FEBRUARY 2018 # 61

the Analytical Scientist™

A Study in Scarlet

Mars 2020's SHERLOC will use spectroscopy to seek molecular traces of life on the red planet.

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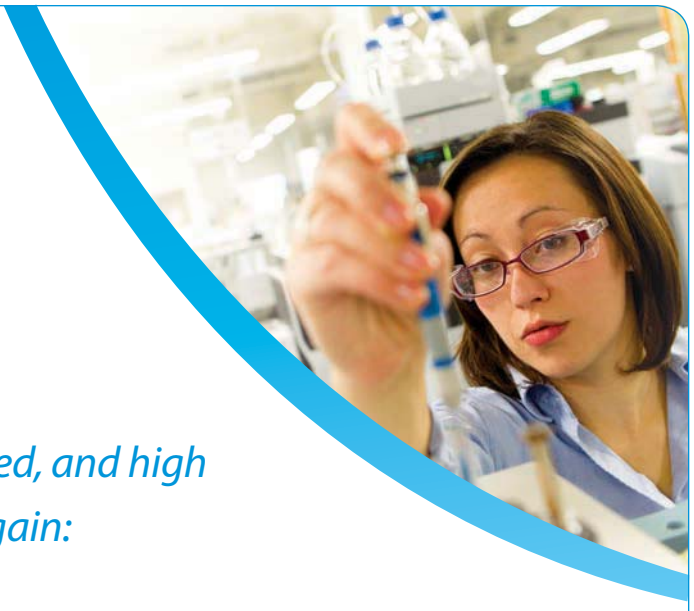

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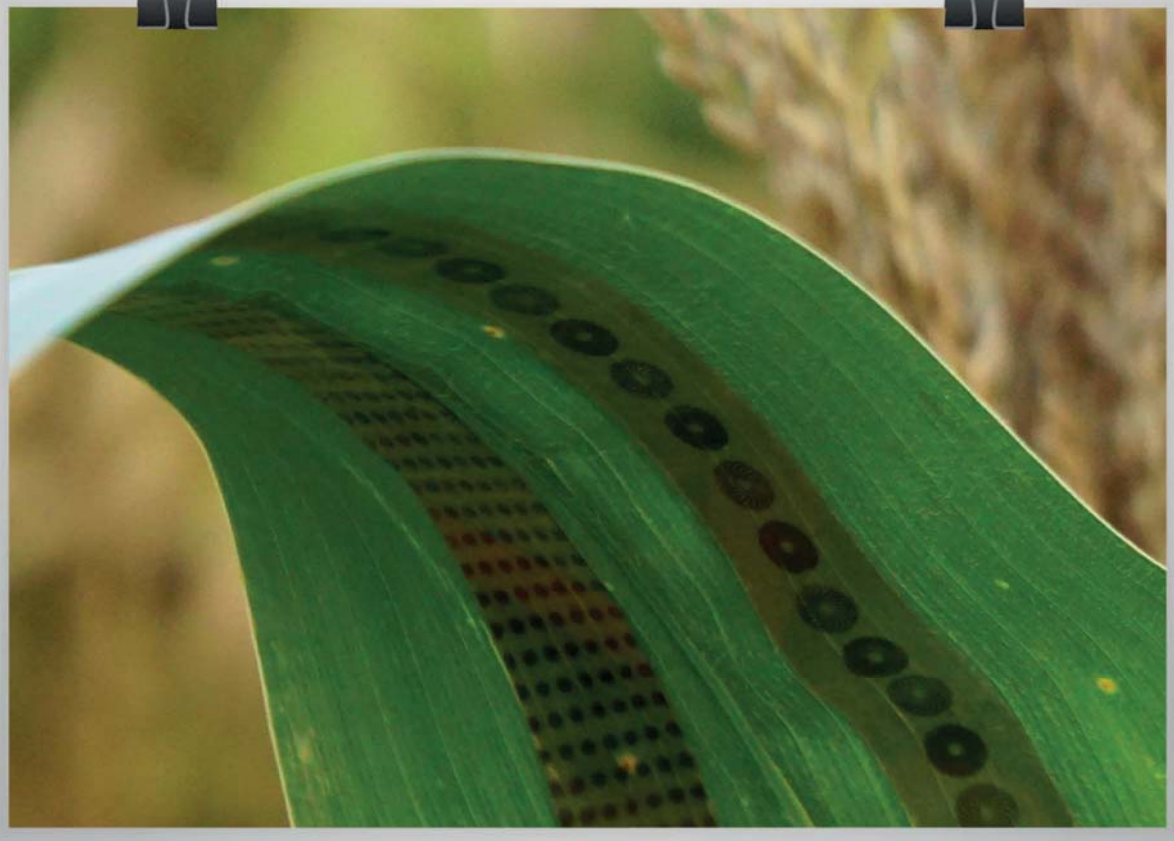
Image of the Month



How Does Your Garden Flow?

It seems that humans don't have the monopoly on wearable sensors – Iowa State researchers have developed a tiny sensor for measuring the speed of water movement in plants. Made from graphene oxide and attached to the plant using adhesive tape, the microsensor can measure transpiration in real time. The authors believe this low-cost "plant tattoo sensor" has potential for diagnostics, testing pesticides on crops, and environmental monitoring.

Credit: Liang Dong/Iowa State University.

Reference: S Oren et al., Adv Mater Technol, 2, 1700223 (2017).

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

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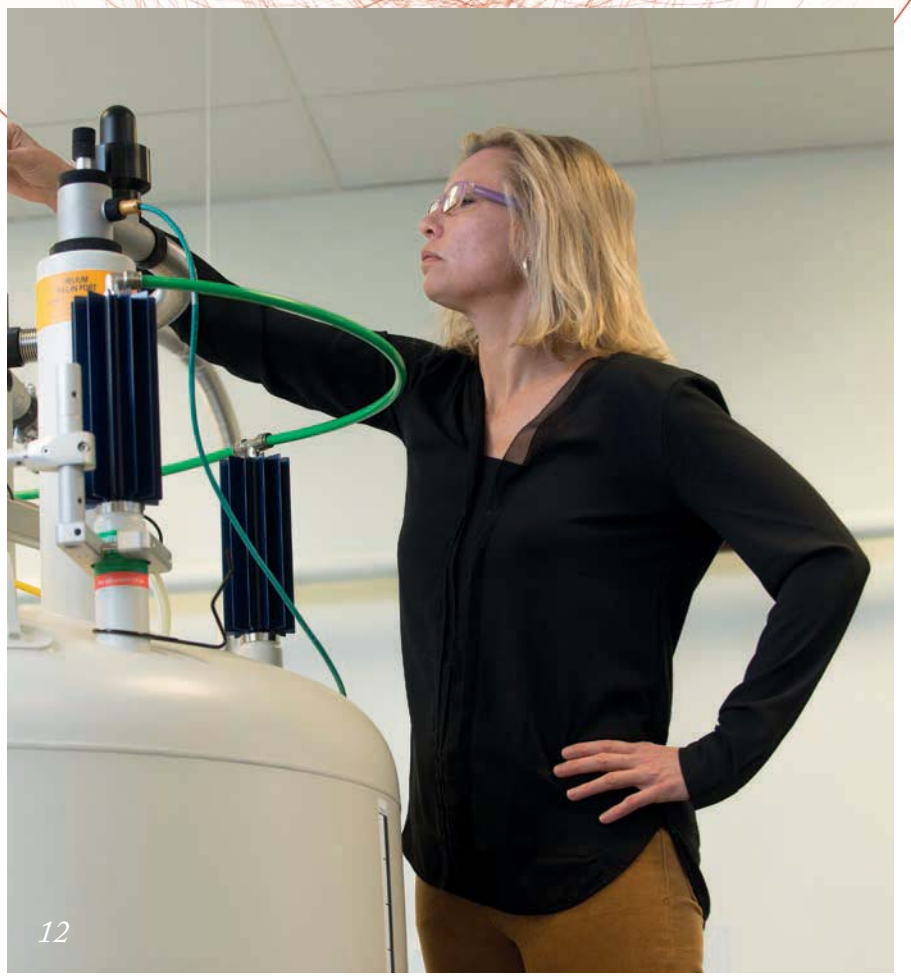

On The Cover

The great detective is the namesake for Mars 2020 instrument SHERLOC (Scanning Habitable Environments with Raman & Luminescence for Organics & Chemicals).

Upfront

the Analytical Scientist

ISSUE 61 - FEBRUARY 2018

Change of address
hayley.atiz@texerepublishing.com
Hayley Atiz, The Analytical Scientist, Texere Publishing Ltd, Haig House, Haig Road, Knutsford, Cheshire, WA16 8DX, UK

General enquiries
www.texerepublishing.com | info@texerepublishing.com
+44 (0) 1565 745 200 | sales@texerepublishing.com

Distribution:
The Analytical Scientist (ISSN 2051-4077), and The Analytical Scientist North America (ISSN 2514-7544), is published monthly by Texere Publishing Ltd and is distributed in the US by UKP Worldwide, 3390 Rand Road, South Plainfield, NJ 07080
Periodicals postage paid at South Plainfield, NJ
POSTMASTER: Send US address changes to The Analytical Scientist C/O 3390 Rand Road, South Plainfield NJ 07080.
Single copy sales £15 (plus postage, cost available on request tracey.nicholls@texerepublishing.com)
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The 42nd International Symposium on Capillary Chromatography (ISCC) and the 15th GC×GC Symposium is a "hyphenated" meeting which will be held again in wonderful Riva del Garda (Italy), from May 13 - 18, 2018. Apart from the most recent advances in the fields of pressure and electrodriven microcolumn separations, and comprehensive 2D GC, This year particular emphasis will be directed to all Comprehensive Separation Technologies and MS Hyphenation and to Capillary Chromatography and 2D GC with various forms of MS from unit-mass to high resolution and from single to hybrid analyzers. Consequently, both the importance and complementary nature of chromatographic and MS processes will be given high consideration. Within the wider context of separation science, great space will be also given to the sample preparation process, in both oral and poster sessions.

The ISCC/GC×GC scientific program will be a rich one, it being characterized by:

- invited contributions from leading scientists reporting the latest most exciting developments
- keynote lectures from promising young researchers
- very active poster sessions
- discussion sessions
- workshop seminars presenting the most recent novelties in scientific instrumentation
- a world-class GC×GC course

Researchers in all areas relevant to the subjects of the symposia are invited to submit abstracts. As is traditional for the Riva meetings, the majority of presentations will be in a poster format and the Scientific Committee will select contributions for oral presentations.

As always, many awards will be assigned in both the ISCC and GC×GC events, recognizing excellence in both established and young scientists, in oral and poster presentations.

Exhibitors and sponsors are a fundamental part of the meeting (without them…Riva wouldn't be Riva!) and are encouraged to participate by reserving booth space, and becoming a sponsor., and to promote the ISCC and GC×GC events.

Last, but not least, the traditional "Riva" social program will be entirely maintained, with one or two events each day: cocktails, the welcome reception, the concert, the wine and cheese evening, and of course, the disco night!

Please keep visiting our web site (www.chromaleont.it/iscc) for new information as it becomes available.

42ND INTERNATIONAL SYMPOSIUM ON CAPILLARY CHROMATOGRAPHY

AND

THE 15TH GC×GC SYMPOSIUM

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CELEBRATING THREE YEARS OF HUMANITY IN SCIENCE

The Humanity in Science Award recognizes and rewards scientific breakthroughs that aim to have a real impact on humankind's health and wellbeing.

HUMANITY IN SCIENCE AWARD

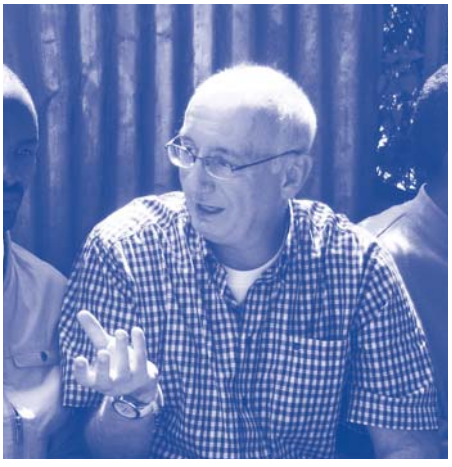
the Analytical Scientist

2015

Peter Seeberger & Andreas Seidel-Morgenstern, Directors at two collaborating Max Planck institutes in Germany, developed an innovative process to manufacture the most effective drugs to treat malaria from plant waste material, air and light.

2016

Waseem Asghar, Assistant Professor at Florida Atlantic University, developed flexible sensors for the rapid and cost-effective diagnosis of HIV – and other infectious diseases – in point-of-care settings.



2017

Richard Jähnke, Global Pharma Health Fund (GPHF), developed and continuously improved GPHF Minilab – a "lab in a suitcase," enabling resource poor countries to rapidly identify substandard and falsified medicines.

Nominations will open soon for the 2018/2019 Humanity in Science Award

www.humanityinscience.com

A Social Network?

We have ways of helping you talk…

Over New Year, I went to a party with non-scientists (it happens), where I was asked the inevitable question: "What do you do?"

"I'm Deputy Editor of a magazine," I threw out, tentatively.

Eyes light up. "What kind of magazine?"

"Analytical science."

Silence.

I was reminded of that earlier exchange in an excellent presentation at HTC-15 last month that focused on the public's lack of emotional connection to chemistry (1). It comes as no surprise. But it's clear to me that the divide does not stem from the fact that analytical science is dull – or that people are unable to relate at all – rather, it's because the general population simply does not know how important it is. Back at the party, when I spoke of its role in forensic toxicology, healthcare, environmental monitoring, or cannabis research (look out for The Cannabis Scientist this month), interest was suddenly re-piqued.

So how do we communicate more effectively with the public? Peer-reviewed journal articles are less likely to be read by people outside the immediate field, let alone the public. (And they are likely to reinforce stereotypes of scientists being preternaturally intelligent, almost alien beings [2].) And while social media is a natural platform to communicate less formally, its impact in (analytical) science seems to have been minimal – the Twitter poster sessions created by Strathclyde's Matt Baker are an exception rather than a rule (3). But things are looking up: many of our interviewees talk about the importance of developing students' communication skills, conferences are increasingly including social media symposia (see page 11), and new research projects are using blogs and Twitter as part of their M.O. (4).

Obviously, we like to think that we're playing our part. In an issue covering space travel, drug discovery, DNA fractionation and analysis of crab urine, I think we shout pretty loudly about the diverse applications of analytical chemistry. And while we may not communicate directly with the public, we do convey complex technical information to scientists in completely different fields. Giving scientists a voice is also important to us; it motivates us in every interview we do, and gives us a thrill when a particularly opinionated In My View comes our way.

So, talk to us, to your students, with the public – to your Twitter followers. If only to save me from awkward party chit-chat.

Joanna Cummings
Deputy Editor

References

1. *http://rsc.li/1fgw4vt*
2. *http://bit.ly/2E3d04I*
3. *www.tas.txp.to/TAS0317/haste*
4. *www.tas.txp.to/TAS0917/foodsmartphone*

Upfront

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

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

Gut Reaction

An experimental electronic pill can analyze the chemical composition of intestinal gases

Gases in the gut – the result of both microbial and chemical activities – are closely related to an individual's state of health. Indeed, specific gas profiles are associated with several disorders, including irritable bowel syndrome (IBS), malabsorption of carbohydrates, and small intestinal bacterial overgrowth (SIBO). Current diagnosis of these conditions can be at best limited, and at worst unreliable – as well as being invasive for the patient.

A collaborative team from RMIT University and Alfred Hospital in Melbourne, Australia believe that the key to better diagnostics lies within the gut itself – and have devised an ingestible pill that can measure gases while moving through the body.

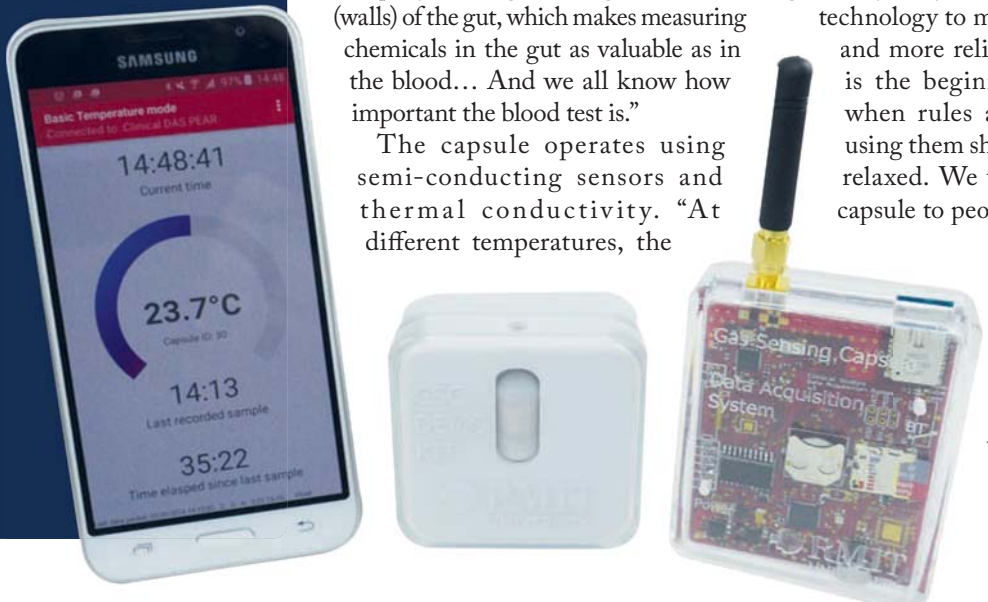
"Ingestible sensors provide easier and less invasive access inside the body," says lead author Kourosh Kalantar-Zadeh (1). "The chemicals of the gut (gases, electrolytes, metabolites, and hormones) are rapidly exchanged through the mucosa (walls) of the gut, which makes measuring chemicals in the gut as valuable as in the blood… And we all know how important the blood test is."

The capsule operates using semi-conducting sensors and thermal conductivity. "At different temperatures, the two sensing elements become sensitive to different gases such as oxygen, hydrogen and carbon dioxide," explains Kalantar-Zadeh. "Then we extract the concentration and type of gases using a data analysis program."

Kalantar-Zadeh and team unveiled a prototype capsule two years ago – but it has undergone considerable development since. "The pill now incorporates an oxygen sensor that shows the passage of the capsule from one segment of the gut to another, improved communication systems to reduce loss of data, a temperature sensor that shows the core temperature of the body and shows when the capsule leaves the body, and modulation of the heater for gas sensors that allows the extraction of several gases at a time and whole capsule reliability," says Kalantar-Zadeh.

The human pilot trial has now been completed, and the group is looking for investors to finalize the Phase II trial. They have also established a company, Atmo Biosciences, to commercialize the pill.

