FEBRUARY 2016 # 37

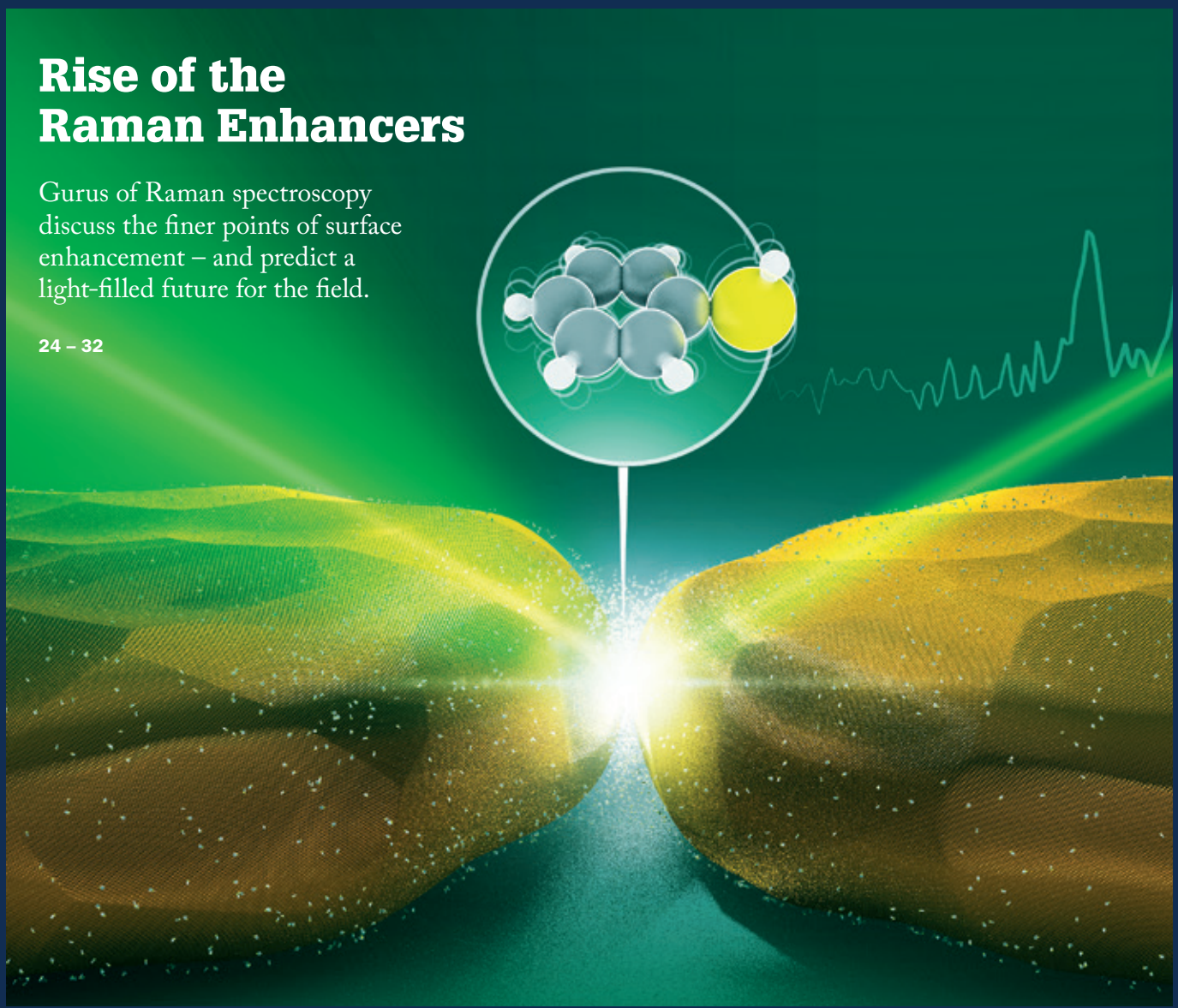
# the Analytical Scientist



## Rise of the Raman Enhancers

Gurus of Raman spectroscopy discuss the finer points of surface enhancement – and predict a light-filled future for the field.

24 – 32



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# Image of the Month


CSI versus PSI: Forensic evidence can be applied to a paper triangle (or the triangle is wetted and used to swab a surface of interest) ahead of paper spray ionization (PSI) on a portable mass spectrometry system. For more information, see page 12.

Photo courtesy of Christopher C. Mulligan, Associate Professor, Analytical Chemistry, Department of Chemistry, Illinois State University, USA.

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

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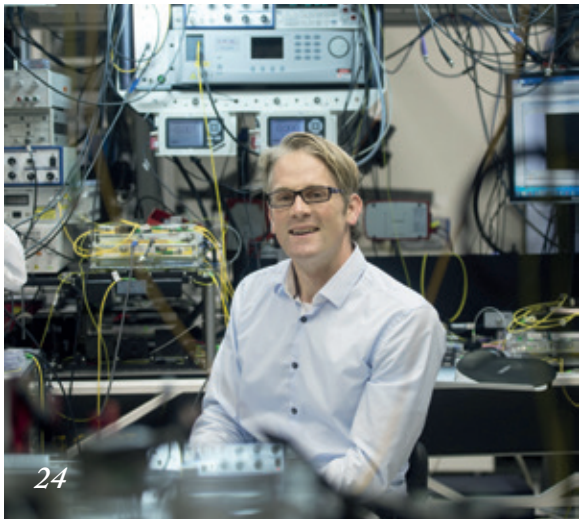




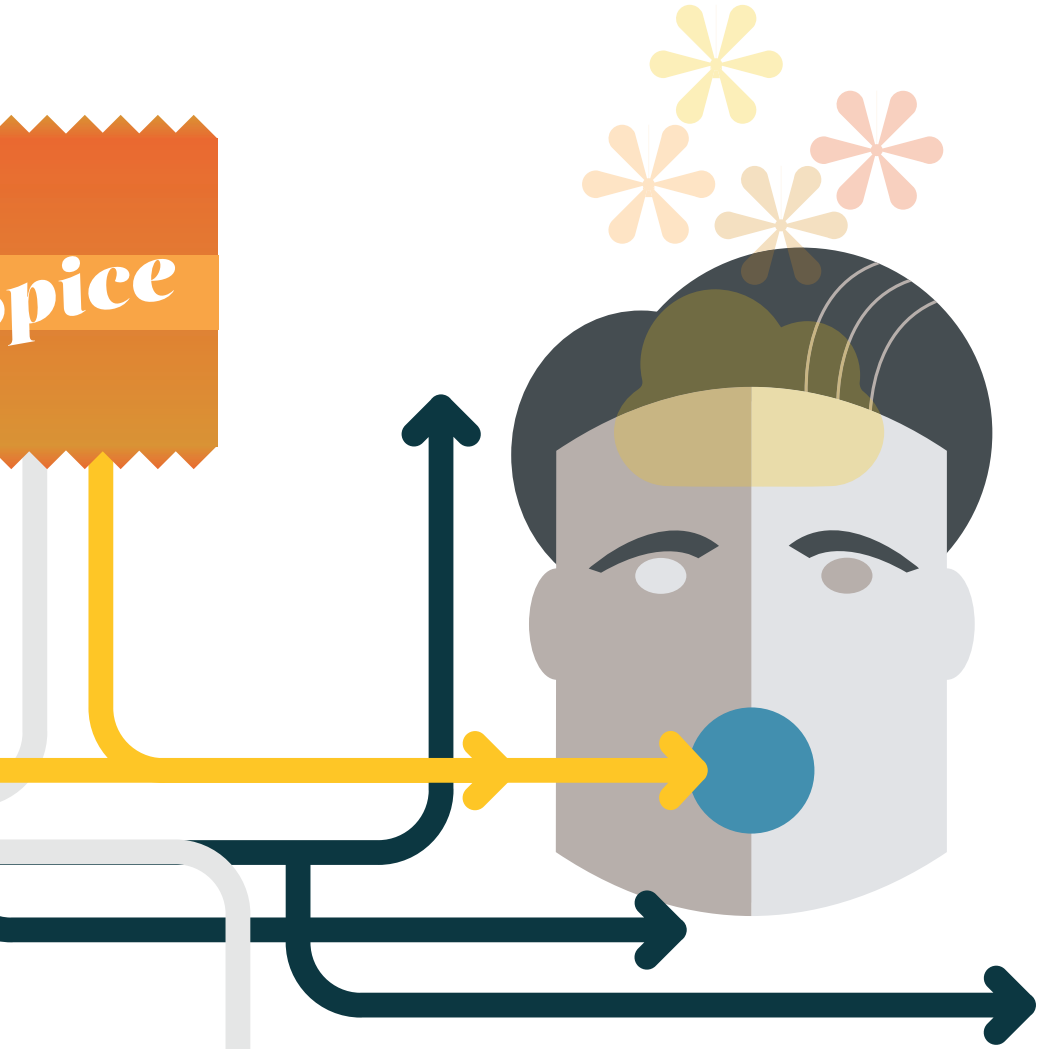

## On The Cover

*Artist's impression of light-mediated detection of a molecule. Photo credit: Nicolas Antille & LPQM / EPFL.*

## Upfront



## In My View

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## Features



## Departments



## Sitting Down With



the **Analytical Scientist**

ISSUE 37 - FEBRUARY 2016



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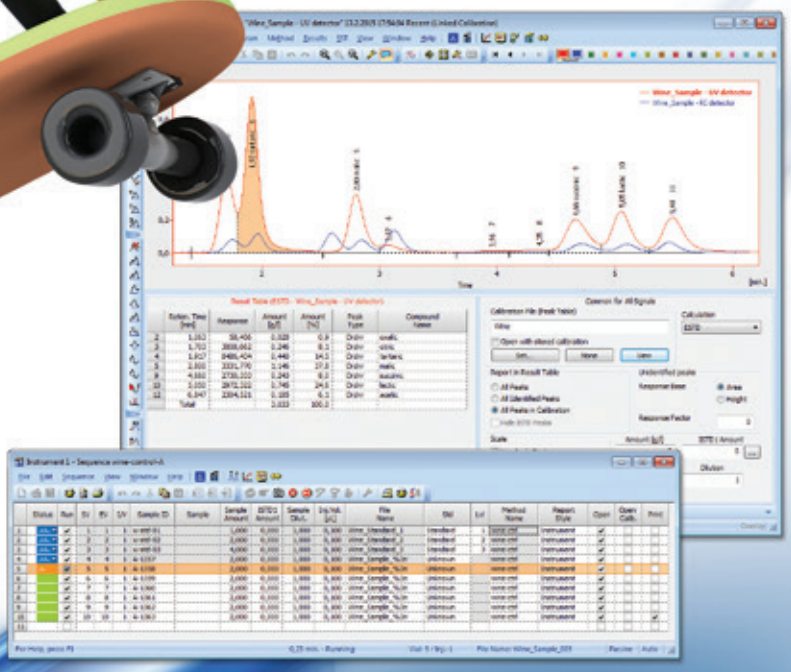


EASY RIDE

**SEE US** at **Pittcon 2016** – booth no. 2863

# G-A-T-T-A-C-A

*Imagine a dystopian society where people are discriminated against not because of their color, race, creed, religion or sex, but because of their genomic predisposition to disease...*

*"My father was right. It didn't matter how much I lied on my resume, my real CV was in my cells. Why should anybody invest all that money to train me, when there are a thousand other applicants with a far cleaner profile? Of course, it's illegal to discriminate – 'genoism' it's called - but no one takes the laws seriously. If you refuse to disclose... they can always take a sample from a door handle or a handshake... Even the saliva on your application form."*
– Vincent (Gattaca, A Screen Play by Andrew M. Niccol).

At university – longer ago than I like to admit (1997) – I remember watching Gattaca at the cinema. I was both spellbound and slightly disturbed by a future world where discrimination was "down to a science".

I watched Gattaca again last night. How different my perception was the second time round – especially having been the editor of The Analytical Scientist for the past three years. The film has not aged well in terms of some technology – the cathode-ray-tube monitors in the high-tech facility make sure of that; it comes across more as 'film noir' than science fiction (no doubt partially intentional). But without giving away the plot (please watch it – even if you already did 20 years ago), I was fascinated most by the portrayal of DNA sequencing. Bear in mind that the film was released before the Human Genome Project was completed in 2003 (1) and only seven years after it started. And yet in Gattaca, citizens take samples to discreet sequencing-while-you-wait holes in the wall to get full genomic profiles. In the workplace, sophisticated 'black-box' instruments with a single button – "analyze" – leave the analysts with little to do in routine urine and blood tests. Indeed, people's lives appear to revolve around routine DNA analysis; high-security buildings require a finger prick test on entry to confirm each and every person's identity, seemingly through DNA biomarkers.

The film begins with the title, "In the not-too-distant future" – and we're getting close... Next-generation sequencing has breached the $1000 human genome barrier (2) – when used at scale. (I recall it cost around $80 in Gattaca). And back in 2008, President Bush signed the Genetic Information Nondiscrimination Act (3).

In April 2015, Nature announced that Chinese scientists had genetically modified human embryos (4) and in February 2016, UK researchers were given the green light to do the same (5).

Welcome to Gattaca.

**Rich Whitworth**
*Editor*


*Sources*

1. *www.genome.gov/10001772*
2. *www.illumina.com/content/illumina-marketing/amr/en_US/systems/hiseq-x-sequencing-system/system.html*
3. *www.genome.gov/24519851*
4. *www.nature.com/news/chinese-scientists-genetically-modify-human-embryos-1.17378*
5. *www.bbc.co.uk/news/health-35459054*

# Upfront

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

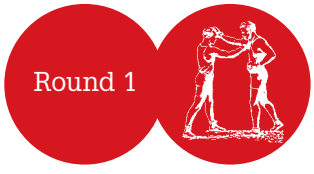
*We welcome information on interesting collaborations or research that has really caught your eye, in a good or bad way. Email:*
rich.whitworth@texerepublishing.com

Image courtesy of Alain Quenderf

## The Gloves Are Off

### What can experts in the analytical sciences do – if anything – to stop doping?

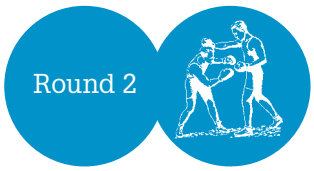
The issue of drugs in sport remains a contentious one, with the forensic, legal and medical industries repeatedly drawn into the fray. In last month's issue, editor Rich Whitworth suggested that analytical scientists need to speak up to avoid being complicit in any controversial decisions made by authorities – especially after Russia accepted a full and indefinite ban from world athletics for "state-sponsored doping" (1). Here, four experts give their views on the role of the analytical community in the war on doping.




Herman Ram, CEO of the Netherlands Anti-Doping Authority

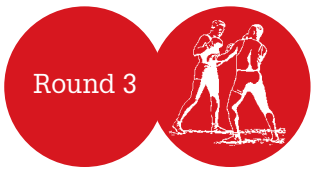
"Analytical scientists can contribute considerably to the fight against doping in sports by developing simpler and cheaper analytical methods (including saliva and dry spot analysis) than the ones that are presently being used. Cost is a major factor in our work, and developing cheaper methods would enable us to increase the number of tests (which at present is decreasing because of financial constraints). Simpler and cheaper methods will undoubtedly be less precise than the present methods, but this is not a major problem, as in many situations, we do not need to look for extremely low concentrations of prohibited substances. Such new methods should not

replace the more sophisticated approaches, but they would be a welcome addition for many situations with a (relatively) low risk of doping use."




