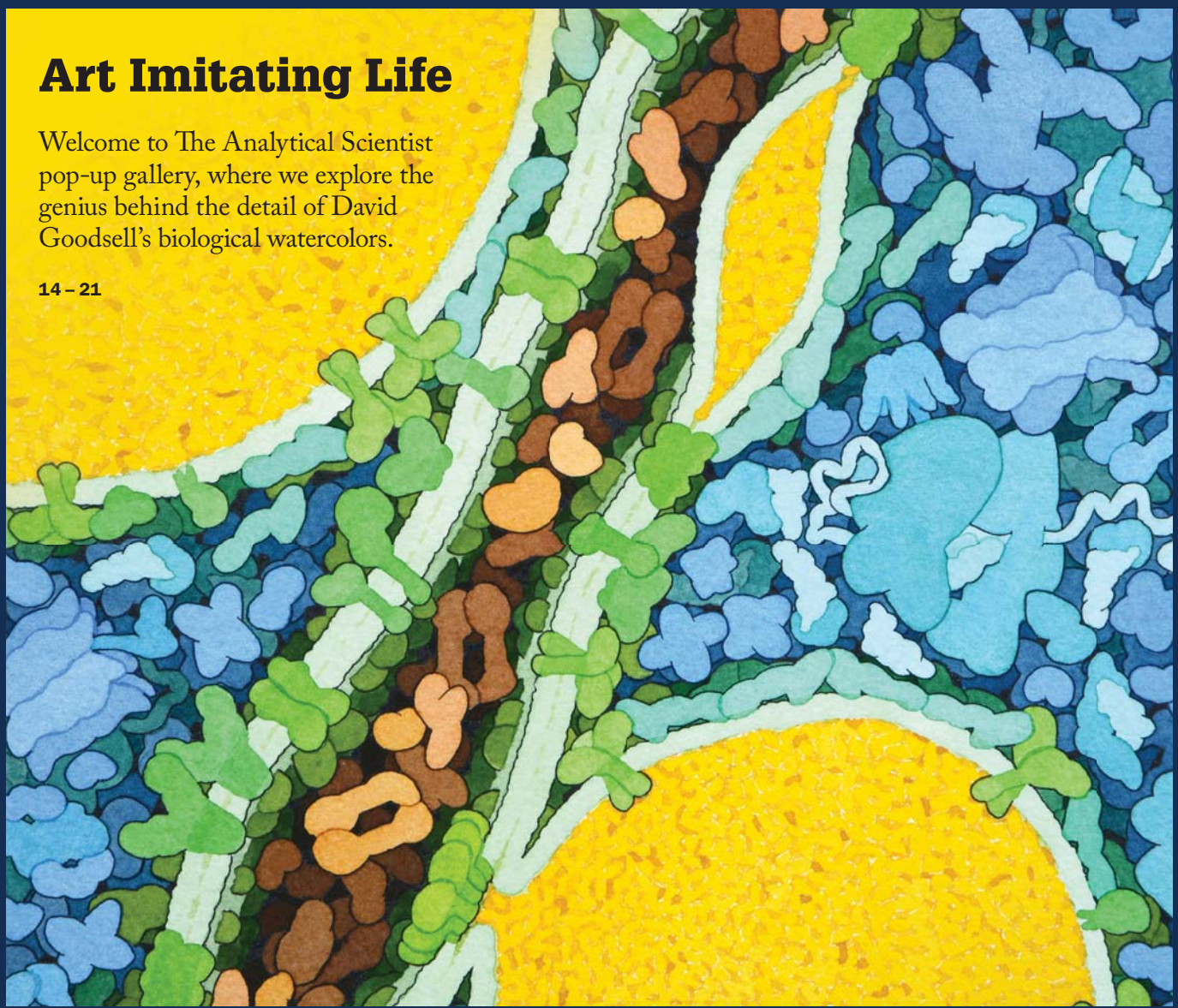
JULY / AUGUST 2020 # 89

the Analytical Scientist™

Art Imitating Life

Welcome to The Analytical Scientist pop-up gallery, where we explore the genius behind the detail of David Goodsell's biological watercolors.

14 – 21



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Daddy, What's the Big Deal with Antibodies?

The (bio)pharma industry is booming, and so is our role in its success

It blew me off my chair when my 12-year-old daughter suddenly showed an interest in my work. Evidently, the question did not arise from the scientific articles and reports that she had spotted on my desk (I really need to find more time to process these), but rather from a discussion on COVID-19 immunity and testing between a virologist and a politician she had heard on television. And so I did what any responsible parent with a background in life science and biochemistry would do: I gave her a short course on antibodies and antigens, immunoglobulins M and G, T-cells, natural killer cells, innate and adaptive immunity…

As I rambled on with great enthusiasm, it eventually dawned on me that she is probably too young for my nerd babble. That is, until she asked THE question: "Why can't we use antibodies as medicines to treat COVID-19 infection?"

In the last two decades, antibodies have reshaped the pharmaceutical landscape and are today amongst the fastest growing and most lucrative therapeutics (six out of the top ten best-selling drugs worldwide are antibody-based). These biotechnology-derived products are being used successfully in the treatment of cancers and autoimmune diseases, amongst others, and are now also being evaluated as COVID-19 therapies.

SPECIAL SERIES
(Bio)pharma

Though antibodies are top-of-mind, the healthcare industry is also welcoming other protein-based products (antibody-drug conjugates, fusion constructs, replacement enzymes), as well as nucleic acid (plasmid DNA, mRNA) and cell-based products. All of these share a common denominator: intriguing therapeutic potential and extreme structural complexity. They are the new kids on the block, gaining rapid popularity amongst pharma visionaries, but remaining somewhat mysterious idols in the analytical community.

Oh, boy! How we love to get analytical autographs from those new rock stars. With all eyes on pharma and biotech as they are today, we live in challenging yet exciting times for analytical scientists. New and increasingly complex therapeutics and vaccines are being developed at an exponential rate. And, amidst this race, our community must move quickly while maintaining an eagle-eye view of our subjects.

In honor of our crucial role in this space, The Analytical Scientist is running a four-month Special Series celebrating (bio)pharma in all its glory. Advances are lurking behind every corner, but which compelling stories will break the mold and make the difference. It's time to explore. You in?

Koen Sandra
CEO, RIC, Kortrijk, Belgium.

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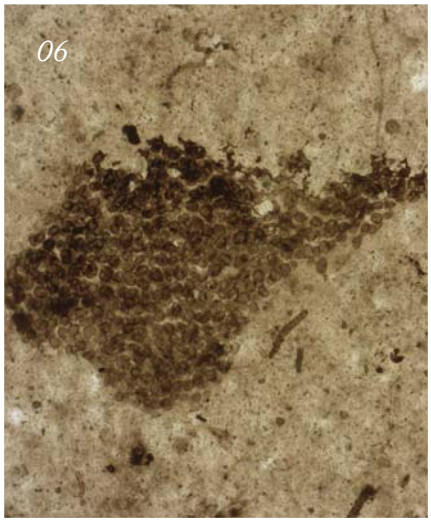

On The Cover

Lipid droplet formation depicted in watercolor – one of many David Goodsell originals currently on display at The Analytical Scientist pop-up gallery

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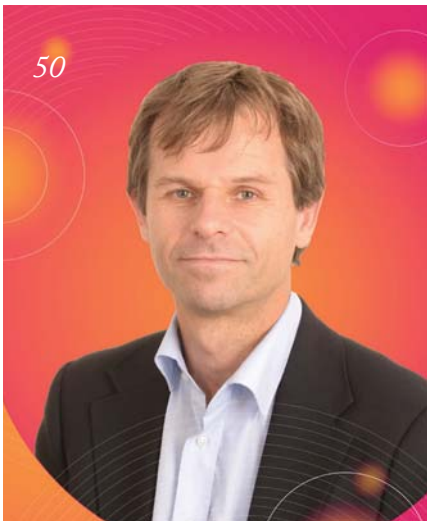

Sitting Down With

the Analytical Scientist

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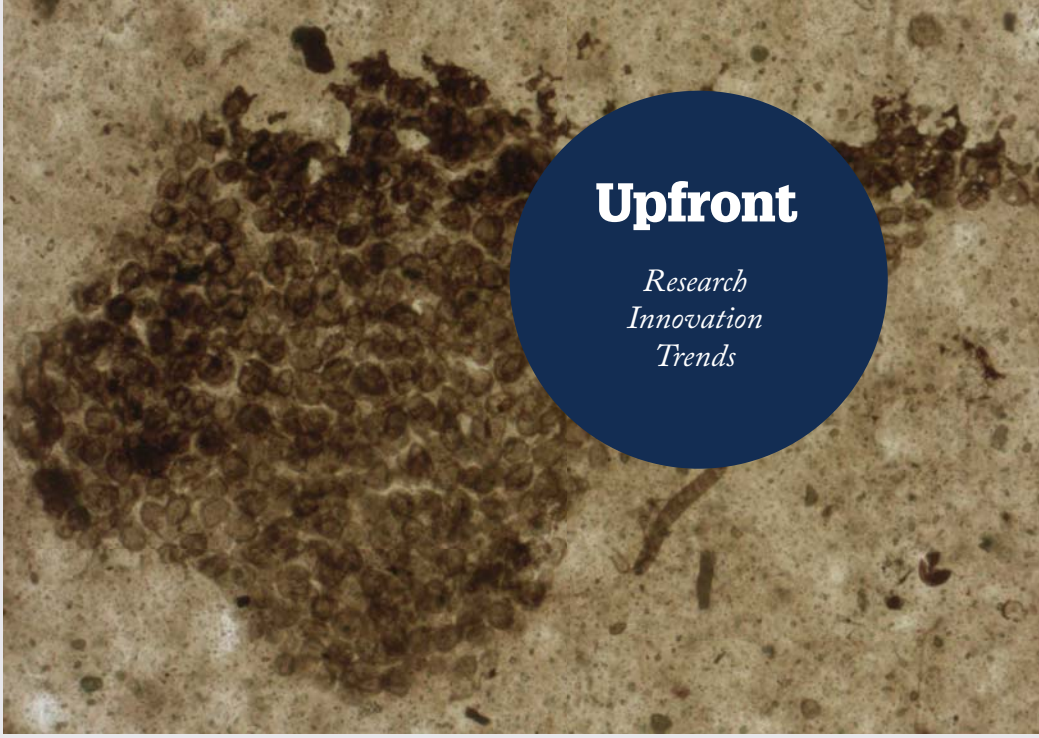

Upfront

Research
Innovation
Trends

Animal Origins

Microspectroscopy could help the search for Earth's first animals

Walk into any natural history museum and you'll see fossils that span the last 550 million years. But what about the first four billion years of Earth's history? Thanks to microfossils, we now know that single-cellular organisms emerged around 3.5 billion years ago. But fossils of this kind are rare – so Ross Anderson and his team from Oxford University set out to uncover the exceptional fossilization conditions that preserve them.

Currently, it is unclear whether microfossil occurrence patterns reflect evolution or simply mirror the distribution of favorable fossilization conditions – information vital to determining when animal life emerged on Earth. "We need to understand how these microfossils are formed so we can narrow our search for ancient life to appropriate rocks and counter the bias in our record of early evolution," says Anderson.

The team focused on kaolinite, an antibacterial clay known to play a role in fossilization. Using a combination of microanalytical techniques, they probed the sediment mineralogy surrounding 800-million-year-old microfossils for kaolinite. Vertical sections of the microfossils were imaged using energy-dispersive X-ray spectroscopy to map the distribution of aluminum (a constituent of kaolinite) across the samples. Synchrotron-based Fourier-transform infrared microspectroscopy was then used to confirm the presence of kaolinite at this microscopic scale.

"We found kaolinite-enriched 'halos' surrounding the fossils, suggesting it is critical to fossilization," says Anderson. The early fossil record may therefore be biased to environments where kaolinite production is high, such as tropical climates. Moreover, the team found no animal fossils in the 800 million-year-old rocks, despite conditions ripe for fossilization. "This would suggest that animals did not originate more than 800 million years ago," Anderson explains.

But the results are not just useful for earthly purposes – they could also help the hunt for life on Mars. "Our search for new microfossils should focus on kaolinite-rich muds," states Anderson. "These criteria may also be valid when exploring other planets for evidence of microscopic life – especially considering NASA's 2020 Perseverance rover mission will land in an area that is rich in clay and maybe even kaolinite."

Ostiana from the Wynniatt Formation, Canada. Either a colony of cyanobacteria or another green alga (an individual cell is ~12 µm). *Credit: University of Oxford/Royal Society.*

Reference

1. *R Anderson et al., Interface Focus (2020). DOI: 10.1098/rsfs.2020.0011*

TIMELINE

Black Chemists Who Made History

Exploring influential Black figures who shaped the field as we know it

Alice Ball (1916)

Ball solubilized the active oil in the chaulmoogra plant, enabling the development of injections to treat leprosy.

St Elmo Brady (1916)

Brady becomes the first African American to earn a PhD in chemistry. Later, he would create the first graduate program in chemistry at a historically Black college in the US.

Percy L. Julian (1935)

Julian successfully synthesized the glaucoma drug physostigmine, making it widely available for treatment of this disease.

BUSINESS IN BRIEF

A roundup of this month's business news, from virtual product launches to industry buyouts

- The Native Antigen Company (NAC), one of the first companies globally to provide antigens for SARS-CoV-2, has been acquired by LGC. NAC will continue to support efforts to fight COVID-19, with the move strengthening LGC's current offering to the molecular diagnostics sector (1).
- Back in March, Thermo Fisher agreed to buy out Qiagen after months of negotiations. But that was when there were fewer than 100,000 COVID-19 cases globally. Now, the demand for Qiagen's diagnostic technologies has skyrocketed, requiring Thermo Fisher to increase their offer. A final deal is expected in early 2021 (2).
- On its 50th anniversary in July 2020, SCIEX launched a virtual product experience enabling customers to explore some of its new software and instruments. This included the next installment of its flagship mass spectrometer, the SCIEX Triple Quad™ 7500 LC-MS/MS System – QTRAP® Ready (3).
- trinamiX, a subsidiary of BASF, has developed a handheld NIR spectrometer that quickly determines the composition of different plastics, aiding recycling processes. The data analysis can be performed anywhere via wireless cloud uploading and is linked to a mobile app for usability (4).
- Waters has announced a new fragmentation technique and imaging option for its high-resolution mass spectrometers, enhancing researchers' ability to probe peptides, proteins, and protein complexes in biomedical and pharmaceutical research (5).

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1. *LGC (2020). Available at: https://bit.ly/2CPDdEe.*
2. *ThermoFisher (2020). Available at: https://bit.ly/2CpQnbn.*
3. *SCIEX (2020). Available at: https://bit.ly/2E5hEAd.*
4. *BASF (2020). Available at: https://on.basf.com/2ZNNZ7a.*
5. *Waters (2020). Available at: https://bwnews.pr/2E5lYiV.*

A Kick From a Rose

MS-based metabolomics reveals how plants defend themselves from insect attack

The complex metabolic patterns of plants have long puzzled scientists. Do they alter their metabolome to more effectively defend against herbivorous insects? Or are the changes random, leaving their attackers unable to adapt?

A team of scientists, led by Dapeng Li, has managed to test these theories for the first time. "Thanks to modern MS-based metabolomics, you can now collect data on as many detected metabolites as you wish without existing knowledge," says Li.

His team used a combination of MS/MS-based metabolomics and statistical principles of information theory to analyze the metabolome of *Nicotiana attenuata*. Their results reveal that plants regulate metabolism in a directional manner, launching a highly-specific defense after insect attack. "In the future, we'd like to explore how circadian and diurnal patterns influence metabolism – a fundamental problem for sunlight-dependent plants," adds Li.

Reference

1. *D Li et al., Science Advances,6 (2020). DOI: 10.1126/sciadv.aaz0381*

Marie Maynard Daly (1947)

Some time after Brady, Daly becomes the first Black American woman to earn a PhD in chemistry.

Mary Elliott Hill and Carl Hill (Mid-1900s)

This husband and wife duo focused on plastics, with Mary designing spectroscopic approaches to track Grignard reactions that form ketenes.

Alma Levant Hayden (Mid-1950s)

A specialist in steroid detection via spectrometry, Hayden became one of the first Black federal scientists to work at the FDA.

Emmett W Chappelle (1990s)

Predominantly interested in bioluminescence, one of Chapelle's key breakthroughs was using laser-induced fluorescence to measure photosynthesis levels – a marker of crop health.

**This list is by no means exhaustive. If you would like to suggest ideas for similar content, feel free to contact our Editor at Matthew.Hallam@texerepublishing.com*

Pandemic Proteomics

MS-based proteomics is providing clues about the huge range of responses exhibited by patients

One of the major challenges we face in the fight against COVID-19 is a lack of knowledge surrounding host responses. Some are asymptomatic; others die – but what biological mechanisms underscore this chasmic disconnect?

Christoph Messner and colleagues are unraveling the mystery (1). Having spent the last few years developing an MS-based, high-throughput proteomics platform at the Francis Crick Institute, the team can quantify over 200 proteins per sample in less than 10 minutes. "It was obvious that our proteomics platform could be used as a powerful tool to study the plasma proteins of patients infected with SARS-CoV-2," Messner says. "We set out to assess protein-level host responses with the hope of identifying markers for disease severity."

And that's just what they did. Samples from the first COVID-19 patients hospitalized at the Charité University Hospital in Berlin were subjected to MS-based proteomics by applying Sequential Window Acquisition of All Theoretical Mass Spectra. Interestingly, the platform uses a standard-flow ultra-high-performance LC system, rather than the usual nano-LC, to reduce run-to-run time and increase robustness. Coupled with semi-automated sample preparation, which allows the preparation of four 96-well plates in parallel, the team can analyze hundreds of samples per day.

"We found 27 biomarkers that classify mild and severe forms of COVID-19, some of which may represent therapeutic targets," Messner says. "These proteins highlight roles for complement factors, the coagulation system, and inflammatory mediators (including proinflammatory signaling molecules up- and downstream of interleukin-6) in the SARS-CoV-2 host response." The hope now is that these markers could be targeted in routine tests, allowing doctors to earmark patients at increased risk of critical illness.

The researchers are currently advancing their work in larger, longitudinal patient cohorts to refine the identified biomarkers and build models to predict COVID-19 progression. At the same time, they are developing multiple reaction monitoring assays to guide treatment decisions in hospitals.

