique. Literature contains numerous examples of quite spectacular results obtained with GC×GC, which of course fuels further interest. Some industries, especially petroleum (1), realized quite some time ago that they could never get such detailed information on their products (in as little time) using alternative approaches – so they adopted GC×GC for routine use. Unfortunately, this is not widely publicized – perhaps because it is considered a trade secret and could provide a competitive advantage. Still, petroleum industry professionals are well aware of the power of GCxGC and often consider it to be an indispensable tool for product characterization.

I suspect this attitude probably permeates to industries using petroleum products as raw materials (including cosmetics and personal care products), which would explain why I received the unusual request mentioned above. Other areas where GC×GC plays a very important role include metabolomics (2) and environmental analysis, the latter being the first to adopt an official GC×GC-based analytical method (3). Overall, for many analytical scientists, GC×GC is now the go-to technique for dealing with very complex samples.

Which brings me to the other side of the coin: if GC×GC is now recognized as being a very powerful technique, why is it not used routinely by anyone outside of a relatively narrow cluster of research laboratories and industries? Why can't someone outside of this cluster simply send a sample to a commercial laboratory

*"Overall, for many analytical scientists, GC×GC is now the go-to technique for dealing with very complex samples."*

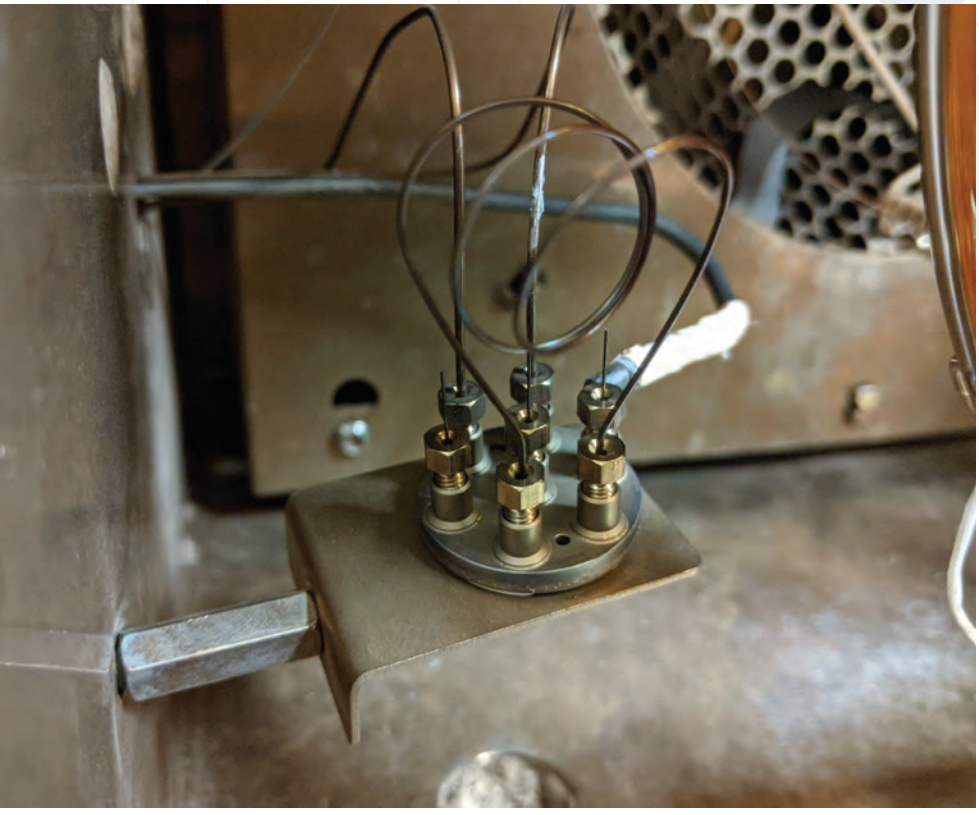


Figure 1. Examples of commercially available modulators

to have it characterized by GC×GC? As practitioners of the technique, we should be asking ourselves why there is so clearly a disconnect between the great promise of the technique and its wider adoption. Having thought about this question quite extensively, I would like to share some of my own conclusions, with the explicit understanding that these may not be shared by all.

## Intricate beginnings

To me, the elephant in the room is the perceived (or real) complexity of the technique. GC is relatively easy to explain to students, and people who use it typically have a reasonably good grasp of the technique. Generally speaking, GC is easy to learn. You install a typical 30 m x 0.25 mm ID x 0.25 µm df column in a GC oven, inject a sample at a relatively low temperature, then program the oven to heat up to a certain final temperature that should be high enough to allow all sample components to elute from the column, but not so high that the stationary phase would be destroyed. If some components fail to separate, one can play with the temperature program and/or carrier gas flow rate. If that does not help, you can try a longer column or switch to a different column – and that's about it.

