September / October 2024
#119

the Analytical Scientist®

The mRNA Big Bang

The success of the COVID-19 mRNA vaccines has supercharged the field of nucleotide-based therapies. How will analytical scientists rise to the challenge?

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The Language of Innovation

Entering a new field is like hearing an unfamiliar language: "You hear the melody and the rhythm without understanding the words"

I've had several conversations about innovation recently, and I've noticed a theme emerging. Chris Enke co-invented the triple quadrupole mass spectrometer in the 1970s – but he wasn't a mass spectrometrist. Similarly, Lloyd Smith, who developed the first automated DNA sequencer used in the Human Genome Project, had little sequencing experience. Another example: Fran Ligler, engineer, biosensor pioneer, and National Inventors Hall of Fame Awardee, has a policy where every five years she tries to "figure out what's the most exciting thing going on in science or engineering and put a foot in that door."

Do these examples simply demonstrate that brilliant people do brilliant things wherever they turn their talents? Perhaps. But Gary Patti thinks there's real scientific benefit to exploring new terrain – he likens it to hearing an unfamiliar language. He says (page 27): "When you enter a new field for the first time, your perspective is completely different to someone who can already 'speak the language.' You hear the melody and the rhythm without understanding the words."

There are plenty of areas ripe for new thinking in analytical science. For example, this month's cover feature explores the characterization challenges in the flourishing field of nucleic acid-based therapies, where "new methods are required," as Thermo's Ken Cook puts it (page 13).

Any takers?

Perhaps we all need to consider stepping outside our comfort zone and turning our ears to the melody of the unfamiliar.

James Strachan,
Editor

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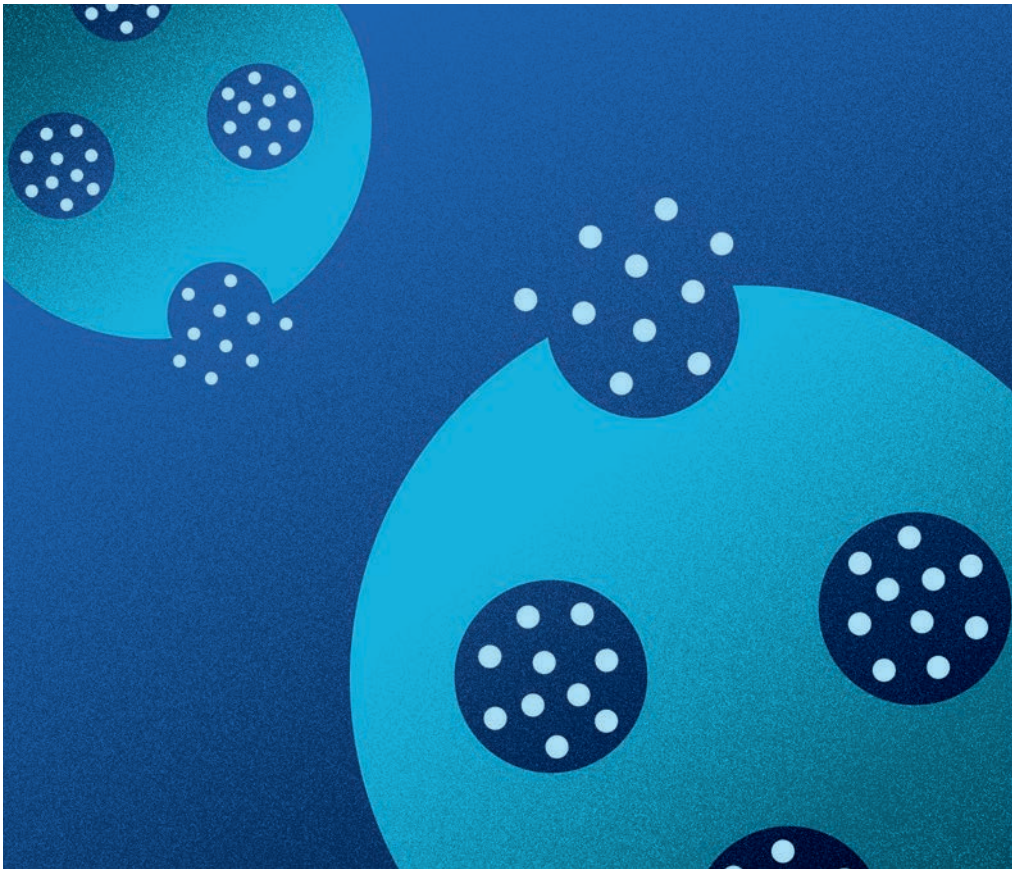
Electrokinetic Sensor Rapidly Diagnoses Brain Cancer

A new biochip diagnoses glioblastoma in under an hour using electrokinetic technology to detect EGFR biomarkers



A new device can diagnose glioblastoma in under an hour using electrokinetic technology to detect active cancer biomarkers on extracellular vesicles (1). Developed by researchers at the University of Notre Dame, USA, the platform uses a biochip with an anion exchange membrane and silica nanoparticle reporters to measure active epidermal growth factor receptors (EGFRs). The biomarkers are overexpressed in glioblastoma and other cancers, and they can be found on extracellular vesicles secreted by cancer cells.

The biochip requires just 100 microliters of blood, costs under $2 in materials, and produces results in less than an hour.

Testing showed the device could detect as few as 30 extracellular vesicles per microliter. Blood samples from glioblastoma patients and healthy individuals were analyzed, revealing a clear distinction in active EGFR vesicle levels.

The device's charge-sensing approach detects voltage shifts when highly charged silica nanoparticles bind to extracellular vesicles on the membrane, allowing it to accurately quantify active EGFRs.

A big challenge for the researchers was to overcome the long incubation time for the pull down of the extracellular vesicles. "With a diffusivity that is 100x to 1000x lower than most molecules, our pull down time required days initially – which right away rules it out as a commercializable diagnostic test," says Hsueh-Chia Chang, Bayer Professor of Chemical and Biomolecular Engineering at Notre Dame – and lead author of the study. "The eureka moment was when we realized that the multivalent capture increases the binding rate so much that the pull down is diffusion controlled. Diffusion controlled pull down is unique in that it will only deplete the analytes within a hemisphere above the sensor with the same radius as the sensor. Thus, we decreased the sensor radius from the usual cm in ELISA assays to hundreds of microns to reduce the assay time roughly 400 fold – from days to 20 minutes."

The researchers are working on improving the device to extend to other cancers and diseases. "The main drawback with the current design is that we need a micropump to drive a high-speed flow to remove non-specifically bound silica-nanoparticles – a controlled wash," says Chang. "We are working on an electric field-based washing technology. If successful, the entire device would be a thumb-drive like chip – an electrochemical lateral flow assay that can be plugged into a laptop."

To extend to other cancers or diseases, the team will need to detect multiple biomarkers on the extracellular vesicles – which translates to scaling up to multiple sensors on their chip. "We are currently working on this multiplex technology and will also develop its manufacturing strategy for large-volume production," says Chang. "Once we are done, it should be a pan-disease platform for all diseases whose biomarkers are enriched on extracellular vesicles."

"We hope our platform will be the first pan-disease extracellular vesicle diagnostic platform that realizes this vision to fundamentally transform healthcare."

Reference

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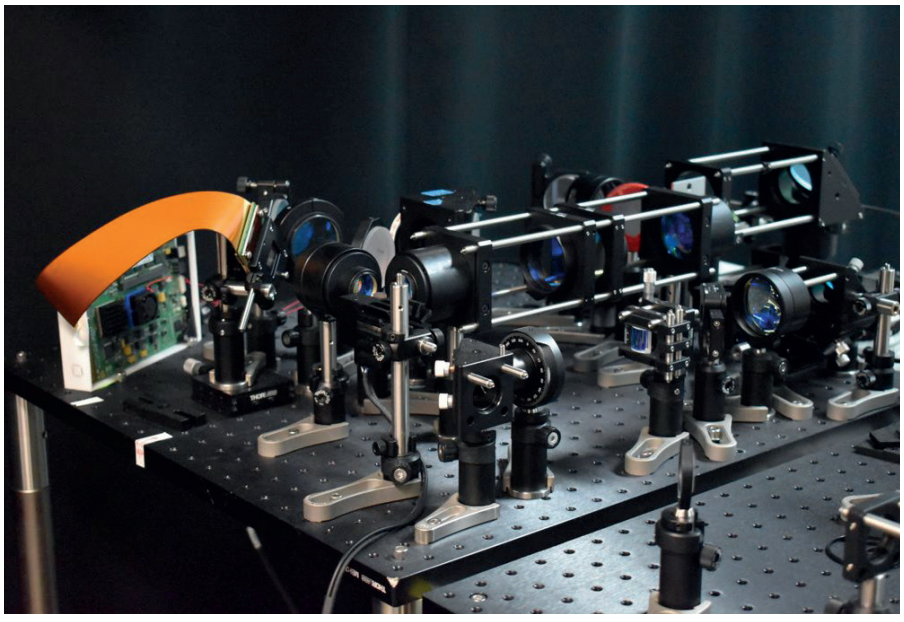

Real-Time Window into the Brain

A new two-photon fluorescence microscope developed by researchers at the University of California, Davis, allows for high-speed imaging at cellular resolution, while minimizing potential damage to brain tissue. Key to this innovation is the use of a digital micromirror device (DMD), which dynamically shapes and directs the laser beam based on the identified regions of interest. This setup allows the system to capture large areas of active neurons in a single pass, significantly increasing the imaging speed. The combination of high-speed imaging and reduced laser power not only protects the brain tissue but also enables the system to focus on the most relevant neuronal activity. The UC Davis team's work holds promise for a wide range of applications, from studying basic neural processes, such as learning and memory, to investigating the early stages of neurological diseases like Alzheimer's and Parkinson's.

Credit: Molly M Bechtel, University of California, Davis

QUOTE *of the month*

"This question about 'the role of analytical science' always surprises me when I see it. There is not a lot of science which can be done without analytical measurement. Everything has to be ultimately measured in some way, whether it's simply for the sake of quantification or whether it's for characterization."