Reference

1. *CB Messner et al., Cell Syst [ePub ahead of print] (2020). DOI: 10.1016/j.cels.2020.05.012*

The Usual Suspect

Every major mass extinction can now be linked to global warming

The cause of the Late Ordovician Mass Extinction (LOME), one of the five largest extinction events recognized by geologists, has long been debated. While the other events were associated with temperature increases, LOME was linked to cooling and glaciation. Now, researchers have found evidence tying it to global warming.

Using MS to analyze rock samples, they looked for signs of the huge volcanic eruptions typical of every other mass extinction. "The data suggests LOME was associated with volcanic activity, which would have triggered global warming and led to an anoxic ocean that suffocated marine life," says Bond, co-author of the paper. "This discovery is extremely important. It proves that, since complex life evolved on Earth, all mass extinctions can be tied to global warming."

Reference

1. *VD Bond, S Grasby, Geology (2020). DOI: 10.1130/G47377.1*

IMAGE OF THE MONTH

The (Enduring) Scream

Edvard Munch's "The Scream" has stood the test of time as a prominent portrayal of our anxiety-laden world, but the painting is beginning to show its age. By analyzing flaking paint using micro X-ray diffraction, micro X-ray fluorescence and micro X-ray absorption near-edge structure spectroscopy, researchers have identified moisture and mobile chlorine compounds as the culprits behind the degradation. They were also able to recommend optimum storage conditions to protect the painting – advice that could help protect the works of other greats like van Gogh and Matisse.

Image credit: Photograph of The Scream (ca. 1910) (Munch Museum, Oslo; catalogue n. Woll.M.896). Irina Crina Anca Sandu and Eva Storevik Tveit, Munch Museum.

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

QUOTE OF THE MONTH

"You too can be an ally. Let's work together to eradicate systemic racism in STEM. Let's work together to clear the air and pave the way for great science to be conducted amongst all races and genders."

Candice Ulmer (@drcandice_u) Clinical Research Chemist at the CDC and co-founder of the Coalition of Black Mass Spectrometrists. Look out for more content from the Coalition in future issues.

A Matter of Precision (Spectroscopy)

High-precision spectroscopy of short-lived radioactive molecules could help solve the mysteries surrounding matter in our universe

Scientists have, for the first time, used high-precision spectroscopy to study short-lived radioactive molecules – research that could push particle physics exploration beyond the standard model.

Copyright: KU Leuven – Rob Stevens.

Their use of collinear resonance ionization spectroscopy yielded highly sensitive measurements of artificially created radium monofluoride (RaF) molecules. RaF is of particular interest in high-precision spectroscopy because it is thought to have an electronic structure appropriate for laser cooling.

"Our results indicate that RaF molecules do, in fact, have a structure that will facilitate their laser cooling," says Ronald Fernando Garcia Ruiz, the study's lead author. "Not only is this the first major step towards high-precision measurements of these subatomic properties, but it could offer a sensitive sensor for future dark matter searches."

Reference

1. *RF Garcia Ruiz et al., Nature, 581, 396 (2020). DOI: 10.1038/s41586-020-2299-4*

In My View

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

Kickstarting Publishing Parity

Women face many obstacles in the worlds of academia and publishing – and it's time that changed

By Emma Wilson, Director of Publishing, Royal Society of Chemistry, UK

Science has the power to solve many of our global challenges, not least the pandemic we currently face. In rising to this occasion, it has never been more important for science to attract, develop, and retain a diverse group of talented people. Why? Because diverse teams deliver better results – and because it's our ethical obligation to encourage inclusivity in science. Unfortunately, many people's personal experiences, and much of the available data, suggest that science still suffers from bias and discrimination against underrepresented groups.

I lead the publishing program for the Royal Society of Chemistry and have always been passionate about the contributions of women in both science and academic publishing. Over the past few years, I have combined these interests through my involvement in developing a framework for action (1). The framework, a tool for editorial decision-makers, is designed to drive progress on inclusion and diversity in terms of gender and beyond. What we need right now is action – and it seems other publishers agree. As a result, we've combined forces with 19 other organizations to act on inclusion and diversity in academic publishing.

Over the years, our community has worked to create change, but we needed to go further – and faster. We began the hunt for more information three years ago, and published two reports (2) (3) detailing data on the lack of diversity. But that's not all. We also identified a need for greater transparency and uncovered a number of barriers women face regarding retention and progression in UK academia. Hungry for more data on these barriers, we realized that we were sitting on a huge pile of incredibly rich information we could mine – our journal publications.

We carried out an in-depth gender analysis of each stage of the publication process, analyzing more than 700,000 manuscript submissions and 141,000 citations of our journals. The data are published in our report – "Is publishing in the chemical sciences gender biased?" (4) – and in a peer-reviewed paper (5). Our analyses showed that gender differences exist at each step of the publishing process. Many appear minor in isolation, but their combined effect puts women at a significant disadvantage. Knowing this, we had to act – a decision that culminated a year later in our framework, which we believe sets the standard for driving change in the academic publishing industry. It's a "go-to" reference guide that outlines the methods by which we can achieve change and how this change can be quantified across the publishing process.

We always planned to roll the framework out across our editorial teams, but thought it was too important to keep to ourselves once it was complete. What we needed was a workshop – so we swiftly organized one via Zoom at the start of lockdown. Eight other publishers joined us, and we found that we had huge areas of common interest, as well as a genuine desire to support positive action in our community. The workshop concluded with a joint commitment (6), now signed by 19 publishers with portfolios in excess of 7,000 journals across many disciplines.

Our pledge: to scrutinize our publishing processes and take action to achieve a minimum standard for inclusion in publishing. It's still early days, but this is important work – and I'm absolutely energized to play a role in it.

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6. *Royal Society of Chemistry, "Joint commitment for action on inclusion and diversity in publishing" (2020). Available at: https://rsc.li/391bM6g.*

Behind Every Instrument...

Analytical scientists are not just another machine in the lab – we must learn from this period of disruption and strive to take better care of ourselves and our colleagues

By Zoë Ayres, Industrial Research Scientist and mental health advocate, UK

With the need for high-throughput, rapid, and reliable testing, analytical science has readily taken its position at the forefront of the COVID-19 battle. With daily updates about the creation of new techniques to help tackle the crisis, it's easy to forget about the people behind the machines. And yet, behind every test, and behind every instrument, there is a human being.

That's why, as we move towards a "new normal" in the post-pandemic world, I encourage you to use this disruptive period as an opportunity to disrupt our thinking about mental and physical wellbeing in science.

Many of us have found ourselves without a lab in recent months. For some, this has provided an opportunity to work from home – perhaps for the first time. While some of us have been counting down the days until we can set foot in our labs again, others have welcomed the opportunity to trial home working. But even when we do return, we should consider the benefits that flexible working brings. Are there ways to schedule data processing days at home, allowing people to save valuable

time? Could meetings be consolidated to a few days a week, allowing people to work from home more often? This could not only improve the workplace experience for many, but potentially increase efficiency in the long run. Flexibility is also likely to promote a more inclusive workforce by allowing people to fit their work around other aspects of their lives, such as caring responsibilities.

The pandemic has also emphasized the importance of mental health. Burnout – emotional or physical exhaustion brought about by overwork or stress – ultimately leads to reduced productivity and output. With the constant pressure to do more and achieve more, and now the added emotional load of a global pandemic, it is vital that we prioritize the wellbeing of our researchers going forward. However, many of us are not comfortable being open about our mental health. This is perhaps not surprising. As analytical scientists, we feel we must be just that – analytical. It's hard to analyze our own mental health and wellbeing or talk about the pressures we face. This needs to change.

"It is essential that we create working environments where people feel supported and can thrive, otherwise we risk losing valuable talent."

If you remain unconvinced by the need to improve mental health awareness and support within the analytical sciences, perhaps it's easier to talk about it in more familiar terms? As analysts, we all understand the importance of calibrations. They are vital for us to have confidence in our results and draw meaningful conclusions. This same logic applies to our own mental health – our internal calibration. If not properly addressed, we cannot perform at our best. It is essential that we create working environments where people feel supported and can thrive, otherwise we risk losing valuable talent.

During this uncertain time, we should acknowledge the opportunity to reflect on and review the culture in our companies and institutions. It is only by creating a more inclusive, compassionate, and supportive environment for all that we will be able to solve the greatest analytical problems of our future.

The Proteins of Prehistory

Skepticism surrounding paleoproteomics is understandable, but unfounded – here's why

By Troy D. Wood, Associate Professor at the University at Buffalo, New York, USA; Connor Gould, graduate student in chemistry at the University at Buffalo; and Emily Sekera, postdoctoral associate at the Ohio State University, Columbus, Ohio, USA.

The hit movie Jurassic Park sparked broad interest in paleontology by raising the tantalizing possibility of bringing dinosaurs back from extinction. In the film, this was accomplished

by extracting dinosaur DNA from mosquitoes preserved in ancient amber. That, however, remains a critical plot element and nothing more; the general scientific consensus is that DNA has a half-life of hundreds to thousands of years at most.

Proteins, on the other hand, have much longer half-lives – hundreds of thousands to over a million years under optimal storage conditions. Plus, we have long known that amino acids can be extracted from fossilized hard tissues. This knowledge, and a desire to look deeper, gave rise to paleoproteomics, an interdisciplinary field that examines ancient proteins to study the molecular-level adaptations of species – and evolution itself.

Crucial evolutionary changes emerged during the age of the dinosaurs: gigantism, endothermy, and the development of feathers, to name just a few. Due to protein degradation, however, conventional wisdom asserts that paleoproteomic investigations carry a very low probability of success. But what if the proteins did not degrade? Dinosaur fossils are intriguing reservoirs of ancient protein because the fossilization process can impede

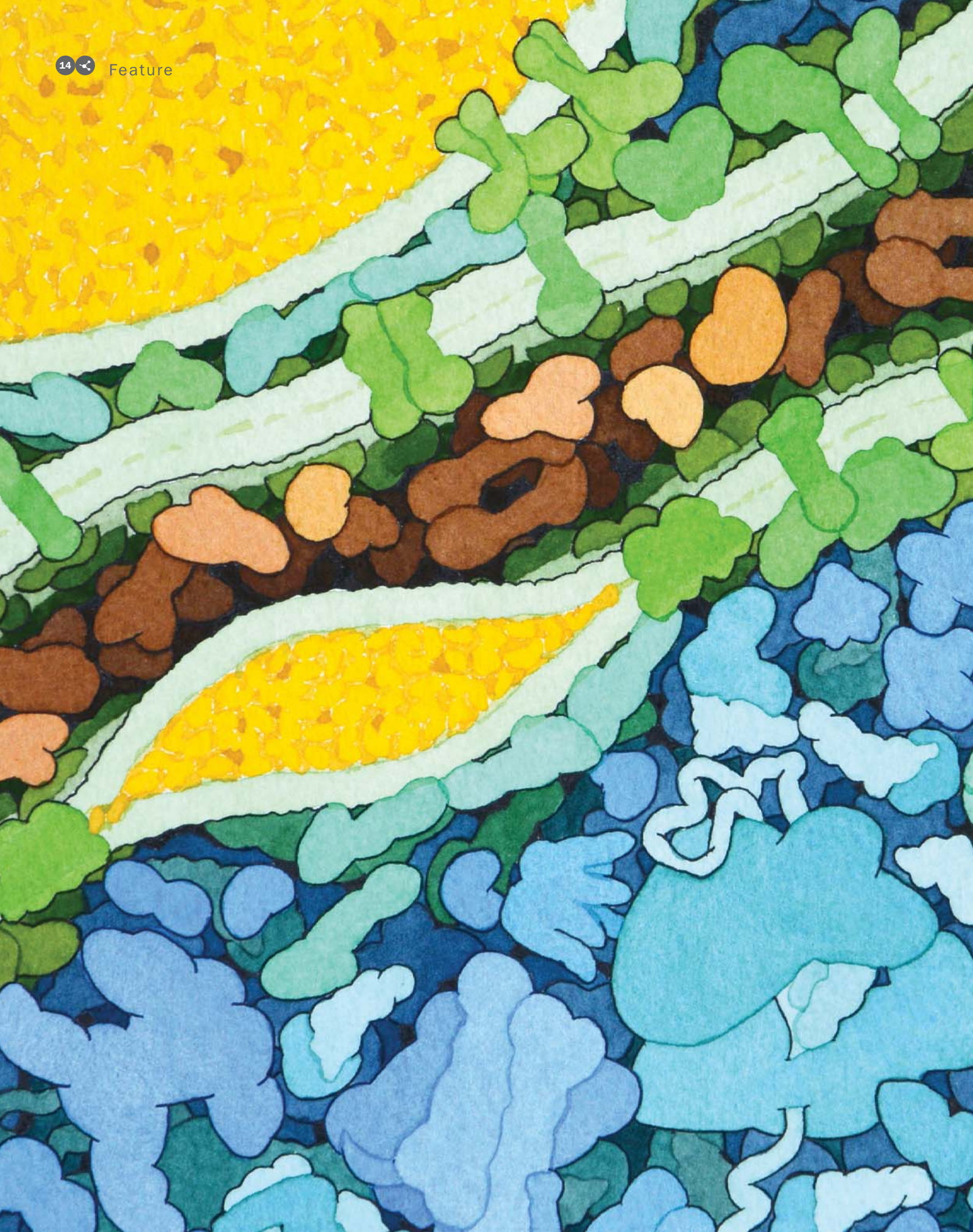
degradation and loss. In fact, reports detail the discovery of proteins such as collagen (the most abundant protein in vertebrates and fundamental to animal evolution) in dinosaur fossils using MS – but, because of the potential for exogenous protein contamination, these reports have been met with skepticism.

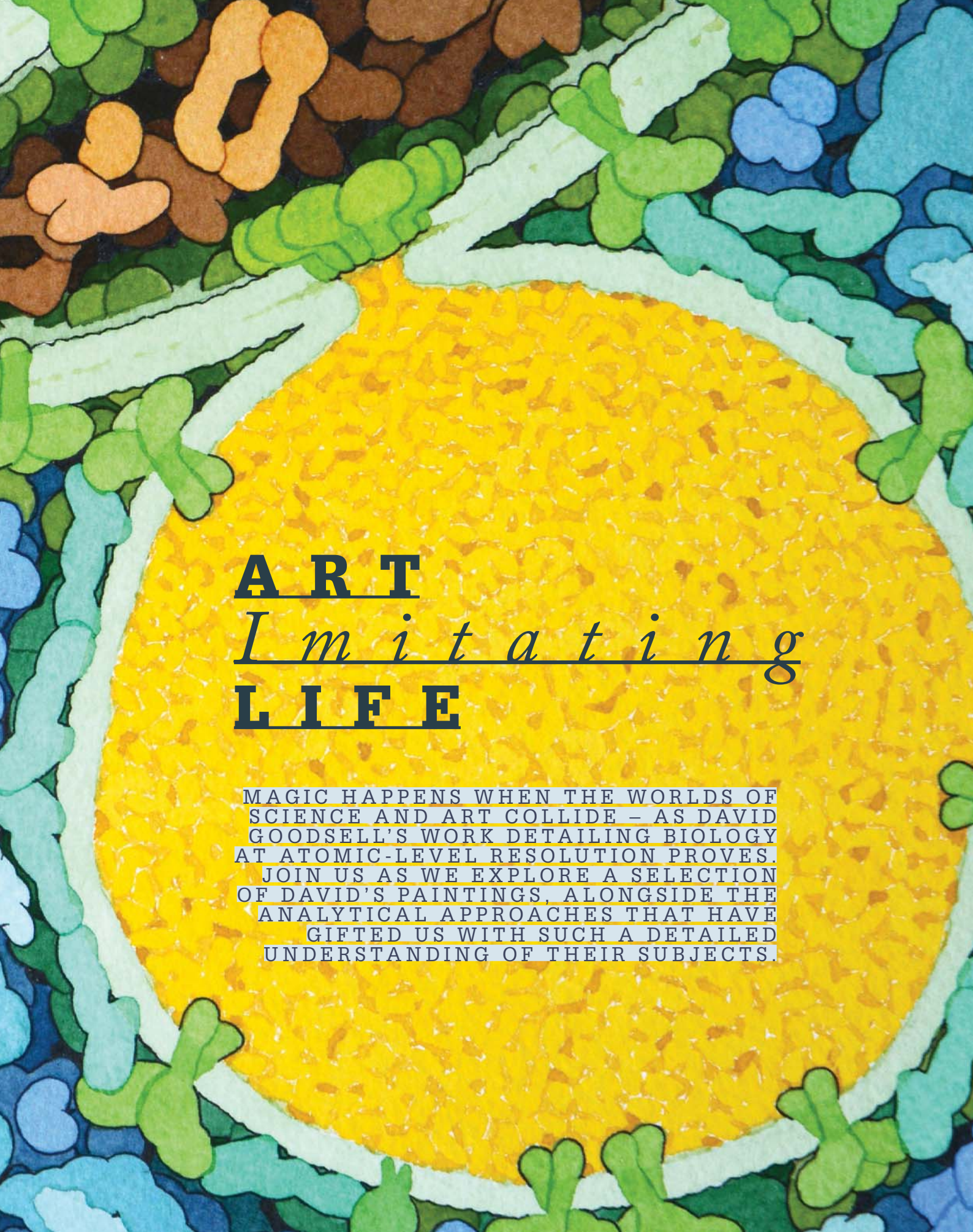
To have detected ancient dinosaur protein is indeed an extraordinary claim – one that must be supported by extraordinary evidence. But we feel that the criticism overlooks one important point: that evidence of a protein's existence does not require the detection of a full protein sequence. Distinctive post-translational modifications (such as extensive proline hydroxylation in collagen) can support its identification in fossils. Blanks and independent methods of analysis can lower the probability of false positives or misinterpreted results due to contamination. Here, we suggest additional criteria to bolster positive reports of protein detection from dinosaur fossils.

Because the genomes of dinosaur species are not known, every putative protein identified from fossils is a unique chemical entity. But even ancient proteins should have molecular relationships to those in modern animals – particularly birds and reptiles – which makes sequence homology with other species essential to these studies. We also advocate the introduction of fresh, immobilized enzyme microreactors to digest extracted protein in the microreactor environment and maximize digestion efficiency; using multiple enzyme microreactors will enhance sequence coverage for such identifications.

What's more, with time, chiral amino acids in proteins will racemize. Although insufficient protein recovery from dinosaur fossil specimens is an issue, we believe that (where sufficient sample exists) measuring the D:L ratios in acid hydrolysates is critical. Low D:L ratios suggest that a protein is not ancient and is likely the result of exogenous contamination.

The greatest discoveries in science are achieved by those willing to challenge conventional paradigms. In our view, there is sufficient, carefully collected evidence from fossils to suggest that protein molecules from dinosaurs are detectable – and just waiting to be discovered!