*"To me, the elephant in the room is the perceived (or real) complexity of the technique."*

Contrast that with GC×GC, where one has to choose not one, but two columns and decide on their geometries. Should I use a 30 m or 60 m column in the first dimension (1D)? Should it be polar or nonpolar? What column length should I use in the second dimension (2D)? What should be its internal diameter? The stationary phase? Most users look for generic methods as the starting point to method development. A typical configuration these days would include a nonpolar column in 1D, and a semipolar or polar one in 2D, but that

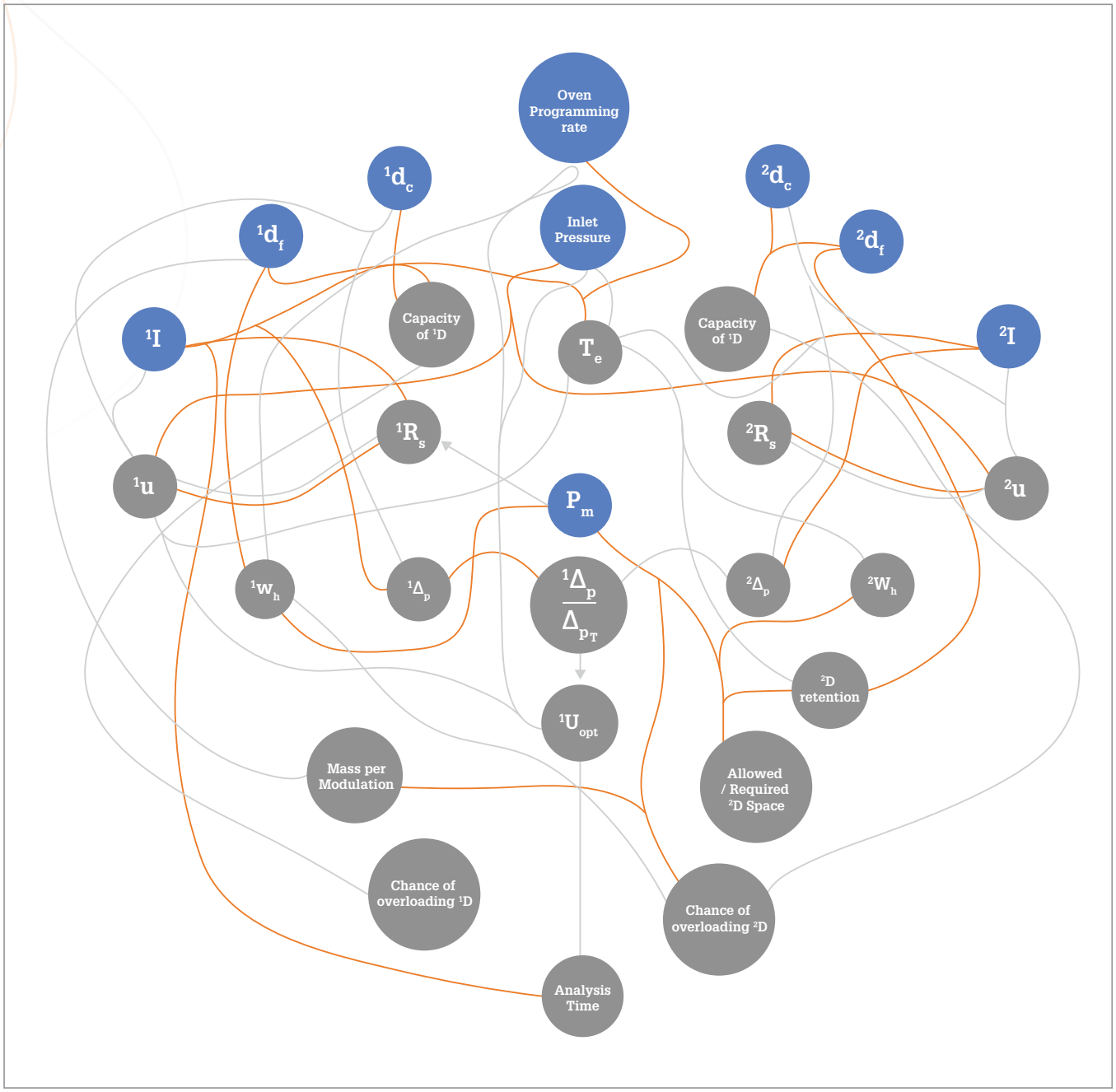
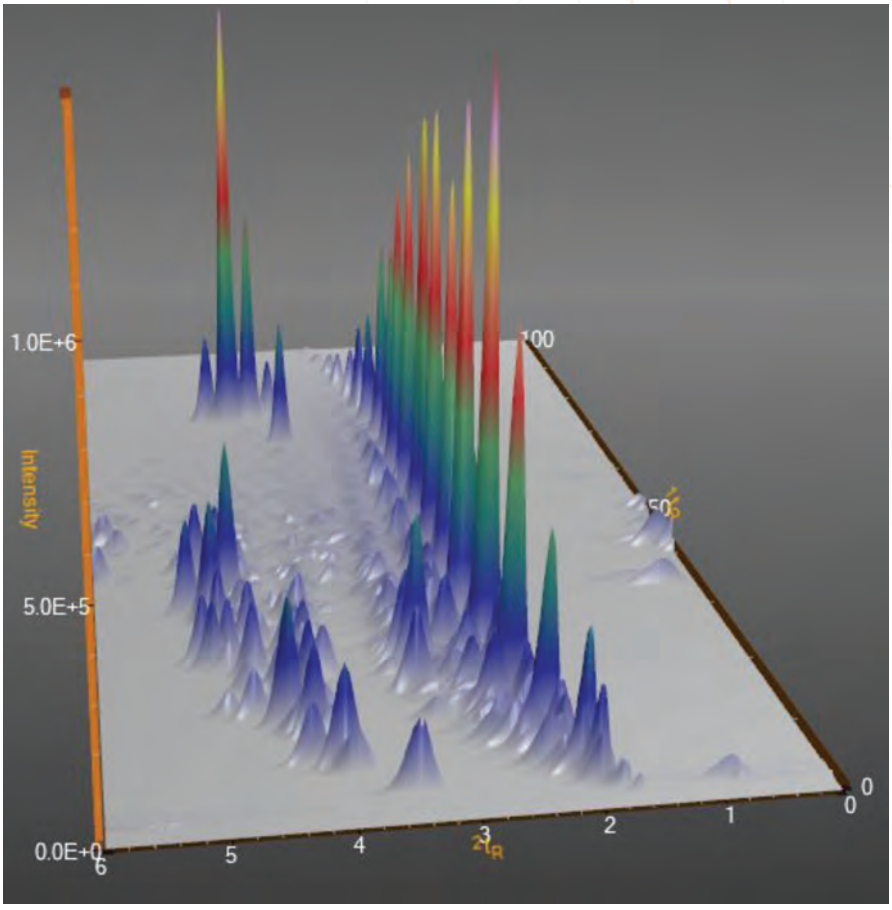