Kalantar-Zadeh says he would next like to target metabolites, and measure additional gases. However, a major hurdle is regulation. "Ingestibles are generally very safe and we have the technology to make them even safer and more reliable," he says. "This is the beginning of a new era, when rules and regulations for using them should be significantly relaxed. We want to deliver this capsule to people in need."



Reference

1. J Kalantar-Zadeh et al., "A human pilot trial of ingestible electronic capsules capable of sensing different gases in the gut", Nat Electron, 1 79–87 (2018).

Something New Under the Sun

We preview Pittcon 2018 to see if it will have you walking on sunshine

Pack your swimsuits, folks – this year's Pittcon is in Orlando, Florida. The lectures and symposia tip a cap both to experienced scientists and early career researchers, and as ever, the networking sessions – covering everything from social media to lab safety – provide plenty of opportunities to share ideas with fellow scientists. There's also a new feature this year: NEXUS, a "hub of interactive presentations providing education, entertainment, networking and more" – including a VR experience and a LEGO car race. As for The Analytical Scientist team? When we're not at stand #3056, we'll be attending the sessions below.

Symposia

IAEAC – Thinking Outside the Box (Monday AM)
The Role of Ultrahigh-Resolution Mass Spectrometry in the Omics Era (Tuesday AM)
Nanotechnology Against Cancer, Heart and Neurological Diseases: A Fight in Progress (Wednesday AM)
Powerful New Ionization Processes in Mass Spectrometry (Thursday AM)

Oral Sessions

Innovations in Teaching (Monday AM)
Sensors – Bioanalytical (Monday PM)
Analytical Applications in Material Science (Tuesday AM)
Data Analysis and Manipulation (Wednesday AM)
Food Safety – Sensors, GC/MS and Others (Thursday AM)

Networking

Getting the Most From Your Pittcon Experience – Pittcon Committee (Monday AM)
Core Scientific Facilities: Challenges and Opportunities – Thayumanasamy Somasundaram (Monday PM)
Student-Faculty-Industry Networking: Getting Students Prepared for Their Careers – Erin Gross (Tuesday AM)
Sharing Science to the Non-Scientist – Doug Carlton (Wednesday AM)
Using Social Media to Reach the Scientific Community – Megan Cavanaugh (Wednesday PM)
Quality in the Cannabis Industry – Roger Brauninger (Thursday AM)
Nanomaterial Characterization – Matthew Linford (Thursday PM)

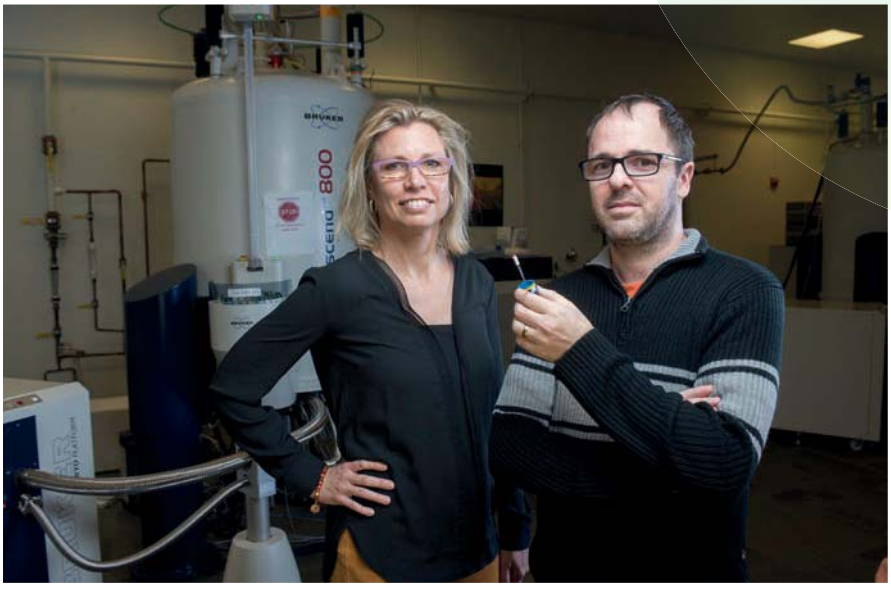
Organized Contributed Sessions

Field Spectroscopic Applications: Point of Care, Safety & Security, and Environmental Scenarios (Monday PM)
Ionophore-Based Chemical Sensors II (Tuesday AM)
Overview of Recent Developments in Ultrafast Chromatography: From Theory to Applications (Weds PM)
Innovations and Trends in Forensic Examination of Seized Drugs and Forensic Toxicology (Thursday AM)

Pittcon 2018 will be held February 26 – March 1 at Orange County Convention Center, Orlando, Florida, USA: www.pittcon.org

A "Nose" for Trouble

What makes blue crab urine so scary? NMR- and MS-based metabolomics has the answer



Crabs are known to have superb chemosensory capabilities, walking upstream towards tantalizing chemical cues to locate food and mates, despite turbulence in the water. These detection abilities are particularly important for the mud crab; its ability to "smell" its main predator – the blue crab – is a matter of life or death. The canny crabs hide themselves away whenever one of its predatory cousins is in the vicinity - but what is it that makes the muddy invertebrates act so... spinelessly? A team from the Georgia Institute of Technology decided to wade in and find out.

"We suspected that the crabs were responding to the presence of predators by sensing chemicals that were being transmitted through the water column – however, little was known about the molecular cues involved," says Julia Kubanek, one of the researchers (1). "We then discovered that when the mud crabs are exposed to the urine of blue crabs, we get the same hiding and hunkering down response that mud crabs exhibit when there is a whole, live blue crab nearby."

The team analyzed blue crab urine to isolate "fear-inducing" chemical cues, using both nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) in an untargeted metabolomics approach. "We had no preconceived notion of what urinary metabolites would be important, so we wanted to apply chemical profiling tools that would allow us to detect the largest number and breadth of chemicals possible," says Kubanek.

"We looked for NMR signals whose abundance correlated with fear-inducing effects, as measured by the behavioral assay," says Kubanek. Signals for two compounds jumped out of the analysis: trigonelline and homarine. "Their concentrations were consistently higher in the urine samples that induced greatest fear. So we tested each compound individually at concentrations found in blue crab urine, and each of these compounds alone, and in combination, recapitulated the effects of whole urine."

Notably, the urine of blue crabs that had been fed with mud crabs resulted in the greatest fear response: the mud crabs were less scared (although still quite scared) when exposed to urine of blue crabs fed with oysters.

The work is only the first (side!) step for the team. "We would like to better understand what chemosensory and physiological pathways are involved in mud crab detection of trigonelline and homarine, and to study how other estuarine animals respond to blue crab urinary metabolites," says Kubanek. "Another interesting avenue for future research would be to study how anthropogenic influences, such as pollution and ocean acidification, affect crabs' ability to perceive chemical cues."

Reference

1. *RX Poulin et al., "Chemical encoding of risk perception and predator detection among estuarine invertebrates", PNAS [Epub ahead of print] (2017).*

Mirror, Mirror…

A tool to predict racemization could help prevent drug discovery dead ends

Many drugs are chiral molecules, which means that they have the potential to "flip" and exist as different enantiomers – non-superimposable mirror images of the original molecule with an identical chemical structure. In some cases, this flipping behavior can occur when an enantiomerically pure drug enters the body – a process known as racemization. Recent research aims to predict racemization (1), so we spoke to co-author Niek Buurma from Cardiff University's School of Chemistry to discover more about the dangers of mirror molecules – and a new tool to remedy the problem.

Why is racemization a problem?

The pharmaceutical action of a significant fraction of all drugs depends on administering the correct enantiomer. When we administer a mixture of enantiomers, one of the enantiomers will act as intended, but the other doesn't fit with the target, which can lead to binding to unintended targets and potentially serious side effects. If racemization is discovered late in the drug discovery process, the compound may turn into an expensive blind alley.

These days, everyone is aware of the need to administer single-enantiomer drugs. But until now, there were no good models to quantitatively predict how these enantiomers would behave once exposed to the aqueous conditions in the body.

How can your model help?

Using circular dichroism and H NMR spectroscopy to measure the kinetics for racemization of 28 compounds, we (along with Andrew Leach, from Liverpool John Moores University) have developed a predictive model that allows efficient quantitative prediction of racemization risk. Essentially, it allows researchers in academia and industry to identify molecules at risk of racemization at a very early stage. Not only does this help avoid dead-end research and development pathways, but it also allows researchers to "design out" racemization risk by exploring the effect of changes to the molecular structure. In addition, we present a series of different experimental approaches to confirm whether a potential drug racemizes under physiological conditions.

What's next?

We are now developing a version of the model that predicts the risk of racemization during typical reaction workup procedures. Our guidelines and predictive models provide a solid approach to predicting racemization, and we would like to see our quantitative predictions and experimental tests incorporated as standard in the drug discovery pipeline.

Reference

1. *A Ballard et al., "Quantitative prediction of rate constants for aqueous racemization to avoid pointless stereoselective syntheses", Angew Chem Int Ed, 57, 982 (2018).*

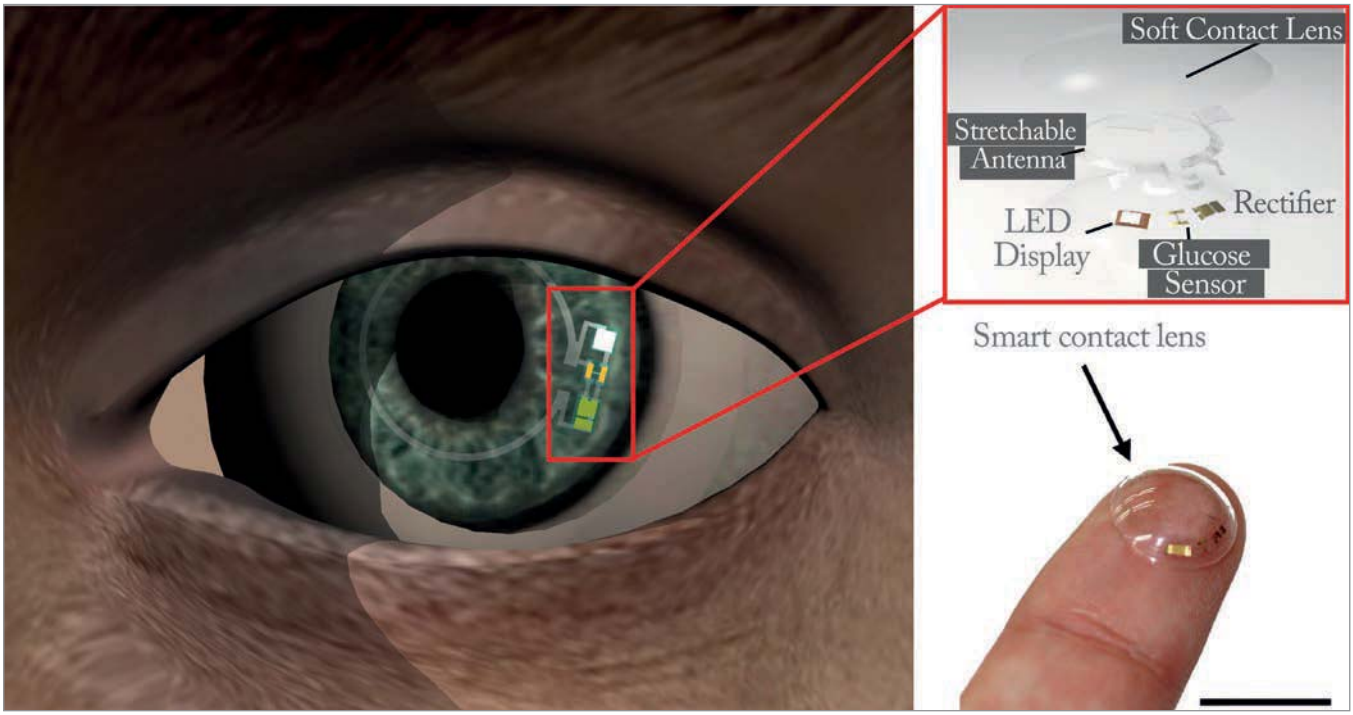


Figure 1. The soft, smart contact lens is comprised of a hybrid substrate, functional devices (rectifier, LED and glucose sensor) and a transparent, stretchable conductor (for antenna and interconnects). Electric power is wirelessly transmitted to the lens through the antenna, and activates the LED pixel and the glucose sensor. If glucose levels in tear fluid rise above the predefined threshold level (0.9 mM), the pixel turns off (1).

Sweet Tears

A noninvasive method to monitor disease in the eye

Many patients with diabetes would be happy to see the back of their blood glucose monitor and the daily finger-prick tests. Enter: a team of scientists from the Ulsan National Institute of Science and Technology (UNIST), South Korea, who have created a means of wirelessly monitoring glucose levels with a soft contact lens.

"Embedded within our smart contact lens are electronic circuits, an antenna, a glucose sensor and LED pixels integrated as stretchable forms," explains Jang-Ung Park (1). "This improves the comfort and wearing-time of the lens compared with previous smart lenses that were hard due to having brittle and more rigid components."

Their sensor comprises a graphene surface to which glucose oxidase (GOD) enzyme is immobilized. Tears (containing glucose) pass through the sensor channel; GOD oxidizes the glucose, which releases electrons in a concentration-dependent manner, which the sensor detects, enabling the glucose concentration to be determined (1). The sensor contains an LED that responds to the changes in resistance (which is coupled to tear glucose concentration). Below 0.9 mM, the LED emits light; above this, the LED pixel is turned off, providing a visible cue that the glucose threshold has been reached (Figure 1).

So far, the team has demonstrated that the device can respond to changing glucose concentrations in rabbit eyes, and they plan to move into clinical tests in humans. But what of its applications for ophthalmology? The team write that their novel system could "provide a platform for wireless, continuous, and noninvasive monitoring of physiological conditions, as well as the detection of biomarkers associated with ocular and other diseases," and drug delivery isn't out of the question.

Reference

1. *J Park et al., "Soft, smart contact lenses with integrations of wireless circuits, glucose sensors, and displays", Science Advances, 4, eaap9841.*

GoldenEye and a Golden Ticket

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

Products and launches

- SCIEX has launched a high-performance mass spectrometry technology device - Citrine™ MS/MS – for use in clinical diagnostics.
- At the end of January 2018, Waters introduced the ACQUITY biosystem, a quaternary LC for the improvement of bioseparation.
- Hitachi High-Tech Analytical Science has added to its range of benchtop analyzers with the FT110A and FT150 series, designed to measure ultra-thin coatings.
- BaySpec has introduced a new range of spectrometers and spectral analyzers, including the snapshot imager GoldenEye™, and the new palm spectrometer Breeze™.

Collaborations and acquisitions

- Ingenza has joined ConBioChem, a translational project focusing on "the development of novel platform technologies for the continuous bio-production of commodity chemicals."
- Agilent has awarded Angiex with a Golden Ticket, which provides funding for a lab bench space for one year. Agilent's Senior VP and CTO Darlene Solomon said the biotech startup had demonstrated "an excellent combination of scientific impact, enthusiasm to collaborate with Agilent, and personal drive to advancing cancer treatments."
- Eurofins has acquired Craft Technologies, Inc., expanding its offering in food and feed testing. The company has also branched out further into the Asian market with its acquisition of Tsing Hua in Taiwan.
- A new collaboration between Waters and Malvern PANalytical aims to improve the characterization of polymers by combining Waters' Advanced Polymer Chromatography System with Malvern's OMNISEC REVEAL for the calculation of absolute molecular weight, intrinsic viscosity and hydrodynamic radius.

Company updates

- Ray Himmel has joined VUV Analytics as Senior Vice President of Sales. Himmel's previous positions include 15 years at Waters Corporation.
- Agilent has been named Company of the Year by Instrument Business Outlook, for "the expansion of Agilent's diagnostics business, entry into new markets such as cell analysis and Raman spectroscopy, and additions to successful franchises in NGS sample preparation and LC-MS, among other key product launches."
- Owlstone Medical has won the Idiopathic Pulmonary Fibrosis (IPF) Catalyst Challenge for its breath biopsy platform. This a non-invasive system finds and analyzes volatile organic compounds in breath to improve diagnosis of IPF.

For links to original press releases, visit the online version of this article at: tas.txp.to/0218/BUSINESS

In My View

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

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

Contact the editors at edit@texerepublishing.com

Keeping it Simple(r) in SFC

Method development in supercritical fluid chromatography is hampered by too much focus on the wrong variables.

By Terry A Berger, SFC Solutions, Inc., Sarasota, Florida, USA.

When I talk to analytical chemists about supercritical fluid chromatography (SFC), they often say that SFC is more complex than reversed phase high performance liquid chromatography (rHPLC) because, unlike the almost universal use of C18 in rHPLC, there are "too many" achiral stationary phases for SFC.

As a side note, there are reasons for the proliferation: historically, polar solutes often tailed badly or did not elute when using traditional polar stationary phases (silica, diol, Cyano, amino), so polar additives were added to the mobile phase. While dramatically extending the range of solutes amenable to SFC, additives also caused problems, particularly with MS detection (1). And so the search was on for new achiral stationary phases that could eliminate the need for additives. Some of these new phases have improved elution but, so far, they have not been successful in completely eliminating the need for additives. The search continues, and that's why recent years have seen the introduction of (too) many new phases for SFC.

Although there are many stationary phases available, choosing one does not have to be a Herculean task. As Lesellier and West point out in their systematic approach to characterizing stationary phases in SFC (2), it is often far simpler to choose – and stick with – one appropriate stationary phase or at least narrow the possibilities to just a few for known solute structures. It's clear that this good message has not reached all users, some of whom now approach SFC stationary phase selection as if it were chiral method development. In chiral method development, there remains no means to a priori predict the stationary phase that will give any, let alone the best, separation. Thus, many different chiral stationary phases are compared using mobile phases with and without additives.

A similar approach is being used in SFC for achiral stationary phase selection with a generic composition gradient at fixed temperatures and pressures. This "stationary phase-centric" view of method development largely ignores variations in the interactions of the mobile phase with both the solutes and the stationary phase. I believe this approach wastes time and resources evaluating inappropriate stationary phases, while minimizing the probability of finding conditions that get anywhere near optimum.

In SFC, method development involves much more than stationary phase selection. Like the stationary phase, the mobile phase should have chemical characteristics similar to, or at least compatible with, the solutes. Pure CO_2 is inappropriate for solvating any but the least polar solutes. Modifiers can dramatically increase the solvent strength of mixed fluids; Snyder's solvent triangle (3) is a reasonable guide to modifier selection. Solvent strength in SFC largely follows the P scale, which allows the appropriate modifiers to be predicted – provided you know the structures of the solutes. After choosing an appropriate modifier, the next obvious question is whether or not an additive is needed, and can be answered by a few quick injections on the chosen stationary phase. Most of the time, the need for (or nature of) the additive can be

"A little bit of thought goes a long way in choosing an appropriate stationary phase, the most likely modifier, and the nature of the additive."

predicted (acid versus base).

