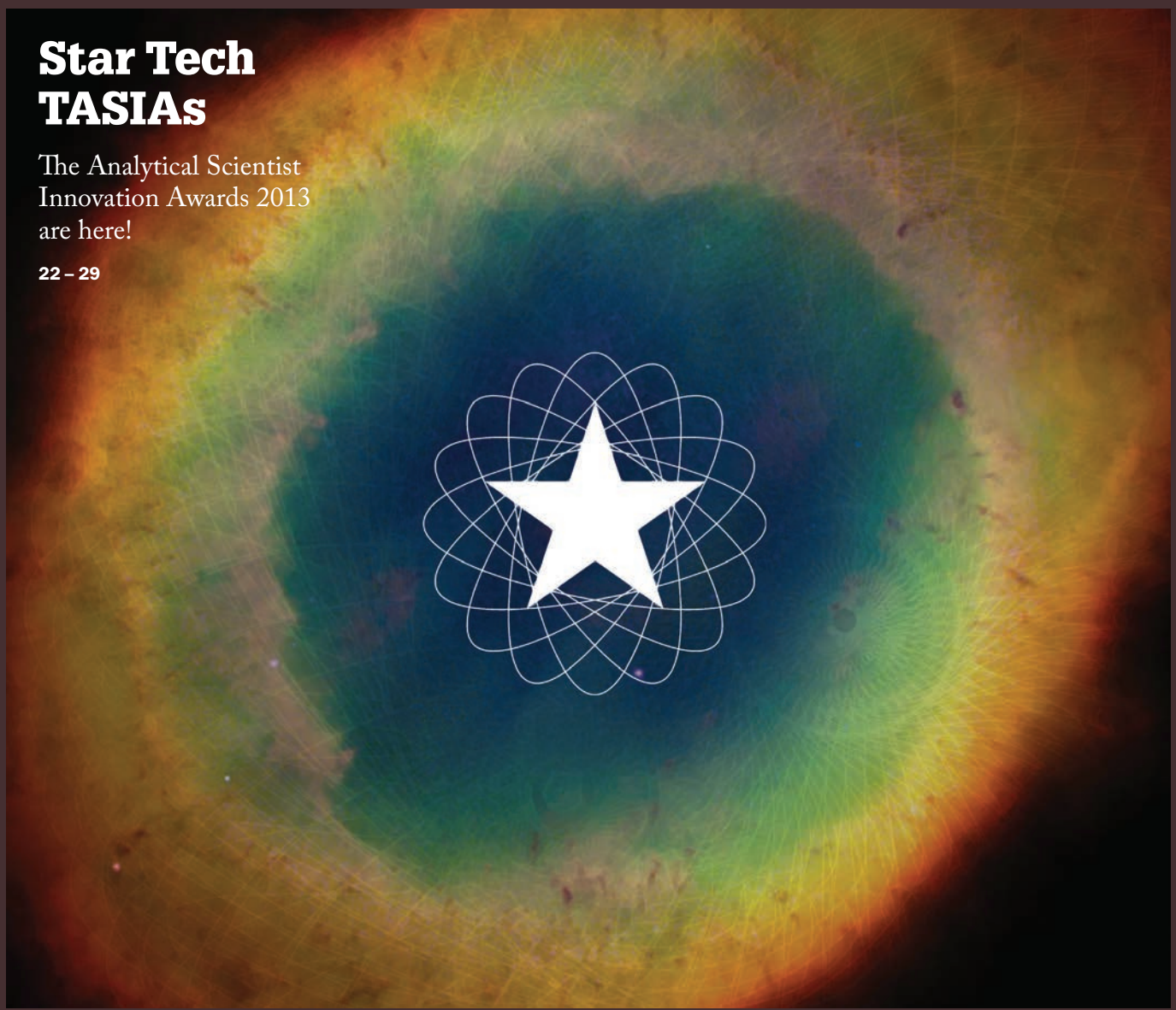
DECEMBER 2013 # 11

the Analytical Scientist



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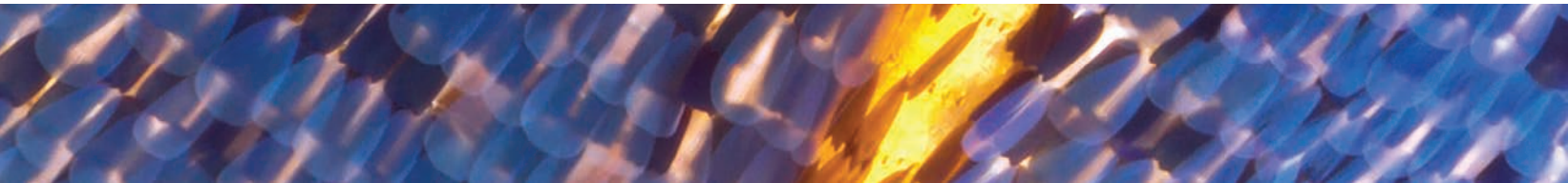

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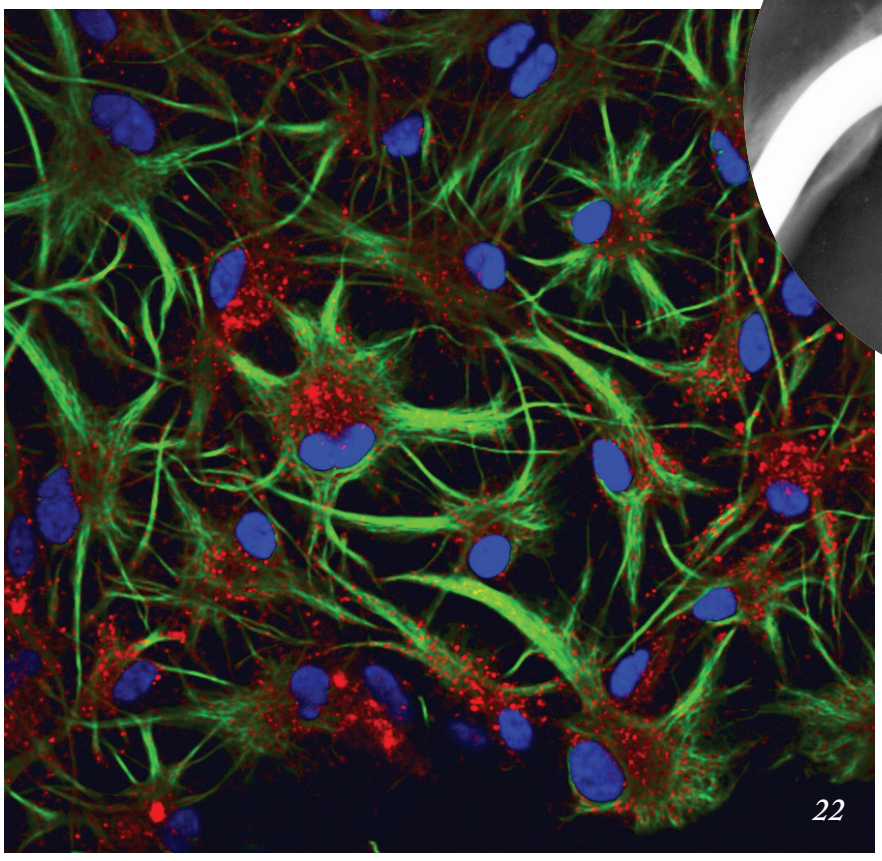
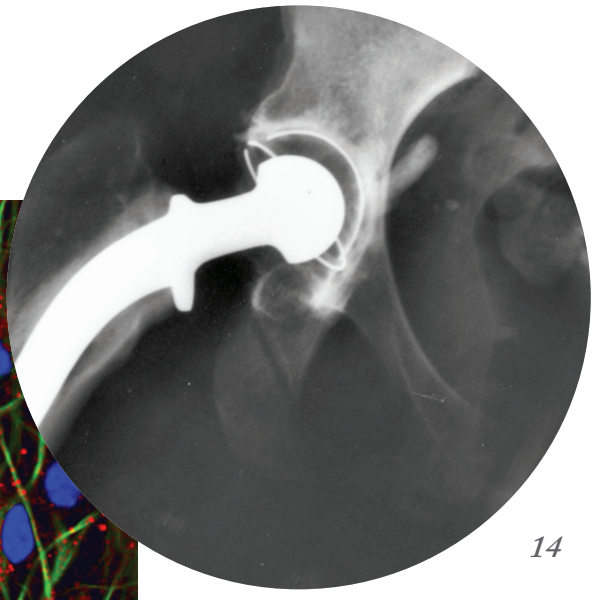
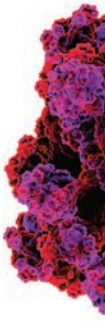
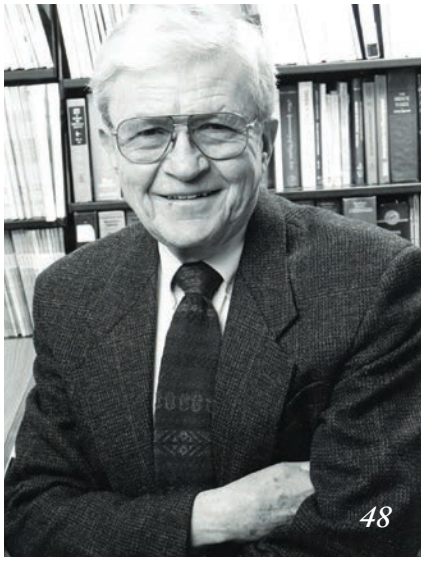


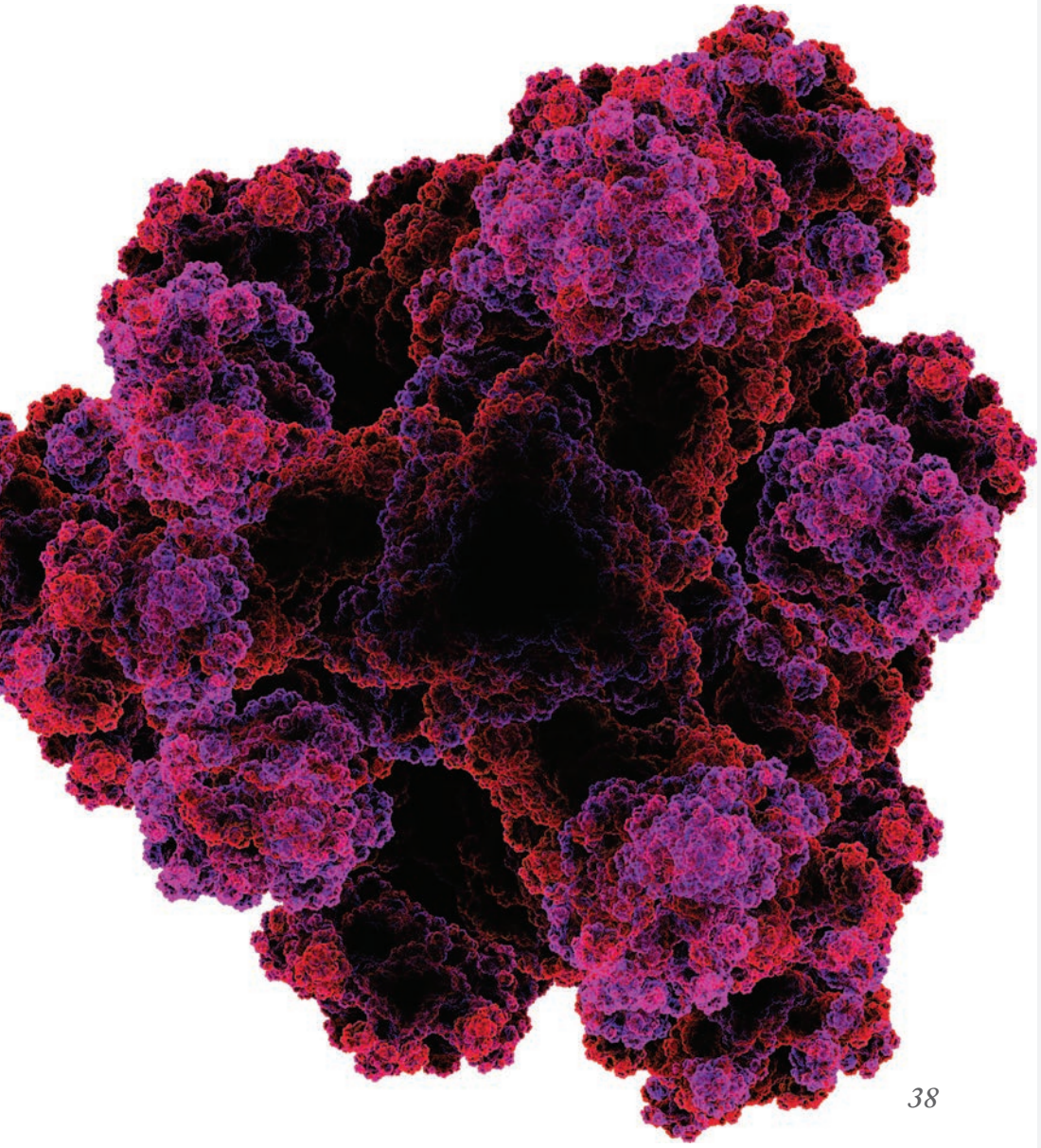






On The Cover

The TASLAs hit warp speed (based on Ring Nebula image courtesy of NASA/Hubble Heritage).



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Features

Departments

Sitting Down With

the **Analytical Scientist**

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Oh, Elusive Innovation!

What is it, why is it, and where on earth is it? The 2013 TASIAs have the answer.

Innovation: the word is very familiar to us all, but appears to lack any truly representative synonym and its actual form can be similarly ethereal. "Innovation is child's play," was an article title in our last issue – yet, in that same issue, Alexander Makarov described in intricate detail just how difficult true innovation can be. Indeed, genuine innovation lurks in the half-light, always shifting shape. Producing one innovation does not guarantee that you have a recipe for success or even the ability to generate another.

Given its slippery nature, the term tends to be overused by marketeers, who have latched onto the power held within those mighty four syllables to insist that every minor product upgrade is an 'innovation', diluting its impact.

In this issue, we present full-strength innovation, in the form of the 2013 TASIAs (The Analytical Scientist Innovation Awards).

The TASIAs showcase the extraordinary range and pace of progress in our field. Drawing on a large list of nominations, 15 winning combinations of invention, vision and utility were selected by three leading contributors to the field and the editors of this publication. The products recognized in the TASIAs all enhance preparation, separation, identification, or quantitation in some way, and endeavor to push the boundaries. After all, limits of detection are the benchmarks of safety, and fast, accurate clinical diagnostics save lives – or, in other words, what we measure today, we can improve tomorrow.

Large corporations and small start-ups are both represented in the TASIAs; perhaps because "Innovation has nothing to do with how many R&D dollars you have [...] It's not about money. It's about the people you have, how you're led, and how much you get it," as Steve Jobs once said.

As with The Power List (published in October), we do not assert that the TASIAs are definitive. The nominations were provided by you and selected by a panel of expert judges; if something great wasn't put forward, it sadly won't be included. We welcome feedback on the winners and on other innovative technologies launched in 2013.

To those whose technologies have been recognized in the first TASIAs (which will become an annual fixture), we extend our congratulations. And to everyone involved in the development of innovations in analytical science, our thanks. Because, to misquote the words of Winston Churchill:

"Without tradition, [analytical science] is a flock of sheep without a shepherd. Without innovation, it is a corpse."

Rich Whitworth
Editor

Hans-Gerd Janssen

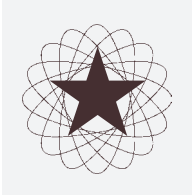
"A chemical engineer is what I wanted to be," says Hans-Gerd, who decided not to go to a "regular, dull" university but to a University of Technology. "I then got annoyed by the approximate nature of chemical engineering and changed direction". Now, combining the chemistries of interactions with the physics of mass transfer to separate compounds is what makes him tick. Hans-Gerd is a professor of analytical chemistry at the University of Amsterdam and a scientist at Unilever. "My research focuses on getting information on increasing numbers of molecules from ever more complex samples," he says.

Robert Kennedy

Robert Kennedy is the Willard professor of chemistry and professor of pharmacology at the University of Michigan. "My research interests are analytical chemistry and its application to neuroscience, endocrinology, and biotechnology," he says. "My group developed instrumentation that couples sampling probes to capillary electrophoresis, capillary chromatography, mass spectrometry, and microfluidic assays for monitoring neurotransmitters in vivo." These methods have been used for studying changes in neurotransmitter concentrations associated with behavior and disease. Robert is associate editor of Analytical Chemistry and director of the Microfluidics in Biomedical Sciences Training Program at Michigan.

Oscar van den Brink

As group leader of spectroscopy and process analysis with AkzoNobel, Oscar van den Brink observed that innovation and human capital were both crucial for success. "Earlier in my career, while combining novel mass spectrometric techniques with the research of works of art, I noticed that the range of application areas that benefit from analytical science can be very broad," he says. As managing director of COAST, Oscar executes its integrated strategic agenda of research, education and infrastructure. "I am very happy to now be in the position to enhance collaboration in analytical science among companies and knowledge institutes in many application areas."



Rich Whitworth, Frank van Geel, Richard Gallagher

Somehow casting aside their regional, ethical, and musical differences for one moment, the editorial team united to cast the fourth vote in the inaugural TASIAs (The Analytical Scientist Innovation Awards). With combined experience (in life) well into triple digits (in years), the Brit, the Dutchman, and the Scot, could agree on one thing – that the nominations were highly competitive. "I thought you said this would only take a 'couple' of hours!" said Frank to Rich, who is notorious for under-estimating editorial resources. Rich shook his head in dismay as Richard simply winked at the daylight-deprived duo from his sunlounger in California. Turn to The Analytical Scientist Innovation Awards on page 22 for the result of much deliberation.