Figure 2. Interplay of parameters in GC×GC separations. Individual parameters indicated by ovals, with instrumental parameters that are directly controllable by the chromatographer shown in blue. Orange arrows point to parameters whose values increase as the input parameter value increases; gray arrows indicate opposite influences (the value of the parameter decreases as the value of the input parameter increases). The relationships shown in the diagram are valid when only one instrumental parameter is changed at a time, with the rest held constant. Symbols: 1dc : 1D column diameter, 2dc : 2D column diameter, 1df :1D column film thickness, 2df : 2D column film thickness, 1l :1D column length, 2l :2D column length, Te: elution temperature, 1u: 1D column linear velocity, 2u : 2D column linear velocity, 1wh: 1D peak width, 2wh: 2D peak width, 1Δp: pressure drop in the 1D, 2Δp: pressure drop in the 2D, 1Δp/ΔpT: pressure drop in the 1D compared to the total pressure drop, 1RS : 1D resolution, 2RS : 2D resolution, 1uopt: 1D column optimal linear velocity. (Reprinted from ref. 6 with permission).



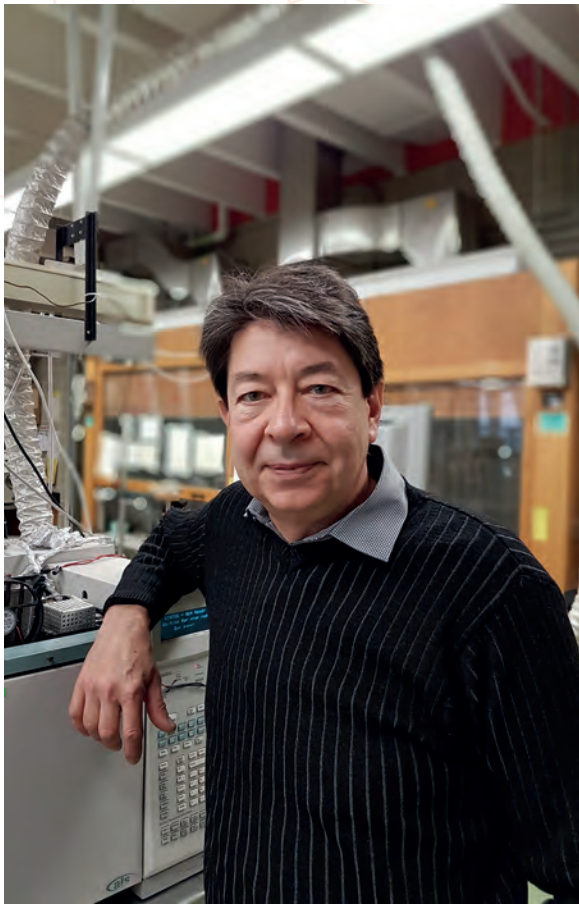



Figure 3: A 3D visualization of a GC×GC chromatogram of a diesel sample

is not always the best combination. One then needs to choose the modulator. In the early days, the choices were rather limited, as cryogenic modulators were the only ones commercially available. The picture is definitely more complicated these days (4). Though cryogenic modulators still dominate, they now face serious competition from thermal and (in particular) various flow modulation platforms (see Figure 1 for selected examples). Each modulator type has its strengths and weaknesses, but none can claim that it is definitely the best for all applications.