Douwe de Boer, researcher at the Department of Clinical Chemistry, Maastricht University

"Analytical scientists play an essential role in trying to stop certain developments in sports doping, because scientific progress is continuously required. Unfortunately, and despite those efforts, sports doping can never be stopped. While the development of scientific knowledge is one of the responsibilities of the analytical scientist, following certain ethical standards is obviously another important responsibility. Nevertheless, finding an adequate balance between both responsibilities is quite a challenge for scientists [...] An essential role of analytical scientists is not trying to stop sports doping at all costs, but to do it in a politically independent, fair and balanced manner."




Mario Thevis, forensic chemist and Professor for Preventive Doping Research at the German Sports University

"It is vital to continue improving doping control test methods in various regards. This certainly includes, amongst others, pursuing the identification of long-term markers that enable enhanced retrospectivity and the early incorporation of new, emerging doping agents into routine sports drug testing programs. Further, alternative test matrices might provide added value for both the testing authorities/laboratories and the athletes in a complementary manner, rather than substituting currently used blood and urine doping control samples. With retrospective accuracy in particular (that is to say, was the drug taken recently/at the time of competition?), using specimens such as dried blood spots could be advantageous, and hair samples have also proven helpful to probe for the duration and amount of exposure to certain drugs relevant to doping controls. Surely, these matrices have their limitations as well; hence, the suggestion that they should be complementary and not substitutes."


Peter Kootstra, analytical chemist, owner of Lab QC and co-founder of Lab-QAcademy

"To be honest, this question is not an easy one to answer. In recent last years, I have reviewed several documentation packages from so-called 'anti-doping laboratories'. The analytical procedures used by these laboratories are rarely validated and are based on assumptions. The accreditation bodies are chained to WADA, and the 17015 assessment may be combined with the WADA accreditation only when WADA selects the auditors. The analysis of the B-sample is just a replicate, with no second opinion sought. Athletes don't have a fair chance. So to answer your question: the anti-doping laboratories should be open and co-operate with other analytical chemists/statisticians to validate and discuss the results in public." *JC*

*Reference*

1. *R Whitworth, "Nice Measurement – Now What?!" The Analytical Scientist, 36, 7 (2016). Online: tas.txp.to/0216/NowWhat*

# Georgia (and Pittcon) On My Mind

**Are you ready for the "Pittcon experience" in Atlanta? Get the most out of the best-attended annual conference on analytical science with our top picks.**

Pittcon is "where innovation goes to play" and there are certainly plenty of innovators among this year's array of speakers. In addition to the diverse selection of cutting-edge technology, plenary lectures, and exhibitions, there will be live product demos on the expo floor – something new for you Pittcon veterans – as well as over 100 short courses. And with plenty of networking sessions you'll be able to catch up with long-lost colleagues – as well as meet members of The Analytical Scientist team; we'll be at booth 2362. If you can't make it, we'll be live tweeting from @tAnaSci. If you are going, here are our top picks. *JC*

## Editorial Top Fives

Symposia

- Wearable and Point-of-Care Sensor Technologies for Biomonitoring (March 6)
- Omics for Environmental and Public Health Protection (March 7)
- JAMIA – The State-of-the-Art Technologies from Japan: Analytical Instruments with/for Nano-Chemistry Technology and Advanced Diagnosis (March 8)
- Vibrational Spectroscopy of Biodegradable Plastics: Evolution, Revolution or Back to the Future (March 9)
- Overcoming the Obstacles to Making Measurements in the Brain (March 10)

Oral Sessions

- Measurement Strategies - Sensors and Spectroscopy (March 6)
- Innovative Approaches to Science Education (March 7)
- Detection of Illicit Drugs (March 8)
- Biomedical: New Technologies for Breath Analysis (March 9)
- Novel Synthesis and Applications of Nanomaterials (March 10)

Organized Contributed Sessions

- R&D to QC: Bridging the Gap (March 6)
- Cell Phone Spectroscopy - Handheld Spectroscopy for the Citizen (March 7)
- High Performance SFC for the Analysis of Pharmaceuticals, Nutraceuticals, Natural Products and Metabolomics (March 8)
- High Throughput Analysis for Food Safety and Cosmetics: Challenges and Validation (March 9)
- Bioanalytical: Fluorescence/ Luminescence Techniques (March 10)

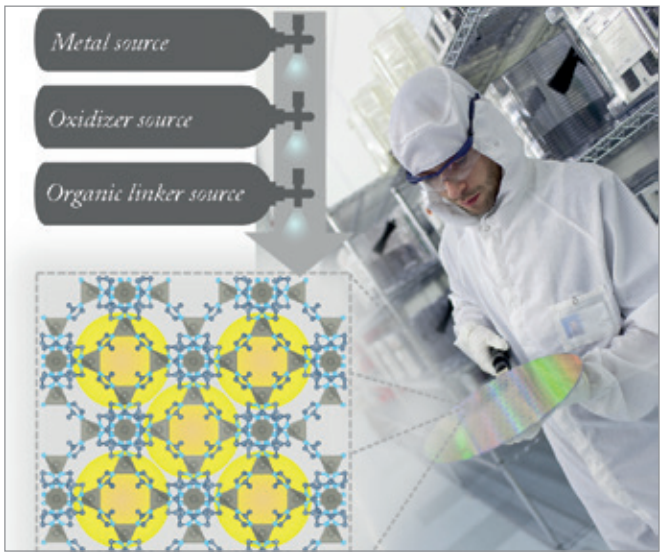
*Pittcon 2016 will be held March 6–10 at Georgia World Congress Center, Atlanta, USA: www.pittcon.org*

# Bringing MOFs Down to Size

## Metal-organic frameworks (MOFs) can now be nanofabricated – but what impact could the materials have on analytical science?

MOFs are porous crystalline materials composed of metal ions and organic molecules. They have excited researchers because of their extremely large surface-areas – the largest of any known material – and the flexibility with which their chemical functionality can be tuned. They are also very robust, with high mechanical and thermal stabilities. However, the current synthesis pathways used to obtain MOFs are incompatible with nanofabrication, meaning they cannot be designed and manufactured at the nanoscale.

Researchers from the Center for Surface Chemistry and Catalysis at the University of Leuven set out to develop a method of synthesizing MOFs that aligns with the way nanoscale devices are manufactured (1). "Vapor phase deposition methods are the cornerstone of nanofabrication," says Rob Ameloot, lead author of the study. "We therefore set out to synthesize a crystalline and porous MOF using vaporized precursors."

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The researchers were able to synthesize the MOFs in a two-step process. "In the first step we rely on an established technology: atomic layer deposition of metal oxides," says Ameloot. "In the second step, we react these sacrificial metal oxide films with a vaporized MOF ligand," he says. "The trick is to get the conditions at the solid-vapor interface just right to enable the formation of a porous and crystalline material, a feat that thus far required the presence of a solvent."

Through the development of a synthesis method compatible with nanofabrication, the researchers have opened the door to a wide range of industrial applications – including some within analytical science. "We are very enthusiastic about applying MOFs to gas sensors, and we're working on it as we speak," says Ameloot. "The perfectly uniform nanometer-sized pockets in the MOF materials are ideal to capture small molecules; important examples include carbon dioxide, for integration in smart AC, and volatile organic compounds (VOCs) such as formaldehyde, acetone, benzene, and so on," he adds. "The latter group of compounds are a major part of indoor and industrial air pollution, and it is becoming increasingly interesting to detect changes in the concentration of these marker molecules in breath, for early diagnosis of diseases such as cancer."

Ameloot and his colleagues believe that MOF-based sensors could have a major impact on small molecule detection and monitoring, especially for applications that need sub-ppm level sensitivity. *JS*

*Reference*

1. *I Stassen et al., "Chemical vapour deposition of zeolitic imidazolate framework thin films", Nature Materials [EPUB ahead of print] (2015).*


# Plug-and-Play Forensic MS

**Portable mass spectrometry readies itself for on-site identification of physical evidence.**

Transporting, processing and finally analyzing crime scene evidence takes precious time, creating a backlog in forensic labs. Three Illinois State University professors have been working on the development and implementation of a portable mass spectrometer that could allow real-time analysis at the crime scene itself. Christopher Mulligan, Associate Professor in the university's analytical chemistry department tells us more.

What makes your device different to other portable MS systems?

We have adapted a FLIR Systems AI-MS 1.2 cylindrical ion trap mass spectrometer, which is a ruggedized, portable MS system made for harsh environments. And we have developed ionization methods that allow one to screen forensic evidence types directly in their native state (what is commonly referred to as "ambient" ionization), yet do not require volatilization for sample introduction. It is common for many of the portable MS systems available to require samples to be in the gas-phase prior to its investigation because of their low-power vacuum systems. Systems are either limited to gas-phase analytes (for example, the KORE MS-2000) or rely on thermal desorption of the sample (for example, the M908 from 908 Devices), which can alter or complicate data.

Since the AI-MS 1.2 employs a true atmospheric inlet (that is, a directly capillary inlet from the external environment into the MS vacuum system) like traditional lab-scale MS instrumentation, it is compatible with a plethora of ionization sources that have shown promise to forensic sample analysis. So we've built a suite of ionization sources that can be used rapidly in a plug-and-play style. In this way, the user can select the most suitable source for the evidence at hand. Currently, we have sources that accommodate solids (for example, trace residues and bulk powders), liquids/solutions, and gases. Most importantly, these sources – as well as the whole MS system – are simple to operate and flexible so that they can be used by non-

technical operators. Another distinction is that our system employs tandem MS (MS/MS) data and fragmentation spectral matching to provide a much more accurate identification of the unknown substances encountered.

A good analogy is the recent success and utility of phone applications. The portable MS itself is like a phone, which can do a few things like make calls and take photos, but the real power and utility of the device is unlocked by compatible apps (like social media, word processing, email...) Having a selection of plug-and-play style ionization sources available is analogous to having several highly useful and effective apps available at the discretion of the user.

### What are the highlights from your research?

It was exciting when we tested the applicability of our system towards clandestine drug laboratory screening by actually making methamphetamine alongside US DEA representatives (1). You really get a feel for the difficult task that today's law enforcement and crime scene investigators face when you see just how easy and rapidly it can be produced (see page 36 for more on the war against drugs). During this exercise, we showed the AI-MS 1.2 was capable of identifying both reaction precursors (for example, pseudoephedrine) and the methamphetamine product regardless of time point of the synthesis. Furthermore, our gas-compatible source based upon atmospheric pressure chemical ionization (APCI) was able to then go through and identify organic solvents utilized for extraction and drying.

### How has the collaborative approach paid off?

It has been transformative. Forensic science is fundamentally an interdisciplinary field, so bringing in broad expertise allows us to investigate new areas of research. Through our recent National Institute of Justice grant funding, I have been collaborating with Jamie Wieland (ISU Department of Technology) to determine the financial viability of the system compared to current evidentiary protocols and Michael Gizzi (ISU Department of Criminal Justice) to examine the legality of using our developed method to prompt "probable cause" searching in traffic control stops. Both of these research directions would have been very difficult to pursue with just my skill set...

### What are the main challenges ahead of you?

Our ultimate goal is to create a MS platform that is readily useable in both crime scene investigation and routine law enforcement scenarios. In these environments, the primary user will not be an analytical chemist, so the burden falls on us to ensure that the instrument is both easy to use, but also robust in terms of data quality when non-technical personnel operate it.


*Reference*

1. *AE O'Leary et al., "Monitoring the clandestine synthesis of methamphetamine in real-time with ambient sampling, portable mass spectrometry", Anal Methods, 7, 7156– 7163 (2015). DOI: 10.1039/C5AY00511F*

# Fish Pharm

**Drugs get a second chance to enter our bodies – via wastewater and fish…**

Blinky the three-eyed fish is quite endearing in The Simpsons, but you probably wouldn't want contaminated fish on your plate. Mohammad Mottaleb, Adjunct Professor in chemistry at Northwest Missouri State University, used GC-MS (with selected ion monitoring) to discover ng/g levels of anti-histamine diphenhydramine (Benadryl), anti-anxiety drug diazepam (Valium), and anti-seizure carbamazepine – and their metabolites – in fresh- and salt-water fish species purchased from regular grocery shops (1). Eleven of fourteen fish investigate were found to be contaminated.

These potentially harmful yet readily available products are released as discharge from sewage treatment plants, having entered the system via human urine and feces or through inappropriate disposal from drug manufacturers and hospitals. And according to Mottaleb, the trend shows no signs of abating. "Continuous loading of the parent compounds and metabolites of pharmaceuticals and personal care products (PPCPs) will reach harmful concentrations that adversely affect the freedom of aquatic creatures. And eating contaminated fish might have consequences for human health effect hazards over period of time," says Mottaleb.

Mottaleb suggests one of the major problems is the lack of effective contaminant removal techniques in water treatment plants, which are not generally designed to eliminate pharmaceuticals. He also notes that reduction in contaminants depends on an assumption of collective responsibility, which calls for increased awareness, beginning with the regulatory authorities and filtering down to health care professionals and consumers. "Consumers need to be aware of the consequences of PPCPs to aquatic organisms and ecosystems, and should follow regulatory agency disposal guidelines to make our environment friendly for all living organisms."

At the same time, Mottaleb suggests, science professionals play a crucial role in addressing the challenge of PPCPs as emerging contaminants. This is particularly important in the light of a more recent discovery: traces of illegal drugs in surface water and river waters. "Scientists and toxicologists should continue to investigate the transport, fate, toxicity and their potential physiological and psychological effects on humans and wildlife as well as the relationship between bioaccumulation and diseases. Our research group is investigating those illegal drugs in fish from these rivers – periodic measurements of exposure level of those compounds are very important." *JC*

*Reference*

1. *MA Mottaleb et al., "Pharmaceuticals in grocery market fish fillets by gas chromatography–mass spectrometry", Food Chem, 190, 529-36 (2016). PMID: 26213006.*

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# In My View

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

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

*Contact the editors at edit@texerepublishing.com*

# The Search for the Ideal Detector

**Will aerosol-based detectors ever meet all the needs of the pharmaceutical industry?**

*By Dorina Kotoni, Analytical Expert - Principal Scientist, Novartis, Basel, Switzerland.*

When developing impurity-profiling methods for pharmaceutical applications, detection and sensitivity can be challenging. On one hand, the high variability of physico-chemical properties of the analytes requires that we use universal detection systems, while on the other hand the expectations of regulators drive the need for higher sensitivity and detectability (for trace analysis, degradation by-products, and so on).

The pharmaceutical industry has been basing most of its release methods on liquid chromatography (LC) with UV detectors and gas chromatography with flame ionization detectors (GC-FID), with the occasional use of fluorescence and mass spectrometry (MS). There is still a reluctance to introduce other "universal detectors". Moreover, pharma's "ideal" detector should be sensitive and robust, ideally showing a universal response independently of the properties of the sample. It should be able to detect and quantify all components, including unknowns for which no reference standards are available (which is typically the case for early phase projects). Ease-of-use, the ability to interface with HPLC and UHPLC instruments, cost effectiveness, as well as a good understanding of the response curves, are fundamental for the purchase and implementation of a new detector.

The truth is, although we are promised great things, we are still waiting for a commercially available and truly universal detector!