ART *Imitating* LIFE

MAGIC HAPPENS WHEN THE WORLDS OF SCIENCE AND ART COLLIDE – AS DAVID GOODSELL'S WORK DETAILING BIOLOGY AT ATOMIC-LEVEL RESOLUTION PROVES. JOIN US AS WE EXPLORE A SELECTION OF DAVID'S PAINTINGS, ALONGSIDE THE ANALYTICAL APPROACHES THAT HAVE GIFTED US WITH SUCH A DETAILED UNDERSTANDING OF THEIR SUBJECTS.

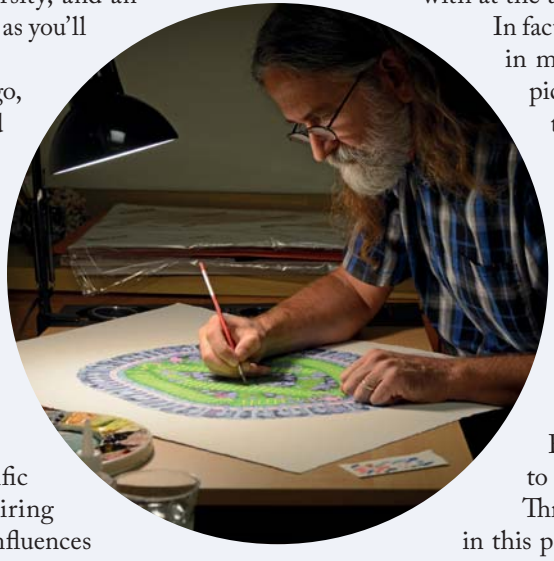
Immunological Synapse, 2020

Hello, and welcome to The Analytical Scientist's pop-up art gallery! I'm Matthew – Editor of The Analytical Scientist– and I'll be your guide on today's tour. If you'd be kind enough to leave any coats and bags in our cloakroom, I'll begin by sharing a short message from the artist behind the pieces we have on show. Over to you, David Goodsell!

FROM THE ARTIST

Thanks, Matty. Hello everyone, I'm David Goodsell, Professor of Computational Biology at the Scripps Research Institute, Research Professor at Rutgers University, and an avid painter of biological phenomena – as you'll see for yourself shortly.

I started in the science world long ago, completing my doctorate with Richard Dickerson at UCLA using X-ray crystallography and computational modeling to explore the structure of DNA. I subsequently completed a postdoc with Arthur Olson at Scripps Research; this portion of my studies focused on molecular graphics and methods for computational drug design. My art training, on the other hand, is purely informal.

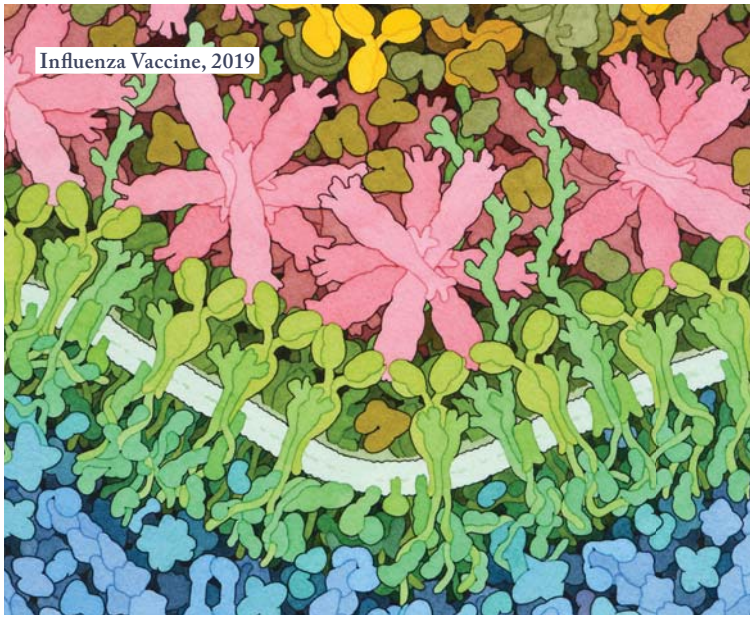


I am a voracious consumer of scientific imagery, and draw from more inspiring examples than I can count. My early influences are the Golden Nature Guides and Time-Life Science Library, and the wonderful work of Chesley Bonestell, Roger Hayward, Irving Geis, and Jane Richardson. Feeding on these inspirations, I started working on my own cellular landscape during my postdoc. The paintings were a creative way for me to reconnect with larger themes in biology, the structural aspects of which I was becoming so involved with at the time.

In fact, I articulated the aim of these pieces in my first paper on the topic: "A clear picture of the interior of a living cell that shows the average distribution of molecules at the proper scale, the proper concentration, and with no missing parts seems to me to be central to the understanding of the working of life." Today, this remains as the clearest description that I can apply to my work, and I integrate information from the RCSB Protein Data Bank, UniProt, EMDataBank, and primary literature to bring my vision to reality on paper.

Three exhibitions of my work are on show in this pop-up gallery at present. I hope you enjoy viewing each of them as much as I enjoyed producing them. Now, I'll hand back to your tour guide; the first exhibition awaits!

Poliovirus Neutralization, 2019



Influenza Vaccine, 2019

THE VAX SERIES

Here it is: the VAX series. This collection hosts paintings that explore the molecular basis of one of mankind's greatest protectors – the vaccine. Let's get started!

Immunological Synapse, 2020

Here, we see David's representation of the immunological synapse – the response-promoting interface between lymphocytes (bottom) and antigen-presenting cells (top). In this case, the painting demonstrates the presentation of a viral component (red) with major histocompatibility complex (MHC) proteins (sky blue) to T-cells through T-cell receptors (green).

Images of the T-cell activation complex were first captured by Don Wiley via X-ray crystallography in 1996. These high-resolution images were quickly hailed as the "holy grail of immunology" – finally demonstrating this critical bodily response in 3D detail and opening the door to untold medical (and in David's case, artistic) opportunities.

Identification of MHC-bound viral peptides (collectively referred to as the immunopeptidome) is crucial for vaccine development, and benefited substantially from the pioneering work of Donald Hunt, who applied data-dependent analysis MS to yield previously untold knowledge of these molecules in the 1990s (1). Today, contemporary techniques enabled by impressive chromatographic advances – such as nano-ultra-performance LC linked to high-resolution MS – enable identification of these peptides from numerous cell types, including cancerous and infected cells, in just two to three days (2).

Poliovirus Neutralization, 2019

The second piece in this collection portrays the aggregation of poliovirus particles by antibodies. These antibodies (yellow) are produced in the bodies of vaccinated individuals and neutralize the virus (pink) by blocking the infection of somatic cells. In the absence of antibodies, poliovirus causes poliomyelitis – a potentially paralyzing condition caused by motor neuron destruction. This now-rare disease was the destructive force that necessitated the iron lung.

The virus' structure was first elucidated by a team led by Rosalind Franklin – today famous for her contribution to discovery of the DNA double helix via Photo 51 – using X-ray diffraction. X-ray crystallographic research in the 1980s then demonstrated the icosahedral shape of the capsid, composed of 60 copies of four different coat proteins: VP1, VP2, VP3 and VP4 (3). These components are represented by the overlapping pink shapes comprising each viral particle in the painting.

Three poliovirus serotypes are known, each the result of differing antigenic sites on their capsid proteins. These serotypes differ in their virulence and can be resolved today using matrix-assisted laser desorption/ionization time-of-flight MS (MALDI-TOF MS) (4).

Influenza Vaccine, 2019

The final piece in our VAX series exhibition depicts a recombinant influenza vaccine (red) based on viral hemagglutinin – a glycoprotein that mediates infection. Binding of the vaccine to B-cell receptors (green) leads to the production of antibodies protective against the influenza virus. Adaptive immunity of this kind underscores the mechanism by which all vaccines work.

Hemagglutinin binds to human cells – namely erythrocytes and those of the upper respiratory tract – via cell-surface sialic acid to facilitate viral internalization and replication. Though X-ray crystallography gave the first demonstration of hemagglutinin's 3D structure in complex with sialic acid (5), nuclear magnetic resonance (NMR) spectroscopy was a driving force in understanding the specific components of sialic acid that are required for hemagglutinin attachment (6). And NMR is still useful for such studies today; its combination with data-driven molecular dynamics simulations can provide essential information for anti-influenza drug design in the case of new pandemics (7).

The same binding mechanism is utilized by B-cell receptors, enabling the antibody-producing binding process depicted in the center of David's piece.

CORONAVIRUS IN TECHNICOLOR

As the information available to us about SARS-CoV-2 expands, we are painting an increasingly vivid picture of its structure and behavior. In this exhibit, David presents these advances through his own artistic lens.

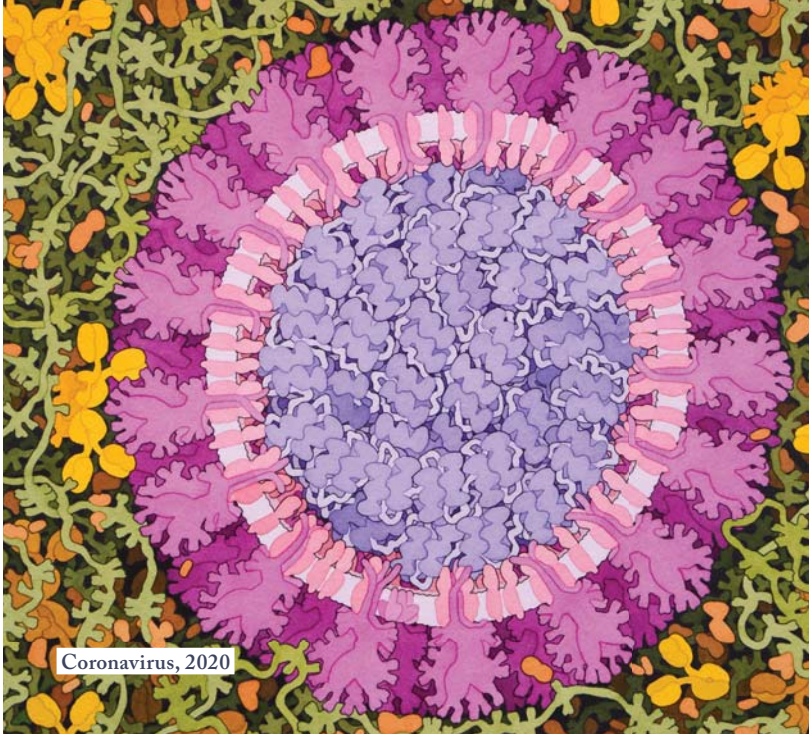
Coronavirus, 2020

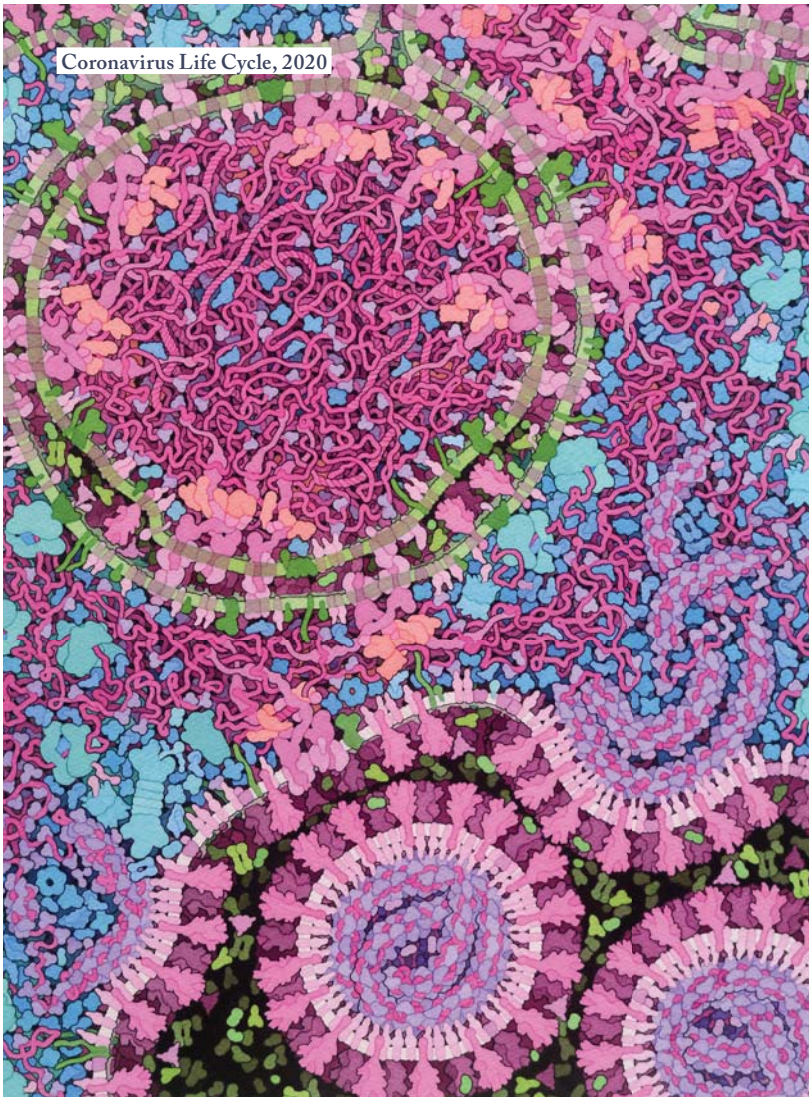
The first of our two coronavirus pieces depicts the virus after entry to the lungs. Here we see the virus (center) surrounded by mucus, secreted antibodies (yellow), and various immune system proteins (orange hues).

Membrane proteins (peach), envelope proteins (small pink proteins contained in the white membrane) and spike proteins (pink) comprise the viral membrane – the latter mediates entry to host cells. The dangerous potential of zoonotic coronaviruses has been long-recognized by the infectious disease community, and the association of ubiquitinated envelope proteins and spike proteins was first demonstrated using tandem affinity purification-MS in 2010 (8). Following the SARS-CoV-2 outbreak, spike protein binding to human ACE2 has been studied in greater detail using biolayer interferometry – providing clues as to its ability to transmit so rapidly (9). Sugar molecules not present in hosts have also been identified in association with the virus' spike protein using LC-MS (10).

But these are just a few of many examples of investigation by the scientific community. The nail-like shape of the post-fusion spike protein was first demonstrated by transmission electron microscopy (alongside a general structure of the full viral particle) (11), for example, and the envelope protein shape depicted in David's painting is based on a structural SARS-CoV E-channel model elucidated by NMR (12).

"As a disclaimer, the painting is based on the current state of our ever-changing knowledge of the virus, meaning portions of it are speculative."

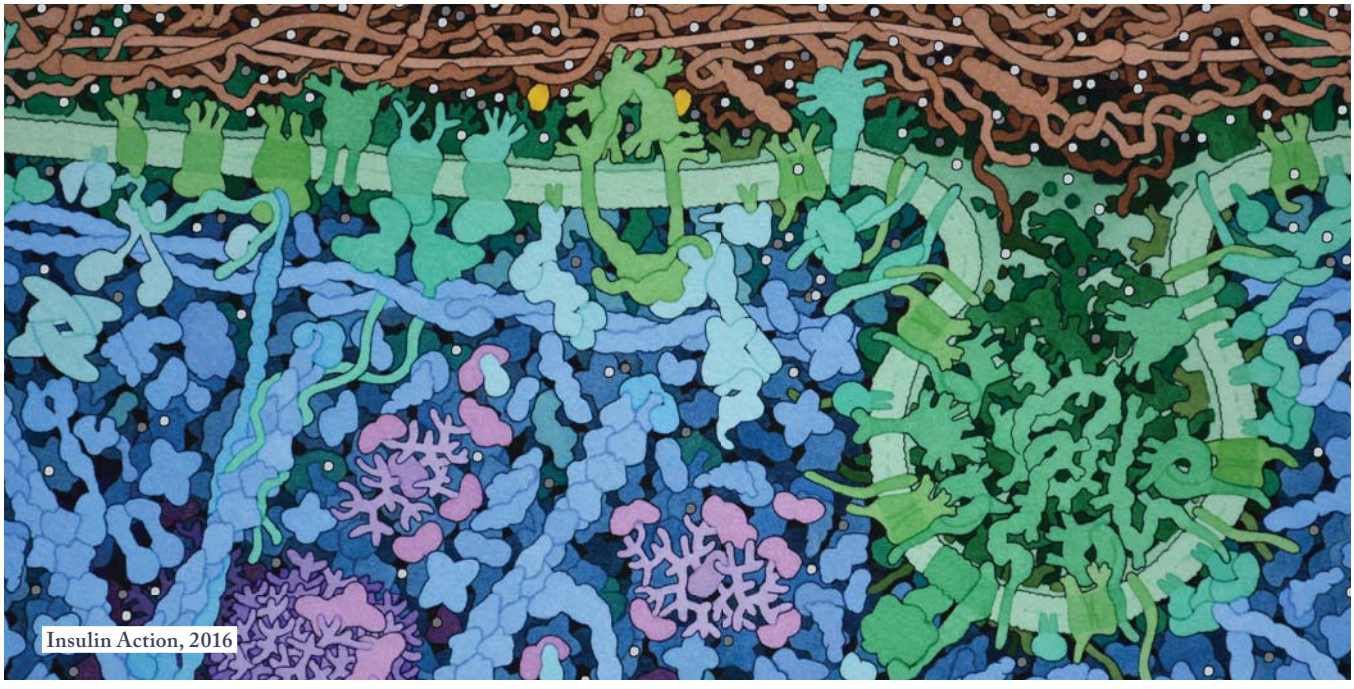

Coronavirus, 2020


Coronavirus Life Cycle, 2020

Coronavirus Life Cycle, 2020

This piece depicts an entirely different view of the virus: a cross-section through an infected somatic cell, showing the replication of an internalized viral particle. Host-cell molecules (blue and green) surround two mature virions (purple and pink, in the bottom right), with a budding virus (pink and blue, representing viral nucleoprotein bound to RNA) protruding from the surrounding membrane. Viral replicase complexes (peach and purple) are associated with host-cell membranes, especially the double-membrane vesicle (enclosed by green in the top left), and work to copy the virus' RNA genome (purple curls throughout the cell). Numerous accessory proteins with roles in virulence are also shown throughout the cytoplasm. As a disclaimer, this painting is based on the current state of our ever-changing knowledge of the virus, meaning that portions of it are speculative.

