JUNE 2016 # 41

the Analytical Scientist

Toward Integrative Omics

Amanda Hummon and Tom Metz point to the power – and inevitability – of multi-omic efforts.

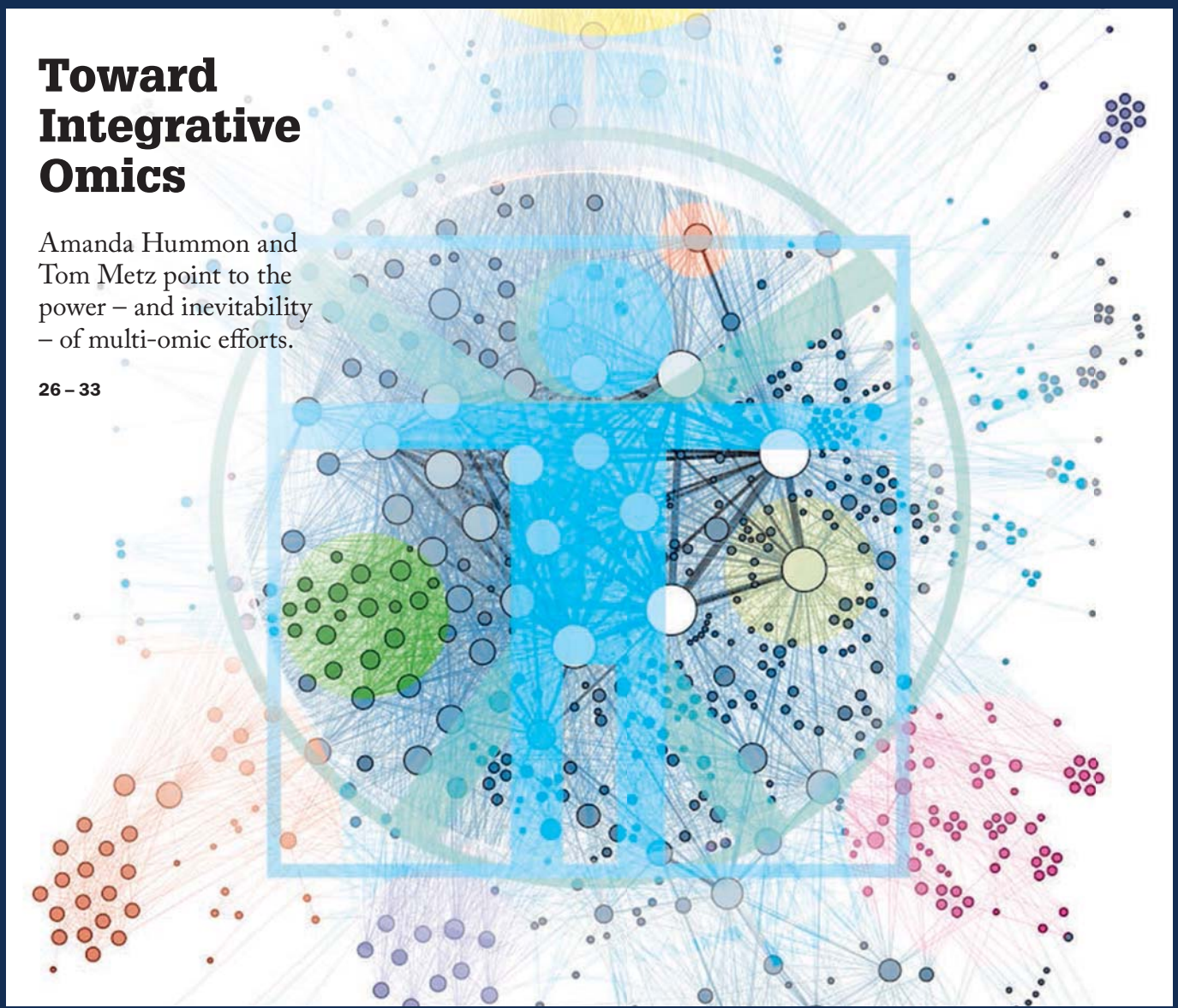

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Image of the Month

Quick Chick Pick

Researchers at the Dresden University of Technology can now determine the sex of a chicken before it hatches – in under a minute. A small hole is cut into the shell and near-infrared spectroscopy is used to identify the DNA content, which is around 2 percent higher in male chicks. The female embryos are then patched up before being returned to incubation, while the eggs containing male chicks are disposed of. Once automated, it is hoped the technique, which has an accuracy rate of 95 percent, will put an end to the current practice of shredding male chicks after birth... Credit: Stephan Wiegand, Dresden University of Technology

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

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On The Cover

A very modern Vitruvian Man (or Woman) surrounded by the complexity of omics information.

Upfront

the Analytical Scientist

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Change of address:
tracey.nicholls@texerepublishing.com
Tracey Nicholls, 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 745200
sales@texerepublishing.com

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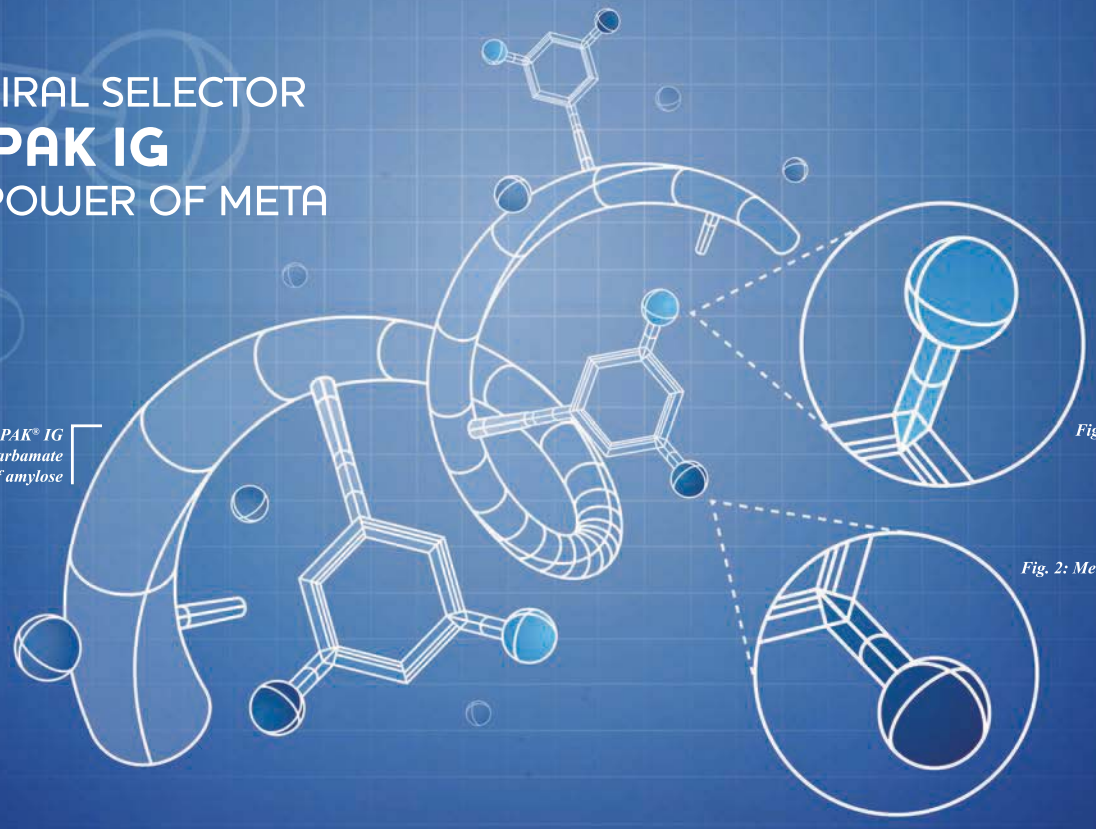

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Fig. 1: Chloro

Fig. 2: Methyl

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Open Access Fraud

The world is in serious trouble when "Photoshop" is added as a major skill on the curricula vitae of scientists.

Oh, the damage one can do with cut, paste and rotate. A recently published paper in Nature's Scientific Reports (1) has attracted a great deal of attention – for all the wrong reasons. With spectacular audacity, the authors share numerous remarkably suspicious confocal microscopy images that feature cloned (by Photoshop not biotechnology) cells. Fortunately, the researchers have not grasped the true potential of photo-editing software – the fake images are easy to spot on second glance. (I am confident that our own talented design team is more than capable of creating images that would hoodwink the keenest of eyes.)

You may struggle to find the referenced paper – the DOI number currently kicks up an "Internal Server Error (500)" message in a classic ostrich-style retraction. I've provided an alternate reference (2) in case you wish to review the paper for your own amusement. The fact that the paper appeared in an open access 'peer-reviewed' journal from an otherwise reputable publisher perfectly highlights concerns that have been aired many times. If authors are "paying to play," are publishers likely to make the peer-review process as rigorous as it should be?

In this case, clearly not. As Derek Lowe notes in his blog post (3), "I don't have a problem with [the open access] model. But it doesn't work if you don't review the damned papers. I don't think that Nature wants to compete head-to-head with all the junk publishers of this world, but if they really want to, this is how you'd do it. Wave everything through, publish crap, cash the checks."

Lowe and numerous others point out additional errors that will be of particular interest to analytical chemists in the supporting information (4), including mystifying NMR spectra – can you fathom the unfathomable? And does that mass spectrometry data seem right?

Yes, the paper under fire appears to be an extreme example of (incompetent) fraud – the authors have used every trick in the book – but they have also published more than one paper (with the same copy–paste images). And where there is fire, there is likely a good deal of smoke. If peer review fails or does not happen at all, the reputation of open-access publishing – and even science itself – is at stake.

What is the answer? Well, the Internet and social media are rising up to be the best peer-review tools we've got (5). But it needs an active community with a critical eye. If you see a paper that just doesn't seem right – I urge you to speak out. Or at least share your concerns with me (6). After all, The Analytical Scientist isn't a (pseudo) peer-reviewed journal; it is a truly open magazine – we don't demand payment to either view or publish content. And that gives you the power to reach a like-minded community.

Rich Whitworth
Editor

References

1. *N Samie et al, "Novel piperazine core compound induces death in human liver cancer cells: possible pharmacological properties", Sci Rep 6:24172 (2016).*
2. *http://tas.txp.to/0616/fraud*
3. *http://blogs.sciencemag.org/pipeline/archives/2016/06/10/crap-courtesy-of-a-major-scientific-publisher*
4. *http://tas.txp.to/0616/supp*
5. *http://retractionwatch.com/2016/06/10/author-denies-accusations-of-blatant-duplication/*
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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:
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Baited Breath

Using PTR-MS to track the chemistry of group emotion

A team at the Max Planck Institute for Chemistry in Mainz, Germany, have discovered that film fans can change the chemical composition of the air around them – with their breath. The team screened 9,500 people for potential emotion signaling molecules during 108 screenings of 16 different films. They found that the chemicals emitted varied from scene to scene, allowing them to pinpoint exact moments in the film that elicited strong emotional responses.

Originally, the team were looking to answer a different question: are the chemicals that we breathe out significant to global atmospheric chemistry? The group decided a trip to the football stadium might help. "With 30,000 people in a confined space you can get a nice average breath spectrum. Based on that we could multiply the average by 7 billion people and see whether the numbers were in any way important on a global scale," says Jonathan Williams,

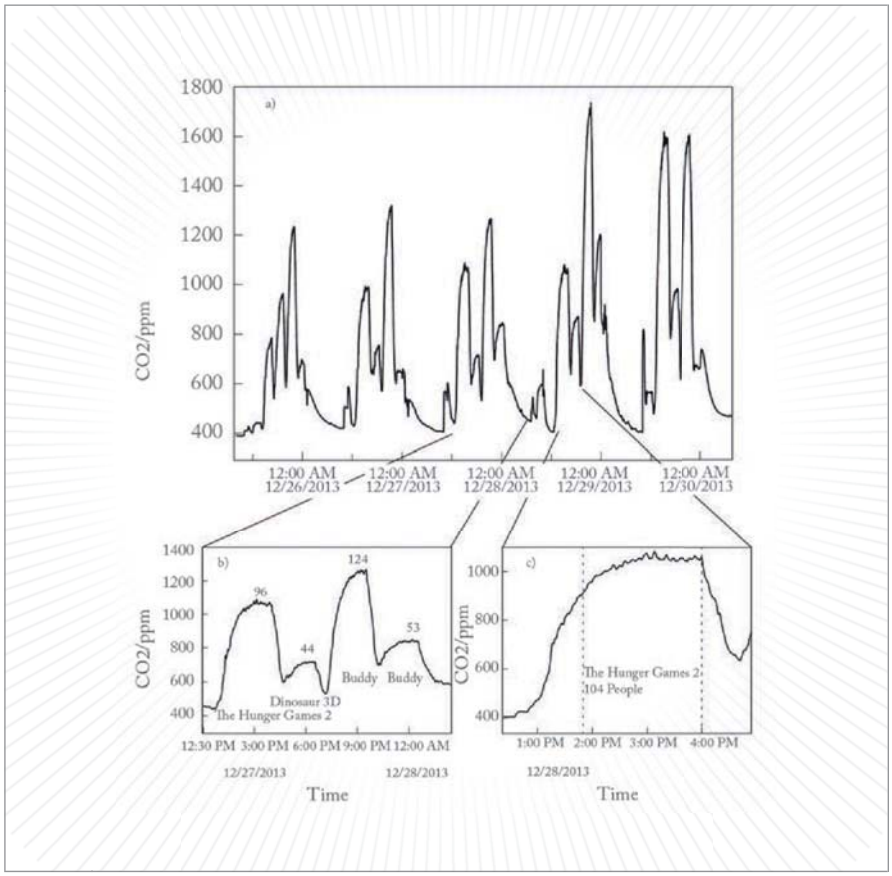


Figure 1. a) peaks in CO_2 over five days of film screening. The levels increase rapidly as audience members enter the auditorium and fall off sharply as they leave; b) CO_2 measurements for four film showings. The higher the CO_2 measurement, the higher the attendee numbers; c) CO_2 measurements throughout a showing of "Hunger Games 2". The small peaks in CO_2 suggest momentary increases in pulse and breathing rate in response to key moments in the film.

from the Air Chemistry Department. From that came the idea of capturing the "chemistry of group euphoria". Williams says, "We thought it would be cool if, when there was a goal and everybody got ecstatic at the same time, we could capture the chemical signature of a goal!"

Unfortunately, the match ended 0-0. The team decided that a movie theater could be an good substitute location. "A cinema is a really elegant way of measuring the potential linkage between what we breathe out and our chemical state of emotion," Williams says. "We essentially got people in a nicely ventilated box and then frightened them or made them laugh. All the time we flowed air over them, and simply measured the sequence of peaks. We were able to measure online, so could see clearly and in real time when everyone was frightened and we got a peak in CO_2." Film events labeled "suspense" or "injury" elicited the strongest chemical changes, leading the team to speculate that this "chemical communication" may function as a form of group signaling beneficial from an evolutionary perspective.

They used Ionicon's proton transfer reaction mass spectrometer (PTR-MS) to track the trace gases. "It runs on water – you essentially pass a lightning bolt through it and that forces the water to carry an extra proton, giving you H_3O+ instead of H_2O," Williams says. "You simply put these reagent ions through ambient air, and they try to pass on that uncomfortable extra proton. Fortunately for us, the proton does not transfer to nitrogen, oxygen, argon – all the main components in the air – so we are able to detect a whole host of organic species with the system – acids, alcohols, aromatic compounds, and so on."

A crucial advantage to conducting the experiment in a cinema was reproducibility. "I looked at the results for The Hunger Games 2 and noticed that when the same film was shown on a different day to a different 250 people, for some of the chemicals the peaks occurred in the same place," says Williams. "Of course, as a scientist – and especially as an analytical scientist – you want reproducible results. In a cinema, you can do that by showing a film multiple times."