The next best thing to a universal detector, according to instrument manufacturers, are aerosol-based detectors, but, in reality, these only deliver what they promise for detecting non-volatile components under isocratic elution conditions. While there have been significant advances in this field, there is still a lot to be understood in the responses obtained with light scattering and charged aerosol detectors – for example, gradient effects, detector settings, influence of compound properties on the detection. Interestingly, a few recent studies highlight that volatility of a compound is not sufficient to explain differences observed in detection. For non-volatiles and semi-volatiles, parameters such as molar volume and diffusivity, as well as net charge of the compound seem to play a role in detection. These new aspects still need further investigation and explanation by theoreticians and instrument manufacturers.

Understanding the response model of aerosol-based detectors under hydrophilic interaction LC (HILIC) conditions can frequently prove even more challenging. Analytes eluted in HILIC often require an alternative detection technique due to low UV absorption (sugars, aliphatic amines, lipids, amino acids). There is ongoing research in the field but so far, most publications describe work done under mainly isocratic elution conditions.

In my opinion, we still have a long way to go in understanding aerosol-based

*"Understanding the response model of aerosol-based detectors under HILIC conditions can frequently prove even more challenging."*

detection under HILIC conditions. A HILIC expert once told me "we never use gradients in HILIC, they are too complex", showing that there is still a big gap to cover, model and understand, both in terms of separation and of detection. A universal response model for non-volatile molecules in HILIC is inherently more complex and needs to consider not only the gradient effects, but also the detector settings, the mobile phase interference, and the polarity of the analytes.

Manufacturers need to investigate the fundamentals of nebulization and particle formation further to be able to design detectors with increased sensitivity and greater uniformity in their response. In some cases, it might not be enough to widen the dynamic range of the detector by data interpolation through algorithms introduced between the analog and the digital output of the signal. As analytical scientists, we need to detect more and better – and I don't think that, in the case of aerosol-based detection, an algorithm (as refined as it may be) will save the day.

I am convinced, however, that when we have a better understanding of the response model for aerosol-based detection under a variety of analytical conditions, the pharma industry will be very happy to introduce them routinely in the analytical lab. The need is there. In the meantime, we are still looking for that ideal detector...

# Is Your Biomarker Analysis Accurate?

**It's time to take the plunge with supercritical fluid chromatography in bioanalytical applications.**

*By James Settlage, senior research investigator, Clinical Lab, inVentiv Health, Princeton, New Jersey, USA.*

Supercritical fluid chromatography (SFC) finally appears ready for prime time with both Agilent and Waters selling viable machines. But, in the bioanalytical field at least, there has been surprisingly little interest shown to date. Might this be due, in part at least, to the fact that chromatography in general has taken a back seat to the selectivity provided by triple sector mass spectrometry(MS) and highly selective antibodies in the case of ligand-binding assays?

*"Not only can we not demonstrate specificity, we are also much less likely to achieve it."*

Indeed, why buy an expensive new chromatographic platform if there is no 'apparent' need? Apparent being the operative word. There is an easy trap for us to fall in to here. All of our confidence in MS/MS has grown while, for several decades, we have been measuring drugs and their metabolites – typically xenobiotics. For analytes not normally found in the matrix of interest, demonstrating the specificity of a method is straightforward. In the absence of the drug, if the matrix generates no signal, it is highly likely that the method is specific for the analyte of interest. And we can often achieve that specificity with little or even no chromatography. However, we are now becoming more and more involved with the analysis of biomarkers.

Our move to biomarkers introduces a very important but not always completely obvious challenge – the fact that a blank matrix is often not available, so it is not possible to demonstrate specificity. Nevertheless, we have developed so much confidence in the selectivity of MS/MS and our antibodies while quantifying xenobiotics, that we are not inclined to be concerned.

Unfortunately, there is a very real reason to be concerned! Biomarkers are created by complex biosynthetic processes that also produce a number of other structurally very similar substances. Not only can we not demonstrate specificity, we are also much less likely to achieve it. Chromatography suddenly becomes a critically important part of our analytical methodology. And we cannot know how much chromatography is required because we can never be sure that our analyte signal is not partly produced by another similar molecule.

I am not saying that every biomarker method should include an extensive and time-consuming chromatographic step – but if accuracy is important, we could benefit by testing our method against one that does utilize the best chromatography available – better still, more than one type of chromatography, which is why SFC can be a very important tool. Not only is SFC comparable to liquid chromatography (LC) in ease of use, but it also tends to produce very different retention times for a given analyte compared with LC. In addition, SFC yields very different retention times on different stationary phases. The orthogonality gained can be as useful as brute force chromatography, benefitting from high numbers of theoretical plates. And it requires only a few minutes to test each stationary phase using SFC. If the intended method (LC-MS/MS or ligand-binding assay, for example) has been used to analyze the amount of the endogenous biomarker in a given lot of matrix, and if we test the same lot using various SFC stationary phases, we will either discover that the intended method is not specific – or we will be as confident as possible that it is.

# Microchip-CE: Smaller is Faster

**Exploring the potential of microchip capillary electrophoresis (MCE) to speed up sample throughput in the pharmaceutical industry.**

*By Friederike Winkhaus, Scientist Development Analytics and New Technologies, and Markus Haindl, Director Development Analytics and New Technologies, Roche Diagnostics GmbH, Penzberg, Germany.*

Monoclonal antibodies are important therapeutic molecules. Increasingly, novel complex antibody formats are replacing classical monoclonal antibodies. To ensure product integrity and product quality, a well-controlled manufacturing process and thorough analytical characterization are required. Continuous process automation, Quality by Design (QbD) studies, and Design of Experiments (DoE) approaches to understand the relationship between critical process parameters and product quality increase the number of samples that need to be processed.

Conventional analytical methods, such as capillary electrophoresis (CE-SDS), capillary zone electrophoresis (CZE) or ion exchange chromatography (IEC) are very precise and reproducible but, unfortunately, have only a low sample throughput. Therefore, there is clearly a need for new high-throughput

*"One sample can be analyzed in only 40 seconds (about 45 times faster than CE-SDS)."*

analytical technologies to handle the increasing number of samples.

We think that microchip capillary electrophoresis (MCE) is a good solution as it offers a high-throughput approach for monitoring antibody quality. In our laboratories, we have implemented the LabChip GXII system (Perkin Elmer), which enables automated analysis of 96 to 384 samples in one run. The system provides three different assays for the analysis of proteins: Microchip CE-SDS (MCE-SDS), Microchip CZE (MCZE), and glycan analysis. In this article, we focus on the MCE-SDS assay.

From our point of view, the MCE-SDS assay offers several advantages compared to our conventional CE-SDS. Our CE-SDS method uses a ~30 cm capillary to separate the antibody samples, which leads to an analysis time of about 30 minutes per sample. With MCE-SDS, samples are analyzed in a separation channel of only 14 mm in length. Thus, one sample can be analyzed in only 40 seconds (about 45 times faster than CE-SDS). Because of the short separation channel on the microchip, we can assume a decrease in the resolution. Surprisingly, for some antibody features – especially in the low molecular weight range – MCE-SDS shows a resolution that is superior to CE-SDS.

With MCE-SDS, the antibodies are labeled with a fluorescent dye and detected using laser-induced fluorescence. The proteins can be stained directly in the capillary using a non-covalent labeling mechanism: the dye, which is provided in the gel matrix, binds to both protein-SDS complexes and SDS-micelles. To reduce the background signal from labeled SDS-micelles, there is a de-staining step at the end of the separation channel. The big advantage of the non-covalent labeling is that the sample preparation is not only quick

*"Our main goal is to use the MCE-SDS assay for GMP (good manufacturing practice) lot release testing."*

and easy but also compatible with a variety of different buffers. Only harvested cell culture fluids require a buffer exchange step during sample preparation. Moreover, the sample preparation can be automated on different robotic platforms, further reducing the analyst's hands-on time. As regards the general system handling, our experience is that the LabChip GXII shows a lower failure rate than the CE-SDS systems and requires less experienced staff. However, with the high sample throughput, data analysis is a bottleneck – even the best automatic integration algorithms cannot replace the manual review of every analyzed sample.

We have implemented the MCE-SDS assay to support our upstream processing (USP) and downstream processing (DSP) departments during process development. Here, we monitor product purity, product-related impurities, in-process controls, as well as product stability. However, our main goal is to use the MCE-SDS assay for GMP (good manufacturing practice) lot release testing. The assay still has some drawbacks compared to CE-SDS in relation to sensitivity and reproducibility, but, together with Perkin Elmer, we are optimizing the assay for use in quality control (QC). By moving the assay to the QC environment, we will prevent any method offset by having the same method for process development, in process testing, process characterization/process validation (PC/PV) studies, critical quality attribute (CQA) assessments and lot release testing at the same time.

In summary, MCE-SDS is a high-throughput assay for monitoring and characterizing classical and novel formats of antibody-based biotherapeutics. And though it is currently used for non-GMP samples only, we think that it has the potential to become a QC assay in the future.

# Waste Not, Want Not

**Isn't it time to share resources to open up more opportunities for metabolic profiling?**

*By Julien Wist, Professor, Department of Chemistry, Universidad de Valle, Colombia, and Elaine Holmes, Professor, Department of Surgery and Cancer, Imperial College, UK.*

When it comes to human waste products, such as urine and feces, the first thing that comes to mind is probably rapid disposal. However, over the last decade, intensive research into their metabolic composition has shown that these waste products harbor a great deal of information relating to diet, lifestyle and risk of disease. Spectroscopic profiling of these samples, typically using mass spectrometry (MS) or nuclear magnetic resonance (NMR) spectroscopy, generates 'metabolic fingerprints' or 'metabolic phenotypes' that are unique to the person producing them. The technology can characterize a wide range of diseases, as well as being able to track an individual's response to dietary and therapeutic interventions (1, 2).

The success of this technology has relied on advances in analytical spectroscopy and progress in bioinformatics. However, there has been much effort but relatively little progress

*"Investigators generally remain reluctant to deposit 'their' data into open-access databases."*

in standardizing methodological profiles, mathematical modeling tools and spectral databases. Some headway has been made in collating databases of reference standards, with the Human Metabolome Database (HMDB; funded by the Canadian Government) and the Biological Magnetic Resonance Bank (BMRB; hosted by the University of

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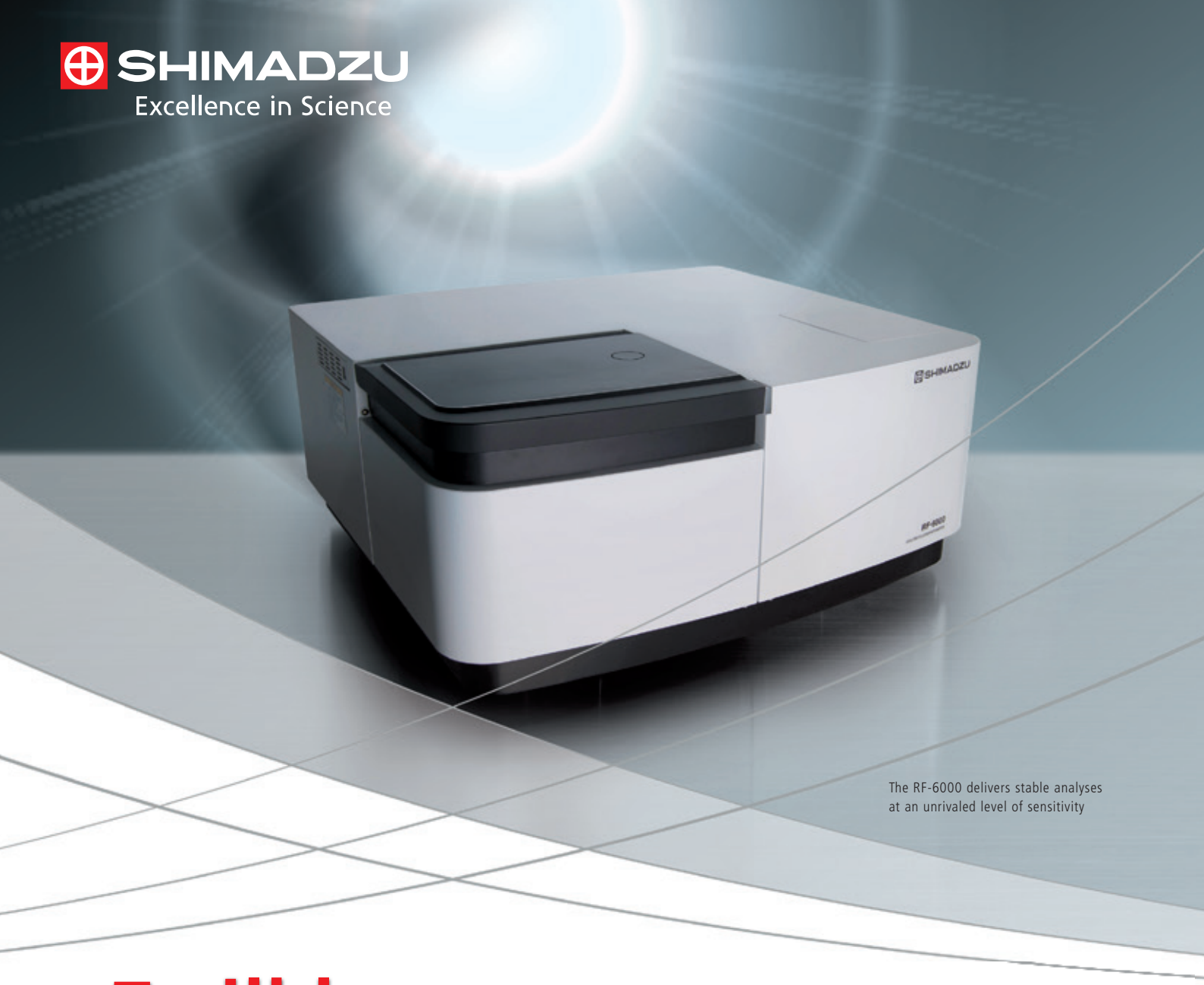

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*"The efforts in technology standardization to date have been driven by analytical criteria."*

Wisconsin at Madison, USA) being amongst the best-known examples. In terms of sharing NMR and MS spectra of real biological samples, there are no large publically-available resources. The European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI; Wellcome Genome Campus, UK) is co-ordinating an initiative to allow storage, exchange, comparison and re-utilization of metabolomics data and has begun to develop a repository for biofluid spectral data (3).

Yet despite these efforts and the drive towards open-source data by funding and regulatory bodies, investigators generally remain reluctant to deposit 'their' data into open-access databases. Combined with variability in analytical protocols, this has severely constrained the utility of the resource. The extent of collaboration, including the practice of data and resource sharing, has reached different levels across academic disciplines. For instance, the proteomic community has a universal standard for reporting data (minimum information about a microarray experiment; MIAME), a plethora of non-commercial freeware (4) and is beginning to provide test-set data with which to validate new algorithms. In principle, the lack of communal facilities does not harm the larger research groups that have sufficient resource to build large in-house databases and data mining tools. However, collaboration across research communities has obvious advantages – the most relevant being to accelerate the pace of discovery.

Barriers to widespread implementation of metabolic profiling technology include the initial capital cost (often prohibitively high for resource-limited research and clinical environments), a lack of trained researchers, a lack of universal standards and annotation, and restricted mobility of both commodities (samples, solvents, equipment) and people. Nevertheless, one of the main attractions of implementing multipurpose metabolic profiling platforms in resource-limited research settings is the conveyance of research flexibility. By focusing a research community or field on critical issues (such as harmonization of experimental design, sample handling, sample acquisition) and by implementing a uniform structure for training and technology transfer, we can begin to both support and benefit from resource-limited research groups on a global scale. The other major initiative required to facilitate success is the sharing of spectral repositories through an accessible framework of databases.