Once the modulator is chosen, the user faces method development. Right from the beginning, it's more complicated than in standard GC. In addition to the parameters that are normally adjusted in a 1D GC method, the user has to decide on the length of the modulation period and the secondary oven temperature offset (if available). Further to this, certain parameters specific to a given modulator platform must be selected. If this were the end of the story, an average GC user would be able to learn how to develop a GC×GC method quite quickly. However, things get more complicated still because of the mutual dependence of many parameters in GC×GC – as we tried to illustrate with the now quite (in) famous spaghetti diagram (see Figure 2) published originally in 2007 (5) and then reprinted in an improved version in 2012 (6). For example, changing the oven temperature ramp changes the elution temperature of an analyte, which in turn affects its retention in 2D. It may also change the 1D peak width, which will affect the number of times a given 1D peak is sampled, and that in turn affects peak resolution and method sensitivity. For an inexperienced user, this may quickly lead to confusion – and lots of hand-wringing!

## The chromatogram and software

Suppose, though, that our hypothetical new user successfully navigates the GC×GC method development waters and is now prepared to get down to some real analyses. Unfortunately, there are

*"To complicate life even further, different software packages deal with the integration differently."*

further obstacles awaiting them! Indeed, acquiring the data is just the first step – getting the required information from the raw data is a whole new ball game.

In 1D GC, the chromatogram is a simple plot of signal intensity as a function of time. Peaks have (more or less) Gaussian shapes, and the peak area is proportional to the amount of the analyte. Problems mostly arise due to incomplete peak resolution and the uncertainties related to determining the start and stop of a peak, especially when it is asymmetrical. Over the many years of development, manufacturers of GC instrumentation managed to develop pretty robust tools that can adequately handle most situations, and manual intervention is rarely required (and generally avoided). The procedures are more or less standardized by now, hence users do not have to worry that a different software package will handle their data differently.

Unfortunately, this is not yet the case with GC×GC. A raw GC×GC chromatogram does not differ from the one obtained in 1D GC – it is also a plot of signal intensity as a function of time. The 2D structure of the data has to be brought out through data processing. The basics are quite simple and are the same for all software packages – the raw chromatogram is "cut" into segments corresponding to the individual modulation periods, which are then placed side-by-side to produce a 2D map on which signal intensity is coded with different colors, or a three-dimensional representation that can be easily manipulated on the computer screen (and which produces the pretty pictures so popular in GC×GC publications – see Figure 3).

What happens next, though, very much depends on the software package being used. When quantitation is required, the areas of peaks of a given analyte in the individual modulation periods need to be added to yield the total area of the peak (which should be the same, in principle, as the corresponding 1D peak area). However, this requires that the peaks belonging to a given analyte in the consecutive modulation periods ("slices") be properly identified. With non-selective detectors, the identification can only be based on 2D retention times.

However, this task is not straightforward, as every consecutive 2D chromatogram is developed at progressively higher temperature in a temperature-programmed run, hence the 2D retention times become shorter in each consecutive "slice." If a peak is well resolved, symmetrical, and sampled the recommended two-to-three times, this is typically not an issue and proper reconstruction of the total peak area is quite straightforward. However, tailing peaks are much more difficult to deal with. The progressively shorter 2D retention times produce the characteristic banana-shaped profiles, and most software packages have problems with accurate assignment of all the component slices in which the tail of a given peak is still present. The job is a little easier when MS is used for detection, but the noise that inevitably creeps into the spectra when the tail becomes small can throw these software packages off as well.

To complicate life even further, different software packages deal with the integration differently. Some stick to integration of the component peak areas in the raw signal and simple summation of them; others might resort to summing of peak heights to reduce the computational

*"There is certainly room for budding entrepreneurs to open dedicated GC×GC laboratories that could handle odd requests."*

requirements. Yet another solution is to try to "reconstruct" the shape of the peak in three dimensions and assign a volume to it rather than an area. All of these approaches have their benefits and shortcomings, but the net result is that the same raw data processed with different software packages might not produce identical results, as recently described by Weggler and colleagues (7).

### It's not all bad…

If you have reached this point of my deliberations, you may wonder whether my purpose is to discourage people from using GC×GC. Let me assure you that this is not the case – in fact the opposite is true. What I am trying to do is figure out the best ways to help new and inexperienced users navigate choppy GC×GC waters, and to finally bring GCxGC where it belongs – into the mainstream!