Williams describes the paper as "a signpost publication" and says he would expect a lot of atmospheric chemists to be interested in developing the findings further. "These temporal changes in chemistry are there to be seen in a group of people in a controlled environment – and you can use those signals for whatever purpose," Williams says. He suggests it may also have uses in biology, film-making, advertising and psychology: "A lot of psychologists try to do experiments where they attach probes or have to wear a mask – which can influence behavior. To be able to non-invasively measure someone's reactions could be very useful." *JC*

Reference

1. *J Williams et al, "Cinema audiences reproducibly vary the chemical composition of air during films, by broadcasting scene specific emissions on breath", Sci Rep 6, 25464 (2016). DOI: 10.1038/srep25464*

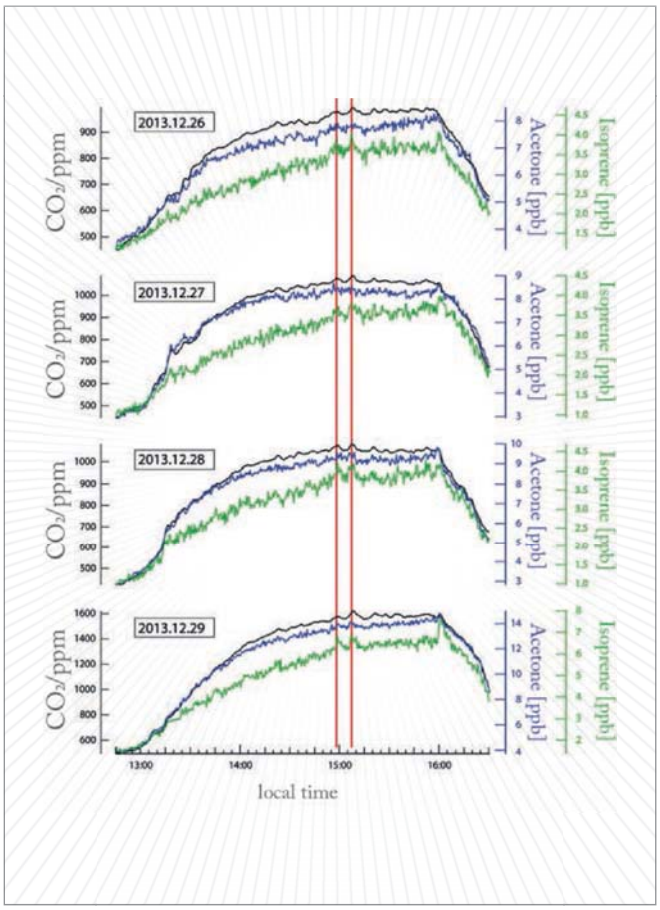
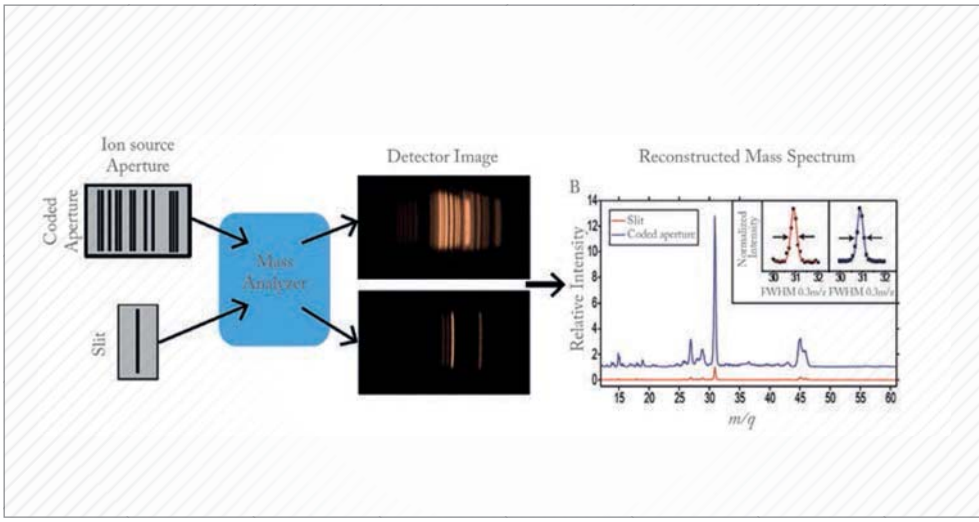


Figure 2: Measurements of CO_2, isoprene and acetone during four separate screenings of "Hunger Games 2". The red markers show moments where audiences had reproducible responses to the same moments in the film. The two peaks in isoprene suggest breath-holding, and correspond with the heroine's dress catching fire and the final battle.

Small and Mighty

Is a new and improved mass spectrometer the "Holy Grail" of chemical sensing?



A "miniaturized" mass spectrometer promising higher resolution and more sophisticated detection is being developed by a team at Duke University, NC, USA. Jeffrey Glass, professor of electrical and computer engineering and lead researcher, has been working on the development of this high-resolution miniature mass spectrometer, and says that the benefits are likely to have a broad reach, from point-of-care applications to space travel. Here he tells us more about the development of coded aperture MS – and why this technology is welcome news.

How did you come to focus on the challenge of size and sensitivity versus resolution?

Miniaturization of a mass spectrometer is considered the "Holy Grail" of chemical sensing due to the broad range of chemicals it can detect and its sensitivity to low concentrations of those chemicals. However, there is a historical trade-off between resolution and throughput when miniaturizing a spectrometer. In order to effectively miniaturize a mass spectrometer, we had to find a solution to this trade-off – hence the development of a coded aperture mass spectrometer.

How does a coded aperture mass spectrometer work?

In a typical sector mass spectrometer, the ions pass through a thin slit. The slit width interacts with the path length the ions take through the instrument to define the system resolution. When you shrink the instrument, you shrink this path length, and so the slit width has to shrink as well in order to maintain the system resolution. This means the throughput passing through the instrument is going to be reduced, decreasing signal intensity and reducing the signal-to-noise ratio (SNR). We got around this issue by using a "coded aperture" – an array of several slits arranged in a particular mathematical pattern. The resulting measurements do not directly resemble the spectrum, but we can use a reconstruction algorithm to undo the mixing introduced by the aperture and recover the spectrum. The net result is that we are able to achieve high-resolution while also maintaining SNR; this breaks the historical trade-off between resolution and throughput and frees us to miniaturize the system without sacrificing performance.

Is the coded aperture restricted to specific types of MS?

We are currently investigating the various types of MS that work with coded apertures. So far, we have shown that they work with simple magnetic sectors and more complex instruments that employ an electric sector and magnetic sector, like the Mattauch-Herzog geometry described in our recent publication (1). In practice, coded apertures allow for a smaller MS system by maintaining the throughput of the spectrometer without compromising the resolution.

How will the system compete against other portable technologies?

Our miniature mass spectrometer has several advantages. First, it is a spectrograph, meaning that it measures the entire spectrum at once; devices like the M908 are serial instruments that measure each mass sequentially. Measuring the entire spectrum all at once allows for more efficient sample use. In addition, due to the coded apertures, our ability to maintain throughput without sacrificing resolution will likely allow us to have a higher resolution instrument than other portable technologies.

What challenges lie ahead?

Although the fundamental proof of concept for coded aperture mass spectrometry has been achieved, there are several engineering challenges to be tackled ahead of making a working portable system. We must miniaturize all of the various components of the mass spectrometer including the ion source, sample inlet, detector, and vacuum pumps. In addition, one of main challenges of developing an instrument is to get all of the various parts working together at the same time!

Reference

1. *ZE Russell et al., "Compatibility of spatially coded apertures with a miniature Mattauch–Herzog mass spectrograph", JASMS, 27 (4), 578–84 (2016). DOI: 10.1007/s13361-015-1323-7*

Holding Back the Years

Using Raman spectroscopy to track hyaluronic acid permeation in skin

Hyaluronic acid (HA) is a highly hydrophilic and remarkably viscoelastic molecule, able to retain up to 1000 times its weight in water – properties that make it a protective structure-stabilizing, shock-absorbing agent in the epidermis and dermis of skin. But the aging process brings with it a decrease in the skin's natural HA content, which leads to loss of hydration, smoothness, and softness. In fact, its decline is even thought to cause wrinkles. Little wonder then that the molecule plays a starring role in cosmetics products and is name-dropped across beauty blogs and YouTube on a daily basis.

But how effective are HA-containing products at getting the beautifying acid where it is needed? A team of scientists at the University of Riems, France, set out to evaluate the depth of HA penetration and provide accurate localization of cosmetic actives in human skin, using samples from plastic surgical intervention (1). According to lead researcher Mohammed Essendoubi, several studies have demonstrated a significant improvement in skin hydration, wrinkle depth, and elasticity by using HA-based creams, but they have not shown HA penetration and localization in the skin layers – something his research managed to achieve. "Topical penetration of cosmetics molecules through the skin structures is one of the main issues in dermatology and cosmetology," says Essendoubi. "Several conventional techniques, such as tape-stripping, are commonly used, but although widely accepted, these methods lack spatial accuracy and cannot be used for molecules where penetration is limited to the outermost layers of the skin."

The researchers used a Labram microspectrometer to assess the permeability of HA, and discovered that Raman imaging combined with chemometric methods are interesting alternative tools for cutaneous permeation tests. "As a laser-based spectroscopic technique, Raman detects the characteristic vibrational energy levels of a molecule. The spectra thereby constitute highly specific spectroscopic fingerprints," Essendoubi says. "It represents an analytical, non-destructive, and dynamic method to improve the evaluation of the skin penetration of exogenous molecules with high accuracy and without particular preparation of the sample."

Essendoubi says this is the first report to prove permeation and spatial distribution of HA with different molecular weight in human skin. "High molecular weights HA (1000-1400 kDa) hydrates the skin by forming a film on its surface and preventing water loss. In contrast, low molecular weight HA (20-300 kDa) can penetrate skin to protect and support epidermal hydration and to moisturize the stratum corneum continuously to assure high quality of the epidermal texture."

Will these discoveries filter down to more effective hydration in cosmetics or will the technique filter out to the study of other topical drugs? Only time will tell… *JC*

Reference

1. *M Essendoubi et al., "Human skin penetration of hyaluronic acid of different molecular weights as probed by Raman spectroscopy", Skin Research and Technology, 22, 55-62 (2016)*

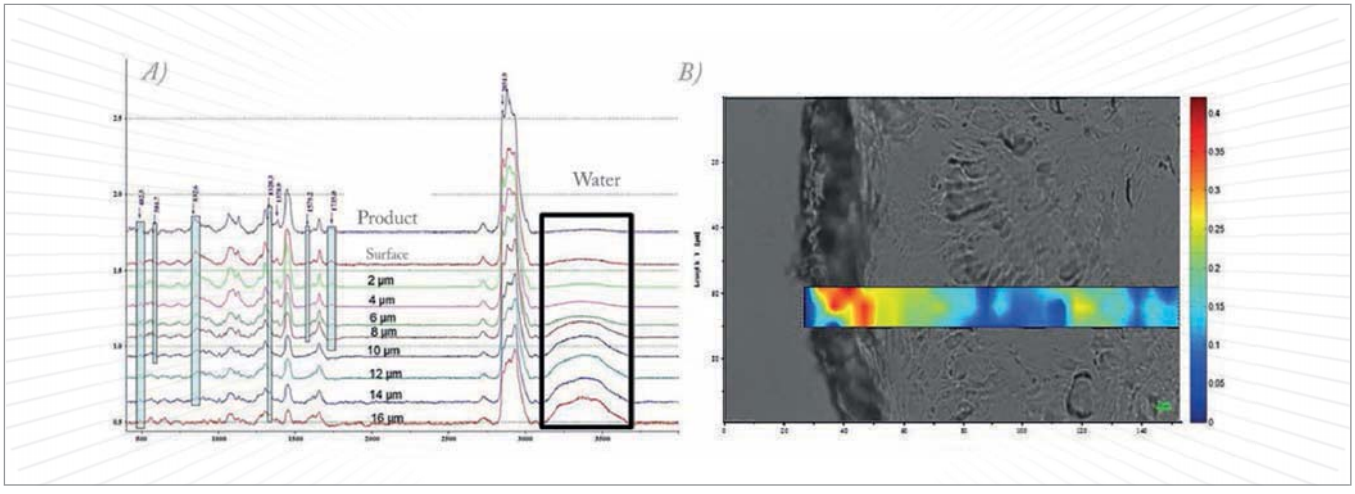


Figure 1. Cutaneous tracking of molecules. A) Confocal Raman microspectroscopy spectra of hyaluronic acid product and water B) Spectral imaging for diffusion and localization of the active ingredient.

Looking for a Lab?

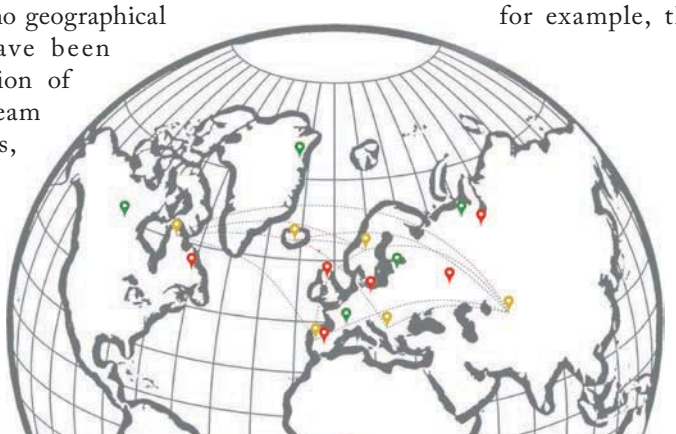
Finding a research partner just got a lot easier

Labs Explorer (www.labsexplorer.com) is a new – free – online tool that launched in April 2016 to support collaboration. We ask founder Stephane Tholander to explain what it's all about.

What? Labs Explorer is a specialized search engine with more than 100,000 referenced labs in all fields of R&D. It is designed to help you find partners for free – and to help labs showcase their expertise. In particular, they can list their collaborations, scientific publications and contract services, so that virtual visitors can get a good overview.

Why? It became clear to the founding team that R&D collaborations are critical for innovation. For instance, the Horizon 2020 European program urges interdisciplinary collaborations and partnerships between private and academic labs. The problem? Finding relevant labs in fields you don't know about is extremely time-consuming or overly expensive. We wanted to provide the R&D community with a free search engine to look for partners.

Where? Currently, most registered labs on the platform are based in Europe – even though no geographical boundaries have been set. The ambition of the founding team (based in Paris, France) is to provide a truly global R&D-specific environment, where labs from all over the world an share their expertise.



Who? Researchers from academic and private labs are invited to create lab pages and manage their own details. Labs from many well known institutions already feature on Labs Explorer; for example, the Fraunhofer MEVIS institute, several units from the French National Center for Scientific Research (CNRS), various groups from the universities of Oxford and Cambridge, and many more.

How? Labs Explorer offers a simple and free alternative to other platforms. The search engine delivers an instant and relevant list of labs for free (and without login or registration). By signing up, users are able to search for R&D partners, request partnerships, and manage their own lab page.

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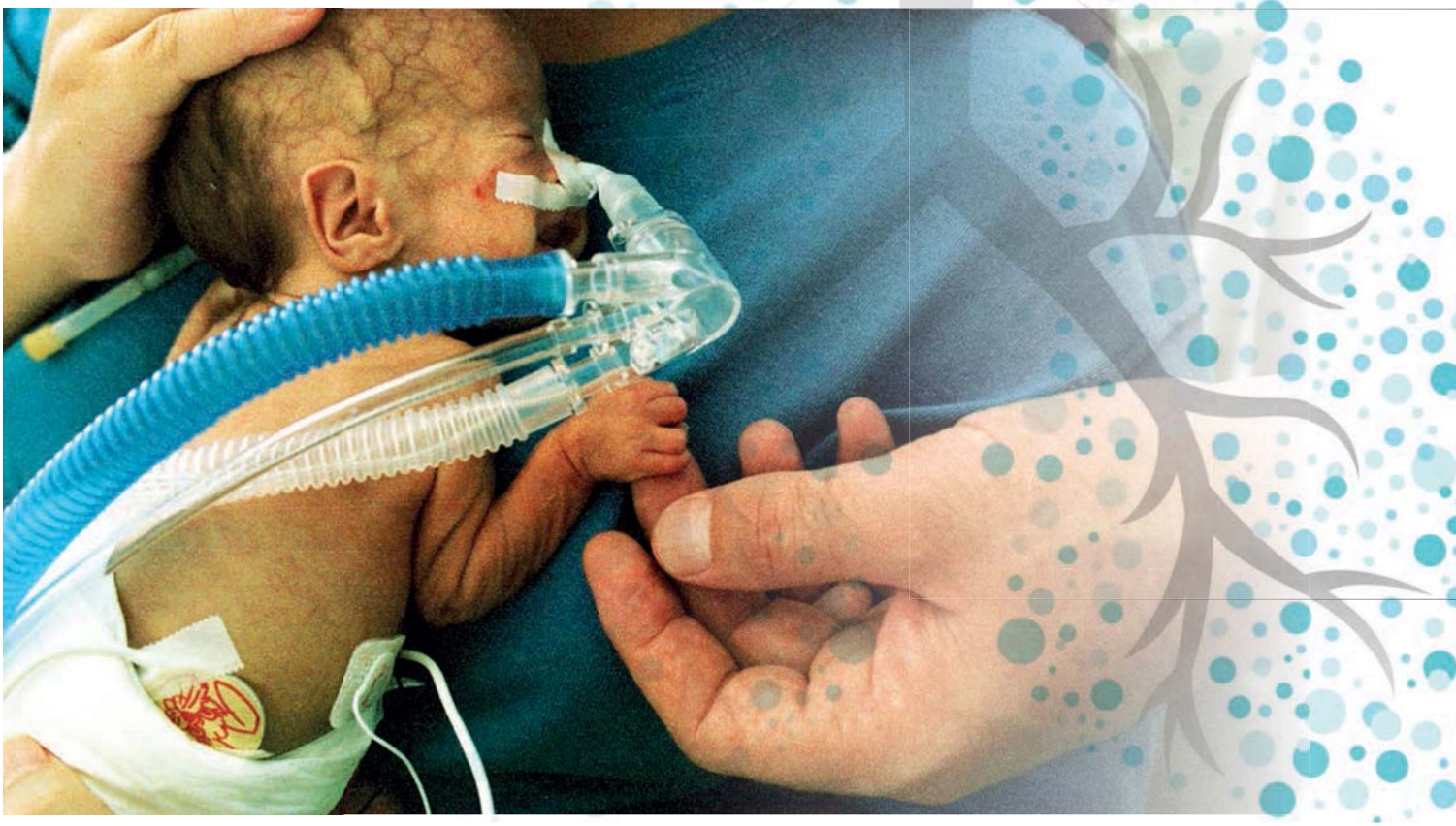


The Lungs of Babes

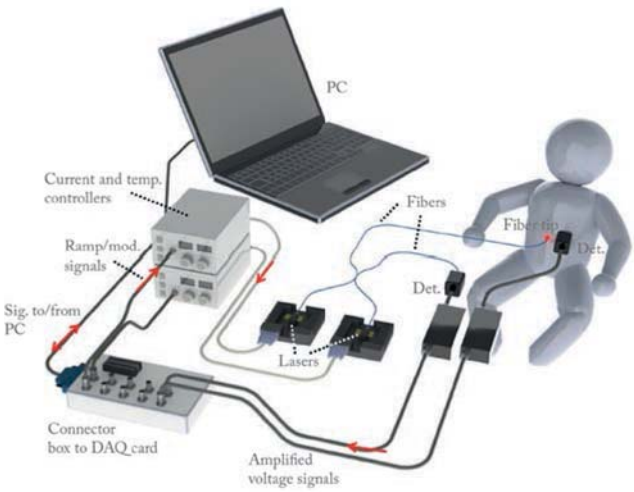
Near-infrared spectroscopy may allow non-invasive monitoring of premature babies

Monitoring patient oxygenation levels in intensive care is key, but prematurely born babies pose a unique problem. "They are very vulnerable, since their organs are immature, in particular the brain and the lungs," says Emilie Krite Svanberg, who specializes in anesthesiology and intensive care medicine at Lund University. "Prematurely born children weighing as little as 500 grams very frequently have respiratory distress syndrome and need strong surveillance – unfortunately, this often demands repeated X-ray recordings." The main aim of Krite Svanberg's work is to reduce the use of damaging X-rays by using near-infrared spectroscopy (NIRS) for 24-hour non-invasive monitoring of lung tissue oxygenation.