There is another difference between SFC and HPLC: temperature tends to have little effect on selectivity in HPLC, whereas in SFC relatively modest temperature changes (+/- 5-10°C) can have significant and unpredictable effects. Even almost identical compounds can respond differently to small shifts in temperature, and such shifts can be used to separate peaks that previously overlapped (change selectivity).

Despite significant over-emphasis in the literature, changes in selectivity are not caused by simple bulk density effects. Indeed, much of the recent literature treats density as a major control variable, but this is deceptive. Most of us think of density as a homogeneous property of the mobile phase; however, pressure and temperature variations have much more impact than simple bulk density. Temperature affects the degree of adsorption of mobile phase components and the solutes on the stationary phase. It also affects the composition of the solvent sheath around solute molecules. These major aspects of SFC are almost completely ignored!

Pressure tends to be a secondary control variable in SFC. Unlike temperature, increasing pressure tends to simply decrease retention to a modest degree. There are seldom peak reversals, indicating only modest selectivity changes. Adsorption and modifier clustering around solute molecules appears to be primarily driven by thermal energy, not pressure.

In my view, using a chiral development system to choose the "best" achiral stationary phases for a particular sample with a "universal" gradient is misguided. A little bit of thought goes a long way in choosing an appropriate stationary phase, the most likely modifier, and the nature of the additive, if needed. After that, the most useful instrumental variable is temperature, followed by pressure. In short, I'm positive that most SFC separations could be optimized on a few similar stationary phases using instrumentally controlled variables.

References

1. *A Grand-Guillaume Perrenoud et al., "Analysis of basic compounds by supercritical fluid chromatography: Attempts to improve peak shape and maintain mass spectrometry compatibility", J Chromatogr A, 1262, 205–213 (2012).*
2. *C West et al., "An improved classification of stationary phases for ultra-high performance supercritical fluid chromatography", J Chromatogr A, 1440, 212–228 (2016).*
3. *LR Snyder, "Classification of the solvent properties of common liquids", J Chromatogr Sci, 16, 223–234 (1978).*

The Road to HPLC2018 Part IV: The Grand Challenge of Whole Proteins

The intact protein separations crucial for top-down mass spectrometry continue to thwart chromatographers – will we see progress in 2018?

By John E. Wiktorowicz, Professor, University of Texas Medical Branch, Galveston, Texas, USA and Neil L. Kelleher, Professor, Northwestern University, Evanston, Illinois, USA.

The essential questions of how and to what purpose we investigate proteomics are becoming ever more pressing as we unravel the complexity of the human genome and its relationship to the proteome. Recent analyses suggest that the human genome contains roughly 20,700 genes (1). However, the complexity of the human proteome also reflects multiple splice variants (2), which yield an estimated 205,000 protein-coding transcripts (3). With over 400 different types of post-translational modifications currently known, and without even contemplating the vast combinatorial universe that implies, there are at least 1,000,000 distinct protein forms within a given human cell (4).

The huge diversity of proteoforms and their post-translational modifications leads us to question what their functional role is. Could their dysfunction underlie many human diseases? Essentially, the entire spectrum of human cellular biology can be traced to protein-level post-translational modifications, but compared with the genome, the proteome is dramatically under-mapped.

Post-translational modifications can decorate proteins at multiple sites simultaneously – the resulting overwhelming complexity presents an unmet challenge for current proteomic approaches. It's not only the sheer number of proteoforms we have to get to grips with – post-translational modifications alter protein properties such as size, net charge, and hydrophobic behavior, all of which are exploited to achieve separations – but also their impact on mass spectrometric sensitivity and peak capacity.

Among the new tools being developed, top-down mass spectrometry (TDMS) analyzes intact proteins, and in doing so

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"There is a growing demand for efficient, rapid, and quantitative separations for intact proteins and top-down mass spectrometry."

is best suited to preserve the complexity of multiply modified proteoforms. However, intact proteins and their complexes from 10 kDa to >5 MDa present significant challenges to TDMS runs in both denaturing and native modes. As a consequence, deep proteomics by TDMS is extraordinarily reliant on highly resolving protein separations, their peak capacities, and their ability to quantitatively recover the proteins that are applied. Moreover, most separation systems historically used for conventional proteomic MS are better suited to separations of peptides, not intact proteins. Hence, there is a growing demand for efficient, rapid, and quantitative separations for intact proteins and TDMS.

No single separation technology can address the demands of proteomics on selectivity, orthogonality, and quantitative recovery. Clearly, systems that employ multiple, orthogonal dimensions, with as little protein loss as possible, will be most successful. Ideally, these would involve liquid-based, non-adsorptive (requiring no elution) formats, exploiting isoelectric point (pI), molecular size (radius of gyration), hydrophobicity, hydrophilicity, and ultimately mass by MS.

Many approaches have been devised, exploiting one or more of the parameters described above, either solely or in combination (for recent reviews, see 5,6,7); however, none have demonstrated their ability to attack the complexity of the cellular proteoform.

The separation science community declared whole proteins a "Grand Challenge" at the HPLC conference in 2012. As such, increased innovation and creativity are being brought to bear from both academia and industry in this growing sub-sector of proteomics. This effort will be critical for both genomics and proteomics to achieve their full potential in addressing the central issues in human health, and to realize the promise of precision medicine.

References

1. *C Southan, "Last rolls of the yoyo: Assessing the human canonical protein count", F1000Res 6, 448 (2017).*
2. *ML Uhlen et al., "Proteomics. Tissue-based map of the human proteome" Science 347, 1260419 (2015).*
3. *Z Hu et al., "Revealing missing human protein isoforms based on ab initio prediction, RNA-seq and proteomics", Sci Rep, 5, 10940 (2015).*
4. *LM Smith, NL Kelleher, & The Consortium for Top Down Proteomics, "Proteoform: a single term describing protein complexity", Nat Methods 10, 186–187 (2013).*
5. *S Stepanova & V Kasicka, "Analysis of proteins and peptides by electromigration methods in microchips", J Sep Sci 40, 228–250 (2017).*
6. *KK Tetala & MA Vijayalakshmi, "A review on recent developments for biomolecule separation at analytical scale using microfluidic devices", Anal Chim Acta 906, 7–21 (2016).*
7. *TK Toby, L Fornelli & NL Kelleher, "Progress in top-down proteomics and the analysis of proteoforms", Annu Rev Anal Chem (Palo Alto Calif), 9, 499–519 (2016).*

High Potency – High Rewards, High Risks

Powerful new drugs are offering new hope to cancer patients, but can pose risks to the workers involved in their development and manufacture – including analytical scientists.

By Houri Simonian, Director, Analytical Operations, SGS Canada.

The hazards facing operators in facilities that manufacture highly potent and other potentially dangerous drugs are widely recognized, and the need for containment and protection is well understood. Yet, these powerful drugs don't just pose risks during manufacturing, but at any point where people might come into contact with the hazardous materials they contain – as such, analytical scientists are particularly at risk.

The greatest safety concerns center on highly potent active pharmaceutical ingredients (HPAPIs). An increasing number of HPAPIs are entering development and reaching the market, most commonly to treat cancer. The very qualities that help make these anticancer drugs so effective – cytotoxicity, high potency and a low daily dose – make them potentially dangerous for anyone handling them. In comparison to over-the-counter drugs, which may contain milligram to gram quantities of APIs, these more powerful drugs are active at micrograms per gram levels. Analyzing such highly potent drugs naturally comes with challenges.

As well as cytotoxic chemotherapeutics, analysts may have to deal with drugs that are mutagenic, teratogenic, or cause other significant biological effects at a low dose, including hormones and immunosuppressants. Regardless of the nature of the API, a similar suite of analytical tests is generally required, and the handling issues for different types of high-potency drugs are broadly similar.

First and foremost, it's crucial to ensure complete protection from the API throughout the analytical process. The importance of using effective personal protective equipment, including correct masks, gloves and protective gowns, cannot be overstated.

The hazards are greatest while the drug is still in a solid form, so until it is in solution or suspension, particular care must be taken to protect both the analyst and the environment. Sample preparation should be carried out in a dedicated laboratory, or a segregated part of a more general laboratory, with additional air handling capabilities that are completely separate from the rest of the facility. Everything that comes into contact with the drug must be securely disposed of using specific cytotoxic waste protocols, and typically double-bagged. Furthermore, a gowning process for the analysts before they walk into the laboratory or segregated area, and de-gowning before leaving, leaves no opportunity for any of the highly toxic material to leave the secure area.

Segregation is also important for the equipment, to ensure the drug remains in a contained environment. For example, when running a HPLC analysis, pre- and post-analysis cleaning will be carried out to eliminate the risk of cross-contamination to both the instrument and surroundings.

"First and foremost, it's crucial to ensure complete protection from the API throughout the analytical process."

Pop-up cleanrooms can be used to prepare samples in isolation, and allow a safe environment to be created in situ without the huge capital costs involved in building and validating a dedicated facility.

In manufacturing, the risks are known and, if correct safety procedures are followed, can be largely mitigated. However, in early-stage development projects, many unknowns remain. The candidate molecule's full efficacy has yet to be established, so the true nature of its potency, and therefore the risks, carry a question mark. Until all toxicology studies have been completed, compounds must be treated as potentially highly dangerous.

With the number of high potency drugs increasing every year, the key for drug companies is putting measures in place at the outset that allow them to be isolated, and quickly deal with any untoward events, such as a spill. Anyone who handles the product must be fully educated on safety procedures – there can be no exceptions.

There is no reason for an analytical scientist to be scared of working with a highly potent drug. As long as all necessary precautions to isolate the material are taken, the risks are minimal. As with most dangers in the lab, it's when safety rules are broken (or even bent) that accidents happen, so maintaining a strong safety culture is paramount.

The Vendor's Vendor

Markus Fuchs shares the secret behind the success of KNAUER's longstanding OEM business.

Tell us about your role in the OEM business...

I have been with KNAUER for 22 years now, and I'm currently a Group Leader in the OEM group, taking care of our third-party customers. In our OEM business, KNAUER sells whole instruments or components in custom-made housing solutions to other instrument vendors. Some companies co-label the technology; for example, international radio-pharmaceutical company Eckert & Ziegler partner with KNAUER to offer liquid chromatography capabilities, while ensuring a quality product for their customers. However, much of our work is private label, and therefore confidential, which can sometimes make it hard to promote this side of the business!

Why do you enjoy working in OEM?

I think it's one of the most exciting jobs in the company, at least for people who like to collaborate. Every day we tackle different topics, different instruments, and different needs. We enjoy sitting down with our customers "backstage" and discussing how we can realize their ideas. Of course, you could say that some of our customers are competitors in other areas of the business, but here in the OEM group we don't look at it that way. We understand that the market is huge, so we think that more – and, in particular, better – instruments on the market is a good thing.

Do ideas from the OEM business ever inform the instrument business?

Absolutely! It's not just the business itself that makes OEM so valuable to KNAUER, but the influx of new ideas that it brings. Our customers often have very different ideas to us about how they arrange things or think about the market, and sometimes that causes us to re-evaluate our own technology and strategies.

Why do customers choose KNAUER as an OEM?

There are many reasons. One is certainly that KNAUER is a financially independent company, not listed on the stock exchange market – we don't have to report to a "higher authority," so we have the freedom to make our own decisions and our own patents. For technology partners, it's often important to work with an independent company with full access to its technology.

Our agile approach is another aspect that customers often comment on. We are a very flexible team; we have time for our customers – that can be a rare thing in today's world. With some companies you have to book meetings months ahead, but we try to be much more flexible, and we're known for making and carrying out development plans quickly and efficiently. We are not just a supplier; we work with the customer and actively suggest ways to make projects run smarter and in a more time effective way.

What makes KNAUER different?

The team here is highly motivated. In larger companies, you often find the wrong attitude; for example, employees might think that talking about instrumentation at great length is boring, but we love it! We are passionate about discovering the active goals of our customers – and then supporting them successfully into the market. It can be a challenge, but every time we succeed, it really motivates us.

Where does your own enthusiasm for analytical chemistry stem from?

I specialized early in instrumental analytics as a result of my studies in biochemistry. My PhD focused on measuring the metabolic processes within an "artificial liver" system, designed to keep patients alive while they wait for a liver transplant. It was certainly exciting work but, more importantly, it gave me a real sense of how analytical chemistry can change people's lives. Taking great pride in our work and the role that we play in the impact of analytical science is shared among all KNAUER employees – whatever the group.


Jupiter
Mars

LIFE AS WE DON'T KNOW IT

SHERLOC – together with its camera sidekick WATSON – will use Raman and fluorescence spectroscopy to seek out the molecular signatures of life on the Mars 2020 mission.

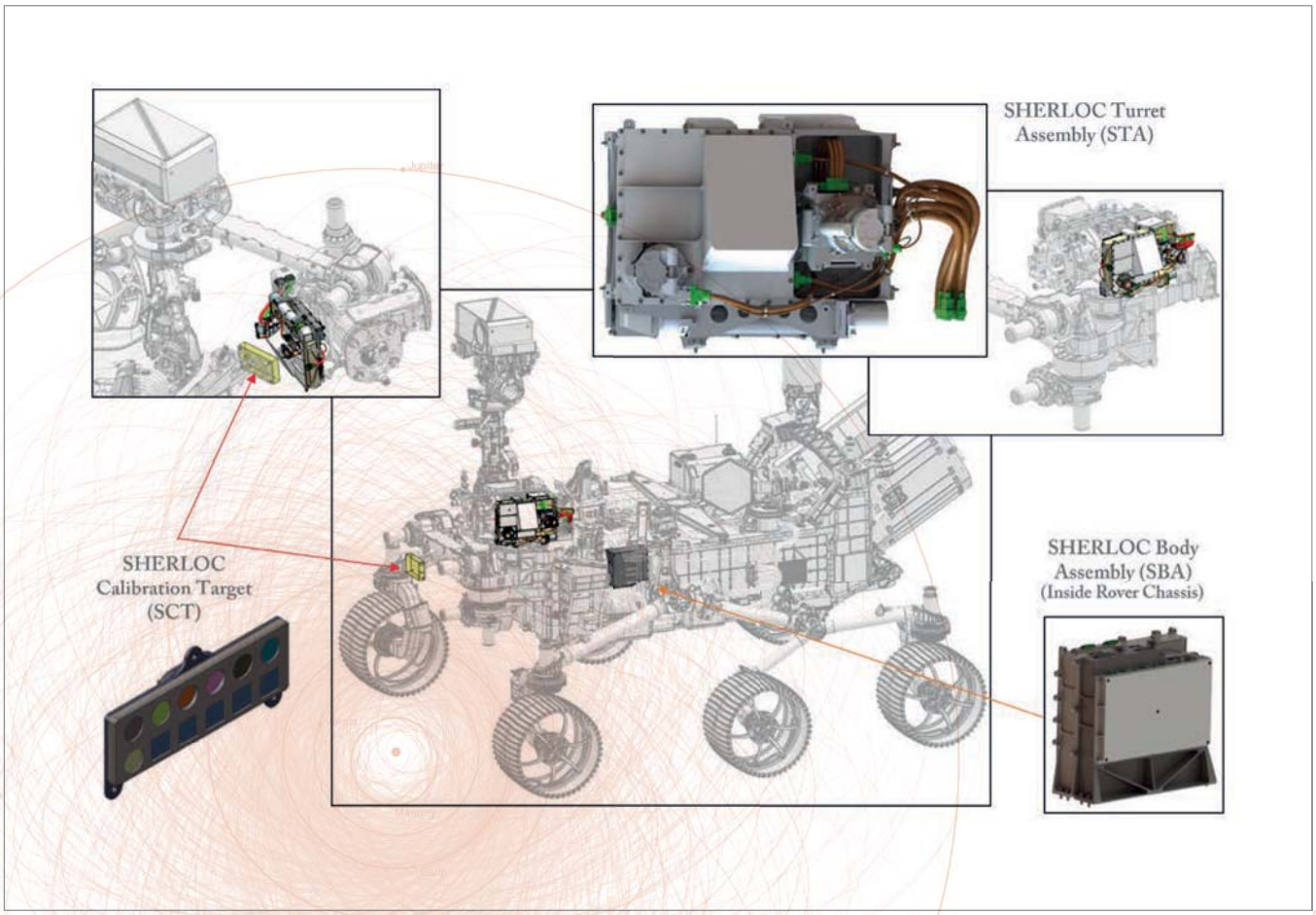
By Luther Beegle, Principal Investigator, SHERLOC, and Deputy Division Manager Science, NASA Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California, USA.

The Scanning Habitable Environments with Raman and Luminescence for Organics and Chemicals (SHERLOC) instrument will be mounted on the robotic arm of the Mars 2020 rover. SHERLOC enables non-contact, spatially resolved, and highly sensitivity detection and characterization of organics and minerals on the Martian surface and near subsurface, with four main goals:

i. To assess the habitability potential of a sample and its aqueous history
ii. To detect the availability of key elements and energy sources for life (for example, carbon, hydrogen, nitrogen and many others)
iii. To determine if there are potential biosignatures preserved in Martian rocks and outcrops
iv. To provide organic and mineral analysis for selective sample caching.

SHERLOC is a resonance Raman and fluorescence spectrometer with a 248.6-nm deep UV laser. Deep UV-induced native fluorescence is very sensitive to condensed carbon and aromatic organics, enabling detection at or below 1 ppm at 100 µm spatial scales, while deep UV resonance Raman enables detection and classification of aromatic and aliphatic organics with sensitivities of 0.01 to 1 percent at 100 µm spatial scales. In addition to organics, the deep UV Raman enables detection and classification of minerals relevant to aqueous chemistry with grain sizes on the order of 20 µm. SHERLOC will be able to map the distribution of organic material against the visible features and minerals that are identifiable with the Raman spectrometer.

It's important to understand that SHERLOC is not a life detection instrument in the classic sense of the word "life." We do not believe a single instrument could find life on Mars, for two reasons: first, there is so much uncertainty as to what life on



Mars might look like that it's hard to imagine a single instrument unambiguously identifying it; second, given what we know of present day Mars, if life "as we know it" were present, it would be very scarce indeed – below the limits of detection of any instrument.

SOWING THE SEEDS

In 1996, a research paper claimed to have found life in a Martian meteorite called ALH84001 (1). The debates that followed amongst planetary scientists and astrobiologists – while ultimately concluding that the meteorite did not contain life – highlighted that we had little clue how to look for life on another planet, or even what to look for.

Intrigued by the problem, the team here at NASA's Jet Propulsion Laboratory (JPL) embarked on a series of projects developing instruments capable of identifying life. SHERLOC came out of that effort. Ten years ago, my colleague Rohit Bhartia was leading a Raman spectroscopy-based project, while I was working on mass spectrometry-based instrumentation – both with the aim of detecting "life" on Mars. By 2008, Rohit and I were talking on an almost daily basis to compare our different strategies, and identify what we would be looking for. We both agreed early on that to definitively identify life, you need multiple instruments that make multiple different types of measurement.