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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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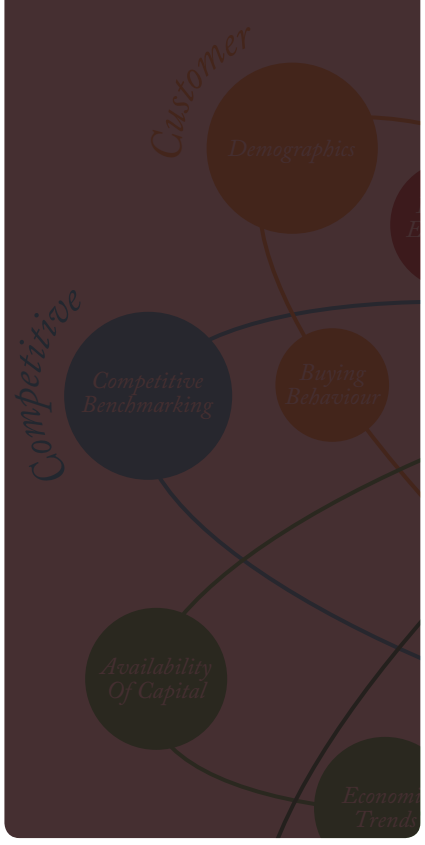


Mass Spec Market Ignites

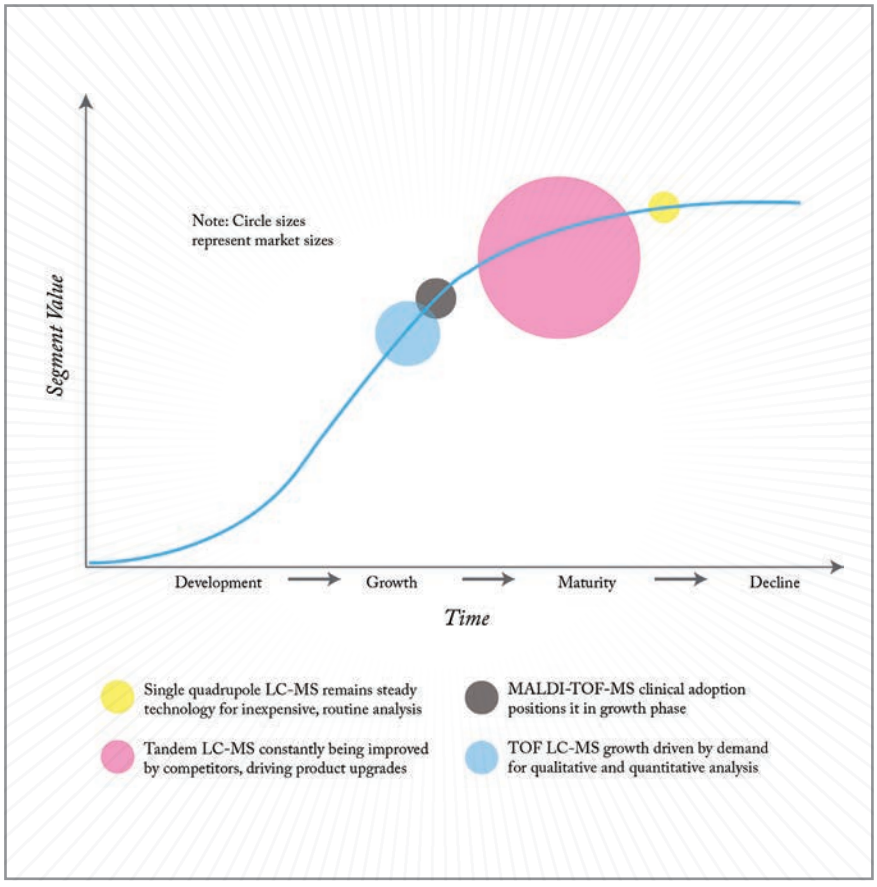
A business consultancy report estimates that the global mass spectrometry market will reach $2.5 billion by 2017

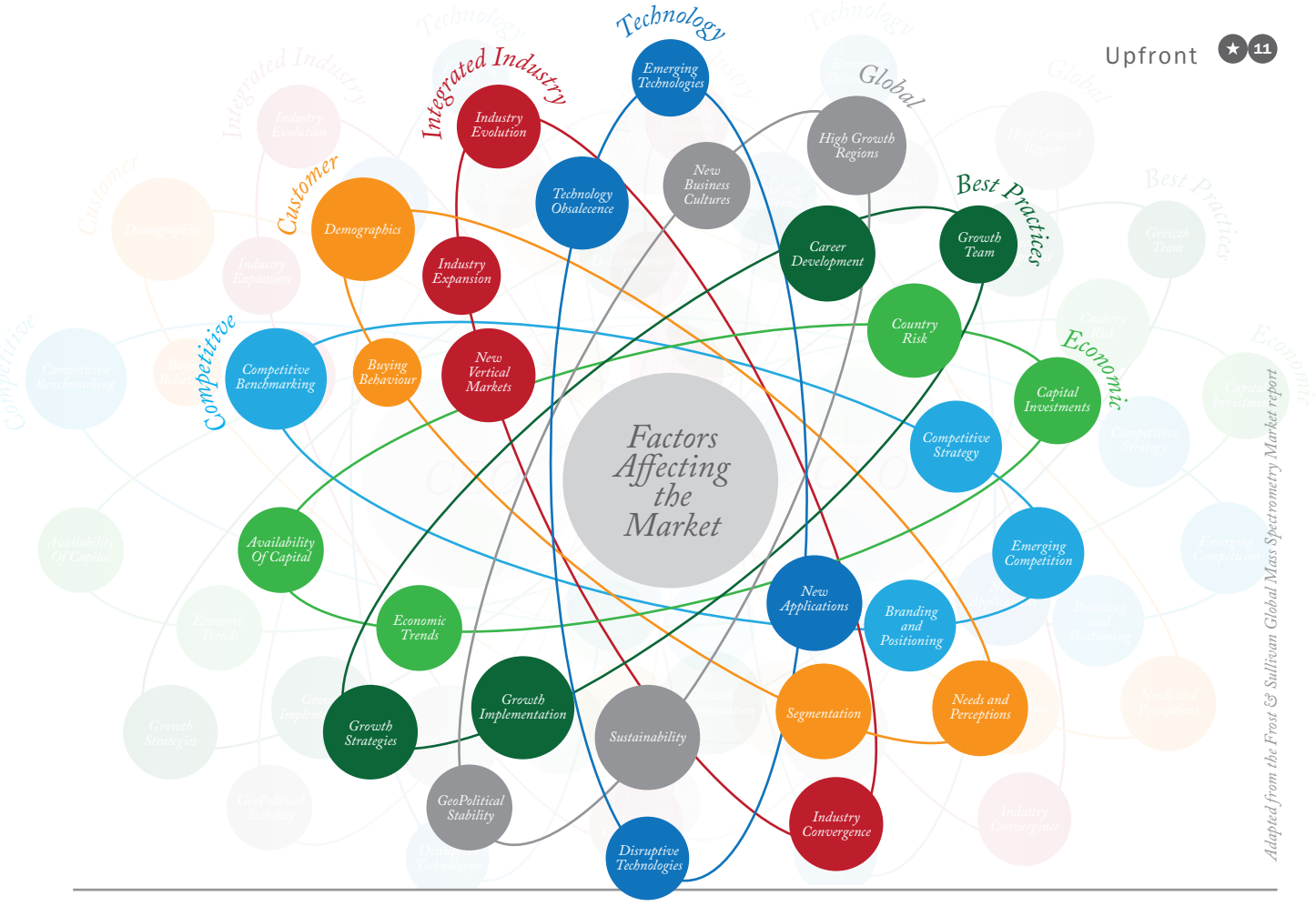
"Strong research and development efforts by equipment manufacturers – a testament to the large market opportunity – have enabled improved qualitative and quantitative capabilities, giving rise to new applications," says Christi Bird, Frost & Sullivan Life Sciences senior industry analyst and author of Global Mass Spectrometry Market (for full report, visit: tas.txp.to/1113/frost).

The report focuses on four key segments – single quadrupole liquid chromatography-mass spectrometry (LC-MS), tandem LC-MS (triple quadrupole and ion trap), time-of-flight (TOF) LC-MS, and matrix-assisted laser desorption ionization (MALDI)-TOF – the combination of which, for the purposes of the report, defines the MS market as a whole.

Here, we present some of the trends that the report identified.

Global Market Lifecycle Analysis





Adapted from the Frost & Sullivan Global Mass Spectrometry Market report

Market Activity

- ▲ Market revenue in 2012: *$1.7 billion*
- ● Unit shipments in 2012: *>6000*
- ▲ Market Size in 2017: *$2.7 billion*
- ▲ 2012 Market Growth Rate: *6.7%*
- ● Customer price sensitivity: *1: low to 10: high* — *6*
- ▲ Degree of technical change: *1: low to 10: high* — *8*
- ▼ Market concentration: *Percentage of market held by top three companies* — *70.2%*
- ● Active market competitors: *companies with > $10 million revenue* — *8*

Key: ▲ Increase ● Stable ▼ Decrease

Top Three Predictions

1. Driven by diversified MS usage, Asia-Pacific and "rest of world" markets are set to surpass the US in 2014.
2. European MS growth above GDP during stagnation indicates the high priority of the technology, but could signify a bounce-back peak in the future.
3. Clinical diagnostics is poised to drive growth, mergers and acquisitions, partnerships, and major changes in market shares.

What the CEOs Say

▶ The triple quadrupole LC-MS market has the fastest growth, despite increased competition from other areas.

▶ Looking ahead, MALDI-TOF-MS is set to be boosted by clinical adoption.

▶ The Asia-Pacific market is growing faster than the global rate; slowing as it becomes more established.

▶ Despite the European economic crisis stifling growth, the MS market is growing stronger than gross domestic product.

▶ The US market slowed down due to sequestration measures; however, offers the most potential for early clinical acceptance through 2017.

▶ Manufacturers should obtain ISO certifications and seek clinical approval.

The Adventures of Titin

How high-speed force spectroscopy (HS-FS) closes the gap between experimental and simulated unfolding of titin

Titin is the largest known protein, at around 3.5 MDa (the etymological link to Titan is intentional). It acts as a molecular spring in muscle and is a prime target for protein unfolding studies. Previously, direct comparison between molecular simulations and single-molecule unfolding experiments was not possible because of the huge difference in pulling velocity exerted on the molecule. Now, researchers in the Scheuring Lab at Université Aix-Marseille have developed a high-speed force spectroscopy (HS-FS) method that stretches titin molecules at speeds more akin to simulations. Lab director Simon Scheuring answers questions about the technique.

How does it work?

The technique is atomic force microscopy (AFM)-based force spectroscopy. The protein molecule is tethered between the AFM tip (at the end of the AFM cantilever) and a solid support that is pulled backwards by a piezoelectric element. When this is done, the protein molecule is stretched and unfolded. The AFM cantilever reports the forces required for this process (See Figure 1).

What were the main challenges?

Compared to conventional AFM force spectroscopy, we used cantilevers that are about 30 times shorter than conventional cantilevers (6 µm versus 200 µm). We also used a novel sample support to minimize hydrodynamic drag and allow very fast electronics. Each of these ameliorations was essential to pull about 1000 times faster than a conventional setup.

Any limitations?

We read the data (the cantilever deflection) out at frequency of 2MHz, or two million data points per second. When we unfold a protein that is 25 nm in length at a speed of 4 millimetres per second, we only retrieve about 15 data points. Therefore, to unfold molecules even faster, we must further improve our electronics. As you can imagine, we also acquire enormous amounts of data during a long experiment.

How important is it to bridge the gap between simulation and experimentation?

Simulations have become very important in modern biology (see "The New Model", page 17). In silico simulations provide full-atomistic movies of what molecules are 'doing'. However, full-atomistic simulations are very calculation-consuming, that's why simulations can only cover nano- to micro-seconds of the lifetime of a protein molecule. I believe this is the first experiment that manipulates a molecule at the same speed as it is done on a computer, hence allowing direct comparison.

Were there any surprises?

If you pull the protein apart faster than a certain speed, about 100µm/s, then the molecule starts behaving very differently than it does at lower speeds. It appears that the molecule is pulled so fast that the imposed trajectory dominates over natural diffusion.

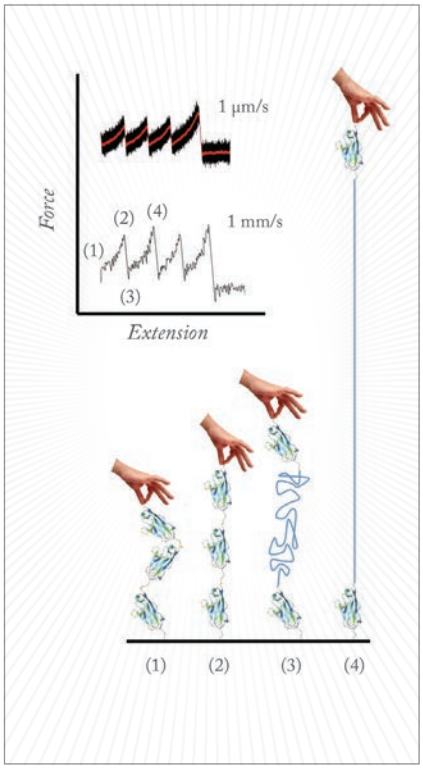


Figure 1. Force-extension curves acquired at retraction velocities of 1 µm/s and 1000 µm/s. The 1 µm/s curve is moving average filtered (red line).

How and where do you envisage the technique being applied?

This is a fundamental biophysics technique and, as such, will allow us to examine any type of single molecule interaction – obvious targets are ligand-receptor pairs.

What next?

Go faster! Not only pulling at faster speeds, but also detecting events faster. We also hope to apply the technique to challenging bio-samples. *RW*

References

1. *F. Rico et al., "High-Speed Force Spectroscopy Unfolds Titin at the Velocity of Molecular Dynamics Simulations", Science, 342 (6159), 741-743 (2013). DOI:10.1126/science.1239764*

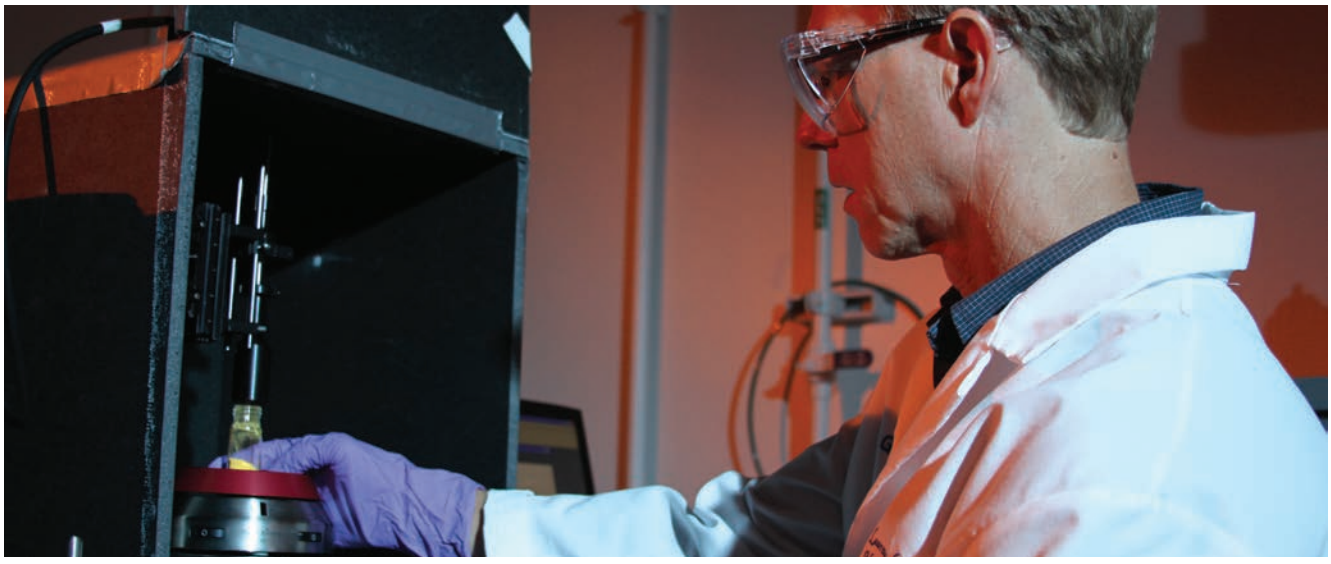


Photo courtesy of Steve Wampler/LLNL.

Scoping Out Yellowcake

Pioneering near-infrared spectrometry for the analysis of uranium ore concentrates

Yellowcake – or uranium ore concentrate (UOC) – predominantly comprises triuranium octoxide (U_3O_8) but different conditions during mine processing can affect the final composition. In the past, a primary method for evaluating different types of UOC was a simple – and clearly, limited – visual inspection of color. Now, researchers at the Lawrence Livermore National Laboratory (LLNL) in California, USA, have applied near-infrared spectroscopy (NIRS) to analyze the chemical composition (1), which can help track a sample back to its origin.

The benefits of NIRS were found somewhat by chance, says Greg Klunder (pictured), a chemist at LLNL's Forensic Science Center and lead author of the paper. He was originally investigating the lab's spectrometer (Labspec Pro, Analytical Spectral Devices Inc.) for use in an objective method to evaluate the color of UOC samples. "Since our spectrometer also covered the near-infrared range, we had that data as well," he says, "The presence of absorption bands in the NIR was both fortuitous and unexpected, as this region classically measures CH, OH, and NH overtones and combination bands." Further investigation by the team demonstrated that UOC samples from different processes have different spectral signatures in the NIR range.

So, why has NIRS not been used in this application before, given its non-contact, non-destructive nature? "Nuclear forensic analysis is a relatively new area and a variety of analytical methods are being pursued to provide information about the samples," explains Klunder. For example, mass spectrometry has been used extensively to provide trace elemental and isotopic information, but requires sample preparation that can significantly compromise the uranium samples. "Since NIR absorption bands are broader and less information-rich, there has not been as much interest in this region," he says. "However, the ability to make non-contact measurements without consuming or contaminating the sample is clearly very useful."