Oxygenation is often measured by blood gas analysis but optical techniques, such as common pulse oximetry (with a clip around the index finger), are also routinely employed. NIRS is still an emerging technique. "Using broad-band spectroscopy it is possible to distinguish between oxygenated blood and non-oxygenated blood. My first studies related to refining such techniques, for example, by working with a time-resolved spectroscopic variety of NIRS, which is less sensitive to the strong light scattering in tissue," says Krite Svanberg.

But other spectroscopic developments from the physicists in the engineering faculty at Lund University were emerging, including narrow-band spectroscopy using tunable diode lasers. "They had already applied the GASMAS (gas in scattering media absorption spectroscopy) technique to construction materials, pharmaceutical preparations, and also for diagnosis of sinusitis," Krite Svanberg explains. "The idea emerged to apply these techniques to newborn children, who are small enough to yield a useful optical signal from their lungs."

How does the technique work? Weak laser light is fiber-optically 'injected' into the body, and scattered light, which has partly passed through air-containing tissues, reaches the body surface, where it is collected by a detector. Two narrow-band diode lasers are employed and scanned through sharp molecular absorption lines – one through an appropriate



oxygen line, and one through a water-vapor line. "The latter is employed for normalization purposes, since the concentration of water vapor is determined by the temperature only, which is known," says Krite Svanberg. "The problem with unknown optical path length through the scattering medium can then be largely eliminated to yield calibrated oxygen gas levels."

In the initial clinical trial (1), clear oxygen and water vapor signals were detected from the chests of a large number of full-term newborn children. There are still issues to be resolved, however. "We have had signal intensity and normalization problems, which we expect to be solved with improved equipment and measurement approaches. This is important before proceeding to studies of premature children."

The next stage? "We need specially designed laser sources and optical arrangements to obtain clinically adapted equipment for routine application," say Krite Svanberg. The project has received EU funding, and will continue in collaboration with three other enterprises (in Sweden, Norway and Germany) and the interdisciplinary research group at Lund University. Krite Svanberg believes the findings so far show great possibilities: "With a new proposal to deliver the light internally in the patient via the endotracheal tube of the respirator – which is frequently already there for the severely ill patients – it might even be possible to extend the technique to adult monitoring." *JC*

Reference

1. *E Krite Svanberg et al, "Noninvasive monitoring of oxygen in the lungs of newborn infants by diode laser spectroscopy", Pediatr Res (2015). DOI: 10.1038/pr.2015.267*

Are you a keen model maker? You may be interested in Bruker's quirky photo competition: tas.txp.to/0616/timsTOF

Launches, Libraries and Lipid Analyses

What's new in business this month?

In our new regular column, we partner with www.mass-spec-capital.com to let you know what's going on in the business world of analytical science. June brought with it ASMS 2016, and a resulting onslaught of mass spectrometry innovations – too many to list in print. For more information on all the news listed here, plus links to other launches and deals, please visit the online version of this article: tas.txp.to/0616/BUSINESS

Products

Sciex announced the QTRAP 6500+ mass spectrometer at ASMS, as well as the new release of BioPharmaView Software 2.0.

Bruker launched the rapifleX MALDI PharmaPulse Solution for uHTS and the timsTOF Mass Spectrometer at ASMS, as well as the MetaboBASE Personal Library.

Waters announced the Metabolic Profiling CCS Library and the Symphony Data Pipeline Software at ASMS. It also introduced its Xevo TQ-XS benchtop mass spectrometer in May.

Dresden-based Lipotype GmbH have started to offer lipid analysis services via a web shop.

Collaborations

908 Devices and Thermo Fisher Scientific have combined ZipChip sample separation with Orbitrap MS systems.

The University of Geneva and Bruker BioSpin, both based in Switzerland, have jointly developed a new superconductive coil able to reach a magnetic field of 25 Tesla.

BIA Separations has appointed Nilsan as distributor in India.

Investments and acquisitions

Thermo Fisher intends to acquire FEI Company for $4.2bn in cash.

Germany-based Stratec Biomedical has signed an agreement to acquire Sony DADC BioSciences GmbH (based in Salzburg, Austria) for €30m.

Swiss-based CovalX AG and Biozentrum Basel have received a CHF436k KTI grant from the Swiss government.

Eurofins has acquired the French food testing firm Agro-Analyses SAS, the environmental testing laboratory EAC Corp in Japan, and Exova's food, water, and pharma testing labs in the UK and Ireland.

bioMérieux has acquired Hyglos GmbH, a Germany-based company specializing in the detection of endotoxins.

Management and board appointments

Proteome Sciences has appointed Jeremy Haigh as CEO, citing the growing PS Biomarker Services business as reason to look for a senior figure strongly connected to the pharmaceutical industry and to split the role of Executive Chairman.

Bruker has appointed Harvard's Cynthia Friend to its Board of Directors.

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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

RIP GC?

When disruptive technology comes along, it can be hard to defend the technique under attack for some applications – even if we have embraced it for decades.

By Ray Perkins, Managing Director, Anatune, Cambridge, UK.

For the first time, I find myself viewing gas chromatography as quaintly passé. It's the same sensation I experienced when, as a Nokia mobile phone user, I picked up an iPhone for the first time. It's the feeling you get when you find yourself staring the future in the face; it has crept upon me as I have become acquainted with selected ion flow tube mass spectrometry (SIFT-MS).

GC has served us well for more than half a century, separating mixtures of non-polar volatile organics and presenting the components to a detector as a time series for identification and measurement. Hyphenated with MS, GC does a good job, but long familiarity kept me blind to some significant faults.

When it comes to small molecules, SIFT-MS can separate and measure pretty much everything that GC can – but unlike GC, it handles polar compounds well. For example, it can analyse BTEXs, carbonyls and acid gases at the same time. In part, this is possible because introducing samples into a SIFT-MS is ridiculously simple – the instrument sucks the sample in through a hole. With GC, your sample has to navigate the GC's injector – one of the most misunderstood, troublesome and error-prone devices known to science.

The second major issue is speed; SIFT-MS is really fast. Not "fast GC" fast (which is still slow) but "getting-the-job-done-and-dusted-in-seconds" fast. This changes things in more ways than you can imagine and leaves GC trailing behind and out of sight.

"The speed of SIFT-MS also opens up new possibilities that are closed to gas chromatography."

Let's begin with method development. With SIFT-MS, the experimental feedback loop is so short you can complete method development typically in a few hours. And that means you can experiment more because it is quick and cheap to do so. The speed of SIFT-MS also opens up new possibilities that are closed to gas chromatography.

In comparison, imagine deploying SIFT-MS on the production line so that every product could be tested in real time. The feedback on pass/fail conditions is immediate, and the incremental cost of additional measurements is pretty much zero. The business can deal with problems the moment they arise and can individually certify every product they make.

Moreover, there are many chemical processes where significant changes

occur at a rate that GC cannot track. GC instruments also have sample size limitations; sampling volatile compounds in air at low levels means having to take each air sample through several cycles of adsorption and desorption to reduce the volume of sample to a volume that the GC can accommodate. In contrast, SIFT-MS can sample air directly and offers pptv detection limits for many analytes. Adsorption tubes aren't needed; instead, SIFT-MS gives a real-time continuous measurement of concentrations in air.

The final issue I have with GC is that you must obtain a standard for each compound of interest and you have to run standards to calibrate the instrument. SIFT-MS, on the other hand, measures concentrations from first principles.

In truth, the overlap between old and new technologies is never 100 percent (compact discs never fully replaced vinyl records) and GC will continue to be an important technique for many years to come. However, the rose-tinted glasses through which I used to view GC have gone forever – replaced by spectacles lightly tinged with Norwegian Blue*.

*If you don't get it, Google it!

The Dose Makes the Poison!

Detecting and controlling mutagenic impurities in pharmaceuticals is challenging. What is ICH M7 and what do you need in your analytical toolbox?

By David Elder, David P Elder Consultancy, UK.

Paracelsus highlighted that "the dose makes the poison," which is the fundamental tenant of modern toxicology. That is to say, toxic substances are harmless at very small doses, and conversely a relatively harmless substance can be deadly if over-consumed. Building on this tenet, Fritz Haber stated that concentration and time are important variables in the toxicity of many materials. Thus, the incidence and/or severity of toxicity is dependent on the total exposure, which is to say, exposure concentration (c) × duration time (t) of exposure (c × t).

Haber's Law, with appropriate caveats (1), is often used in defining exposure guidance for toxic substances, but it results in absolute exposure limits (≤ x µg/day), rather than the more familiar relative exposure limits (≤ 0.2 percent). Haber's law is applied in the setting of safe limits for less than lifetime (LTL) exposure of mutagenic impurities (MI) in pharmaceuticals (ICH M7) (2). The LTL limits have a wide range – 120 µg/day (≤30 days), 20 µg/day (≥1-12 months), 10 µg/day (≥1-10 years), 1.5 µg/day (≥10-lifetime) – and are applicable in both clinical development and for marketed products.

From an analytical chemistry perspective, MIs are often challenging to detect and control. Due to their reactive nature, they generally have poor stability in the analytical matrix. Very high sensitivities are typically required (often ppm or even ppt levels). As a class, MIs have very diverse structural features and physicochemical properties, which often preclude a generic analytical strategy. Selectivity is typically challenging as high levels of the active pharmaceutical ingredient (API) will be present, along with the MI(s), which can cause significant matrix interference. The issue is magnified in drug products, where, in addition to the API, there will be excipients that can also potentially interfere. Lastly, there can be interference from the impurities that

"From an analytical chemistry perspective, MIs are often challenging to detect and control."

are present in both API and excipients. Notably, the synthetic route for the API is often evolving and knowledge of likely impurity profiles are limited during early development. The MI methods need to be rapidly developed and aligned with aggressive development timelines (3).

Method selection is often based on the volatility of the MI and demands a reasonably big toolbox. For volatile but thermally stable MIs, gas chromatography (GC) is favored, with direct injection, headspace or derivatization. Detection is typically via mass spectrometry – either using single ion monitoring mode (SIM) or MS/MS selective reaction monitoring mode (SRM). For non-volatile analytes, high performance liquid chromatography (HPLC) is used – once again typically coupled with MS (SIM) or MS/MS (SRM) detection. There is a role for HPLC-UV detection

when MIs are controlled via in-process tests. And in addition to standard reverse phase HPLC, hydrophilic interaction liquid chromatography (HILIC) for polar alkylating agents and ion chromatography for a limited sub-set of MIs (for example, hydrazine) have found favor (3). MI methods, during early development, are typically not fully validated (as per International Council for Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines, ICH Q2(R1)) (4), but are used as limit tests, with validation involving specificity, accuracy/recovery and LOD.

ICH M7 defines four different control strategies that are applicable to MIs that are present in APIs. The first approach is control of the MIs on the API specification (option 1). This test can be included on the specification as a limit test, but is generally not favored by the pharmaceutical industry as it involves transfer of sophisticated, sensitive HPLC-MS or HPLC-MS/MS methods into production. The MI impurity can also be specified as a control test in a registered starting material, reagent, and so on (option 2), and is used for materials that are incorporated near the completion of the synthetic pathway, where purging arguments may not be justifiable. The MI can also be included as an in-process control (option 3). Here, there is knowledge of the downstream purging ability of the synthetic process (often supplemented by spike and purge experiments), that allow upstream controls, often at much higher levels (for example, 0.5 percent).

Finally, theoretical, non-empirical arguments can be made that the MI is so reactive and is introduced sufficiently far enough upstream from the final API that there is no likelihood of it carrying over into the API (option 4). So, in conclusion, the introduction of ICH M7 for the control of MIs, although challenging from an analytical chemistry perspective, has been well received by the pharmaceutical industry and contributes to our overall efforts to ensure patient safety.

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Long Live the Microsample

When it comes to microsampling for quantitative bioanalysis, the devil is certainly in the details. But we should learn from our mistakes – not retreat back into convention.

By Neil Spooner, Spooner Bioanalytical Solutions Ltd and School of Life and Medical Sciences, University of Hertfordshire, Hertfordshire, UK.

There has been a recent growth of interest in approaches for obtaining quantitative measurements of analytes in very small volumes of biological fluids, such as blood and plasma, particularly for drug development. The approach is broadly termed "microsampling". The growth has been fuelled by increasing considerations and requirements around collecting smaller blood volumes for the benefit of animal ethics (the 3Rs – refinement, reduction and replacement), development of medicines for children, desires for simplified approaches to blood sampling (finger/heel prick, compared to venous), drug trials in remote areas (neglected/tropical diseases), therapeutic drug monitoring and the ability to collect samples in a non-centralized location (at pharmacies or in the home). The renewed interest has been facilitated by developments in analytical technology (increased sensitivity from modern LC-MS systems, capillary separations, smaller LC particle sizes), which has enabled the analysis of small samples, whilst still delivering the required quantitative assay sensitivity.

Approaches to microsampling are many and varied, including wet and dried formats of blood, plasma, serum and myriad other more rarely analyzed matrices. Whilst some people may consider that there is nothing novel about these smaller matrix samples, the majority take a more conservative approach, particularly in the highly regulated environment of therapeutic drug development. It is in this environment where the blossoming spring of microsampling was almost

stopped before it had begun.

The issues brought to light around hematocrit and homogeneity associated with dried blood spot (DBS) sampling meant that when a fixed size sub-sample was taken from a non-volumetrically collected sample for quantitative bioanalysis (such as has been traditionally performed for established plasma sampling) it was not necessarily considered reflective of a known volume. Understandably, those responsible for regulating drug development and the registration of new medicines demanded that concordance be shown between conventional wet and DBS samples in patient studies. In turn, pharmaceutical companies backed away from implementing this technology in clinical studies, because of the added complexity and its related increased expense (development and validation of two analytical methods and analysis of two samples), despite the well recognized and significant benefits.

All could have been lost, but for the passion and dedication of a small number of bioanalytical scientists, animal technicians, clinicians, academics and vendors, who were determined to overcome the issues and realize the benefits of microsampling. Many of the significant innovations and increased levels of understanding have come through these scientists (from different backgrounds) coming together in an open, non-competitive environment, learning from each other, and understanding each other's requirements for the technologies.

The renaissance in alternative microsampling approaches has led to a number of commercially available technologies in addition to those just emerging from the drawing board. The innovators – and the early adopters of the new technologies – are well aware of the recent history of conventional DBS microsampling and its potential pitfalls, so new technologies have been developed by understanding the journey of the sample and by considering the impact of sampling on the analyte of interest. After all, good-quality data can only be obtained from a good-quality sample that is reflective of the subject's state at the point of collection.