Varun Gadkari (page 17)

Ancient Bubblegum

Our Mesolithic ancestors could have suffered from periodontitis due to frequent chewing of "gum" (red fox fur), suggests metagenomics analysis

Credit: Created using Adobe Stock images

Our Mesolithic ancestors used to chew on pitch materials not only to create an adhesive glue for their tools – but also for fun when they were bored. However, this habit might have been detrimental to their oral health, according to a study that investigated oral microbiome compositions from 10,000 years ago (1).

These chewed pitch materials contain ancient DNA (aDNA) from human cells and microbes found in the mouth of our Mesolithic ancestors – making them an ideal target for genetic and genomic analysis. The catch? "aDNA is a degraded material. It often breaks down into small fragments and contains nucleotide lesions," explains Emrah Kırdök, Assistant Professor at Mersin University, Department of Biotechnology, and corresponding author.

By extracting, then aligning this DNA with reference genomes and adapting analysis parameters, the team found a higher abundance of periodontitis-associated microbes and even red fox DNA.

"Our study suggests that the frequent pitch chewing could increase the risk of collecting periodontitis like microbes, thus increasing the risk of having oral disease," says Kırdök.

Reference

1. *E Kırdök et al., Sci Rep, 14, 22125 (2024). DOI: doi.org/10.1038/s41598-023-48762-6.*

Credit: Arian Khoshchin

Work Hard, Network Harder

Tips and tricks for a happy (and healthy) PhD journey

By Isabelle Kohler, Assistant Professor at the Division of Bioanalytical Chemistry at the Vrije Universiteit Amsterdam, The Netherlands; and Founder of NextMinds

PhD candidates often don't know what it really means to "do a PhD." A common feature of young scientists interested in a PhD is a strong interest for research – but research is only one of the many aspects of a doctorate. Often, they aren't fully aware of all the tasks that compose a PhD trajectory and can feel overwhelmed when tasks start to pile up – including those they weren't expecting. That's not surprising to me, as there's only little information given to students about the PhD trajectory.

In fact, hard work is not directly correlated to success and output in a PhD – and that is a hard pill to swallow. A PhD trajectory is hugely influenced by other factors, which you often cannot fully control. This could be supervisors (for example, how supportive they are, how busy they are, their network and mentoring skills, and so on), the infrastructure (for example, access to state-of-the-art instrumentation, troubleshooting, training opportunities, and so on), and collaborators (especially if the PhD project is part of a bigger consortium, where PhD students depend on each other's results).

Doing a PhD or any type of study/research in a field like analytical science can be a rollercoaster, with a lot of ups and downs. Having colleagues and friends who can be present and supportive during these moments will make a huge difference. I spent years troubleshooting my instrument during my PhD, wondering why I couldn't get the results I wanted to have. That was frustrating and very demotivating. However, I could always count on the support of my three PhD office mates – we cried and laughed a lot together. They were a crucial aspect in my PhD trajectory, and we're still friends more than 10 years later.

When I was a PhD student, I didn't truly realize that I was only at the beginning of my career, and that the entire journey would be much more exciting than the end destination. I thought that I had to have my career set in stone in the first years afterwards – I couldn't have been more wrong! When I discuss the future with our Masters and PhD students, I feel their stress in finding the perfect first job – the one that "ticks all the boxes." As Mark Nepo said: "There are no wrong turns, only unexpected paths."

Ultimately, the best advice I can give is to talk to people. Talk to your colleagues and peers, your supervisor(s), teachers, and other possible mentors to seek guidance and support when going through challenging periods or having questions about their future. Also, talk to other scientists during symposiums or conferences to broaden your professional horizons. Be careful to actually listen to their stories to get insights and inspiration! Sharing experiences, fears, ambitions, and ideas among peers and mentors brings not only possible solutions or answers to questions, but also the feeling of belonging to a community. That's why I really believe in the power of networking.

At the beginning of my career, I was also very intimidated as an introvert having to network in a foreign language that I didn't master. However, I quickly understood that what people called "networking" simply meant partake in discussion with another human and express genuine interest in them.

To overcome networking challenges, I usually give the following advice: first, consider networking as a conversation between two (or more) humans. It's not different from this! Next, if you're intimidated by approaching a big "superstar" during a conference, start with approaching other young scientists during poster sessions or workshops. Simply introduce yourself and ask whether they can tell you more about their work. The first few minutes may feel awkward, but once the conversation has started, you'll feel yourself start to relax. Also, consider contacting people through LinkedIn, and even invite them to have a coffee and small chat with you the next time you are at the same event. And finally, be as authentic as possible. There's no need to play a role; just be yourself! I promise that you'll meet people from all ages and all cultures – people who will enrich your life beyond the professional aspects.

EME Turns 20

Recently commercialized, amenable to operation in multi-well plates and microfluidic devices, and fundamentally green – has electromembrane extraction (EME) reached an inflection point?

By Stig Pedersen-Bjergaard, Professor, University of Oslo, Norway

In the late 1990s, my former colleague Knut Rasmussen and I were working on hollow-fiber liquid-phase microextraction (HF-LPME). The idea was to develop a robust formate for miniaturized liquid-liquid extraction, where the analytes of interest were extracted from aqueous sample, through an oil membrane and into an acceptor. The acceptor was either an organic solvent or an aqueous buffer. We mainly worked with pharmaceuticals, and these were extracted into aqueous buffer based on a pH gradient. For basic pharmaceuticals, the sample was made alkaline, while the acceptor was acidic. The basic analytes were extracted as neutral species across the liquid membrane, and into acidic acceptor. Here the molecules were trapped due to protonation. HF-LPME provided excellent cleanup and enrichment, acceptors were injected directly into LC-MS after extraction (no evaporation and reconstitution), and the consumption of organic solvent was limited to a few microliters per sample. However, a lengthy (30-45 minutes) extraction was required to reach equilibrium.

In the autumn 2004, we began discussing the prospect of controlling and accelerating the extraction by applying an electrical field across the liquid membrane, provided that the analyte was charged both in the sample and acceptor. In our old introductory organic chemistry courses, we were told that organic substances with charge have no partition across water-oil interfaces. But what happens, if a strong electrical field is applied across the water-oil interface? As curious experimentalists, we tested different oil membranes. Initially, we weren't successful. But, when we tested 2-nitrophenyl octyl ether (NPOE) – one of the solvents available in our laboratory – as an oil membrane, we surprisingly obtained very fast extraction.

After a couple of more days of successful experiments, including extraction from human plasma and urine samples, we understood a new extraction technique was born. We then checked the literature for similar extractions, but we were unable to find any. We then contacted the technology transfer office at the University of Oslo, and they decided to file a patent application. This was followed up by our first article on EME published in 2006. We initially termed the technique "electromembrane isolation" but this was later replaced by "electromembrane extraction" (EME).

On November 4 this year, we can celebrate that EME is 20 years old. Over the past two decades, more than 500 papers have been published on EME – spanning applications, fundamental understanding, and technical formats. About 20 percent of the EME papers originate from our laboratory in Oslo; the rest have been published by more than 200 scientists from 45 different countries. All experiments up to date have been done with laboratory-built systems. Very recently, however, a Norwegian company launched commercial equipment for EME, based on the use of conductive vials. Thus, the traditional use of platinum electrodes has been omitted, and the electrical field is now coupled directly through the containers. From my point of view, this first-generation commercial equipment represents a huge step forward for EME, because the technique now becomes available for all laboratories, and experiments reported with this equipment of industrial standard can easily be repeated by other laboratories.

Based on the papers in the literature, and due to the release of commercial equipment, I foresee increased interest and use of EME in the near future. The new activities may go in two different directions. In one direction, EME may be considered as a new and alternative extraction tool for laboratory use in areas such as pharmaceutical, environmental, food, and beverages analysis. Development of EME in this direction may be justified by greenness (due to its low solvent and sample consumption), and next-generation analytical scientists will definitely have a strong focus on this. Very efficient sample cleanup, and acceptors directly injectable in LC-MS are additional advantages in favor of EME for pharmaceutical, environmental, food, and beverages analysis. Development of 96-well plates for the commercial EME equipment is in progress, and I expect this will accelerate the implementation of EME in the laboratory direction.

In a second and highly innovative direction, one can look into EME much more fundamentally, as a separation principle based on transfer across a liquid-liquid interface under the influence of an electrical field. My feeling, after 20 years of EME experiments, is that this principle can be used for much more sophisticated separations and applications than reported up to date. This is a core part of our research – taking EME to the next level of sophistication via new and innovative experiments. The rest of our research is currently in the laboratory direction, devoted to the development of robust generic EME methods for a variety of chemical substances and biomolecules, according to their charge and polarity.

I hope many scientists will join in the years to come, either using EME as a green sample preparation technique in routine applications, or developing EME into systems of very high sophistication.

References available online:

The Future of Forensics

With additional forensic-specific analysis software, nanopore sequencing platforms look set to revolutionize the industry

By Roxanne Zascavage, Assistant Professor, University of North Texas Health Science Center, USA

Human remains identification (HRID) is important in both crime scene investigation and in live human identification. Methods used to test degraded remains can also be applied to traditional cases involving DNA left by victims and perpetrators. Additionally, HRID is used to identify victims of mass disasters and military operations. To give an idea of the scale of this task, as of December 31, 2022, the US National Crime Information Center reported 8,242 active unidentified persons cases, as well as 546,568 open cases for missing people (1).

In forensic investigation, current pitfalls mostly lie with trying to type damaged or degraded samples. The most common practices for human identification revolve around short tandem repeat sequences, which require intact fragments of nuclear DNA. However, this is often not accessible for human remains, as time and environmental exposure break down DNA. Other methods are available; for example, exploring mitochondrial DNA, but this is not an easy piece of evidence to work with. Many labs aren't equipped to perform mtDNA testing and those that are cannot individualize with DNA of this kind.