The efforts in technology standardization to date have been driven by analytical criteria. For smaller research groups the imperative to justify expensive technology with practical application is stronger. Initiatives such as the Latin American Metabolic Profiling Society (LAMPS, http://lamps.yo-que.ch), which brought together academic spectroscopists from more than 10 countries, supported by industrial partners, such as Airbus, Bruker Biospin, Waters, Agilent and Metabometrix, have arisen in direct response to the need to accelerate research by building networks of chemists and application scientists. The goal is to create active research networks that can foster and deliver collaborations across local research groups in biomedical sciences.

In addition to the chemical requirements for conducting high-quality research, the group has also identified biological areas of interest across the community, including bariatric surgery and dengue infection, whereby the community can reach a critical mass of data more quickly and has an inbuilt structure for validation of disease-associated biomarkers and metabolic networks. Rather than constraining metabolic phenotyping to just a few of the worlds best-equipped chemistry laboratories, we should work towards reducing the 'waste' of locally owned and hidden spectral resources (by identifying opportunities for sharing of facilities, mobilizing scientists and consumables, organizing training and technology transfer networks and creating annotated data repositories and reference databases). Overcoming these barriers will help fulfill the potential of metabolic profiling as a mainstream tool in terms of diagnostics, prognostics and monitoring in both patient- and population-centered frameworks, thereby building a bridge between analytical scientists and clinicians to drive translational healthcare initiatives.

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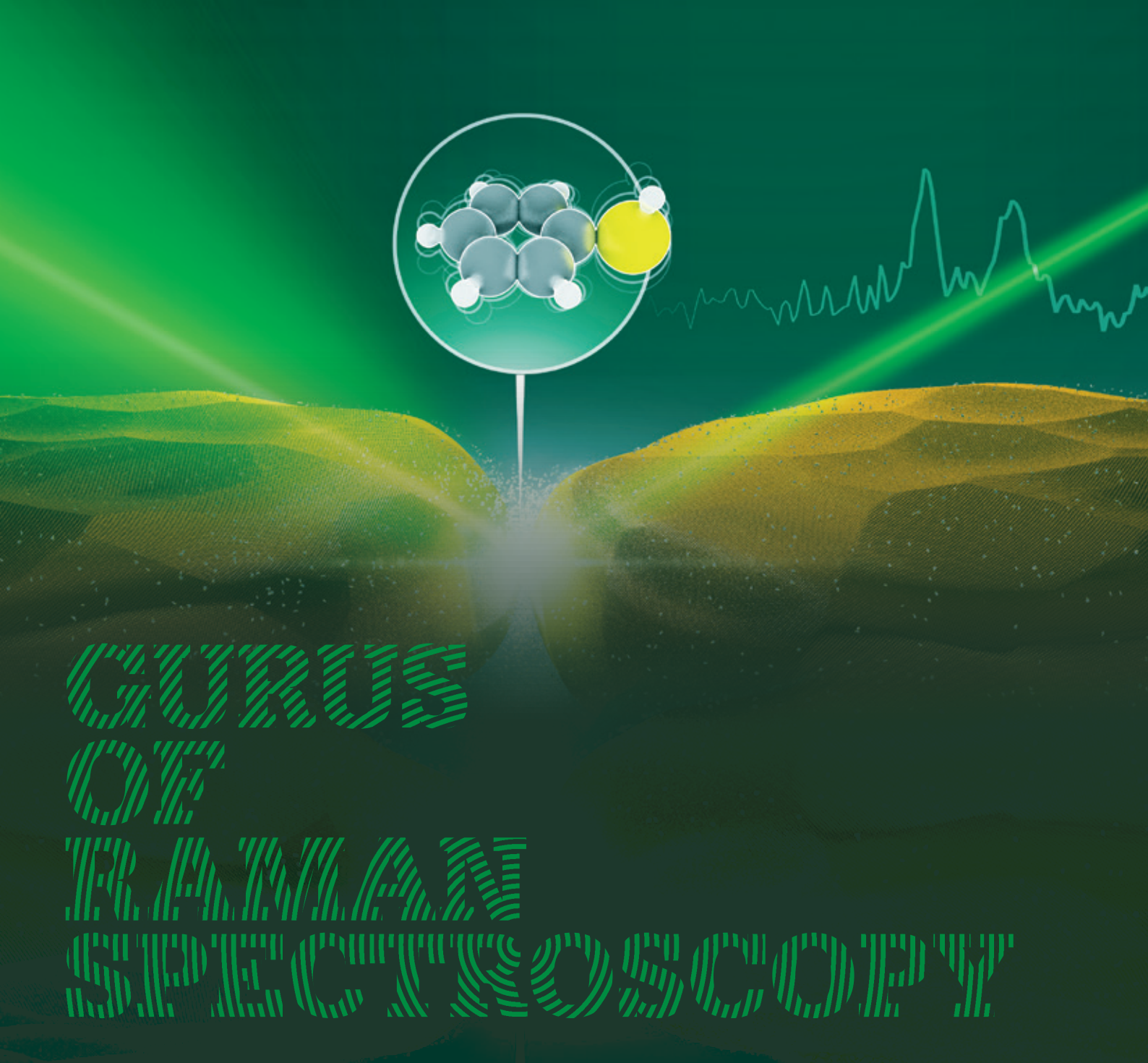


# GURUS OF RAMAN SPECTROSCOPY

A group from the École polytechnique fédérale de Lausanne in Switzerland has proposed a potentially disruptive new model – dynamical backaction amplification (DBA) of molecular vibrations – to explain unexpected observations in surface-enhanced Raman spectroscopy (SERS). And they believe the work will open up opportunities for novel systems that further enhance the detection capability of SERS. However, the model has not been fully accepted by the spectroscopy community, with other researchers heading in different directions. Volker Deckert (University of Jena and Leibniz Institute of Photonic Technology, Germany) and Duncan Graham (University of Strathclyde and Renishaw Diagnostics, UK) offer their own thoughts on the future of Raman spectroscopy.

## The DBA Lowdown

Christophe Galland and Tobias Kippenberg – co-authors of the new paper (1) with Philippe Roelli and Nicolas Piro – discuss the group's findings.

The set of vibrational frequencies of a molecule constitutes its unique fingerprint. Vibrational modes that are "Raman-active" interact with incident laser light in an inelastic scattering process. This results in secondary photons with a frequency shifted from the incident ones by the vibrational frequency. Raman spectroscopy leverages this process to optically measure the vibrational spectrum of a molecule (or a material) and thus reveal its chemical identity.

Unfortunately, Raman scattering has a very small cross-section and it is impossible to detect single molecules with a conventionally focused laser beam. A route to overcoming this limitation was discovered in 1997 independently by K. Kneipp et al. and S. Nie and S. R. Emory (2, 3). Using surface-enhanced Raman scattering (SERS), the teams dispersed individual molecules on rough metallic surfaces, which served to focus light down to molecular dimensions. The incident light generates localized oscillating electric fields in the metal, which are called plasmons. With the rapid progress of micro- and nano-fabrication techniques, researchers are now able to tailor the properties of these plasmons in a variety of metallic nanostructures, which has led to rapid improvements in sensitivity and unexpected observations such as nonlinear effects and anomalously large enhancement factors, which could not find explanations within the conventional models of SERS.

## Christophe Galland

Christophe obtained his PhD from Ataç Imamoglu's group at ETH Zürich (Switzerland) in 2010. He then spent two years as a postdoctoral fellow at Los Alamos National Lab (New Mexico, USA) with Victor Klimov, where he developed a novel spectroscopic technique to study the fluorescence of single nanocrystal quantum dots in an electrochemical cell. In 2012, he joined Michael Hochberg and Tom Baehr-Jones at the University of Delaware (USA) to implement quantum optical devices and experiments with silicon photonic integrated circuits. Since mid-2013, he has been the holder of a fellowship from the Swiss National Science Foundation in Tobias Kippenberg's group. Christophe's current research with PhD student Philippe Roelli focuses on the theory and experimental realizations of optomechanical systems based on molecules coupled to plasmonic cavities.


*"Our findings question some widely accepted guidelines used to optimize SERS."*

### A different angle

Our group has an atypical approach to Raman scattering, since our expertise is not in molecular or crystal spectroscopy but rather in cavity optomechanics. Our field deals with carefully engineered micro-fabricated systems in which a mechanically compliant element modulates the resonance frequency of a high-quality optical cavity. When a laser excites such a system, a Raman process can generate or annihilate one quantum of mechanical excitation (that is to say, a phonon), in analogy to what happens in a molecule or a crystal. Yet the oscillation of optomechanical systems are typically at much lower frequencies and feature much lower damping rates and much longer relaxation times.

From our perspective, Raman scattering in optomechanical cavities is thus a tool to manipulate mechanical motion with light. In particular, you can optically damp mechanical oscillations, a technique that has been used in quantum optics to cool trapped atoms or ions to their motional ground state. The work we are doing, therefore, opens a new way to investigate molecular vibrations in the quantum regime using the concepts of quantum optics.

In our latest theoretical research, we developed a radically novel framework to model the plasmon-enhanced Raman interaction and calculate the Raman signal. We demonstrate that a vibrating molecule coupled to a localized plasmon is formally equivalent to an optomechanical cavity, a generic system in which a mechanical oscillator modulates the resonance frequency of an optical cavity, consisting of, for example, two facing mirrors. The radiation pressure of photons bouncing on the mirrors can be used to optically amplify their oscillating motion in a process called dynamical backaction amplification (DBA).

Our research shows that DBA can be used in SERS to amplify Raman-active molecular vibrations and thus provide a new mechanism for signal enhancement. In other words, light is more than a passive analytical tool; it also acts on the internal molecular degrees of freedom and drives molecular vibrations. The light-induced force, analogous to radiation pressure on a mirror, can thus be used to excite a particular vibrational mode of a molecule well above its thermal motion. The extra excitation greatly enhances the Raman signal, which is proportional to the vibrational energy.

## The implications?

The practical consequence of our findings is to question some widely accepted guidelines used to optimize the substrates and excitation schemes in SERS. Until now, researchers have obtained large signal enhancement by using broad plasmon resonances, which overlap with both the incoming laser and the outgoing Raman light. Considering DBA, we find two counter-intuitive conditions for obtaining amplification that is even more efficient. First, one should strive for narrower plasmon resonances (more precisely, the width of the resonance should be smaller than the vibrational frequency). Second, the excitation laser should be blue-detuned from the plasmon by exactly the vibrational frequency of the mode to be amplified.

Following these new guidelines, researchers and engineers should be able to design and fabricate a new generation of plasmonic devices to push the detection limits of SERS and its analytical capabilities even further.

The frequency shift between the laser and the plasmon resonance determines which particular vibrational mode is amplified, which opens a route toward higher resolution spectroscopy and mode-specific amplification. By applying our method, it should be possible to drive one particular vibrational mode far out of thermal equilibrium, and thereby study the dynamics at a molecular scale, quantifying the degree of anharmonicity (deviation of a system from harmonic oscillation) of each mode and their inter-mode couplings.

In practice, if the conditions for efficient DBA can be achieved in a commercial device, it would enable very specific detection of given types of molecules by carefully choosing the laser wavelength with respect to the plasmon resonance. Moreover, this parameter is easily tunable, allowing the device to be reconfigured for different molecules. On the detection side, if mode-specific amplification is reached, you could dispense with using high resolution spectrometers to distinguish between different modes, as a single frequency should be amplified and contribute to the signal.


### Tobias Kippenberg

Tobias Kippenberg has been a full professor in the Institute of Condensed Matter Physics and Electrical Engineering at EPFL, Switzerland since 2013. Prior to EPFL he was Independent Max Planck Junior Research group leader at the Max Planck Institute of Quantum Optics (MPQ), Garching, Germany, in T. W. Haensch's laser spectroscopy division. There he demonstrated radiation pressure cooling of optical micro-resonators, and developed techniques with which mechanical oscillators can be cooled, measured and manipulated in the quantum regime that are now part of cavity quantum optomechanics. His group also discovered the generation of optical frequency combs using high Q micro-resonators. Tobias is the recipient of several awards, including the EFTF Award for Young Scientists (2011), the Helmholtz Prize in Metrology (2009), the EPS Fresnel Prize (2009), ICO Award (2014), the Swiss Latsis Prize (2015), the Wilhelmy Klung Research Prize in Physics (2015) and he won first prize in the 8th European Union Contest for Young Scientists in 1996.

# Exploring C. V. Raman's Nobel Legacy

Volker Deckert and Duncan Graham discuss the current state-of-the-art in Raman spectroscopy and share insights into current and future research directions.

## What is your current research focus?

Volker Deckert: I've been working with Raman spectroscopy since my diploma thesis and I've always appreciated the different directions that this technique allows one to move in. Even though more and more commercial systems are available, ranging from scientifically flexible systems to handheld systems for all kind of applications, there is still space for instrumental progress in the academic environment. Such instrumental development has been always one of my favourite areas. Improving detector and spectrometer capabilities, developing difference spectroscopy for multichannel detection, adapting spectrometers for "terahertz Raman" spectroscopy before it was even given the name – I loved doing that. Certainly this playing (fooling?!) around was an important aspect when developing near-field Raman systems and TERS. The latter two are most probably the most defining techniques for me and my group and continue to challenge us even after all this time. Pushing the lateral resolution limit of Raman spectroscopy was and still is my favorite subject. Currently, my work has moved towards more applied scientific cases and the challenge is shifting from the instrumental details towards sample preparation, data evaluation and even theoretical issues. Still, we've seen several surprises in recent years and I am looking forward to the next few!

Duncan Graham: We use chemistry to functionalize nanoparticles and design new methods of analysis based on surface-enhanced Raman scattering (SERS). We have a heavy emphasis on the role of the surface chemistry to enable the controlled enhancement of the Raman scattering and have developed a number of unique assays based on the properties of nanoparticles combined with SERS. There is a heavy emphasis on the analytical performance of our assays and we have been interested in the detection of biomolecules, such as DNA, proteins, and small biomarkers, as well as looking at cells, tissue and in vivo systems. We have also covered areas of interest in security applications (for example, trace detection of explosives), but our main focus is on life science and clinical applications.