The important characteristics that have been built into these novel sampling approaches are mainly focused around the ability to simply collect a high-quality sample – the simplified analysis of these samples takes an understandable back seat. It is hoped that users will consider these new technologies carefully and judge them on their merits, and not reject them out of hand due to the recent history of DBS sampling in regulated drug development. I firmly believe that, given time – and the sharing of usability and outcome data – microsampling approaches will become more regularly used in drug development and other applications. In drug development, it is likely that more progress will initially be made in discovery and non-clinical applications (where there is less regulatory oversight). In the clinical arena, priority will be placed on applications where microsampling technology offers measurable benefits over the collection of traditional samples – or where it enables the collection of samples that would not previously have been possible. Areas of focus will include pediatrics, studies in remote areas, therapeutic drug monitoring, home sampling and collection of samples during clinical events.

The development and implementation of microsampling in regulated bioanalysis has been a tricky road – and there have been a few mistakes along the way. But isn't that how we learn? As we say in the UK: let's not throw the baby out with the bathwater! Microsampling is clearly an important part of the future of bioanalysis.

Neglected Micro LC

Is now the right time for HPLC's diminutive cousin to make a comeback in routine analysis?

By Thorsten Teutenberg, Head of Research Analysis, Institute of Energy and Environmental Technology (IUTA), Duisburg, Germany.

It's clear that good ideas do not always end up in the form of real applications – indeed, this is often true whenever a 'new' technology emerges. One good example (or bad example, depending on your perspective) is supercritical fluid chromatography (SFC), which – after its glorious future had been predicted – fell into oblivion for quite some time. The same demise was suffered by micro liquid chromatography, which was identified early on as a key technology to reduce toxic organic solvents while speeding up the separation process.

It's reasonable to question why this happened. But the answer, when you think about it, is reasonably simple: the technological aspects of micro-LC were poorly developed back then, and high-efficiency separation media were not commercially available. With the introduction of modern UHPLC instruments in around 2004, column technology also took a giant step forward; after all, sub-2-micron particles are key to improving the efficiency of small i.d. columns. Nevertheless, there are still some strong prejudices against micro-LC that center around the robustness of the technology, which is claimed to be very poor. Moreover, the common opinion remains that micro-LC columns cannot be packed very efficiently. And yet, if we look to the scientific literature, the available data is not able to fully support the thesis or antithesis of this statement. To that end, a much wider base of research is needed to prove (or disprove) the hypotheses. Either way, column manufacturers will need to focus on developing more reliable packing protocols for small-scale columns. Unfortunately, if the demand is low, then the incentive to do so is also low.

Another micro-LC myth is that dedicated instrumentation is not commercially available. Clearly, that is not true. I can only assume that several small- and medium-sized companies with excellent products in their portfolio have been overlooked. In fact, microfluidic flow control has been commercially available for at least ten years, and such platforms have extremely low system volumes.

A further advantage of micro-LC is that hyphenation with mass spectrometry can be very easy. Some manufacturers offer the possibility to simply replace the emitter tip within the ion source, meaning that no further adjustments on the hardware are necessary if you wish to access micro-LC. And if MS detection is unnecessary or unwanted, UV- and fluorescence type detectors with extremely low cell volumes (just a few nanoliters) are also commercially available.

So in fact, it seems that the technology is in place, which means that the biggest hurdle to overcome is an area that we can address immediately: education. Here, academic institutions and universities should grasp hold of their responsibility by using state-of-the art technology rather than old-fashioned equipment.

"So in fact, it seems that the technology is in place, which means that the biggest hurdle to overcome is an area that we can address immediately: education."

I'm really flabbergasted when I think about the technological advances in telecommunications over the last ten years. Today, we all manage our lives with smart mobile phones – all despite tremendous security issues that still have to be resolved. In stark contrast, the 'modern' laboratory sometimes reminds me of some relic from a bygone era – where the introduction of modern technology is still hampered by the opinion that the re-validation of methods renders the transfer over to improved techniques or systems impossible. If this is the common opinion, then why should there be any need to invest in modern technology at all?

High Hopes for High Resolution

Then & Now, with Amadeo Fernández-Alba, Professor at the University of Almeria, Spain.

Then: one sunny day in 2006...

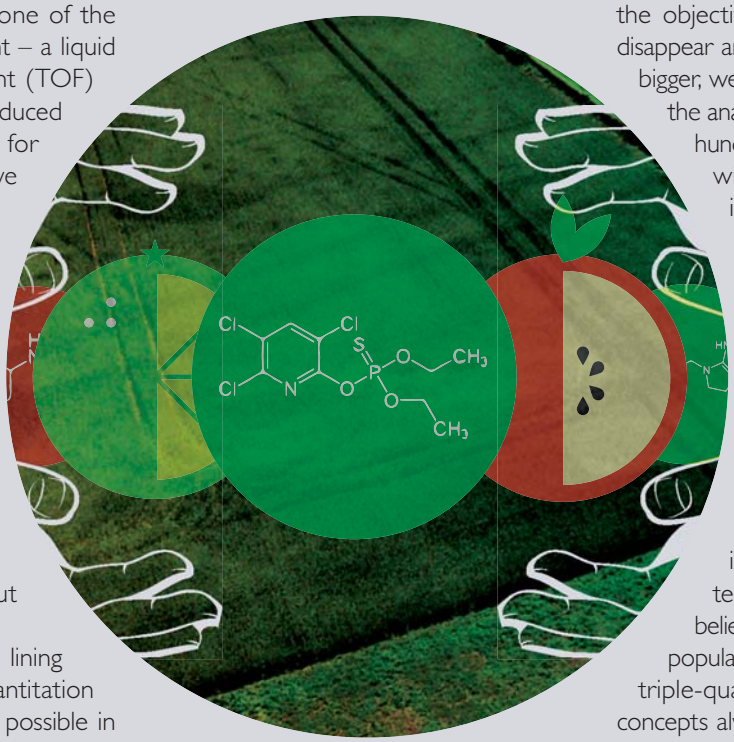
Ten years ago, we started working with accurate mass, high-resolution mass spectrometry (MS). It was one of the first times such an instrument – a liquid chromatography-time-of-flight (TOF) MS system – had been introduced into a routine laboratory for pesticide residue analysis. I have to say, it was really exciting to see how we could detect and identify a compound simply by inputting its molecular weight or identify new compounds by comparing molecular weights with a database – a great prospect for food safety, as we could detect banned pesticides for which there were no analytical standards. I was truly enthusiastic about the new capabilities.

Unfortunately, every silver lining has a cloud... Credible quantitation on the new system was not possible in many cases – and in pesticide residue analysis, reliable quantification is essential. As a consequence, our conclusion on that time was that accurate-mass, high-resolution MS could only really become a complementary technique (to triple-quadrupole instruments) in food safety analysis; for example, when we had only one transition on the triple-quads for specific compounds or if there was a very strong co-elution of matrix with an isobaric transition.

We had a new tool – but it wasn't quite the revolution I was hoping for. There was a dark side!

Now: June 2, 2016

Over the last ten years, the situation has changed and technology has improved tremendously – and improvements to system software have also been pivotal. Today, good sensitivity, good linearity and good reproducibility – coupled to incredible resolution and excellent mass accuracy (the Q Exactive™ Focus Hybrid Quadrupole-Orbitrap™ Mass Spectrometer provides a resolving



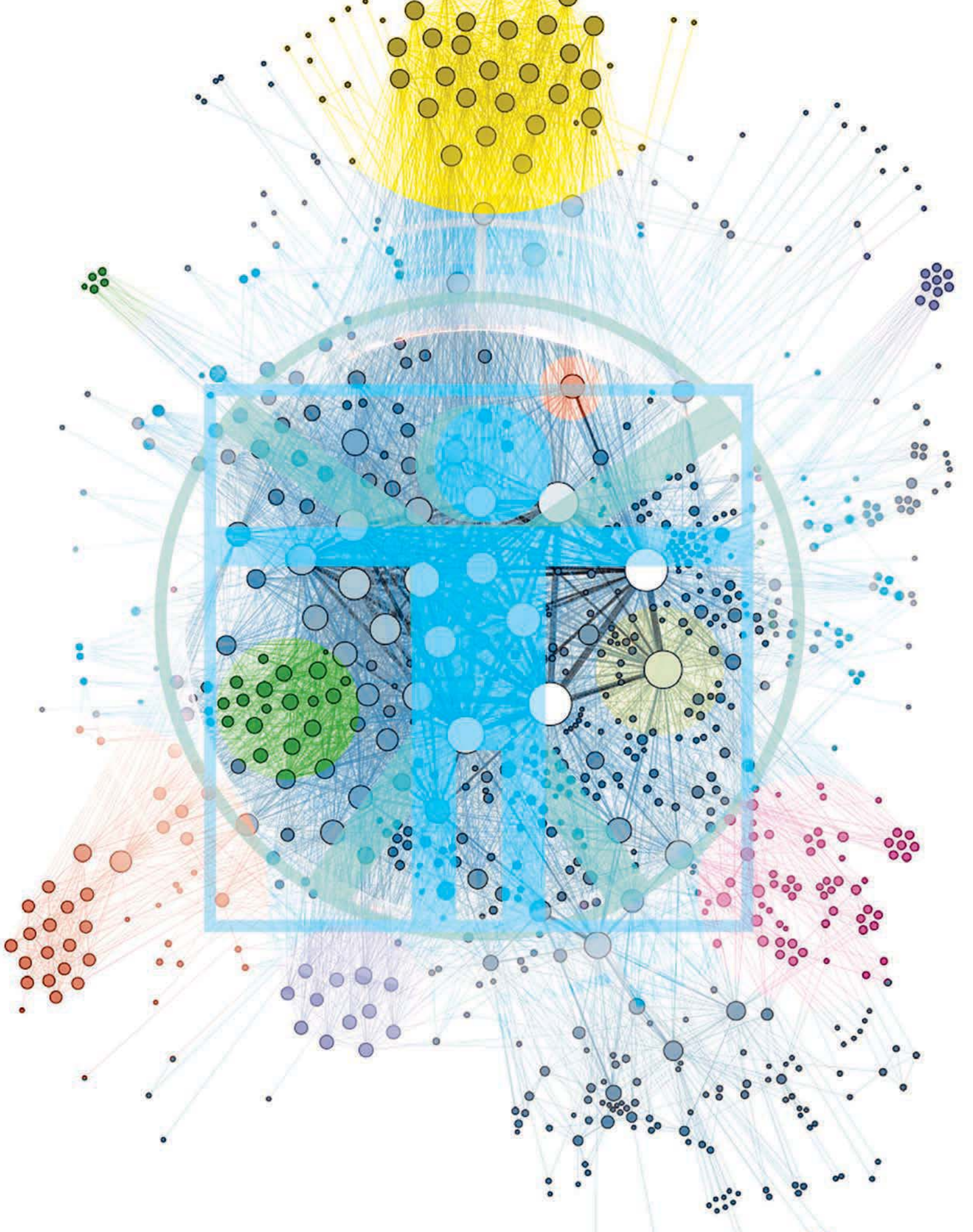
power of 70,000 at m/z 200 in full-scan mode and 1 ppm mass accuracy) mean that HRAM platforms have developed from a complementary technique to the technique of choice. And that's not a statement I make lightly. I am sure we will see more incremental improvements in the future, but we've already reached the point where identification capabilities are higher in HRAM instruments, and where quantitation is comparable for pesticide residues in food. It's true that the sensitivity can be a little lower than the newest triple quadrupole systems – but it is high enough. And after thousands of samples, I can state that the robustness is excellent.

In addition, new identification options are open to laboratories: we can now analyze samples in a retrospective way to detect, identify and quantify new unexpected compounds – even without analytical standards.

In reality, the requirement for HRAM MS systems (LC or GC) will depend on the objectives of each lab. But as labs disappear and those that remain become bigger, we can expect that the scope of the analytical challenge (which covers hundreds of different commodities) will only grow. Moreover, an increasing number of target compounds (and an awareness of untargeted contaminants) in increasingly complex matrices is a clear trend; being able to efficiently cope in this new world will become a real differentiator for routine labs.

Right now, I would guess that around 10 percent of labs in my field have adopted HRAM technology. But in 5–10 years, I believe that HRAM-MS will be highly popular, perhaps even outnumbering triple-quadrupole instruments. New concepts always take time to catch on – and for Orbitrap technology, GC was the missing link; laboratories were perhaps wary of switching over to a new concept of analysis for LC but not for GC. With the introduction of the Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS system, the situation has changed.

Our primary driver is to protect consumers, so we must always strive to achieve the best possible pesticide residue control in food. The advanced capability of HRAM-MS systems, such as those based on Orbitrap technology, represent a very important step in that direction.



Toward Integrative Omics

Cancer is incredibly complex, posing enormous challenges beyond the biological field. Taking a multi-omic approach can help us make sense of this diverse set of diseases – and, ultimately, allow us to better understand ourselves as human beings.

By Amanda Hummon

I started researching colorectal cancer for multiple reasons, but a significant part of my interest was triggered by grief; a member of my immediate family died as a result of metastatic colorectal cancer, despite having the access to the best medical care. I wanted to understand more of what had happened and why.

Reading about colorectal cancer, it was apparent that while the genomics and transcriptomics of the disease had been well studied, the proteomic changes that accompany the disease were not as well understood. I believe the prejudice is related to the tools that were/are available to tackle the problem. After realizing how much remained to be done in the field of cancer proteomics, I decided to devote my career to studying the molecular changes that underwrite colorectal cancer. The more I work in this field, the more I recognize how truly deep understanding – from genotype to phenotype – is the only way we can tackle cancer.

THE CATERPILLAR AND THE BUTTERFLY

Multi-omics approaches attempt to make sense of the genome, the transcriptome, the proteome, and the metabolome all together. If you look back to the articles that were written around 2000 (and the publication of the human genome) you will find a glimpse of what we could achieve with this information. With a greater understanding of our genes, transcripts, proteins, and metabolites, we can better understand how the 'blueprint' corresponds to reality.

The classic example that I give to my students is the caterpillar and the butterfly. Both have the same genome, but the phenotype of the two animals is shockingly different. That striking physical difference is the result of the transcriptome, the proteome and the metabolome at work.

Multi-omics has of course been gaining traction for the last decade. The major developments that have brought it to the forefront are: i) the completion of the Human Genome Project and ii) the development of high-throughput methods to analyze the transcriptome (first microarrays, later next gen sequencing) and the proteome (mass spectrometry). Multi-omics studies are now everywhere. I would bet that for any major disease there are several manuscripts characterizing the genome, transcriptome, proteome, and/or metabolome of healthy versus diseased tissues. Similarly, it is now routine for the chemical characterization of any organism to start with the sequencing of the genome. When I last checked (April, 2016), the NCBI genome archives held over 75,000 genome sequences, and many of those species will have also been analyzed for transcriptomic and proteomic contents.

In cancer, chemical analysis is highly complex because you are dealing with very different types of molecules that appear at different points in time and in space. For example, a specific transcript or protein may only be needed at certain points in the lifecycle of the organism. If it is only synthesized in a few copies for a short window of time, it can be extremely difficult to measure. Again, I refer to the example of the caterpillar and the butterfly.

Another enormous challenge is the incredible dynamic range of the molecules. Some molecules are produced abundantly at all times, making it hard to see around them. Albumin is the classic example; it makes up more that half the protein content of human blood. What that effectively means is that researchers

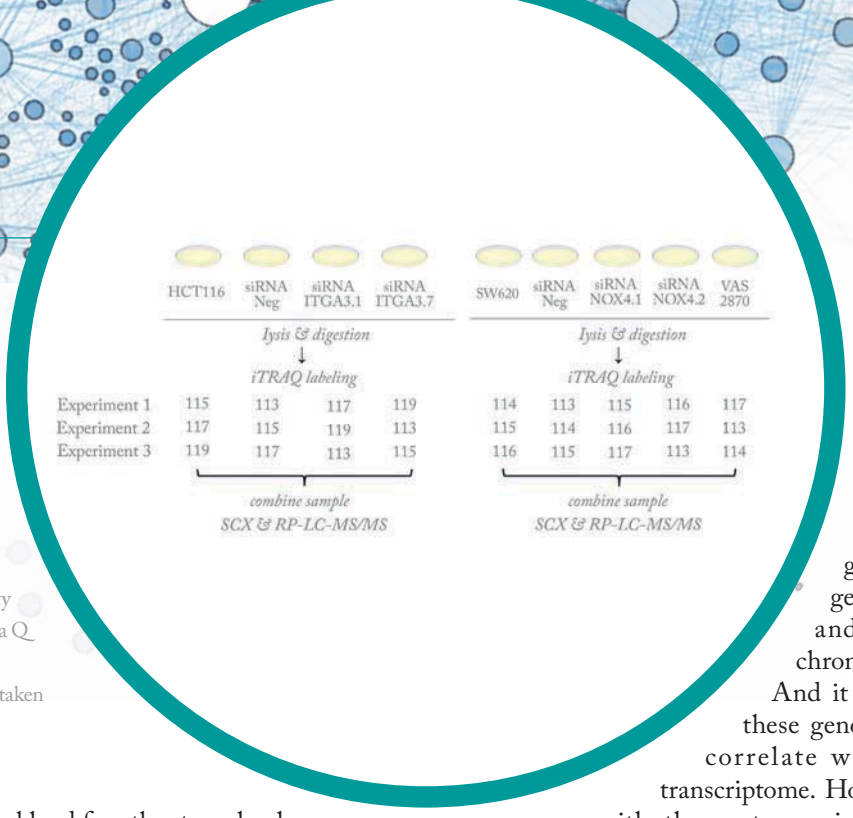


Figure 1. Workflow of experimental design. In brief i) HCT116 cells were silenced with siITGA3 siRNAs ii) NOX4 was inhibited in SW620 cells with siRNAs or VAS2870. Samples were analyzed with a nanoAcquity UPLC (Waters) coupled to a Q Exactive mass spectrometer (Thermo Scientific). Figure taken from reference 1.

trying to analyze human blood for other trace level proteins must first deplete albumin before they can conduct any other analyses to see the lower abundant "more interesting" stuff. Separation is the key.