First and foremost, I believe education is the foundation of future success. GC×GC courses offered at different conferences can be a great introduction to the technique, but due to their (necessarily) short duration, future users will not learn how to navigate GC×GC waters effortlessly. Some instrument manufacturers offer great training courses, but they are limited to their customers only. I would love to see manufacturers and leading experts in the field joining forces to organize in-depth training courses, including hands-on experience, that are open to everybody. I am not sure of the best way to accomplish this, but as they say, "where there's a will, there's a way."

For industry to widely adopt the technique, we need robust, reliable, and fully validated turn-key solutions. As much as my researcher's gut hates the "black box" concept, I believe this is exactly what the industry needs. Some manufacturers are trying to do it already, and that is very encouraging.

Conquering the commercial laboratories is a much taller order, because official methods are desperately needed. No commercial laboratory will invest a single penny into a technology that produces results that will not be accepted by regulatory agencies or are not defensible in the court of law. The task of changing this falls on the shoulders of both the manufacturers and the users of the technique. If you have developed a fantastic method that solves a real problem, maybe you should think about taking it a step further and turning it into an official method. It may be lot of work, but it would be a great service to the GC×GC community – and, ultimately, society as a whole.

Finally, there is certainly room for budding entrepreneurs to open dedicated GC×GC laboratories that could handle odd requests (like the one that inspired this feature), routine analyses, and consulting services. With a little marketing, I am quite confident they would not sit idle.

I have to emphasize once again that these are my personal opinions; the true path to bringing GC×GC into the mainstream might be completely different. If you have different ideas, I would love to hear them. Maybe they will form the basis for a follow-up article!

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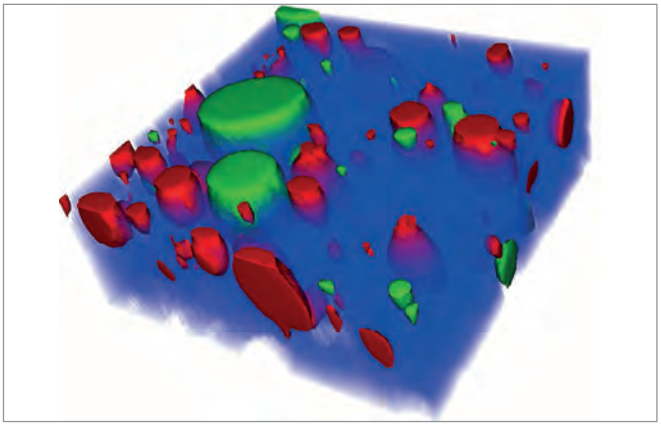
# Experience a new Level of 3D Raman Imaging

Confocal Raman microscopy is a powerful tool for analyzing the chemical composition of samples. The Raman effect is based on inelastic scattering of excitation light by the molecules of gaseous, liquid or solid materials. The interaction of a molecule with photons causes vibrations of its chemical bonds, leading to specific energy shifts in the scattered light that can be identified in its Raman spectrum. Any given chemical compound produces a particular Raman spectrum when excited and can be easily identified by this individual 'fingerprint'. When Raman spectra are collected at each measurement point using a confocal microscope combined with a spectrometer, a Raman image can be generated that visualizes the distribution of the sample's compounds. Due to the high confocality of WITec Raman systems, volume scans and 3D images can also be generated from 2D images from different focal planes. A new application note presents



a collection of best practice examples from various fields of research, such as pharmaceutics, food science, semiconducting materials and geology.

*Read the full application note here:*
*https://www.witec.de/assets/Literature/Files/WITec-AppNote-3DRamanImaging-WebVersion.pdf*

# Using the Empore™ EZ-Trace SPE Workstation for Efficient, Reproducible Extractions of SVOCs

## Extraction of SVOCs from water samples with the Empore™ SDB-XC disk by standard EPA method 525.3

*Xiaohui Zhang, Michael Apsokardu, Guotao Lu*

This application note demonstrates how the Empore™ EZ-Trace SPE System is used to perform multiple extractions simultaneously while achieving clean and reproducible results using EPA Method 525.3 as an example application.

The Empore™ EZ-Trace (Figure 1) is a manual, vacuum-controlled extraction workstation designed to perform up to 4 extractions simultaneously to improve efficiency in a clean and reproducible manner. The Empore EZ-Trace workstation is universally compatible with all Empore 47 / 90mm disks and cartridges. The unique independent channel design ensures that each extraction is precisely controlled, preventing cross-contamination with the other channels. High-throughput flow path switches provide a unique, environmentally friendly extraction by collecting organic and aqueous wastes in separate reservoirs. This is demonstrated here using an example application of EPA Method 525.3, which is designed for identification and quantification of SVOCs such as PCBs, PAHs, and pesticides. Under this method, SVOCs are extracted from three reagent water samples having different concentrations and one sample of ground water, with 4 replicate measurements required for each sample. Methods such as EPA 525.3 require ways to improve the efficiency when numerous extractions are needed.


Figure 1: Empore EZ-Trace extraction workstation for multiple extractions using Empore disks and cartridges.