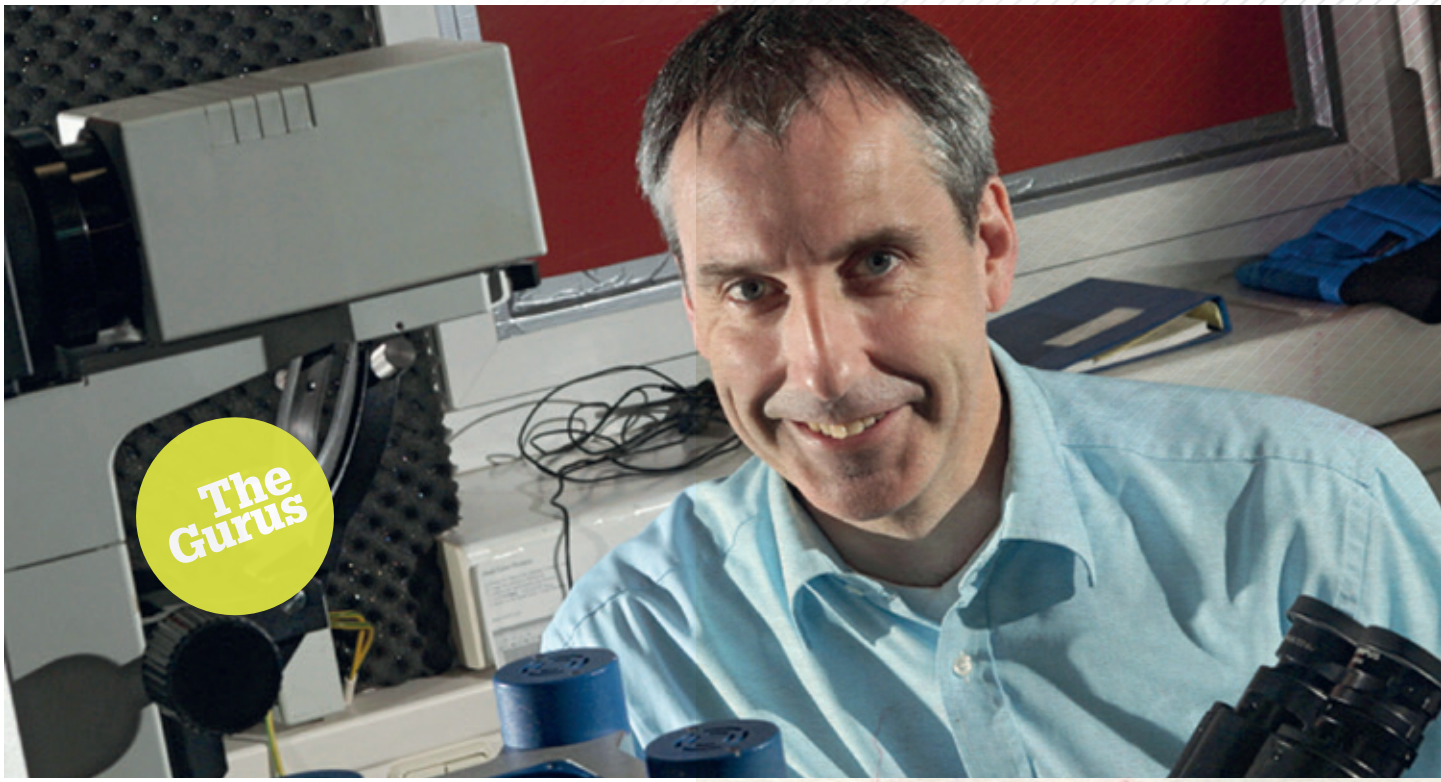
Additionally, we work with the end users of our analytical techniques and breakthroughs from an early stage in terms of designing projects and attempting to translate them into actual use. To date, our lead example is a high-throughput assay for fungal infections using SERS; the research took place a few years ago but it is now being commercialized (through Renishaw Diagnostics Ltd) and has just been awarded CE marking for use in European hospitals. It proves that SERS can be used in a bioanalytical assay in a clinical setting if there is enough understanding of the underlying chemistry to obtain suitable analytical performance.

## What are the main recent Raman milestones?

VD: The typical timeline begins with the application of the laser in the 1960s – and electronic recording more or less at the same time. In the 1970s and 1980s, the advent of Raman microscopes and multichannel detectors. All these technologies – and many more "small" developments – have helped to improve measurement time by orders of magnitude, changing acquisition times from hours and even days to sometimes only a few seconds. Modern Raman microscopes allow the acquisition of large areas so quickly that adaptation for clinical analysis is on the near horizon. Here, the intrinsic advantage of Raman as a label-free technique might change the way how diagnosis is being done in the future (either because of replacement with Raman or by pushing competing techniques to be developed further).

Particularly important to my favorite technique – near-field Raman – was of course the discovery of surface-enhanced Raman spectroscopy (SERS) effect by Fleischmann, van Duyne and Creighton in the 1970s. Indeed, SERS is a branch of Raman spectroscopy that is still a driving force for innovations in the field. A milestone was definitely the discovery of single molecule sensitivity in the 1990s and beginning of the new century by Nie, Kneipp, Etchegoin and again van Duyne. With the advent of tip-enhanced Raman spectroscopy (TERS) in 2000, the basic principles of SERS allowed a confinement of the lateral resolution far beyond the diffraction limit. All these advances culminated in the recent detection of sub-molecular units by Dong et al and Deckert et al. And though the reason behind the increased resolution is still controversial, experiments clearly indicate sub-nanometer resolution, which is, in turn, driving theoretical developments to explain the facts.

DG: The big milestones in Raman have been written about in several reviews by other authors (4, for example), but they tend to feature breakthroughs that are fundamental rather than applied. In terms of applications, Shuming Nie's first paper on the use of SERS for in vivo analysis in 2008 prompted a lot of subsequent activity (5). Another game-changer is Pavel Matousek (Rutherford Appleton Laboratory) who has been developing spatially offset Raman scattering for security applications. His work has now moved onto the analysis of living systems where he can penetrate deep into tissues (6).




From a personal perspective, our biggest breakthrough was in 2008 when we showed that a nanoparticle assembly could be used to turn on the enhancement of Raman scattering using biological interactions. That work has now been expanded into a variety of studies (7). And, more recently, a collaborative paper with Ewan Blanch at the Royal Melbourne Institute of Technology, Australia, has shown the first through space transfer of chirality in surface-enhanced Raman optical activity, which has implications for the study of the chirality of biomolecules at low concentrations (8).

### Where does Raman sit in the world of chemical analysis?

DG: Raman is being used a lot at the moment in terms of chemical analysis. It is gaining a position in process analytical technology for online analysis of chemical reactions with feedback loops to allow improvement of scaled up chemical synthesis. I think analytical chemists are very aware of the potential for Raman scattering to be used in various scenarios; however, the weak scattering from a number of molecules tends to limit this technique to bulk analysis. In terms of trace analysis, advanced techniques such as SERS and TERS can play a role and they are starting to gain traction in the chemical and bioanalytical sectors. Reproducibility can be an issue and I believe that this

### Volker Deckert

Volker Deckert is the head of the "Nano Spectroscopy Group" at the Institute of Physical Chemistry, University of Jena, Germany. The Group's research goal is to push the lateral resolution of vibrational spectroscopy. The main tool for related projects is tip-enhanced Raman scattering (TERS). Current research with TERS includes biopolymer sequencing, fundamental reactivity on plasmonic assisted reactions, membranes and membrane proteins (studying the nanoscale composition of membranes with respect to lipid distribution and inclusion of proteins, shedding light on intermolecular and intramolecular interactions of protein structures, and examining the basic mechanism of drug delivery), and fast and efficient diagnostics of viruses to establish an ultra-sensitive technology for the qualitative analysis of different viruses and their specific antibodies.


## Duncan Graham

Duncan is Research Professor of Chemistry and Director of the Center for Molecular Nanometrology at the University of Strathclyde in Glasgow. He is currently Chair of the Editorial Board of Analyst and will serve in that role until 2018. He has been awarded numerous awards for his research including the RSCs SAC Silver medal (2004), Corday Morgan prize (2009), a Royal Society Wolfson Research Merit award (2010), the Craver Award from the Coblentz Society (2012), Fellows Award from the Society for Applied Spectroscopy (2012) and was elected to the fellowship of the Royal Society of Edinburgh (2008). He is a cofounder and director of Renishaw Diagnostics Ltd (2007) and has filed 15 patents with license deals on most of his portfolio. He completed a PhD in organic chemistry at the University of Edinburgh (1996) and his interests are in developing new diagnostic assays based on nanoparticles and spectroscopy with target molecules including DNA, RNA, proteins and small molecule biomarkers.

*"The main challenge for SERS is to find the right application for the technique where it offers something beyond other analytical techniques."*

can be achieved for SERS, and also for TERS with appropriate (and sufficient) research and effort.

VD: The number of instrument makers increases at every exhibition, so it seems that more and more applications arise. My guess is that those applications are mostly analytical. And if such applications reach a routine stage they might be extremely successful; however, from an academic point of view those applications are not necessarily in the focus of research and might be less visible, for instance, at conferences. The number of attendees at the recent International Conference on Raman Spectroscopy, clearly indicates an increasing interest in the field both in academia and in industry. In areas where the science case is still close to the potential application (for instance, bio-medical research), I dare say that Raman is getting more and more attention on the clinical side. So I can see that the analysis in this field will profit from the benefits of Raman spectroscopy, but because of elaborate medical trials, it will certainly take several more years until Raman becomes as commonplace as it is, for example, in geology.

### How do you see the role and impact of SERS developing?

DG: The main challenge for SERS is to find the right application for the technique where it offers something beyond other analytical techniques. There are a number of two and three-dimensional surfaces commercially available that are interesting from a research perspective, but I am not aware of a killer application for nano-structured surface-based SERS technology, despite there being a growing number of vendors. In terms of solution- or suspension-based approaches where nanoparticles are used, SERS is gaining an advantage over other techniques. And I believe that this will move into the chemical and bioanalytical sectors. Again, the technical challenge really

lies in the reproducibility that comes from having a solid and in-depth understanding of the surface chemistry; everything has to adsorb on the surface to be enhanced. Once you can control this, you get reproducible SERS. Much is said about having the right substrate and the right particles, which is of course important, and you can make nanoparticles very reproducibly, which will give a consistent enhancement – but only if your surface chemistry is robust and reproducible. This has an impact on imaging where enhancement from nanoparticles needs to be reproducible and similar, in particular, when looking at biological systems. There is a lot of work going on in this sector right now and I think this is an area of great interest for the SERS community. The effects will be felt in the life science and clinical sectors.

VD: Wherever high sensitivity is required, SERS is a potential option. At the moment, there is a big discrepancy between laboratory substrates that can reach extremely high enhancement factors and commercially available substrates that show, in general, much lower enhancement, but are reliable and potentially also comparable. Some consolidation is required along with the understanding that high enhancement factors usually come at a cost. I am sure that SERS in combination with handheld instruments will have a big share in the market when sensitive qualitative analysis is required. Quick tests for drugs could be an example. Solving the quantitative issue seems to me much more challenging, but in combination with lab-on-the-chip technologies this challenge can probably be solved as well.

From an imaging point of view, SERS imaging is somewhat comparable to MALDI imaging. As a substrate has to bind onto a nano particle/nano-structured array, thermodynamics and kinetics must also be considered so a preferential binding can cause false readings. As in MALDI imaging, this issue must be solved, at which point SERS imaging could become a competing technique.

### How do you see the role and impact of TERS (tip-enhanced Raman spectroscopy) expanding?

DG: I'll leave this one to Volker!

VD: TERS is an interesting case – and my view on it cannot be neutral for obvious reasons. It is still experimentally demanding and most fundamental and application related research is found in academia. As it is one of the few techniques that allow us to address chemical composition with nanometer resolution, TERS is without alternatives in many cases. My expectation is that TERS will be tested on many different samples in the near future and either we will really learn about the desired nanoscale composition or equally important, learn about fundamental issues that prevent TERS detection in specific cases (diffusion, stability and so on). Clearly, TERS needs to move towards more general specimens with "normal" Raman scattering cross-sections, which will in turn have an impact on TERS mapping. In the near future my expectation is that most experiments will still be point measurements or profiles of specific areas of interest in combination with larger scale Raman and AFM images. Why? Because of fundamental physical laws in these dimensions. Positioning probes with sub-nanometer precision for a long time literally comes at a cost, but it could change dramatically when UV-TERS systems become available. Then an additional resonance Raman effect potentially decreases the measurement time such that imaging at a reasonable speed becomes feasible. In this regard, the instrument makers, in general, are heading in a very positive direction. And though their influence on the tip material and design has been low, the features of the instruments that allow a fast determination of potential areas of interest – whether Raman or AFM imaging – have improved a lot over the last few years. My expectation is that such developments will continue to help the development of novel TERS modes simply by focusing on the location of interest.

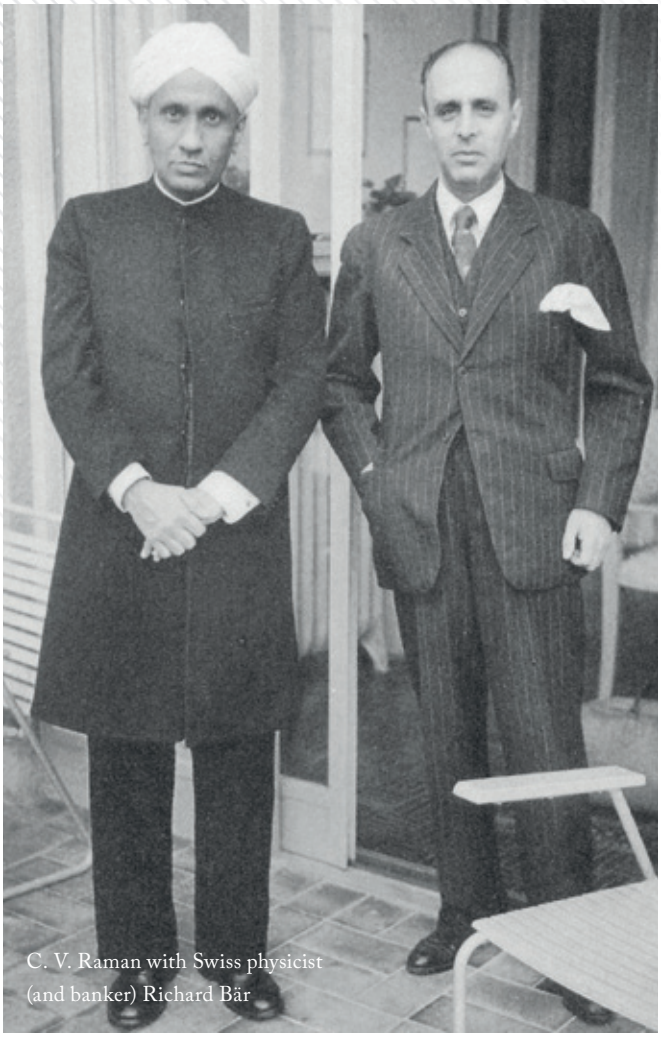
### Considering the work done at EPFL, what do you think are the implications for analytical sciences?

VD: The EPFL approach is off the beaten track and provides a new route to the understanding of SERS. The challenge for all theoretical models for SERS is the intrinsic variability of the substrates. In this respect, most substrates for SERS were not produced using first principles, but rather in a trial and error fashion, which was, in some cases, extremely successful (for example, the proverbial Lee-Meisel colloid). Any directed method of SERS substrate manufacturing is of course very desirable and I am looking forward to seeing how this paper will affect practical SERS substrate design.

DG: The use of optical cavities could be very useful in SERS and it will be interesting to see the experimental SERS community building on this paper where some very exciting data is presented. However, SERS is a very complicated process and it does seem that the exact system being used or modeled can be unique, so it's not always the case that data obtained or predicted in one SERS setup is transferable to others. The most obvious example of this is where several papers have shown a lack of dependence on matching the excitation frequency to that of the plasmon – but where as many papers show the opposite.