I was lucky enough to have the opportunity to work with nanopore sequencing technology when the instrument was first released to a small group of researchers for testing. Given its unique characteristics, I saw its potential to revolutionize the industry. For example, the cost of the instrument is minimal compared with other sequencers, making it accessible to crime labs on a budget. Additionally, nanopore sequencing technology has the potential for in-field use thanks to its small size and weight, which would help in reducing backlogs and turnaround times. Some instruments also have limitless data generation, enabling simultaneous assessment of multiple targets. And that means we can make the most of our samples by performing various analyses, traditional short tandem repeat typing alongside single nucleotide polymorphisms, mtDNA, or epigenetic analysis, even when traditional methods yield unreliable results.

The gold standard in forensic analysis would be capillary electrophoresis (CE) and perhaps mtDNA sequencing, but I'm working to develop a more streamlined process that doesn't rely on clunky traditional methods. With my team at the University of North Texas Health Science Center, I use nanopore sequencing technology for both whole genome and targeted analysis (post-PCR using standard commercially available forensic kits) (2).

We also designed RNA baits to target regions of interest – something that is new, but has resulted in increased enrichment of our target regions. Our results have shown improved discriminatory power from traditional methods because we are able to resolve isoalleles (alleles with the same length-based designation, but different sequence) that are indistinguishable through CE. One remaining challenge here is aligning allele names across different kits or platforms, which can lead to inconsistencies in our results compared with historical nomenclature standards. This remains a hurdle, as consistency in databases is essential in forensic work.

Working with a new technology has also given us plenty of bugs to work through, such as new basecallers and sequencing chemistry being released. Luckily, these aspects have stabilized over the past few years. We've also had to modify our library preparation methods to accommodate short DNA fragments that were clogging the nanopores. After undertaking several rounds of design and testing during development, we believe we now have the right baits to optimize results.

Moving forward, my team aims to expand upon our work with nanopore sequencing technology in forensics – working on a specific assessment of methylation for age estimation and body fluid identification. Nanopore sequencing technology platforms are able to conduct these tests without DNA modifications, which will help generate leads for HRID. However, for these systems to be a true competitor in the forensic community, they need a plug and play option for data analysis.

After working in this area for so many years, I still believe in building the capabilities of nanopore sequencing technology and its potential in forensics. I'd like to see a focus on resolving nomenclature issues in the future (which is currently being discussed), as well as consistent guidelines to be implemented across platforms. As this area progresses, I hope to see less of the same instruments in labs. Instead, with different specialized options available, labs will be able to select instruments that best fit their needs to ensure proper validations and cross-platform comparisons.

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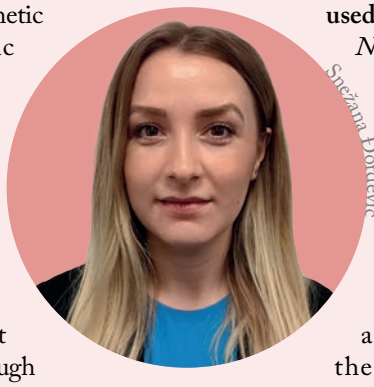
PITTCONNECTIONS LAST A LIFETIME

Navigating Medtech Polymer Analysis – Together

Experts from TOSOH and the Galien Paris-Saclay Institute collaborate to overcome increasingly complex characterization challenges in biopolymer analysis


Julien Nicolas


Snežana Đorđević

Medtech polymers are used for various biomedical applications – as drug carriers, in tissue engineering, or even as building blocks for medical devices. Medtech polymers can be natural (for example, peptides and polysaccharides) or synthetic (for example, aliphatic polyesters and vinyl polymers). But whatever the origin, they must be biocompatible to avoid immune reactions and toxic side effects, and biodegradable for some applications. To ensure medtech polymers meet these requirements, thorough characterization is crucial.

To explore the challenges – and solutions – in medtech polymer analysis, we spoke with experts and collaboration partners Julien Nicolas, CNRS Director of Research at the Galien Paris-Saclay Institute, France, and Snežana Đorđević, Application Scientist at TOSOH Bioscience.

Why is it important to characterize medtech polymers?

Julien Nicolas: Medtech polymers are designed with the goal to be administered and used in a living organism, for instance under the form of nanoparticles. So, it is important that we first ensure they are of extremely high purity and quality – which is why we put an emphasis on their characterization. We need to make sure that there are no impurities or any residual solvents. Characterizing these polymers can also help us determine if they have the properties we are looking for; for example, in terms of functionality, composition and mechanical properties. For instance, if polymer chains are too long, they may accumulate in the body and lead to toxicity; but if polymers are too short, they may be excreted too rapidly, without inducing any therapeutic effects. We have to be certain they have all the right properties.

What analytical techniques are commonly used in the field?

Nicolas: In our lab, we use nuclear magnetic resonance (NMR) – specifically proton and carbon NMR. This technique works well for characterizing polymers in terms of global composition and modifications of the chain ends. In some cases, if we do not have visible protons from the chain end, we can have access to their chain length, which is a crucial parameter.

We also employ size exclusion chromatography, which enables us to study the distribution of the different molecular weight species. Another method we use in our lab is asymmetrical field flow fractionation – which is a good alternative to SEC because it offers additional versatility and flexibility; however, it is limited in other ways.

What are the main analytical challenges in medtech polymer characterization?

Nicolas: Polymers are a mixture of macromolecules, which makes the characterization rather complex. The challenge is to have access to the most accurate data possible. For instance, make sure they have the same characteristics from one polymer chain to another, to minimize heterogeneity as much as possible. Another challenge is to determine their purity as precisely as possible, which is also very important from a regulatory perspective.

How does TOSOH Biosciences help tackle challenges in medtech polymer characterization?

Snežana Đorđević: When it comes to polymer analysis, we offer more than just instruments. TOSOH was originally a chemical company – we have 90 years of in-house experience in polymer synthesis, which means that we are fully aware of the challenges faced by scientists like Professor Nicolas. In fact, these challenges led us to develop our SEC instruments. Initially, these instruments were just for internal use, but, when we realized how robust our instruments were, we decided to make them available for everyone. Today, we are able to offer full support to our clients: from whole instrumentation sets, down to smaller analytical components, such as our SEC columns, which can be adapted for the separation of a wide range of polymers. Our application scientists also enjoy sharing their polymer analysis expertise and experience with customers.

How does TOSOH partner with industry and academia in medtech polymer analysis?

Đorđević: This field is complex and it's growing really fast. We're seeing an increasing number of new polymer types, which are getting more difficult to characterize. And that's why it's really important for us to collaborate with academics like Professor Nicolas – to break new ground with SEC. We also work with

"We're seeing an increasing number of new polymer types, which are getting more difficult to characterize."

graduate and postgraduate students, who collaborate with our application scientists as part of their projects. In this way, we can share our knowledge and expertise in SEC with academia and help them answer their research questions in biopolymer analysis; in return, they provide us with really important feedback on our products. Indeed, this approach allows us to continue improving instruments and developing new models.

What tips and tricks – or lessons learned – can you share here?

Đorđević: The first piece of biopolymer analysis advice I offer is: "Do not shake or vortex your samples!" This process can lead to degradation – especially if you have a really high molecular weight polymer, like hyaluronic acid. I also advise using a combination of techniques – do not rely on just one. When it comes to the analytical methods, it is typical to start your analysis with NMR, but I would suggest screening your data with DLS and finally confirm your results with SEC. And last but not least, take care of your instruments.

We understand that polymer analysis requires a lot of time – and patience. And that's why we always emphasize communication with our clients; the help that we provide with regard to instrumentation maintenance and troubleshooting often saves our users a lot of time in the long run.

Any final thoughts on the future of the medtech polymer field?

Nicolas: There is a plethora of new and exciting polymers in development, but, given that we are dealing with materials that are ultimately intended for administration in patients, I would like to emphasize the vital importance of accurate characterization. Accuracy is the key to understanding whether or not a new polymer has the right properties – whether it has the potential to ultimately change a patient's life. To do this, we need fast, easy to use, and robust instruments to produce high-quality analytical data.

Đorđević: I totally agree on the importance of accurate instrumentation for polymer analysis! I would also add that it is equally important to know which techniques to use and when. Unfortunately, in a field as complex as biopolymer analysis, it isn't as simple as injecting a sample and reading the results. You need to understand at a high level the core of your technique and the ultimate goal of your research to get reliable results. If you're not quite there yet, that's not a problem – you just need to know who to ask! TOSOH is here to help; we have a range of experts focusing on polymer analysis – including natural and synthetic polymers. We will always find a solution – whether in the form of products or in the form of technical support from an application scientist like me. There is no harm in recognizing that you are not an expert in a certain area – just ask for assistance and we will offer you all the support you need!