At around the same time that ALH84001 ignited a debate about life on Mars, there was a revolution in how we think about life here on Earth. Microbial life was found in an array of seemingly unlikely places – several kilometers underground, in the dry valleys of Antarctica, in saline lakes, and in highly alkaline environments. It proved that life can exist in the most inhospitable places, and Rohit and I talked a lot about how we would find life in those harsh environments, and how that might translate to finding life on Mars.

In 2013, when the Mars 2020 Science Definition Team (who are responsible for defining the objectives and capabilities of the mission) announced that instruments would need to interrogate at the 100 micron spatial scale, with minimal sample handling, we ruled out mass spectrometry and turned all our efforts towards Rohit's spectroscopy-based concept.

"It's important to understand that SHERLOC is not a life detection instrument in the classic sense of the word 'life'"

BIRTH OF A MASTER DETECTIVE

We spent the first part of 2013 putting together the concept so that it could be ready for when the instrument Announcement of Opportunity was released that summer. It helped that we could develop the concept with an idea on how things worked on the Mars Science Laboratory (MSL) mission, which landed in August 2012. For example, we knew that the robotic arm had inaccuracies in placement of up to a cm, so an autofocus mechanism would be key; we talked to the developer of the Mars Hand Lens Imager (MAHLI) on the MSL and figured out how to incorporate their hardware. By leveraging existing hardware, we could concentrate on developing the "higher risk" items, such as the laser.

We went through a lot of internal JPL reviews, and naturally everyone had an opinion on what we should or should not include, with plenty of contradictory advice – all of which we listened to (and some of which we followed!). We had an

MISSION NAMECHECK

SHERLOC has three charge-coupled devices (CCD) and three optical paths, along with multiple power supplies, two separate computers, two moving parts and multiple electronics assemblies. It's a huge task and includes the work of five subcontractors:

1. Malin Space Sciences: ACI and WATSON hardware
2. Los Alamos National Laboratory: CCD, command and data handling
3. Left Hand Design Corporation (LHDC): scanner mirror
4. Photon Systems: laser
5. Johnson Space Center: calibration target

SHERLOC THROUGH THE YEARS

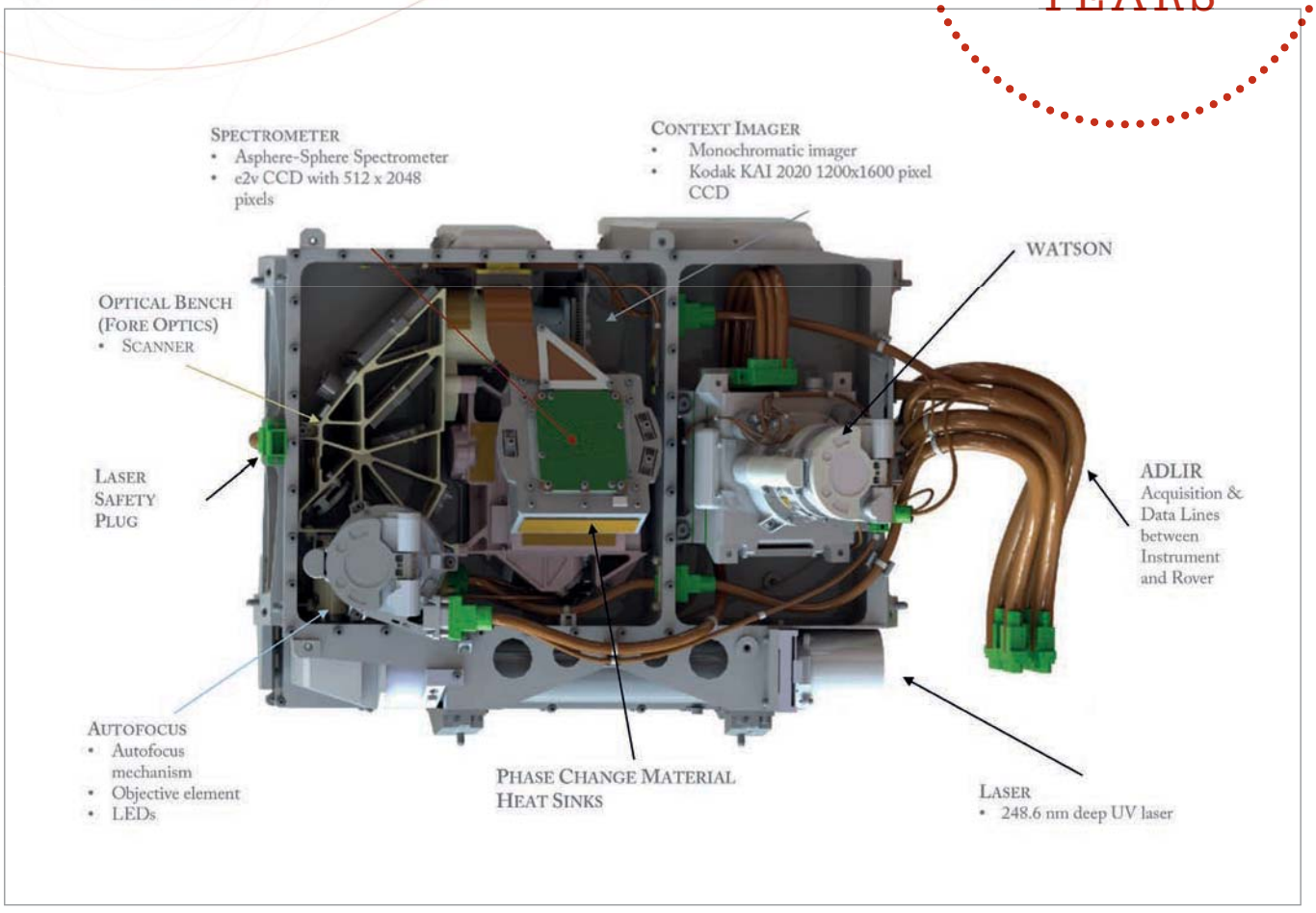


Figure 1. Major SHERLOC assemblies.

excellent team to help us put the concept together, including an awesome system engineer, Lauren DeFlores, who made sure that everything fit together into a neat little package.

In July 2014, I got the call to say we'd been selected for inclusion in Mars 2020, while on vacation with my family in Breckenridge. It was lucky that I was away from JPL, or I'm not sure I could have survived the 24-hour embargo on sharing the good news.

"Our main challenges come from Mars itself – an inhospitable place for scientific instruments."

A LOOK INSIDE

Our main challenges come from Mars itself – an inhospitable place for scientific instruments. It is an optical instrument attached to a vibration-inducing coring mechanism at the end of a robotic arm that experiences 100 °C temperature variations on a daily basis – in a very dusty environment. Plus, we have to operate with low power and limited data volumes.

There are two optical assemblies in SHERLOC (see Figure 1), the one on the right is called WATSON, and is a reflight of the MAHLI, which is currently on MSL. WATSON is a variable focus color imager that can take microscopic images of science targets as well as images for engineering purposes, such as

1998
Initial development begins

1998 –2013
Proposals and scientific papers published

2014
Selected for inclusion in Mars 2020

2015
Integration review determines that we will fit on the robotic arm

2016
Preliminary and critical design reviews

Summer 2017
Start building flight hardware

October 2017
Instrument integration begins

October 2018
Deliver the flight instrument to the rover

Summer 2020
Mars 2020 launch

February 18, 2021
Land on Mars

recording wear on the robotic wheels. The Autofocus Contextual Imager (ACI) on the left allows us to obtain a black and white image of a surface, as well as autofocusing so we can do laser spectroscopy. The laser spectroscopy takes place at a working distance of 48 mm from a surface, plus or minus 7 mm.

There are two other pieces of hardware – an electronics box inside the rover and a calibration target. The calibration target allows us to verify the instrument's performance on the surface of Mars and includes a piece of a Martian meteorite and several pieces of spacesuit material of a type that would be used when humans eventually make it to Mars; by exposing the material to the environment of Mars, we can assess if it degrades with time.

SHERLOC is one of seven instruments being developed for Mars 2020. We share the arm with the PIXL instrument, which produces elemental maps of surface material. There are two instruments on the mast, MastCam-Z and SuperCam. MastCam-Z takes color stereo images of the surface, and SuperCam has the ability to take Raman spectra at a distance of up to a few meters, laser induced break down spectroscopy (LIBS) from up to 10 meters and IR spectra from even further. All these measurements will help us understand the geologic context of a sample, over multiple spatial scales.

In this mission there is very little ability to do sample handling, because the caching system is so complex. On MSL, samples can be obtained through a rotary percussive drill that pulverizes a sample, feeds it through a sieve and then into a measuring tube, before it is delivered to the Sample Analysis at Mars (SAM) instrument. It was not feasible to incorporate all that hardware on Mars2020. Plus, Mars has a significant amount of perchlorate on the surface – on heating, the perchlorate destroys organics, meaning that conventional GC-MS destroys the very molecules that you are looking for. SHERLOC is a very low power instrument, and we can see organics and perchlorates without causing a chemical reaction.

WHAT ARE WE LOOKING FOR?

The two different types of spectroscopy in SHERLOC have different but complimentary uses. Fluorescence spectroscopy (with a 248 nm laser) detects poly aromatic hydrocarbons (PAHs) – a very strong signal that allows for sub-ppm detection; the fluorescence signature starts at around 273 nm and continues into the visible.

Raman spectroscopy allows us to identify aliphatic organic material as well as study mineralogy. The Raman region starts at 252 –273 nm and the signal is up to six orders of magnitude weaker than fluorescence. The use of the 248.6 nm laser allows us to get the Raman and fluorescence on the same charge-coupled device (CCD), thereby doubling the amount of information we obtain with each laser pulse.

We will look for aromatic organics using fluorescence and aliphatic organics using Raman spectroscopy. We will also search for minerals that are astrobiologically relevant because they were created in aqueous environments, such as carbonates, phyllosilicates, and so on.

Potential biosignatures could include:

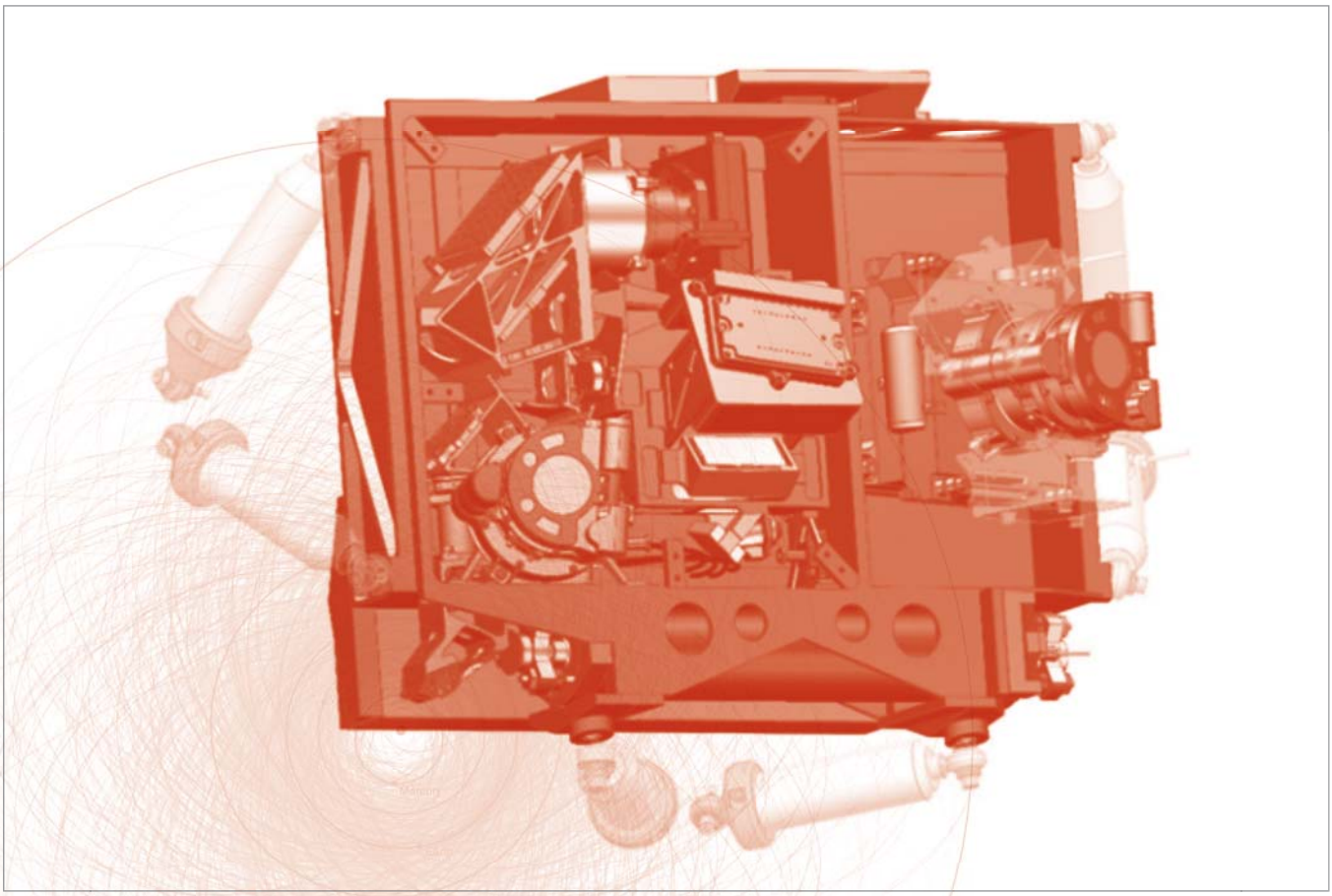
- A significant amount of organics in conjunction with minerals that formed in aqueous environments
- A significant deposit of aliphatic organics right under the surface
- Layers of organic molecules that correspond to specific elements that PIXL quantifies.

It is a real challenge to tell the SHERLOC story without generating sensational headlines about "life on Mars". We are able to detect organic molecules in very trace amounts in very small areas, and this will inevitably cause some people to think we will have found life. Even today, there are a handful of researchers who believe that they found life on Mars in the 1970s, during the Viking mission, while others insist that ALH84001 shows genuine signs of life. While we are excited about the possibility of what we will find, we believe that extraordinary claims require extraordinary evidence and that definitive proof of life requires multiple lines of evidence from multiple instruments.

MISSION STATUS

Currently, we have around 30 full-time employees, including our project managers Ed Miller and Randy Pollock. The team consists of system engineers, and cognizant engineers for subsystems, such as optics, electronics, data handling and so on – as well as integration and test teams who actually build the instrument. We also work with a number of external companies and agencies (see "Mission Namecheck" on page 25).

Right now, our major challenges are twofold: firstly, getting each subsystem to operate under all the conditions that we will encounter for two Martian years (three Earth years); and secondly, to make sure that all the hardware from each vendor and subsystem comes together at the right time so that it can be integrated together. We have a very compressed period of



"We started developing the concept in 1998 and SHERLOC itself in 2013. When it lands on February 18, 2021, we will have been working on it for seven years."

integration and if a part is delivered late, it can really affect other subsystems, no matter how small that part is. We deliver the instrument for integration into the rover on October 31, 2018. Until then, we are building, assembling and testing the instrument to demonstrate that it will survive the planet's harsh environment.

We started developing the concept in 1998 and SHERLOC itself in 2013. When it lands on February 18, 2021, we will have been working on it for seven years. It has been a very exciting (and stressful) four years so far and we now have less than a year left before we deliver the flight instrument to the rover. There is a JPL video called "Seven Minutes of Terror" that describes entry, decent and landing for MSL (http://bit.ly/1DJywCZ), and we like to joke that this project is putting us through our own "Seven Years of Terror"! (My coping strategy is running – three years ago, I could barely run a mile, and in November 2017 I finished my first marathon!) For now, SHERLOC takes up about 20–30 percent of my time, but that will rise to 100 percent by the time we land and for the first few years on Mars.

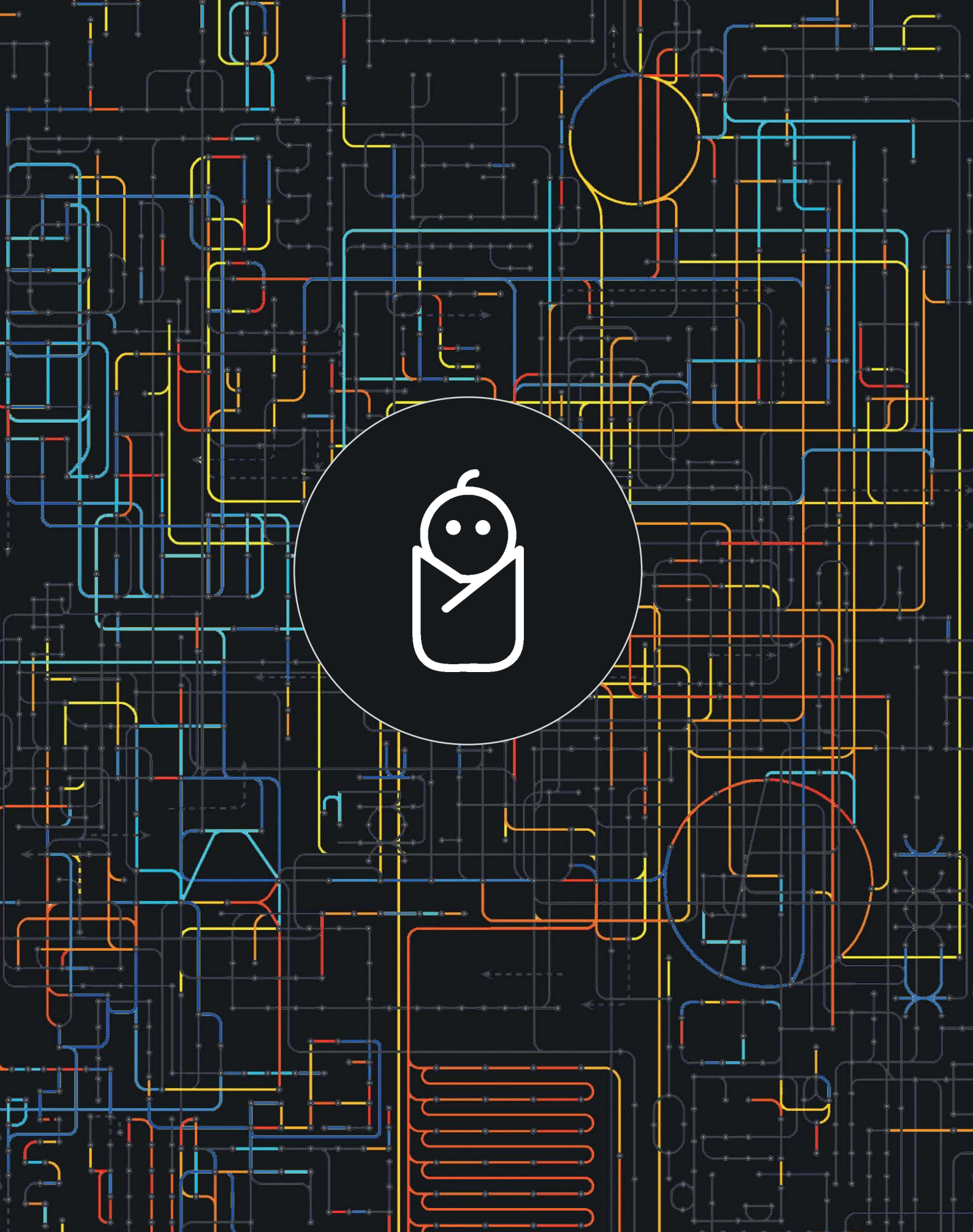
All our families share our excitement (and nerves). During the MSL landing, my 10-year-old son held my hand tighter than I could have imagined, because he knew that I had put four years of hard work into this project, and that if something went wrong it would be the loss of my dream. My son is now 6 feet 4 inches tall; by the Mars 2020 landing he will be a college student – and I hope he will hold my hand just as tightly.