Four extractions were done simultaneously using the Empore™ EZ-Trace system (catalog #: 8000) by following EPA 525.3 extraction procedures to extract SVOCs onto an Empore™ SDB-XC 47mm disk (catalog #: 2240). The extract was then evaporated, diluted, and then analyzed with Shimadzu GC-2010 using a Restek Rxi-5Sil-MS (30m x 0.25mm x 0.25µm) column for separation and MS QP2010 for detection. In this method, 125 analytical calibration standards, 3 surrogate compounds, and 4 internal standards are efficiently separated under this method (Figure 2). The recoveries and concentrations of 125 analytes were determined from four replicate measurements using a five-point calibration curve. For all compounds except for 2, the acceptable range of recoveries is 70-130%. The accepted range for the other 2 compounds is 60-130%. The combined recovery results are summarized in Table I. For each sample, the average recovery is > 89.9% with < 7.4% RSD. These results demonstrate how the Empore™ EZ-Trace provides a clean, efficient, and reproducible extraction.

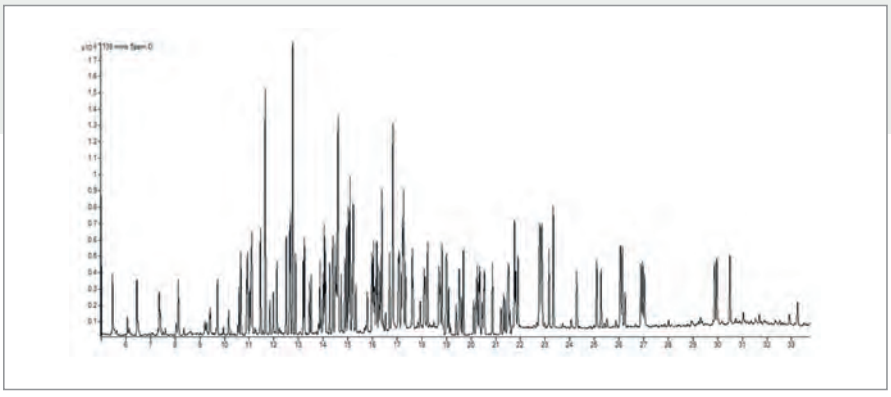


Figure 2: Chromatogram showing the separation of 125 analytical, 3 surrogates, and 4 internal standards compounds extracted from a reagent grade water sample under EPA Method 525.3.

| Water | Reagent Grade | | | Ground |
|---|---|---|---|---|
| µg $L^{-1}$ | 0.2 | 2.0 | 5.0 | 2.0 |
| ≥ 70% | 118 | 123 | 123 | 123 |
| avg Rec. (%RSD) | 89.9 (7.4) | 90.9 (5.8) | 94.4 (3.4) | 93.4 (4.5) |

Table I: Recovery data for 3 samples of reagent grade water with different concentrations of standard compounds and one sample of ground water. ≥ 70% refers to the number of compounds having a recovery greater than 70%. The average recovery of those compounds is shown below that along with the %RSD. Each sample was done in 4 replicate measurements.

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*465 Limestone Rd, Oxford, PA 19363*
*tel. (800) 541-6593*
*Contact: Michael Apsokardu*
*Phone Number: 610-998-4072*

# Determination of true molar masses of collagen peptides

Collagen peptides are relatively small proteins with typical molar masses of less than 10.000 g/mol. They exhibit a molar mass distribution, MMD, which influences their properties.

A monograph by GME describes a procedure how to determine true MMDs for collagen peptides using standard Liquid Chromatography, LC, equipment (1). The LC setup comprises an isocratic pump, an injection system, GPC/SEC columns and an UV/VIS detector.

Column calibration is a two-step procedure. A base calibration is established using one CNBr-Peptides sample. Applying broad standard calibration using 5 different broadly distributed collagen peptide reference materials allows transforming the calibration curve into a chemically matching collagen peptide calibration curve to obtain true molar masses (2).

PSS WinGPC UniChrom software offers a convenient wizard for broad calibration, which guides the users through each step of the calibration and automatically adjusts the base calibration curve such that best agreement with target values is obtained. The resulting calibration curve allows for the determination of true molar masses of collagen peptides.

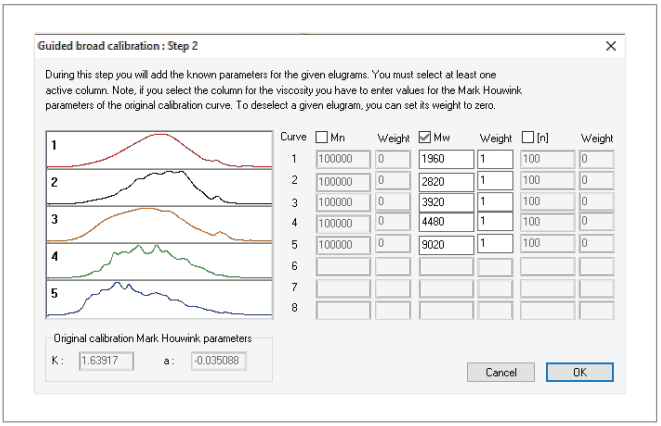


Figure 1: Step 2 of the guided setup processing the overlay of chromatograms of the five collagen peptide reference samples with assigned weight average molar masses