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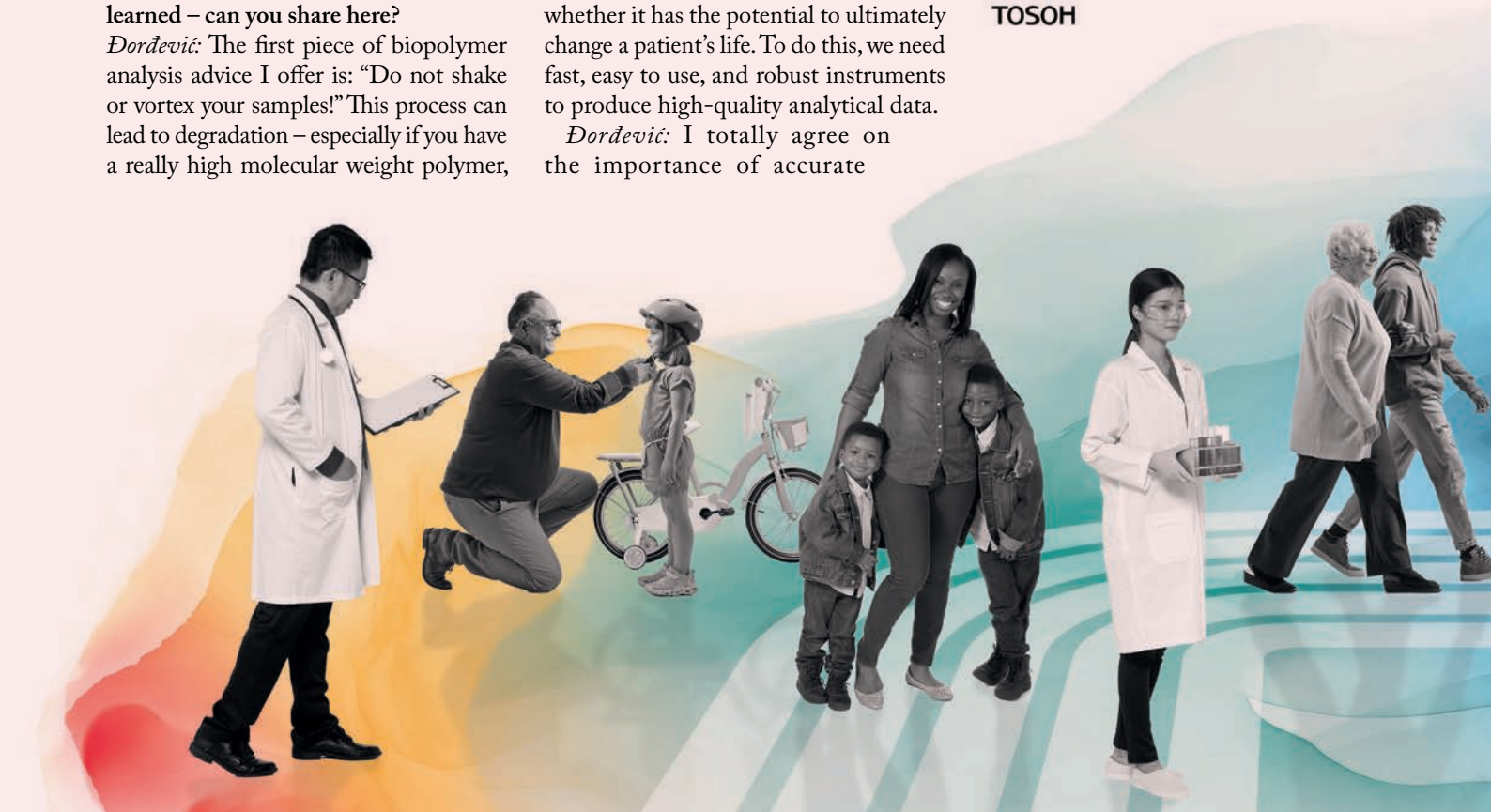
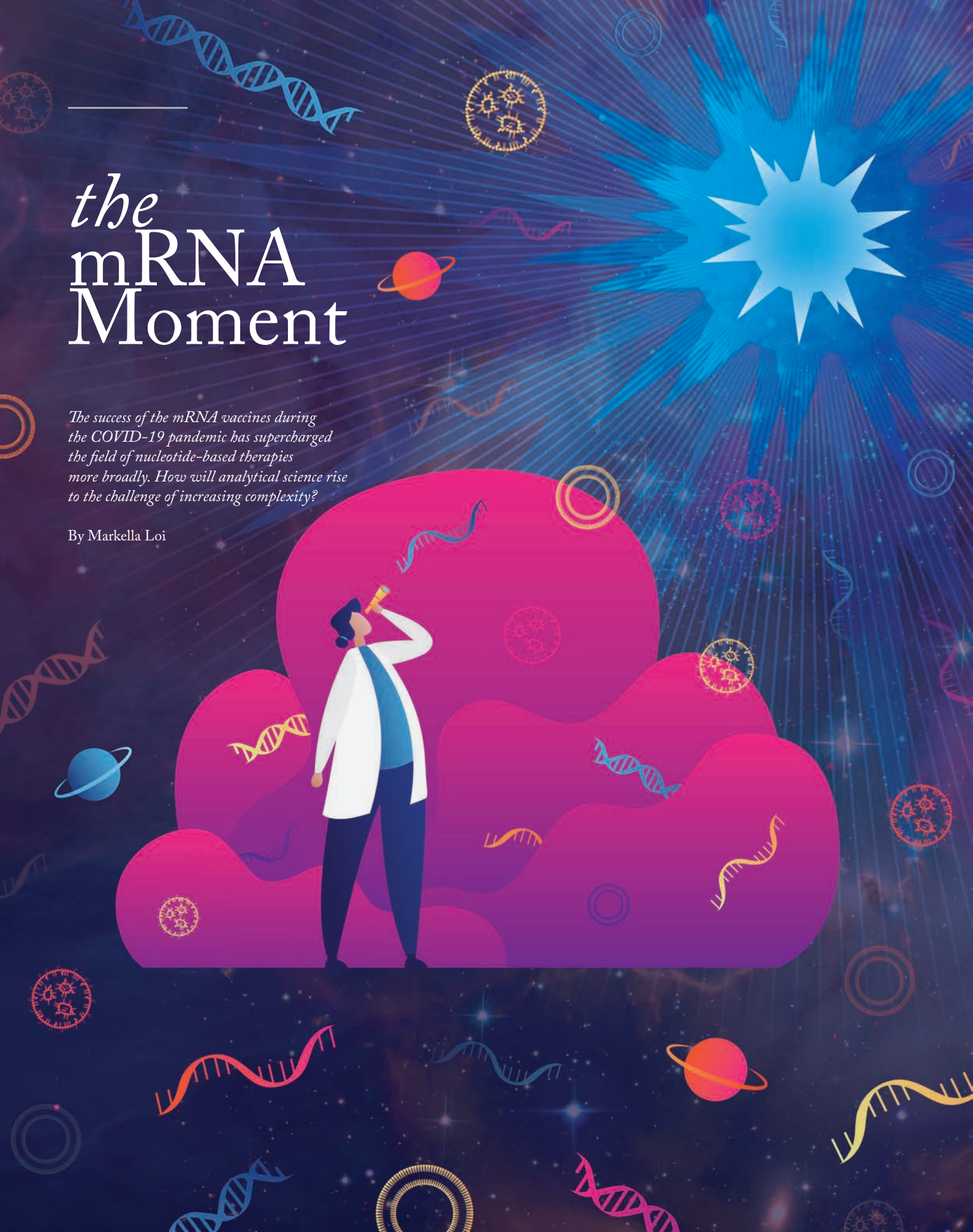


the mRNA Moment

The success of the mRNA vaccines during the COVID-19 pandemic has supercharged the field of nucleotide-based therapies more broadly. How will analytical science rise to the challenge of increasing complexity?

By Markella Loi



Nucleic acid-based therapies have been the subject of research and discovery for over three decades. After all, the prospect of fully harnessing the unique properties of DNA and RNA to instruct cells to produce or block the production of key proteins is tantalizing – potentially opening the door to personalized medicine.

Several barriers to their viability as biopharmaceuticals have been overcome, including progress in formulation, stability, and drug-delivery – notably, with the introduction of lipid nanoparticles, as well as tissue targeting conjugate molecules. But it was the rapid – yet successful – development and deployment of mRNA vaccines during the COVID-19 pandemic that really took these medicines to the next level.

"In the face of a tremendous public health crisis, we saw emergency use authorizations for mRNA vaccines which effectively 'normalized' RNA medicine overnight," says Varun Gadkari, Assistant Professor, Department of Chemistry, University of Minnesota, USA. "Four years later, these therapies are now a fixture in day-to-day life and far more easily accessible."

Bifan Chen, Principal Scientist at Genentech, adds, "I think people saw the tremendous potential of mRNA vaccines over the pandemic years and realized how rapidly science has been advancing."

This increased interest and demand is now being reflected in the number of startup businesses working on the development of nucleic acid therapies – companies that, according to Bingchuan Wei, Senior Principal Scientist at Genentech, are "taking over San Francisco." Interestingly, their focus extends beyond mRNA and its delivery to the development of antisense oligonucleotides and siRNAs.

"We have observed the success and growth of antibody therapies during the last few decades as they opened up more ways to treat disease. I think scientists will always be looking for creative ways to treat disease and reduce patient suffering – now these new therapeutics open up even more avenues to improve patient health," says Christina Vanhinsbergh, Senior Scientist, AstraZeneca, UK.

According to Ken Cook, EU Bio-Separations Manager at Thermo Fisher Scientific, the success of the mRNA vaccines has – quite rapidly – shifted biopharma's focus away from monoclonal antibodies and redirected it to nucleic acid therapeutics, and their daughter oligonucleotide therapies. In fact, he argues that the proven ability to "trick" the body into making a protein also opens up treatments for genetic diseases that result in the production of non-functional proteins. "This has opened up a new field of medicine," he says.

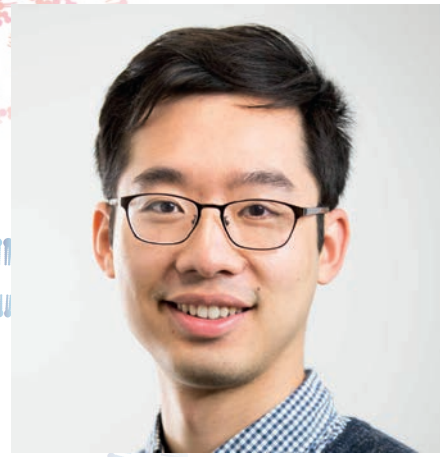
With great promise comes great complexity…

What's clear is that the demand is not going away – rather, it is only growing. "There's now a huge demand from the chemistry side as these therapies move into clinical trials. We need to understand if we're delivering the best materials to our patients, which is quite different from traditional small-molecule or protein drugs. It's an entirely new beast," says Wei. As momentum increases, it becomes clear that traditional approaches to drug development and quality control may not be fit for purpose when it comes to mRNA- and oligonucleotide-based therapies.

"These therapeutics and vaccines are new modalities, which require techniques to characterize them to ensure their safety and efficacy," explains Cook. "Chromatography on its own cannot deliver a full estimation of all the impurities present in the drug products. New methods are required; in fact, mass spectrometry-based methods are already in development. These methods will eventually have to filter into the QC department as well as the development laboratories."