Reference

1. *McKay et al., "Search for past life on Mars: possible relic biogenic activity in Martian meteorite ALH84001", Science, 273, 924–930 (1996).*

Acknowledgement

The research was carried out at the Jet Propulsion Laboratory, California Institute of Technology, under a contract with the National Aeronautics and Space Administration.



The Birth of MS/MS Screening

Advances in metabolomics drive a parallel surge in metabolite discovery – and inspire new platforms able to measure increasing numbers of compounds in blood, plasma or urine. Our ability to perform such analyses at the microsample scale – a single drop of blood dried on filter paper – has given rise to modern newborn screening. Here, I describe how this revolution in metabolomics and clinical diagnostics all started with the analysis of amino acids and acylcarnitines 20 years ago.

By Donald H. Chace

Determination of L-carnitine and its derivatives is the basis for newborn screening for several potentially deadly metabolic disorders – a prime example of how analytical science affects all our lives, from the day we are born.

L-carnitine is unique and fascinating molecule. With some chemical similarity to amino acids (perhaps even an ancient precursor to amino acids – see "The Primordial Group"), L-carnitine is a quaternary ammonium compound – a rare class in the human body. It plays a crucial role in fat metabolism, helping to break down fatty acids (through beta oxidation) into a form that the mitochondria – the cell's powerhouse – can use as fuel.

Beta oxidation is facilitated by coenzyme A, but coenzyme A and its acyl-bound fatty acids (acyl-CoAs) cannot always cross the mitochondrial membrane. Short- or medium-chain acyl-CoAs can traverse the membrane, but very-long-chain acyl-CoAs cannot, so they must be temporarily transferred to another substrate for transport across the membrane via a translocase "tunnel." L-carnitine is that substrate. Figure 1 shows how L-carnitine transports fatty acids through the inner mitochondrial membrane in the form of acylcarnitine. Once inside the mitochondria, the fatty acids are transferred back to coenzyme A for delivery to beta oxidation enzymes. Using a train analogy, coenzyme A can be thought of as a small engine moving a few train cars at a time around a rail yard, while L-carnitine is a bulkier locomotive that can pull longer trains over greater distances.

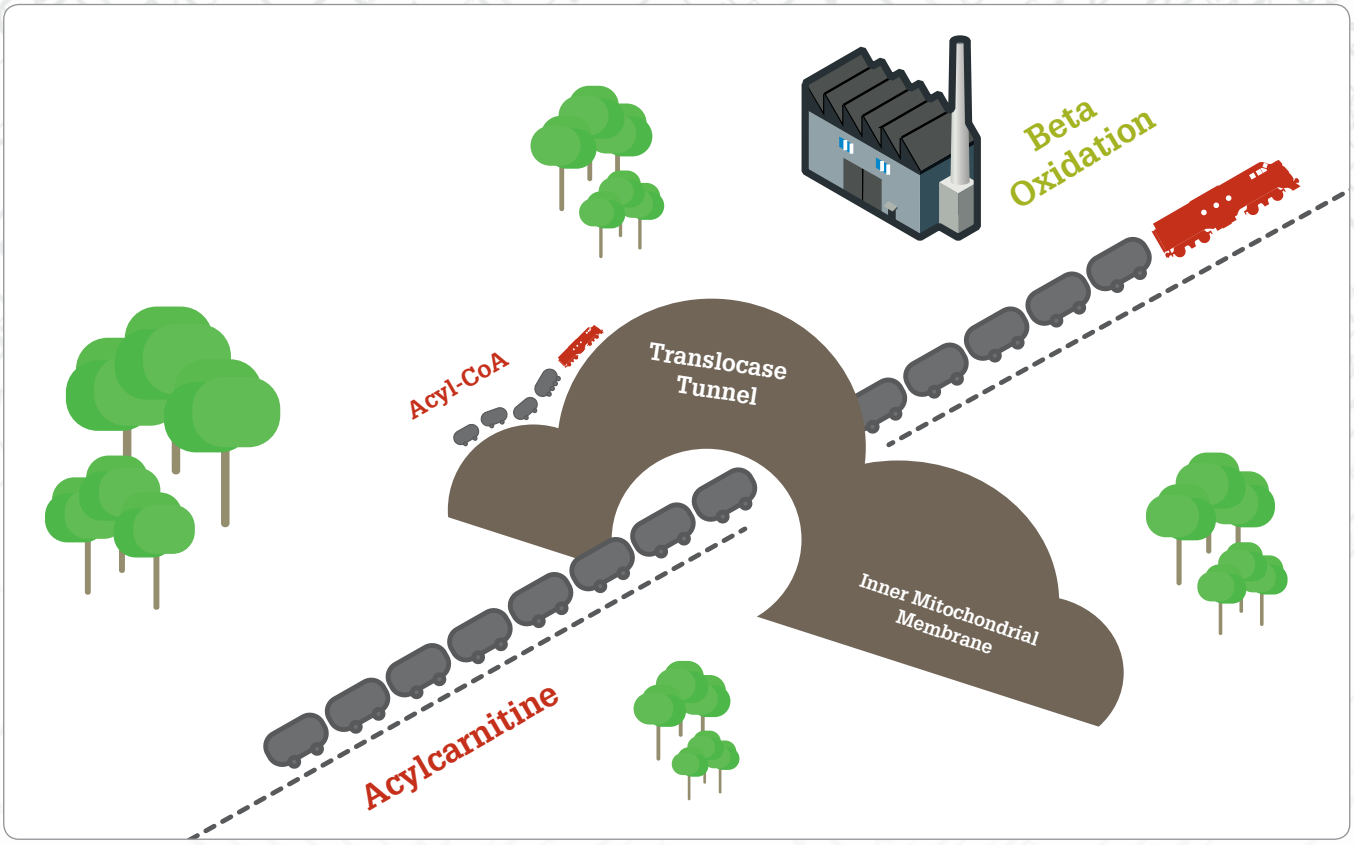


Figure 1. Transport of very-long-chain fatty acids across the inner mitochondrial membrane. Short- and medium- chains have sufficient ability to penetrate the membrane (cross over the hill) as acyl-CoAs. However, very-long-chain fatty acids require L-carnitine via formation of an acylcarnitine and transport via translocase (tunnel). The long-chain fatty acids are transferred back to acyl-CoA for beta oxidation (fatty acid oxidation) in the mitochondria.

There is an additional role for L-carnitine: if an excess of acyl-CoAs has accumulated in the mitochondria, it will transport these out into the cell and onward to the blood for elimination. And that's why a metabolic disorder of beta oxidation can lead to a deficiency in L-carnitine – if certain fatty acids cannot be metabolized they build up in the mitochondria and have to be eliminated as acylcarnitines in urine or bile, leading to gradual loss of the "engines" that deliver the fatty acid cargo. Such disorders are highly unusual but very dangerous. Provided there is sufficient glucose to fuel metabolism, fatty acids simply accumulate, causing fatty liver but often no outward signs of toxicity; but if blood sugar drops, there is no "safety net" in the form of fat metabolism. Muscle weakness followed by coma and death can progress rapidly and without warning. These tragic consequences can be avoided relatively easily with diet and medication, but by the time symptoms occur, it may be too late. Both the understanding of the biochemical role of L-carnitine and also its potential in clinical analysis evolved rapidly and in parallel.

Which came first, the chicken (disease) or the egg (method)?

L-carnitine is a powerful example of what I like to call the clinical chemist's feedback loop: technology changes medicine, which changes technology. Analytical chemists in the 1980s were faced with a problem – L-carnitine and acylcarnitines had great potential as markers of fatty acid metabolism, but these non-volatile compounds could not be analyzed using gas chromatography-mass spectrometry (GC-MS) – the most common tool for metabolic research of the time. To overcome this hurdle, they developed complex sample preparation procedures to remove the quaternary amine and make L-carnitine volatile for GC, while keeping the molecule structurally informative. But these methods were limited to measuring free and total L-carnitine; it was impossible to identify individual fatty acylcarnitines. Consequently, there was still relatively little data on acylcarnitines until the mid-1980s, when significant advances in LC-MS interfaces were made

"As we uncovered beta oxidation disorders in sick patients, in unexplained deaths, and from family history, it became obvious that a newborn screening test could save many lives."

(thermospray and liquid secondary ionization methods, such as fast atom bombardment, FAB). As L-carnitine is a preformed cation with at least one positive charge in aqueous solution, it is the perfect substrate for positive-ion LC-MS.

With further MS advances and the availability of less expensive tandem MS (MS/MS) systems, clinical investigations expanded, and the role of carnitine in fatty acid metabolism was better understood. Clinical investigators studying infants with inherited disorders in fatty acid metabolism had a new and better analytical tool, and were quick to make use of it. Using analytical data from urine, plasma and blood analysis and numerous biomarkers from organic acids to acylcarnitines, it was possible not only to understand mitochondrial fatty acid oxidation but also to develop a test to detect beta oxidation disorders shortly after birth, while they are still treatable.

Ultimately, the technology set the stage for an important and significant expansion of newborn screening. Early MS/MS used cumbersome ionization techniques (FAB or fast ion bombardment, which require a difficult insertion and manual analysis) but, soon after, new ionization methods were established; for example, electrospray, which enabled automation and easier population analysis. Today, pathology laboratories in most developed nations screen infants using MS/MS. Even now, the technology is still being expanded to include new diseases – spawning even more advancements. In my view, it is this feedback loop (Figure 2) that makes analytical science key to discovery and better health.

A revolution in newborn screening

The fundamental problem with most clinical diagnostic tests is that you only test once symptoms appear, so you run the risk that the disease will have already caused irreversible damage or even death before treatment can be started. Still, in the technology loop described earlier (Figure 2), it is a necessary step prior to developing a screening technique and is extremely important in confirming disorders detected by screening. Information from the metabolic profiles of a diagnostic test tells us what to look for in developing early screening.

As we uncovered beta oxidation disorders in sick patients, in unexplained deaths, and from family history, it became obvious that a newborn screening test could save many lives, especially as treatment is often simple and noninvasive – a low-fat, high carbohydrate diet (a literal "spoonful of sugar") and supplemental L-carnitine.

Though the treatment for fatty acid disorders may be simple, the evolution of newborn screening of acylcarnitines was anything but. Tandem MS was a game changer when it was introduced in the 1990s, but it took at least another 10 years for its use to be accepted and widely implemented for newborn screening. One of the first MS/MS screening tests was developed for MCAD deficiency. A genetic survey for the most common gene associated with the disorder showed that

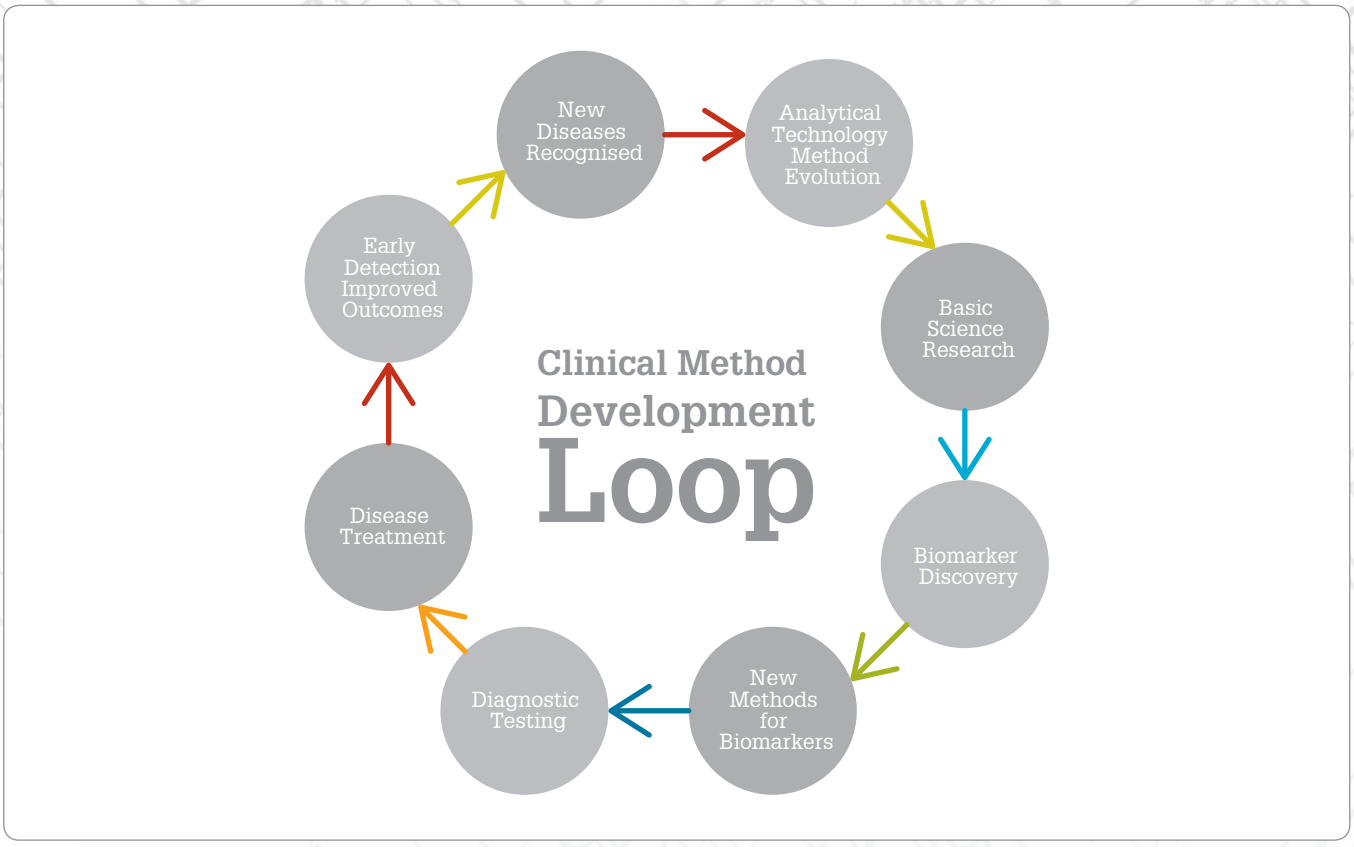


Figure 2. How analytical technology evolves with each new round of discovery.

the frequency of the mutation was 1 in 10,000–15,000 (a similar frequency to phenylketonuria, a disorder of phenylalanine metabolism that was the first disease screened in newborns more than 50 years ago). MCAD deficiency is a disorder of the first steps (dehydrogenation) of beta oxidation for medium-chain (6–12 carbon units) fatty acids; if the enzyme is deficient (or defective), the oxidation of these fatty acids does not occur and precursors accumulate in the mitochondria. The precursors are coenzyme A adducts octanoyl (C8), hexanoyl (C6), decanoyl (C10), and decanoyl (C10:1), which cannot be measured directly in the blood because they are never transported out of the mitochondria. As mentioned above, one of the roles of carnitine is to remove excess acyl-CoAs from the mitochondria (to keep it functioning) and transfer them to blood for elimination in urine and bile; thus, we can measure acylcarnitines as a substitute for acyl-CoAs. In MCAD deficiency, we observe elevated medium-chain acylcarnitines in a unique pattern, with all four peaks clearly increased as shown in Figure 3. In normal metabolic states, C8 concentrations are barely distinguishable from noise; in MCAD deficiency, it often becomes the most significant peak in the mass spectrum.

Before newborn screening, a diagnosis of MCAD deficiency required either urine organic acid analysis or a repeat acylcarnitine profile after intravenous L-carnitine supplementation. Why? Acylcarnitine formation clearly depends on the availability of L-carnitine, but as beta oxidation defects (such as MCAD deficiency) deplete L-carnitine, the concentrations of these metabolites decline. Fortunately, L-carnitine is not generally deficient at birth so metabolites are easily detected in newborns.

The use of acylcarnitine profiles in newborn screening has saved the lives of many children born with inherited disorders. Such screening shows the power of metabolomics in practice – there is no better example of how clinical research can evolve to become standard practice. But what about alterations of metabolism not due to inherited disease?

"Newborn screening shows the power of metabolomics in practice – there is no better example of how clinical research can become standard practice."

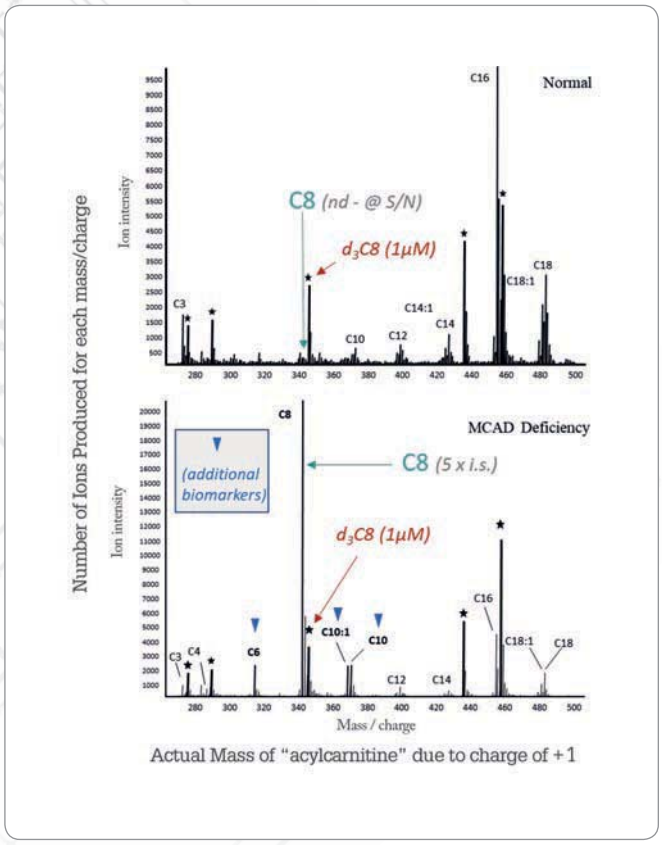


Figure 3. Acylcarnitine "metabolic" profile by MS/MS. The upper mass spectrum is from a healthy newborn and the lower spectrum from a newborn with MCAD deficiency. The analysis is from a dried blood spot collected at two days old. The acylcarnitine metabolites are labeled according to the number of carbons in the fatty acid attached to L-carnitine. The starred peaks are stable isotope-labeled internal standards used as a reference for quantification. The MCAD deficient newborn has a concentration of C8 fivefold higher than the internal standard.