*Read the full application note here: https://bit.ly/3ca56pY*

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# *Spotlight on...* Technology

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LECO's new Multi-Mode Source™, in the Pegasus GC-HRT+ and HRT+ 4D, combines the power of EI, PCI, and NCI/ECNI modes in one source. One source providing three modes gives the benefits of better peak shapes and alignment between modes and higher selectivity without needing to change any of your hardware.
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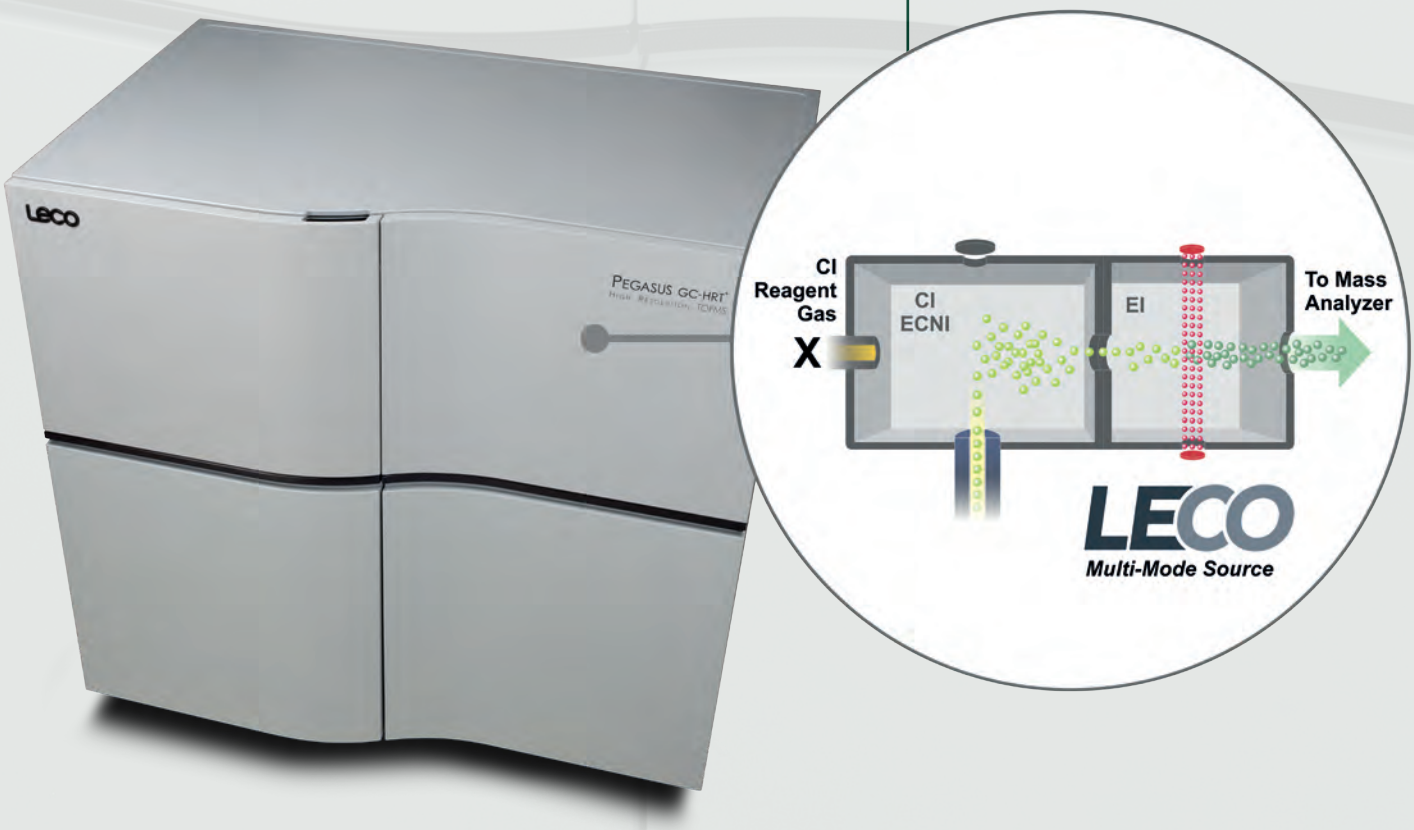