Specifically, oligonucleotides are highly charged molecules, making traditional methods like HPLC and mass spectrometry more difficult. "The solid phase synthesis based manufacturing process for oligonucleotides is complex, with each step adding potential impurities. Modifications are also needed to make them stable, as enzymes in the body can quickly degrade them. Developing methods to monitor these chemical impurities and diastereomers is tough but exciting," explains Wei. For mRNA therapies, the primary challenge is delivery. "The mRNA itself is already difficult to analyze, but the lipid nanoparticles used to encapsulate it add even more complexity. Characterizing the particles – ensuring the right particle size and encapsulation efficiency – is crucial. We're still developing innovative ways to measure, analyze and deliver these therapies."

For Gadkari, many of the analytical challenges stem from the chemical complexity and structural heterogeneity of nucleic acids themselves. For example, mRNA therapeutics have structured untranslated regions (UTRs) that are as important to the efficacy/stability of the mRNA molecule as the translated region of the molecule. "Characterizing the intact mRNA structure is an important step in ensuring that the molecule will function as intended," says Gadkari. "This highlights one of the major challenges: RNA structure elucidation is typically a challenging endeavor, and it is especially challenging when the molecules of interest are thousands of bases in length, as most mRNA vaccine molecules are." He believes that once these challenges are addressed, the next challenge would be adapting the analytical tools for different size



Bifan Chen

Christina Vanhinsbergh

Varun Gadkari

Bingchuan Wei

Ken Cook

Daniel Meston

nucleic acid therapeutics – which requires experience gained from analysis of other nucleic acid molecules (in other words, adapting a method designed for 20 nt oligos up to analyzing 100 nt single guide RNAs).

Vanhinsbergh agrees that knowledge from smaller therapeutics method development could be shared and applied to the analysis of mRNA. Moreover, she says, "Developments in tissue targeting techniques (conjugation and smarter drug product formulations) apply more ubiquitously so will hopefully benefit mRNA therapeutics in line with smaller oligonucleotides." However, as the structural chemistry, size, and heterogeneity of mRNA is individual, Vanhinsbergh believes that therapeutics should be treated as an individual class of sample from an analytical perspective.

Analytical solutions emerge

The inherent complexity and heterogeneity of nucleic acids have energized the search for improved separations and mass spectrometry-based methods.

"I am seeing broader adoption of photodissociation methods, such as ultraviolet photodissociation (UVPD) for tandem MS of nucleic acids, substantially improving sequence coverage of larger molecules," says Gadkari. "I am also excited to see the emergence of top-down and native mass spectrometry in this space as two MS based methods that yield not only sequence information, but also provide structural context, which is just as important as sequence for therapeutic molecules."

According to Daniel Meston, Research Associate Professor in Chemistry, Gustavus Adolphus College, multidimensional chromatography is becoming increasingly important in the elucidation of highly structural specific impurities in early phase drug discovery. "Heartcutting 2D-LC is emerging as an extremely useful part of the analytical scientist's toolbox; we have seen a number of pharmaceutical companies begin to install at least one 2D-LC system in their upstream research labs," he says. "We have been largely interested in pushing the performance of chromatographic methods to improve the separation power as well as investigating the unique challenges that 2D-LC faces when transferring these biomolecules onto complementary modes of LC."

Aware of these challenges in oligonucleotide analysis, Chen and his team have developed a specialized LC-MS technology for oligonucleotide analysis – focusing on guide RNAs used in CRISPR-Cas9 gene editing systems, which are necessary for developing targeted cancer therapies. "We are using a top-down mass spectrometry strategy for the 100-mer guide RNA without any sample preparation and digestion. The key feature is the introduction of large oligonucleotide samples to the mass spectrometer through a small pore, high-pH resistant reversed phase column in a size exclusion mode," Chen explains. "In a short 10 min LC-MS run, we were achieving nearly 70 percent sequence coverage in our previous work for the 100-mer guide RNA; now, we are nearly at 100 percent sequence coverage with the improved dissociation strategy."

For Cook, high resolution mass spectrometry (HRMS) is at the heart of these advancements – thanks to its added sensitivity and accuracy. "Using a novel magnetic bound nuclease for controlled partial digestion with high-resolution MS/MS for the analysis and new software tools for the data analysis, we can now directly sequence the mRNA vaccines to confirm the correct sequence in the product," he says. "These tools are also being used with the smaller synthetic RNA drug candidates."

Data analysis is another improving aspect of oligonucleotide/mRNA analysis, with most of the major vendors tackling challenges across arduous and complex processing, high numbers of large data files, and multiple signals to extract and integrate. "I have worked with numerous vendors to give feedback on processing improvements to help design these tools for the analytical community," says Vanhinsbergh. "By making the data more accessible, analysis turnaround times will reduce and will hopefully become more cost effective." Vanhinsbergh also emphasizes sustainable and environmentally friendly analytical approaches for the developments of these therapeutics – an aspect often neglected. "Simplifying the mobile phase and using less toxic reagents will be a fantastic move towards sustainability improvements in analysis," she says. "It will also assist with the introduction and transfer of analytical methods internationally, where additional safety controls may be placed on existing mobile phase constituents." Cross collaboration with both industry and academia experts has proven central to her efforts towards achieving her sustainability goals – using ion-pair free separations, such as hydrophilic liquid chromatography (HILIC).

Meanwhile, Chen underlines miniaturization and high-throughput screening as some of the biggest emerging trends in the field that help researchers overcome technical challenges. Chen also notes that machine learning and AI may well come into play – aiding data analysis

Through the Eyes of a Biopharma Pioneer

Koen Sandra shares his thoughts on the future of mRNA and oligonucleotide analysis

The approval of mRNA vaccines did not only help us confine the COVID-19 pandemic, but has also urged the development of proper analytical methods to study various critical structural properties associated with mRNA – which compared to protein and antibody analytics are far less mature. I believe we are now at the point with mRNA that we were with antibody analysis 15 years ago, which creates plenty of opportunities – and challenges – for us analytical scientists!

For example, intact mRNA is currently tricky to measure with MS, although some recent attempts seem promising. MALS, mass photometry, and CD-MS are furthermore relevant to obtain MW information on these molecular giants. Capillary electrophoresis and chromatographic modes like SEC, AEX, IP-RPLC and HILIC provide information on mRNA integrity and fragmentation, as well as SEC on covalent and non-covalent aggregates.

LC-MS following ribonuclease digestion is gaining a lot of traction to assess various attributes of mRNA, such as sequence, 3' poly tail length, 5' capping and post-transcriptional modifications. Ribonucleases are used to generate oligonucleotides amenable to LC-MS using either IP-RPLC or HILIC as chromatographic mode. The use of low adsorption flow paths is critical, hence, instrument and column vendors have introduced various solutions, such as biocompatible or bio-inert instrumentation, PEEK-lined or deactivated stainless steel columns. This is especially important for HILIC but to a certain extent also IP-RPLC. HILIC is heavily explored as an alternative for IP-RPLC because the latter suffers from the use of sticky (contaminating) alkylamines and HFIP, which is categorized as a PFAS (I'm not sure if there will be restrictions in the near future on the use of HFIP). MS sensitivity is, however, much higher when using IP-RPLC compared to HILIC.

In addition, mRNA is typically formulated in LNPs – creating an additional analytical need. Techniques like SEC(-MALS), field flow fractionation (FFF) and DLS come in the picture here.

For synthetic oligonucleotides, phosphorothioate diastereomers are particularly troublesome. Do we want to reveal these or push them in one chromatographic peak – bearing in mind that they might have different pharmacological properties? In any case, a wide range of, often co-eluting impurities need to be tackled. Nowadays, oligonucleotides are also coupled to antibodies or antibody fragments. Here knowledge of protein and oligonucleotide analysis is key – and completely new analytical challenges arise.

In conclusion, these nucleic acid-based medicines present a lot of fun for us analytical scientists!

Koen Sandra is CEO & Co-owner, RIC group, Kortrijk, Belgium; and Visiting Professor, Ghent University, Belgium

and helping us make faster, more informed decisions. Wei agrees, RNA-based therapies are "perfect candidates" for this approach because they have well-defined sequences and building blocks, which makes it easier to apply AI and machine learning techniques.

But as one complexity challenge is addressed, another pops up in its place; for example, conjugate technologies that enable drug targeting add an additional dimension to the analytics, according to Cook. The development of lipid nanoparticles and conjugation of GalNAc to target liver hepatocytes has enabled better drug delivery and recent registration of therapeutics with these formulations or conjugations demonstrates regulatory acceptance. "I think we will also likely see other types of conjugations to oligonucleotides, which will further complicate the analytical strategy as these conjugates will exhibit their own impurity profiles. To ensure these drugs are safe for patients, analysts will need to continue to develop methods that cope with more complicated samples and impurities," suggests Vanhinsbergh.

"I think it's safe to say we're squarely into the era of nucleic acid medicine," states Gadkari. To him, the possibilities of nucleic acids as therapeutic molecules are tremendously promising and analytical science will continue to play a strong role in ensuring that these therapeutics reach their potential. "This question about 'the role of analytical science' always surprises me when I see it. There is not a lot of science which can be done without analytical measurement. Everything has to be ultimately measured in some way, whether it's simply for the sake of quantification or whether it's for characterization," he says. "Because of this, I feel like I can never think of a scenario where analytical science would not play a role in the advancement of any promising science."

The stakes – and potential reward – have never been higher…

Evidently, the growing demand for nucleotide-based therapeutics, especially mRNA and oligonucleotide therapies, has spurred a wave of analytical innovation, with researchers developing advanced methods to meet characterization challenges – at all stages of the biopharma pipeline. "Their influence goes far beyond the bench," says Meston.

Regulatory bodies must also keep pace with rapid scientific progress to ensure that safe and effective treatments reach patients quickly. Indeed, the balance between innovation and regulation will be crucial to embracing the era of nucleic acid medicine. "To reach full potential quickly, the analytics and [regulatory] approval processes need to be in place quickly," advises Cook. "Many companies and regulatory bodies are working together to ensure these products reach the market faster than traditionally expected."