Beyond newborn screening

In my opinion, detection of inherited disorders is just one piece of the jigsaw. In particular, measurement of acylcarnitines, together with amino acids, may be important in understanding metabolism, growth and nutrition in premature infants. Unlike detection of inherited metabolic disease, changes in metabolism in preterm infants are temporary and result from indirect factors, such as immature organs. The neonatologist's toolbox for metabolic investigations has historically been limited by a reluctance to move away from older "tried and tested" technology, the need for rapid turnaround (an HPLC amino acid analysis or urine organic acid could take several days) and, most importantly, the amount of blood that can be safely taken from an infant who may weigh less than 1 kg.

However, better metabolic tests are sorely needed in these smallest of patients. For example, preterm infants require extra protein compared with a full-term baby, to mimic the

"Because the level of acylcarnitines depends in part on the availability of L-carnitine, it can create confusing results from an analytical point of view."

high level of amino acids they would be exposed to in the womb. The gut of a 22–28-week-old premature infant is not able to extract the protein they need from their diet, so they require intravenous amino acids (parenteral nutrition) to provide the building blocks for growth. The challenge is knowing how much of this amino acid mixture to administer to provide the fuel for growth – too often this is based on guesswork.

By now, you may be asking yourself – what does this have to do with L-carnitine?

In full-term newborns, we have observed that acylcarnitines decline rapidly after birth, stabilizing at 1-week-old to near-adult levels. It is thought that after the stress of birth and the sudden "cut off" from nutrition provided by the mother via the umbilical cord, newborns rely on fatty acid metabolism until they switch to a steady glucose supply in the form of milk. Regardless of the cause, acylcarnitines decline, but L-carnitine is relatively stable. In preterm infants, acylcarnitines also decline but free L-carnitine levels depend on the actions of their neonatologist. Modern bioinformatics have revealed two distinct populations of preterm infants: those who received L-carnitine as part of their IV nutrition and those who did not (1). The infants receiving supplemental L-carnitine had high levels of free L-carnitine, as well as higher acylcarnitine concentrations. Infants who were not administered L-carnitine saw levels decline over the first week of life until they were close to a deficiency. In all cases, once infants moved from parenteral nutrition to feeding normally, L-carnitine increased back to near-normal levels.

Because the level of acylcarnitines depends in part on the

L-Carnitine: The Little Engine That Could

What is the role of L-carnitine in fatty acid metabolism? And what happens when things go wrong?

The biochemical role of L-carnitine is to transport very-long-chain fatty acids across the inner mitochondrial membranes into the mitochondrial matrix. It might be helpful to imagine that L-carnitine is the engine (locomotive) that transports its cargo (oil tankers/fatty acids) into the mitochondria. Figure 4 illustrates this "train" analogy from a structural perspective.

The mitochondria are the power houses of metabolism and ATP generation. They are also the site of beta-oxidation of fatty acids. Figure 5 illustrates the metabolic pathways of fatty acids and the role of L-carnitine and acylcarnitines. Note the large number of enzymes and proteins involved in transporting fatty acids across the inner mitochondrial membranes and processing them once inside – at each of these enzyme or protein steps, a defect in the protein will cause metabolic disease. As in most metabolic disorders, the metabolite normally processed by the defective enzyme or protein accumulates, while metabolites further down the pathway may decline.

Take a disorder in CPT II (carnitine palmitoyl transferase type II), the enzyme responsible for transferring the fatty acyl group from the long-chain acylcarnitine back to acyl-CoA – the substrate involved in beta oxidation. When CPT II is defective, acylcarnitine is not released to acyl-CoA and accumulates in the membrane and inner mitochondria; short chain acyl-CoAs are not produced and hence beta oxidation declines. Only those acylcarnitines that are small enough to penetrate the membranes without the assistance of carnitine are metabolized (see Figure 1). Blood tests in those with the disorder show excess long-chain acylcarnitines with very low L-carnitine because it is all bound up in the long-chain acylcarnitines. This high ratio of long-chain acylcarnitines to L-carnitine is the basis for detection of a metabolic disease by MS/MS.

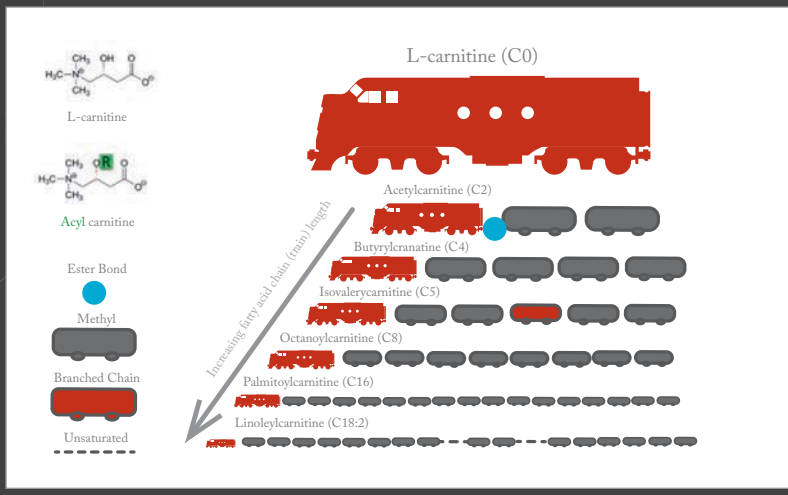


Figure 4. L-carnitine and acylcarnitine can be thought of as locomotives. The engine is L-carnitine, to which fatty acids of various lengths are attached to form an acylcarnitine.

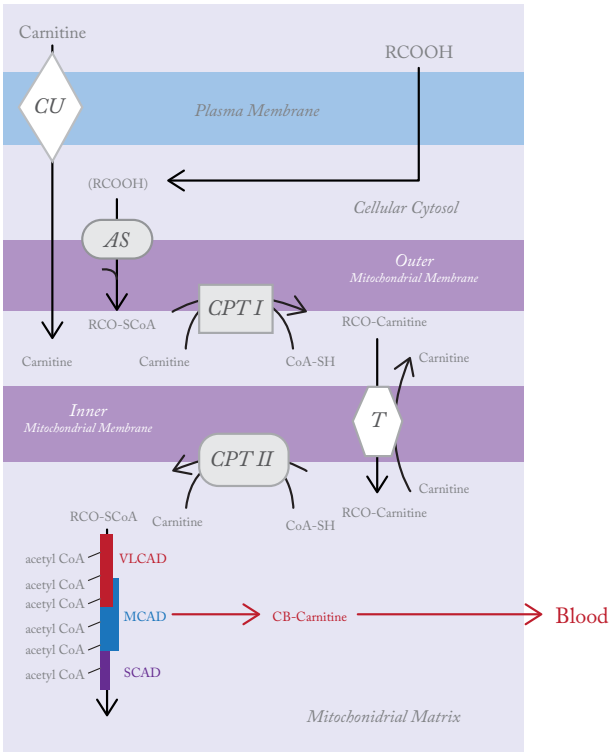


Figure 5. Beta-oxidation and the role of L-carnitine in fatty acid metabolism. L-carnitine is transported into the mitochondria via a carnitine uptake protein (CU) in the plasma membrane. Fatty acids freely diffuse into cells and are attached to coenzyme A (AS) and transported into the inner/outer mitochondria membrane space. The long-chain fatty acids are transferred to L-carnitine, forming an acylcarnitine by a transferase enzyme (CPT I), then transported across the inner mitochondrial membrane via translocase. The carnitine transfers its long-chain fatty acyl group back to coenzyme A where the process of beta oxidation begins. Beta oxidation is a series of four oxidation steps that ultimately lead to removal of acetic acid and a fatty acid chain length shortened by two carbon units. There are three main sets of these four enzymes – one for very-long-chains, one for medium chains and one for short chains. The first step, a dehydrogenase, is implicated in three diseases, VLCAD, MCAD and SCAD (very long, medium and short chain acyl-CoA dehydrogenase) deficiencies. There are additional diseases caused by defects in each of the intermediate steps at each fatty acid chain length level. When acyl-CoA accumulates in metabolic dysfunction, the excess is transferred back to L-carnitine and the resulting acylcarnitines are exported to blood for elimination in bile or urine. This is the basis for MS/MS analysis of acylcarnitines in the blood and the detection of dozens of disorders of fat metabolism.

The Primordial Group

Could L-carnitine provide clues into our biochemical evolution?

A scientist at a major pharmaceutical company once told me that L-carnitine should be thought of as a "prehistoric" amino acid. The idea of L-carnitine as a primitive amino acid immediately appealed to me for several reasons. First, both amino acids and L-carnitine contain basic amine and carboxylic acid functional groups, which become charged (zwitter ions) in water. Both amino acids and L-carnitine have a central carbon that separates the amino and carboxylic acid functional groups. This central carbon also contains a variable "R" group that changes the molecule both structurally and functionally. Figure 6 shows "generic" structures for L-carnitine, acylcarnitines and α-amino acids.

We assume that early life (in the primordial soup) required more reactive functional groups as pH and temperature conditions were widely variable in Earth's early history. With time, environmental conditions and pH stabilized and more efficient metabolic processes evolved, catalyzed by enzymes. The idea of carnitine as a "prehistoric" precursor to amino acids comes from the strong ionic nature of the quaternary amine (four carbons attached to nitrogen), making it permanently charged in aqueous solution regardless of pH. By comparison, amino acids are primary amines (one carbon attached to nitrogen), and are only charged in aqueous solution at physiological pH (7.4).

To further this "ancient history" concept for L-carnitine, it is worth noting that it has a unique role in our most ancient organelle, the mitochondrion – among other more important functions like energy production, the mitochondria house our ancient and conserved DNA. The evolution of mitochondria and role of ancestral DNA has attracted attention recently, and thus it is quite natural to wonder about the evolutionary role of L-carnitine and why it plays a vital role in mitochondrial fatty acid metabolism.

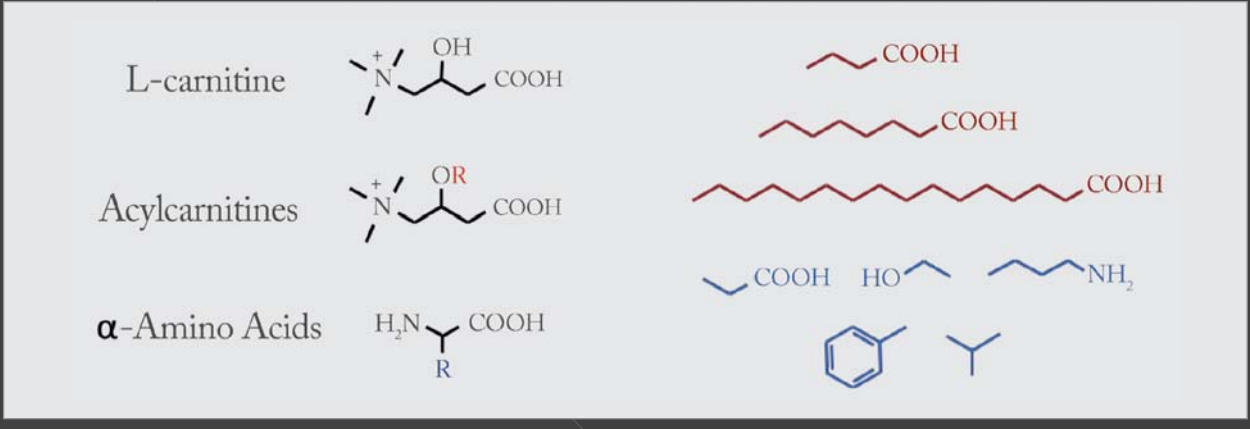


Figure 6. Comparison of L-carnitine, acylcarnitines and amino acids, and associated R-groups. These R groups are variable chain length fatty acids for L-carnitine (red) and a variety of functional groups for α-amino acids (blue).

availability of L-carnitine, it can create confusing results from an analytical point of view. For example, monitoring the catabolic product of leucine, isovaleric acid (which forms an acylcarnitine), is unhelpful because its levels may be higher or lower depending on the levels of L-carnitine. Further, organic acids for amino acid catabolism and fatty acid catabolism merge in beta oxidation. Lipids are also supplemented in parenteral nutrition, and high doses of both amino acids and fatty acids can create a perfect storm of metabolites in beta oxidation and overflow the system, causing toxicity. Currently, neonatologists monitor ammonia but this only reflects amino acid catabolism and not fatty acid oxidation. The solution is to normalize all levels of acylcarnitines to L-carnitine and that is currently being investigated from a bioinformatics perspective.

What's next for L-carnitine?

L-carnitine is still not well understood beyond its role in fatty acid metabolism and the obvious problems associated with a deficiency. That doesn't stop it being widely sold as a supplement in the vitamin aisle – previously called Vitamin B_T (it is not a vitamin), it is used frequently in body building and sports for the perceived benefit of metabolizing fat more efficiently and improving muscle function. These claims are dubious, at best. But could L-carnitine have qualities we are not yet aware of?

With the detection of hundreds of infants with rare metabolic disorders, the prophylactic administration of L-carnitine became a topic for debate. The rationale behind using L-carnitine for metabolic disease is to correct a deficiency and by doing so keep beta oxidation functioning more efficiently, especially at times when fats are burned faster or glucose is used more quickly. Diseases like MCAD lead to acute illness when glucose becomes depleted – the reasoning is that supplemental L-carnitine can keep the dysfunctional fatty acid oxidation functioning slightly longer and better, until glucose can be replenished. It is also believed to help keep muscle functioning more efficiently in terms of fat metabolism, preventing or reducing cardiomyopathy in some metabolic states. It is not difficult to see how the scientific and medical use of L-carnitine has been translated into the pseudoscientific claims made by supplement companies. It seems unlikely that L-carnitine will have a significant impact on muscle function in healthy adults, who produce more than enough L-carnitine for their needs. However, it's not inconceivable that there may be roles for L-carnitine in metabolic states that are highly reliant on fat metabolism, including brain biochemistry and disease such as stroke. I wouldn't be surprised to see this multifaceted molecule reimagined once again, as new roles and functions are discovered.

Donald H. Chace is Chief Scientific Officer, Medolac Laboratories, Lake Oswego, Oregon, USA.

Reference

1. *RH Clark et al., "Impact of L-carnitine supplementation on metabolic profiles in premature infants", J Perinatol, 37, 566-571 (2017).*

Metabolomics: the Superglue of Omics

As the profile of metabolomics soars, we offer our thoughts on the future of the field.

By Martin Giera, Mary E. Spilker and Gary Siuzdak

> *"The metabolome is the closest link to the phenotype and hence at the forefront of personalized diagnosis and therapy."*

Our understanding of cellular and molecular processes has increased exponentially during the last century. Several key questions about the function of our cells and, to a broader extent, their interactions with entire organisms have been answered, with the most prominent findings being the elucidation of our DNA structure and the sequencing of the human genome (1). The key outcome of 20th century biomedical and biological research was the knowledge we gained about the coding, content and flow of information within our cells and body. The concept that our cells contain our hereditary information in the form of DNA, which is transcribed into RNA and translated into proteins that are ultimately involved in the modulation of our metabolome is the central dogma of biology, and a comprehensive understanding of this process represents the fundamental goal of omics technologies (2).

But beyond a better understanding of ourselves, what has been the impact of these findings and technologies on our lives? Genomics (DNA sequencing) has certainly enhanced diagnosis and treatment of inborn errors and hereditary diseases. Transcriptomics (the analysis of RNA transcripts) complements genomics by allowing us to decipher gene expression, again helping to identify undiagnosed cases of genetic diseases. Proteomics (the comprehensive analysis of an organism's proteins) would ideally complement genomics and transcriptomics; however, several ongoing analytical challenges are still hampering comprehensive protein analysis. Nevertheless, we believe advances in proteomics happening today will allow for a more efficient use of proteomics tomorrow, so that it may become a complementary diagnostic approach to other omics techniques (3).

Metabolomics rules

Ultimately, it is the metabolome – the underlying biochemical layer of the genome, transcriptome and proteome – that reflects all the information expressed and modulated by all other omic layers. The metabolome is the closest link to the phenotype and hence at the forefront of personalized diagnosis and therapy (4, 5). This concept is now widely accepted and has enhanced the application of metabolomics in biomarker research, but it has also blurred our view of its broader implications. A sea change

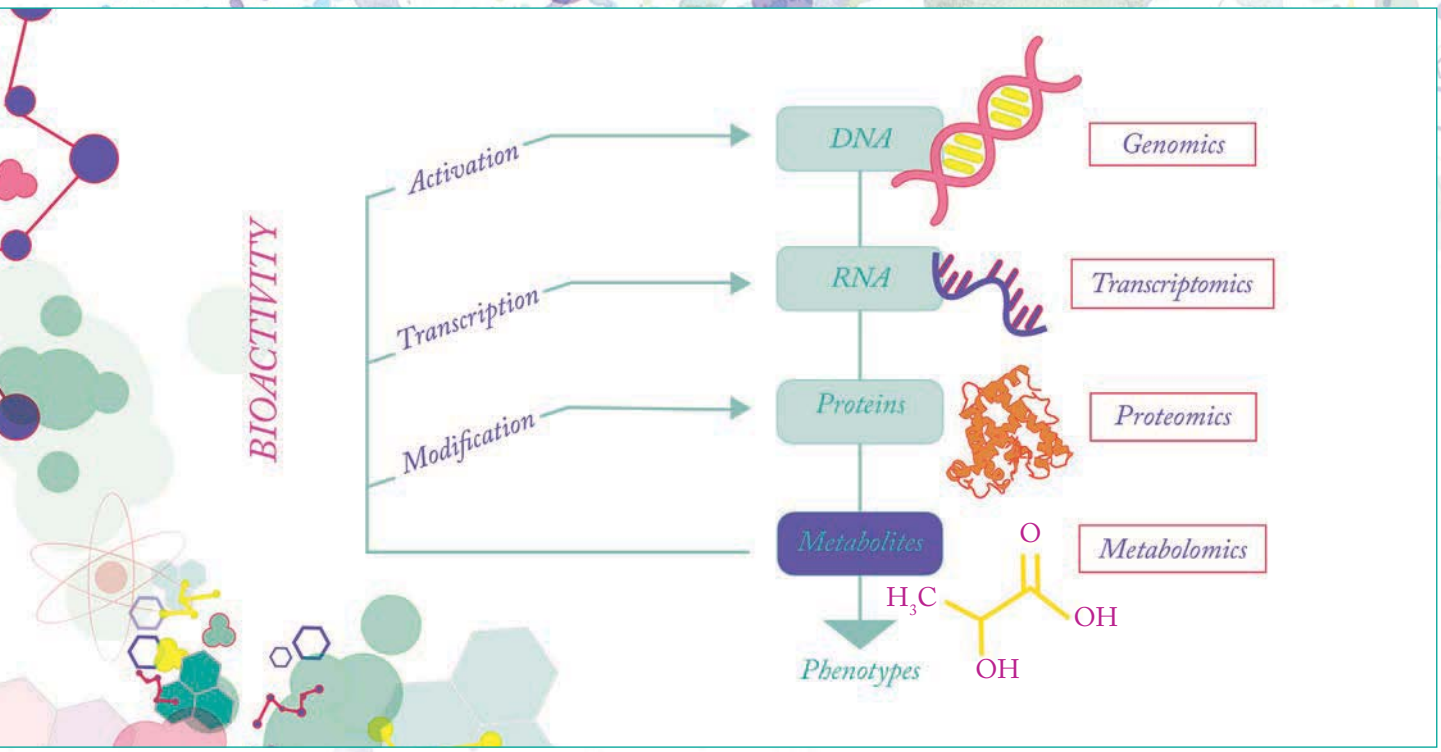


Figure 1. Metabolic activity among the different omic layers.

is taking place, which defines the metabolome not only as an organism's phenotype, but also includes the biological activity of metabolites and their respective control functions (6-8).