NIRS is not a trace analysis technique and can only detect components in the low percentage range; clearly, it is not intended as a replacement for mass spectrometry. Rather, Klunder believes, the method is a valuable addition to the identification toolbox in a rapidly growing area: material composition analyses in the field. Raman spectroscopy, another non-contact field technique, has difficulties with dark materials or those that have fluorescent components.

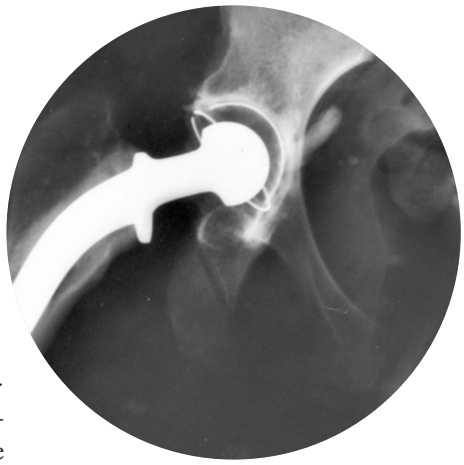
The team at LLNL hopes to develop the technique further by improving the sample library and identification protocols. In the future, says Klunder, "NIR imaging could also provide spatially resolved chemical information for more heterogeneous samples". *RW*

Reference

1. *G. L. Klunder et al., "Application of Visible/Near-Infrared Reflectance Spectroscopy to Uranium Ore Concentrates for Nuclear Forensic Analysis and Attribution", Applied Spectroscopy, 67 (9), 1049–1056 (2013).*

Ti Tox Test

A new method can determine ultra-trace levels of titanium in clinical samples

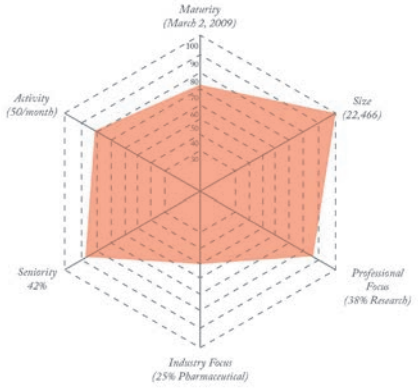


Over the last 20 years, metallic joint replacement devices have been used in several million patients all over the world with excellent results. Titanium is one of the most frequently used metals in prostheses because of its high strength, low weight, corrosion-resistance and biocompatibility. But, health authorities – and patients – are increasingly being confronted with unexplained pain and soft tissue reactions following implant insertion. The question is: "could this be attributed to metal toxicity?" Metal ions and particles generated by wear and corrosion of the prostheses is one cause for concern, but even in patients with well-functioning prostheses, the consequence of prolonged systemic exposure to metal remains unclear.

Concentrations of metals in clinical samples are typically in the (sub)-µg/L range. Now, researchers of the Department of Analytical Chemistry at Ghent University, Belgium, and the University of Zaragoza, Spain, have developed a novel method for the determination of titanium at ultra-trace levels, based on the use of a triple-quadrupole inductively coupled plasma - mass spectrometer (ICP-QQQ), operating in MS/MS mode (1). The accurate determination of very low levels of titanium in human body fluids is

Scanning the LinkedIn Landscape

The omnipresent professional networking site seems to have a group for everything. Here, we throw the radar over the six biggest analytical offerings in terms of activity, seniority and focus.



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traditionally hindered by spectral overlap of the signals obtained for the different titanium isotopes and those of matrix-based interfering ions. However, the presence of a reaction/collision cell (located between two of the quadrupole analyzers) in the ICP-QQQ instrument means it can be operated in MS/MS mode. This set-up gives enhanced control over the chemical reactions taking place in the reaction cell. The conversion of Ti^+ ions into $Ti(NH_3)^{6+}$ cluster ions by NH_3/He reaction gas enables interference-free conditions for accurate and precise determinations of very low titanium concentrations in human serum samples. The instrumental limit of detection (LOD) is 3 ng/L, which is the lowest LOD ever obtained for Ti using quadrupole-based instrumentation. It means that titanium concentrations in control samples from healthy individuals can also be determined accurately.

In the initial study performed in Ghent by Frank Vanhaecke's group, serum samples from four healthy individuals and four patients with four-year old Ti-6Al-4V hip prostheses were collected and analyzed by ICP-QQQ. The typical basal titanium level in control serum samples was under 1 µg/L while for implant patients, values in the range of 2-6 µg/L were observed. These results indicate that implants can cause elevated titanium levels in body fluids, even if the device appears to be functioning correctly. Further investigations may help predict prosthesis malfunction and toxicity, in which case accurate and highly sensitive analytical methods for titanium determination in clinical samples will be invaluable.

By Lieve Balcaen

References

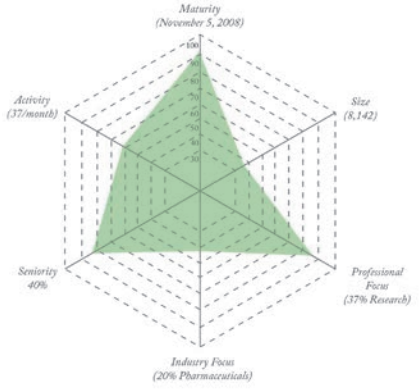
1. *L. Balcaen et al., "Accurate determination of ultra-trace levels of Ti in blood serum using ICP-MS/MS", Analytica Chimica Acta (2013) (DOI: 10.1016/j.aca.2013.10.017) – in press.*

For each of the six groups the following information is shown:

Maturity: date created
Size: numbers of members
Activity: the number of discussions in November 2013
Seniority: by percentage of "senior" members
Industry/academia focus: percentage of industry members and main industry represented
Professional focus: percentage of members who are researchers.

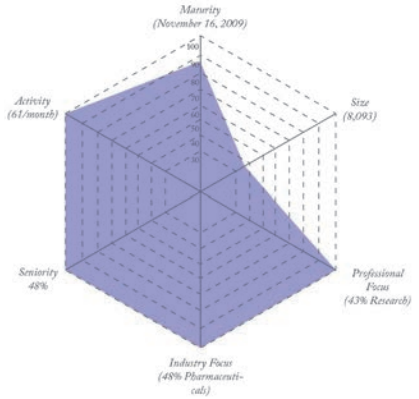
Comparative radar plot data are percentages of the maximum score in each category. For example, "Analytical Chemistry" is the biggest group with 22,466 and so receives 100 percent in that category.

RW



Chromatography & Mass Spectrometry (LC, MS, LCMS) User's Forum

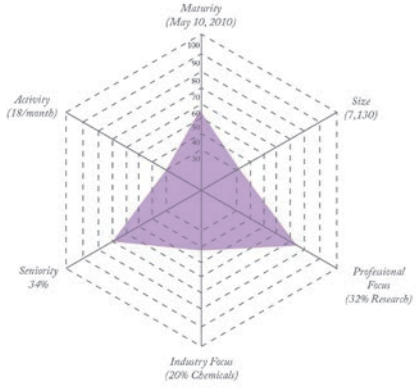
"Networking among Chromatography and Mass Spectrometry (LC, LCMS, MS) end users."



Analytical Chemistry – Method Development

"To spread and share the knowledge in the area of Analytical Chemistry"

To learn how the Group Owner grew this successful group, see "Analytical Links" on page 20.



Drugs / Toxicology / Analytical Chemistry

(Sub-group of Forensic Scientists Online)

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

March of Progress

Where does clinical biomolecular mass spectrometry (MS) sit on the steep slope between hype and hope?

By André Deelder, Center for Proteomics and Metabolomics, Leiden University Medical Center (LUMC), The Netherlands.

45 years ago, when I was a master's student, schistosomiasis was detected by immunodiagnosis: a patient's antibody to the parasite was detected on frozen sections using a second, fluorescently-labeled antibody. Fluorescence rapidly faded and quantitation was based on brightness from – to +++, which was open to personal interpretation. I aimed to develop quantitative assays using "purified" antigens and, after a few years' work, Bas Ploem and I implemented assays based on schistosome protein antigens coupled to agarose beads – the DASS system. A variation on this theme, this time in collaboration with Han Streefkerk, was our quantitative immunoperoxidase assay, marking the arrival of enzyme-linked immunosorbent assays (ELISAs). The perceived impact of these assays was not too high; in 1975, at a presentation that I gave on ELISA, a famous malariologist remarked, "Remember young man: her first name was Eliza, but her family name was Doolittle." So much for the rapid acceptance of novel technologies…

As antibody detection in schistosomiasis reflects active infections poorly, if at all, I began to focus on detection of schistosome antigens in the circulation of the host. Initial assays were not sensitive, detecting only heavy experimental infections, and therefore not useful for human infection diagnosis. Over the years, we painstakingly transformed these assays into highly sensitive and specific tests by evaluating immunoassays, inspired by hybridoma technology's ability to generate thousands of specific and reproducible monoclonal antibodies. Assays became more and more sensitive and we conducted large-scale immunoepidemiological studies, even demonstrating schistosome antigen in a 5,000 year-old Egyptian mummy. Today, a lateral flow assay based on upconverting phosphorescence labels can detect just one pair of worms in a urine sample.

To study the unusual characteristics of glycan moieties, Hans Kamerling, Hans Vliegenthart and I used nuclear magnetic resonance (NMR). This led to the study of antigen synthesis, biomolecular interactions (both surface plasmon resonance and fluorescence correlation spectroscopy), and crystallization studies on Lewis X-antibody interactions, but also to the purchase of our first mass spectrometer – an ion trap instrument – some ten years ago.

Working at a university medical center, I realized that there was a huge and largely unexploited potential for the application of mass spectrometry (MS) in clinical studies and, ultimately, in the clinic. I also realized that we must switch from our initial approach, which was targeted to particular antigens, parasite life-cycle stages, or defined cell populations, to less targeted discovery studies using multi-sample cohorts. With Rob Tollenaar, I began to develop reliable and robust MS-based assays

"At my presentation on ELISA, a famous malariologist remarked, 'Remember young man: her first name was Eliza, but her family name was Doolittle.'"

to define serum profiles and enable detection of colorectal and breast cancer. Our interest and infrastructure quickly grew and the medical center now houses a special unit for research on (glyco)proteomics, metabolomics and MS imaging; we work in close collaboration with clinical colleagues, using a suite of MS systems and a dedicated NMR metabolic profiler platform. Conducting large-scale studies with thousands of samples has made us painfully aware of the importance of sample preparation standardization – something we have heavily invested in.

If I extrapolate my experiences as a medical parasitologist, it seems we are halfway up the slope – but with a long way to the summit. There is now a rapidly growing interest, that is matched (and initiated) by increasingly sophisticated MS instruments, in the quantitative measurement of multiple proteins or metabolites in patient body fluids.

While huge steps have already been made in this area, much work remains, such as the improvement of inter-laboratory reproducibility. And although specific algorithms for complex data analysis are rapidly becoming more powerful, the concept of (ultimate) multi-parameter measurement versus unimarker measurements still largely needs to be integrated into clinical diagnostic procedures. For the analysis of translational modifications and, in particular, glycosylation, the field is still wide open: despite significant progress, the analysis of (aberrant) plasma protein glycosylation is still largely unexplored.

It is interesting to see how, in conjunction with MS, immunoaffinity procedures are increasingly being used to highlight low abundant peptides and proteins. Likewise, I believe MS-analysis will significantly contribute to the further immunoassay optimization. ■

The New Model

What the 2013 Nobel Prize in Chemistry says about predictive sciences

By Adrian Stevens, Senior Manager, Accelrys Limited, Cambridge, UK.

The 2013 Nobel Prize in Chemistry, announced on October 9, 2013, was awarded to Martin Karplus (Harvard), Michael Levitt (Stanford) and Arieh Warshel (University of Southern California), "for the development of multiscale models for complex chemical systems." It recognized their work in bringing together classic Newtonian molecular mechanics and quantum mechanics methods to help scientists model large complex chemical systems and reactions. Given the magnitude of the achievement, this recognition was widely welcomed and, arguably, overdue.

What fascinated me was the associated information provided by the Nobel organizing committee, in particular, this short statement that emphasizes the wider impact: "Today chemists experiment just as much on their computers as they do in their labs." The Nobel chemistry committee is highlighting the value that predictive science adds to our understanding of how molecules interact in chemistry and biology. It is appropriate to do so: the predictive multiscale models and methods developed by Karplus,

"Predictive multiscale models and methods have fundamentally changed the way scientists innovate in bringing new drugs to market."

Levitt, Warshel and others have fundamentally changed the way scientists innovate in bringing new drugs to market. For instance, many pharmaceutical modeling tools are based on CHARMM (chemistry at Harvard macromolecular mechanics) – a molecular dynamics simulation and analysis program that was first developed by Karplus' group decades

"It's not so long ago that we were using Orbit kits or the metal Dreiding kits to build molecular models."

ago. My company's product, the Accelrys Discovery Studio, contains the only commercial version of this widely used program.

It's not so long ago that we were using Orbit kits or the metal Dreiding kits to build molecular models. In the 'good old days', these tools helped us to understand the underlying physics of molecular structures but couldn't tell you anything about how molecules moved, what forces of strain they had within them or, crucially, how they interacted with other molecules. Fortunately, while we were playing with sticks and balls, mathematical models of molecular systems quietly but steadily evolved, progressing from purely theoretical tools that were used by just a few highly specialized computational scientists into robust and broadly accurate predictive tools that are readily applicable to discovery research applications. These have transformed the way that many areas of scientific research are now conducted. A much broader population of scientists can now routinely access and use them; indeed, such tools are now starting to make an appearance on smart phones and tablet devices.

To demonstrate the paradigm shift from theoretical tools into everyday research aids, cast your mind back to the heady days when computational chemists ran simulations on huge (and usually very hot) computing platforms. These were typically power-hungry, expensive machines that were hidden away in broom closets and far removed from "real chemists." Today, if you walk around any lab you will see those same chemists actively collaborating with their project team counterparts using new generations of the same tools. They might, for example, be calculating the properties of a macromolecule or predicting how a compound may bind to a protein target. Today, we consume predictive science directly; it is no longer segregated from the discovery process.

An area where this is of particular value is biotherapeutics. Many of modern medicine's most valuable tools for treating and preventing illness are biologics and topics such as the development of novel antibody technologies have become key research pursuits. However, biotherapeutics also present unique challenges. Their storage and administration typically requires high-concentration solutions with specific biophysical profiles pertaining to solubility, thermal and chemical stability, and low aggregation propensity. Testing for these properties can be time-consuming and expensive. To accelerate development and reduce costs, researchers are increasingly applying predictive methods to identify and optimize the best biologic leads early.

By enhancing direct experimentation, the multiscale modeling techniques developed by this year's Nobel laureates in Chemistry can make these and many other hitherto unsolvable problems solvable. ■

Free Innovation Accelerator!

Open-source scientific hardware is a reality. It gives researchers more freedom to experiment with design and can open new doors in research. What's stopping you?

By Joshua M. Pearce, Department of Materials Science & Engineering and Department of Electrical & Computer Engineering, Michigan Technological University.

Ever finished your meal at a fancy restaurant and realized that you've forgotten your wallet? I had that same sinking feeling when I saw the price of our first round of heat exchanger prototypes. Our heat exchangers use plastic instead of metal and make up for their relatively weak thermal conductivity with very thin layers between microchannels. The numerical simulations were promising so we wanted to optimize the design and verify experimentally. Sadly, we realized that it was impossible to conduct the comprehensive study needed to drive the innovative technology to market at the prices quoted. Buying a commercial laser welding system was out of the question (budget!) and designing one

"Countless microchannels later, the device has saved us tens of thousands of dollars on that one project alone"

from scratch was entirely unacceptable (time!). We turned to the free and open source hardware (FOSH) community for a solution. FOSH is publicly available hardware with designs that can be studied, modified, distributed, fabricated, or even commercialized, which I covered in greater detail in the first issue of this magazine.

Fortunately, graduate student Peter Jansen (McMaster University, Ontario, Canada) had already developed an open-source laser cutter for about 5 percent of the cost of a commercial system; it uses 3D printable mechanical components. We downloaded his schematics, adapted them to our own needs, and created a digitally-controlled polymer laser welder, which we had up and running shortly after the laser arrived. Countless microchannels later, that one device, which allowed us to quickly test innovative designs, has saved us tens of thousands of dollars on that one project alone. Our group now routinely uses, develops and shares scientific FOSH for scientific equipment – everything from rapid prototyping systems to analytical equipment – to allow others to innovate rapidly as well.

We recently completed an open-source mobile water quality-testing platform, based on an open-source desktop colorimeter. We adapted it to be 3D printed and powered by multiple mobile sources, and expanded its ability to include nephelometry (measurement of turbidity). In shoot-outs with commercial systems we've found comparable accuracy and reproducibility for about one tenth of the cost.

Clearly, we are not the only lab to realize the opportunity of FOSH. The concept of open-source scientific hardware is catching on like wildfire all over the globe, as I document in my book, "Open-Source Lab: How to Build Your Own Hardware and Reduce Research Costs". Lower cost, faster innovation cycles, and reduced time investment are great, but the primary advantages of open source hardware are customization and control. Rather than rely on what is on the shelf, you can create scientific instruments that meet your exact needs and specifications, which is particularly helpful to those on the bleeding edge who need never-before-seen equipment to make the next great discovery.

There is a deeper benefit to developing open-source hardware: scientific good karma. People from all over the world have made suggestions and built upon the hardware our group has shared, which, in turn, makes better equipment for us. It's

"We downloaded his schematics, adapted them to our own needs, and created a digitally-controlled polymer laser welder"

"Jansen's current work (as a professor) is the development of a hand-held open-source tricorder. Yes, Star Trek fans, that kind of Tricorder."

like having a global R&D firm with thousands of engineers all working for you. The better they are, the more help you get. And that brings us neatly back to Jansen's current work (as a professor), developing a hand-held open-source tricorder (tricorderproject.org). Yes, Star Trek fans, that kind of Tricorder. It already has a dizzying array of research-grade sensors to measure atmospheric temperature, humidity, magnetic fields, light intensity, color, pressure, location, acceleration, and even non-contact infrared detection. The next generation of tricorders will also include a sophisticated water quality-testing module, which he did not have to spend a penny developing – all the source code for the hardware and software is free, compliments of my lab's work.