UNDERSTANDING COLORECTAL CANCER

When I was a postdoctoral researcher at the National Cancer Institute, almost every member of my lab had lost a family member to cancer. Most of the students who walk into my office tell me they are there because they want to contribute to cancer research. It is a complex problem that affects so many people. From a molecular perspective, it is both fascinating and incredibly motivating. I am hopeful that with greater understanding, we can do a much better job of treating colorectal and other cancers.

Colorectal cancer is a good research target for several reasons. First, it follows a sequential path of genomic instability – more so than other soft epithelial cancers. In colorectal cancer, there is a common pattern of mutations and genomic instability that is observed in approximately two thirds of all colorectal cancer patients. We and others have hypothesized that this similar pattern of genomic instability would result in conserved patterns of proteomic changes – and we are still investigating this phenomenon. Second, though it is one of the most common types of cancer, colorectal cancer is not as well studied as some other cancers. I wonder if the functions of the organs involved result in people being less interested in this disease – unless they have a personal connection. Finally, like many other cancers, colorectal cancer is linked to obesity, meaning that it has the potential to be an increasing health burden in the future.

DRIVEN BY MUTATIONS

Like many soft epithelial cancers, colorectal cancer starts with a few driver mutations – that is to say, a few mutations that push the cancer along. In fact, there are five critical genes – Pi3K, APC, p53, TGFB, KRAS – that are part of several pathways and have been causally linked to changes in the genome.

Colorectal cancer cells frequently show gross changes in the genome – amplifications and deletions of entire chromosomes are common. And it has been shown that these genomic changes directly correlate with changes to the transcriptome. However, the correlation with the proteome is much less clear. In some of our recent work, we have demonstrated that the amplifications in the genome, while resulting in upregulation of transcripts, do not necessarily result in higher corresponding protein abundance.

Our current understanding of the disease reflects the tools that we employ to detect cancer chemically. For example, to examine changes in the genome, either spectral karyotyping or comparative genomic hybridization are effective analysis strategies. Changes to the transcripts can be assessed by many different high throughput strategies. Microarray analysis is commonly used to survey the expression levels of thousands of transcripts, but are increasingly being replaced by more global next-generation sequencing methods, such as exome and RNA sequencing.

Fundamentally, cancer is a single term used to describe a huge range of diseases. And though the chemical component is very important and dictates the behavior, it's the phenotype we care the most about at the end of the day. The final definition of whether something is cancer or not is defined by how the cell behaves. Does it grow, proliferate, spread, and metastasize? Although chemicals enable these processes, it is the processes themselves that define the cancer.

PUSHING PROTEOMIC KNOWLEDGE

We are examining how protein expression in colorectal cancer differs from normal colon tissue from many different angles. And we are also considering how these expression patterns change over time – as the disease progresses. We use 3D cell cultures to examine spatial differences in expression patterns in tumor mimics.

Our hypothesis is that the genomic changes that are so evident and pervasive in the colorectal cancer genome also play out in the colorectal cancer proteome. As proteins are the action molecules

in the cell, they are the best chance we have to develop rational strategies to turn off a cancer-associated signaling pathway.

As we are looking at protein expression, we primarily use mass spectrometry – applying different platforms based on the specific question we are asking. For example, when we are examining the global differences in protein expression level between a normal and a cancer sample, we use quantitative labels and nLC-MS/MS to perform a quantitative comparison (1) – see Figures 1 and 2. When we are examining the differences in spatial distribution in a 3D sample, we employ either imaging mass spectrometry or serial trypsinization to harvest sequential concentric rings of cells for nLC-MS/MS.

We are also interested in gaining a better understanding of how to make treatments more effective. To that end, we've developed a powerful imaging approach to visualize drug penetration in tumor mimics, which allows us to see how and where a drug is metabolized. Our approach has been adopted by a couple of European pharmaceutical companies and I hope it will also be implemented by US companies. I believe it could help get more therapies on the market quicker.

We have another project in the lab where we are examining the molecular changes that occur with fasting, also known as caloric restriction. That work has led to some tantalizing evidence that fasting can improve the efficacy of chemotherapies. Now, we are trying to figure out why that is and how it could be implemented clinically.

We've also had some extremely rewarding results in the transcriptomics space (see sidebar: The power of transcriptomics and collaboration).

Finally, we are striving to gain a better understanding of why metastasis occurs. The vast majority of cancer deaths result from cells spreading throughout the body. The critical step in the process is the ability of the cells to insert themselves into the secondary location. We are working with Pinar Zorlutuna, a bioengineer, to model a tumor in proximity to a potential secondary site. We have designed the system so that we can manipulate both the chemical and physical stresses. We then evaluate whether the cell succeeds in metastasizing and also evaluate the chemical environment that facilitates or hinders metastasis. I would be delighted if we can decipher a combination of physical and chemical properties that promote – or better yet reject – a metastatic cell. Such information would be incredibly valuable. Thus, five years from now, I hope that we will be applying this knowledge to make potential secondary sites less hospitable for a metastasis.

Cancer is an incredibly complex disease. You can't effectively treat a disease if you don't understand it. Our current methods to treat it – radiation, chemotherapy and surgery – are blunt measures. Those in the field share the same hope that with better understanding of the pathways, we can improve diagnosis and therapy.

THE POWER OF TRANSCRIPTOMICS AND COLLABORATION

One of our most striking results comes from a transcriptomics study. We have been working with Steven Buechler, a statistician here at Notre Dame. Steve performs bioinformatics analyses and, a few years ago, he noticed a striking trend in some of the published colorectal cancer microarray studies. In his analyses, he showed that the gene expression patterns were extremely distinct on the right versus the left side of the colon. The colon is a large organ and initially develops in different parts of the embryo, resulting in differential gene expression patterns. The right side of the colon includes the ascending and transverse segments, while the left colon includes the descending colon to the rectum. The two sides of the colon are very distinct; polyp formation differs significantly between the right and the left.

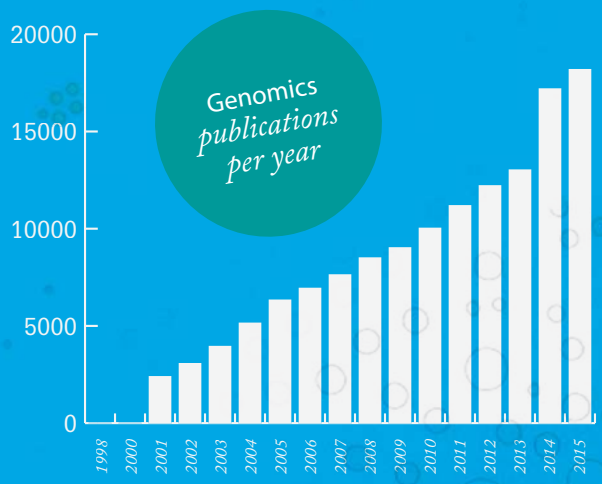
Though it was known that the gene expression patterns between the right and left sides of colon cancer were distinct, the result had never been used clinically. Steve's lab – in collaboration with my lab – discovered that the gene expressions on the two sides were also prognostic of relapse. We identified a panel of five genes on each side of the colon that can be used to predict whether a patient will have a relapse in the next five years. We've been working together over the last few years to validate the expression of these genes in numerous samples (both cell lines and primary tissues) and we hope to translate this information into a clinically actionable test. We are in the process of publishing these results and patenting the tests, so I can't say more at this moment, but I am extremely excited about this work. It's a project that could make a real difference to people's lives.

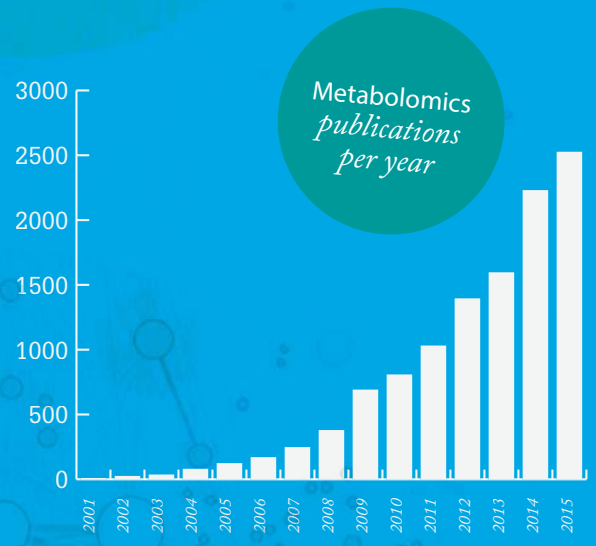
We couldn't do the project without Steve Buechler; he brings the statistical expertise and we have the bench-top know-how. The project only works when we both work together. In fact, many of the current projects in the labs are only made possible through collaboration with other research groups.

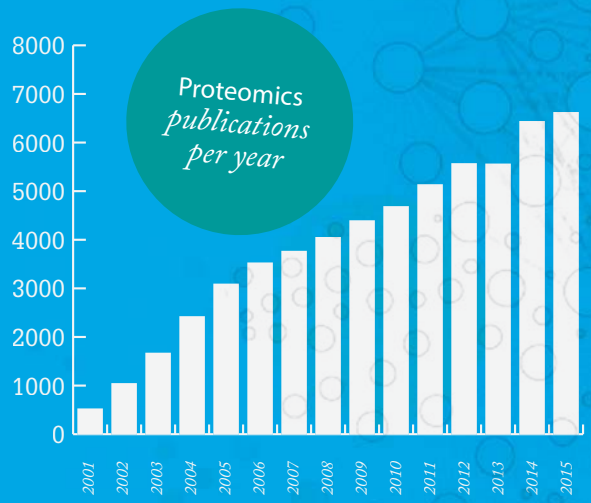
OMICS IN THE LITERATURE

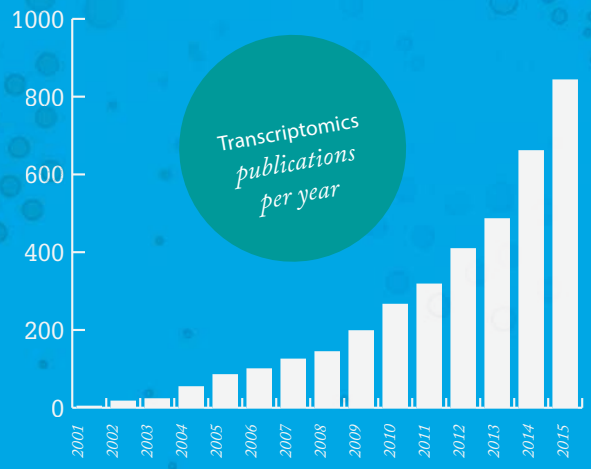
What does analysis of the last 15 years of literature on the different omics tell us about the growing importance of the field, and the move towards a more integrated approach?

PubMed was searched for "genomics", "metabolomics", "proteomics", "lipidomics" and "integrated omics" with a date filter of 2001 to 2015. The data were then analyzed in Microsoft Excel 2013.









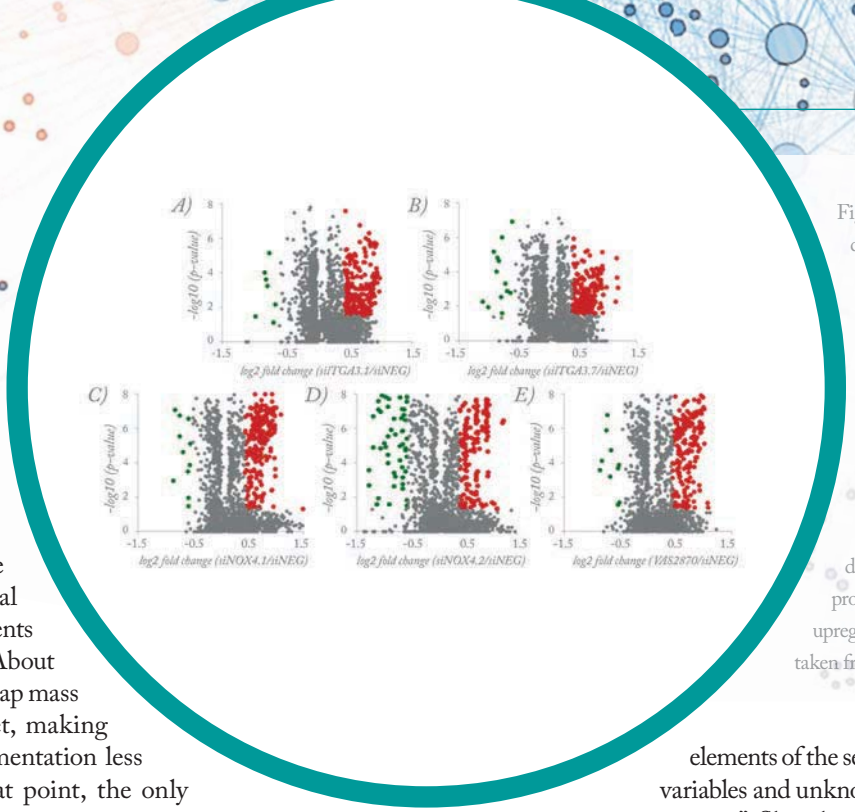


Figure 2. Volcano plots displaying protein expression changes that are statistically significant. (A, B) Proteins changed in expression with ITGA3 silencing. (C–E) Protein altered in expression with NOX4 gene silencing or chemical inhibition. Green data points = downregulated proteins; red data points = upregulated proteins. Figure taken from Reference 1.

ENABLED BY TECHNOLOGY

It's clear that advances in next-gen sequencing are driving genomics. But for those of use placing an emphasis on proteomics, the most important technical advances are improvements in mass spectrometers. About ten years ago, the Orbitrap mass analyzer hit the market, making high-resolution instrumentation less expensive. Prior to that point, the only available high-resolution instruments were ion cyclotron resonance MS systems, which were prohibitively expensive for most labs. And though Orbitrap technology is expensive, it is relatively more affordable and has enabled global proteomic analyses in a way that wasn't possible a few years ago.

To truly enable advances in the field, mass spectrometry must be paired with really smart data analysis. So I'd like to acknowledge the incredible importance of database searching algorithms, such as MASCOT and SEQUEST. Using such search tools, we can rapidly identify thousands of peptides, and thus proteins, from a complex mixture.

The development of these tools was really seminal for the field of proteomics – a fact that becomes more evident when you consider the current state of metabolomics. In metabolomics research, the separations and mass spectrometric analyses are similar to proteomics research. However, the databases and the search algorithms are not yet mature. The current standard practice to confirm identification is to test your compound of interest against a known standard, which is expensive, labor intensive and low throughput. As a result, while the field is growing, there isn't a widespread consensus on how to identify features. I anticipate that within the next few years, someone will develop an approach that enables rapid confirmation of mass spectrometric metabolite datasets, which would be transformative for the field, gain great traction and have a huge scientific impact.

OUR OMICS-DRIVEN FUTURE

Going back to multi-omics, there is an excellent article from Shelia Jasanoff, in which she compares the human genome to the US Constitution: "Like the Constitution of the United States, the human genome turned out to be a sparse document, containing fewer genes than expected. This means that, as with the Constitution, the genome's meanings will evolve over time, as scientists, lawmakers, and [the public] make sense of the fixed elements of the sequence in relation to the variables and unknowns in the surrounding environment." She also addresses some of the criticism that has been leveled at the Human Genome Project and the fact that it has not resulted in fast medical breakthroughs: "A decade is not nearly enough time to measure the impact of a scientific revolution [...] It is too soon to tell whether cures for genetic disease were oversold [...] What matters is that we found a powerful new way to represent human identity, and the moral implications of that re-representation are just beginning to unfold."

I like these analogies. I think the problems we are trying to answer are incredibly complex and it is unrealistic to think that huge sweeping medical changes will result immediately. That being said, there are already some medical changes occurring. Just in the last couple years, it has become possible for pregnant women to learn about the genomic status of their fetus through circulating fetal (cf) DNA sampled from a blood draw. That is an enormous advance and I anticipate that within a few years a range of tests will be available on cfDNA and other valuable samples. Similarly, another area of research that I think is on the cusp on making a breakthrough scientifically is the analysis of circulating tumor cells.

Tumors shed cells into the bloodstream and researchers are making great strides in their ability to enrich for these cells and perform omics analyses on them. Success in this arena would have a huge impact on cancer diagnoses in the next few years. Both of these developments fall under the umbrella of personalized diagnostics. And I anticipate that we will see many more of these important developments in the near future.