## Award-winning new PTR3-TOF 10K Mass Spectrometer – Trace VOC Analyzer

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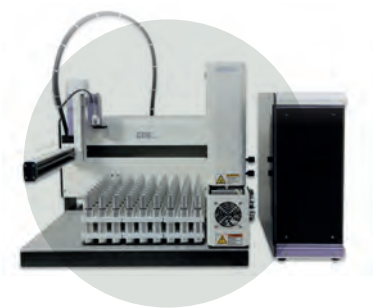
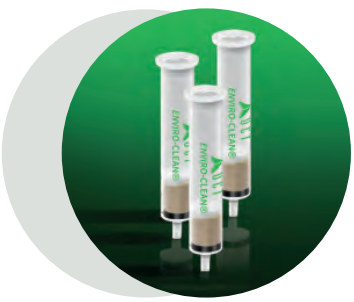
*https://www.ionicon.com/blog/2020/the-ptr3-scores-prestigious-innovation-award*



## CDS 7550S Thermal Desorption Autosampler

The CDS 7550S is a stand-alone Peltier and Pre-heat dual mode focusing 72 position thermal desorption autosampler. Equipped with the 2nd generation autosampler platform. The system is capable of offering a sample split up to 10,000:1, thermal slicing and automated tube conditioning.
*https://www.cdsanalytical.com/thermal-desorption-7550s*



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# Expanding the Anti-Cancer Arsenal

**Sitting Down With… Amanda Hummon,** Associate Professor, The Ohio State University, Columbus, Ohio

Why analytical chemistry?

The moment that my high school chemistry teacher introduced the periodic table was a turning point for me. I'm a compulsive organizer, and the periodic table is the most exquisite form of organization in existence. The notion that you can organize everything in the universe through this single system is incredible, and coming to this realization was a real eureka moment.

As an undergrad, I worked in synthetic chemistry, but I (embarrassingly) booked incorrect plane tickets when visiting grad schools. I was supposed to visit the University of Illinois for a day and half, but ended up there for an additional day. I used that extra day to meet additional faculty and students, including a bunch of students from my soon-to-be-advisor's group. They were so excited about their work (they were studying neuropeptides in slug brains, which sounds random, but we share many of these with our slimy friends) – and that made me excited, too!

And what inspired your focus on cancer?

It's a focus that evolved over time. Back in grad school, I was involved mostly in method development, but I soon realized my passion for work related to human health – particularly cancer. So I started working at the National Cancer Center to study the mechanisms behind cancer evolution after obtaining my PhD.

I – like many – have lost an immediate family member to colon cancer. One of my coping mechanisms was understanding the underlying science. Plus, in 2004, nobody was applying MS in cancer research when I finished my graduate studies; there was a lot of genomic and transcriptomic research, but little focus on proteins. I thought I could fill that void!

And how do you apply MS?

We develop many MS-based methods with cell lines, but we also work with organoids grown from patient biopsies. These organoids allow us to probe how and why specific patients might respond to specific drugs, allowing us to adapt treatment pathways in line with individual needs. The drive is towards more personalized medicine.

Omics is a powerful tool for looking at cancer holistically. The ability to monitor the identity of and changes across thousands of molecules in an untargeted fashion conveys clear advantages in this complex disease, and MS has become the core way in which we conduct these studies. When I started in analytical chemistry, MS was just a small part of the field. Now, around half of all analytical chemistry relies on the technique.

You must work with a talented team…

I certainly do! Half of my group comes from an analytical chemistry background, and the other half are more motivated from the oncology side – often due to personal experiences. Whatever their motivation, all of my students become well versed in both MS and the biology we are applying it to. Witnessing this growth is one of my favorite parts of the job. By their fourth or fifth year, my students transition into fully fledged scientists with their own ideas and directions for research. It's a transition I've been fortunate to witness many times, having overseen the graduation of over 10 PhD students.

My lab usually hosts around 12 to 15 students at a time (a very nice number, I'd say), and – when the world isn't in pandemic mode – we spend a lot of time together outside the lab. That is, when I'm not busy making stained glass windows or reading. The former has been a long-time hobby that I treated myself to starting after passing my candidacy exam as a grad student.

What breakthroughs are you particularly proud of?

Our work on imaging MS to characterize new drugs is a great example. It took us 10 years to perfect, but it is now used by our partners at Ohio State University to test the anti-cancer drugs they are producing in terms of the distribution and metabolism of new drugs in tumors and tumor cell culture models.

A few years ago, one of my former students also demonstrated that eating fewer calories increases the efficacy of some chemotherapy drugs (such as topoisomerase inhibitors) against cancers. Our next steps for this project will be to move these studies into mouse models. That, like many areas of research, remains on hold for the time being!

And what do you foresee for the future of the field (and your lab)?

There's more of a focus on the front end of analysis right now, especially better separations using approaches like ion mobility, which allow us to extract more information from samples. Looking forward, stronger tools for data analysis will be essential. Omics studies produce huge volumes of data and we must work to handle these data in a standardized way.

I also foresee growth in imaging MS, particularly at single-cell resolution. As things stand, sensitivity represents a significant hurdle for these single-cell techniques, but I'm sure they will become more widespread as time goes on.

As for our lab, we're currently working with clinicians at Ohio State University to develop banks of organoids to study patients who initially respond to immunotherapy but develop resistance over time. The hope: to improve treatment for such patients.

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