Vanhinsbergh notes recent progress in this regard; for example, the EMA has released a draft of the "Guideline on the development and manufacture of oligonucleotides" – which can help analysts understand what regulators expect to see during submissions.

As analytical scientists across the world continue to rise to meet new challenges, our community must not be shy in celebrating the essential role we play in shaping and delivering next generation therapies. The future is exciting, but the path forward demands continued collaboration, creativity, and agility.

MASS SPEC

Going Native

Native MS has become essential in biochemistry labs. So what is preventing more researchers from "going native?"

By Joseph Loo

The term "native MS" was coined in 2004 by Albert Heck, but many researchers have performed electrospray ionization (ESI) MS experiments to detect interactions between non-covalently bound molecular partners since it was first demonstrated in 1991 by a group at Cornell University. Indeed, I've been developing and applying native MS for over 30 years.

With improved sensitivity and increased mass range, native MS can now address larger protein complexes – up to the megadalton range. However, we already know the potential of this technique for measuring the size, heterogeneity, and binding affinities of large protein complexes – they were already established in the 1990s!

In our lab, we've looked at answering relatively simple questions, such as: "Does this protein bind to another protein? How many proteins does it bind? Are there other molecules bound to the protein?" All of which we were able to address by a molecular mass measurement of the complex – native MS. For example, we found that a protein complex is a hexamer, not a trimer or tetramer as previously suggested in earlier models or lower resolution techniques, such as size exclusion chromatography or centrifugation. Detecting small molecules bound to the protein, such as lipids, metal ions, and ATP, is straightforward because the MS mass measurement provides the information directly.

In addition, drug binding studies are quite amenable by native MS. Though not reaching capacity levels of screening platforms in industry and academic screening labs, the throughput of the measurements can be moderately high with automation to identify molecular scaffolds by screening targeted or focused compound libraries for further drug development. While working at Pfizer, we used native MS to detect co-binding of a potential drug and an ATP molecule to a protein kinase, which was a target for a potential cancer treatment. This revealed that the drug was binding in a distinct pocket away from the ATP-binding site, showcasing a unique mechanism of action for a potential cancer drug. This highlights one of the benefits of native MS – the capacity to provide specific molecular details of biomolecular interactions without relying on time-consuming and high sample-consuming methods, such as crystallography, NMR, and electron microscopy.

More broadly, native MS has been a useful tool for research groups in labs and institutions, and for some fields it is an essential method. Membrane protein structural biology has opened up with the addition of native MS, with Carol Robinson's research group at Oxford paving the way forward. Structure determination of membrane proteins is challenging due to their hydrophobic nature, requiring specialized solubilization methods, such as detergent-based micelles and nanodiscs. Because membrane proteins are potential targets in drug discovery, native MS provides binding and structural details, including the role of specific lipids in stabilization.

The megadalton range of native MS has also expanded to measure viral assemblies, including pathogenic viruses and those used in gene therapy and drug delivery. In the 1990s, detecting a relatively simple protein like the intact 64 kDa human hemoglobin was a challenge. But now, native MS allows labs to measure hemoglobin from a single red blood cell to determine the molecular mass of jumbo-sized molecules, like a virus. It's a remarkable advancement!

However, the disadvantage is that you need a high-performance mass spectrometer – and they aren't cheap. They also aren't easy to automate for native MS and not every sample behaves in the same way. Some expertise is required to run these machines, but, as the field matures, I hope that these issues will disappear. We need to democratize native MS to progress.

A biochemical no brainer?

A reliable technique that provides knowledge on whether or not molecule-X binds to Y-numbers of Z-molecules should be present in every biochemical analysis facility. And if native MS can routinely determine binding affinities, who wouldn't want it in their lab?

Currently, there's a demand in large institutions to establish or enlarge structural biology labs to include as many cryoelectron

microscopy (cryoEM) platforms as they can afford. CryoEM and cryo-electron tomography (cryoET) are game changers in the structural biology domain because of their unprecedented capability for obtaining atomic level structures of large macromolecular machines.

Native MS compliments cryoEM – without replacing it, of course – by assisting with screening sample preparations before they move onto subsequent labor-intensive cryoEM experiments. It could be used to assign features in cryoEM-derived structures that were unanticipated, such as a post-translational modification. Native MS could also identify additional proteins or small molecule ligands that bound to the complex. Relative to the cost of a high-resolution cryo-electron microscope, an additional native MS instrument is a mere drop-in-the-bucket. CryoEM vendors should make mass spectrometers an accessory within purchase orders.

Additionally, a few labs are making efforts to essentially physically marry native MS and cryoEM. Protein ions going through a mass spectrometer can be filtered so that only those of interest are deposited onto EM grids for subsequent high-resolution structure determination. This technique, called soft landing, could well be the future marriage of cryoEM and native MS.

In my view, native MS will continue to expand its applicability across different areas of research as mass spec technology improves in sensitivity, accuracy, resolving power, and so on. However, there are other barriers to native MS adoption; for example, sample prep has been a consistent issue for novices in particular. When combined with ESI, native MS doesn't tolerate the usual buffers and salts that most biochemical labs use – and each protein system can behave differently. This is where additional education on sample prep practices would be useful.

Another challenge for biopharma companies is a relative lack of automation in today's most common native MS platform, which uses nanoESI needles or emitters and can result in low sample throughputs. The Wysocki Lab at Ohio State University developed a relatively simple strategy that combine sample desalting via a mini-size exclusion chromatography column with a slightly higher sample injection flow rate compared with nanoESI. It can be automated in principle.

"I believe that every biochemistry core facility should have a native MS instrument. It should be easy for a student researcher to use and achieve a reliable result in a matter of minutes."

I feel that the relatively high price tag for a mass spectrometer suited to native MS experiments is particularly challenging for academic labs. Why can't vendors design a relatively simple-to-operate, low cost instrument with moderate resolving power and mass-to-charge range that addresses most routine native MS applications? More advanced labs with sophisticated instruments and expertise can manage heroic instruments that demand higher-end resources.

My advice? Talk to the experts. Visit their labs and watch them perform a native MS measurement. It's hard to replace seeing an experiment performed in person. Even though I no longer have the time to conduct experiments with my own hands, I watch my students in the lab and visit other institutions' labs – especially if I'm interested in a new type of mass spectrometer.

Attending mass spec conferences that include native MS talks – for example, the American Society for Mass Spectrometry (ASMS) meeting are also highly beneficial. Students can also find summer classes on mass spec that involve native MS. Simply approach an expert and put your questions forward. They won't bite (at least most of them won't, I think).

Time to go native?

I believe that every biochemistry core facility should have a native MS instrument. It should be easy for a student researcher to use and achieve a reliable result in a matter of minutes. Currently, we have walk-up matrix-assisted laser desorption/ionization (MALDI)-MS instruments. And of course GC-MS and LC-MS instruments are present in most chemistry facilities.

I also hope that native MS will be used to complement high resolution structural biology experiments. It's already becoming indispensable for membrane protein research, such as for G-protein-coupled receptors (GPCRs). The application of native MS for characterizing viruses is already filling a role in drug research and discovery, and pharma companies have been using native MS for protein-ligand and drug binding measurements. No doubt it could be further integrated with future automation. The combination of native MS with on-line ion mobility offers enhanced insight into protein folding, conformations, and the impact of ligand binding. Given that biological processes rely on molecular interactions, native MS can serve as a go-to tool, providing direct readouts of these processes.

Joseph Loo is Professor of Chemistry and Biochemistry at University of California, USA

CHROMATOGRAPHY

Pushing the Boundaries of Bioprocessing

Biopharma tech continues to advance so why is downstream processing such a headache? Chromatography, in particular, is an expensive process – but improvements are being made.

By Jungmin Oh

Downstream processing in biotechnology and pharmaceutical industries has undergone significant advancements in recent years. We've seen increased adoption of single-use technology, which can reduce contamination risks, lower capital costs, and increase manufacturing flexibility. Additionally, continuous processing has gained traction over traditional batch processing because of its productivity and footprint advantages. Advanced chromatography techniques have also evolved, resulting in higher purity levels, increased throughput, and enhanced efficiency in purification. Furthermore, there's been a focus on process intensification, employing higher capacity resins, multi-column chromatography systems, and integrated process trains. Automation and digitalization technologies have also been integrated to improve process control, data management, and real-time monitoring of critical parameters.

And yet, despite these notable advances, downstream processing continues to present challenges. The complexity of biologics necessitates meticulous purification processes, often involving multiple downstream steps. Companies also need to consider equipment scalability, process transferability, and maintaining quality at larger scales. All of this demands substantial investments in process optimization.

At the same time, downstream processing is inherently expensive. Raw materials and consumables can all be pricey – and the costs increase significantly as production is scaled up. Low productivity and yields are also commonplace because of inefficient recovery and purification processes, leading to suboptimal manufacturing efficiency that increases costs even further. Complex purification processes with multiple steps, including chromatography and filtration, require careful tuning to balance purity, yield, and productivity. Ensuring product stability and shelf-life while removing impurities such as host cell proteins and DNA demands robust purification strategies and precise control over storage conditions.

Focusing on chromatography processes

Chromatography is a pivotal technique in downstream processing. Here, the key challenges include achieving sufficiently high selectivity to separate the target biopharmaceutical from impurities (particularly for complex molecules with similar properties) and obtaining adequate resolution between closely related species to ensure desired purity levels (as just one example, consider the problems many biopharma manufacturers now face in separating empty/full capsids of AAV particles).

The limited binding capacity of chromatography columns can hinder throughput and increase processing time, especially in large-scale production, while scaling up processes poses challenges related to column packing and flow dynamics. Chromatography is also an expensive part of the downstream process – the resins, buffers, and the hardware itself can all come with high price tags.

However, chromatography equipment and resin technologies are continuously evolving. Advancements in resin technology are focusing on creating novel stationary phases with improved selectivity, capacity, and stability – enabled by clever chemistry, such as surface modifications and ligand immobilization techniques, tailored to specific biomolecules and purification challenges. Multi-modal and mixed-mode resins integrate various chromatographic functions into a single stationary phase, providing enhanced selectivity and flexibility to purify complex biomolecules. Continuous chromatography systems are also gaining traction as an alternative to batch chromatography, offering higher productivity, reduced buffer consumption and a smaller footprint.