The structure of the viral capsid bound to RNA (the nucleocapsid, visible in the association of the blue and pink coils with viral membrane) has been determined at 2.7 Å detail using X-ray crystallography (13). Binding of the nucleocapsid protein to RNA is mediated by electrostatic interactions between genetic material and an RNA-binding pocket at the protein N-terminal; given the key role of the nucleocapsid in several stages of the virus life cycle, researchers believe this interaction may represent a promising drug target. Similar hopes also surround expanding knowledge of the viral protease (14) and polymerase – elements of the SARS-CoV-2 replicase. Remdesivir, which is currently receiving much attention in the press for its potential anti-COVID-19 activity, targets the RNA-dependent RNA polymerase.



Insulin Action, 2016

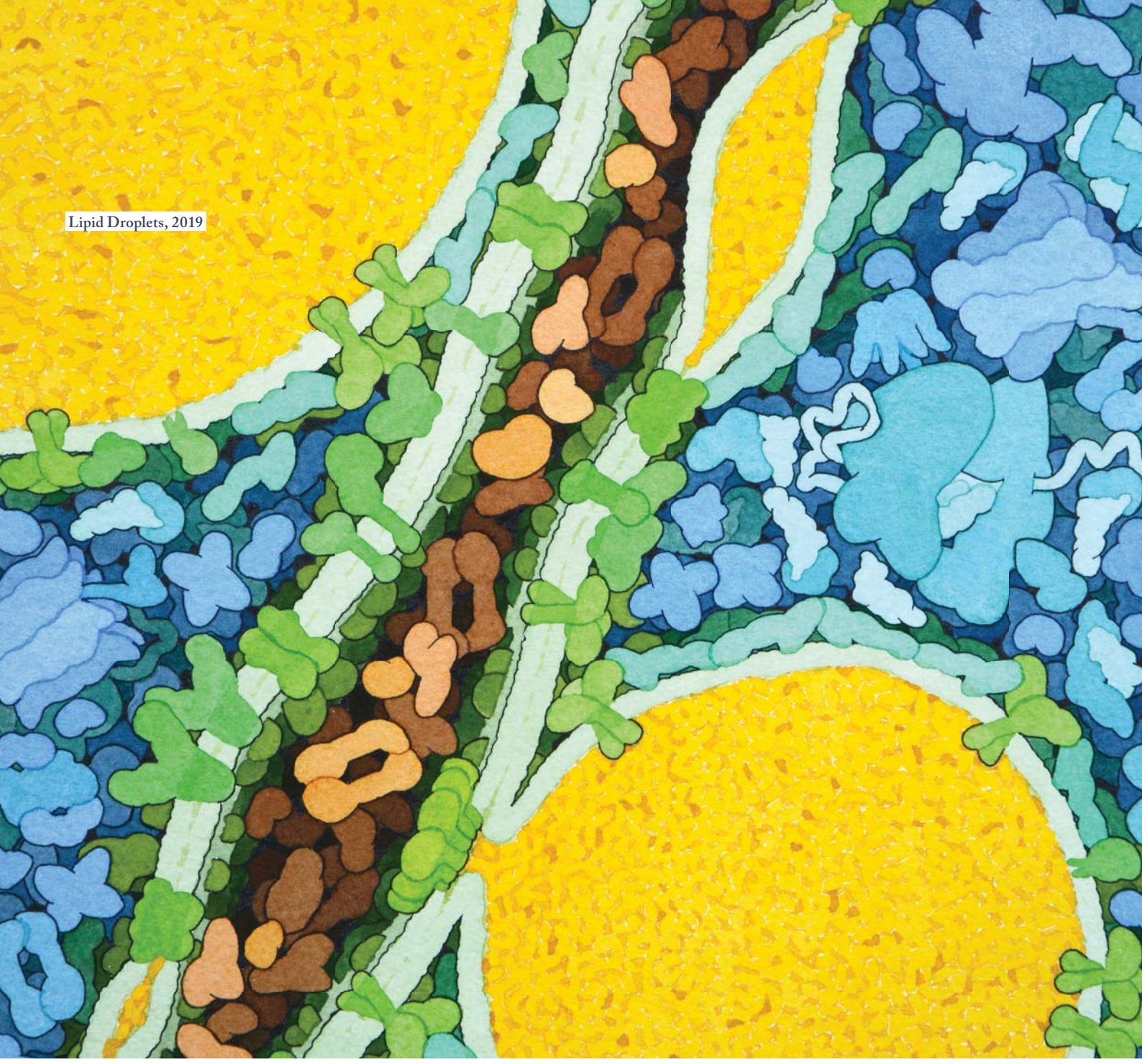
THE BODY IN ACTION

The three bodily actions we've chosen to take aim at in this final exhibit range from the infamous to much lesser known, and each has their own important implications for functioning and disease. If you turn to your right, we can begin with painting number one: "Insulin Action."

Insulin Action, 2016

This painting shows insulin molecules (yellow, at the top) binding to insulin receptors (green complex within the membrane), and the subsequent cell signaling pathways and events that are induced.

The resulting signal cascade is represented by the complexed grey-blue shapes associated with the insulin receptor. In response, glucose transporters (green membrane channels) move to the cell surface to facilitate glucose import; some is



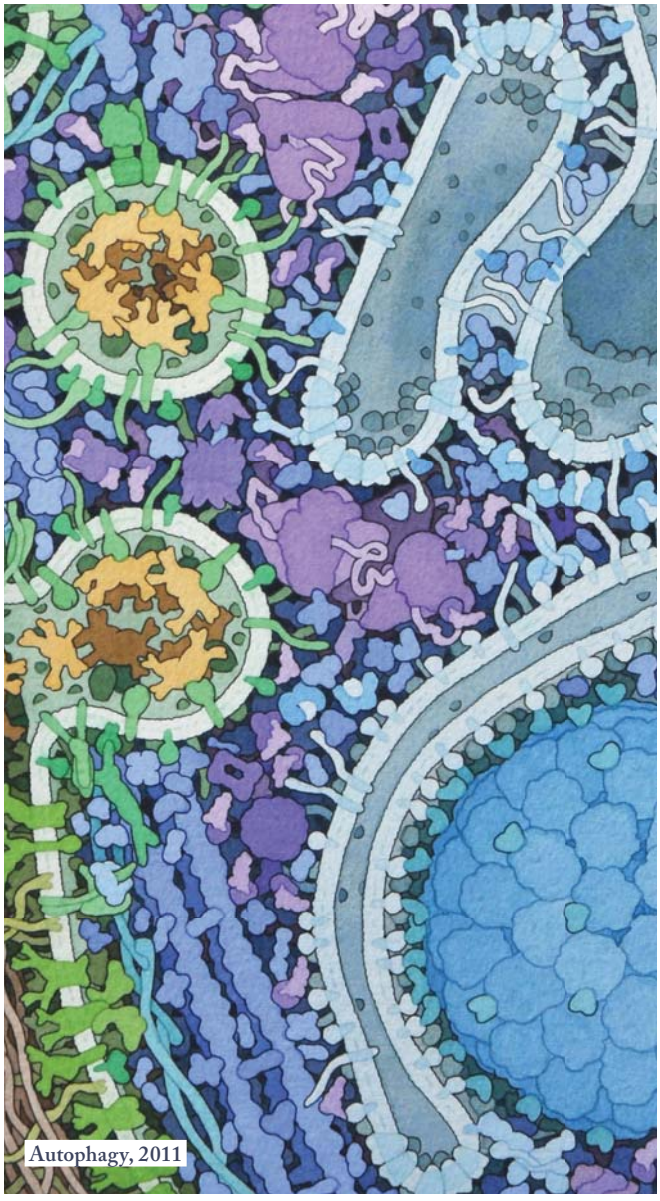
Lipid Droplets, 2019

used for cellular processes, but much is converted into glycogen (pink-purple) by glycogen synthase (pink shapes associated with the glycogen) to be broken down again and used later.

The discovery of insulin in 1921 was one of the greatest medical discoveries of the century; previously, type 1 diabetes patients rarely survived over a couple of years. Today, we are well acquainted with insulin's structure in both crystal (via X-ray diffraction) and solution (via NMR) . These approaches were key in determining that insulin is stored as a hexamer stabilized by zinc, but that it exerts its biological effects as a monomer (15). The secondary structure of the transmembrane domain of the insulin receptor was also deciphered in solution using C^{13} NMR, and paramagnetic relaxation enhancement using N^{15}-labeled samples indicates the exposed nature of residues following this domain to aqueous solution (16).

Lipid Droplets, 2019

Here we see the process of lipid droplet formation in somatic cells. The painting shows the lipids (yellow) being enclosed by leaflets of the lipid bilayer of the endoplasmic reticulum (running


Autophagy, 2011

diagonally through the center). Droplets then bud from the bilayer and migrate into the cytoplasm, where they serve as oil-based reserves of metabolic energy and membrane components.

Seipin protein (the green shapes connecting the lower droplet to the central membrane) assists with this budding process. This role, carried out in association with membrane-shaping protein Pex30, has been investigated in eukaryotic cells using nano-LC-MS (17); these experiments showed toxic triacylglycerol accumulation in cells lacking Pex30 and seipin – highlighting the importance of the process.

"Autophagy is involved in many disease states, and is one of many cellular obstacles hijacked by human cancers."

The role of lipid droplets in sequestering triacylglycerol and ceramide has since been confirmed in mouse models of non-alcoholic fatty liver disease by LC-MS/MS-based targeted lipidomics (18). These droplets are also thought to have roles in insulin resistance and type 2 diabetes, meaning that better understanding of their structure and contents could support the development of treatments for these disorders in humans.

Autophagy, 2011

And that brings us to the final piece in our pop-up gallery: Autophagy, which describes a type of cellular cannibalism, by which our tissues consume and break down redundant cellular components. This process is involved in many disease states, and is one of the many cellular obstacles hijacked by human cancers. The painting shows formation of the autophagosome (bottom right), with golgi apparatus shown in the top left and mitochondria (blue) in the center.

Historically, this process has been studied by genetic manipulation of cells, but Raman microspectroscopy was shown to have utility in quantitative assessment of the conditions triggering autophagy in conditions of cell starvation in 2012 (19). In 2018, advances in Raman spectroscopy and associated technologies facilitated the tracking of autophagy-mediated apoptosis (cell death) in human oral squamous carcinoma cells in real time (20).

GOODBYE FOR NOW

I'm afraid that brings us to the end of our tour. I hope that you enjoyed the exhibits. Do you have a favorite? Or even multiple favorites? Well, you're in luck – each of the pieces is available as a print in our gift shop on the way out. Please do take a look if you have the time. We hope to see you again soon.

Please see references online at: https://bit.ly/2Pk5aqR

Improving GC Reliability with a Hydrogen Gas Generator

Hydrogen gas generators are increasing in popularity across the globe – but why should you switch from a traditional cylinder?

More and more labs are switching to hydrogen gas generators. Why? The main drivers are safety, convenience, and reliability. Combine these factors with the scarcity of helium in recent years and the case for carrier and fuel gas generators becomes even stronger. We spoke to Timothy Fassette, Senior Forensic Toxicologist for Henderson Police Department Crime Lab, to find out why he's gone down the generator path – as well as his experience as a Peak Scientific customer.

What does your role involve?

Our lab conducts DUI (alcohol and drugs) testing with ante mortem (living) samples. We test these for five key volatiles – ethanol, acetone, isopropanol, methanol, and acetaldehyde – and about 100 different drugs.

As a Senior Forensic Toxicologist, my main job is to review case work and validate any new methods we implement in the laboratory. I also help manage quality assurance and quality control for our toxicology and drug analysis programs.

Equipment reliability is extremely important because, as a forensic science laboratory, everything we do is checked through the legal system. Every analysis we run must be examined for accuracy and precision to ensure we maintain the required standard for our field.

We also have to work quickly to provide results to different law enforcement agencies. Without us, they often can't make progress on their cases – so it becomes troublesome if we have to wait days or weeks for instrumentation that isn't working properly.

Why did you switch to a hydrogen gas generator for your lab?

Our gas generator has two main applications. The first is as carrier gas, and the second is for flame support – but we have six or seven other applications in our lab.

Previously, many of our GC applications ran on helium, but we're suffering a worldwide shortage at the moment. We had to switch our GC-MS instruments onto hydrogen generators to ensure that we didn't have to worry about downtime while waiting for helium cylinders...

Reliability was also a big draw. We don't have to perform much maintenance because the units are relatively self-sufficient and we can rely on them to perform well. The continuous gas flow also means that we enjoy a pure, consistent, and reliable hydrogen supply – without any of the safety issues associated with having a hydrogen cylinder in the lab. And because the generator only requires a single delivery for installation – rather than regular cylinder deliveries – logistical costs are also minimized.

"We don't have to perform much maintenance because the units are relatively self-sufficient and we can rely on them to perform well."

Plus, we no longer need to worry about issues like leaky regulators – a trial we've come up against in the past; it's a newfound luxury for us to arrive each day knowing that our instruments will run instead of finding our gas supply completely drained!

PEAK SCIENTIFIC

"It's a newfound luxury for us to arrive each day knowing that our instruments will run instead of finding our gas supply completely drained!"

What are the advantages of Peak Scientific's hydrogen gas generator?

A key benefit has been the ability to stack the Precision Hydrogen Trace units in conjunction with other generators. Each of these units is about one foot wide, so the ability to stack them vertically saves valuable lab space.

The consistency of gas purity has also been fantastic; you don't need to worry about the impact of batch-to-batch variation on your chromatogram – because there is none. The generator gives us consistent results across the board, day in and day out. And having one service engineer available to support us across all of our units means we're not having to manage multiple people around the lab – one person can do all our servicing in one go!

How has the pandemic affected your work?

Even before COVID-19, the helium supply was uncertain. With Peak Scientific's help, we've been able to take control of our own gas supply and avoid the unpleasant surprises of potential pandemic-induced price fluctuations. It's also great to have everything on site; not only does it mean we can minimize contact with non-members of staff through cylinder delivery or installation, but it also ensures our gas supply is available at all times. There's little risk of disruption through delayed delivery or maintenance.

How would you describe your experience with Peak Scientific?

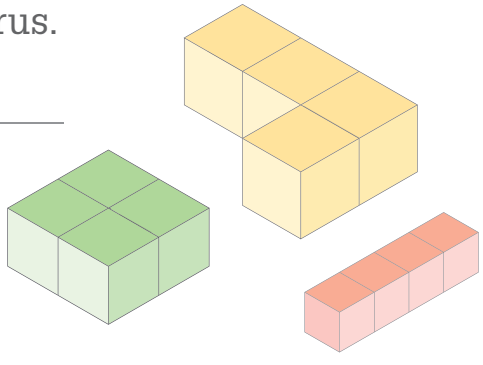
Their service department is incredible and we have a fantastic relationship with our service engineer. We can contact him directly for advice on routine changes that we can handle ourselves, which has minimized our downtime significantly.

The organization itself is very responsive. When we first installed the systems, we realized we had ordered a generator with insufficient capacity. We contacted Peak and they immediately came to the lab, assessed the issue, and agreed that we needed a higher-grade system. They treated the situation as a priority, ensuring the replacement arrived as soon as possible. Everything was up and running at the required level in no time – an experience I haven't had with any other supplier.

SPECIAL SERIES
(Bio)pharma

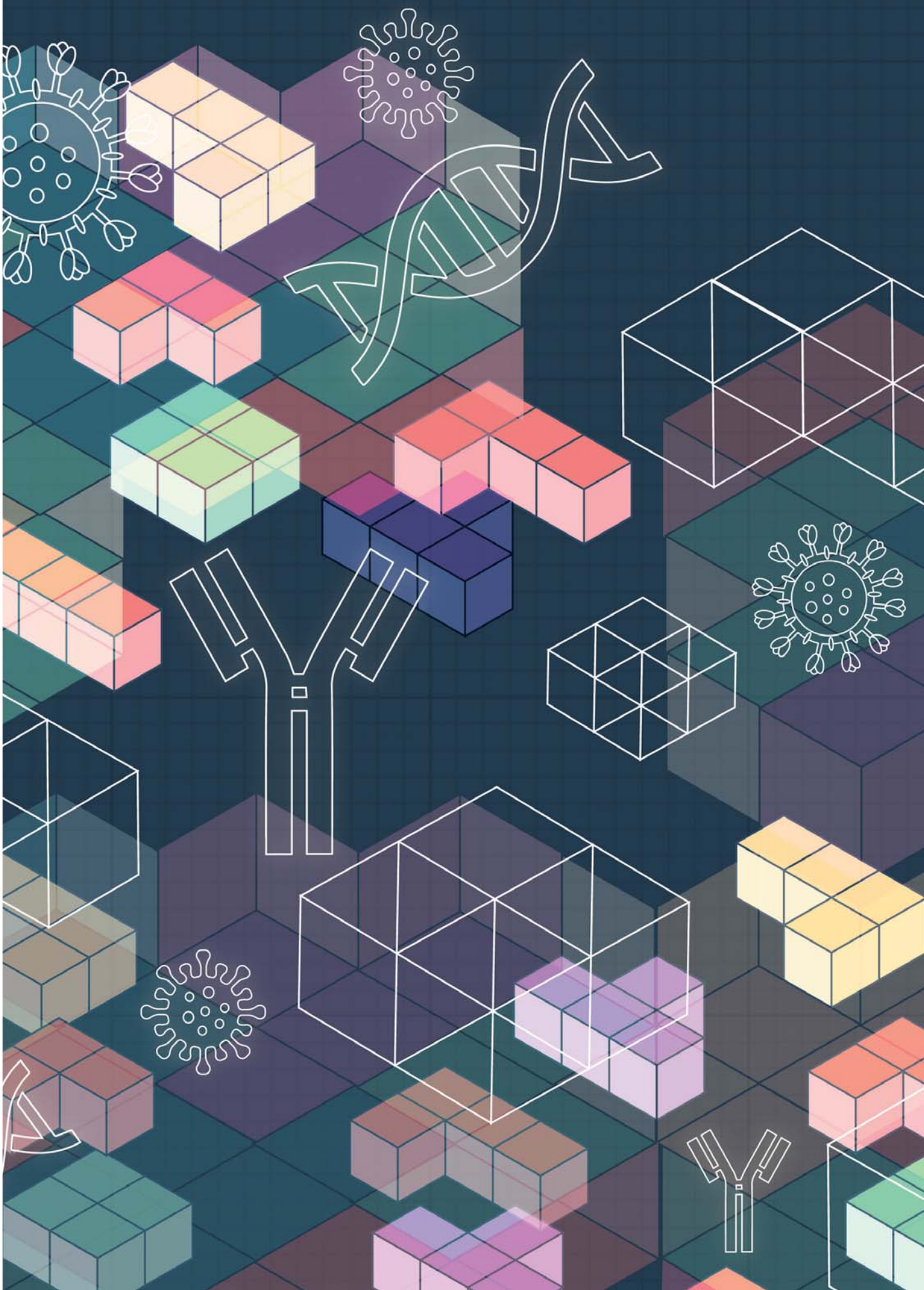
THE COVID-19 ARMS RACE

Scientists across the world are both collaborating and competing in efforts to combat the COVID-19 pandemic. Here, three analytical scientists describe how they are exploring and targeting key components of the virus.