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The 'One Pot' Approach

Tom Metz, Integrative Omics Scientist and Metabolomics Technical Lead at Pacific Northwest National Laboratory (Washington State, USA), selects six papers that exemplify the power of multi-omics.

When people select one technology over another it's usually because they "grew up with it", which is either dependent on where they did their PhD work, or which kind of -omics they happened to apply first to their area of biology. So many molecules are detected with -omics technologies that the false positive rate is likely higher than we expect given today's tools and metrics. When basing subsequent hypotheses and publishing results on single-omic studies, there is bound to be misinformation put forth. Being able to perform additional -omics experiments will help constrain that to some extent. For example, if someone performs a transcriptomic study and has complementary proteomic data (or other -omics data), they will be able to check if what they thought might be going on at the transcript level had propagated through to the protein or the metabolite level.

PIECES OF THE PUZZLE

For one thing, transcriptomics doesn't give you any information at all about post-translational modification of proteins, such as phosphorylation and signaling; the only way to capture such information is to do the appropriate proteomics analyses. If resources are not a limitation, then I would suggest that a multi-omics approach should be taken (when reasonable). Clearly, it depends on what questions are being asked, but if the questions are open-ended, then the more data you have, the better.

Here and there, multi-omics is taking the place of a single -omics. You can tell how seriously we take it at PNNL – we have an integrative -omics group! The Department of Energy Office of Biological and Environmental Research has funded many large programs to study both isolated microorganisms and microbial communities. The goal of these programs is to achieve a systems-level understanding of these organisms and communities such that they could be engineered or otherwise manipulated for the benefit of society, such as for improved biofuels production or carbon sequestration. And the National Institute of Allergy and Infectious Diseases (NIAID) has sponsored the Systems Biology for Infectious Diseases Research Program since 2008. We have been part of that program since its inception and (like others who were funded) have been using transcriptomics, proteomics, metabolomics, and lipidomics to study pathogenic bacteria and viruses. I would say there have been enough papers using multi-omics approaches for me to describe it as in vogue...

OMIC EFFICIENCY

In essence, with a "one-pot" sample extraction, you're going to save time and effort – one of the big drivers for me and my collaborators in the NIAID systems biology project. It's also likely that it reduces overall experimental variability, because you're no longer trying to integrate data that came from separate cells – you've now got protein, metabolite and lipid data from the same cells. If you were to include a step to extract the genetic material, as others have done, then you could also combine the DNA/RNA data sets.

With microbial communities, for example those bacteria that reside in soils and particularly in association with plant root nodules, the scientific community in general is realizing that we need to get beyond 16S sequencing to discover which organisms are there, and instead focus on what those organisms are doing

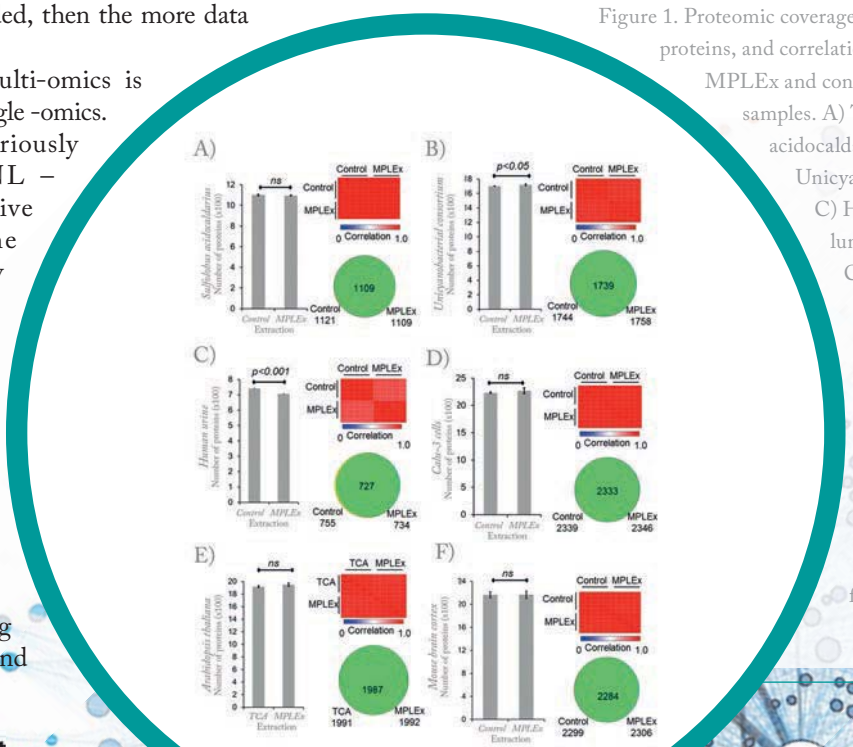

Figure 1. Proteomic coverage, number of identified proteins, and correlation over five replicates for MPLEx and control for different samples. A) The archaeon S. acidocaldarius. B) Unicyanobacterial consortium. C) Human urine. D) Human lung epithelial cell line Calu-3. E) A. thaliana plant leaves. F) Mouse brain cortex. Abbreviations: MPLEx: metabolite, protein, and lipid extraction; Control: no-extraction control; TCA: trichloroacetic acid extraction. Taken from paper 1.

metabolically. That's not only what metabolites they might be producing and releasing into their respective environments, but also what proteins they're producing and how the microbiota are interacting with each other and their environments, including any hosts. This is also the case for the gut microbiome. Could metabolites and proteins that might be released in the lumen of the gut act as signaling or hormone molecules and affect the health of the host? There have been many very cool studies showing that certain populations of microbiota are associated with certain diseases. Now, we need to mechanistically understand why particular phenotypes are associated with those populations of microbiota – and that means looking at the genes that are being expressed, and the proteins and metabolites that are being produced. It's a very complex problem – but multi-omics is best suited to unraveling all of these questions.

PAPERS PUSHING MULTI-OMICS

1. ES Nakayasu et al., "MPLEx: a robust and universal protocol for single-sample integrative proteomic, metabolomic, and lipidomic analyses", mSystems, 1, 1-14 (2016).
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5. J Tisoncik-Go et al., "Integrated omics analysis of pathogenic host responses during pandemic h1n1 influenza virus infection: the crucial role of lipid metabolism", Cell Host & Microbe, 19, 254-266 (2016).
6. L Valledor et al., "A universal protocol for the combined isolation of metabolites, DNA, long RNAs, small RNAs, and proteins from plants and microorganisms", The Plant Journal, 79, 173-180 (2014).
7. W Weckworth et al., "Process for the integrated extraction, identification and quantification of metabolites, proteins and RNA to reveal their co-regulation in biochemical networks", Proteomics, 4, 78-83 (2004).

Being an experimentalist at heart, I appreciate those multi-omics papers that focus on methods for "one-pot" preparations of samples to enable extraction of all the molecules necessary for multi-omics analyses. Because my primary area of research is metabolomics and lipidomics, I've chosen the papers that go beyond genes and proteins to cover metabolomics, lipidomics and other small molecule data. Transcriptomics and proteomics are more mature, whereas metabolomics and lipidomics are still developing and evolving to become more robust. I like it when investigators try to bring in additional data on other small molecules. Why? Even though it's riskier (because the platforms are less mature), I don't think it is appreciated just how valuable such information can be.

WASTE NOT, WANT NOT

For a long time, we've used a protocol to prepare metabolites and lipids from samples by using a mixture of organic solvents. A liquid-liquid bilayer of polar metabolites and non-polar metabolites (which are the lipids) forms, and then precipitated proteins are located in the middle after centrifugation. In metabolomics studies, we would discard the protein because we weren't necessarily doing proteomics. I figured that was wasteful and wondered if we could use the precipitated protein for proteomic analyses. I wanted to make sure that we could get just as good data with the precipitated protein as we would with a traditional proteomics preparation. And so we went through a pretty extensive literature search to see who had done it before, in what context, and with what biological samples. We found that there had been initial demonstrations of using precipitated proteins, but no one had really assessed the reproducibility or quality of the proteomics data. It had also never been applied very broadly – so we thought we'd give it a shot. Of course, we could have been even broader in the sample types and conditions we investigated, but again we're always limited by perhaps the most important -omics of all: economics! The funding only goes so far... In any case, the result of our efforts was MPLEx (metabolite, protein, and lipid extraction, see Figure 1).

We found that precipitated proteins, for the most part, give proteomic data that is comparable with the data obtained from working up proteins using traditional methods. And it turns out that the coefficients of variation (CV), which is a measure of reproducibility, are just as good as the standard approach – sometimes better. We found statistical differences in certain cases, but that was almost assuredly due to the nature of the proteins in those particular sample types. We're certainly very comfortable using the approach to gather multi-omics data. Certainly, our MPLEx method is not the only way to gather all the molecular types needed for multi-omics – and I welcome other investigators to rise to the challenge by adopting or adapting other protocols.

The next step is to also add the genomics data. The authors in the ISME paper – H Roume et al. – included a pre-step where they could isolate genetic material (DNA and RNA). And the paper by W Weckworth does the same for one of the plant samples. I see no reason why one cannot get all the molecular types necessary for a complete multi-omics study from DNA all the way down to metabolites. I believe that the main limitations are the resources and technical skills that the investigator has at their disposal.

LESSONS I'VE LEARNED

with Jenny Van Eyk

From hardcore peptide biochemist to translational scientist, it's been an unconventional journey for Jennifer Van Eyk, Director of the Advanced Clinical Biosystems Research Institute at Cedars-Sinai Medical Center, Los Angeles. Here, Jenny tells us what she's learnt along the way.

There is much we don't know

I was born in Northern Ontario, Canada and went to a small high school. There were just three students in my advanced calculus class and hardly anyone went to university, but I got a place studying chemistry at the University of Waterloo. For a long time, I thought that you went to university simply to study the traditional subjects – I didn't even know that you could become a researcher or engineer. In my first year, I was taking chemistry courses and, as I'd expected, the things we were learning had been known for hundreds of years. Then one day I took a biology course, during which the professor told us about the newly published fluid mosaic model for lipid membranes. He said "we think this is right, but we don't know for sure." I was amazed – it hadn't occurred to me that there were things even professors didn't know, and that I might be able to add to our collective knowledge. I switched my course to biochemistry and fell in love with discovery.

The power of peptides

I took some persuading to embark on my PhD. I was happy working as a technician in a great lab, publishing papers, and I didn't want anything to get in the way of my science. But my PhD in peptide chemistry (with Bob Hodges at University of Alberta) allowed me to publish my first landmark paper; I created a synthetic peptide made up of just 12 amino acids that could replace cardiac troponin I (cTnI) in the muscle contractile apparatus.

After my PhD, I spent four months in Heidelberg, Germany, studying the physiology of muscle fibers. The Heidelberg group used skinned muscle fiber bundles – a perfect model for muscle contraction, created by peeling away the membrane of muscle fibers to expose the contractile proteins, and adding calcium to make them contract and relax. It was a short placement, but the timing was fantastic – I arrived just as the group discovered how to remove just cTnI from the muscle fibers. We were able to use the 12 amino acid peptide I developed during my PhD to replace cTnI and were delighted to find that the fibers would contract and relax as normal. It showed me how powerful amino acid sequences are. If you hit the right sequence of just a few amino acids, you can replace the function of whole proteins.

The work was a big departure from the basic science I had been doing at Alberta. I wanted to connect the pieces of the puzzle and relate the biochemistry of muscle contraction to real physiological mechanisms. Such multidisciplinary research was very rare at the time – most scientists stayed

within their own narrow field. Luckily for me, cTnI became a major biomarker in cardiology, and I was mentored by some great clinical chemists and cardiologists working with emerging medicines.

Science changes lives

Another short post doc early in my career had a profound effect on me. I was back at Alberta, looking at cTnI as a biomarker in heart transplantation. Part of my job was going to the hospital to collect blood samples and on one of my visits I met a transplant patient who really stuck with me. He was a young man, but when I went to collect a blood sample the day before his surgery he was lying in bed, unable to move. His damaged heart simply couldn't pump enough blood to his muscles to allow them to move. When I returned, just a couple of days after surgery, he was up and walking around the room. I was blown away by the change – he'd gone from being close to death to looking forward to an almost normal life. We all say we are in translational science to help people, but that was the moment I saw what that really means.

A leader brings out the best in others

My next post doc was another physiology lab, this time in Chicago, with John Solaro. I went there not just to learn science but to learn how to lead. John is a fantastic leader and my time in his lab really reinforced my views on what was important. He showed me that wherever you are, you have to be there completely. When you are home with your family, be 100 percent focused on them, but once the kids are in bed and you sit at your computer to work, totally focus on your work. You have to learn to segregate your brain – and to live in the moment.

The most important thing he and Bob taught me was that being a leader is all about the people that you surround yourself with. You need to create an environment that lets them be at their best – an environment where they are supported and where knowledge is freely shared – not one where you are thinking of your own success. Those lessons stood me in good stead once I became the head of a proteomics center at Johns Hopkins.

Bigger data need better tools

Leaving Canada to take up a position at Johns Hopkins as Director of the Proteomics Innovation Center in Heart Failure allowed me to continue to expand the scope of my research. I wanted to be able to work directly with physicians and physician–scientists so that I could better understand clinical processes and where we could best focus our efforts. I didn't just want to look at a single diagnostic, I wanted to map out a whole clinical domain and look at how we can make the entire continuum better.

It quickly became apparent that while data is getting bigger, it won't help you unless it is a) really quantitative and b) you have enough samples to attempt to look at whole populations.

I decided that I wanted to start a research institute to focus on how we train and equip the next generation of scientists. We need to go beyond case–control animal studies, where (n) might be as low as six. Instead, we need tools that let us vary animal systems in many different ways over time. That requires workflows for sample prep, mass spectrometry and computation. We had been working on those tools for years, but we really needed a dedicated center (and collaborative industry partners) to look at how we can take biomarkers right through the pipeline and into a commercial product.

I moved to Cedars-Sinai in 2014 to achieve that ambition, as Director of the Advanced Clinical Biosystems Research Institute. Research at Cedars-Sinai is firmly embedded into the hospital, so it's an ideal place for a cross-discipline approach. My current role is allowing me to build a research institute from the ground up – and that means hiring in people with diverse skills, but who are all focused on the end goal of clinical translation. We want to do great basic research, but we must always ask "Why?" And if the answer isn't to improve patients' lives, we need to go back to the drawing board.

Collaboration is crucial

Translation is all about bringing people together. A research or industry scientist may have spent 10 years learning how to do mass spectrometry analyses, while a physician spent the same 10 years learning how to treat patients. You can't expect them to then spend another 10 years learning each other's trades as well, so we need to form partnerships early on. But we do need each partner to gain just enough understanding of other fields to work effectively together, otherwise projects are likely to be derailed by false assumptions. It's not just scientists versus clinicians: even within science we all speak different languages – the language of GWAS is very different to that of clinical chemistry or computational science. I want to create an environment that breaks down those barriers for next-generation scientists, because that is the only way we are going to get new products onto the market quickly. At the Institute we share pipelines and technology, and bring in new faculty to add to our collective expertise. When we bring in new postdoctoral fellows, we don't assign them to one group, we put them between groups, to build bridges. Thankfully, it's getting easier and easier to find people who share that vision. Every generation is getting more comfortable with multidisciplinary work.

You can speak to 100 clinicians and hear about 100 solutions they desperately need to improve patients' lives. How do you decide where to focus your energy? Often, I think it comes down to your environment; who are the scientists and clinicians around you and what are the compelling questions they are facing? For example, an area I'm particularly focusing on now is women's heart health. Cardiovascular disease is the number one killer of women worldwide, and at the Cedars-Sinai Heart Institute I'm surrounded by people working on women's health in the Barbra Streisand Women's Heart Center – from populations, to epidemiology, to basic science.

"We want to do great basic research, but we must always ask 'Why?' And if the answer isn't to improve patients' lives, we need to go back to the drawing board."

An unexpected joy for me has been the strength of the regenerative medicine program at Cedars-Sinai. I now work with Clive Svendsen in our regenerative medicine program, and other groups from John Hopkins and USD Gladstone, on induced pluripotent stem cells. We take cells from patients with neurological disease and transform them into motor neurons for in vitro studies. My group has been involved with ensuring quality control of the cells. Now, we're starting to think about biobanking – banking induced pluripotent stem cells from large numbers of patients and healthy individuals and using them to investigate the pathways involved. It's a new paradigm for us, so we're having to think about how we can best apply the tools available to us. It's an exciting new slant to my research.

Don't lose your humanity in science

Being a scientist is an amazing job. In fact, it's not even a job to me – it's a passion. But science can be a really hard business to be in. You are always being told you aren't good enough – grants get rejected, peer reviewers criticize your work. It's a very negative environment in some ways. That's why it's important not to lose sight of the human side of science. For example, we can't continue to lose (mostly) female scientists just because they have a child – we need to be more flexible. Those few years when a parent has young children at home are

so short in the context of a whole career. You have to accept that life is complicated and scientists are human beings just like everyone else. No-one is perfect. Can you imagine how boring and unapproachable you would be if you were? All I expect from my team and collaborators is to do their best, don't be afraid to ask questions and respect each other. I look for people who share my values – if you shout at a member of my team, I probably won't choose to work with you.