If you get the feeling that application of FOSH to science is just getting started, that's because it is. What great innovations will the next generation of open-source scientists develop, with tricorders in their back pockets and 3D printers under their beds? It's exciting to imagine the possibilities. ■

Analytical Links

How I grew an active LinkedIn group of over 7,500 members with a single aspiration – to make all our jobs easier.

By Dev Kant Shandilya, TEVA Pharmaceuticals, India

Let's begin with the obvious: analytical chemistry is a diverse and rapidly expanding field. Every day, the identifications and measurements we make directly affect the safety of living things. Therefore, we – by which I mean analytical scientists – have a fundamental obligation to fully sharpen our analytical skills to better serve society. But that sounds daunting and difficult, doesn't it? How could I help fulfill this basic need more easily? It was with such a question that I decided to create the LinkedIn group "Analytical Chemistry – Method Development" (AD-MD) at the end of 2009 (see "Scanning the LinkedIn Landscape" on page 14).

All I needed was an Internet connection, a computer, some time and the motivation. LinkedIn (www.linkedin.com) is the world's largest professional network. It has 225 million members, who can choose to join various online forums (groups) that match their interests. I positioned the AC-MD as a global platform for sharing and learning. In particular, it has a focus on analysis with advanced instruments – an area that can prove challenging when one is faced with the need to learn a new technique or when a sample is not playing by the rules.

Sharing problems and trouble-shooting isn't always easy – it can be embarrassing and might show a chink in a person's own knowledge. But I believe that we are all slightly more comfortable doing it knowing that we will receive attention from an experienced and diverse group of application specialists who we will most likely never meet in person. I think that the group also enables members to develop analysis skills beyond what they may – or can – learn in the classroom or work place. It's collaborative education. And it's free.

Online groups also leverage the two greatest assets of the Internet – speed and reach. Platforms like mine allow users to receive expert advice very quickly, often with international and/or multiple viewpoints. It also gives people the chance to correspond on breaking news that may affect their work in an informal but professional setting – something I hadn't considered at the beginning. In some parts of the world, professional advice may only be down the hall or at the next coffee break. But in less developed countries, access to expertise is not always so easy to come by. By creating a truly global and open network of analytical scientists, the playing field is leveled and everyone online has the ability to ask questions.

Since the beginning of 2010, AC-MD has attracted 40-60 new members per week – including application scientists, research scholars, consultants, quality control analysts – and generates, on average, four queries each week, with each receiving around

"By creating a truly global and open network of analytical scientists, the playing field is leveled and everyone online has the ability to ask questions."

five responses. It is a considerable success, in my book.

There are few ground rules, but we request that members only share:

- Analytical skills for method development and recent advances
- Data interpretation and data analysis skills and challenges
- Knowledge gathered from past experiences
- Thoughts on new analytical techniques
- Literature and useful links related to analytical chemistry
- Education links related to analytical chemistry
- Workshop and webinar information

My plan is to augment the group by creating subgroups for discussion of specific techniques – especially in areas where further expertise is required – for example, biosimilar analysis, hyphenated mass spectrometry, ion chromatography and capillary electrophoresis. This will only come to pass with the continued engagement and positive support from the community. I am very thankful to the members of AC-MD and hope that they have been able to take as much from the group as they have given. ■

The TASIAs Are Here

Accurate measurement is the very essence of science; better measurement technologies are the fuel of progress. Here, we recognize a year's worth of innovation in The Analytical Scientist Innovation Awards (TASIAs) 2013. The 15 TASIAs enhance sample preparation, separation, identification, quantification, analysis, or a combination of these. How might you use them to develop your own innovative solutions in 2014?

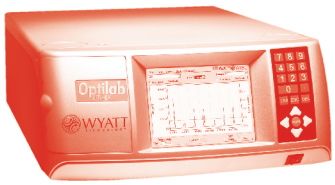
15

1260 Infinity Automated Purification System

An automated LC-MS system that provides pure fractions without method development or scale-up work

Produced by: Agilent Technologies (www.agilent.com)
Detail: Scientists can analyze samples with this generic ultra high-performance liquid chromatography/mass spectrometry (UHPLC/MS) method to confirm successful synthesis of a target compound in a few minutes. On the same combined analytical and preparative system they can start the purification of that sample up to gram quantities without any calculations, method translation or vague guesses about optimum conditions. A focused solvent gradient targeted at the compound of interest ensures maximum column loadability, purity and recovery for the purification step. Picking up the fractions and recovery collection is all that needs to be done.
Impact: Without in-depth training on preparative chromatography, chemists, biologists, or engineers can finally benefit from state-of-the art separation efficiency. Specialized chromatographers can move away from routine purification work to focus on truly difficult separations. Economics of using preparative chromatography are improved as maximum efficiency can be achieved with every sample.

14



The Optilab UT-rEX

A refractive index detector for ultra high performance liquid chromatography (UHPLC) systems

Produced by: Wyatt Technology (www.wyatt.com)
Detail: Specifically designed as a refractive index (RI) detector for use with UHPLC systems using columns packed with small beads, this instrument combines miniaturized components, semiconductor photodiode technology and computer algorithms. It can be operated either stand-alone, as the sole on-line detector or in combination with another detector, such as UV/Vis absorption. The temperature regulation capabilities allow control of the flow path above and below ambient, providing wide dynamic range and the absolute refractive index measurement of solutions, improving resolution, sample and solvent quantity.
Impact: The Optilab UT-rEX adds RI for UHPLC applications, augmenting MS and evaporative light scattering. The instrument's low dead volume of <1.5µl, offers superior resolution. The full range of instrument detection is fully sensitive and always present, while stable RI baselines and RIS signals further enhance sensitivity.

13

3D Preparative Gas Chromatography (GC) System

A preparative multidimensional (MD)-GC system that collects pure compounds from complex samples

Produced by: Department of Analytical Chemistry, School of Pharmacy, University of Messina, Italy
Detail: The instrument consists of three GC systems, equipped with three Deans switch transfer devices, between two subsequent columns and the collection system. The switching elements, located inside the ovens, are connected to three advanced pressure control systems, which supply helium carrier gas at constant pressure. The GC systems are equipped with split/splitless injectors and a flame ionization detector. A lab-constructed collection system, based on a heated aluminum block (300 °C) equipped with a PT-100 sensor, is located within the GC oven roof.
Impact: Structural elucidation of molecules is challenging and requires the isolation of pure compounds. An exemplar: When a single apolar column was used, an analyte purity of 88.6 percent was attained; this was raised to 92 percent when a bidimensional "apolar-polyethylene glycol" instrument was used. The addition of a third analytical column, plus a third transfer device to direct the GC effluent to the collector, gave 99.1 percent purity. The MD-GC is a promising tool for the characterization of unknown molecules, as well as for the collection of known components from complex samples.

12

Chromaster Ultra Rs

UHPLC system with high resolution and sensitivity

Produced by: VWR/Hitachi (www.vwr.com)
Detail: Users wanted a UHPLC system capable of delivering the performance necessary to couple two high-resolution columns, resulting in a column with an equivalent length of 500 cm, a 3 mm internal diameter, and 1.9 µm particle size. However, with a backpressure of 1350 Bar at a flow rate of 0.5 ml/min, a 1400 bar pump had to be designed. In addition to new components, Liquid Beat Technology (LBT) was specially designed to enable highly stable, very high pressure pumping. The plunger is controlled with a changeable stroke, based on calculated solvent compressibility and a sensor on each pump head enables stable pumping.
Impact: The engineers at Hitachi were given a challenge: to design a system capable of providing the resolution and sensitivity needed for related compound analysis in order to meet potential challenges in regulated laboratories. The Chromaster Ultra Rs is the result.

11

ASCENT 2.0

Automated chromatography data review and analysis expert software

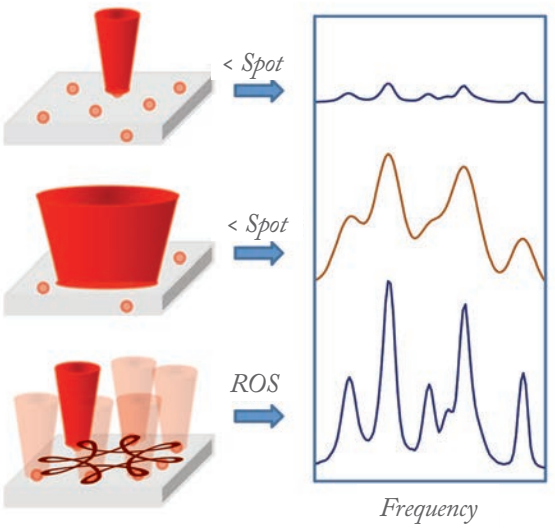
Produced by: Indigo Biosystems (www.indigobio.com)
Detail: ASCENT 2.0 gives labs the ability to practice true review-by-exception, bringing labs closer to full auto-verification. Key features include expert peak quantitation, consistent baselines, automatic calibration, and comprehensive, user-configurable quality rules. Specifically, its expert peak quantitation uses a one-of-a-kind peak picker based on a digital noise filter algorithm. ASCENT uses exponentially-modified Gaussian distribution (EMG), which minimizes peak distortion while simultaneously maximizing the signal-to-noise ratio of the peak area. It can be used with any instrument data and integrated into laboratory Information management systems (LIMS).
Impact: Customers are reporting a 90 percent reduction in the number of peaks that require review, a 75 percent drop in labor costs for data review, and a 50 percent drop in sample rework. Also, with the secure hosted environment, users are able to review their data anywhere, anytime, giving chromatography reviewers a new sense of autonomy. These benefits align to move chromatography/mass spectrometry analysis towards full auto-verification.

10

Inert Flow Path Solutions

Eliminating the weakest link in GC-MS: activity within the flow path

Produced by: Agilent Technologies (www.agilent.com)
Detail: GC and GC-MS detector technology continues to advance, with parts-per-billion, indeed parts-per-trillion, detection levels becoming common. However. since detectors can only detect analytes that reach them, even the most powerful detector technology is at risk to the GC sample flow path. Activity within the flow path can adsorb or catalytically decompose sensitive or labile analytes, causing peak tailing, signal loss or totally preventing them from detection. Inertness of the complete sample flow path is critical to accuracy and sample residence or contact time, along with surface area, are the most likely contributors to activity, with the GC column and inlet liners the largest potential source of active sites. New chemistries and demanding test protocols for inertness dramatically reduce sample loss due to activity in columns and liners and investigation has turned to the remaining components in the flow path that can mask or hide active compounds.
Impact: The combined impact of specialized surface chemistries for varying materials with a solid understanding of GC design and component functionality results in an inert flow path for GC. With minimal compound loss and degradation along the flow path, the sample entering the detector is truly representative of the sample injected, ensuring more accurate quantitation of active analytes, especially at trace levels.
The Judges Say: *"Reduces impact of active surfaces significantly."*



9

Raster Orbital Scanning (ROS) Sampling for Raman Spectroscopy

A tightly-focused laser beam is scanned over a sample surface to acquire Raman spectra with high resolution and increased sensitivity

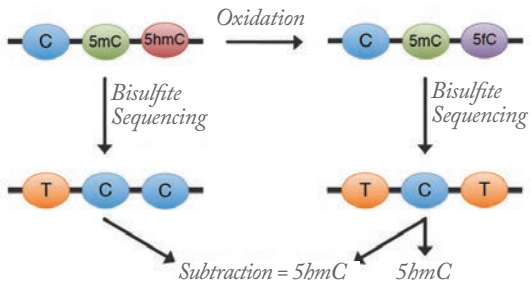
Produced by: Ocean Optics (www.oceanoptics.com)
Detail: Improved sampling of non-homogeneous mixtures by raster scanning a tightly focused beam across the surface allows ROS to provide a five- to ten-fold increase in sensitivity without sacrificing resolution. Raster scanning gives accurate measurement of surface enhanced Raman spectroscopy (SERS) samples by sampling more Raman-active compounds and decreasing the background signal. The larger sampling area allows common surfaces to be used for SERS, which is typically reserved for pristine surfaces. Improving the robustness of substrates extends the applicability and utility of SERS, allowing more analytes to be measured under more demanding conditions.
Impact: The novel ROS sampling technique increases the reliability of Raman measurements by eliminating the need for rigorous sample preparation, driving development of low cost tools that extend Raman into mainstream applications. This novel Raman sampling technique improves the reliability of traditionally demanding data acquisition and interpretation. Raman spectroscopy can now extend into medical diagnostic and biological applications. Answers are more reliable, and complex samples can be analyzed with less sample preparation. For example, ROS enables the use of low-cost, common paper as a SERS substrate for a point-of-care diagnostic test that can be read by a technician without extensive spectroscopy training.
The Judges Say: *"Allows us to better see where molecules actually are!"*

8

Oxidative Bisulfite Sequencing (oxBS-seq)

Quantitation of covalent DNA modifications at single-base resolution

Produced by: Cambridge Epigenetix (www.cambridge-epigenetix.com)
Detail: Gene sequencing at its most basic level identifies the four bases (G,C,A, and T). For some time, cytosine (C) has been known to have a variant, 5-methylcytosine (5mC), often called "the fifth base". In 2009, a further variant of the C base, 5-hydroxymethylcytosine (5hmC) – the sixth base – came to light. Rapidly increasing research interest has shown that 5hmC is an important epigenetic marker in cell differentiation, neurodegenerative diseases, cancer, stem cell dynamics and a variety of other processes, with distinct functions from 5mC. However, traditional sequencing methods cannot discriminate between 5mC and 5hmC. Oxidative bisulfite sequencing (oxBS-seq) is the first reliable technique to quantify 5mC and 5hmC at single-base resolution.
Impact: Discrimination between 5mC and 5hmC at single-base resolution opens up new avenues in epigenetic research that could finally explain why identical genomes don't create identical organisms and how life on earth continues to diversify so quickly and extensively.
The Judges Say: *"This could be very important in light of increasing interest in epigenetics."*



7

LCMS-8050

Speed and sensitivity for qualitative – quantitative analysis in MS/MS

Produced by: Shimadzu (www.shimadzu.com)
Detail: This triple quadrupole LC-MS/MS features the world's fastest data acquisition rates as well as high sensitivity and high data quality for trace-level quantitation in clinical research and other markets. Attogram-level sensitivity is achieved by the UFsweeper III collision cell, which enhances collision-induced dissociation (CID), and a heated electrospray ionization (ESI) source, which improves desolvation while enhancing ionization efficiency. The high voltage power supply enables a maximum scan rate of 30,000 u/sec with a 5 msec polarity switching time. It is now possible to include 1,000 events with up to 32 channels each for a maximum of 32,000 MRMs per analysis.
Impact: The LCMS-8050 sets a new benchmark in terms of analyses speed, sensitivity and cost of ownership. Thanks to ultra fast polarity switching (UFswitching) and ultra-high-speed scanning (UFscanning), customers are able to reduce the number of analyses per sample and, therefore, reduce cost per sample.
The Judges Say: *"I am a big believer that scan speed and sensitivity is essential for MS. I like this direction."*

6

ACQUITY QDa Detector

A small, easy-to-use and affordable mass detector that brings high-quality mass spectral data to chromatographic separations.