COVID-19 waits for no one. The pandemic has swept the rug from beneath modern society, and researchers and drug companies across the globe are frantically working to provide solutions for patients. Molecules that are essential to the virus life cycle – namely, the (in)famous spike protein (which mediates host-cell infection), the viral envelope proteins, and replicases – all represent highly tempting therapeutic targets. But which could provide a route to effective treatment?

Many teams are working to provide an answer to this question, and unparalleled interest surrounds their results. With regular promises of movement towards treatments and dominating headlines, we decided to explore the crucial (and arguably under-appreciated) role that analytical scientists are playing in this arms race. Listen closely as our three invited researchers discuss the techniques and potential drug candidates being used to understand and attack these viral elements.



SMALL, BUT MIGHTY

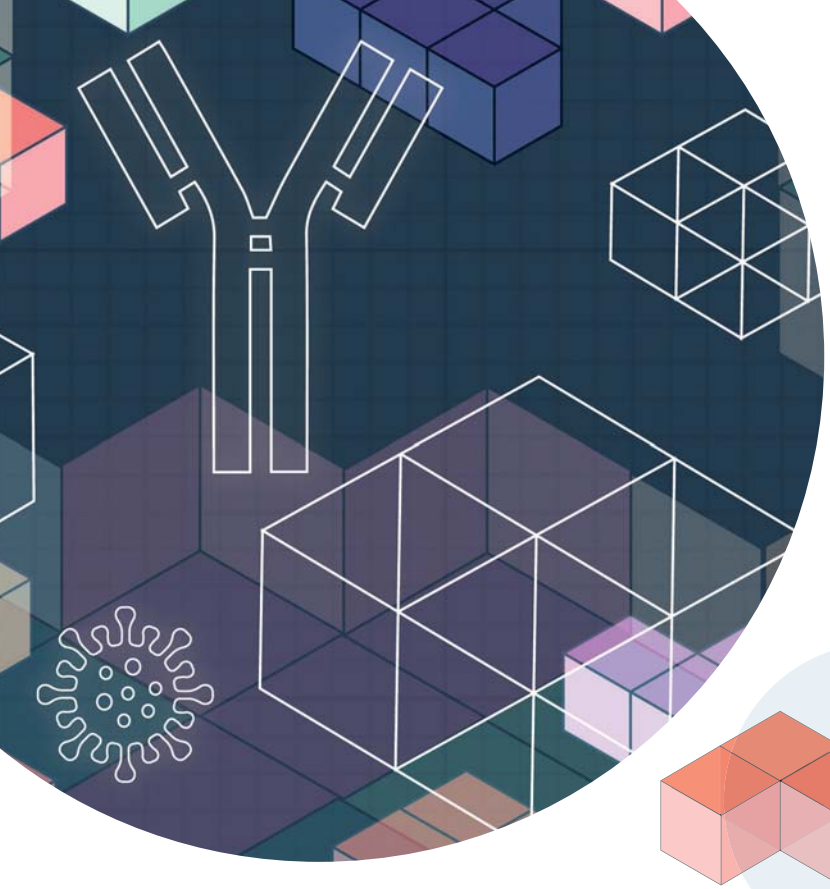
Vaccines may hog the limelight, but small-molecule inhibitors could prove to be the secret weapon that addresses the pandemic

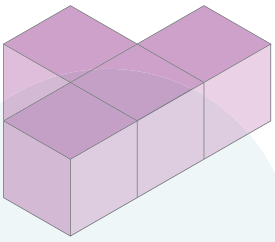
By Brent R Stockwell, Professor of Biological Sciences and Chemistry, Columbia University, New York, USA

In recent months, my research group has been involved in a large collaborative effort with the labs of David Ho, Alex Chavez, Tom Rovis, Farhad Forouhar, Brandon Fowler, John Hunt, Chuck Karan, and Waters. Together, we are aiming to develop small molecule inhibitors targeting the main protease of SARS-CoV-2. My lab normally focuses on the molecular mechanisms involved in cell death – specifically ferroptosis, which my lab discovered in 2012 – but we also conduct broader work related to drug discovery for cancer and degenerative diseases. When the pandemic hit, our focus turned to coming up with a viable therapeutic option for this enigmatic new virus.

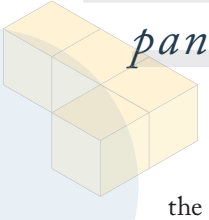
While the media focus in on vaccine development, the most effective treatments for other devastating viral infections has historically been small-molecule protease inhibitors. For example, despite a massive effort over many years, there's still no vaccine for HIV. What turned the virus from a death sentence into a treatable disease? Protease inhibitors. We decided to explore a similar approach for SARS-CoV-2.

After infecting a cell, SARS-CoV-2 genomic RNA is translated into proteins. Two large polyproteins are key to the virus' lifecycle, but these massive polyproteins must be cleaved at more than 11 different sites in order to function as individual proteins. Inhibit this cleavage process, and you can block viral replication. Previous research on the original SARS-CoV virus told us that there are two proteases that do this cleaving. One of these, the main 3CL protease, has a substrate binding





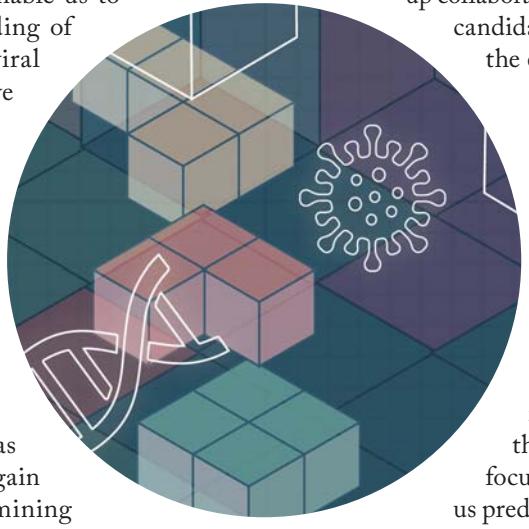
"Some of us predict that small-molecule inhibitors will ultimately be the breakthrough that brings back some degree of pre-pandemic normalcy to the world."



pocket that is similar across 12 different coronaviruses – a promising target for our research.

We've been applying chromatography and MS techniques to understand small-molecule inhibitor binding sites in the protease active site. As part of this effort, we've employed some creative digestion and modification experiments in conjunction with native size-exclusion chromatography (SEC) and reverse-phase separations.

These chromatography methods enable us to gather rich information on the binding of small-molecule candidates to the viral protease, while MS detection means we can determine where on the protease the small molecule has bound irreversibly. For example, using MALDI and LC-MS, we were able to observe the formation of a 1:1 complex when compound 18 (one of our most promising candidates) was mixed with the 3CL protease – confirmation that it is binding and inhibiting selectively as we'd hoped. Isothermal titration calorimetry has also added to the information we can gain from these MS analyses: by determining thermodynamic parameters, we can understand the specificity and energetics driving the interaction between drug candidate and protease.



The data gathered thus far have been incredibly useful in prioritizing candidates—time will tell if any of these have the potential to become a breakthrough medicine. After characterizing a number of different interactions, the compound GC376 seemed particularly promising. However, we still need to comprehensively evaluate ADME (absorption, distribution, metabolism, and excretion) properties of these compounds and their analogs.

The next hurdle will be getting an animal model set up for testing the optimized compounds. Mouse models for SARS-CoV-2 are only just emerging, and we're setting up collaborations with other groups to test the first candidate in this way. We hope to do this by the end of summer.

The analytical community can make a big difference in the fight against COVID-19, in testing and beyond. Whether that difference will manifest itself in the form of small molecule inhibitors, viral proteins or biologics, we are yet to see. Despite the billions beings invested, we know that safe, long-lasting and effective vaccines are in many cases not feasible. While most of the world's attention and funding is still focused on vaccine development, some of us predict that small-molecule inhibitors will ultimately be the breakthrough that brings back some degree of pre-pandemic normalcy to the world. In this sense, protease inhibitors for COVID-19 may perform the same task as penicillin in 1928 – turning an otherwise lethal infection into an inconvenience.

CHARGE DETECTION FOR VIRUS PROTECTION

An emerging charge-detection technique allows MS analysis of super-sized molecules – including the SARS-CoV-2 spike protein

By David Clemmer, Distinguished Professor & Robert and Marjorie Mann Chair of Chemistry, Indiana University, USA

Our work, in collaboration with Martin Jarrold's group at Indiana University, is centered around a powerful new technique: charge-detection MS (CDMS), which works by detecting exact charges of individual particles. As readers may know, determination of mass from electrospray-based measurements of the mass-to-charge (m/z) ratio requires that each different m/z peak can be resolved and assigned as a specific charge. As particle mass increases, the ability to resolve and assign m/z peaks becomes prohibitively difficult – especially for complex mixtures. By detecting both the m/z and z for each ion, CDMS eliminates this limitation – allowing the determination of masses for particles in the megadalton to gigadalton size range, and beyond.

The CDMS detection technology that we use was developed by Martin Jarrold's group in the early 2010s, and is now undergoing commercialization via a startup company (Megadalton Solutions) founded by Martin and me in 2018, along with Martin's former graduate student Benjamin Draper.

Since the pandemic hit, our research groups have collaborated to measure the exact mass of the SARS-CoV-2 spike protein via CDMS. The spike is a protein trimer responsible for recognizing host cell receptors and facilitating infection; there are hundreds of spikes on each virus particle, and each monomer is associated with 22 N-glycosylation sites. Knowledge of spike-bound glycan molecules may prove essential in targeting the spike protein's "sweet spot" and disabling infection. At first glance, one might think this a straightforward task, but it is complicated by incredibly heterogeneous glycan profiles.

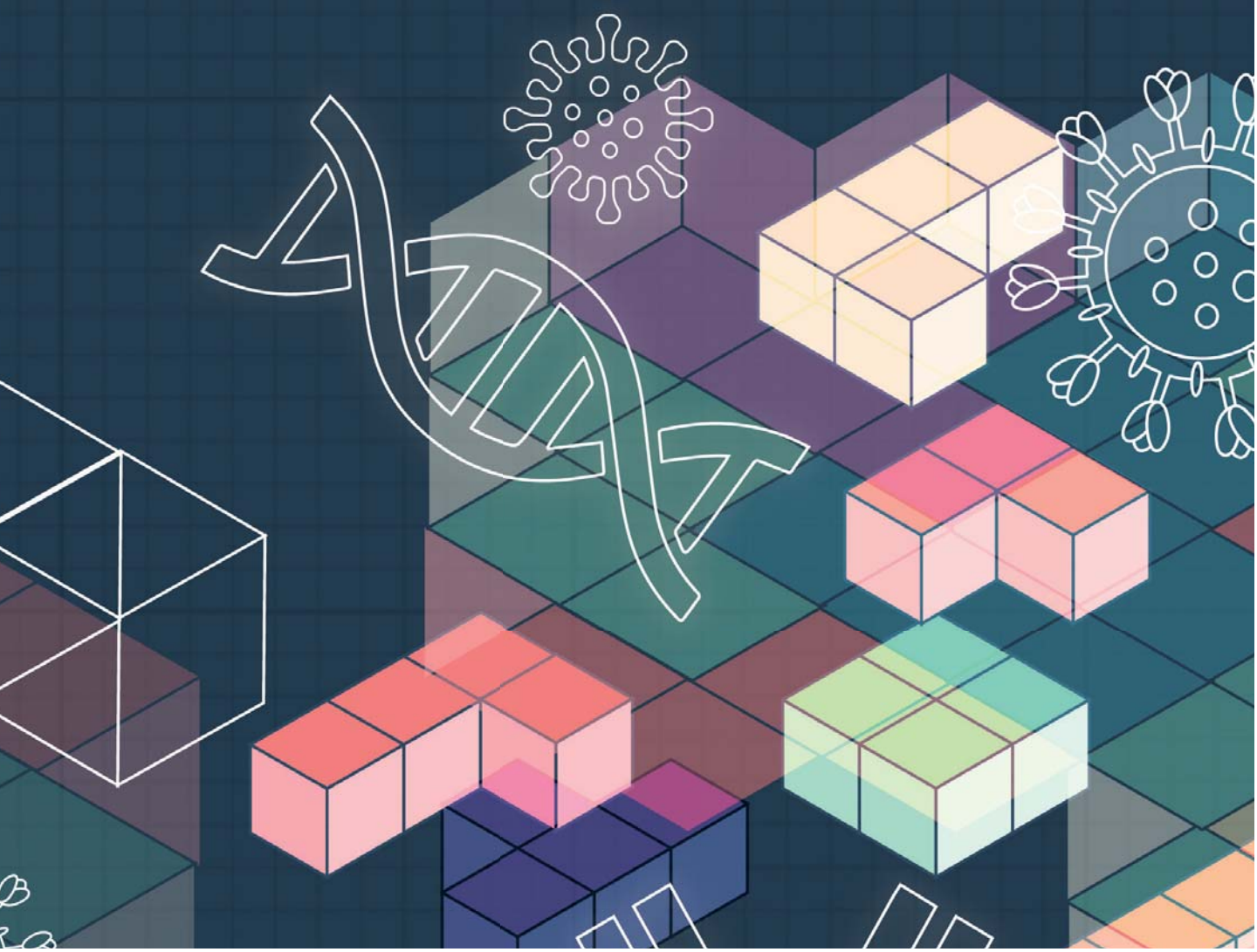
Amidst this pandemic, the spike protein region is attracting considerable attention as a potential target for therapeutics and vaccines. Other groups are also exploring a range of alternative angles to develop a vaccine; for example, placing the viral genome in an AAV capsid (or another vector) for delivery. In all of these treatment and vaccine endeavors, the ability to measure the mass of the huge particles involved will be of great benefit.

Another interesting application of CDMS lies in the field of gene therapy. These treatments hold the potential to counteract some devastating – and often rare – diseases; in 2018, ~2,400 gene therapies were at some stage of clinical trial. Our startup, Megadalton Solutions, uses CDMS to check whether or not adeno-associated viruses designed for therapeutic purposes contain the correct genome, by measuring the precise mass of the filled and empty capsules. These therapies generally have masses below 10 megadaltons, but we have recently measured viral vectors of up to hundreds of megadaltons.

At present, Martin's CDMS measurements can be made in only a few academic labs around the world. There is, however, clear interest in creating commercial instruments and we look forward to seeing it in widespread use in the future. Think of the story of coupling ion mobility spectrometry (IMS) with MS; Waters commercialized IMS-MS in 2006, and commercial IMS instruments are now used for countless applications in labs around the world. Yet, CDMS is a very different technology in that it enables measurements in a mass region that has never been explored. As such, it has the potential to transform our understanding of a number of booming areas of research, ranging from therapeutics to plastic degradation, and phenomena like virus assembly and antibody aggregation.

But right now, our focus is to contribute in any way we can to the global fight against SARS-CoV-2. While I recognize the tremendous complexity and challenge that this virus presents, I am cautiously optimistic that the combined efforts of talented scientists will lead to better treatments – and potentially safe and efficacious vaccines. I also recognize that the associated societal changes are difficult for many; however, every week that we delay major outbreaks is critical for advancing treatments.

"[CDMS] has the potential to transform our understanding of a number of booming areas of research, ranging from therapeutics to plastic degradation, and phenomena like virus assembly and antibody aggregation."



LET'S GET BIOPHYSICAL

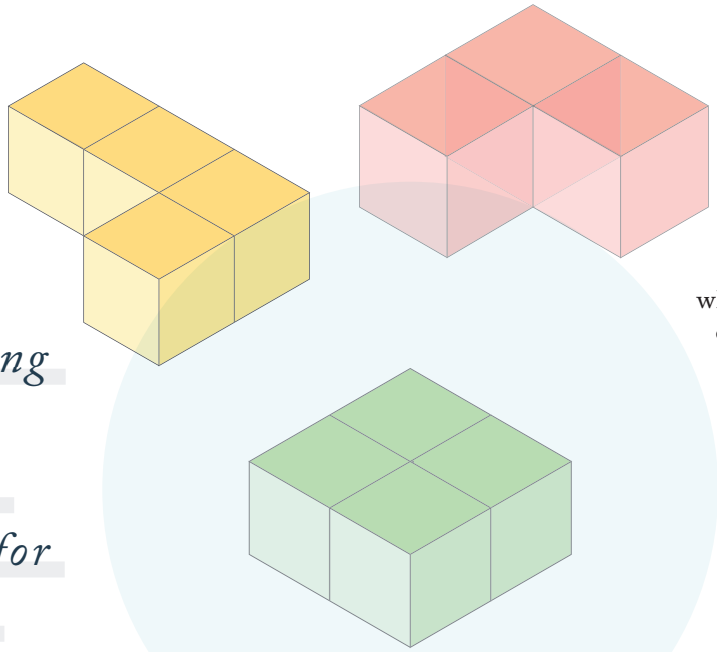
Nuclear magnetic resonance and hydrogen-deuterium exchange are uncovering key information to facilitate small molecule targeting of the SARS-CoV-2 envelope protein

By Chad Petit, Associate Professor, Department of Biochemistry and Molecular Genetics, and Peter Prevelige, Professor, Department of Microbiology, the University of Alabama at Birmingham, Alabama, USA

The SARS-CoV-2 envelope protein is a small transmembrane protein found in the viral lipid envelope. In other coronaviruses, the envelope plays key roles in viral assembly, morphogenesis, and virus-like particle formation and release. And although the envelope protein is not solely responsible for replication, coronaviruses unable to express it are severely attenuated. In fact, envelope protein inhibition can severely mitigate replication in a number of other coronaviruses – these findings indicate that the envelope protein is a viable target for not only the development of antivirals, but also the generation of attenuated strains of the virus for vaccines.

With this in mind, we set out to analyze the envelope protein and use its structural information to identify small molecules that can impede its function. In doing so, we are focusing on small molecules that are approved for the treatment of other diseases; by repurposing them, we hope to identify novel therapeutic options that are already available to patients. This will dramatically reduce the time needed to get these treatments to COVID-19 patients.

So how do we obtain the protein information we



"We are focusing on small molecules that are approved for the treatment of other diseases... This will dramatically reduce the time needed to get these treatments to COVID-19 patients."

need? First, we obtain high levels of envelope protein using a bacteriophage T7 expression system. This is subsequently purified using a combination of fast-protein LC and high-performance LC, giving us samples of envelope protein at over 95 percent purity. For our biophysical experiments, we need milligram quantities of the protein.