When taking a closer look at the involvement of metabolites in gene expression, transcription and translation control, it becomes evident that the metabolome is not simply a cellular information sink. Rather, the metabolome is the omics superglue, providing biochemical feedback across all omic layers (see Figure 1). Metabolites are essential in the control of gene expression, with several transcription factors controlled by specific molecules, including the liver X receptors, estrogen receptors and thyroid hormone receptors. In addition, as metabolic control has become better recognized (9), numerous other cellular receptors activated by metabolites have been discovered, including G-protein coupled receptors that are involved in signal transduction. Beyond these noncovalent protein–metabolite interactions, metabolites are also active substrates and products of enzymatic reactions, as well as participants in protein post-translational modifications, such as acetylation and palmitoylation. This activity even extends to metabolites shaping the metabolic environment to influence protein function, as illustrated by membrane proteins (10).

Clearly, the metabolome interacts with, and hence coalesces, all the omic layers. Therefore, it is not surprising that scientists in the field have started to emphasize the biological activity of metabolites as an integral part of metabolomic studies (6-8, 11). When systems biology evolved as a biological discipline, with the express purpose of comprehensively describing all the biochemical reactions of an organism, metabolomics was rapidly recognized as an important component, allowing us to confirm computational predictions and shed light on biochemical pathways (12). However, it is only recently that the concept of metabolite activity screening (MAS) has started to integrate metabolomics, systems biology, and bioactivity (7). The field is in its infancy, yet the practical impact of identifying

"It is not surprising that scientists in the field have started to emphasize the biological activity of metabolites as an integral part of metabolomic studies."

novel bioactive metabolites and characterizing their associated control, function, and mechanisms of action is exciting.

Critical MAS

Omega-3 poly-unsaturated fatty acids (n-3-PUFAs) and their corresponding metabolic products exemplify the power of directly connecting metabolites with health. Evolving from the observation that Inuit populations – with their n-3-PUFA rich diets – have a lower incidence of heart disease, today, n-3-PUFAs can be considered one of the most important and scientifically proven dietary supplements, with numerous beneficial health effects (13). Charles Serhan's group at Harvard University has subsequently elucidated how n-3-PUFAs and their downstream metabolites function as immunologically active molecules, founding the field of resolution physiology and opening up novel strategies for the treatment of (chronic) inflammation (14). While the n-3-PUFA field originally sprung from a 1970s epidemiological study and the comparison of dietary habits, it is now at the cutting edge of metabolomics.

The combination of systems biology and MAS (systems-MAS), will allow us to systematically elucidate novel pathways and key metabolites, which might subsequently be used as novel therapeutics. An example is the recent systems-MAS finding that taurine significantly enhances drug-induced oligodendrocyte precursor cell differentiation and facilitates the in vitro myelination of co-cultured axons (15). This is especially interesting because metabolites are generally safe, inexpensive, readily available and can rapidly impact a system, be it a cell or an entire organism.

As with any new scientific endeavor, systems-MAS needs to overcome several significant challenges, including the need to develop effective approaches to identify active metabolites and generate libraries cataloging their bioactivity. This process is challenging because metabolic activity will likely vary depending on the organism and the phenotype of that organism. The hope is that when enough biochemical pathway and biological activity data have been correlated, we will be able to shape the metabolic landscape to our own ends. It's possible we could even harness metabolism by stimulating the other omic layers, allowing us to better understand and manipulate physiology, to develop new therapeutics or stimulate or inhibit bacteria (influencing the microbiome). We find this concept particularly intriguing as it transforms our view of the metabolome, which changes from a phenotypic descriptor into a phenotype modulator (7). Perhaps, following on from the success of therapeutic antibodies, metabolites may evolve as a new means to "fix biology with biology".

Martin Giera is Associate Professor and Head of the Metabolomics group at the Center for Proteomics and Metabolomics, Leiden University Medical Center, Leiden, The Netherlands.

Mary E. Spilker is Associate Research Fellow at Pfizer Worldwide Research and Development and is currently a visiting scientist at the Scripps Center for Metabolomics at the Scripps Research Institute, La Jolla, California, USA.

Gary Siuzdak is Professor and Director of the Scripps Center for Metabolomics at the Scripps Research Institute, La Jolla, California, USA.

References

1. *International Human Genome Sequencing Consortium, "Initial sequencing and analysis of the human genome", Nature, 409, 860 (2001).*
2. *F Crick, "Central dogma of molecular biology", Nature, 227, 561 (1970).*
3. *PE Geyer et al., "Revisiting biomarker discovery by plasma proteomics", Mol Syst Biol, 13, 942–957 (2017).*
4. *M Jacob et al., "Metabolomics toward personalized medicine", Mass Spec Rev, 2017, 1–18 (2017).*
5. *I Kohler et al., "Analytical pitfalls and challenges in clinical metabolomics", Bioanalysis, 8, 1509–1532 (2016).*
6. *T Huan et al., "Systems biology guided by XCMS Online metabolomics, Nature Methods, 14, 461 (2017).*
7. *C Guijas et al., "Metabolites that modulate phenotype can be identified by metabolomics activity screening", Nat Biotechnol (2018) (Accepted manuscript).*
8. *M Giera, F Branco dos Santos, G Siuzdak, "Metabolite-induced protein expression guided by metabolomics and systems biology", Cell Metab (2018) (Accepted manuscript).*
9. *AS Husted et al., "GPCR-mediated signaling of metabolites", Cell Metab, 25, 777–796 (2017).*
10. *DA Los, N Murata, "Regulation of enzymatic activity and gene expression by membrane fluidity", Science's STKE, 2000, pe1 (2000).*
11. *F Branco dos Santos et al., "Probing the genome-scale metabolic landscape of Bordetella pertussis, the causative agent of whooping cough", Appl Environ Microbiol, 83, e01528-17 (2017).*
12. *DB Kell, "Metabolomics and systems biology: making sense of the soup", Curr Opin Microbiol, 7, 296–307 (2004).*
13. *JH O'Keefe, Jr, WS Harris, "From Inuit to implementation: omega-3 fatty acids come of age", Mayo Clinic Proc, 75 607–614 (2000).*
14. *CN Serhan, "Pro-resolving lipid mediators are leads for resolution physiology", Nature, 510, 92 (2014).*
15. *BA Beyer et al., "Metabolomics-based discovery of a metabolite that enhances oligodendrocyte maturation", Nat Chem Biol, 14, 22–28 (2018).*

Solutions

Real analytical problems
Collaborative expertise
Novel applications

Go With the Flow

Our continuous flow separation microchip offers faster and more affordable fractionation of DNA.

By Burcu Gumuscu, BIOS Lab-on-a-Chip Group, MESA+ Institute for Nanotechnology, MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, the Netherlands.

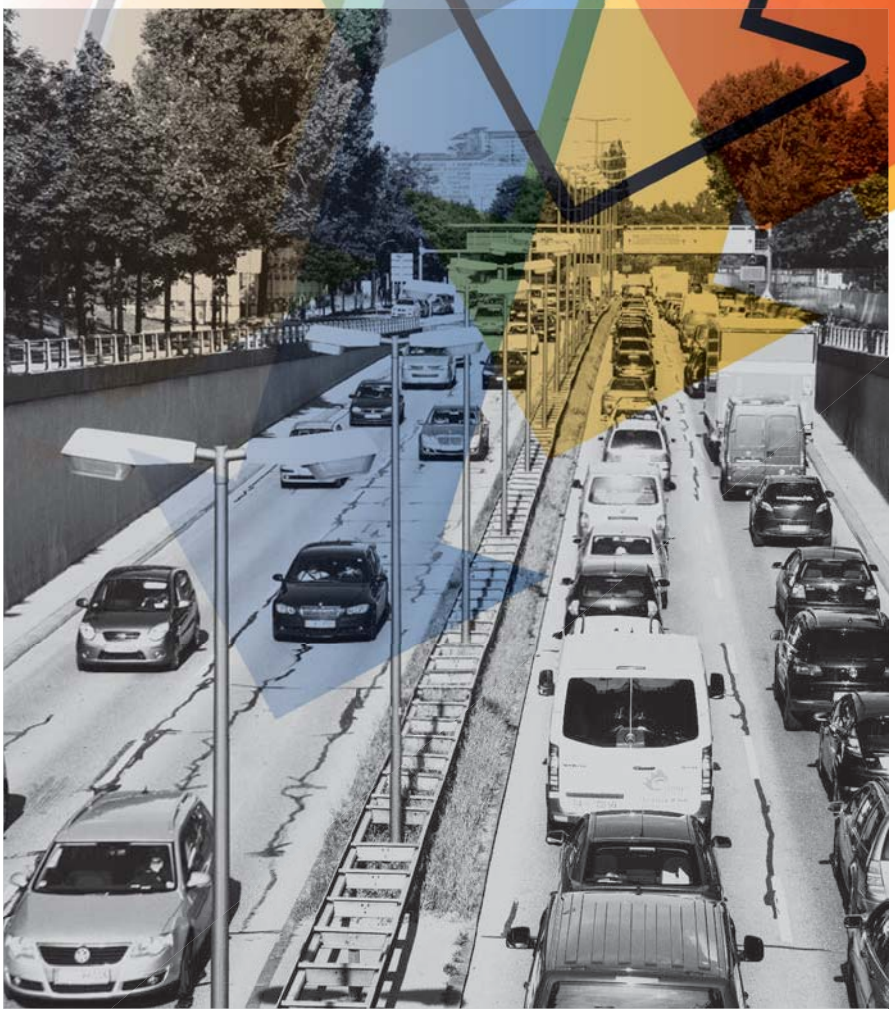
The problem

Standard gel electrophoresis has been intensively used for DNA fractionation in various genotyping and sequencing applications, and has the advantages of great simplicity, versatility, and reproducibility. However, it suffers from long processing times – many hours or even days; for instance, a typical pulsed-field gel electrophoresis (PFGE) device takes 25 hours to perform fractionation of 5–120 kbp DNA. Given trends towards second-generation sequencing, replacing standard gel electrophoresis with microchip-based systems is an attractive option to minimize processing time and optimize DNA fractionation. We set out to develop a new method to improve sample throughput and recovery compared with current technology.

Background

The development of DNA electrophoresis, using both slab gels and capillaries, enabled the first-generation DNA sequencing and genotyping technologies required for the Human Genome Project. However, only minimal sample numbers could be separated and identified, at very high cost. In recent years, efforts have been directed towards reducing the cost and analysis time of DNA genotyping in second- and later-generation sequencing tools. To achieve this, sample preparation methods, such as electrophoresis, must be optimized to increase the throughput and efficiency of the analyses via user-friendly, portable and functional platforms. In some devices, traditional DNA separation gels have been replaced

"2D nanostructures are easier to manufacture, but intrinsically yield low sample throughput."



by microfabricated post arrays for separation of genomic-length DNA. However, defect-free fabrication of 3D nanostructures, such as nanopost arrays and crystalline nanoarrays, is extremely challenging. 2D nanostructures are easier to manufacture, but intrinsically yield low sample throughput. An ideal sieving matrix should have simple design and fabrication steps, yet provide high-resolution, high-throughput separation. And that's why we opted for gel-based devices. People had originally considered continuous flow separation in such devices impossible, but we showed that it could work.

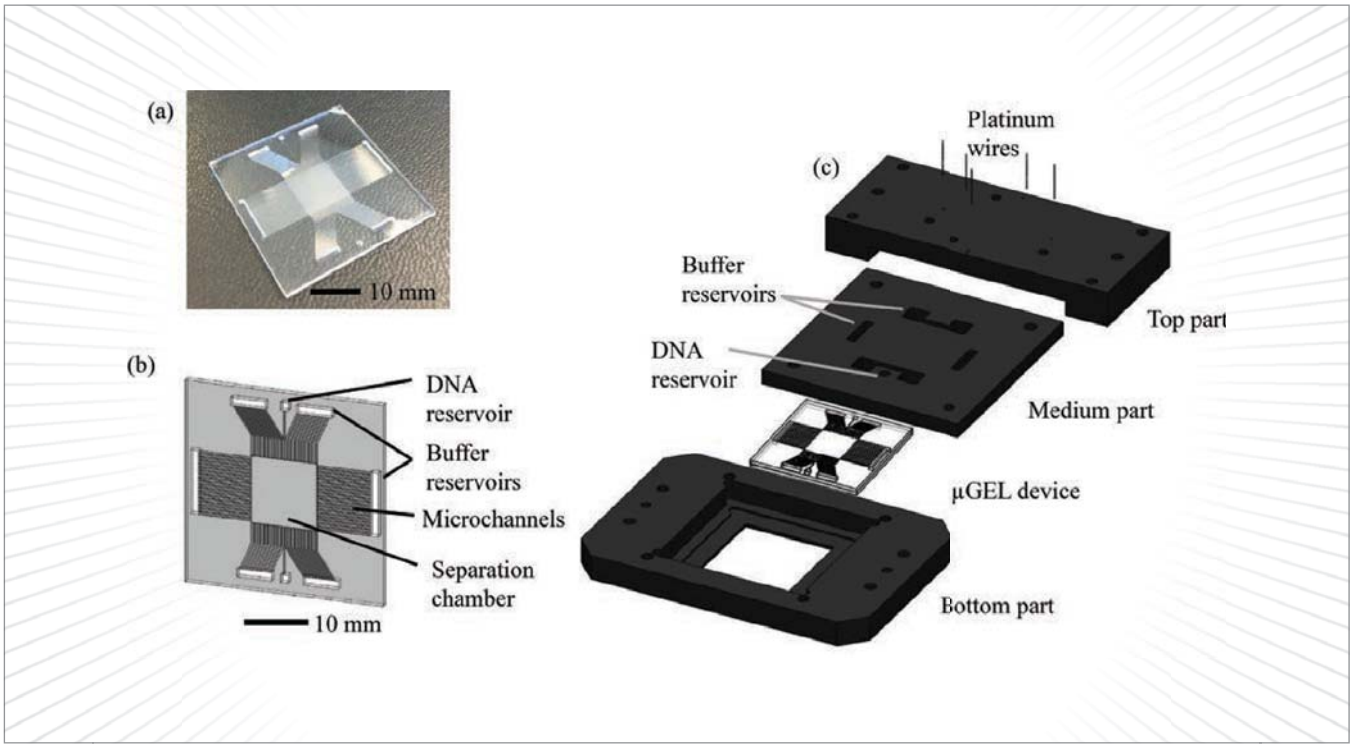


Figure 1. (a) The fabricated microchip made of glass. (b) The microchip layout. (c) The in-house made chip holder made of Delrin.

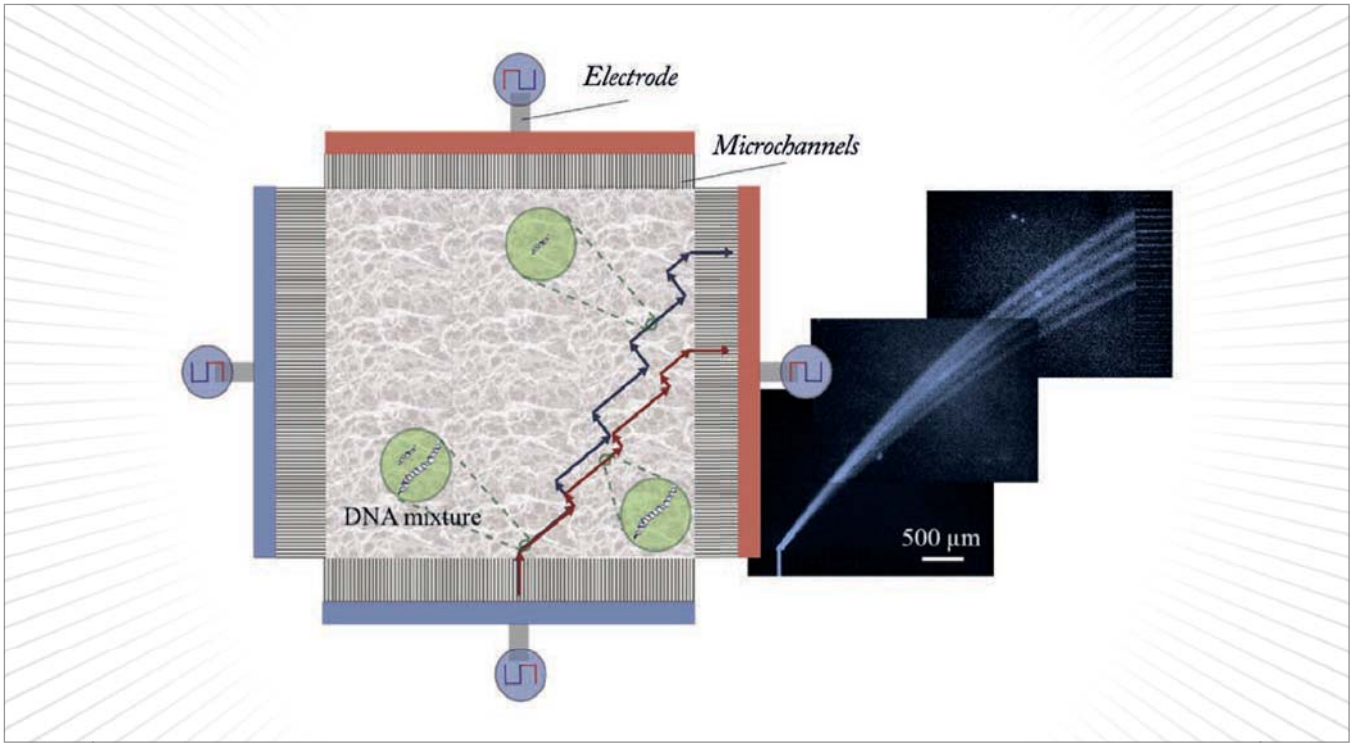


Figure 2. The snake-like flow of DNA molecules through the chip (left) and the resulting fluorescence images (right).