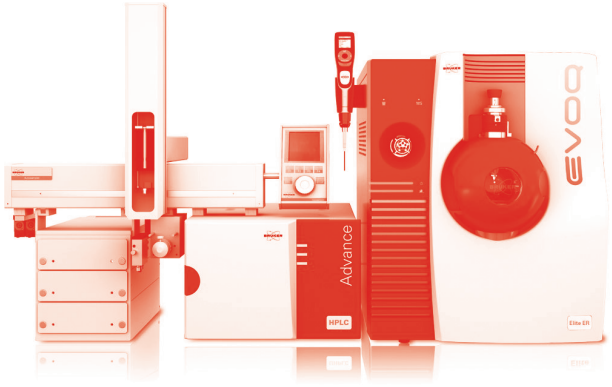
Produced by: Waters (www.waters.com)
Detail: The ACQUITY QDa Detector brings MS analysis to all chromatographers. With on/off simplicity, the detector fully automates sample analysis and eliminates sample-specific adjustments for certainty in results, from user to user and system to system. Robust and reliable enough for routine use, it generates the high quality MS data expected of a single quadrupole MS in a mass detector no larger than – and as easy to use as – a more familiar photodiode array detector.
Impact: Armed with MS data, separations scientists can be more valuable and more confident contributors to the laboratory. The ACQUITY QDa Detector answers many of their questions: Did I synthesize what I intended to and how do I know? Is this peak really pure? Have I accounted for all components in my sample? What is the component represented by this peak? Will my method pass validation? The result: greater productivity.
The Judges Say: *"Turns MS into a regular detector that can be operated by all users. Important improvement in terms of reliability of the analysis."*

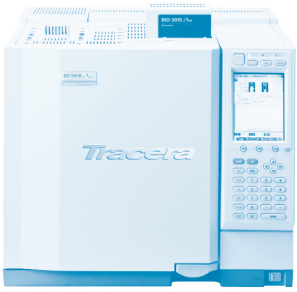
5

EVOQ Elite ER

A liquid chromatography mass spectrometer with extended mass range for proteomics

Produced by: Bruker (www.bruker.com)
Detail: Offering the unmatched proteomics testing that LC-MS TQ technology delivers, together with additional benefits to improve productivity in the routine laboratory, the Elite ER is the latest model in the EVOQ liquid chromatography mass spectrometry range. With an extended mass range of 2,000 m/z, it delivers the high sustainable sensitivity and specificity needed for rigorous proteomics quantitation over thousands of samples. Moreover, the EVOQ incorporates a number of targeted innovations for routine proteomics testing in both a discovery and hospital environment. It delivers the fastest sample-to-report time and exceptional robustness, for maximum productivity.
Impact: A range of unique hardware and software features support laboratories undertaking routine proteomics testing. The Active Exhaust provides exceptional robustness by eliminating the recirculation of sprayed gases, to reduce chemical noise and allow longer exposure to harsh matrices: less time is spent cleaning and more time running samples. PACER software delivers exception-based data review, decreasing sample-to-report time by flagging any runs that fail to meet preset criteria. EVOQ is the first solution to offer the best quality LC-MS proteomics testing in a fast and easy-to-use format, ideally suited to routine use.
The Judges Say: *"Will be a big boost for proteomics."*





4

BID (Barrier Ionization Discharge) Detector

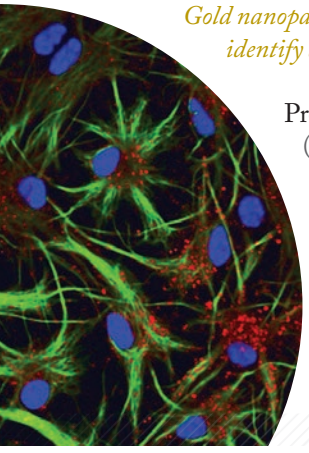
A new GC detector design based on innovative plasma technology

Produced by: Shimadzu (www.shimadzu.com)
Detail: The Barrier Ionization Discharge Detector (BID) is a novel universal GC detector designed for trace analysis. Using new technology, helium plasma is generated inside the BID. A quartz glass tube is used as a dielectric barrier between helium plasma and the detector electrodes, shielding them from all kinds of contamination. With the exception of helium and neon, all types of components are ionized by the light emitted from the helium plasma and are measured on 'kovar alloy' collection electrodes. The result is a detector capable of ultra trace analysis in combination with unsurpassed long-term stability.
Impact: The flame ionization detector (FID) is popular in gas chromatography due to its outstanding combination of long-term stability, precision and sensitivity. Unfortunately, its detection spectrum lacks permanent gases, such as nitrogen, oxygen and carbon dioxide. The BID detector fills this gap. It is more sensitive than the FID but comparable in precision and long-term stability. This makes it an ideal tool for trace measurements of permanent gases and light hydrocarbons, especially volatile oxygenated organic components.
The Judges Say: *"Can replace the most important GC detector: the FID. It is better and easier to use, with a novel measurement principle."*

3

SmartFlare live cell RNA detection technology

Gold nanoparticles that enter cells naturally and identify desired RNA targets in live cells

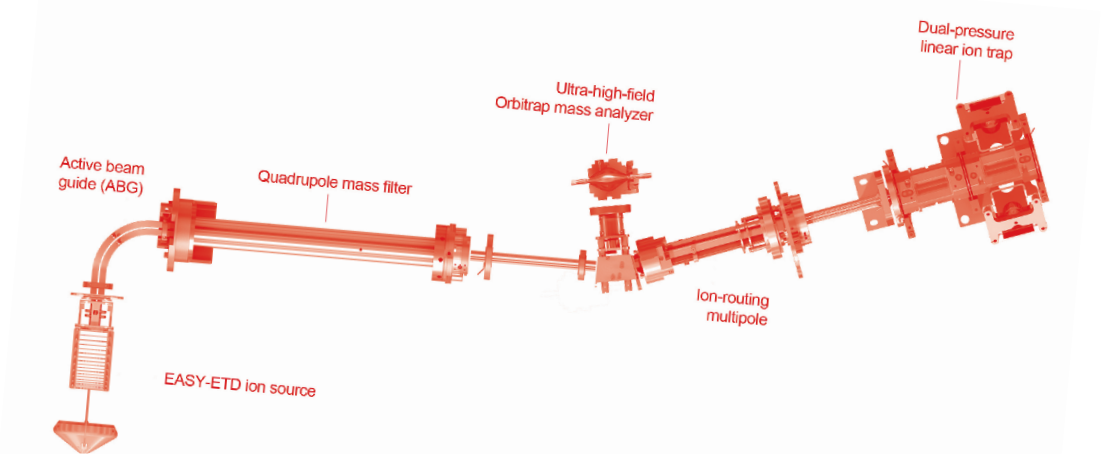


Produced by: Merck Millipore (www.millipore.com)

Detail: Gold nanoparticles with duplexed oligonucleotide strands complementary to target RNA sequences, SmartFlare probes enter live cells through endocytosis. They bind targets via hybridization, consequently releasing a fluorescence signal which is measured to analyze the expression level of the a specific RNA target. Afterwards, SmartFlare probes exit the cells over time, leaving the cells unharmed, unchanged, and ready for downstream assays.

Impact: With SmartFlare detection probes, researchers can get a real-time glimpse of gene expression in live cells, and can further characterize those same cells with downstream assays. SmartFlare probes enable correlation of protein and RNA of the same cell, providing more physiologically-relevant data. Being able to sort cells based on gene expression and reuse those cells for downstream assays aids work with difficult targets or looking at the impact of modulating RNA in live cells.

The Judges Say: *"This technology is fantastic and will be of significance for a great deal of biological research."*



2

Orbitrap Fusion Tribrid LC-MS system

Three different mass analyzers working in harmony to create a new class of mass spectrometer

Produced by: Thermo Scientific (www.thermoscientific.com)

Detail: The Tribrid architecture provides unprecedented resolution, scan speed and versatility, letting scientists look deeper into proteomes, identify lower-abundance analytes in more complex samples, make quantitative comparisons over more experimental conditions, and elucidate structures faster. Demonstrated resolution, greater than 450,000, is an order of magnitude higher than previous instruments. It also features two built-in ion sources: one for the main ion stream and a second internal source for electron transfer dissociation (ETD), an a internal calibration feature.

Impact: The system is already impacting biological research. The Josh Coon Lab at the University of Wisconsin-Madison, for example, recently achieved comprehensive analysis of the yeast proteome in just one hour; four times faster than the field's previous best time. Coon has said that this speed and quality can transform proteomics and will "doubtless open new avenues of research," and predicts that comprehensive analysis of the human proteome in three to four hours is within reach.

The Judges Say: *"It is a truly powerful tool!"*

1

M908

Introducing handheld high pressure mass spectrometery

Produced by: 908 Devices (www.908devices.com)

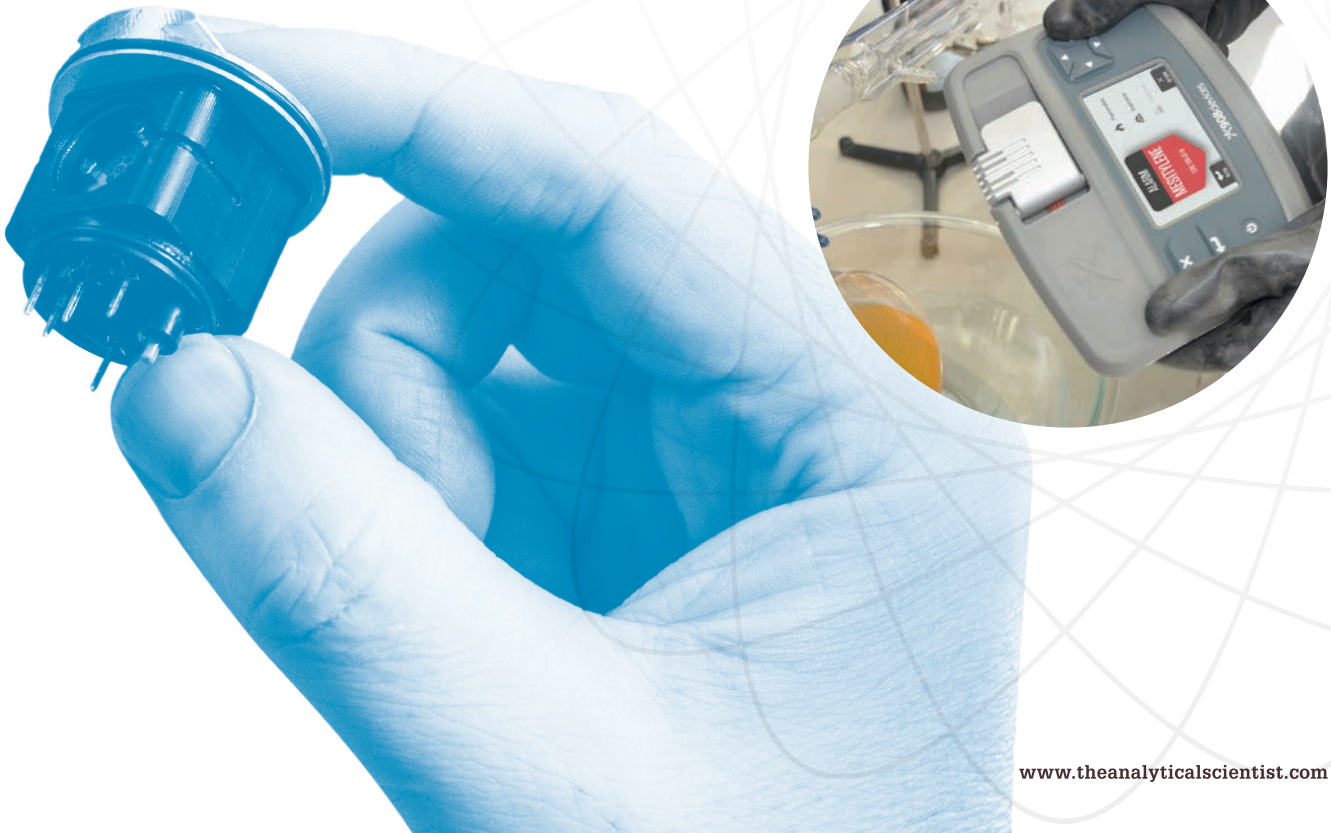
Detail: Using high pressure mass spectrometry (HPMS), this chemical detection and identification tool is handheld, battery-operated and ten times smaller and lighter than any commercial mass spectrometer device. Operating much closer to atmospheric pressures than conventional MS systems, M908 uses micro-scale ion traps in a package designed to US defense-standard (MilSpec) ruggedness and reliability. HPMS technology combined with M908's algorithms and integrated software provides unprecedented levels of selectivity and sensitivity at low ppb levels for actionable intelligence with push-button operation.

Purpose built for safety and security applications, M908 detects and identifies trace levels of chemical weapons, TICs, explosives and drugs. M908 is:

- Simple to operate
- Quick, with one-second analysis and rapid clear down time
- Highly resistant to interferents and false-positive readings
- Rugged, with MilSpec design for extreme environments and rough handling
- Battery powered
- Light, with a total weight of 1.7kg

Impact: M908 frees the powerful capabilities of mass spectrometry from the confines of the central laboratory. It provides accurate and immediate identification of the increasing range of chemical and explosive threats; existing handheld chemical detectors work for only a handful of compounds and are plagued by false alarms. Beyond safety and security, HPMS offers a broad analytical platform for life science research, food safety testing and a host of other applications that will benefit from small footprint analysis on the workbench or handheld, field operation.

The Judges Say: *"Revolutionary technology, will be an inspiration for new generations."*



Innovation Showcase

Sponsored Section

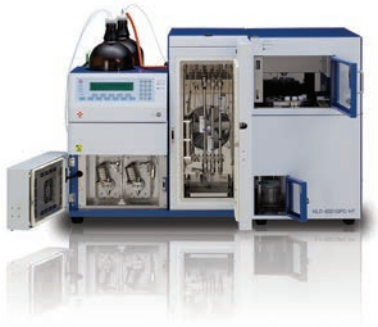
EcoSEC-HT – Unmatched Stability for High Temperature GPC

Tosoh Bioscience launched a compact high temperature system for the GPC analysis of engineering polymers and a set of suitable TSKgel GPC columns.

The EcoSEC-HT, a new all-in-one high temperature GPC/SEC system, provides thermostatization up to 220 °C. Autoinjector, pumps, and a dual-flow refractive index (RI) detector with an independent temperature control are integrated in a compact system including safety features, such as auto-lock doors and gas sensors. The combination of a high performance heating system and the extremely stable RI detector speeds up equilibration time and reduces the baseline noise. The optional sample processing unit can process up to 24 samples at temperature programs ranging from 40 to 220 °C. The TSKgel GMHHR- HT2 series of GPC columns was developed to complement the HT GPC system when using the maximum temperature range up to 220°C.

Tosoh Bioscience, a global leader in the field of bioseparation, offers a comprehensive line of biopurification media, (U)HPLC columns and SEC/GPC instruments.

TOSOH BIOSCIENCE



www.ecosec.eu

Setting New Benchmarks in Antibody Analyis

Tosoh Bioscience expanded the renowned TSKgel SW series of SEC columns by new silica-based (U)HPLC columns covering different aspects of antibody analysis.

TSKgel SuperSW mAb HTP supports high throughput antibody analysis and was developed for easy transfer of SEC methods, which have been developed on conventional gel filtration columns, to fast UHPLC analysis. TSKgel SuperSW mAb HR, designed for high resolution monoclonal analysis, provides superior resolution for the analysis of fragments, monomers, and aggregates in one run. TSKgel UltraSW Aggregate covers the range of higher molecular weights and best separates antibody dimers and higher aggregates. The new columns are ideally used in UHPLC instruments, even though the columns will not create back pressures exceeding the pressure limits of standard HPLC systems.

Tosoh Bioscience, a global leader in the field of bioseparation, offers a comprehensive line of biopurification media, (U)HPLC columns and SEC/GPC instruments.

TOSOH BIOSCIENCE

www.tosohbioscience.de

Select-eV – Revolutionary Variable-energy Ion Source Technology for GC–MS

Select-eV allows the analyst to adjust the energy used for electron ionisation on a sliding scale down to 10 eV.

Offering variable-energy electron ionisation with no loss of sensitivity, Select-eV is poised to revolutionise the way analysts approach the identification of challenging samples by GC–MS. When ionisation at 70 eV doesn't discriminate between similar compounds, Select-eV allows effortless switching to the lower ionisation energies that preserve the molecular ion and structurally significant fragments needed for reliable assignment.

Available with the ALMSCO BenchTOF series of mass spectrometers and due to launch in late January 2014, Select-eV technology allows the analyst to harness the power of soft ionisation without the need to use reagent gases or switch ion sources.

Since 1997, Markes International has led the way in developing instrumentation to enhance the measurement of trace-level chemicals by GC.

MARKES international

www.markes.com

Chromolith® HighResolution HPLC Columns

Speed, packing shape, and reproducibility – Nothing to lose, everything to gain

Chromolith® HighResolution columns are the ideal alternative to sub 3 µm particle columns: At a 1 ml/min flow rate a chromatogram run on a Chromolith® HighResolution column looks almost identical to the same chromatogram run on the 2.6 µm ID core-shell particle column, but with significantly less back-pressure. In addition, Chromolith HighResolution provides a min 30% longer column lifetime.

Merck Millipore's monolithic Chromolith® columns are made from a single rod of high-purity monolithic silica, rather than from particles. Their unique bimodal pore structure allows two-three times faster analysis then particle-packed columns. Method transfer from particulate to monolithic Chromolith® columns is easy since the chromatographic selectivity of Chromolith® columns is very similar to that of many modern particulate HPLC columns.

Merck Millipore is the Life Science division of Merck KGaA of Germany and offers a broad range of innovative, performance products, services and business relationships that enable our customers' success in research, development and production of biotech and pharmaceutical drug therapies. Merck Millipore operates as EMD Millipore in the US and Canada.

www.merckmillipore.com/chromatography

Innovation Showcase

Sponsored Section

Robustness Testing with DryLab®4

The Robustness Module tests the tolerance limits of your working point by evaluating how small fluctuations in up to six method parameters affect the critical resolution of the separation.

Robustness testing is a vital stage of method development that determines the success of a method and the likelihood of Out of Specification (OoS) results.

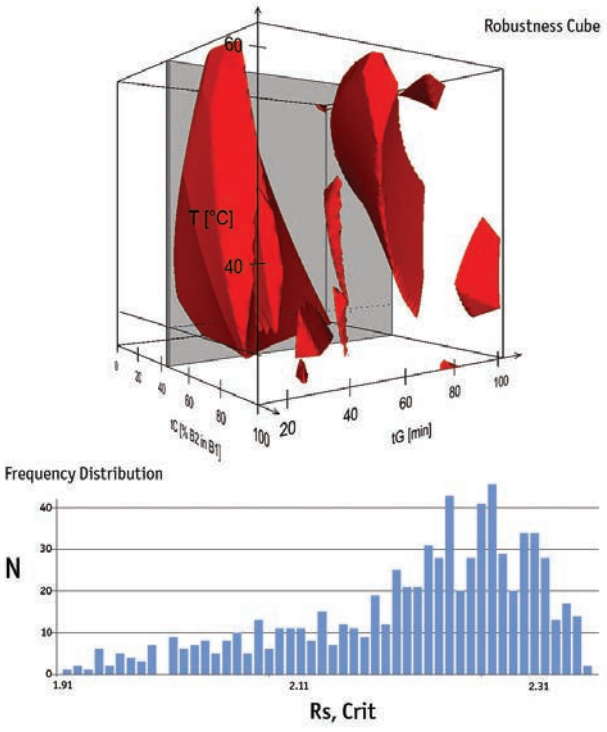
Due to small unpredictable inconsistencies in experimental conditions, this testing is necessary to ensure that a statistically safe set point is chosen during method development.

Our new robustness module aims to reduce the risk of OoS results by calculating the number of successful experiments that result from given variabilities in six of the most influential parameters of the method.

It also determines which factors are especially sensitive to variations and must be strictly controlled to maintain the desired critical resolution.

These calculations take under one minute, and based on the outcome, the set point can be easily changed to get safer results immediately.

By employing this module, users can quickly find methods that have a 100% success rate in routine applications!



Molnár-Institute develops DryLab®4, the next generation (U)HPLC modeling software. With its comprehensive resolution and robustness mapping abilities, DryLab®4 offers an intuitive and visual solution for method development, optimization, trouble-shooting, robustness testing, and training. To learn more, visit our website: www.molnar-institute.com.