Nuclear magnetic resonance (NMR) and hydrogen/deuterium exchange MS (HDX-MS) are our methods of choice for probing the secondary and tertiary structures of the envelope protein to identify binding sites for potential small molecule inhibitors. This approach can measure the dynamics of the protein at atomic-level resolution over a range of timescales – and, because it is solution-based, it can measure dynamic events that are inaccessible to alternative methods like X-ray crystallography. If necessary, we believe we could use NMR to solve the high-resolution structure of the entire envelope protein.

Understanding the envelope protein will allow us to generate hypotheses regarding the specific roles it plays in SARS-CoV-2 replication. To aid with this, we are also developing a platform to generate mutant SARS-CoV-2 viruses, which will further our understanding of the envelope protein's role in the viral life cycle and support our biophysical findings. We plan to complement our NMR studies with HDX-MS, which extends the timescale over which we can extract dynamic information.

Overall, we are optimistic about the scientific community's efforts to provide us with effective treatments and vaccines against COVID-19. The sheer volume of work is encouraging – and, when such a monumental effort is made toward a single goal, we often see results sooner than expected. For example, the Altimmune vaccine developed at the University of Alabama at Birmingham is showing promising preclinical results – as does the vaccine from the UK'S University of Oxford, which appears to induce immunity with relative safety.

the Spectroscopist

INSIDE

Taken at a symposium that the University of Idaho hosted upon Peter's retirement 12 years ago. Peter can be seen holding up an inscribed beamsplitter (the key component of an FT-IR spectrometer), presented to him by his PhD graduates.

A Series of Fortunate Events

Spectroscopist Peter Griffiths tells us about his life in the field – and the serendipitous moments that led him there

If you've ever performed Fourier-transform infrared (FT-IR) spectrometry, then you've likely read – or at least heard of – the bestselling reference book on the subject by Peter Griffiths and James de Haseth. With a career spanning more than 50 years, Griffiths has pioneered the use of many techniques in vibrational spectroscopy, published over 300 papers, and was editor-in-chief of Applied Spectroscopy from 2009 to 2012. We spoke to him about his (initially on-off) relationship with spectroscopy, what he foresees for the field, and some of the strange but fortuitous events that have led him where he is today.

Why did you first get into vibrational spectroscopy?

Because I found that I wasn't a very good physical organic chemist! I had originally wanted to pursue a career in that field, but discovered that it was most likely not my calling during my final exam on advanced organic chemistry at Oxford University – I couldn't answer a single question on the topic! Luckily, there were a lot of other areas I could write about – but my original career aspirations came to a grinding halt. Instead, I decided to pursue a PhD in physical chemistry, and I ended up working on FT-IR spectroscopy in Sir Harold Thompson's lab (or "Tommy," as we knew him).

What happened after your PhD?

It may seem surprising, but I developed quite a strong aversion to FT-IR during my PhD. The spectrometer sometimes took hours to measure an interferogram and the data required extensive computing – you can imagine how tedious that process was during the 1960s. In the end, I decided I didn't want to make spectroscopy my career – strange as this may seem now.

If I hadn't been handed an opportunity in 1966, I'm not sure where I'd be today. I feel my life has been somewhat determined by a series of fortuitous events – this time in the form of a croquet game. A year before I earned my PhD, Tommy organized a conference on far-infrared spectroscopy that was attended by spectroscopists from all over the world. One afternoon, Tommy approached my friend Roger Lake and me and said, "Lord and Lippincott want to learn how to play croquet. Go and show them!" By this, he meant Dick Lord, a professor at MIT, and Ellis Lippincott, a professor at the University of Maryland – two of the biggest names in the field at that time. A year later, Roger was a postdoc with Dick Lord at MIT and I was working

with Ellis Lippincott at the University of Maryland!

Lippincott sent me a list of nine projects I could work on as a post-doc; eight were spectroscopy-based. As you can guess from what I said before, I chose the ninth! The project involved trying to simulate conditions under which biologically important molecules could be formed on planets similar to Jupiter or its moons. As it happens, I missed collaborating with Carl Sagan on this project by one year! While in Lippincott's lab, I did some research with an early FT-IR spectrometer and once my main project ended, the company who manufactured it offered me a position as product manager for a "new and improved" instrument that was under development. This instrument turned out to be the basis of all contemporary FT-IR spectrometers.

When did you decide to pursue academia?

I didn't really get on with the president of my division at this new company. Among other things, he often ignored my input – but one time, he didn't. He asked me for a rough estimate of a parameter related to the sensitivity of the instrument we were developing. I gave him a number off the top of my head and he put it into a new brochure without consulting me further. Funnily enough, over 50 years later, I saw another company using exactly the same number for their new FT-IR spectrometer!

But what really pushed me in the direction of academia was something that happened while I was a technical consultant on a sales trip – another fortunate coincidence. We were talking to a chemistry professor who had received a National Science Foundation grant that allowed him to purchase infrared spectrometers for two different wavelength regions. I asked him about his planned experiments, and it turned out I knew someone who had already published on the subject matter of his new grant. After we left his office, my colleague told me I had completely screwed up the sale – in slightly different words. I said I had simply told him about something he had missed; the sales guy replied, "No, you told him he was stupid!" Needless to say, we didn't get the order – but I started to rationalize that, if that professor could receive a large grant for something that had already been done, I could do just as well – or maybe even better. So I started to apply for academic positions!

You mentioned consulting work. Are you still doing that?

I've been asked a number of times to act as an expert witness for pharmaceutical patent cases, starting in the 1990s. The first was around a patent dispute over generic versions of Paxil, a blockbuster antidepressant. The case depended on a combination of infrared spectroscopy and chemometrics, which happen to be my specialties. I was also involved in a case concerning the conversion of coal to a form that was untaxed. Infrared spectra were needed to prove whether the treated coal was different from freshly mined coal.

I'm still involved in these types of projects today. An interesting recent one is addressing the impact of particulate matter below 2.5 microns on miners – particularly silicosis (lung fibrosis caused by silica inhalation). In the past, the preferred method was to collect particulate matter on a filter and run the samples through X-ray diffraction or infrared spectroscopy. However, you'd typically have to wait several days for the results – not very useful when you want to find out whether or not a miner has just been exposed to dangerous materials. Ideally, you need an instantaneous readout – and that's what we've been working on.

How has the field changed during the course of your career?

What I've been most amazed by are the computational advances – computers are getting smaller and more powerful all the time. The components of an infrared spectrometer haven't changed all that much, but the computers used to fill up a large room. It is remarkable that there

are now handheld instruments in which the optics and electronics take up only a shade more space than a mobile phone.

So what are the areas to watch?

The hot topics in FT-IR spectroscopy right now are microscopy and imaging using array detectors. Although both techniques have been around for well over 10 years, they have reached the point where some beautiful work is being carried out on some very significant topics, such as tumor characterization. Being able to see the stage of a given cancer quickly and objectively could be a game-changer. The technology isn't quite there yet, but some superb work is being done in this area.

Of arguably equal importance is the work being done in infrared microscopy below the diffraction limit. These instruments are expensive, but so were FT-IR spectrometers in the early days – and we can expect further developments in the near future.

Another exciting area is tip-enhanced Raman spectroscopy (TERS), which has even higher spatial resolution than is possible by any IR technique. In fact, I recently reviewed a 60-page manuscript covering TERS applications for Applied Spectroscopy. In one of the papers discussed by the author, the Raman spectra of different regions of a single porphyrin molecule were shown!

Are there any developments you'd still like to see?

I think the full story is yet to be told on this one, but I am intrigued to see just how important tunable IR lasers, such as quantum cascade lasers (QCLs), will become. Today, QCLs only cover a relatively short wavelength range – although that is changing every year – and they have limited stability. But if you can make a stable laser that can be rapidly tuned across the full mid-IR spectrum, then you've got the basis of an instrument that can do more than a standard FT-IR spectrometer. The currently short tuning range of QCLs limits the information content of the spectrum of, for example, pathological samples, such as biopsies. With a greater spectral range and higher sensitivity, it should be possible to measure spectra that allow the routine analysis of tissues and tumors at micrometer scales. Developments of QCLs and other mid-IR lasers over the last 15 years have been quite remarkable and we can expect more in the future.

While on the subject of lasers, I would be remiss if I didn't mention the potential use of UV lasers for Raman spectroscopy. Because the intensity of Raman scattering is proportional to the fourth power of the excitation frequency (λ^{-4}), lasers of a shorter wavelength have an advantage over visible lasers. They are also often less susceptible to fluorescence interference than visible lasers. As is often the case when only a few people

use a technique, UV lasers are expensive. But, hopefully, as demand increases, the cost of these instruments will fall.

What part of your career are you most proud of?

That's a difficult question. I've dabbled in a fairly large number of different applications, but I think one of my more useful contributions has been in the interfacing of chromatographs and FT-IR spectrometers to identify the components of mixtures. MS has always been a step ahead in this area, but infrared spectra still have an important role to play in distinguishing isomers. The other areas are probably my work on diffuse reflection, open-path atmospheric spectroscopy, and surface-enhanced infrared spectroscopy.

I am also proud that the papers and books I've written have been cited over 10,000 times – and, 12 years after I retired, I'm still getting about eight citations per week. It's great to know that other scientists have found, and still find, my work useful.

But I'm probably most proud of the fact that, during my time as a professor, 52 graduate students earned their PhDs under my supervision. The students were often "diamonds in the rough", and I have always been happy to see so many of them go on to successful careers, usually in industrial labs.

What would you say to anyone starting out in spectroscopy?

Look to see what's hot and where it's going. Don't get stuck doing the same old stuff over and over again, but have fun doing what you're doing. The other thing I would say is to go to as many conferences as you can (this is perhaps even more possible now that so many are online) and join scientific societies. Societies allow you to meet people in your field and gain a grasp of the big things that are happening before they appear in print.

Most importantly, have the courage to go and talk to the big names in your field! Every vibrational spectroscopist that I have met is very personable and there's little competition between academic groups. You'd be surprised how many people really love talking with young, enthusiastic scientists who are just getting into the field, so why not give it a try?

Solutions

Real analytical problems
Collaborative expertise
Novel applications

A COVID Coalition for the Masses

The COVID-19 MS Coalition is a new initiative bringing together analytical scientists from across the globe to fight the pandemic

By Perdita Barran, Professor of Mass Spectrometry at the University of Manchester, UK

As I closed down my lab at the start of lockdown, I felt thoroughly dejected. I thought about all the amazing equipment sitting unused inside, in other empty labs around the world and the scientists sitting at home – so much potential going to waste. Once the initial feelings of despair had subsided, it dawned on me that perhaps this wealth of instrumentation didn't have to go to waste after all. Why couldn't we leverage it in the fight against COVID-19?

I recognized that MS could provide vital information about the SARS-CoV-2 virus – particularly at the metabolite and protein level. Though most current tests are based on genomic information, other "omics" approaches have tremendous potential to advance our knowledge of COVID-19. For example, proteomics and metabolomics offer insight into host responses to infection, and lipidomics is useful for profiling the inflammatory components of this response. In this way, MS offers rapid, precise, and reproducible results to inform our molecular understanding of the virus, which is vital for vaccine design.

I approached my colleagues with the idea and asked how we could contribute. The result: the COVID-19 MS Coalition (covid19-msc.org).

Written in the stars

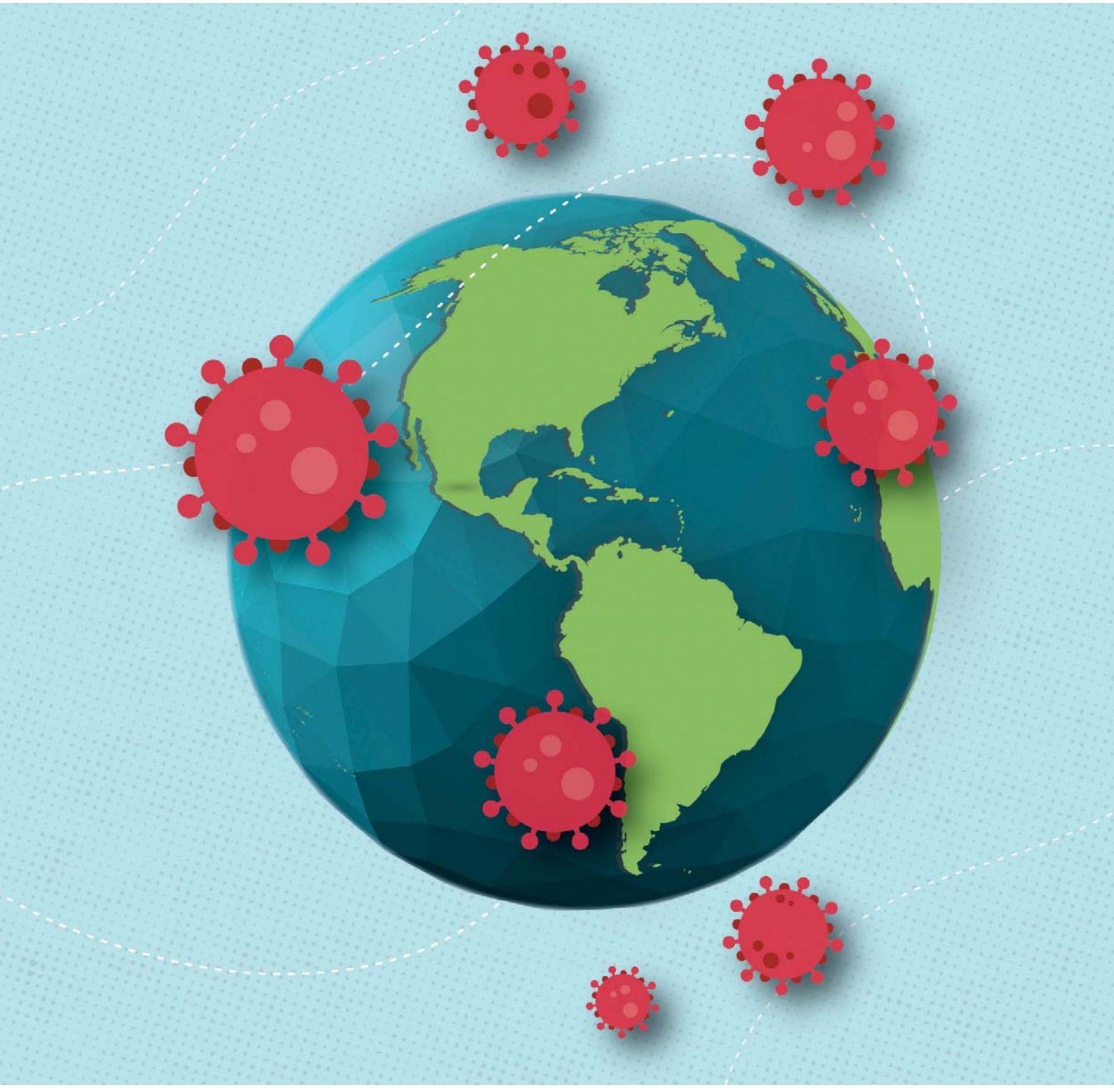
Some fields, such as meteorology, are well-versed in sharing methods and data, but this is less common than I'd like in analytical chemistry. If there is a positive lesson we can take from this pandemic, it is that we can work together more – as scientists, organizations, or even pharmaceutical companies. And that is the basis on which we formed our coalition.

In brief, the COVID-19 MS Coalition is a collective effort to provide molecular-level information about the virus through MS, with the aim of reducing the harm caused by the disease. As analytical scientists, we understand the importance of replicate studies. By combining our resources, we are able to conduct replicate analyses at pace, maximizing the benefit of research conducted worldwide. More than 500 scientists from 28 countries are already contributing to the effort, and this number is growing all the time. We are also fortunate to receive support from Bruker, Thermo Fisher Scientific, Sciex, LGC, Pfizer and Waters, as well as other relevant organizations.

> *"It dawned on me that perhaps this wealth of instrumentation didn't have to go to waste after all. Why couldn't we leverage it in the fight against COVID-19?."*

A deeper understanding

We've focused on a number of key areas where MS can make a difference,



The key aims of the COVID-19 MS Coalition:

- To use MS to minimize the harm caused by COVID-19
- To form a coalition of MS labs with national points of contact
- To share methods and protocols and all data
- To map the viral antigens in blood and other biofluids to inform serological testing
- To assay (QC) compounds produced for serological tests kits
- To use MS to inform vaccine and therapeutic developments (mapping viral proteins and their interactions)
- To develop methods to determine disease prognosis
- To develop methods to determine the lifetime of infective particles in the environment.

including omics, protein dynamics, in situ analysis, and data generation and processing (1). The aim is to understand why the disease affects some people more than others, to learn about its spread, and identify prognostic markers. Though structural studies of the virus are useful, they don't tell us about the glycan content or dynamics of the virus antigens. Being able to understand not just the structure, but how this structure changes, is a question that MS is well-placed to answer.

It is possible to examine individual infection responses using multi-omics approaches. By developing our understanding of why some individuals react more to the virus than others, we can identify prognostic biomarkers that may allow hospitals to profile patients going forward. In particular, biomarkers that signal the transition from a healthy to a harmful immune response will be valuable in identifying those at risk of experiencing complications such as cytokine storms. Finding biomarkers that predict an individual's response to the virus will also allow us to track disease progression and uncover vulnerabilities that will ultimately help prevent future pandemics.

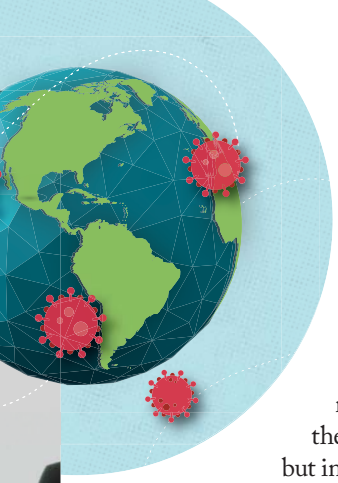
We are also exploring the SARS-CoV-2 viral spike glycoprotein, which is key for host–cell attachment. It represents a major target for vaccine development, but the functional role of these viral spike glycans remains undetermined. Advanced MS methods can provide information about the glycosylation of this protein and associated conformational dynamics. Coalition members will work on "quality control" experiments on the recombinant forms of the spike protein being used to develop antibody tests.

Action plan

How is the coalition facilitating this important work? First, we are selecting "national champions" to streamline the research process and rapidly disseminate best practices across the group. We recognize the difficulty of obtaining patient samples and differing ethical guidelines surrounding this practice between countries; we hope that sharing success stories will help others benefit from the available information.