### How do you see the role of Raman developing in the near future?

DG: Raman has the opportunity to produce ultrasensitive analysis through some of the advanced surface enhanced or tip-enhanced Raman spectroscopy options. And because of



C. V. Raman with Swiss physicist (and banker) Richard Bär

instrumentation developments, low cost and portable Raman spectrometers are now available at a range of wavelengths, which opens up a range of new scenarios; for example, in vivo analysis, use outside laboratory environments, and use in the clinic at an acceptable cost. As noted, the key to the analytical performance of SERS lies in understanding and controlling the surface chemistry of any analyte species. This statement also applies to imaging techniques, where new microscope systems offer incredibly high performance when it comes to resolution and speed, but bring challenges in terms of data complexity, which demands the expertise of statisticians and the need for image processors. Raman and SERS imaging and mapping rival fluorescence in terms of the data; however, the techniques still acquire the images more slowly – but with the advantage that they can do so without labels. Ultimately, moving forward I suspect that a combination of fluorescence, Raman and SERS will start to be a necessary addition to the armory of the life or clinical scientist interested in studying single cells, tissues and complex biological systems.

VD: As indicated, I think the main impact of Raman will be in the field of biomedical analysis. Quick determination and identification of bacteria, viruses (potentially with TERS) will allow a faster response for infections. Compared to normal cultivation approaches, patients can gain up to several days. Similarly, for spectroscopic identification or supported identification of tumors during surgery, Raman is potentially very powerful. The challenges are similar to any other new treatment – trials take a long time and are very costly.

Normal Raman imaging is unlikely to compete with fluorescence-based techniques with respect to speed and lateral resolution. Raman imaging is competitive only if labeling is not possible or prohibitive. In terms of analytical challenges, we can also take from this that if little is known about a sample a-priori, Raman imaging is the best approach to get a first idea about a sample. Having said that, Raman can be more demanding than its competitors (IR, fluorescence) from an instrumental perspective. I will say that the stiff competition between the three has and will continue to be beneficial for them all. And I am pretty sure that none of these techniques will go out of business soon...

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| | |
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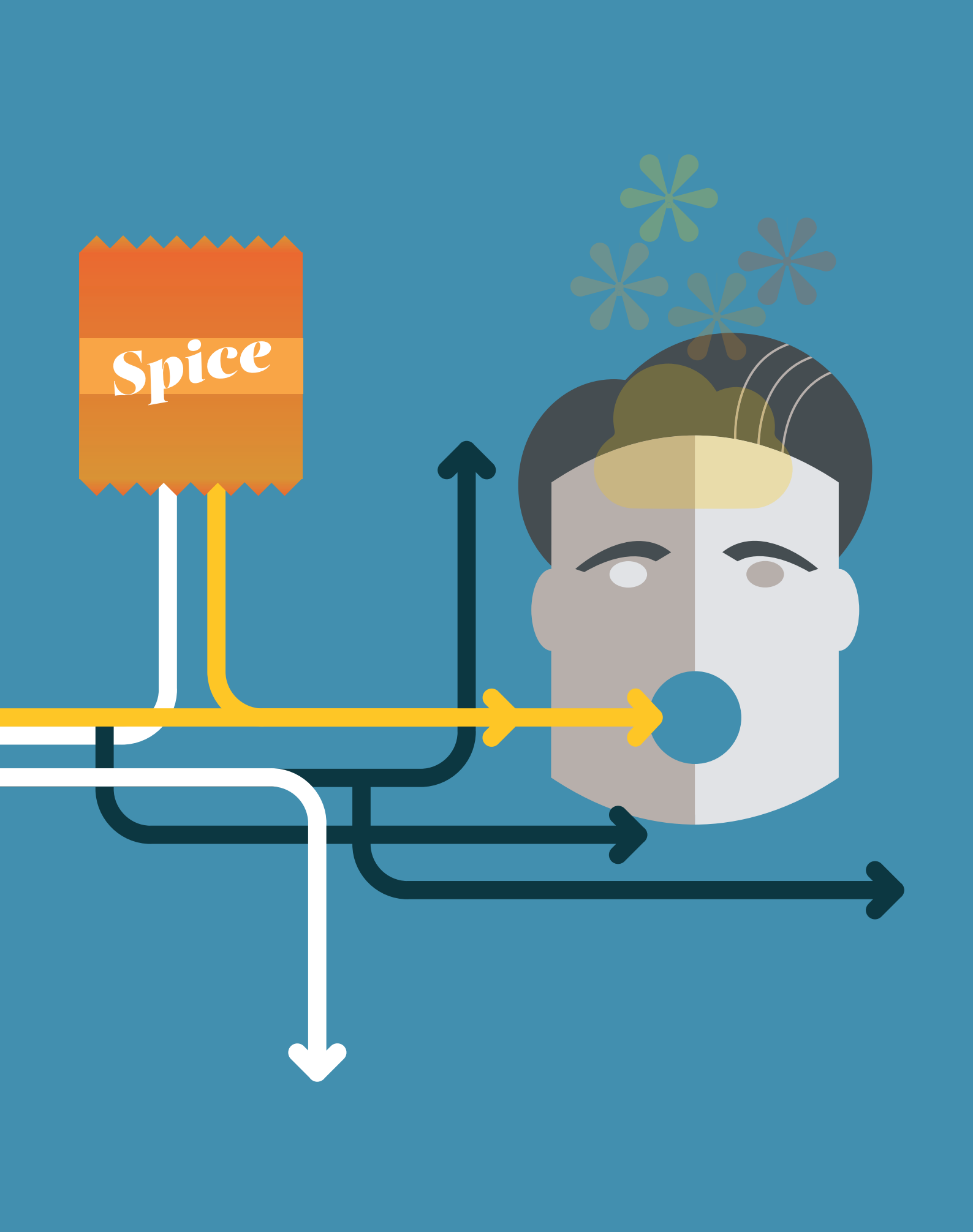

Spice

# Legal Highs & Lows

As new psychoactive substances (NPS) flood the market and "designer drug" sales are on the rise, we are faced with significant – and growing – social and analytical challenges. Here, I offer an overview of a quietly unraveling crisis.

*By Amira Guirguis*

While serving as a hospital pharmacist, I frequently encountered patients with marijuana hidden in their belongings; often, they declared them as personal "herbal medicines". At the same time, the use of "designer drugs" was increasing and it was not uncommon to encounter numerous admissions with unknown sources of toxicities. The lack of available clinical guidelines for treating designer drug-related toxicity made it challenging and led to the provision of supportive adjunct treatment as well as treating the symptoms themselves. Furthermore, symptom control was based on local formularies used by different hospitals rather than national guidelines (1). These challenges inspired me to undertake a PhD research project to study and investigate the new flood of "designer drugs". This has led me into contributing to two EU-funded projects that focus on the problem of NPS: EU-MADNESS (www.eumadness.eu) and Enhancing Police Skills regarding NPS (www.npsproject.eu).

## No control = crisis

NPS are synthetic drugs made for recreational use that are not subject to international control (2). To circumvent the law, NPS manufacturers slightly modify the structure of established drugs of abuse, while retaining their pharmacological effects (3) (Table 1). NPS are sold and marketed with labels that do not reflect the actual content of the product. Indeed, they are often branded with confusing and frankly ridiculous names such as "Dr. Booga Shooga" or "meow meow", labeled as "research chemicals", "legal highs", "food supplements", "bath salts", "plant food", or "herbal highs", with complex acronyms, such as AH-7921 or 251-NBOMe (or Nbomb). The ban of one drug has been shown to be associated with rapid replacement by an alternative "legal and uncontrolled" drug and has actually resulted in the proliferation of the NPS market, with increased diversity of products, users, distributors and risks.

In contrast to the limited number of traditional drugs of abuse, such as cocaine, heroin and ecstasy, the number of NPS is

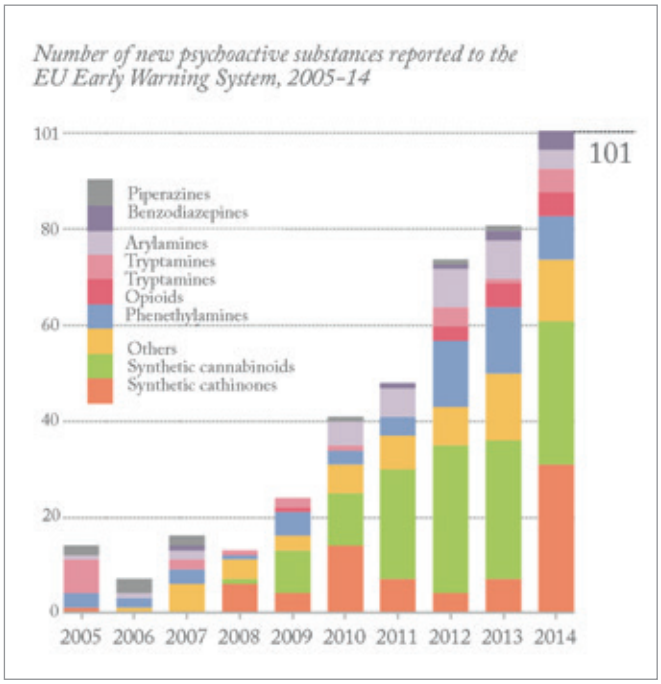



Figure 1. New psychoactive substances in Europe. An update from the EU Early Warning System (March 2015, Reproduced with permission from the EMCDDA)

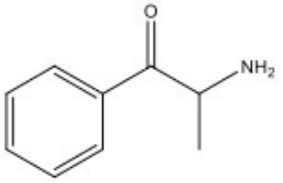
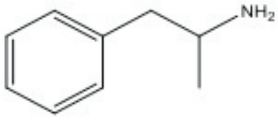
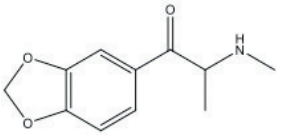
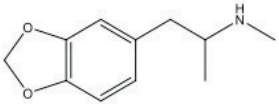
| *NPS* | *Traditional drugs of abuse* |
|---|---|
|  |  |
| Cathinone | Amphetamine |
|  |  |
| Methylone | Ecstasy |

Table 1: Structural similarity between emerging NPS and traditional drugs of abuse

increasing dramatically and there is no sign of the market slowing down. Internet sales and the ease with which new online markets are created contribute to the crisis. Currently, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) is monitoring more than 500 NPS. The EMCDDA has also reported that more than two drugs appeared on the market each week in 2014. And the reported number of NPS seizures in Europe has also increased seven-fold between 2008 and 2013 (4).

The EMCDDA categorizes NPS as piperazines, benzodiazepines, arylamines, tryptamines, opioids, phenethylamines, synthetic cathinones, synthetic cannabinoids and others (see Figure 1). Synthetic cathinones and synthetic cannabinoids have been the most popular classes of NPS in Europe since 2009. According to the EMCDDA report (March 2015), the NPS market has expanded to include "legal highs" (marketed with bright packaging and attractive brands, and sold in headshops and over the Internet); "research chemicals" sold over the Internet "for scientific research" (with labels imitating safety data sheets). Such NPS are more attractive to "psychonauts", who like to try "new stuff" rather than get addicted to one drug. NPS also include food supplements sold in fitness shops and over the Internet and "designer drugs" sold as traditional drugs of abuse by drug dealers in the illicit drug market. The products often contain one or more NPS but can also contain diverted prescription medicines sold by drug dealers in the illicit drug market.

The term "legal highs" is undesirable and misleading. It gives the impression that these products are legal and safe when they may contain controlled substances (drugs or their precursors) and harmful contaminants. It also gives the impression that these NPS induce "highs", when in fact some of them are depressants or have overlapping pharmacological effects.

In addition, there is evidence that NPS are used in combination with traditional drugs of abuse and/or alcohol. In other cases, NPS replace the use of traditional drugs of abuse; for example, heroin injectors are now moving onto injecting mephedrone because it is cheaper but has similar psychoactive effects.

## A recipe for social disaster

NPS emerged after the drug cookery books written by Alexander (Sasha) and Anna Shulgin became available in the late 1990s (5–6). In 2009, NPS appeared in huge numbers and became internationally popular for different reasons primarily because they are legal, cheap, "undetectable", and provide desired effects such as euphoria, increased sociability, elevated mood, hallucinations, increased libido and so on. Users of NPS include people of all ages

and persuasions: students, members of the LGBT community, people interested in 'chemsex', heroin users, clubbers, gym-users, the homeless population, prisoners, and users of substance misuse and needle-and-syringe-exchange schemes. Knowledge of the patterns of NPS abuse mainly depends on anecdotal self-reported user surveys and user experiences shared in drug fora, for example Erowid and Bluelight. NPS can be snorted, smoked, injected, ingested (sometimes via bombing, in which the drug is placed in paper, rolled into a ball and ingested) or dissolved in alcohol.

## Toxic ignorance

Information regarding NPS intoxication is very limited due to the lack of data from hospital emergency departments, walk-in centers, and so on. Nevertheless, the EMCDDA issued 16 public health alerts in 2014 for NPS associated with serious harm, such as hospitalisation and death. Published case studies highlight some of the toxic effects of NPS and include UK cases of sympathomimetic toxicity from the intake of mephedrone (8), attempted murder resulting from the combined intake of 3-methoxyphencyclidine (3-MeO-PCP) and methylenedioxypyrovalerone (MDPV) (9), lethal serotonin syndrome after the combined ingestion of methylone and butylone (10), and death from taking the diet pill, 2,4-dinitrophenol (or DNP) (11). NPS effects on different body systems such as the sympathetic nervous system, cardiovascular, or neurological systems is dependent on the amount taken, and the number and type of drugs co-ingested/injected, all of which have different clinical implications.

The 2011/12 Crime Survey for England and Wales (CSEW) has reported that ketamine users generally have high rates of simultaneous poly-use (12). Ketamine has also been linked to significant sexual health risks (13) and the prevalence of premature death among the drug-injecting population (14). For mephedrone alone, a synthetic cathinone, presentations for treatment rose in England from 839 in 2010/11 to 900 in 2011/12 amongst clients aged 18 years and over (15). And the number of mephedrone-related TOXBASE accesses (a primary clinical toxicology database) was 7061 in 2013/14, 8432 in 2011/12 and 6169 in 2011/12 (16–18).

The explosive emergence of NPS, the anonymity of Internet sales and the emergence of "cryptomarkets" are posing great challenges for policy-makers and law-enforcement agencies. NPS are not currently under any international control, although many countries have established permanent control measures for some NPS or issued temporary bans. For example, in April 2010 the UK classified cathinones as controlled drugs under Schedule II of the Misuse of Drugs Act 1971 through a generic definition i.e., banning all emerging NPS that are made by slightly modifying the generic chemical structure of cathinones. Conversely, selected cathinones were placed under temporary orders in the USA such as the cathinone 4-MEC (4-methylethcathinone), which was only classified as a Schedule I controlled drug in 2014. The downside of the generic definition ban of classes of NPS is that some new analogs have fallen outside of the generic definition and became legal analogs to controlled drugs. Naphyrone, which emerged to the market following the ban in July 2010, is an example. Therefore, the generic definition was modified to include it. This was followed by the emergence of bk-2C-B, which remains a legal cathinone in the UK.

"NPS may exhibit stimulant, depressant, empathogenic, aphrodisiac, dissociative, hallucinogenic, entactogenic and psychotropic effects."

## Root of the problem

India and China produce large quantities of legal high products. And the fact that they can also be manufactured from legal precursors of known tested pharmaceuticals facilitates large-scale production. The drugs are exported legally to Europe, where they are cut and distributed across the vast Internet market. The drugs are typically cut with a variety of controlled or uncontrolled active ingredients, prescription medicines and inert substances. Figure 2 shows example chemical structures for NPS categorised by EMCDDA.