MOLNÁR-INSTITUTE
for applied chromatography

www.molnar-institute.com

Innovation Showcase
Sponsored Section

Agilent Inert Flow Path Solutions – Eliminating the Weakest Link

Agilent Inert Flow Path solutions ensure a reliably inert GC flow path for higher sensitivity, accuracy, and reproducibility… especially at trace levels.

Regulatory agencies are continuously lowering requirements for GC and GCMS detection limits of increasingly active analytes. To achieve these part-per-million, or part-per-trillion levels, you cannot afford adsorption caused by flow path activity. For labs that need to perform trace level analysis on very active compounds, Agilent Inert Flow Path solutions minimize activity along every step of the flow path and ensure a reliably inert flow path for higher sensitivity, accuracy, and reproducibility. These analyses can now be done with the utmost confidence because the inert flow path ensures that analytes reach the detector to achieve the exceptionally low detection levels offered by the latest detector technology.

Creating an inert flow path requires identifying then deactivating all internal surfaces of the flow path that could come in contact with the sample. The combined impact of specialized surface chemistries for varying materials with a solid understanding of GC design and component functionality results in an extremely inert flow path for GC.

For more info:
www.agilent.com/chem/inert.

AGILENT INERT FLOW PATH

Agilent Technologies Inc. (NYSE: A) is the world's premier measurement company and a technology leader in chemical analysis, life sciences, diagnostics, electronics and communications. www.agilent.com.

Agilent Technologies

www.agilent.com/chem/inert

Innovation Showcase

Sponsored Section

Clarity MS Software Solution

DataApex integrated MS capabilities into their chromatography SW package; users can benefit from simplified workflow

Clarity Chromatography Software version 5.0 newly includes the MASS SPECTRA (MS) data processing module. It has been designed to provide seamless integration between the MS detector and chromatograph. Clarity MS enables users to evaluate MS spectral and conventional chromatography data together in one software. Spectral data, together with chromatograms, add a third dimension to analytical data analysis. The software provides interactive spectral analysis, peak purity analysis and compound identification based on spectral library search. Clarity MS is currently offered primarily as an OEM solution. The first of the supported MS systems is Advion's Line of Expression CMS.

DataApex is an independent chromatography software vendor, recognized for reliable, easy-to-use products and extensive customer support.

DataApex

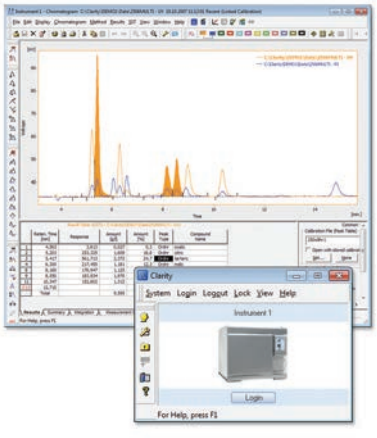
Clarity Chromatography Software Can Control More Than 450 Instruments

Flexible solution that provides unified interface for multi-vendor instrumentation. The portfolio of controlled instruments is constantly growing.

DataApex provides tools for GC/HPLC/MS detector manufacturers, allowing them to easily develop control drivers for their instruments and easily join the established independent platform of Clarity Chromatography Software. Besides extensive functionality, Clarity brings easy operation, unmatched free user support, including free SW updates and optional extensions for specific applications (PDA, SST, GPC, NGA, CE etc.) Clarity SW is available in English, Chinese, Russian, Spanish, French and German localizations. Recently introduced, Clarity2Go is a free application for both Android and iPhone mobile phones. It allows for online monitoring of the status of the analyses. A free demonstration version is available from the DataApex website.

Clarity Chromatography Software is sold in over 80 countries and is also available for OEM.

DataApex



http://www.dataapex.com/?promotion=MKT00966

941 Eluent Production Module: Inline Eluent Preparation for Ion Chromatography

With the 941 Eluent Production Module, Metrohm integrates Inline Eluent Preparation into the 940 Professional IC Vario and 930 Compact IC Flex ion chromatographs.

It is even possible to integrate the continuous supply of ultrapure water that the system requires. To this end, any standard tap water connection can be linked to an ELGA PURELAB flex5 ultrapure water system, which is then connected to the 941 Eluent Production Module. This means that ion chromatography can be performed using ordinary tap water!

The benefits of Inline Eluent Preparation using the 941 Eluent Production Module include:

- Stable retention times, contamination-free working, and reliable measuring results
- Free choice of eluent, concentration, and composition
- Easy to integrate new and existing applications
- Uninterrupted system operation for up to one month, maintenance-free
- Fully automated Inline Eluent Preparation replaces time-consuming, error-prone, manual working steps in everyday laboratory routine

Metrohm

scTRACE Gold – New Sensor for Straightforward and Reliable Determination of Arsenic in Water

Metrohm is pleased to introduce the scTRACE Gold, a new sensor for the straightforward voltammetric determination of arsenic in water.

The scTRACE Gold is simply plugged in the electrode shaft; as there is no preconditioning required, users can start measuring immediately after. Analysis time per sample is approximately 10 minutes only and detection limit is well below the 10 µg/L stipulated by the latest legislation in the US and the EU.

The scTRACE Gold combines all three of the electrodes needed for the measurement in one sensor. Screen printing makes it very affordable, which means that regular replacements are not a problem. This ensures reliable results at all times. Moreover, the scTRACE Gold does not need any maintenance.

Another benefit of voltammetric measurements with the scTRACE Gold is the ability to discriminate between different oxidation states of arsenic, notably the highly toxic As(III) and the less toxic As(V). The scTRACE Gold can be used with any Metrohm voltammetry measuring stand.

Metrohm

http://www.metrohm.com

Innovation Showcase

Sponsored Section

Precision Series Gas Generators for GC Instruments

Peak Scientific has launched the Precision Series, a beautifully designed modular system offering a GC gas supply solution specific to your lab.

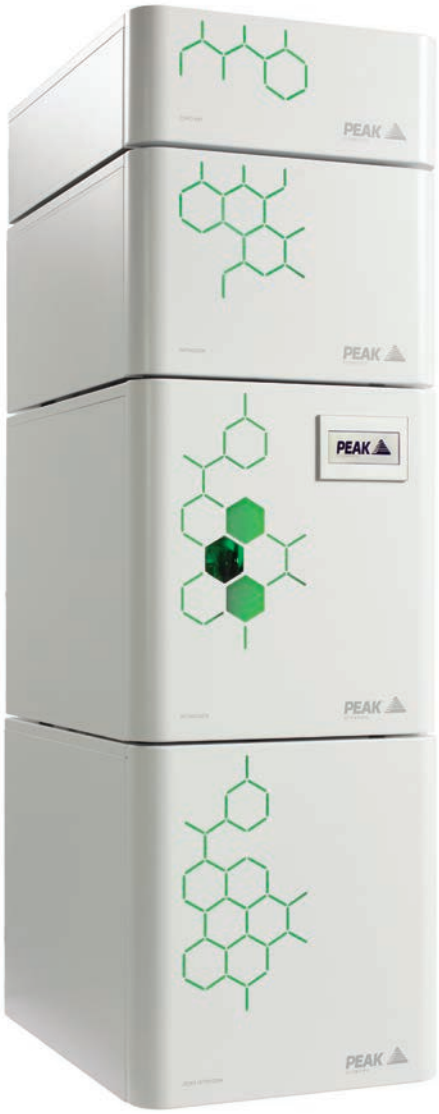
The range consists of Nitrogen, Hydrogen and Zero Air generators for both Carrier and Detector gases including MS, FID, ECD and TCD. Whatever the application, we understand that the lab is a unique environment demanding precision, accuracy, reliability and design steeped in functionality. The precision Series will keep your gas flowing with consummate accuracy and reliability whatever your needs.

The Precision Series incorporates a space saving stackable design, allowing individuals to make the most of their lab space. Additionally the modular design allows for different combinations to suit single and multiple GC applications. The Precision series can also accommodate Helium to Hydrogen conversion as natural resources run low and costs rise. It also eliminates the inconvenience of gas cylinders: no more changing over, no more supply issues, no safety worries. As a result the range is extremely low maintenance and provides long term cost stability.

Peak Scientific are a world-renowned hydrogen generator and nitrogen generator manufacturer. Our range of products includes the Genius, Infinity and Precision generators, which are small to large scale nitrogen and hydrogen gas systems for the GC and LCMS markets.

PEAK SCIENTIFIC

www.peakscientific.com



Innovation Showcase

Sponsored Section

ACE® SuperC18™ - Ultra-inert UHPLC/HPLC Columns with Extended pH Stability

Encapsulated Bonding Technology (EBT™) dramatically increases the ligand coverage of the silica surface to increase stability and effectively eliminate the negative effects of unbonded silanol groups on UHPLC/HPLC separations.

ACE® SuperC18™ UHPLC/HPLC columns are ultra-inert and offer excellent stability across an extended pH range (pH 1.5 to 11.5), allowing chromatographers to routinely exploit beneficial selectivity changes at low, intermediate and high pH. These columns can be used with both MeOH and MeCN mobile phases and offer rapid column equilibration without memory effects. They are designed for use with LC/MS compatible buffers and offer ultra-low bleed for improved LC/MS compatibility. Analytical columns of 2µm, 3µm and 5µm particle sizes are supplied in dual compatible UHPLC/HPLC Excel hardware and are stable up to 1,000bar/15,000psi. A 10µm particle size is also available.

ACE UltraCore SuperC18 and UltraCore SuperPhenylHexyl are now also available - offering all the proven advantages of ACE UHPLC/HPLC columns in new solid-core (superficially porous) particles.

Advanced Chromatography Technologies Ltd

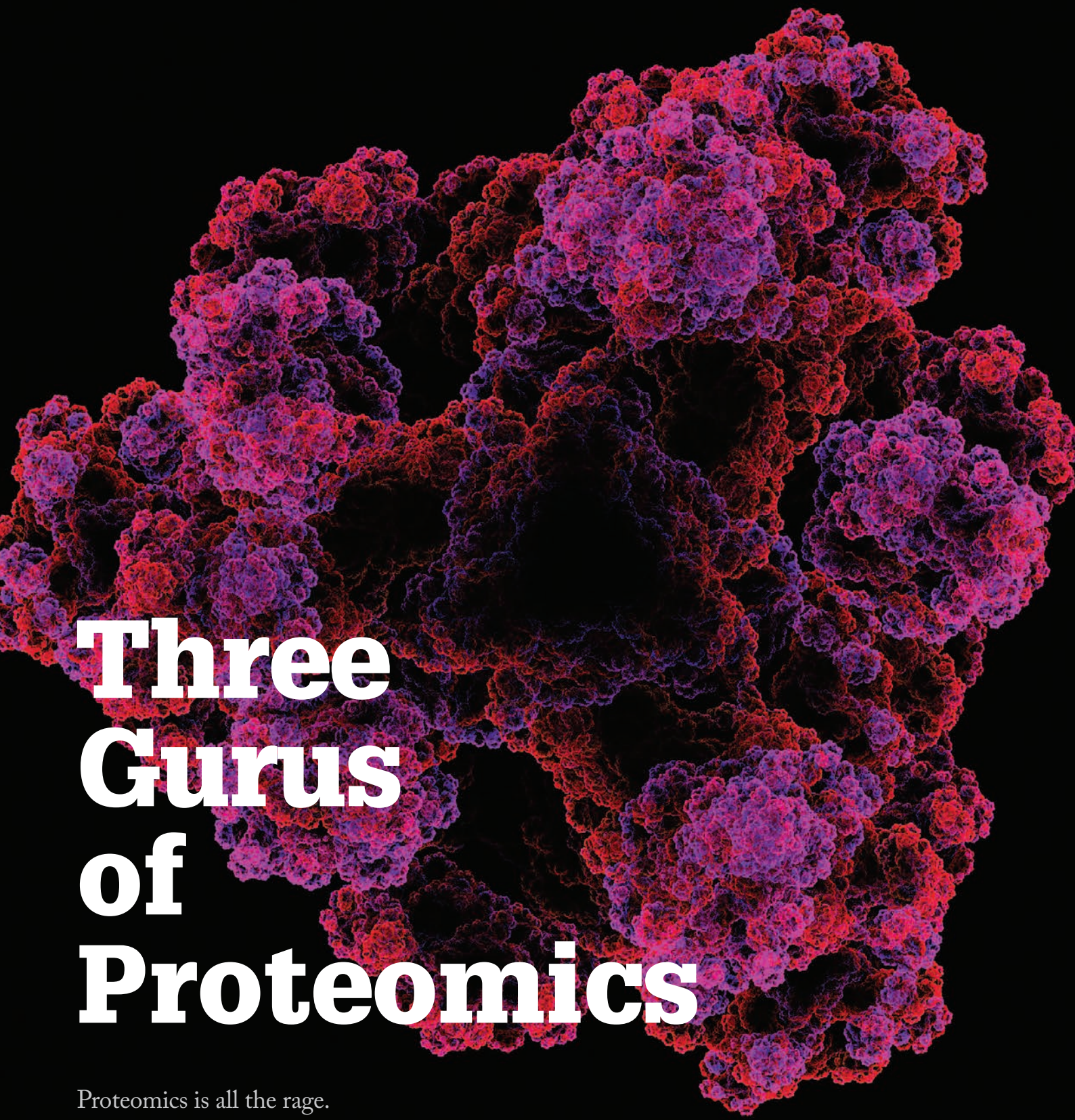
New ACE® C18-Amide and ACE CN-ES phases for UHPLC and HPLC

Advanced Chromatography Technologies launched new ultra-inert phases with alternative selectivity to provide "Extra Resolving Power" for UHPLC and HPLC method development.

ACE® C18-Amide and CN-ES are new phases that incorporate the latest developments in LC stationary phase design to provide chromatographers with more options for changing selectivity - without compromising stability, robustness or reproducibility. Both new phases complement existing phases, which include the novel ACE C18-AR and ACE C18-PFP chemistries. The unique ligand design of these products incorporates an extended alkyl chain spacer between the silica surface and the functional group, which gives extra phase stability and increased column lifetime. All phases can be used in 100% aqueous mobile phase conditions. Particle sizes of 2, 3, 5 and 10µm are available.

ACE ultra-inert, high performance UHPLC/HPLC columns are available globally through our extensive approved distributor network.

http://www.ace-hplc.com



Three Gurus of Proteomics

Proteomics is all the rage. Here, three leaders in the field – Christian Huber, Barry Karger and John Yates – go beyond the hype to discuss where the field is going and what might hold progress back.

Fascinated by the diversity of biological macromolecules, Christian Huber strived to find biomedical applications for the analytical methods he developed. After becoming chair of analytical chemistry at Saarland University, he moved into proteome analysis. More recently, he joined the University of Salzburg's molecular biology department in Austria. "It is fascinating how state-of-the art analytical technologies, most of all chromatography and mass spectrometry, can be used to study the molecular biology of cancer, nanotoxicity, or drug integrity," he says.

Barry Karger is Director of the Barnett Institute of Chemical and Biological Analysis and James L. Waters chair in analytical chemistry, Northeastern University, Massachusetts, USA. "I find science exciting and I am interested in not only analytical chemistry but the problems it must address". Barry integrates various technologies into separations and detection, especially LC- and CE-MS. "I try to understand what's important in the areas where we apply our technology, for example, biotechnology or clinical research."

John Yates received a PhD in Chemistry from the University of Virginia in 1987 and is now a professor in the department of cell biology at The Scripps Research Institute in California, USA. There, John's group uses information yielded by tandem mass spectrometry to identify proteins in complex mixtures. John, who came out top in our 2013 Power List, was asked what excites him about his role: "As new questions come up in biology, it often requires technology development to get a real insight, and that's made life fun over the last 20 years."

On the Major Challenges in Proteomics

Christian Huber: As in all analytical areas, coverage and reliable quantification are major issues for proteomics. It is currently impossible to cover the complete proteome, either qualitatively or quantitatively, due to the very large number of proteins present in proteomics samples and their wide dynamic range. The result is that there are potential blind spots involving proteins that are of real interest. Moreover, it is difficult in repeated proteome measurements to always detect the same proteins, so finding the important parameter in all samples is not a straightforward task.

Another issue is obtaining and preparing representative biological/medical samples. The observed differences in proteomic measurements may be due to biological or individual dissimilarities rather than real differences in the biological processes under observation.

These problems will likely be addressed by the very rapid ongoing development of methods and instruments. Nevertheless, we may continue to employ a toolbox of different approaches to address proteomic samples of high informational content and, unfortunately, this significantly increases cost and effort. What is needed is complete new workflows, devised by teams that integrate expertise. These workflows should start with the selection of samples that represent the biological state to be measured; continue with experimental design, sample preparation, identification and quantification measurements, and bioinformatic data analysis; and finish with biological interpretation of the data.

The biggest challenge of all, in my view, is the treatment of the data. In a typical experiment, information has to be extracted from tens of thousands of mass spectra; to do this, we rely on appropriate software tools that do the job automatically. But this automation has a risk of identifying artifacts, which can lead to false conclusions. In addition, the translation of analytical data into biological information remains difficult and can only be addressed through interaction among analytical chemists, bioinformaticians, biologists and clinicians.

John Yates: Some of the issues that Christian raises are being addressed, others less so. Actually, I feel that proteomics is pretty well evolved at this point. It is having (and has had) a huge impact on the biological sciences. However, most of that happens behind the scenes because the "front page" stories are all about the discovery and not how it was accomplished. Certainly, bottlenecks persist at each of the stages of proteomics – sample preparation, separations, mass spectrometry (MS) analysis, and data analysis – but that is not to say that these issues are serious impediments, rather that improvements in these areas will advance the state of the art.

Funding is an issue. As I see it, when funders are challenged by decreases in their budgets it affects their funding priorities, which adds a burden to proteomics research. In tight money times, the focus is on the core mission; it requires the strong argument that advancing technology is key to a better understanding of a disease to get a technology-oriented grant funded.