We will also share sample collection and processing protocols on our website, as well as the data obtained from experiments. We've put a lot of energy

"We're not limited to a particular kind of lab – anyone who wants to be part of the coalition is very welcome."



into making a simple protocol that will work across many labs, not just in the current crisis situation but in the long term, too.

Reproducibility is another important focus for us. Under normal circumstances, scientists might prefer to publish a paper and then see if others can repeat the results, but the current situation necessitates much more rapid data and method sharing. Our coalition will assist in this.

The final part of our initiative is to establish what we refer to as a "data catalog." This open data repository, hosted on our website, will allow access to information generated from international studies, aiding the understanding of antigen response mechanisms, informing vaccine development, and enabling antiviral drug design. We won't be holding all of the data ourselves, but instead listing the accession numbers. We suggest collaborators use repositories such as PRIDE for proteomics datasets and MetaboLights for metabolomics. Once the data is deposited, we will record the accession number and the requisite metadata for identification. This will allow comparisons between different labs, countries, and patients.

Get involved!

The support we're receiving from instrument and pharmaceutical companies means we're not limited to a particular kind of lab – anyone who wants to be part of the coalition is very welcome. We want to be able to promote and collect all clinical MS studies of COVID-19, so we encourage anyone who would like to contribute to our initiative to get in touch. Simply reach out to the representative from your country, or contact us directly to let us know about any published data or pre-print data via the website (covid19-msc.org/join)

This pandemic has proven just how vulnerable our society is – not just in terms of our health, but our economy. All around us, we face powerful common threats – I hope that realization will galvanize the scientific community to find new ways to collaborate and share data.

References

1. *W Struwe et al., "The COVID-19 MS Coalition—accelerating diagnostics, prognostics, and treatment", Lancet, 395, 1761 (2020). PMID: 32473097*

Ion-Pair Reversed-Phase Analysis of an Oligonucleotide Ladder Standard with Avantor® ACE® Oligo

Oligonucleotides are short lengths of DNA/RNA which are increasingly being used as therapeutic agents for the treatment of genetic disorders and cancers.

With oligonucleotide treatments, it is possible to synthesize a single-strand of DNA/RNA which has a complementary sequence to a disease-causing gene. Once this therapeutic DNA sequence has bound to the target gene in the body, the gene is deactivated. This method of treatment is known as antisense therapy.

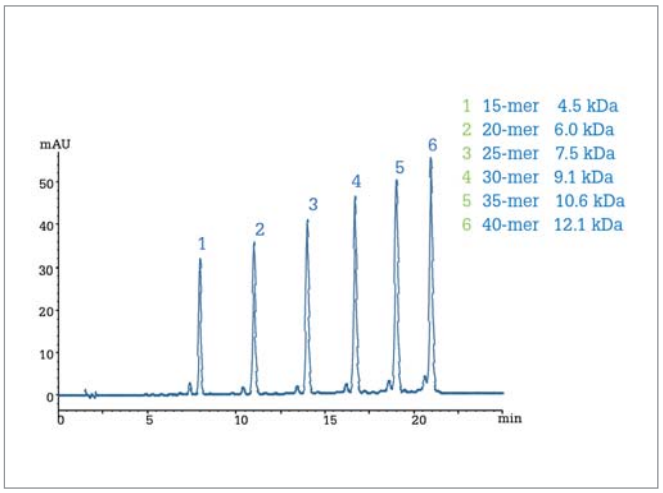


Figure 1. Separation of thymidine oligomers

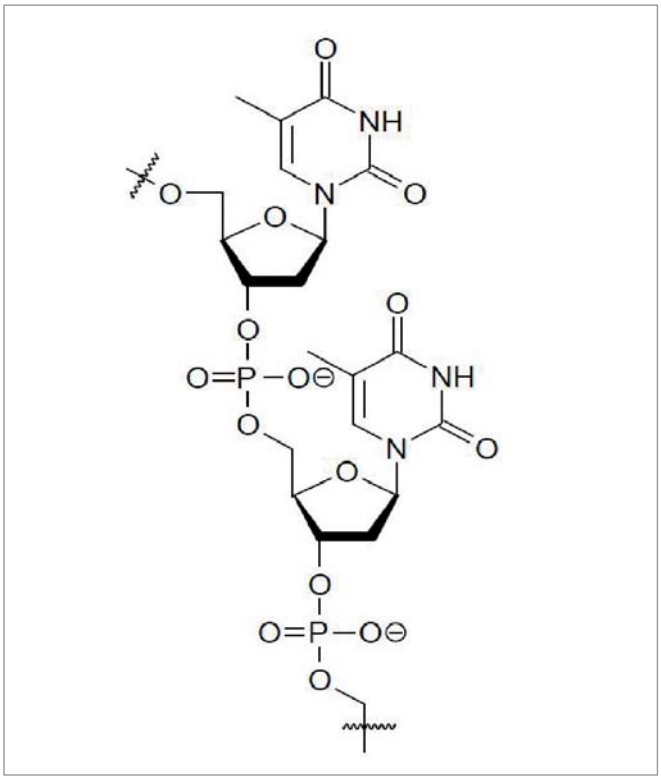


Figure 2. Structure of thymidine oligonucleotide.

Currently, over 100 oligonucleotide-based therapies are in the clinical pipeline with many more in the pre-clinical development stage.

Several impurities can be produced during oligonucleotide synthesis (e.g. failure sequences), due to a less than 100 percent efficient process, and these need to be removed from the desired product.

This purification process can be performed using ion-pair reversed-phase HPLC (RPLC).

Download at https://uk.cmd.vwr.com/rq/ddl/ace_020

Multi-Step Pyrolysis of a Meteorite Using a Pyroprobe with GC/MS

In the search for extraterrestrial life, finding organic molecules has been a topic of special interest. NASA's Curiosity rover equipped with the Sample Analysis at Mars (SAM) Suite Investigation in the MSL Analytical Laboratory is designed to address this interest. The main analytical chemistry hurdle that SAM resolved is using pyrolysis to 1: thermally extract organic matter with low molecular weight from a piece of inorganic matter sample, which is usually insoluble into common solvents; 2: thermally break carbon bond in organic matter with high molecular weight; and 3: send to GC/MS for identification. As the inventor of the first commercial pyrolyzer for a GC/MS system, CDS Analytical contributed to the development of SAM in the Curiosity rover (1), which was launched on November 26, 2011 and landed on Mars on August 5, 2012.

This application note demonstrates the operation of SAM by thermally extracting 15 mg of powdered meteorite in a multi-step sequence of 120˚C, 200˚C, 280˚C, followed by a flash pyrolysis at 610˚C to evaluate its organic matter content.

Experimental Parameters

A small piece from Murchison meteorite, which fell in Australia in 1969, was powdered, and 15 mg of the powder was added to a Drop-In-Sample Chamber (DISC) tube, to run on a Pyroprobe 6200.

Pyroprobe

DISC chamber:	120˚C	15 min	200˚C	15 min
	280˚C	15 min	610˚C	30 sec
Trap rest:	40˚C			
Trap final:	300˚C	4 min		
Interface:	300˚C			
Transfer line:	300˚C			
Valve oven:	300˚C			

GC/MS

Column:	5% phenyl (30m x 0.25 m)
Carrier:	Helium 1.25mL/min 25:1 split
Injector:	300˚C
Oven:	40˚C for 2 minutes 10˚C/min to 320˚C
Ion source:	230˚C
Mass range:	35–600amu

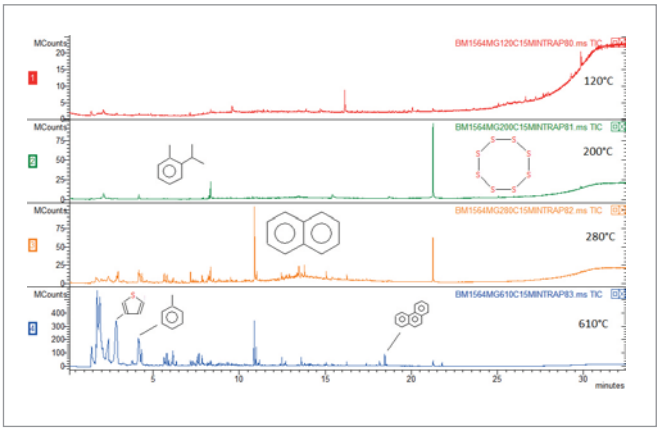


Figure 1. Meteorite, 15mg multi-step at 120˚C, 200˚C, 280˚C and 610˚C.

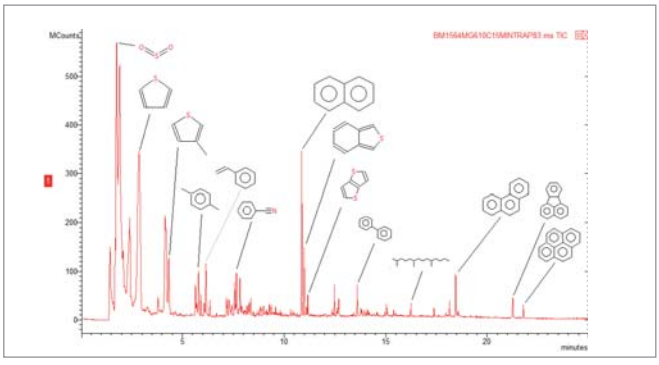


Figure 2. Powdered Meteorite, 610˚C, after multi-step extraction at 120˚C, 200˚C, and 280˚C.

Results and Discussion

All four temperature runs are summarized in Figure 1. The run at 120˚C did not yield organic compounds at enough concentration to be identified. Aromatics started to emerge at 200˚C, along with cyclic sulfur. At 280˚C, aromatics at higher boiling point, like naphthalene and some hydrocarbons become more abundant. Sulfur compounds such as thiophenes also were extracted.

The last flash pyrolysis run at 610˚C in Figure 1 was zoomed in Figure 2 to show more details. Pyrolysis at 610˚C revealed sulfur dioxide, more thiophenes, hydrocarbons, and polycyclic aromatic hydrocarbons, including phenanthrene.

This application note demonstrated thermal extraction and pyrolysis analysis of powdered meteorite using a 6200 Pyroprobe with GC/MS.

Reference

1. *R Navarro-González et al., "The limitations on organic detection in Mars-like soils by thermal volatilization–gas chromatography–MS and their implications for the Viking results," Proceedings of the National Academy of Sciences, 103, 16089 (2006). PMID: 17060639.*

Incorporating CCS Values to Enable 4-Dimensional Annotation of Metabolic Features

Reliable annotation of metabolites for LC-MS/MS-based data requires the adept combination of many parameters. To extend our parameter portfolio we analyzed trapped ion mobility spectrometry (TIMS) data.

By Ulrike Schweiger-Hufnagel[1], Matthias Szesny[1], Aiko Barsch[1], Melvin Gay[2], Torben Kimhofer[3], Joel Gummer[3], Luke Whiley[3], Jeremy Nicholson[3];
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[2] Bruker Pty. LTD., Australia, Preston, Australia
[3] Australian National Phenome Centre, Murdoch University, Perth, Australia.

MS	timsTOF Pro
Source	Apollo II ESI source
Ionization	ESI(+), 4500 V Capillary Voltage ESI(-), 4200 V Capillary Voltage
Scan range	20–1000 m/z
Calibration	Internal mass calibration through automation, sodium formate, Mobility calibration before sequence using Agilent Tunemix
PASEF	Collison energy 20/50 eV
LC	**Elute UHPLC (in Bremen), Waters I-Class (in Perth/AU)**
Column	LC column kit "T-ReX Elute M-column kit: RP", containing Bruker Intensity Solo 2 C18, 2.1 x 100 mm and Waters Acquity UPLC BEH C18, 1.7 µl Van Guard Pre-column 2.1 x 5 mm
Column Oven Temp.	35°C
Mobile phases	A: water with 0.1 % formic acid B: acetonitrile with 0.1 % formic acid
Wash solvents	Wash 1: Solvent A Wash 2: acetonitrile / methanol/ water (1:1:1)
Gradient	0 min 1% B, 0.25 mL/min 2 min 1% B, 0.25 mL/min 17 min 99 % B, 0.25 mL/min 20 min 99 % B, 0.25 mL/min 20.1 min 1 % B, 0.35 mL/min 22 min 1 % B, 0.35 mL/min 28 min 1 % B, 0.35 mL/min 28.1 min 1 % B, 0.25 mL/min 30 min 1 % B, 0.25 mL/min
Injection volume	2 µl

Table 1. LC-TIMS-MS conditions.

Introduction

We investigated the reproducibility of CCS values for intra- and inter-lab measurements. Furthermore, we compared the measured CCS values to those from literature, and we applied these to a sample comparison. From the present results, we conclude that CCS values serve as an excellent additional filter for metabolite annotation.

Experimental

Sample preparation

Urine (Bremen): A sample was collected from a volunteer. The sample was centrifugated (20817 g for 10 min at 4°C) and filtered (0.22 µm sterile syringe filters with MCE membrane SLGS033SS from EMD Millipore).

NovaMT sample: Hydrophilized urine (www.novaMT.com, TRX-3178-R) was dissolved in 200 µl LC-MS grade water, vortexed and centrifugated (20817 g for 15 min at 4°C). After adding 250 µl methanol the sample was vortexed and centrifugated (20817 g for 5 min at 4°C). 600 µl LC solvent A was added to the supernatant.

Retention time standard (TRX-2101, NovaMT) prepared according to protocol from NovaMT.

Data acquisition

See Table 1.

Data processing

Data were processed in MetaboScape® 2021 (preliminary version), resulting in a table of features, so called buckets. An Analyte List was created combining the retention times from the T-ReX LC-QTOF solution (https://www. bruker.com/products/mass-spectrometry-and-separations/ms-software/metabolomics-solution.html), CCS values from the Unified CCS Compendium (https://mcleanresearchgroup.shinyapps.io/CCS-Compendium/) and fragment spectra from the Bruker HMDB Metabolite Library 2.0 and the Bruker MetaboBASE® Personal Library 3.0 (https://www.bruker.com/products/mass-spectrometry-and-separations/ms-software/metabolomics-spectral-libraries/overview.html). For each system the retention times were checked and corrected using a retention time standard. Using MetaboScape, features in the bucket table were putatively annotated based on an Analyte List of known compounds.

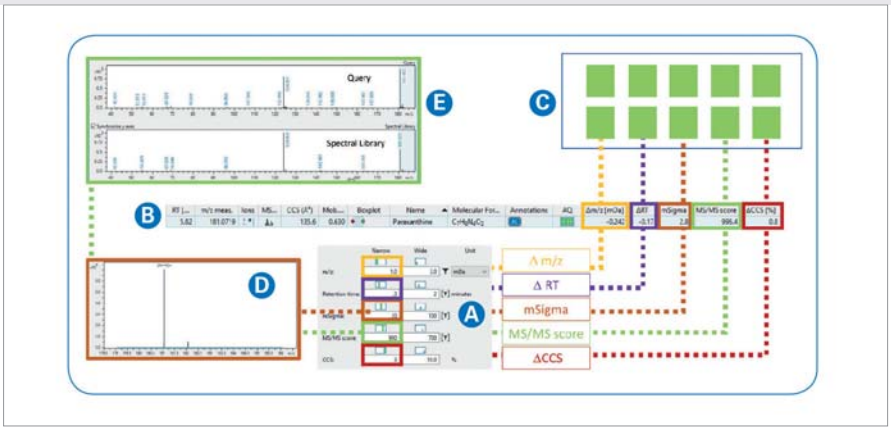

Figure 1. Annotation of paraxanthine. A: Narrow and wide filters set for annotation. B: Bucket table entry for paraxanthine. C: Visualisation of AQ_scoring. D: TIMS cleaned MS spectra for precursor m/z 181.072. E: PASEF-MS/MS spectrum in comparison to Spectral Library spectrum.

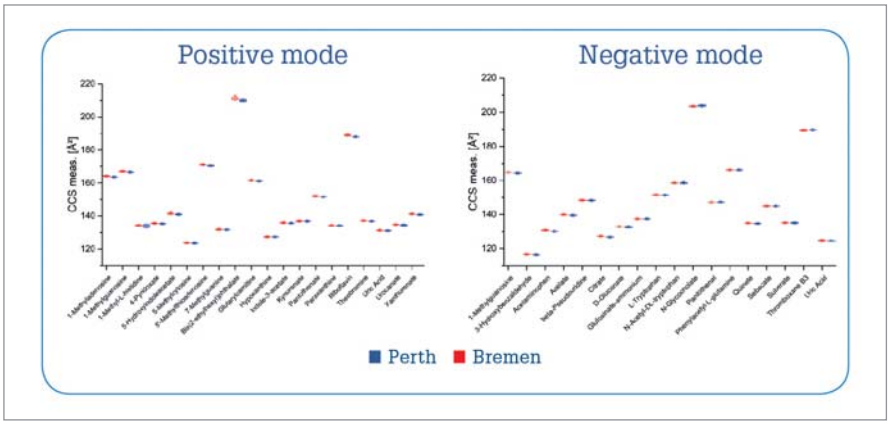

Figure 2. Reproducibility of measured CCS values. Box Plots CCS values obtained from data files.

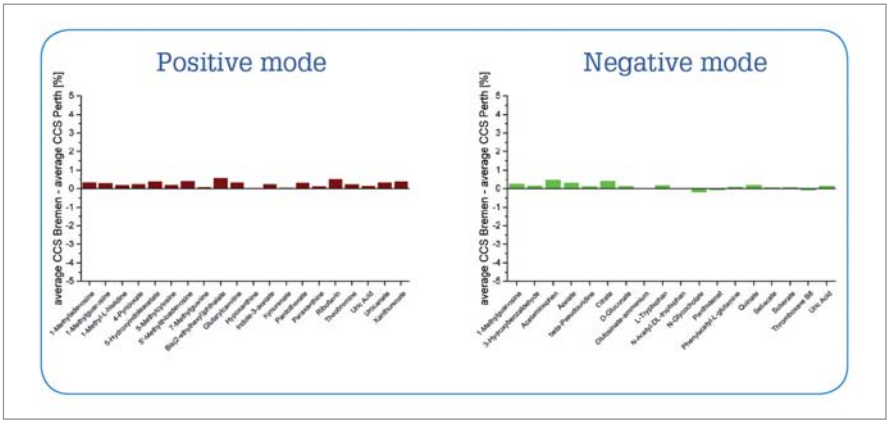

Figure 3. CSS variations across two laboratories in Bremen and Perth. The difference between the average CCS values for four replicate values is shown.