Companies can enhance their chromatography processes by focusing on key areas such as process characterization

"The future of chromatography technology in biopharma is poised for significant advancements and transformations. My predictions include a surge in automation and integration fueled by robotics, AI, and real-time monitoring, and more streamlined operations."

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and understanding, in-line dilution and buffer management, and process modeling and simulation. Understanding biomolecule properties and using multi-mode resins can improve impurity separation, and optimizing buffer management with in-line dilution systems can enhance reproducibility and scalability, while reducing operating costs. Additionally, employing computational modeling tools aids in predicting process performance and troubleshooting, ultimately maximizing efficiency, productivity, and reliability.

Additionally, process intensification strategies, including continuous chromatography and high-throughput chromatography, aim to improve efficiency and sustainability. By integrating sustainability considerations into process design, operation and technology development, the biopharmaceutical industry is actively working towards making chromatography processes more environmentally friendly and socially responsible, contributing to a sustainable future for biopharmaceutical manufacturing.

What lies ahead

The future of chromatography technology in biopharma is poised for significant advancements and transformations. My predictions include a surge in automation and integration fueled by robotics, AI, and real-time monitoring, and more streamlined operations. Automation streamlines processes by minimizing manual intervention and errors, performing tasks, such as column packing, sample loading, and fraction collection, with high precision and consistency, while AI and machine learning could optimize chromatography by analyzing large datasets, predicting optimal process parameters, and facilitating adaptive process control. The result? Faster development, fewer experimental iterations, and improved scalability. Additionally, I expect progress in miniaturization technology and the development of tailored resins that can help improve throughput and selectivity for microfluidic systems and innovative stationary phases. Miniaturization technologies, including microfluidic devices and microscale chromatography columns, offer advantages such as smaller sample volumes, reduced reagent consumption, and faster analysis times – again contributing to sustainability efforts. Real-time monitoring and control capabilities, powered by sensor technology and advanced data analytics, further optimize chromatography processes, ensuring consistent product quality and yield.

Overall, the future of chromatography technology in biopharma holds promise for enhancing efficiency, productivity, and sustainability in downstream processing. Embracing emerging technologies, innovative approaches, and collaborative partnerships will enable the biopharmaceutical industry to overcome current challenges and achieve new levels of excellence in chromatography-based purification of biologics.

Jungmin Oh is Manager, New Product Development, at Avantor

SPECTROSCOPY

Through the Spectroscopic Glass

What does the future hold for optical spectroscopy and diagnostics?

By Richard Crocombe

Optical spectroscopy (excluding fluorescence) has not, until recently, been a major player in the clinical field. It generates complex spectra that are hard to interpret, and the instruments are bulky and expensive. However, portable spectrometers and spectroscopic sensors are poised to change that paradigm, by providing rapid answers at the point of need – significantly enhancing efficiency, quality, safety, and reduced cycle time.

Unfortunately, the spectroscopic market is much smaller than sectors like optical telecommunications, consumer electronics, and automotive, so it doesn't attract major investment for new clinical components, instead relying on advancements in these other fields. With light detection and ranging (LiDAR), I believe that the potential for high-volume commercial production of small, rugged optical components can significantly reduce costs. For example, VCSELs (solid-state lasers used in smartphone facial recognition) now cost just a few dollars to manufacture. LiDAR technology, such as fast swept-source lasers, might also be used in near-infrared spectroscopy in the future.

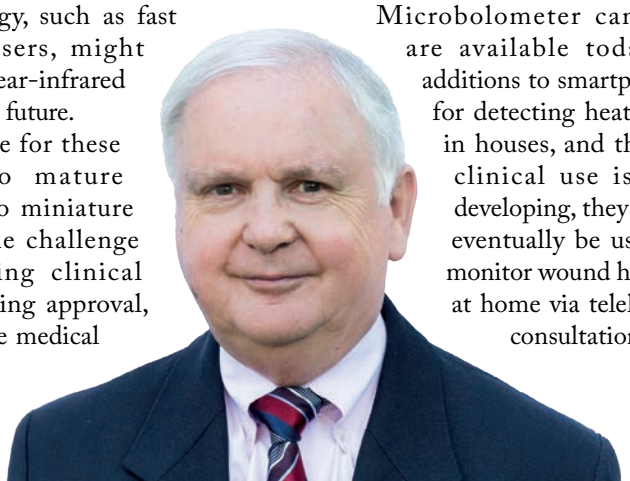
It will take time for these technologies to mature and be applied to miniature spectroscopy. The challenge lies in developing clinical applications, gaining approval, and convincing the medical field of their effectiveness in improving patient outcomes.

Looking ahead to newer technologies, such as photonic integrated circuits (PICs) and planar optics, we have the potential to enable much smaller spectrometers, manufactured on a large scale using semiconductor techniques. Of course, reducing size and cost provides opportunities across various applications. In the short term, this includes "wearables" and "ingestibles."

Miniature and lightweight wearable sensors have the ability to monitor heart rate, blood pressure, and blood oxygen levels – transmitting data to a monitoring station. Ingestibles are able to monitor "from within" – equipped with a camera, a spectroscopic sensor, and an ablation device for treating digestive conditions. Using a small multispectral or hyperspectral camera could also provide support in surgery, determining tumor margins. These are just a few of the possibilities this technology could bring to the clinic.

Health-related smartwatches and rings have already reached popularity, with some professional sports teams taking advantage of this technology. Manufacturers are hoping to add more features, including a non-invasive optical blood glucose monitor. Unfortunately, this application has been incredibly difficult to achieve, despite 30–40 years of research from well-funded groups.

We've even seen studies showcasing the capabilities of smartphones as thermometers using microbolometers. Some healthcare systems are already taking advantage of the telehealth applications – allowing for easy photo taking and sharing of skin conditions among clinicians. Microbolometer cameras are available today as additions to smartphones for detecting heat leaks in houses, and though clinical use is still developing, they could eventually be used to monitor wound healing at home via telehealth consultations.



Another concept, which is slightly bizarre, is a "smart toilet" for analyzing stool and urine using imaging and spectroscopy to provide health insights and diet suggestions. Scientific papers continue to delve into this topic and a product has been developed from a start-up – leading to discussions by established plumbing companies at events like the Consumer Electronics Show.

Technologies are advancing rapidly, as described above; however, a large amount of mathematical work (chemometrics) is required to turn the volume of data recorded by a spectroscopic sensor into usable information. This isn't required for the more specific tests already in use across the clinical community; for example, based on antibodies, which give immediate results. In pathology, visual inspection of stained tissue sections is the established method. But spectroscopic researchers are using infrared and Raman imaging to replicate the results without stains. The challenge is to produce results that clinicians recognize and trust – and then obtain regulatory approval.

Looking towards the future of diagnostics, it's likely that optical spectroscopic techniques will be available in cost-effective, miniature packages with an emphasis on the consumer rather than the clinical space – such as smart watches and other wearables. Of course, clinical applications are guaranteed to follow, but I believe we're at least 10 years from this integration.

Looking even further into the future, I expect spectroscopic sensors will become a key part of the home without us even realizing it – in smoke detectors, vacuum cleaners, refrigerators, and washing machines.

Richard Crocombe, Crocombe Spectroscopic Consulting, LLC, Winchester, UK

References available online:

Analysis of Chlorinated and Brominated Acetic Acids in Water Matrices by HPLC-ICPMS/MS

Halogenated acetic acids (HAAs) are among the most common water disinfection byproducts. Presumably harmful to health, the US Environmental Protection Agency (EPA) regulates the levels of five haloacetic acids to an overall maximum of 60 µg/L. The regulations of the European Union include another four haloacetic acids, with a maximum concentration of 80 µg/L for all nine HAAs combined (1).

This application note will demonstrate the analysis of nine HAAs by using a YMC-Triart C18 column and inductively coupled plasma tandem mass spectrometry (ICPMS/MS) for detection, which is less prone to matrix effects compared to commonly used ESI-MS/MS (2). Different types of water samples from Austria were analysed: tap water from Graz, groundwater from the Leutschach well and river water from the Mur.

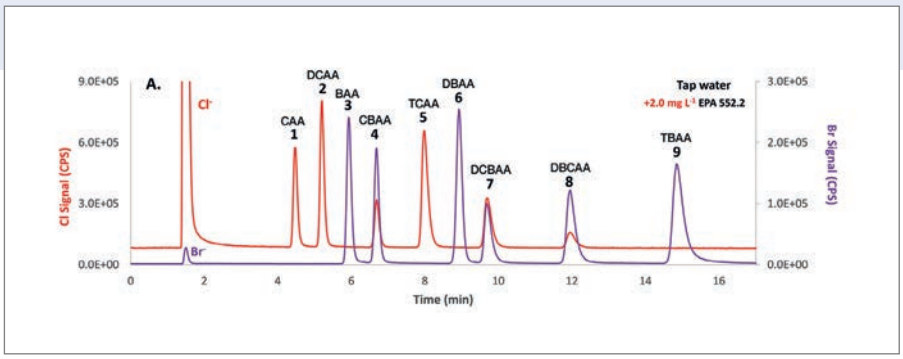


Figure 1: Separation of haloacetic acids in tap water spiked with the EPA 552.2 certified reference material (2).