Industrial research efforts often follow the money (or where they think the money will be) rather than identifying the technical limitations and challenges, and solving those. Mass spectrometry (MS) manufacturers, however, have been fairly strategic in their instrument efforts over the last decade and, as a result, they have driven many of the advances in proteomics.

Finally, I should add that we certainly need better methods for top-down proteomics (the analysis of intact proteins), which remains a huge challenge with many technical issues.

Barry Karger: It is remarkable how much the field of proteomics has advanced in the past five years. At the same time, it is clear that we are limited in the problems that we can solve. In bottom-up proteomics, for example, we measure peptides, not proteins. Let's say we quantitate five or more peptides: the result we get will be an average of all the proteoforms of that protein; an average of the post translational modifications (PTMs), mutations, splice variants, and clipped forms. But it may well be that only specific forms are functionally active. On top of this, even if modified peptides, for example, phosphorylated peptides, are studied, in cases where there are multiple sites of modification, we don't know what constitutes specific protein molecules; only specific forms may be functionally active. Thus, one major issue is to analyze intact molecules (top-down proteomics) using liquid chromatography (LC)- or capillary electrophoresis (CE)-MS. Full quantitative analysis of all proteoforms of a protein is not currently available, except for low molecular, simple proteins; improvements in MS instrumentation, fragmentation processes, data analysis and separation are required. Since this is an area of current interest in the field, we can certainly anticipate advances over the next few years.

A second major challenge is refining proteomic analysis of trace levels of biological samples down to hundreds or tens of cells and, ultimately, down to single cells. There are many instances where this capacity would be a crucial advance: to analyze cells following a needle biopsy, for instance, or in the analysis of stem cells or circulating tumor cells where vanishingly small numbers of cells can be obtained. Here, integration of effective capture of the cells, sample preparation with minimal sample loss, narrow bore LC with sub 25 nL/min flow rates, high sensitivity/resolution MS, and data processing are required. As with top-down MS, work is on-going in ultra-trace analysis, but further advances are necessary.

"The translation of analytical data into biological information remains difficult and can only be addressed through interaction among analytical chemists, bioinformaticians, biologists and clinicians."

"The impact of proteomics is behind the scenes because the 'front page' stories are all about the discovery and not how it was accomplished."

On Driving Proteomics Progress

CH: Because of the complexity of proteomic samples, separation is key in proteome analysis. Two-dimensional gel electrophoresis is the gold standard, although chromatographic technologies and multidimensional combinations thereof represent real alternatives in terms of separation selectivity and peak capacity. MS will continue to be the key technology for the high-throughput identification of protein peptides. Here, the newest technologies, including (ultra-) high-resolution Orbitrap or time-of-flight mass analyzers, usually implemented in hybrid instruments, are enabling protein identification at unprecedented speed, sensitivity, and dynamic range.

However, as I mentioned earlier, incomplete proteome coverage will remain the most challenging issue, and can only be resolved by a combination of greater resolving power of the separation methods; higher scanning speed and lower detection limits of mass spectrometers; and increased identification success rates for database-searching algorithms.

JY: Big advances in proteomics have come from improvements in MS and LC. Faster scanning and more sensitive instruments have worked well with ultra high pressure LC (UPLC), for instance. Data analysis is pretty robust, but the bottleneck is primarily in translating data into knowledge, so interfacing the results of proteomic experiments to the other knowledge sources available on the Internet will be key to solving this problem. I expect smaller gains will come from better sample preparation methods, but these will be necessary for proteomics to be truly comprehensive.

Are There Mature Areas of Proteomics?

BK: I would not use the term mature; there are, however, some areas that are more advanced than others. An example is the use of two dimensional LC separations coupled to MS, which has been published many times. Some state-of-the-art labs can identify and quantitate close to 10,000 proteins with this approach, and many can do the same for at least 4,000 – but only if enough sample is available. Data processing is becoming easier and better with the emergence of spectral libraries. Faster mass spectrometers will also increase the number of peptides and proteins that can be identified.

A second advanced area is multiple reaction monitoring (MRM) for quantitation of specific peptides, and thus of proteins. Triple quadrupoles continue to improve sensitivity and the availability of isotopically-labeled standards mean that the approach is widely used.

CH: I believe that all areas are still in rapid development and that none can be regarded as mature. The more classical approaches of high-throughput shotgun protein identification and differential quantification are now being complemented by high-throughput targeted and untargeted absolute quantification using selected reaction monitoring or data-independent fragmentation. Proteomic analysis that includes or targets posttranslational modifications, such as phosphorylation, N-terminal acetylation, or glycosylation, are gaining importance.

On the Role of Separation Science in Proteomics

BK: Sometimes I feel that researchers focusing on separations and those studying mass spectrometry are in two different universes. Many in the separations field consider only the separation system – the column type, optimized LC or CE conditions, and so on. Their studies often only involve UV or fluorescence detection. In parallel, MS specialists focus mainly on instrumentation or data processing and consider the separation system to be a plug-in device. Since our goal in proteomics is to solve a problem, we need to view the whole platform from an integrated approach. For example, the flow rate from the LC or CE column can dramatically affect the ionization efficiency of electrospray, and thus the signal. If high sensitivity is required, then low flow rates are important. On the other hand, the duty cycle of the MS must be considered along with the speed of separation. Beyond separation-MS coupling, sample preparation ahead of injection must be integrated into the analytical scheme. Sample preparation can so often be the Achilles' heel of the analysis.

Of course, optimized separation systems will be different for bottom-up or middle/top-down analysis. While chromatographic columns are excellent, and getting better, for peptide (bottom-up) separations, the need for improved separation of intact proteins in complex mixtures is all too clear. Perhaps CE-MS will turn out to be an effective tool, given that the separation efficiency and recovery from the open tube column should be high. Even for individual proteins, the number of proteoforms can be in the hundreds, and some of them will be isobaric. If one pulls down a protein complex, the numbers will again be high. Some researchers are attempting top-down analysis of full proteomes. Complexity here will be extreme, so the separation challenges are huge in the top-down arena. Multi-dimensional separations, along with ion mobility separations, will be necessary. In fact, top-down proteomics offers major challenges and, therefore, opportunities for separation science.

CH: The primary goal of sufficient separation of all compounds in the sample mixture remains valid for proteome analysis. However, sample complexity in proteome analysis is usually so high that a full separation is impossible; MS must aid in deconvoluting sample complexity. Increasing separation efficiency and peak capacity are high priority goals. Since ionization, especially electrospray, is influenced by sample complexity, I cannot imagine successful proteome analysis workflows without an integrated separation step.

On directions for Mass Spectrometry

CH: MS will remain the priority platform for protein identification. However, I can foresee a shift away from the peptide-oriented methods to top-down proteomics.

JY: MS has been a central driving force in proteomics for two decades. Without the technological advances over the last decade (for example, Orbitrap) proteomics would not have moved as rapidly as it has. Areas where mass spectrometers have improved and need to continue to improve are scan speed, sensitivity, and dynamic range. Mass resolution and mass accuracy have probably gone as far as they need to go for effective peptide analysis.

BK: Most workers would agree that operating an MS system is very complicated: it requires expertise in the selection of MS conditions, the variety of fragmentation approaches, the interpretation of the data (both manual and automatic), quantitation, and reproducibility. At leading proteomics laboratories, an entire team of specialists gets involved. Add to this the fact that at each American Society for Mass Spectrometry (ASMS) meeting, new instrumentation is introduced that changes the performance to such a dramatic extent that one continually feels the need to purchase new equipment. And yet, it is obvious that MS must be simplified in the near term as broader applications emerge, for example, biomarker quantitation for the clinical lab, protein quantitation as a substitute for Western blotting, and lot release in biotechnology. It is interesting that the recent Human Proteome Organization (HUPO) meeting in Japan also emphasized this point. There has been some effort in simplification of the MS front end, such as microfluidic devices for separation that feature plug-and-play designs, but mass spectrometers themselves are, if anything, getting more complex. I hope that the instrument manufacturers will devote greater resources to the difficult task of simplifying MS, to allow non-specialists to operate the instruments on their own.

On Future Directions

BK: Ion mobility will be used in the complex mixture analyses of proteomics: we will undoubtedly see higher resolution ion mobility systems and improved sensitivity. Note that one-dimensional LC-ion mobility-MS is actually a three-dimensional separation system – all on-line. One or more off-line separations could be added to this system as required. The number of separation steps will depend on the complexity of separation and the speed (duty cycle) of the MS. We will see more on-the-fly MS with feedback to select particular ions for further study; at the moment we are in the early stages of this kind of analysis.

From a data analysis point of view, we need new bioinformatic approaches to combine the proteomic data we generate with other omics data, particularly genomic, but also, metabolomic and lipidomic. The integration of all omics information will provide a much more detailed picture of biological processes, a necessary advance for better understanding specific diseases. We must always remember that proteomics, while essential, is but one piece of the puzzle.

The years ahead will be exciting ones. The whole community will play a role in the many forthcoming advances.

JY: Personally, I'm watching one method and working with another that has promise… I think CE has great potential for the analysis of intact proteins with MS. There are still some technical issues, but I've been impressed with separations and detection limits. A method I'm watching carefully is UV photodissociation (PD) of peptides and proteins. Progress in this area has been staggering. When I was in graduate school, the Hunt laboratory (people.virginia.edu/~dfh/) was trying UV PD on a fourier transform-MS instrument and it was producing awful results. I didn't think it would go anywhere. However, recent progress in the Brodbelt (brodbelt.cm.utexas.edu/research), Reilly (reilly.chem.indiana.edu), and Julian (www.faculty.ucr.edu/~ryanj/) laboratories have gotten me excited about the technique – looks like it has a great future.

CH: Using digested proteins, a lot of analytical information is lost because of the incomplete sequence coverage. A dramatic shift towards intact protein analysis is inevitable – after all, the intact molecules are the ones providing biological function. Proteins are more individual than peptides, so the analytical methods being developed are moving away from the more generic shotgun approaches that are applicable to peptide analysis. I believe that more effort will be required to tune and optimize these methods for specific proteomic problems.

"We need new bioinformatic approaches to combine the proteomic data we generate with other omics data, particularly genomic, but also, metabolomic and lipidomic."

Solutions

Real analytical problems
Collaborative expertise
Novel applications

Pet Food Perseverance

How heart-cutting two-dimensional gas chromatography aided in the identification of hidden odorant peaks to save the day – and the dog's dinner.

By Andrew Ward

The Problem

Reliable identification and quantification of components present at 'undetectable' levels in extremely complex matrices without laborious sample preparation protocols – and without breaking the bank.

Background

No doubt, you've been here: "my old instrument isn't going to do the job; it isn't selective/sensitive enough." At this point, you've started looking for ways to justify the expense of that shiny, new instrument you've had your eye on – the one with the state-of-the-art detector… only to be stopped in your tracks by limited budget. When you hit that wall, it's worth remembering that a little innovation can go a very long way.

The identification and/or quantification of very low-level components in complex matrices is a common challenge. Co-elution is something every experienced chromatographer has faced, and the approaches used to overcome it are numerous. However, de-convolution can only take us so far. In a sample that contains large amounts of 'component X', which co-elutes with trace levels of 'component Y', reliable identification and quantification of both components can be extremely difficult, even with laborious sample preparation protocols. In such circumstances, using higher-end instrumentation can be a logical choice. But it is not the only one.

A good client of ours has been working on odorant identification for some time. One clear problem for them is the subjective nature of odorant detection, and so they were keen to explore analytical options that would facilitate a more objective approach. Here, our main contact 'Mike' hit a situation familiar to many of us: "I know something else is there, but I just can't see it". Mike had confirmed that odorants were present in his samples by using a much more selective and sensitive detector than is commonly available for your chromatographic system of choice – the human nose. However, he could not detect – let alone identify – them as peaks in the chromatogram with the tools that he had available. Mike needed a way of (i) identifying and separating very low levels of components in very complex matrices, while (ii) improving sensitivity to such a degree that components present at 'undetectable' levels could be reliably identified and even quantified.

The Solution

Working with pet food samples, which Mike casually informed me, "really don't taste that bad," he likened the 'hardware' of the human nose to dynamic headspace sample preparation with direct detection, and so paired direct MS with olfactory detection. However, it soon became apparent that it was not possible to correlate the identity and importance of hundreds of ion masses to the associated

odorants, and that gas chromatography (GC) was required.

Next, Mike tried to use GC to separate the volatile organic compounds and combined it with three detectors: an olfactory detection port (ODP) to detect odor-active peaks, a pulsed flame photometric detector (PFPD) to detect the presence of sulphur-containing moieties, and a single quadrupole mass selective detector (MSD) to identify components. Yet, even following sample preparation by solvent-assisted flavor evaporation (SAFE) extraction and distillation, the resulting chromatograms were very crowded and showed a high level of background noise due to the complex nature of the matrix involved Furthermore, the approach came with its own set of problems: odors were being detected where only odor-inactive compounds could be identified in the mass spectrum or where there were no peaks at all (on the MSD or PFPD). Having ruled out misalignment of the detectors, the conclusion became clear: co-eluting peaks in the MSD were masking odorants and some odorants were present at levels below the detection limit of the MSD and PFPD.

To reduce the complexity of the chromatograms, Mike fractionated his sample extracts into basic, neutral and acidic fractions, thus reducing the number of components present in each injection and reducing the chance of co-elution. This approach confirmed the presence of co-eluting peaks, and furthermore the presence of co-eluting odorants. It is important to note that the human nose is capable of detecting more than one odor at once.

Now, the situation becomes even more challenging: according to the odors emitted from the olfactory detection port, multiple, co-eluting odor-active components are present at levels not detectable on the current system.

It was at this stage that Mike approached us, armed with the information he had gained from his work to date and his knowledge of the workings of the human nose and odorants. He told us that he needed to find an objective analytical system equivalent to the human nose. In simple terms, the problem can be broken down as follows: the sensitivity of a single quadrupole MSD is in the region of approximately 1pg (scan); the sensitivity of PFPD is approximately 1pg (spec); and the sensitivity of a high-speed ToF-MS is in the region of approximately 0.1pg. The human nose can detect some compounds down at levels of approximately 40ag, way below that of commercially available GC detectors. We needed to develop a system that could identify unknown compounds present at very low levels in extremely complex matrices, which may co-elute both with each other and with other components present at levels many orders of magnitude higher.

"We hit a couple of snags. Bryan is anosmic – having no sense of smell makes this kind of work particularly difficult."

The solution proposed by Bryan White (UK country manager for JSB) was to heart-cut peaks or regions of interest – where odors were detected – in the chromatogram onto a second column with a different phase for a second dimension of chromatographic separation in isolation. This helped with separation of co-eluting peaks, but the key to improving sensitivity was the addition of a cryo-trap after the heart-cut. Using an Agilent 7890B GC with an Agilent 5795C MSD, we initially used an Agilent Capillary Flow Technology modulator plate to heart-cut regions from column one into a SIM Ice Cube cryo-trap packed with glass beads at -20 ºC to trap the components. This has the advantage of allowing multiple injections into column one, heart-cutting the same region each time and concentrating the components in the trap. When a second analytical method heats the trap the components are released and separated on column two before being detected by the MSD (and ODP).

Results from initial tests using a mixture of alkanes and a sample extract looked promising, then we hit a couple of snags. Bryan is anosmic – having no sense of smell makes this kind of work particularly difficult, which is why I took over. I developed the GC method to separate the alkanes to allow calculation of Kovat's retention indices on column one and set about doing the same on column two by cutting all of the alkanes into the trap. However, it became apparent that glass beads at -20 ºC would not trap the full required range of alkanes (and therefore all of the components of interest based on their retention indices). Reducing the temperature was not an option with a Peltier cooling system. Instead, I replaced the glass beads with Tenax and solved the issue. The system was ready.

We successfully cut out a single peak from the alkanes mix and concentrated it on the trap using repeat injections and heart-cuts. The peak area of the component eluted from the trap after five injections was five times that of the same peak in an un-cut sample, demonstrating that the trapping was linear. The system worked. And I wanted to push it.

Mike gave me a sample of what he described as 'a very difficult co-elution' of known components. This was a mixture of four odorants that all co-eluted with

levels of acetic acid often found in his samples. It was in fact five odors across one large, ugly peak. Other than the acetic acid, the odorants could not be identified by standard GC-MS. Initially we tried three heart-cuts of the peak onto the trap and eluted the components. This allowed us to identify three odorants, but two peaks were still missing. So we did 24 heart-cuts and eluted the trap contents. This allowed the other two components to be easily identified. But was the system linear at such low levels?

The components of this sample were:

Component	Concentration
Acetic acid	*1000 mg/l*
Furanmethanthiol	*10 mg/l*
1-octen-3-ol	*10 mg/l*
3-(methylthio)propanal	*2mg/l*
2-ethyl-3,5(6) -dimethylpyrazine	*0.2 mg/l*

Looking at the furanmethanthiol (TIC) peak areas, the ratio of the peak area after 24 heart cuts to that after three heart cuts was 8.3:1. The corresponding value for the 1-octen-3-ol peak was 7.9:1. Promising results.

So how could the system be improved? The oven was looking more than a little intimidating with all of the connections inside. I changed both columns to low thermal mass (LTM) units so they sat outside the oven, which aids in column changing and has the advantage of independent temperature programming for the two columns. I also connected a flame ionization detector (FID) to facilitate quantification.

A schematic of the final system is shown in Figure 1.