"My career has spanned from biochemistry to clinical translation, and even to entrepreneurship, and I can't wait to see where it takes me next."

Tomorrow's scientists will be better than ever

I wasn't trained in proteomics – if anything I was a reductionist, replacing proteins with a few-amino-acid-long peptide. That is powerful, but what was missing from my training was to look at the broader impacts, and understand the system as a whole. Nowadays, we train proteomics students to think about the bigger picture. We're seeing a new breed of scientist – they go where the data drives them, even if that means crossing disciplines or specialties, and they feel confident in working with different experts to pinpoint the right questions and make sure their data are robust.

I hope that the Institute will change over time. In fact, I think it would be terrible if it didn't. The new Research Institute is all of us – what it becomes over the next five or ten years will be a combination of all of our dreams and aspirations.

Keep changing threads

Someone once asked me "Where do you want to be in five years' time?" I hope I can never answer that question completely, because that would mean I'm not open to change. I do know that I want to have an impact on medicine – to help people to survive longer and with a better quality of life, whether that is by saving hospitals time and money in diagnosis or developing biomarkers for targeted therapies. My career has spanned from biochemistry to clinical translation, and even to entrepreneurship, and I can't wait to see where it takes me next.

When I was a young girl, I read a story that has stayed with me ever since. The author describes the fabric of our lives as the thread of an ornate rug – every time we make a decision or change direction, we introduce a new color or pattern. Some people rarely change course – their rugs are even and symmetrical – while others are a riot of different colors and patterns, constantly growing and changing. I always thought that was the type of life I wanted to have – unique, colorful, joyful… and perhaps a little eccentric!

First published in The Translational Scientist (www.thetranslationalscientist.com), a sister publication of The Analytical Scientist.

Publication Picks

1988 - *J.E. Van Eyk and R. S. Hodges, "The biological importance of each amino acid residue of the troponin I inhibitory sequence 104–115 in the interaction with troponin C and tropomyosin-actin", J. Biol. Chem. 263(4), 1726–1732.*

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2014 - *J. A. Kirk, "Cardiac resynchronization (VRT) sensitizes the sarcomere to calcium by reactivating GSK3 beta", J. Clin. Inv. 124, 129.*

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2015 - *D. I. Lee et al., "Phosphodiesterase 9A controls nitric-oxide-independent cGMP and hypertrophic heart disease", Nature 519, 472.*

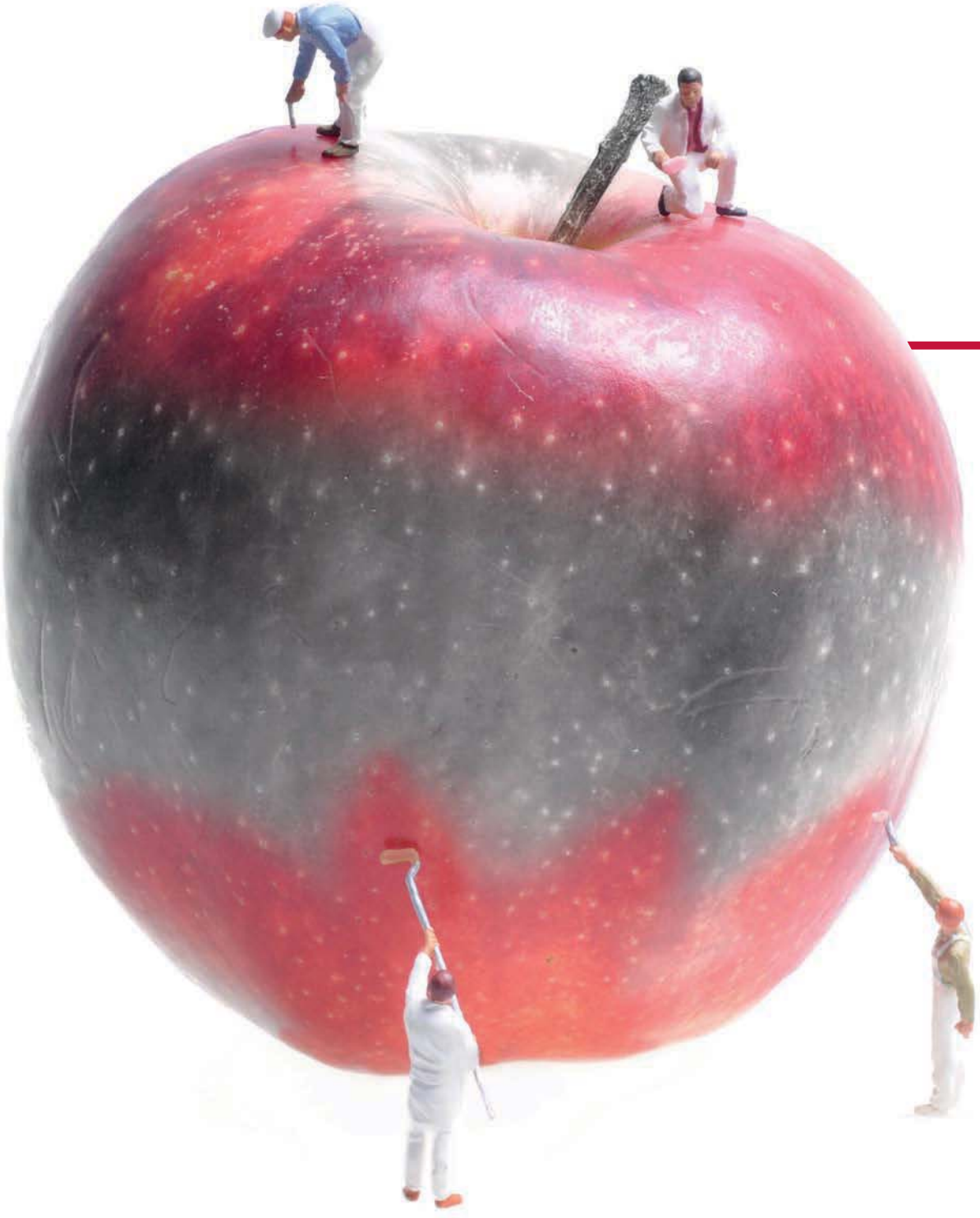
Sildenafil - an inhibitor of PDE 5A - is in clinical trials for heart failure. In this paper, we show that PDE 9A might be a better target.

2016 - *Q. Fu et al., "An empirical approach to signature peptide choice for selected reaction monitoring: quantification of uromodulin in urine", Clin. Chem. 62(1), 198-207.*

Illustrates that current algorithms used to help develop targeted mass spectrometry based assays do not always work and provides an empirical pipeline to increase success rates.

Key:

Blue = cTnI/biomarkers
Pink = mechanism/drug targeting



CASTING A WIDER NET

When fighting food fraud, there are problems that analytical science alone cannot solve. We must work together to take a more inclusive and multidisciplinary approach that ensures true food integrity.

With Petter Olsen

The psychologist, Maslow, visualized a hierarchy of needs as a pyramid, using it to describe a method of problem solving – starting at the bottom and gradually working your way to the top, using known methods. However, this doesn't include the "real" analysis of the problem. In 1966, he also said, "When all you have is a hammer, everything looks like a nail," indicating that not all problems can be fixed with one tool.

Ditching the golden hammer

As an analytical scientist, you may sometimes be tempted to use this "one tool" approach – especially when you've worked hard to try and establish a method; unfortunately, using it makes you biased, whether intentionally or not. You will look at a problem and choose your favorite method to try and solve it – perhaps because you've used it before, or because it's undervalued or unused and you really need to prove its relevance to those who put the problem to you in the first place – after all, you've invested a lot of time, effort and money in its development.

Clearly, such thinking isn't limited to analytical scientists. I come from information technology and computer science, with information logistics being my specialty. I am well aware that computer scientists have their favorite programming languages and typically want to use them, even if there are approaches that are more suitable for the job.

What if we place this narrow thinking into the context of food fraud? We're reading more and more reports these days about how organized crime is playing a greater role. Of course, there is an important role for analytical science in tackling the problem, but simply selecting an analytical approach because that's the way it's always been done before is not a solution.

"If we focus on the food safety issue only, then we are missing a big part of the problem"

Food safety and food security in some contexts are identical, whereas with food fraud you are looking for something that indicates that there is a biochemical property of the food that can be detected analytically. Therefore, there is a need to enhance analytical methods to enable more testing – investing in techniques that are cheaper and faster.

Food fraud is an issue that crosses country borders; therefore, we need to be able to replicate methods readily, so that we are able to identify instances where the country of origin has been changed intentionally or where there is over-production in areas that have strict quotas. There is also the issue of economic fraud, where producers claim that food comes from one country or area, but – to avoid tax or sustainability problems – it's being produced elsewhere. All of these are huge problems that analytical science by itself cannot solve. For example, I am not aware of any analytical methods that can detect whether slave or child labor has been involved, or whether workers are paid on time – both of which are classic indicators for social sustainability.

Essentially – if we focus on the food safety issue only, then we are missing a big part of the problem. We need to look at it as a whole and analyze it before deciding the next steps; we all need to be reminded to revisit what we're being asked to do so that we can find a complete solution. For example, it may be that we are being told that there is a major problem with meat fraud within the retail market. We need to start at the top of that pyramid, looking at it in great detail, and then work our way down gradually to decide on the different methods that will help with the analysis. Analytical scientists can only be expected to analyze the samples they have in front of them. They can't be everywhere at once. On the other hand, if you track "paper trails" you may discover that in reality there should only be 100 tons of a certain food product in the supply chain, but there appears to be 200 tons. That would clearly indicate fraud…which should help with obtaining samples for biochemical analysis, for example. By taking this approach we will identify what we can do – and crucially, those areas where we need to involve others with different competencies.

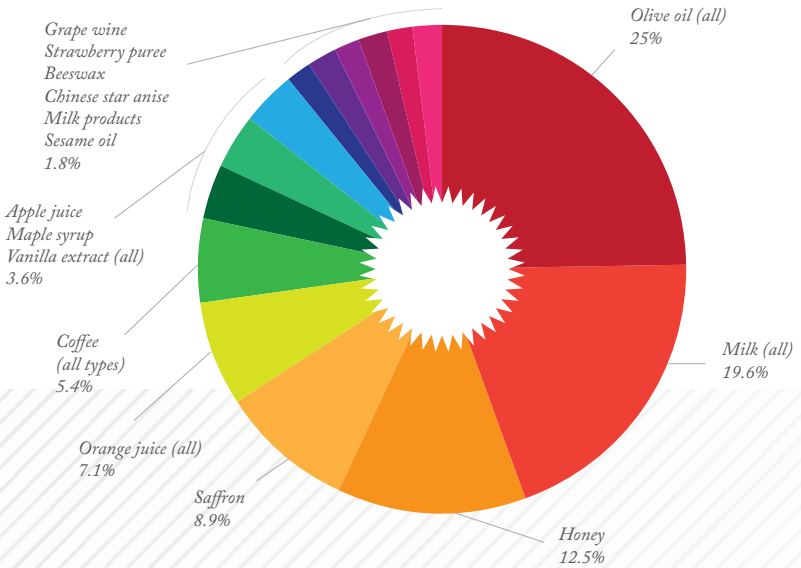
Why is seafood a special case?

- Seafood is traded internationally more than any other foodstuff: often seafood is processed and then traded
- More than 1700 species of fish are traded internationally
- For many species of fish, there is no internationally agreed upon commercial name; the same name is used in different countries to refer to completely different species
- Seafood is a valuable commodity with great potential for economic differentiation between species and products
- Between 14 percent and 33 percent of captured fish (FAO estimate) is from illegal, unreported and unregulated (IUU) fisheries, and fraudulent claims related to origin routinely occur to enable this fish to enter the normal and legal supply chain and be sold there
- There is great concern relating to sustainability of many fish stocks; sustainability claims are valuable
- Seafood is in the Top Three wrongly described foodstuffs

Source: Petter Olsen 04/11/15 ©NofimaMarket

Fifteen most problematic ingredients for economically motivated adulteration

Source: UPS Food Fraud Database



Again, we need to go back and address the problem, not begin with the methods.

Fish in a bigger pond

There is most certainly a need to involve economics in the solution. Chris Elliott (Queen's University, Belfast), speaking at the Recent Advances in Food Analysis (RAFA) conference in Prague, reported that food safety has been forgotten and is underfunded – something which is hitting those laboratories that have invested heavily in methods and technology and are not as active as they once were.

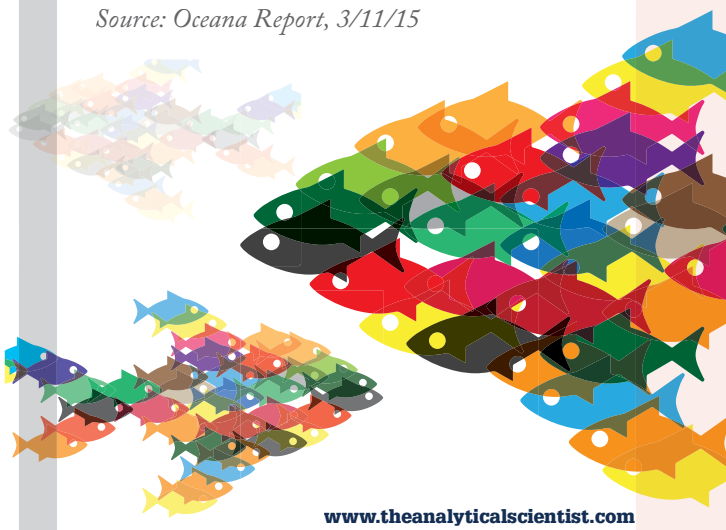
Each year, the EU defines a number of societal challenges for food, and each challenge will receive between one and ten million Euros. For 2016, the catalog contains about 90 challenges. But these project awards are not simply to fund science itself; the EU is looking for answers to the whole problem of food fraud and that means involving all kinds of people, including analytical scientists, information logisticians like myself, economists, socio-economists, and so on. Working as a multidisciplinary team, we can find the underlying cause of why the fraud happens, how it happens, and how it impacts the economy.

I understand the EU project funding system well. During RAFA, I presented our food integrity project, which I believe is the biggest of its type and has the clear multidisciplinary

Seafood fraud in Brussels

- 380 seafood samples taken in restaurants in Brussels
- 15 percent of these from EC and EP restaurants
- 32 percent total mislabeling (wrong species)
- Not Bluefin tuna – 95 percent
- Not cod – 13 percent
- Not sole – 11 percent
- Pangasius common

Source: Oceana Report, 3/11/15



Food Fraud in the News

From fake alcohol to tainted meat, there have been several high profile food fraud cases in the last few months alone.
Source: www.foodintegrity.eu

MARCH
Tainted Cooking Oil

The ex-chairman of Wei Chaun Foods Corp was jailed for four years after it was discovered his company had diluted cooking oil with cottonseed oil and coloring agent copper chlorophyllin – a banned substance. Taipei district court said that Wei had "falsely labelled the products and sold adulterated food to defraud and obtain profits". 11 other employees also received jail terms.

APRIL
Adulterated Chili Powder

The Punjabi Food Authority seized 3000kg of adulterated chili powder as part of a clampdown on food fraud. Both sets of powder were found to be cut with rice flour, but 1000kg was also mixed with bran while the other 2000kg was mixed with oil and artificial color.

APRIL
Beef-laced Lamb

The tasting of a lamb burger by Environmental Health Officers led to the arrest and fining of a Manchester-based Halal butcher. Samples taken from burgers supplied to their customers were found to contain only 50 percent lamb and between 30 and 50 percent beef – legal only if produce is correctly labelled. The company was fined £15,000 for trying to profit by "deliberately misleading the public".

APRIL
Fake Baby Formula

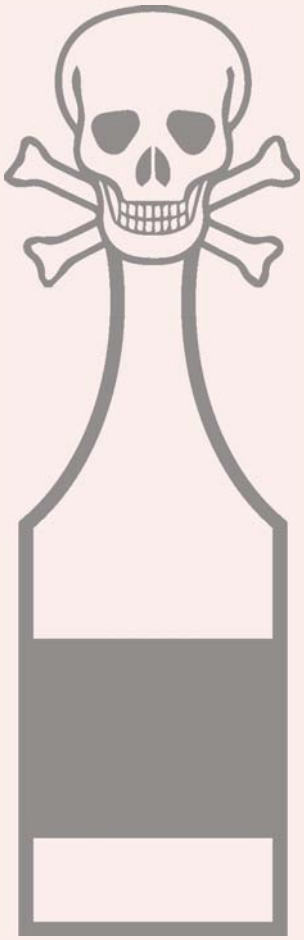
In the wake of the melamine-tainted baby milk scandal of 2008, a new scandal led to the arrest of nine people in Shanghai, when it was discovered that baby formula sold under the brands "Similac" and Beingmate" was fake. The police seized approximately 1,000 cans of milk powder, over 20,000 empty cans and 65,000 fake "Similac" trademarks, but over 3000 cans are still unaccounted for.