Full method details can be accessed here: https://ymc.eu/d/brDqw

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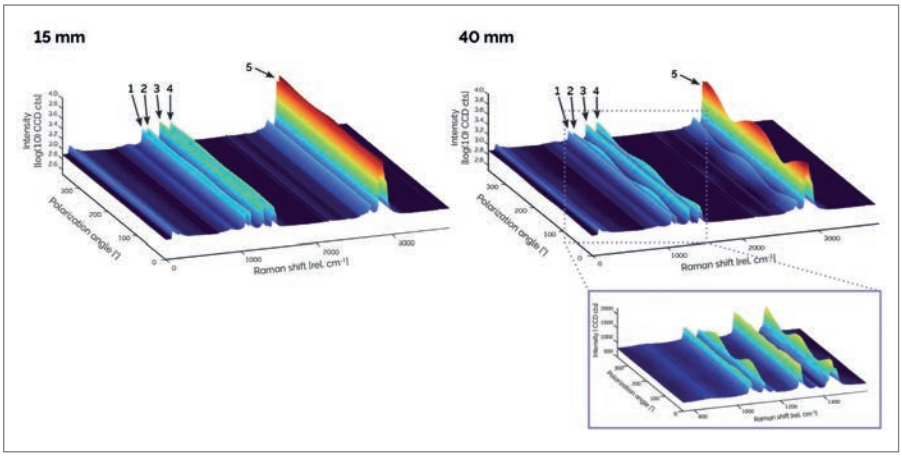
Polarization-Resolved Analysis of a Stretched Polymer Foil

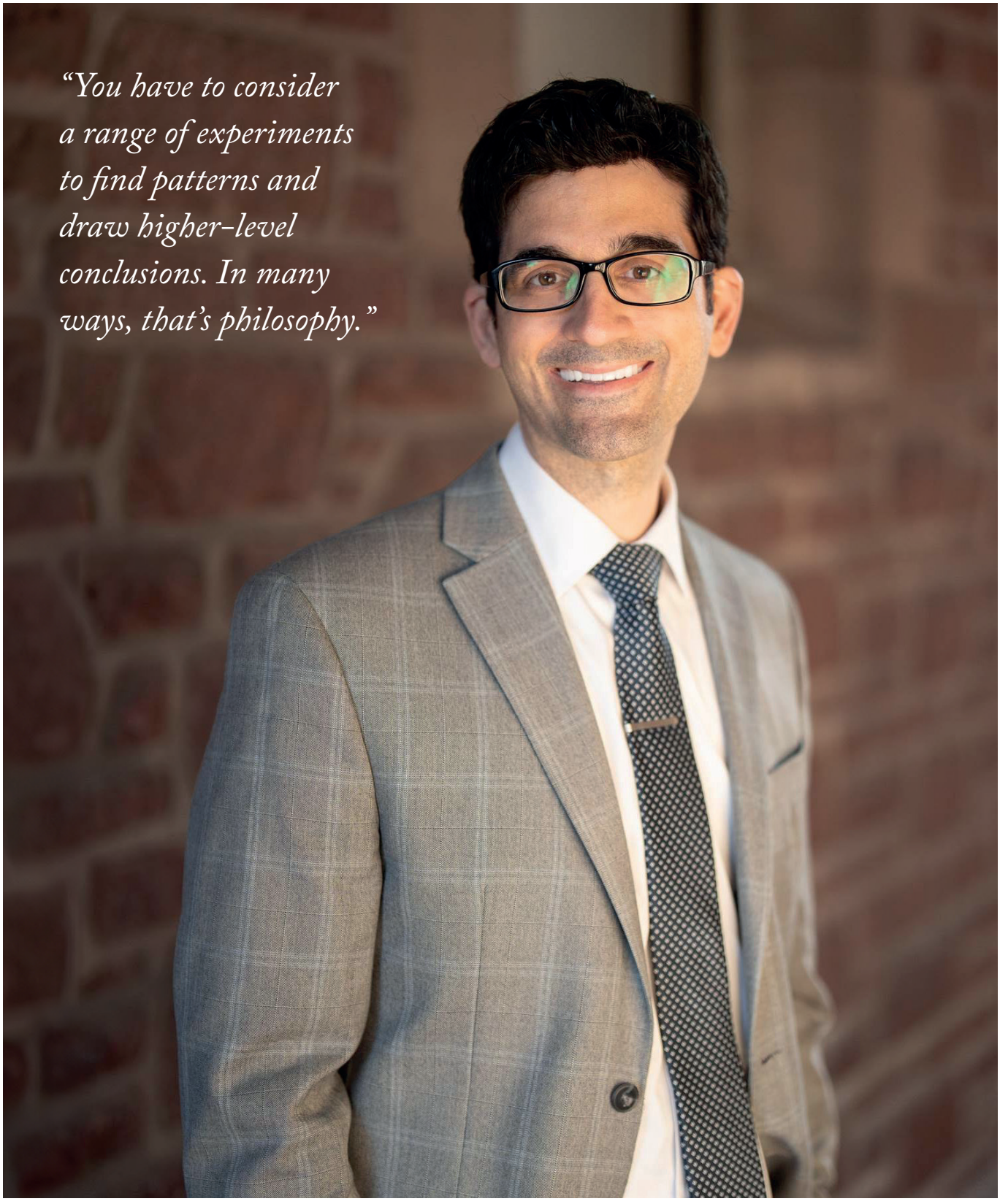
Applying mechanical force to a polymer film aligns its molecules, which act as a polarizing filter for transmitted light. Here, this change was induced in a polyethylene (PE) foil with a modular force stage and analyzed with polarization-resolved Raman microscopy.

The PE was stretched from an initial 15 mm to 40 mm. The Raman modes for C-C stretching, CH_2 bending, and C-H stretching showed polarization-dependent intensity changes in the fully stretched condition.



Plots of Raman spectra at the foil's initial and final dimensions were generated. The five marked spectral peaks show polarization-dependent changes: [1] ν_{as}(C-C) at 1063 rel. cm^{-1}; [2] ν_s(C-C) at 1130 rel. cm^{-1}; [3] $\tau(CH_2)$ at 1297 rel. cm^{-1}; [4] $\delta(CH_2)$ at 1417, 1441 and 1464 rel. cm^{-1}; [5] $\nu_{as}(CH_2)$ and $\nu_s(CH_2)$ at 2849 and 2883 rel. cm^{-1}.

For details, see our Tech Note: https://raman.oxinst.com/assets/uploads/raman/materials/WITec_TechNote_Polarization-web.pdf


"You have to consider a range of experiments to find patterns and draw higher-level conclusions. In many ways, that's philosophy."

The Analytical Philosopher

Sitting Down With… Gary Patti, Michael and Tana Powell Professor, Senior Director, Center for Mass Spectrometry and Metabolic Tracing, Washington University in St. Louis; CSO, Panome Bio, St. Louis, Missouri, USA

Did you always want to be a scientist?
I've always been interested in science and found it intriguing. In many ways, I think we're all scientists – we're all experimenting, generating hypotheses, and testing them. My grandfather, who was a chemist and a significant influence in my life, encouraged me to think about doing experiments – even when I was very little! But as I grew older, I developed interests in other areas – I was drawn to philosophy in particular. I think if more jobs were available in philosophy and it were more practical, maybe I would have pursued that. I also pursued medicine as it seemed like applied science, but I realized that routine aspects of medicine – seeing patients in the clinic – was not as focused on experimentation as I'd hoped. So, my path to scientific research was definitely roundabout.

Do you find that your philosophical interests influence your scientific work?
Absolutely. I think the field of omics is quite philosophical because it involves abstract thinking – what is a metabolite? How big is an "ome"? Does the "dark metabolome" really exist? Science requires two levels of inquiry: one is detail-oriented and evidence-based – you perform experiments and gather data. But if you're a good scientist, you don't operate just at that level. You have to consider a range of experiments to find patterns and draw higher-level conclusions. In many ways, that's philosophy – looking at a bunch of details, and from those details, extrapolating a pattern that can be generalized to something much bigger.

Reflecting on your time at Scripps, what were some key lessons you took away?
One major lesson was learning to challenge established ideas. As a postdoc, I remember going into Richard Lerner's office to discuss the results of an imaging experiment. It wasn't particularly well designed or well executed, but the result was seemingly unexpected. I showed him this black page with a little green speckle, expecting him to tell me it was complete rubbish. But he instead told me how amazing it could be – that it might mean we have to rethink everything we know about neurotransmission. The way Richard – a world renowned scientist – evaluated my seemingly low-quality piece of data in terms of its potential revolutionary impact was extraordinary, and really stuck with me. He wasn't telling me to publish right away, but he did encourage me to be open to new ideas and test them further.

It seems like you've been open to new directions in your career, like transitioning from medical school to mass spectrometry. How do you reflect on these changes?
I've basically always followed what's interesting. You can't really contain science, try as we might. There are practical concerns of course. If you have a lab with a certain set of tools and expertise in the team, it makes sense to utilize your available resources. But that can limit your horizons. I've always tried to focus on the question and see where we end up. You might end up in a totally unfamiliar area, but that's exciting and can be lots of fun! I love to learn new things.

I also think there's a real scientific benefit to exploring new terrain. I once read that when you hear an unfamiliar language, you perceive a pattern that is inaccessible to someone who speaks the language. In the same way, when you're embedded in a scientific field, it's often very difficult to get outside of established paradigms because you get trapped into conventional thinking. But when you enter a new field for the first time, your perspective is completely different to someone who can already "speak the language." You hear the melody and the rhythm without understanding the words. That's why I always tell students when they're first starting a new project to carefully journal and log all of their reflections, because those early impressions can be the most transformative.

What would you say is the most exciting discovery you've made in your career?
When we think about metabolism, especially in the context of metabolomics, we tend to imagine every cell operating with its own autonomous metabolic program. However, what's really interesting to me is realizing that these pathways aren't just occurring autonomously in every cell. Instead, cells share metabolic burdens among themselves as a community.

Understanding this cell-level metabolic synchronization is critical, and it's been particularly fascinating to study in the context of cancer. A few years ago, we demonstrated that a single localized tumor can alter the metabolism of cells throughout the entire body, which I still find astonishing. It's not just the cancer cells with altered metabolism as we typically think; the presence of a tumor impacts every other healthy cell too. Understanding how this works is a huge challenge, but it's something we're very excited about exploring.

Do you have a main ambition for the next five to ten years?
I think the next big step is to move beyond static snapshots of metabolism to capturing its dynamic nature. We need to understand what molecules are being exchanged between which cells and tissues. This will require developing better technologies to track metabolic processes in real-time, leveraging isotopes to trace the origin and flow of metabolites. The goal is to get a holistic view of how metabolism operates within the body and which tissues are chemically coupled to one other.

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