Limited information is available on the pharmacology and toxicology of NPS (19, 20). NPS may exhibit stimulant, depressant, empathogenic, aphrodisiac, dissociative, hallucinogenic, entactogenic and psychotropic effects. Most NPS exhibit psychoactive and sympathomimetic features (21), with the possibility of NPS classes overlapping and sharing one or more psychoactive effects (22) because most street drugs are sold as racemic mixtures.

Potencies, toxicities, pharmacokinetics and pharmacodynamics depend on whether the drug exists as a single enantiomer or a mixture of both enantiomers. If both enantiomers exist, their dynamics may be altered due to potential synergistic or

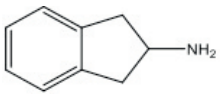
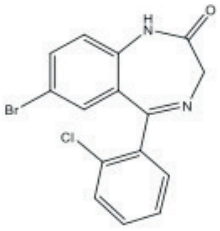
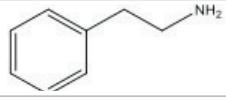
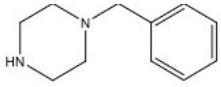
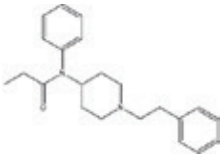
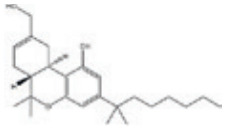
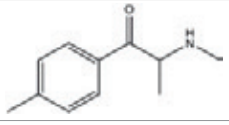
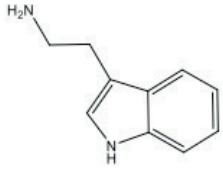
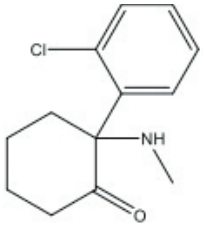
| | |
|---|---|
| Arylamines |  |
| Benzodiazepines |  |
| Phenethylamines |  |
| Piperazines |  |
| Opioids |  |
| Synthetic cannabinoids |  |
| Synthetic cathinones |  |
| Tryptamines |  |
| Others e.g. Ketamine |  |

Figure 2: Example chemical structures for NPS categories (EMCDDA, 2015)

competitive actions. Additionally, the enantiomeric fraction may change over time because of preferential metabolism of one over the other (23). Let's take just one example: cathinone analogues exhibit various pharmacological effects, which include stimulant, empathogenic and anti-depressant effects (24, 25). Coppola et al (2012) and Cozzi et al (1999) showed that cathinones might exert their stimulant effect by inhibiting the enzymes tyrosine hydroxylase and tryptophan hydroxylase, which are responsible for the synthesis of dopamine (DA) and serotonin (5-HT) (26, 27). Cathinones also inhibit the re-uptake of the neurotransmitters DA, 5-HT and norepinephrine (NE) by the monoamine transporters, which then reduces clearance of neurotransmitters from the synaptic cleft. Additionally, they induce the release of newly synthesised monoamines from the cytoplasm as well as stored monoamines from the synaptic vesicle stores. These pharmacological effects result in reduced concentrations of monoamines in the frontal cortex, hippocampus and neostriatum. Reduced catecholamine concentrations have been shown to extend for up to 30 days leading to the destruction of monoaminergic neurons (28, 29).

## Specialized, portable, in-field analysis

The global proliferation of NPS is posing international public health risks and a pronounced burden for first responders. In-field detection of NPS is crucial for law enforcement agents, where the identification of unknown compounds is important for making decisions (for example, making an arrest). Fortunately over the past decade, handheld techniques have become available, which have the advantage of bringing the lab to the sample (7). A recent development includes the use of surface-enhanced Raman spectroscopy (SERS – for more, see page 24) for the detection of mephedrone, with a limit of detection of 1.6 µg mL-1 (30). Presumptive tests have also been used for the identification of cathinones (31), but because such tests depend on the presence of a functional group, they can lead to false positives. Other rapid tests include immunoassays, which are commonly used for in-field detection of drugs of abuse (32) and NPS in biological matrices, such as urine (33). These kits are limited by the fact that they must be developed for known specific drugs and cannot be used for unknown, new NPS (33). In addition, their excellent selectivity prevents the identification of NPS analogues due to low cross-reactivity (34), which may yield false negative results (32, 35). An on-the-spot screening instrument was recently developed and involved the use of disposable electro-analytical sensors for identifying three cathinones (36). Yet, the latter studies investigated NPS samples in solution rather than in solid state.

Handheld techniques employing Fourier transform infrared or Raman spectroscopy are the main analytical techniques currently employed by forensic scientists for the screening and

Oliver Sutcliffe (Senior Lecturer in Psychopharmaceutical Chemistry at MMU) holds an electrochemical NPS sensor. (Photo courtesy of Manchester Metropolitan University)

identification of NPS (see "War on NPS" on page 41 for more). Gas chromatography-mass spectrometry (GC-MS) is considered the main "wet" chemical technique used in forensic labs for the analysis of seized NPS or NPS and their metabolites in body fluids such as urine. So, should a sample test positive in the field, it may be transported to a forensic lab and tested using GC-MS. High performance liquid chromatography (HPLC) is also used for quantification of selected NPS.

## We need you!

Certainly, analytical chemistry plays an important role in screening, identifying and quantifying NPS products; rapid identification and quantification ensures swift arrests/confiscations, aids in treatment decisions in healthcare settings, and assists with preventing the widespread distribution of harmful substances. However, we still need more analytical developments to overcome the challenges of "designer drugs". For example, we lack reference standards for many drug samples because of the pace at which new drugs emerge on the market and the cost of synthesizing them. To evade detection and circumvent the law, clandestine chemists have been able to produce heterogeneous NPS products (37). And as mentioned earlier, NPS are often intentionally branded and mislabeled, which adds complexity and makes the identification of NPS and the discrimination between NPS and excipients difficult. Other challenges faced include the presence of contaminants, coloured powders and unknown constituents and limitations of conventional in-field immunoassay kits. For example, signals resulting from excipients intentionally mask and hinder NPS identification. Finally, NPS products may contain controlled drugs, which requires analytical labs to hold specific expensive licenses, and thus hinders "designer drug" research.

Traditional harm reduction techniques are difficult to apply to NPS because they are continuously emerging with fantasy names, different mixtures, novel analogues, precursors and diverse chemical structures. It is crucial to develop evidence-based harm reduction services by raising awareness and educating the public. Exchange information between different countries through the projects like the European Early Warning System is also indispensable. Despite being debatable, new policy is essential when it comes to tackling the NPS problem; the coming into force in April of the UK's Psychoactive Substances Act 2016 is aimed at effecting a "blanket ban" on NPS without hindering NPS-related research, but it may simply lead to increased clandestine activities.

So far, clandestine chemists have always been one step ahead. In addition to developing more efficient on-site testing

to avoid false positives and false negatives, analytical chemists need to collaborate with forensic and law enforcement agencies and service providers to predict future generations of NPS. We need methods that unambiguously identify these drugs before they can cause harm.

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# The Analytical War on Novel Psychoactive Substances

*By Craig Banks and Jay Smith*

"Have you tried plant food?" Why anybody would eat plant food let alone abuse its use is bemusing. And yet, this is one of the many names given to "legal highs" or, as they're more formally known, novel psychoactive substances (NPS). These designer drugs are produced in clandestine laboratories and given mercurial names to circumvent drug legislation – and they are not going away. "We have got to the point where young people think they are safe because of the word 'legal,'" said the mother of a victim of NPS abuse. We tend to agree with her.

## Analytical challenges

The nature of their underhanded production, purposely designed to evade international drug legislation means they are intrinsically marketed and sold as legal highs and as such, are considered safe. But there are no assurances to the customer of these NPS products that the contents are the same as advertised. For example, mephedrone was detected in products sold as naphyrone or NRG-1 in the UK even after its ban in 2009. The dangers of NPS abuse become clear when one considers that each new designer drug, designed to circumvent drug legislation, has not been evaluated for its effect on human health – the testing comes in the field, customers are test subjects, guinea pigs, at the mercy of potentially fatal side effects. There are many instances reported in the press where the use of NPS has unfortunately resulted in death (1). Analytical chemistry is key to combating these dangers and thus avoiding preventable deaths, crippling addictions and the ruination of lives.

The United Nations Office on Drugs and Crime (UNODC) and European Monitoring Centre for Drugs and Drug Addiction

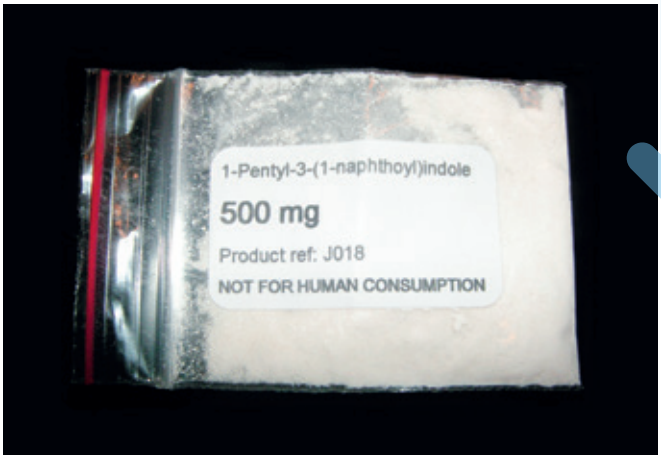


Image by Psychonaut

"One approach to address the growing need is the use of presumptive testing, which has been commonly used for existing drugs of abuse."

(EMCDDA) detailed the following sub-categories: synthetic cannabinoids, synthetic cathinones, ketamine, phenethylamines, piperazines, plant-based substances: Khat, Kratom, Salvia divinorum and miscellaneous: aminoindanes, phencyclidine, and tryptamines. Abuse of NPS has been increasing since 2009 and represents a fast-growing market; something reflected in the online marketplace (the number of online vendors in the UK increased by more than 300 percent between 2010 and 2011). New materials made available for abuse appear rapidly and, at times, can gain a 'foothold' in the market. Mephedrone is one such example. In 2014, 101 new substances were reported for the first time to the EU early warning system (EWS) run by the EMCDDA up from 81 in 2013 (it was 74 in 2012). The findings of the EWS revealed synthetic cannabinoids are the most frequently discovered, with 102 detected between 2005 and 2013.

Given their rapid appearance on the market, the principle challenge facing analytical scientists is to be 'one step ahead' of the clandestine drug manufacturers. We recently reviewed the current state of the "analytical fight" against legal highs/NPS (2).

Current approaches, as expected, are generally laboratory based and take advantage of a range of standard analytical approaches, such as LC-MS/MS and so on. These have been applied successfully for the detection of a range of NPS in a number of different matrices and suspected NPS samples (such as powders). Of course, such approaches are useful because they can be applied to diverse samples, being selective with no interferents, and because they provide exact confirmation of the target analyte within the sample. And though this type of analytical approach has its place (and associated pros and cons), there is a growing need for rapid, cheap, small, portable and analytically useful methods where a near-instantaneous response is required, such as in a clinical or law enforcement setting, as noted by Guirguis on page 39.

## Presumptive testing

One approach to address the growing need is the use of presumptive testing, which has been commonly used for existing drugs of abuse. A recent study explored the presumptive testing of cathinone derivatives as per United Nations recommended guidelines (3). In these approaches, color changes highlight the presence and absence of the target drug/analyte and have been deemed consistently effective for cathinone derivative screening. However, this is not the case for all legal highs/NPS. Selectivity is an issue, so false positives make the analysis of an unknown NPS powder/sample ambiguous. Another approach relies on microcrystalline presumptive tests. A sample of the NPS solution is mixed with a reagent that and then placed onto a glass slide; the resulting crystal structures are then used to identify the unknown NPS. This microcrystalline approach has been successfully used for the identification of MDAI, mephedrone and N-benzylpiperazine (4). The study evaluated purchased "legal high" samples and applied the microcrystalline presumptive test approach, which was then collaborated with GC- FTIR/MS. But there a couple of drawbacks: the reagent solution is costly (sometimes gold chloride is used) and simple changes, such as temperature, can significantly alter the test. Currently, microcrystalline presumptive tests cannot be used for the wide range of NPS.

Immunoassays have also been explored towards the screening of several NPS. A recent study explored 16 different ELISA reagents to determine the cross-reactivity of 30 designer drugs, including 24 phenylethylamines (including eight cathinone derivatives, three piperazines, and three tryptamines) (5). Cross-reactivity towards most drugs was <4 percent in assays targeting amphetamine or methamphetamine. Compounds such as MDA, MDMA, ethylamphetamine, and α-methyltryptamine demonstrated cross-reactivities in the range of 30–250 percent, but the data was found to be

consistent with both manufacturer claims and the published literature. When tested against the commercially available Randox Mephedrone/Methcathinone ELISA kit, cathinone derivatives demonstrated cross-reactivity at concentrations as low as ng/mL levels.

Other approaches recently developed have reported a novel sensing protocol based upon electrochemical methods and are similar to glucose sensors that use disposable carbon-based strips with a small electronic reader.

Researchers have shown the sensing of cathinone substitutes to be comparable to HPLC; detection of mephedrone and 4-MEC was possible in seized street samples (NRG-2), so the method could potentially offer on-the-spot analytical screening (6, 7). However, once again the approach is not without its limitations. If multiple NPS are present, the sensor is unlikely to be able to distinguish between them since selectivity is limited. Even in our recent work, we have now moved to using HPLC with electrochemical detection (8). However, while this provides a new laboratory-based approach, a selective and sensitive NPS sensor is still required and needs developing.

## Work in progress

From the above quick summary, it is clear that a rapid in-the-field sensor covering all subcategories outlined by the UNODC would be ideal; however, this has yet to be realized. And though we've seen some promising research, we must not forget that such screening tools need to be accurate – a point that was proven in a recent case in Australia, where a routine roadside drug test reported positive for methamphetamine – a drug the victim said he had never touched (9). The roadside drug test on the victim's saliva was positive, which allowed further testing in a portable testing station where the second test was negative. However, a further sample was sent for laboratory analysis, which came back as positive two weeks later. Luckily, the accused challenged the result and further retests were negative.

Saliva is a complex matrix and the tests are looking for minute amounts of drugs, which makes the results unreliable. Clearly, development must focus on reducing false positives (and negatives) to ensure robustness. What is surprising in this case is that the laboratory "confirmatory" test also got it wrong...

Whichever analytical protocol is taken forward, the variety within each sub-category of NPS is huge – particularly between different generations. Another issue faced by analytical scientists regarding the detection of NPSs is the presence of adulterants within a sample. Our research showed that "street samples" contained caffeine and benzocaine in addition to the active ingredients. Other groups have also found novocaine, lidocaine sugars and many other compound classes. These ingredients can be randomly changed, which adds to the complexity of testing – in most cases, real sample testing and the effect of interferents is merely an afterthought. In summary, there is much work for analytical scientists to do in combatting the war on NPS. In particular, a portable, rapid, cheap yet sensitive and selective sensor is still required. Sadly, we are aiming for a moving target. As one legal high is made illegal, sensors are developed accordingly, and these are rapidly changed as others emerge. On a high note, it certainly means that this area of research remains both exciting and challenging...