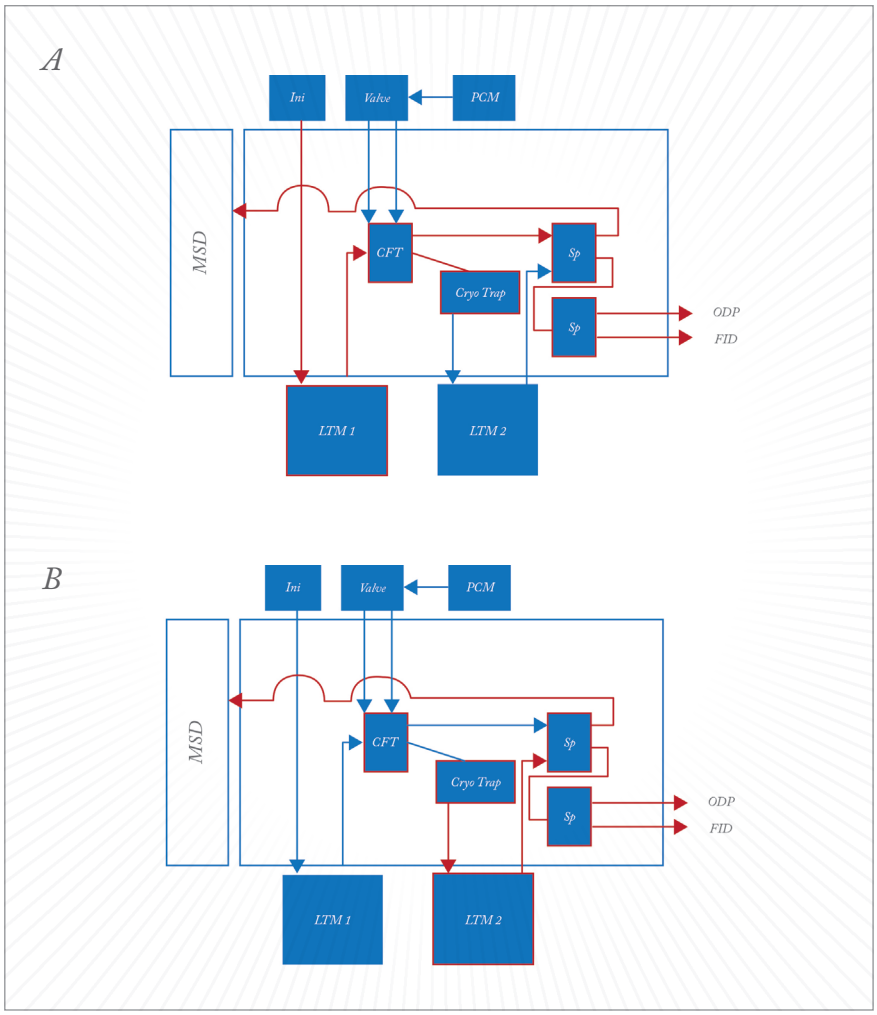


Figure 1. A. Schematic showing heart-cut flow path. B. Schematic showing trap elute method flow path. LTM: low thermal mass column, CFT: capillary flow technology modulator, PCM: pneumatics control module, Sp: flow splitters, Inj: injection, MSD: mass selective detector, ODP: olfactory detection port, FID: flame ionization detector.

Beyond The Solution

I think we can claim success with this solution. It is easy to implement, adaptable (by the simple exchange of low thermal mass columns), doesn't require vast quantities of cryogen, and, most importantly, the potential applications of the system are absolutely vast – essentially any application looking at low level components in complex matrices. The system is also useful for particularly difficult co-elutions (chiral columns can be used to separate enantiomers, for example). Another potential application would be automated sample screening for very low-level components, using sequential runs to take different cuts and concentrating components eluting in regions of interest. But that is for another day!

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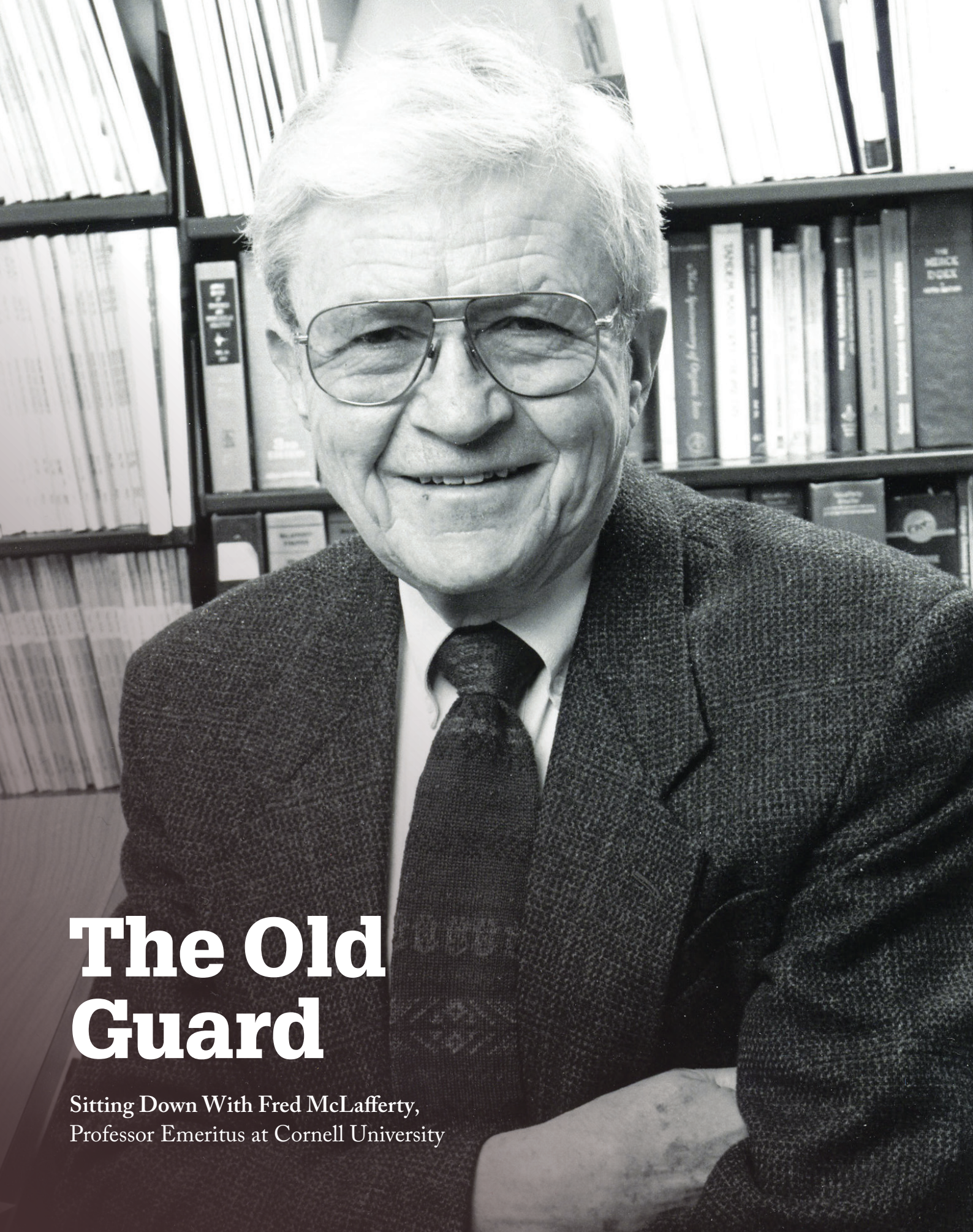
The full version of this article, with figures and further insight, is available online: tas.txp.to/1113/Petfood



The Old Guard

Sitting Down With Fred McLafferty,
Professor Emeritus at Cornell University

What was your first publication and can you pinpoint the highlight of your career?

I published my first independent paper on what's been called "the McLafferty rearrangement" in March 1956 when I was 32 years old. That's a long time ago! Yet I was at a chemistry seminar last week when the young visiting speaker said, "Oh! Fred McLafferty – the McLafferty rearrangement!" To tell you the truth, I'm so much more excited about what we've done many times since, it's kind of funny what is remembered. Not that I'm complaining.

I have a hard time pinpointing one particular highlight. Probably, it's the fact that I've had so much research freedom. I joke about the time when Dow had to shut down polystyrene production because it had black specks in it. Practically everyone was working on a solution. My mass spectrometer showed that 300 ppm carbon tetrachloride had gotten into the styrene monomer when transported in tank cars that hadn't been washed out. Boy! I was a total hero – even though seeing the mass spectrum of carbon tet was the easiest thing you could do by mass spec. The infrared lab was one of the best in the world at that time but they couldn't see it because carbon tetrachloride is transparent in the infrared. Soon after that episode, I needed a new mass spectrometer, and virtually got one thrown at me. In 1964 when I went to Purdue, it was just after Sputnik so, of course, I could get funded by the National Science Foundation and National Institutes of Health. I've always been lucky…

Before setting out on a career in science, you experienced combat in World War II. How did it help shape you?

The best way of answering your question is that, at the age of 21, it was a very maturing experience. I was in an infantry rifle company on the front line. And while I've won several medals, it had more to do with the fact that my particular company experienced very difficult and unusual battles and included great people that deserved recognition. I just happened to be there. Yes, I got a Purple Heart – that means I was wounded. But there are 193 men in a rifle company and we got 194 Purple Hearts. What it really means is that we saw a lot of action. The highest US award is the Medal of Honor – I think there were 384 given in World War II all told – and we had a guy in our company, Johnny Crews, who received one. I was just along for a nice picnic compared with what he did. We had 35 killed in action, so I was also very lucky. Those are the people that really deserve to be honored, of course.

"Boy! I was a total hero – even though seeing the mass spectrum of carbon tet was the easiest thing you could do by mass spec."

That whole chapter of my life gave me confidence, commitment, and even a future excuse. I have a short fuse when it comes to people who don't pull their weight, quickly pointing out their "deficiencies", but I've been able to get away with it because people know I've seen tremendous achievements in times of adversity. The American Society for Mass Spectrometry recently helped me celebrate my 90th birthday, and it was nice to realize that those who spoke had forgiven me!

I wouldn't take a million dollars for my infantry experience, but I sure wouldn't do it again for a million dollars either.

You've had many honors bestowed upon you by the science community. How do you feel about those?

They are very nice to have, very flattering and good for confidence, but come with the danger of increasing one's ego too much. In science, confidence is essential. You've got to try new things and have the intuition to "get lucky". Such recognition is a great help with that, although I'm not sure how well I've controlled my ego.

Disagreements in science are wonderful for progress because they make us all work much harder. But get too carried away, and it can really hurt your whole career. Both confidence and perspective are important – as is not worrying too much about being wrong. I've published things that haven't turned out to be 100 percent correct – but my standard comment is: "Well, correcting this gave me two publications!" That's a lot easier to say with a little name recognition…

On reflection, one of the many nice things about having a wonderful wife and five children to bring up is that they are more than happy to point out my flaws. If you asked me about the most important thing in my life, it's certainly my family and what they've done for me.

How has analytical chemistry changed over the years?

I was at the Dow Chemical Company from 1950 to 1964, and I could see from the outside what a terrible time

analytical chemistry was having in academia at that time. Most of the best chemistry departments turned their noses up at it. The reason I went to Purdue University from Dow is because my good friend "Buck" Rogers went there after being pushed out of the Massachusetts Institute of Technology (MIT). He was a good guy and did a great deal for analytical chemistry. I had a strong analytical chemistry heritage to back me up – but not many "good" universities wanted research on volumetric or gravimetric analysis. Still, I was always proud to call myself an analytical chemist.

Faced with such difficulties you might need a big ego…

Ha! The last I heard, 20 percent of the American Chemical Society's members were analytical chemists. And, with all due respect, the National Academy of Sciences has about two percent or less who call themselves analytical chemists. Some people get around the potential stigma by calling themselves something else… But I've always insisted that I'm an analytical chemist.

Sometimes it's our fault. We can get too involved in a color test that tells us about x, y and z in strange plants over 20 years of research, while forgetting that nobody really cares about x, y and z in strange plants any more. We have to keep in mind that our methods must help solve important problems. But it's a two way street: sometimes the problem isn't recognized, if there isn't a good analytical method to investigate it. On the other hand, a good analytical method isn't recognized, if it doesn't address an important problem.

What's your take on the situation now?

Today's analytical scientists have come a long way from the problem of being second-class citizens in academia. The standing of analytical chemists and the interest in them from the best chemistry departments are both very refreshing. The trouble with these young analytical chemists is that they are too darn smart…

"In science, confidence is essential. You've got to try new things and have the intuition to 'get lucky'."

The downturn in funding is difficult. But that's a cyclic complaint. I think the very best people always get funded, with more or less people dropping by the wayside as a result – it's a competitive world. If the funding situation is holding someone back, I have sympathy but also believe that determination is essential for an independent research career. I exhort them to work harder and ignore the worst problems – the people I meet are wonderfully talented and a certain number have simply got to become successful.

What's the most exciting area in MS today?

Probably top-down proteomics. Bottom-up proteomics involves breaking up the protein before analysis. I always want to do things differently. For styrene or carbon tetrachloride, what did we do? We put the whole molecule into the MS. We didn't break it up and waste all that connection information. So I've always put the whole protein in the MS and tried to separate protein molecular ions and then break them up to get their sequences separately, which is harder to do and you need a better MS. Or rather, the better MS you've got the better job you can do. Probably the loudest voice in top-down proteomics right now is Neil Kelleher at Northwestern University. He got his PhD from me, so I'm very proud of his achievements. We initially used FT-ICR-MS for top-down proteomics, which has sufficiently high resolution but is very expensive. It also uses a liquid helium-cooled superconducting magnet, which is an added expense and a real nuisance. In more recent years, while Thermo's Orbitrap doesn't have some of the performance of the FT-ICR-MS, its high resolution has been great for top-down proteomics, especially because so many more people can do it financially.

What, for you, have been the biggest mass spectrometry (MS) milestones?

I like to emphasize that up until the late 1940s, MS was all about isotopes and elements. Progress over the last 70 years has especially been in molecular MS. Over that time, we got very good at looking at small molecules. But our biggest limitation was that we couldn't look at molecules that didn't have some vapor pressure. Then electrospray ionization – and MALDI – came along so, simply from an increased application point of view, these have got to be the greatest advances.

Are you still active in the scientific community?

I still go to meetings and write papers. But my last coworker left over a year ago, so I miss him. Worse yet, he's my grandson (and now a graduate student with Neil Kelleher). Unfortunately, just because he's smarter than me, it doesn't mean he's guaranteed a good job. I have ten grandchildren in all, so it's nice that one of them is doing mass spectrometry.

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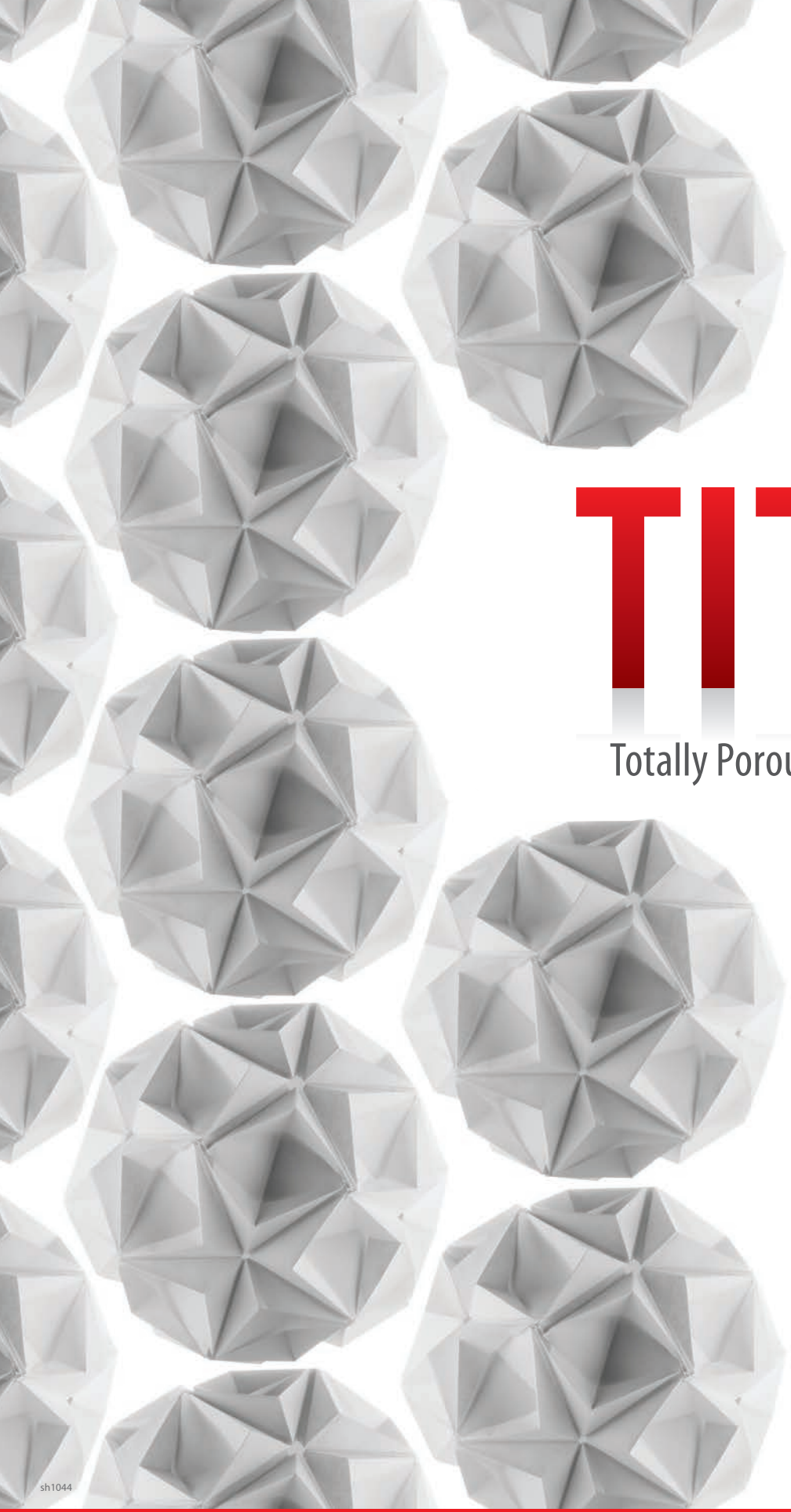

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