For this annotation five criteria were considered: precursor mass accuracy, retention time accuracy, isotopic pattern of the precursor (mSigma), MS/MS score and accuracy of the CCS value.

Statistical analysis (PCA and t-test) was performed in MetaboScape. For pathway mapping the caffeine and theobromine metabolism pathway (https://www.wikipathways.org/index.php/Pathway:WP3633) was imported in MetaboScape to illustrate differences in the urine samples.

Results

Investigation of CCS values

Data from one urine sample (NovaMT) was acquired on two timsTOF Pro instruments in Perth (ANPC, Australia) and Bremen (Bruker, Germany). These type of MS instruments combine advantages like high mass accuracy, sensitivity, dynamic range and mobility separation, and are thus highly suitable for this workflow. At both places, identical LC setup and methods for LC and data acquisition were used (Table 1). Data were processed using MetaboScape software. To ensure a high and reliable quality of annotation an approach combining filter criteria in five dimensions was selected. This was achieved by using the Unified CCS Compendium list, which has been extended by retention time from the Bruker HMDB Metabolite Library 2.0 and the corresponding MS/MS spectra. CCS values from the Compendium are now available for compounds searched for in PubMed and show good correlation with those observed using TIMS.

Annotation confidence is indicated in the Annotation Quality symbol (Figure 1). Each feature in the bucket table is categorized by applying criteria to measure the deviation in m/z, retention time, isotopic fit (calculated as mSigma), MS/MS score and CCS as compared with known values. Narrow filters indicate the highest possible fit, whilst wider filters can be applied to expand the number of possible annotations. The criteria can be refined by the user (Figure 1 A) and is visualized for each compound (Figure 1 C). MS and MS/MS data quality can be inspected within MetaboScape if required (Figures 1D and E). When

TIMS or PASEF data are processed, the corresponding MS and MS/MS spectra shown are cleaned by mobility, allowing noise to be removed. For example as shown for paraxanthine, a bucket table results all information in detail (Figure 1 B), e.g. ΔCCS is 0.8 percent, and Δm/z is -0.242 mDa, for which both is excellent and is represented by two green bars in the AQ symbol. The quality of isotopic pattern of the precursor mass – compared to a theoretical pattern obtained for the resulting molecular formula and calculated as mSigma value – is also used. mSigma scores of <20 indicate high isotopic overlap. The MS/MS spectrum, which is clean due to mobility filtering applied during PASEF acquisition, overlays with a high score (max 1000) to the library spectra, which is also displayed.

For the investigation of CCS values described below, data from four technical replicates have been acquired. 20 buckets with high-quality annotation (positive and negative mode) from Perth and Bremen were selected.

CCS values in the range between 120 and 210 Å² have been determined. To confirm the reproducibility of CCS values, Box plots were created (Figure 2). For both instruments and polarities, the narrow boxes indicate the high stability of CCS values for each measurement. Small average standard deviations were determined:

Polarity/lab	*Perth*	*Bremen*
Positive	*0.27 Å²*	*0.37 Å²*
Negative	*0.21 Å²*	*0.18 Å²*

Also striking is the small absolute difference between the CCS values determined in Perth and in Bremen, for all compounds in positive and negative mode.

As shown before, the CCS values in Bremen differ only slightly from those obtained in Perth. The average |ΔCCS| for Bremen versus Perth is 0.25 percent for positive mode and 0.15 percent for negative mode. The absolute value for the difference

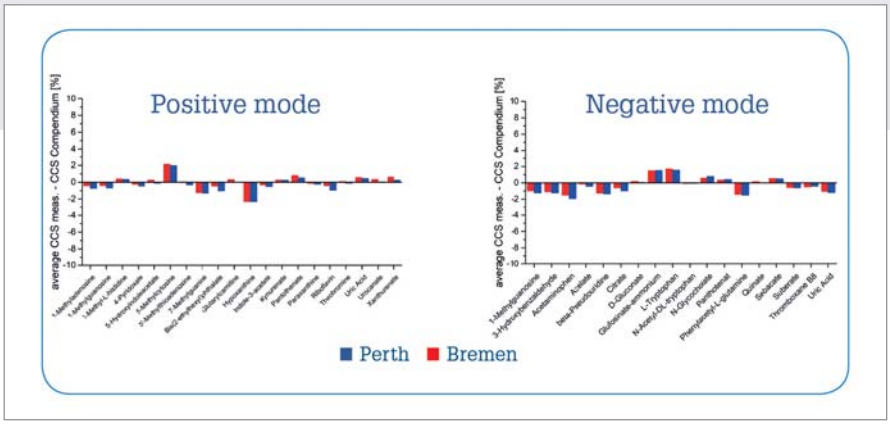

Figure 4. Differences between CCS values determined in Perth/Bremen and CCS Compendium.

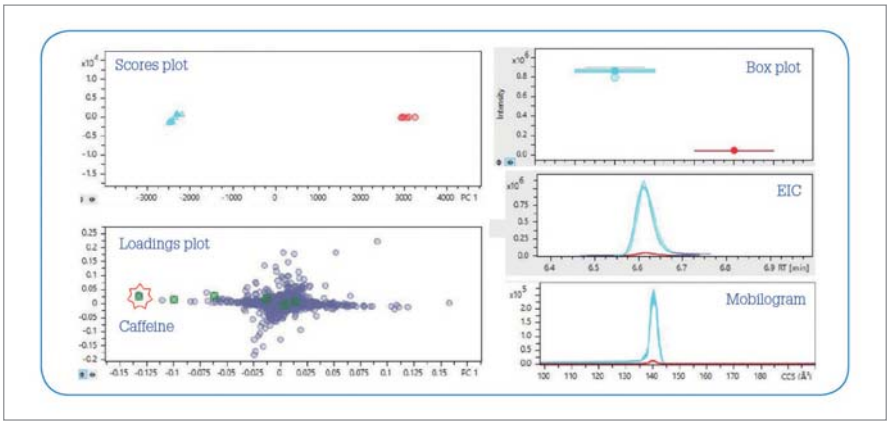

Figure 5. Comparison of two urine samples, eight technical replicates each. PCA results with Scores and Loadings plot (left). The metabolites investigated in Figure 6 are highlighted. Box Plot, Extracted Ion Chromatogram (EIC), and Mobilogram for caffeine (right). Red NovaMT, turquoise Bremen sample.

in average CCS value is positive in most cases (Figure 3), which might arise from slightly different mobility calibration.

Comparison to reference CCS values

The measured CCS values were similar to the publicly available values from the Unified CCS Compendium as shown in Figure 4. Here, average differences between the measured and Compendium CCS value were plotted versus the metabolites for positive and negative mode data from Perth and Bremen. Small Average |ΔCCS| were determined:

Polarity/lab	*Perth*	*Bremen*
Positive	*0.94%*	*0.93%*
Negative	*0.88%*	*0.79%*

For all analyzed metabolites the deviations are <2 percent.

CCS values were compared to retention times in respect of usability for use as filter for metabolite annotation. One disadvantage of retention times is the low transferability from one system to the other. When acquiring data in Perth and in Bremen, no additional effort was required for the comparison CCS values. Calibration of CCS was applied automatically in MetaboScape, whereas retention times had to be adapted to accommodate different LC methods. We also observed that the average CV for CCS values (0.11 percent) was better than for retention times (0.25 percent), as determined for positive mode data.

The high stability of CCS values shows its advantage compared to retention time, demonstrating that CCS is a highly valuable characteristic for the annotation of the features. The availability of CCS values makes this a more desired attribute to characterize metabolites with more confidence.

Comparing urine samples

The NovaMT and Bremen urine samples

Name	Position methyl group	p-value	Fold change NovaMT/Bremen	CV NovaMT [%]	CV Bremen [%]
Xanthine	none	9.05E-03	1.4	11.6	11.6
1-Methylxanthine	1	2.00E-06	-1.6	10	10.3
7-Methylxanthine	7	1.20E-04	1.5	9.1	14.8
Paraxanthine	1, 7	< 1E-08	-9.1	9.3	5.1
Theobromine	3, 7	< 1E-08	-4.6	5.5	10
Caffeine	1, 3, 7	< 1E-08	-19.6	5.9	3.3

Table 2. Fold changes and CV values for metabolites shown in Figure 6.

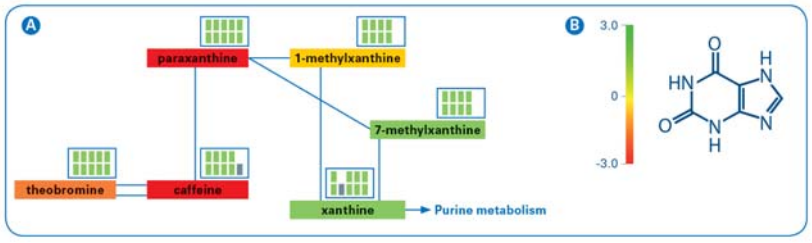

Figure 6. Caffeine and theobromine pathway, presented in a reduced form. Only xanthines are shown, which have been annotated with a high degree of confidence, represented by the Annotation Quality symbol. Two urine samples were compared "Bremen" and "NovaMT." The color coding of the metabolite name boxes indicates different intensities. Red color for higher intensity in the "Bremen" sample, green for the "NovaMT" sample A. B shows the structure of xanthine and the positions for potential methyl groups.

were investigated, and the Scores plot shows clear differences (Figure 5). The differences mainly arise from caffeine and other xanthines and are highlighted. For caffeine, the Box plot, the Extracted Ion chromatogram and the Mobilogram clearly indicate a big difference in intensity between the two urine samples.

Caffeine and theobromine pathway

For relative quantitative analysis, a t-test was performed to confirm suitable low CV values for all samples (Table 2). In Figure 6 we illustrate how two urine samples (NovaMT, Bremen) differ in respect of xanthines (with 0, 1, 2, and 3 methyl groups) in the Caffeine and Theobromine Pathway. To show the reliability of the annotation, the AQ symbols for the investigated metabolites are shown in the Figure. While the amount of xanthine, 1- and 7-methylxanthine are mostly comparable, the amounts for theobromine and caffeine and paraxanthine differ strongly by factors 5, 9 and 20 respectively (Table 1). This difference for theobromine, caffeine and paraxanthine in the Bremen sample clearly indicates consumption of coffee by the proband, which has not completely been degraded. In NovaMT urine, a smaller amount of caffeine was also detected (Figure 6 D and E). We further see that in NovaMT urine the amount of xanthine, a later degradation product of caffeine, is slightly higher than in the Bremen sample. This might result from sampling at a later stage of coffee consumption.

Conclusion

For metabolomics the reliable annotation of features is essential. In the current approach we demonstrated how ion mobility can support this:

- CCS values from the timsTOF Pro instruments show high intra-lab reproducibility for CCS values with a standard deviation <0.4 Å^2, low inter-lab differences in measured CCS values <0.3 percent, and high accordance of measured CCS values to those from Unified CCS Compendium with an average | ΔCCS | <1 percent. This allows to use CCS values as filter for 4D annotation – in addition to retention time, precursor mass, isotopic pattern and MS/MS spectrum.
- PASEF spectra benefit from ion mobility separation, since cleaner MS/MS spectra are obtained using an on-the-fly mobility filter. This improves ID in small molecule workflows.
- The application of using CCS values was shown for pathway mapping of metabolites of the Caffeine and Theobromine metabolism

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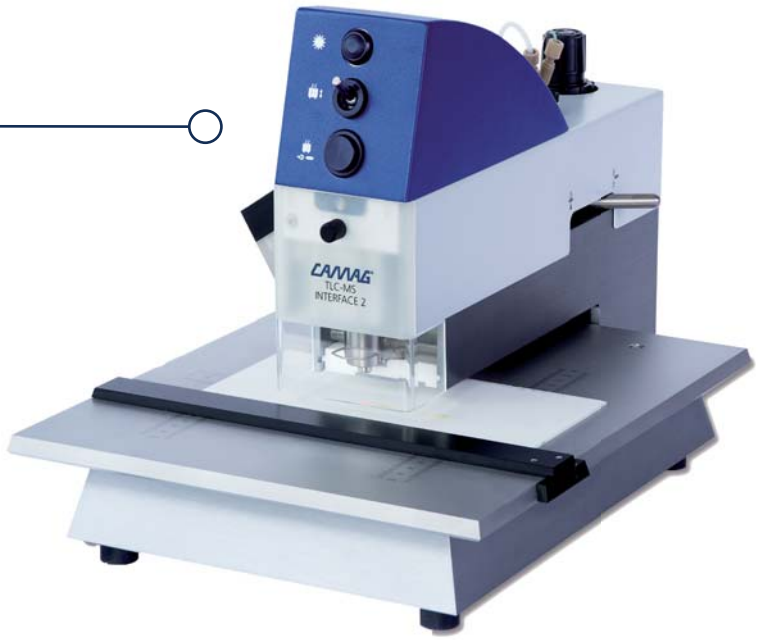

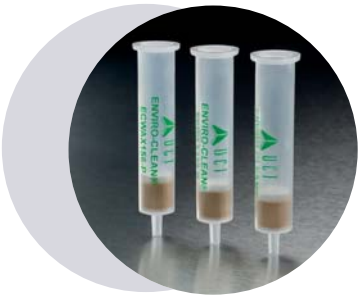

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Sitting Down With...
Michael Lämmerhofer,
Professor of Pharmaceutical (BIO-)Analysis, Institute of Pharmaceutical Sciences, University of Tübingen, Germany

You're a noted proponent of bioanalysis. Tell us more.

It's an ever-growing field that presents a number of interesting challenges for analytical chemists. Biomolecular networks are extremely complex, dynamically regulated and difficult to understand. Too often we have a narrow focus – just a spotlight on a specific pathway or small set of molecules, but practicing a wider perspective is a major challenge. Rather than use a single technique, a complex array of approaches is required.

The clear link to clinical application makes it a fascinating field; samples are obtained from patients and we derive as much important information from them as possible. This insight can be fundamental to achieving correct diagnosis, and subsequently for delivery of the correct treatment. Personalized medicine of this kind can rely on many sets of omics data, making life in this multi-disciplinary field (requiring knowledge of biochemical background and technical analytical chemistry) a serious – but exciting – challenge.

Do you find bioanalysis demanding?

It requires a particular set of skills; it's not enough to simply know how to operate an instrument – you must also understand sample preparation and handle the data produced. That can be challenging for many students; very few tend to get much, if any, bioinformatic training during their regular studies. They must quickly acquaint themselves with these approaches during their PhD. That's crucial – many modern projects involve a considerable amount of data processing. While the "wet chemistry" portion of the project may only take a week, the resulting analysis could take many months!

How do you keep your students inspired?

I like to work with students in the lab because it's much more personable – you're open to one-to-one, in-depth discussion. My motivation is clear – I want to help talented students develop and grow. Combining my own experience with the student's skills (they are often very tech savvy) has produced some very encouraging results. When you're standing in a lecture hall, you can't have that same level of individual connection. In the lab you get to know one another, you work together, and you provide each other a source of inspiration.

How were your own experiences as a student?

My school experience certainly left a lasting impression. I attended a boarding school that was part monastery, part high-school. I learnt about the importance of discipline, which has stood me in good stead throughout my career, and found all the sciences equally fascinating, opting to pursue pharmaceutical sciences because it was so interdisciplinary. Later, I focused solely on chemistry, which appealed to me because it was so understandable and logical. I was fortunate to undertake a PhD with Wolfgang Lindner – a titan of the field – and then follow him to Vienna when he gained a qualified professorship position.

You're also particularly interested in chirality – why?

I've worked on chiral separations for much of my career, and have developed a number of chiral stationary phases. The interesting thing about chiral separation is the depth of molecular recognition and its core in the molecular creation path. How can a small molecule distinguish between stereoisomers just as a ligand recognizes a receptor? What particularly fascinates me, however, is the development of functional materials that can be modified to induce a change in selectivity.

What does the future hold for you?

Over the next five years we hope to develop complementary technologies that will allow us to carry out robust clinical-level lipidomic studies within a reasonable timeframe. It will be important to incorporate seamless data processing into those protocols in order to ensure that clinicians are appropriately supported. This will involve working with around 50 PIs – mostly from the clinic – to ensure we produce a friendly operating structure; we're aiming to automate as much of the system as possible to accelerate output. We're also hoping to share what we learn with researchers at our institution in order to foster new collaborations and developments.

And what about HPLC 2021?

Interaction with colleagues supports our work as scientists, so the cancellation of conferences amidst the pandemic is very unfortunate. Sadly, this included HPLC 2020 in San Diego. Next year, HPLC will be back (we hope), taking place in Düsseldorf, Germany (www.hplc2021-duesseldorf.com).

We hope that many scientists will be keen to attend the meeting to present their results, exchange ideas and discuss them with colleagues. HPLC 2021 will be Europe's largest separation science-focused meeting in 2021, taking aim at MS-hyphenated techniques and their applications in fields from pharma to bioanalysis, the environment and forensics. We will spotlight hot topics, like multidimensional separations, ion-mobility MS, personalized medicine and food quality, but also more industry-centered topics, such as digitalization in the analytical lab. Besides these, traditional topics related to fundamentals of chromatography and new separation materials will also take center stage. What's more, there will also be a large exhibition in which vendors of analytical instruments and products will showcase their latest developments.

We hope many will join us… After all, the great connections you make at 2021, could well guarantee perfect peaks in the future!

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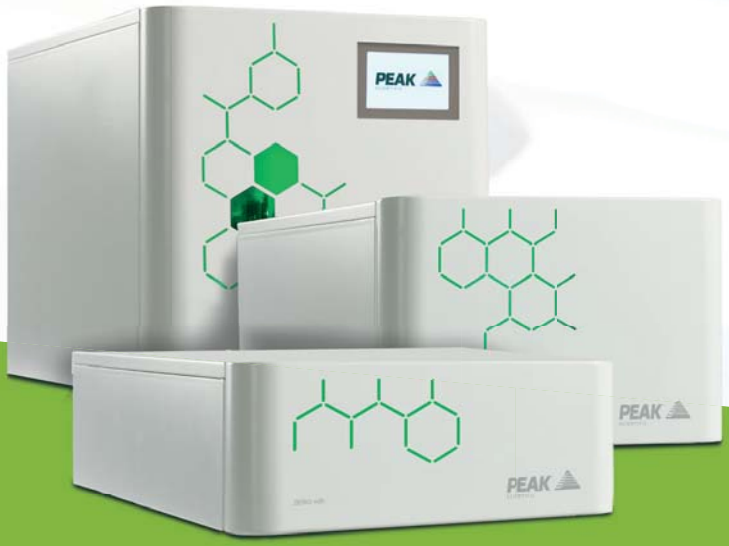

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