*Craig Banks is a full Professor/Personnel Chair in nano and electrochemical technology and Jay (Jamie) Smith is a PhD student at the Manchester Metropolitan University, UK.*

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# Solving Problems for the Greater Good

One year on – and just as judges deliberate over the winner for 2016 – we speak with the exceptional runners up from the 2015 Humanity in Science Award.

*By Joanna Cummings*

The Humanity in Science Award – a collaboration between Phenomenex and The Analytical Scientist – recognizes breakthroughs in analytical science that have the potential to improve human lives. In 2015, Peter H. Seeberger and Andreas Seidel-Morgenstern won top prize (read about their project here: tas.txp.to/0216/HISA2015), but they were in excellent company; the judges commended three other teams for their exceptional contribution to the field. Twelve months on (and just ahead of the announcement of the 2016 winners next month), we find out what those teams have been doing since – and ask why 'humanity' is such a fundamental part of what they do.

## CE Specialists: Serge Rudaz & Julie Schappler

Remind us of your project...

Serge Rudaz: Our project focused on the development of a low-cost capillary electrophoresis (CE) system for counterfeit medicine evaluation in emerging countries. We believe that CE is the perfect technique for emerging countries because it's cost effective, there is no solvent constriction, and it's quite easy to manage. So here at the University of Geneva, in collaboration with the School of Engineering of Fribourg, we have tried to develop a very low cost, very robust prototype, dedicated to counterfeit analysis. Pharmelp – a non-profit association – helped us to build eight prototypes that were sent to Africa and Cambodia to demonstrate that CE is an effective tool for detecting counterfeit medicine. We are also trying to develop very simple methods for analytics as well as e-support to help people with CE, as it is not a well-known technique.

How important is it for your work to have a positive impact?

SR: We are pharmacists, so we believe

that quality in a substance is a human right. For us, the most important aspect is not that a particular compound is copied, but the level of quality control – and we should be helping emerging countries develop their own quality control. Liquid chromatography and other regular techniques are really expensive. In Africa, they have money to buy one instrument – but the problem is the maintenance, solvent costs and so on. With CE, they can have it for a lifetime. We have had success with the prototypes, for example, we already have four PhD theses in capillary electrophoresis in Senegal. We think that as occidental pharmacists, it's our responsibility to give solutions to people and colleagues in emerging countries in this particular field.

It is interesting for us in the lab to have a project that is more people-oriented or global health-oriented. We want to propose problems that can be solved on a smaller scale in terms of tech and larger scale in terms of importance. Also, a lot of students want to develop their skills in a humanitarian context.

Where is your project one year on?

Julie Schappler: After the award, we had lots of ideas, but we needed to make them concrete, so with a team of experts, we worked on the device from a technical point of view, to enhance and improve some of the parts. We also needed to handle specific issues in Africa; for example, there are problems with data integration that make it difficult to identify the location of counterfeit drugs. We're working on a smartphone kind of device that allows us to integrate 'regular people' into the project, so patients themselves can help to tackle counterfeit drugs.

SR: We are now collaborating with the EPFL, a very important engineering school in Switzerland, to miniaturize the detection. We believe there is still room for improvement regarding the technique. We are working with Masters students here in Geneva on methodological development and also have two PhD theses in Africa regarding the use of CE as a tool for drug quality control for natural medicine – plant extracts and so on. We have also a very good relationship with the University of Geneva and the Rector is really interested by our projects. However, it's difficult for us to redevelop a completely new device because it's not our core business. We would like to push the project forward with an open-source device that can be built with very low cost components. Essentially, we want

our project to involve citizen science or at least large-scale collaboration so that it can move forward to meet our initial goals.

What kind of support have you had from the analytical community?

SR: Many of our colleagues have been really interested in supporting the project. Now, we really need support in the countries themselves now; education is really important in that regard.

How has the Humanity in Science Award helped?

JS: Going for an award has been a good way of disseminating information and talking about our projects – especially those not at the fundamental or high expert level.

SR: Author reach is really important for this kind of project. It's really difficult to publish in Science or the Journal of Chromatography, so it's really helpful to have the support of The Analytical Scientist.

*Serge Rudaz & Julie Schappler, (School of Pharmaceutical Sciences, University of Geneva), "Low-cost analytical device based on capillary electrophoresis (CE) for counterfeit drug detection and sub-standard drug quality control." Read the full nomination here: tas.txp.to/0216/Rudaz*

## Critical Translation: Don Farthing

Could you briefly describe your work?

Our translational research focuses on the critical medical need of a new biomarker and analytical method for rapidly detecting acute cardiac ischemia, at the very early onset of a heart attack. Using HPLC and mass spectrometry techniques, we determined that plasma inosine and hypoxanthine were promising candidate biomarkers for indicating acute cardiac ischemia. However, LC-MS technology does not easily lend itself to use in or outside of the hospital emergency room environment. Therefore, we developed a rapid and sensitive chemiluminescence test for use on a microplate luminometer, which can qualitatively determine plasma levels of inosine and hypoxanthine in less than a minute.

Our group at VCU Medical Center performed pre-clinical research using a mouse model of cardiac ischemia, followed by human sample evaluations from ER non-traumatic chest pain patients. Clinical studies are ongoing to evaluate hospital cardiac patient plasma inosine and hypoxanthine levels, in conjunction with cardiac troponin levels, to better understand their diagnostic potential for alerting cardiac ischemia, several hours prior to the markers of ischemia-induced heart tissue necrosis being released into the blood stream of the heart attack patients. If inosine and hypoxanthine are clinically validated for diagnoses, their use and the rapid chemiluminescence test can potentially save thousands of lives, as well as millions of dollars in hospital cost each year. Having global impact, the ultimate goal of this translational research is to miniaturize the US patented chemiluminescence test, for use in a point-of-care handheld medical device.

Translational research is inherently humanitarian...

The purpose of our heart research is to address an unmet critical medical need - the early detection of a heart attack, the leading cause of death for industrialized countries such as the US and Europe. In our humble opinion, there is no stronger humanitarian aspect than saving human lives, and our heart research certainly has the potential to do this.

Additionally, the incidence of heart attacks has been increasing exponentially in several of the world's most populated countries (for example, China and India), because of rapid industrialization and lifestyle changes over the past three decades. The high mortality and morbidity of heart attacks in these developing countries is mainly due to a deficiency of diagnostic equipment and advanced medical facilities. Therefore, the goal of developing low-cost and handheld devices for early detection of a heart attack will undoubtedly benefit at least one third of the global human population.

Where is the project today?

The heart research is still ongoing at VCU, but unfortunately I am no longer

*"In our humble opinion, there is no stronger humanitarian aspect than saving human lives."*

at the VCU Medical Center to support the research because of a lack of sufficient funding. VCU Medical Center is currently collecting and analyzing blood samples from cardiac patients suspected of undergoing acute cardiac ischemia (prior to heart attack). These samples will clinically validate our proposed biomarker to be used in conjunction with cardiac troponin I, the gold standard biomarker indicating a heart attack. We have not performed any development work on the handheld device at this time, pending clinical validation and funding for the technology development.

What kind of support have you had from the analytical community?

To date, we have not solicited for support from the analytical community, but it may be something that we should pursue in the foreseeable future. The Humanity in Science Award competition was a great venue for us to showcase some of our group's research. We have written several grants that were not funded; however, grant money has been very limited over the last five years, plus there have been a large number of highly competitive grant applications from other research groups.

We'd like to thank The Analytical Scientist and Phenomenex for this wonderful recognition. In this day and time when many important scientific breakthroughs are reported out each year, it was quite an honor for our translational research to have been selected as a runner up in the competition.

*Don Farthing, H. Thomas Karnes, Lynne Gehr, Christine Farthing, Todd Gehr, Terri Larus, & Lei Xi (Virginia Commonwealth University), "Translational research on the use of a rapid analytical methodology for detecting acute cardiac ischemia, at early onset of a heart attack." Read the full nomination here: tas.txp.to/0216/Farthing*

## New Generation Chemistry: Frantisek Turecek

What's the history behind your project?
The project has been going almost 17 years now with two lines of research: one of them being the development or optimization of enzyme assays for lysosomal storage disorders, the other basically transitioning the technology we develop here in the lab to a newborn screening lab for trial programs.

How important to you is the humanitarian impact?
The procedures we develop are meant to help with diagnostics. A child can be born and look perfectly ok, but in the months or years that follow, diseases can present themselves. The child stops thriving, deteriorates, and then dies, which is obviously very sad. It also has a huge impact on the parents. Because these are rare diseases – prevalence is about 1 in 7500 – they are basically overshadowed by the most common ones. Physicians aren't trained to recognize them. So parents go from place to place, from clinic to clinic, from doctor to doctor and it takes a long time before the child is diagnosed. That's also very emotionally taxing for the parents. With screening, the treatment can start as early as possible – and before symptoms kick in, which means the child can develop under much improved conditions compared to an untreated child.

Do you have any updates one year on?
We have NIH-funded research for what we call 'new generation chemistry' for this newborn screening of lysosomal storage disorders. So far, we've developed a total of 15 assays for 15 enzymes that are responsible for the disorders; seven are diseases treatable with various therapies. We've been focusing on the treatable diseases with a view to large-scale screening. These are high throughput operations where we must optimize the technology so that it can be used on a large scale and at a low cost.

I've mostly been involved in developing the analytical part (mass spectrometry). My colleague, Mike Gelb, is a bio-organic chemist and a specialist in enzyme chemistry. C. Ronald Scott is a senior pediatrician, so he knows everything about the medical side of these diagnostics.

What kind of support have you had from the analytical community?
We have published our work in journals, and we won the Esselen Award for Chemistry in the Public Interest three years ago from the American Chemical Society. It's a niche area compared to mainstream biology or diseases like cancer or heart diseases, but it's been growing in importance. Some of the larger states now have mandatory screening for lysosomal storage disorders, and this set of diseases – at least those that are treatable – have been added to the standard metabolic disorders that are screened for. We're just adding more – and using different technology.

How do you feel about the award?
I have a lot of strong opinions about awards. They come more or less at random, and have their own dynamics. I think there's a certain special level for awards where they are recognized by a committee and some fall through the cracks. I don't think it has helped us get more recognition or exposure. It also depends how they're advertised, of course, and some may be more visible because they appear in professional literature or newspapers. I think it's nice that TAS is interested in this though!

*Michael H. Gelb & Frantisek Tureček, (Department of Chemistry, University of Washington, Seattle), "Analytical chemistry in newborn screening." Read the full nomination here: tas.txp.to/0216/Turecek*

*The 2016 Humanity in Science Award Winner will be announced in the March issue of The Analytical Scientist, and the award will be presented at a celebration gala dinner at the Hotel Bayerischer Hof in Munich on May 10, during Analytica 2016.*

HUMANITY IN SCIENCE AWARD

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2015 Winners
Andreas Seidel-Morgenstern (left)
and Peter H. Seeberger (right),

# Now the judging begins...

Nominations are now closed for The Humanity in Science Award 2016.

Analytical science has the power to change human lives for the better, but rarely receives the same fanfare as other scientific disciplines. The Humanity in Science Award was launched to recognize and reward a recent breakthrough in analytical science that has truly made the world a better place.

The 2016 award will be presented on May 10 in Munich, Germany.

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# Defying the Data Tsunami

**Sitting Down With… Lutgarde Buydens,**
Professor, Analytical Chemistry: Chemometrics,
Radboud University, Nijmegen, The Netherlands.

*Photo courtesy of Radboud Universiteit/Dick van Aalst*

Your cover feature – "Towards Tsunami-Resistant Chemometrics" – stirred up quite a bit of interest…

Yes, it did. I've had a lot of responses, especially at conferences. Now, "tsunami" is used regularly to describe large amounts of data... That's really quite remarkable.

Everyone is beginning to realize that large amounts of data present problems – the big data issue has exacerbated the situation. Governments and large science organizations see it as one of the major issues that need addressing through "data science", a new umbrella term that includes chemometrics. In fact, I now tag on "chemical data science" whenever I use chemometrics as it helps those unacquainted with the field. But if we stop using chemometrics, we lose part of our identity.

Does increasing commercial interest help boost awareness?

Well, consider any infrared spectroscopy instrument; you wouldn't expect to see one without PLS software onboard – but that's only good when people realize it! Other analytical instrument manufacturers offer free downloadable software packages to preprocess and extract data in the correct format. Hopefully, more usage and discussion will raise awareness of our field's importance.

The data tsunami is a tricky problem…

It's certainly not easy! But we are beginning to see efforts to develop robust data fusion methods able to handle numerous, very large datasets. The objective is to extract the variations in the data relevant to the problem. But it's tricky – and chemometricians are publishing more and more papers that try to provide solutions. Data fusion is a really hot area, right now.

And these methods provide ways to clean up the data?

Not exactly. Cleaning suggests the majority of your data is relevant, so you'd just remove part of the data. However, when we deal with big data, it's the opposite – we are faced with finding the needle in the haystack. Indeed, 99.999 percent of the variation is probably not relevant to your problem. Cleaning up the data is something that was very typical in classical chemometrics with normal size datasets where you have irrelevant "noise" that you can ignore. But, with big data, we have to throw away the haystack, which is something quite different to cleaning data. The key may be 'less is more'.

Where else can chemometrics have an impact?

Chemometrics could be the kickstarter for a whole new range of instrumentation. The widely lauded added value that chemometrics has brought to the spectrometer may be extended to many other fingerprinting methods, such as metal oxide sensing, fluorescence and low-field NMR. It may also be essential to extend the functionality of established methodology, for example, flow cytometry. I believe there is a bright future for chemometrics in instrument development – and Jeroen Jansen, an assistant professor in my department, is very much involved.

The group currently consists of post docs, PhD, masters and bachelors students. We also host two visiting scientists from industry (MSD and Douwe Egberts) who provide the essential support to keep chemometrics attached to the industrial workflow, which has been the inspiration for so many cornerstone developments in the field. I am constantly amazed by the enthusiasm, creativity and motivation of our bachelor and masters students, which bring in a lot of complementary expertise from areas like biology and informatics. They are a lot of fun to work with, and it is a joy to see them grow in the field as emerging specialists.

What about your own beginnings and mentors?

I actually began in a very different field: pharmacy (in Brussels), but later I embarked on an internship and a PhD with Desiré Luc Massart – one of the founding fathers of chemometrics. The actual logistics of studying in Brussels (I had to change from one campus to another twice daily) meant I couldn't do proper experiments; nor was I particularly gifted at experimental work! Instead, I became fascinated with chemometric techniques such as QSAR (quantitative structure–activity relationships), which is related to pharmacy. I loved being able to look at the data and predict outcomes.

So given my newfound interest, I was lucky to later find myself under the careful watch of Massart and also Leo Kaufman, a renowned statistician. I learnt a lot from them both – new methods, such as pattern recognition, PLS (partial least squares), and all kinds of multivariate regressions. In time, I was able to extend my work to all kinds of other data and it has continued from there.

You have strong views on collaboration...

That's right; I have to collaborate to be able to do research! One example is ESPRIT (expert systems for chemical analysis) – a project that brought together people from the University of Brussels and University of Nijmegen. In fact, that's how I ended up in The Netherlands. There's also COAST, which is a private/public consortium that brings together major chemical companies and academia to develop research programs in the field of analytical chemistry. I really like the fact it makes a strong link between chemometrics and analytical chemistry. Industrial partners provide us with datasets that exemplify specific problems and we use them for fundamental research, which ultimately provides a solution for their practical problem. Who says you can't have fundamental and applied science working together? The one cannot exist without the other!

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