"An ideal sieving matrix should have simple design and fabrication steps, yet provide high-resolution, high-throughput separation."

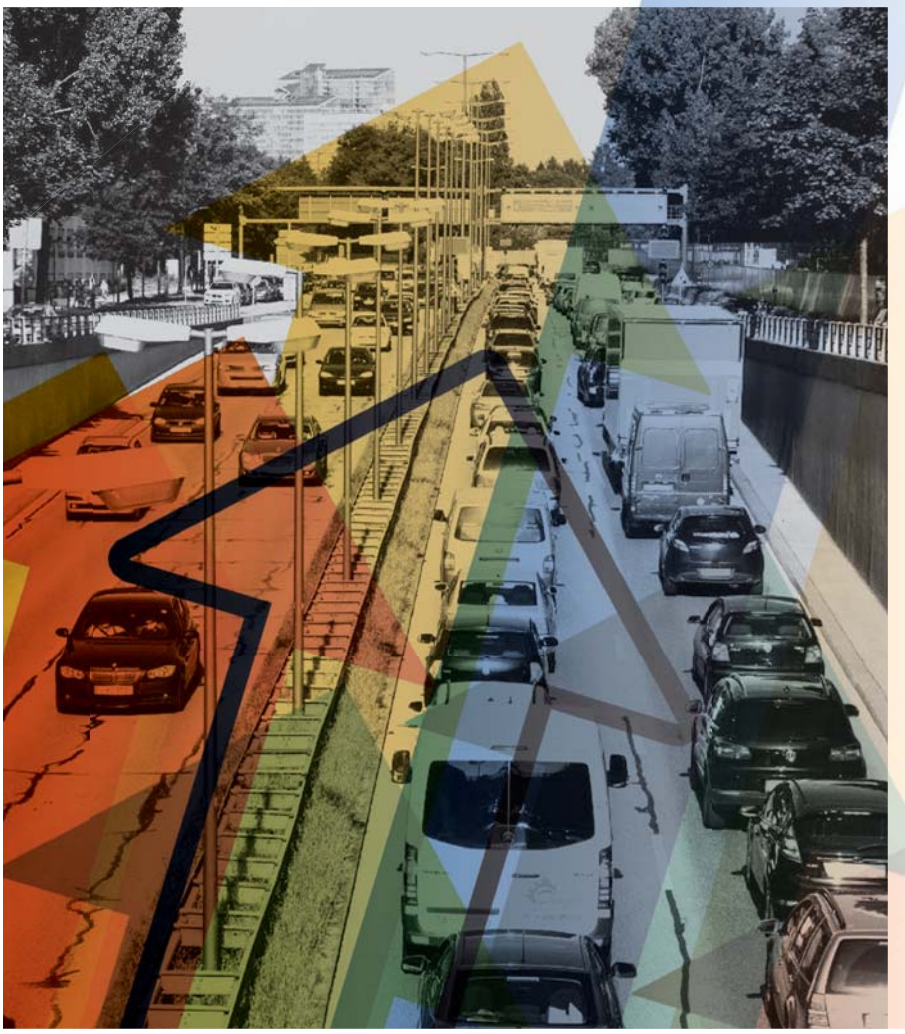
The solution

In the classic DNA electrophoresis approach, an electric field is continuously applied in one direction in a gel matrix and DNA fragments with different base pair numbers (different sizes) are separated in that direction. In PFGE, electric fields of the same strength are switched between two different directions – larger DNA fragments are slower to realign when the field switches, so the fragments become separated by size, but it can be a slow process. In our microchip, we use alternatingly applied orthogonal electric fields of different strengths. Larger fragments will move more slowly than smaller ones in the low field, but both sizes will move at the same speed in the high field. As a result, separation is achieved in the low-field direction and the DNA molecules are rapidly transported through the chip by the high field. The DNA molecules don't all move in the same direction, as in the classical 1D separation, but in various size-dependent directions somewhere in between the two applied field directions (a snake-like motion). Our new chip separates DNA fragments within a few minutes, in high resolution, and purifies the fragments by separating contaminant salts from the fragments.

The chip is made of glass and has a square separation chamber of 1 cm^2, with microchannels on the sides to properly direct the electric fields. The chamber is filled with agarose gel, a standard medium for DNA separation. There are also reservoirs for sample injection and electrodes for applying the fields (see Figure 1). The chip is easy and cheap to produce using basic cleanroom techniques. It is also versatile, as the type and concentration of gel and the properties of the electric fields can be adjusted to the application.

From the beginning of this research, we focused on using a "continuous flow separation" method for fractionating DNA. However, getting to the final concept was a process of trial and error. First, we tried to separate a DNA ladder in a polydimethylsiloxane (PDMS) device with T-shaped separation channels, by applying increasing electrical field in one direction. The device was filled with different agarose gel concentrations to achieve a direction-dependent velocity; however, we could only achieve baseline separation for two different fragment sizes, so it was back to the drawing board. Next, we tried to apply an electric field continuously in an agarose gel-filled 2D separation device in two directions without changing the field strength. Lower electric field strengths did not give successful separation owing to diffusion and frontal separation effects. Neither did applying higher electric fields, due to the similar mobilities of small and large DNA fragments. But by combining the two, we could separate DNA without band broadening problems at low electric field strengths, and simultaneously drive the DNA fragments towards the outlet by applying higher electric field strengths,

"The idea of introducing a new separation mechanism to the mature field of gel electrophoresis is exciting."



resulting in a method that unites speed and good resolution. The potential was obvious.

However, before realizing our idea we had to address a few challenges – including designing a 2D separation matrix that could enable the generation of parallel electric field lines during the separation. In separation matrices with uneven electric field distribution, the separation cannot be performed because DNA fragments will always follow the field lines. This was solved by the addition of the side microchannels. Another great challenge was to produce a single device to cover the separation of a wide range of base pair sizes. In our device, pore size of the gel determines the base pair range in which good separation resolution is achieved, unlike previous DNA separation platforms where isotropic arrays were created using micron-size post arrays (for very long DNA separation), or colloidal crystals (for shorter DNA separation). However, redesigning the post arrays to the base pair range of interest is both laborious and expensive. We solved this problem by basing our designs on the use of inexpensive hydrogels, the pore size of which can be easily adjusted by changing the concentration.

Beyond the solution

The idea of introducing a new separation mechanism to the mature field of gel electrophoresis is exciting. I believe the technology has great potential to be commercialized; for example, it could help diagnose genetic disorders using a much smaller sample volume compared to conventional devices. We believe the microchip will be of broad interest for next-generation sequencing and clinical diagnosis applications, as it can achieve similar separation resolution in much shorter separation times compared to currently available devices. In particular, detection of pathogenic diseases by sorting pathogenic nucleic acids would open new avenues for the market. The device is designed to serve for sample preparation, which is crucial to eliminate false positive/negative results. Importantly, applications of the microchip can further be extended to protein gel electrophoresis by replacing the agarose gel with polyacrylamide gel.

Reference

1. *B Gumuscu et al., "Exploiting biased reptation for continuous flow preparative DNA fractionation in a versatile microfluidic platform", Microsystems & Nanoengineering, 3, 17001 (2017).*

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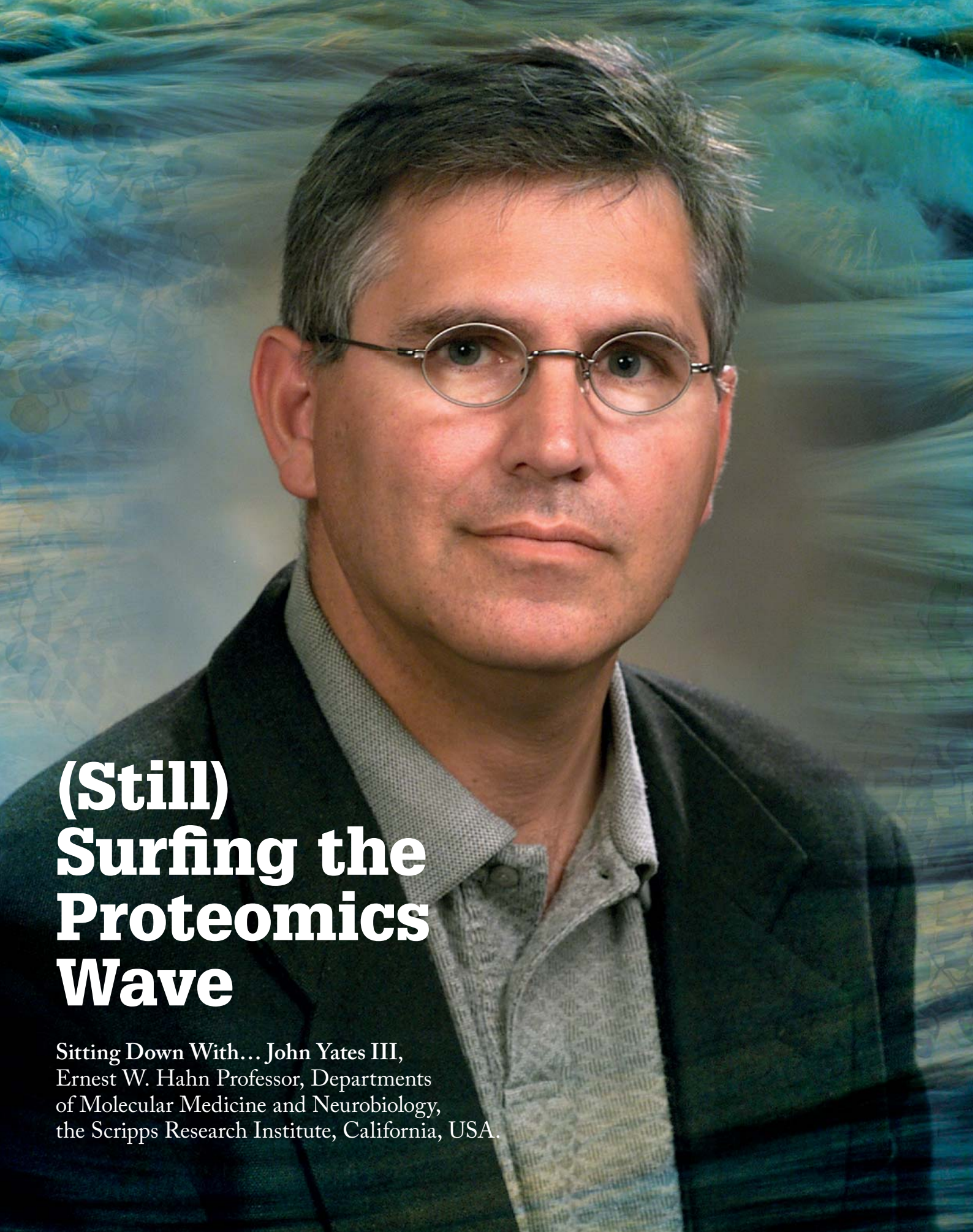


(Still) Surfing the Proteomics Wave

Sitting Down With… John Yates III,
Ernest W. Hahn Professor, Departments of Molecular Medicine and Neurobiology, the Scripps Research Institute, California, USA.

On your Twitter profile, you describe mass spectrometry as "the coolest method on the planet" – why is it so compelling?

The simple answer: because you can do almost anything with it – help develop new pharmaceutical drugs, uncover cheating in sports, carry out life-saving newborn screening, and the list goes on...

When did you see your first mass spectrometer?

In 1981 – it was a Hewlett Packard GC-MS and it was love at first sight. Though I must admit, I was mainly fascinated by the fact that there was a computer attached to it. PCs were not commonplace back then, and I thought it was so cool that it had a computer, with libraries where you could match up compounds.

Where do you get your inspiration?

I like going to meetings outside my own field, where I have a chance to meet new people and hear new ideas. It's thought-provoking to hear about challenges in other fields, and think about how mass spectrometry might fit in. The only problem is that I'm constantly spotting new potential projects, and I have to be careful not to get distracted from the work I already have underway.

How important is it to communicate your science?

In my view, publication is still incredibly important, and it's something I emphasize to my lab members all the time – your science essentially does not exist until you publish it. I believe innovation moves at the speed of publishing.

How can we speed up publication?

I'm Editor of the Journal of Proteome Research (JPL) and we have made speed a real priority. Right now, the biggest bottleneck in the review process is finding reviewers and getting them to respond in a timely manner. So I'm very interested in new ways to do peer review, including the use of artificial intelligence to pre-review papers. Publishers already use software to spot plagiarism; one day I hope we will be able to automate other aspects of copy-editing, leaving the peer reviewers free to focus on core scientific issues of significance, data analysis, and so on.

What else do you hope to achieve as Editor?

Part of the mission of JPL is to encourage researchers to include biological validation of results coming out of proteomics experiments. We don't want authors to be satisfied with publishing a list of protein differences between A and B; we want them to show that the change is contributing to the phenotype.

Looking to the future – where do you see proteomics going?

Single-cell analysis is certainly a hot topic right now. The recently launched Human Cell Atlas project (www.humancellatlas.org) aims to uncover the transcriptome of over 10 billion cells throughout the body using RNA-Seq. It would be great to do the same at the protein level. There are a number of young researchers in the mass spectrometry proteomics field who are looking at strategies for single-cell analysis, with some very exciting results so far. It's also possible that new technologies will come along that don't rely on mass spectrometry at all – there have been some interesting experiments looking at applying nanopore technology used for DNA sequencing to intact protein analysis.

Proteomics hasn't been as well funded as genomics – is that changing?

Proteomics tools are used for almost all protein-related research so grant money is being spent on them, but we haven't seen funding for dedicated proteomics centers as we have with genomics, and I'm not sure if that is likely to change anytime soon, as proteomic driven studies are inherently more complicated than genomics.

Are you concerned about the future of science funding in the USA?

There was a lot of concern among scientists after the current administration proposed large cuts to the NIH for 2017/18. Thankfully, the US Congress pushed back and actually raised the NIH budget for the first time in six years. When budgets are tight, the people who suffer most are young researchers – they are next generation of scientists that we should be nurturing, not chasing out of the field.

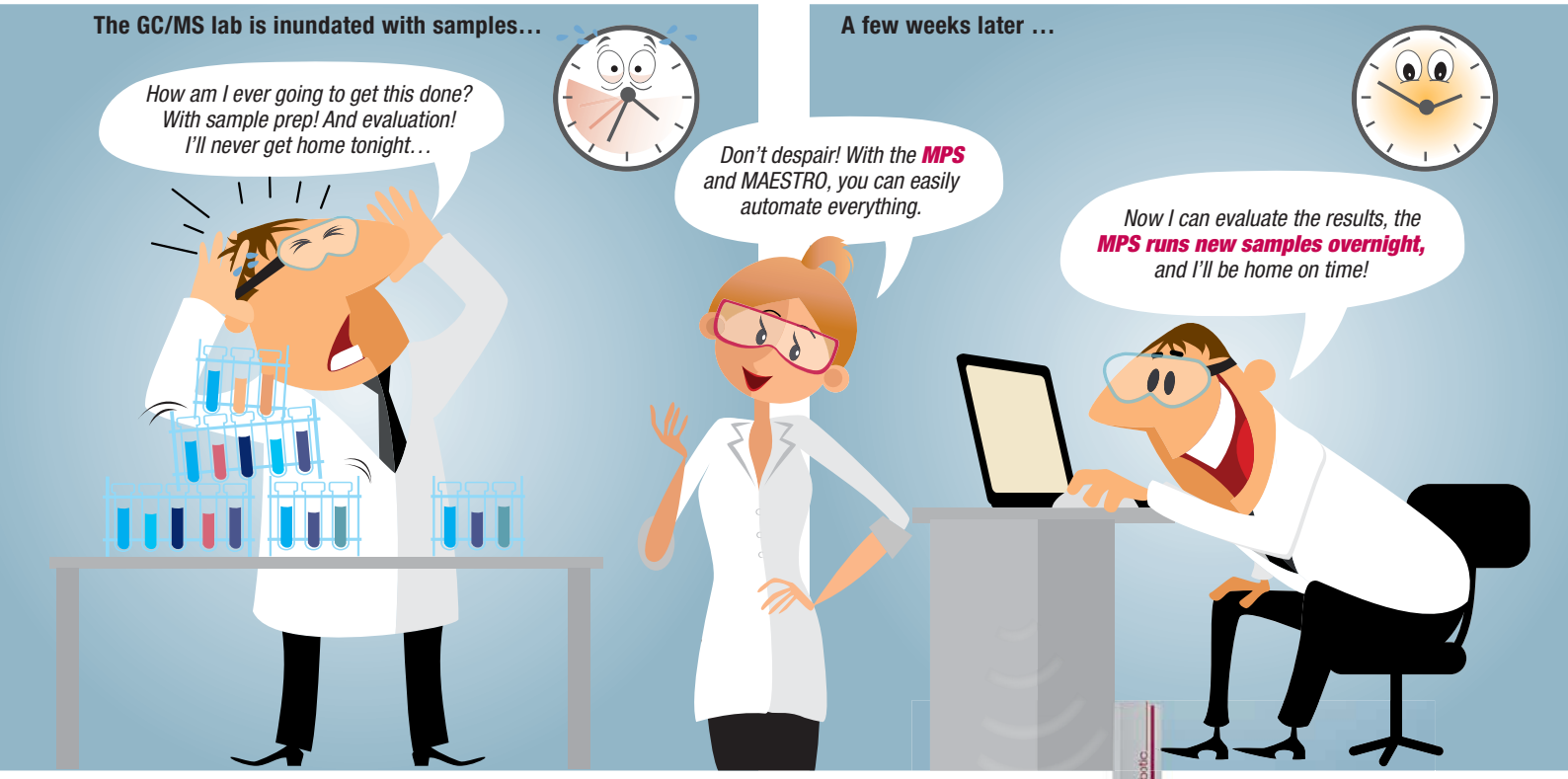
You were recognized for your leadership in the 2017 Power List – what makes a good leader?

First, I make sure my team members have everything they need in terms of money, support and expertise. And then I give them the freedom to be creative and explore. A case in point was when Michael MacCoss and Christine Wu came to me with the idea of introducing stable isotope-labeled amino acids into mice that could be used as internal standards for quantitative proteomics in animal models of disease. It was an expensive experiment, and a gamble – we had no idea if it would work, or if the animals would even eat the labeled feed. But it did work, and proved a valuable tool.

Who are your scientific heroes?

My PhD supervisor Donald Hunt was (and still is) a very imaginative and creative scientist, and my postdoc mentor Leroy Hood gave me a vision of how technology can drive science – both were important in shaping my view of the scientific world and my place in it.

Run, Run, Run!



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