MAY
Fake Jellyfish

Jellyfish are considered a delicacy in China, but demand exceeds supply. Police discovered two "jellyfish workshops" which are thought to have supplied 10 tonnes of fake meat and brought the producers 170,000 yuan (£18,100; US$26,100) in profits. The fake jellyfish were made by mixing three chemicals including ammonium alum, which is particularly harmful in excessive doses.

APRIL
Illegal Alcohol Websites

A series of hospitalizations and deaths caused by fake alcohol led to more than 150 Russian websites being taken down – in an attempt to curtail illicit sales. About half of Russia's vodka sales are estimated to be of illegal alcohol, something believed to be due to price controls and excise tax increases.



"If we develop a new method or instrument, we need the industry to understand how it will benefit from using it"

approach the EU looks for when awarding projects and funding. Over the last 20 years, I've been working within international projects and I think that what the EU expects makes sense. Of course, the EU does fund science through the European Research Council, for example, but those projects are not on the same scale. In the larger "societal challenge" projects, the EU recognizes that science is necessary… but also that it is only part of the solution.

With my own institution – NOFIMA – we have two economics departments, both of which contain socio-economists. They have identified that virtually all EU projects now have an economic and a socio-economic component. It didn't used to be the case. Current projects are all moving away from the narrow, targeted analytical science approach to work that paints a bigger picture and provides answers to food fraud problems on a broader scale – which may involve social sustainability, eco-labeling, and so on. Clearly we need to involve specialists from many more areas, with analytical (and other) scientists creating technological solutions as part of the overall package.

During my experiences of working on EU food fraud projects, I have found that if I get involved early on in the discussions, the consortia members are almost exclusively analytical scientists. I then have to stand up to say we can't just do science, and that we need to bring in people who understand stakeholder engagement. We need to co-create the solution with the industries we expect to put it to use. We need to engage them, discover what their motivations are. Only then can we get their precise requirements and feedback – and involve them in implementing the solution to the societal challenge.

If we develop a new method or instrument, we need the industry to understand how it will benefit from using it – as opposed to continuing with old equipment or approaches. It can be difficult (even after going through cost–benefit calculations) to convince people that they need to change; again this calls upon other specialists within the project team. The same strategy can be mirrored in many other areas.

Imagine we have a problem with species mislabeling. We could take the ten best DNA analysis labs in Europe and ask them to do some profiling. Then we could add a couple of economists to figure out the economic impact. But once again, it would be the wrong way around. We'd be beginning with the method again and not really addressing the bigger problem being raised by the EU, which brings us back to my main message: start by analyzing the problem and not narrowly focusing on the method(s) you want to use because you feel they'll give you a result.

Bigger fish to fry

Working for NOFIMA entails working with food projects in a more general sense. Norway may be a small country but when it comes to fish, we are a world leader. That includes fish caught by our fishing fleets and those produced in aquaculture. We are important exporters of seafood to the world's markets, and we import everything else we need other than fish. In fact, seafood is one of the most internationally traded foodstuffs. And that means we need a lot of knowledge about food supply chains and food fraud, safety and security. You may not be surprised to learn that Japan is one of the biggest consumers of Norwegian-sourced fish – and that makes the supply chain long and complicated. We need to know precisely what is going on along the way (and it also means I need to do a lot of traveling).

We've even incorporated "citizen science" into a food integrity project. Miguel Angel Pardo at AZTI (a marine and food research center) in Spain oversees this. Anyone can join in by contacting Miguel (mpardo@azti.es) and he will send you ten sample tubes for collecting small pieces of fish claimed to be a particular species. Each time you decide to claim something is cod, tuna or some other species of fish, you just need to place a small piece of the fish into the tube and send it to his group for analysis. He then translates the findings into a map showing whether the fish was genuine – a green dot – or fraudulent – a red dot – onto a map of Europe, giving a view of the distribution of the problem and adding to the bigger picture. Again, we come back to this analysis being part of the overall solution we are working towards and not the whole answer.

Similar studies have been conducted by the research organization, Oceana (see sidebar: Seafood Fraud in Brussels). In November 2015, following DNA testing by KU Leuven, Oceana discovered that about 30 percent of seafood served in restaurants in Brussels did not correspond to the species ordered by the consumer.

Based on Petter Olsen's presentation at RAFA 2015 and a subsequent interview.

Business

Economic drivers
Emerging trends
Business strategies

Icebergs Ahead

Are you ready for the "unthinkable" – your laboratory computer systems suffering a titanic failure? Disaster recovery plans are crucial insurance against the unexpected.

By Tony Lisi

Google's self-reported 99.984 percent uptime and the non-existent interruptions claimed by other major providers encourage us to take the availability of laboratory information management systems (LIMS) and other critical software tools for granted. The known risks – upgrades, planned downtime for system maintenance, and the occasional power outage that lasts long enough to test the effectiveness of a fossil fuel-powered generator backup – may be included in a company's disaster recovery (DR) plan. However, when was the last time you checked it? And perhaps more importantly – have you planned for the unexpected?

With record-retention mandates differing worldwide, organizations cannot be too risk averse in their backup retention policies. The risk of not doing so can have both legal and financial consequences. For example, laboratories that monitor air quality and waste output need to be able to produce records and data readily whenever the Environmental Protection Agency (EPA), Occupational Safety and Health Administration (OSHA), and other regulatory authorities request it. Failure to do so can result in fines, shutdowns, loss of revenue and even legal action.

Audits and virtual machines

I recently audited a small laboratory that was not using virtual machines. Upon completion, I found multiple deficiencies that left the lab vulnerable to data loss (whether from a power outage or, more commonly, operator error). Below are a few of the findings with recommended action items to correct the offense:

Unmaintained battery backups

A preventative measure for avoiding data loss is to test battery backups and verify that they will last long enough for either the backup generator power to kick in or for a graceful server shutdown.

Incorrect user permissions

Without correct permissions, the door is open to accidental data loss or updates. I found that users had the ability to edit data throughout the LIMS even though their work was only focused on a subset of the LIMS functionalities. Moreover, I've found that "operator error" is the most common cause of record loss. Addressing this shortcoming can prevent the need for many last minute record recoveries.

Single point recovery failure

When reviewing the DR process, I found that there were multiple single points of failure with the current backup strategy. A single backup method was used, which was a full daily backup of all files on the server. Though it allowed for single file recoveries, it did not allow for server recovery because the open records on the server were not being backed up. In fact, it would only have been valid if there were recovery scripts for a rebuild of the server that would allow file recovery. I determined that a full backup should be run on the weekend, and then

"With record-retention mandates differing worldwide, organizations cannot be too risk averse in their backup retention policies."

incremental server backups run daily during the week. Additionally, recovery scripts were created to rebuild servers. Finally, the tape rotations allowed for many recovery options.

Invalid backup schedules

There were scheduling conflicts with the database backups to disk and the server file level backups. I found that the database hot or cold backups and exports were active during the backup of the server files, which meant that the database was never fully backed up on tape. The corrective action taken was mapping out the start and end times for each database and server file backup to determine if they overlapped and to change the times to prevent scheduling conflicts. If a conflict could not be avoided, other approaches would have been needed, such as rolling backup times or backing databases up directly to tape.

No tape rotations

I found that backup tape rotations were inconsistent. There are a number of reasons for tape rotations. One is to save money by reusing backup tapes, and another is to set aside weekly or monthly tapes for the remainder of the accepted retention period. To correct this, you need to issue a policy where certain tapes are set aside for an agreed period. For example, one tape a week was stored for an entire month and then after six months only one tape a month was stored for the duration of the company's retention period.

Missing "test and verify" backup plans

The DR plan executions were never run to verify that the backups could be recovered. What good would the effort and money spent on developing a DR plan be if backups can't be used to recover a lost system months or years down the road? Make sure the plan is tested and verified to work properly.

There really is no excuse for not putting some level of backup policy in place. Recently, many new backup strategies have been used, especially with the popularization and use of virtual machines (VM) for emulating particular computer systems. With the advancement of today's technology – as well as past developments in database technology that enable multi-master replication and other real-time backup solutions – it is now possible to take a live snapshot of your server. And though this doesn't necessarily provide you with file-level recovery, it does provide another viable backup strategy.

Write a comprehensive DR plan

When a company runs its business on a paperless or near paperless platform, its successful operation relies on its servers and systems availability. You should be asking yourself a number of serious questions:

- What happens if servers or systems are unavailable?
- How long can you run your business "in the dark"?
- Are there adequate backup and recovery plans in place?
- Is your business risk-averse enough or do you take uptime for granted?
- Is working with no net acceptable?
- Are there contingency plans in place for a total loss to the server room or a breakdown of legacy analytical equipment?
- What about the loss of just one server?
- Have you checked to make sure you can actually recover from your backups?
- Have you identified where you will acquire the parts for equipment that is no longer supported by the OEM?

Answering these questions is the true value of a disaster recovery plan, which should provide documented steps to help you take action and limit your losses.

"There really is no excuse for not putting some level of backup policy in place."

No one really appreciates the value of insurance until it needs to be used. Similarly, the benefit of a DR plan is only realized when something happens. Developing and putting in place a working DR plan won't increase revenues, but doing so is great insurance for a company's future. It can be hard justifying the effort needed to develop a working DR model, especially if nothing has ever happened in the past. However, when the unexpected does happen and no DR model is in place, it can cause a business to lose customers, lose revenue, halt growth, downsize, shut down, and even face fines and legal action.

"There is no danger that Titanic will sink. The boat is unsinkable, and nothing but inconvenience will be suffered by the passengers."
– Phillip Franklin, White Star Line vice-president, 1912

Tony Lisi is a laboratory informatics consultant at CSols, Inc. (www.csolsinc.com), Delaware, USA.

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Core Collaborator

Sitting Down With... Jessica Prenni, Director of Research Core Facilities, Director of Proteomics & Metabolomics Facility, University of Colorado, USA.

What sparked your interest in analytical science?

I've always been interested in science, in part because that's what came easy to me. I started out as an engineering major, but found chemistry and basic science the most compelling. I got my first taste of analytical science and mass spectrometry when I did an internship at the US Fish & Wildlife Service Forensics Laboratory. They had a great set-up and we used one of the early electrospray instruments for species identification from dried blood spots collected at crime scenes – apparently it was and still is the only lab in the world dedicated to crimes against wildlife.

The experience gave me the research bug and so I switched gears for graduate school and embraced analytical chemistry. I had a fantastic advisor – Kathy Rowlen (University of Colorado Boulder) – who taught me how to be a good scientist. And my post-doc at Scripps Institute was where I really got serious about mass spectrometry – and where I got my feet wet with omics technologies.

How did you end up as a core facility director?

When I moved to Colorado State University, they didn't have the same kind of omics infrastructure on campus that I had experienced during my post-doc. I was charged with establishing a proteomics service – with one instrument. I had a good handle on the workflows and technology, but running a core facility is a lot like running a small business. You have to figure out what the users need, and I quickly found out that most of the users didn't know what they needed! I also learnt that data must be presented in the right way, and that the quality of the data is super important; once it leaves your hands, researchers can do what they want with it.

Today, my lab is split into proteomics and metabolomics projects – but more and more, we're finding that the two are coming together. One current challenge is integrating those two data streams. Over the years, we've grown substantially, and can be working on 60-70 projects at any one time.

Can you give us an example?

We have a very long-standing collaboration with an epidemiology group at Uppsala University. The really cool thing is that they have access to huge biobanks of clinical samples – we've probably done metabolomics analysis on more than 6000 serum samples over the years by both LC-MS and GC-MS. They have multiple cohorts for population studies (one of which is a twin study) – so we're getting the chance to process some really interesting data. One recent paper focused on coronary heart disease and we found a couple of lipids that turned out to be potentially more predictive than what's currently used.

Are there any tools missing?

The technology that we have in our lab is amazing. The limitation is not in the analytical tools. Downstream is where more development is needed – so we're back to informatics. That said, I know that there are new tools coming out all the time and very smart people working on the problems. In fact, that's the reason we developed RAMClust – an open-source clustering method that facilitates annotation of metabolomics data in an unbiased way.

We're also open to working with instrument manufacturers to ensure that we get the tools we need! In fact, we were involved in the development of the Waters ionKey/MS system, which I believe has the potential to be particularly transformative in proteomics.

New technology that we're hoping to implement in the lab is ion mobility mass spectrometry, which adds another key – and highly consistent – physical parameter that we can measure and add to our metabolite annotation workflows. In metabolomics, full transparency in the identification process is the only way the field can move forward.

How else will metabolomics evolve?

Right now, metabolomics tends to take a 'snapshot' approach – and that certainly gives us interesting information, but it doesn't give us the full picture. Ultimately, we need to move toward flux measurements. I think this area will really blossom – and that will place even more emphasis on informatics. The real key for translational success will be integration with the other omics.

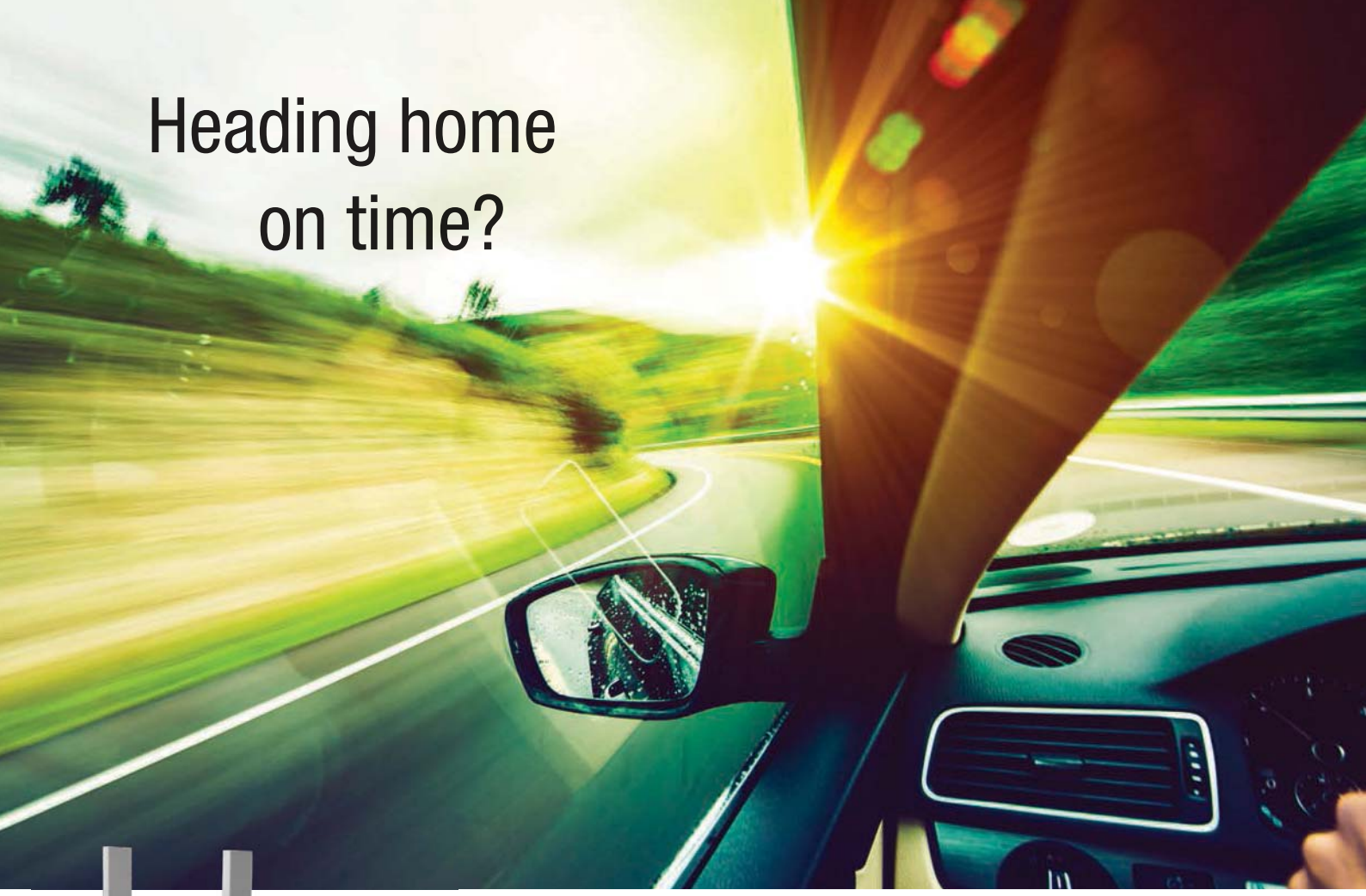
"The real key for translational success will be integration with the other omics."

How do you stay motivated?

I can see the huge potential and I know where we need to be – so it feels like we're on a very positive journey. And every project gets us a little closer.

I feel very fulfilled by the collaborative path I've chosen. And I strongly believe that core facilities with concentrated expertise in increasingly sophisticated measurement technology are essential for real success in translational science. Making a mass spectrometer easy enough for anyone to use is great for certain applications, but experience – and understanding the limitations – is essential, especially for complex samples. I'm not a biologist and a biologist is not an analytical chemist – and we might both need informatics support. In other words, we need to work together. Modern science is very much a team game.

Heading home on time?



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Example: ALEX-GC-MS/MS-System for QuEChERS, Metabolomics ….

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