DAVID MUDDIMAN

Jacob and Betty Belin Distinguished Professor
W.M. Keck FTMS Laboratory for Human Health Research
Department of Chemistry. 603 Cox Hall
2620 Yarbrough Dr.
North Carolina State University. Raleigh, NC 27695-8204
david_muddiman@ncsu.edu
http://www4.ncsu.edu/~dcmuddim/index.html

CONFERENCIA PLENARIA

Innovations in Mass Spectrometry Platform Technologies for Epithelial Ovarian Cancer Research

Mass spectrometry offers the most robust platform to discover and characterize new diagnostic, prognostic, and therapeutic biomarkers for ovarian cancer across all molecular classes. Moreover, a systems biology approach will allow the underlying biology of ovarian cancer to be understood. This presentation will discuss the challenges specific to the study of epithelial ovarian cancer (EOC) in humans and how these challenges have directed our thinking, in terms of the development of model organisms and mass spectrometry-based bioanalytical strategies. First, to augment the human model, we developed the domestic hen model of spontaneous EOC, which allowed us to longitudinally sample the rapid onset and progression of the disease in a controlled environment. Second, we developed bioanalytical tools to characterize structurally challenging analytes that are critical to a systems-level analysis. To increase the electrospray response of *N*-linked glycans, perform stable-isotope relative quantification, and semi-automated data analysis, we synthesized novel hydrophobic tagging reagents (INLIGHT^{TM}). Furthermore, we developed a novel ionization technique for tissue imaging of lipids and metabolites. This unique model organism has and continues to provide new insights into the biology of ovarian cancer; combined with other –OMICS data obtained through these novel bioanalytical approaches, we will understand the origin of ovarian cancer and ultimately translate that knowledge to humans.

SHORT BIOGRAPHY

David C. Muddiman is the Jacob and Betty Belin Distinguished Professor of Chemistry and Founder and Director of the W.M. Keck FTMS Laboratory for Human Health Research at North Carolina State University in Raleigh, NC. Prior to moving his research group to North Carolina State University in 2006, David was a Professor of Biochemistry and Molecular Biology and Founder and Director of the Proteomics Research Center at the Mayo Clinic College of Medicine in Rochester, MN. Prior to this appointment, David was an Associate Professor of Chemistry at Virginia Commonwealth University. It was there that he began his professional career as an assistant professor with an adjunct appointment in the Department of Biochemistry and Molecular Biophysics and as a member of the Massey Cancer Center in 1997. These academic appointments followed a postdoctoral fellowship at Pacific Northwest National Laboratory in the Environmental Molecular Sciences Laboratory under Richard D. Smith from 1995-1997. David was born in Long Beach, CA in 1967 but spent most of his formative years in a small town in Pennsylvania. David received his B.S. in chemistry from Gannon University (Erie, PA) in 1990 and his Ph.D. in Analytical Chemistry from the University of Pittsburgh in 1995 under the auspices of David M. Hercules. Dr. Muddiman is Editor of *Analytical and Biological Chemistry* and Associate Editor of the *Encyclopedia of Analytical Chemistry* as well as on the Editorial Advisory Board of *Mass Spectrometry Reviews*, *Molecular and Cellular Proteomics*, *Rapid Communications in Mass Spectrometry*, and the *Journal of Chromatography B*. He also serves on the advisory board of the NIH Funded Complex Carbohydrate Research Center, University of Georgia and the Yale/NIDA Neuroproteomics Center, Yale University. Dr. Muddiman has served as a member of the ASMS Board of Directors and Treasurer of US-HUPO; he is currently the President of US HUPO. His group has presented over 500 invited lectures and presentations at national and international meetings including 20 plenary/keynote lectures. His group has published over 250 peer-reviewed papers and has received four US patents. He is the recipient of the 2015 ACS Award in Chemical Instrumentation, 2010 Biemann Medal (American Society for Mass Spectrometry), 2009 NCSU Alumni Outstanding Research Award, the 2004 ACS Arthur F. Findeis Award, the 1999 American Society for Mass Spectrometry Research Award, and the 1990-1991 Safford Award for Excellence in Teaching (University of Pittsburgh). Dr. Muddiman's research is at the intersection of innovative mass spectrometry technologies, systems biology, and model organisms for diseases and bioenergy, and is funded by the National Institutes of Health, the National Science Foundation, the Department of Energy, and The United States Department of Agriculture.

ANSWERS TO QUESTIONS:

1.- If you had to highlight major breakthroughs in your research field for the last 10 years, which would be your choice?

A. The conception and reduction to practice of the novel ionization source, IR-MALDESI, for mass spectrometry imaging and direct analysis; broad applications of this platform technology.

B. Development of the INLIGHT strategy for the relative quantification of glycans; applications to ovarian cancer.

C. The discovery of a novel un-templated proteoform of SOD1 and its link to ALS and the environment.

2.- Could you envisage the major or most predictable and desirable outcomes within your area of work for the next 10 years?

A. Linking the environment to disease, from fire retardants to neurotoxins - these are things that one can not predict from a gene sequence.

B. Taking IR-MALDESI to the masses as an effective tool from screening to imaging.

C. Unraveling the role of glycosylation in ovarian cancer by continuing developed of a novel model organism and chemical tagging reagents.

EMMANUEL DELAMARCHE

IBM Research
Zurich, Saumerstrasse 4,
8803 Rueschlikon, Switzerland
emd@zurich.ibm.com
http://researcher.ibm.com/researcher/view.php?person=zurich-emd

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Programming flow in capillary microfluidics: from concepts to assays

Diagnostics are ubiquitous in healthcare because they support prevention, diagnosis and treatment of diseases. Specifically, point-of-care diagnostics are particularly attractive for identifying diseases near patients, quickly, and in many settings and scenarios. One of our contribution to the field of microfluidics is the development of capillary-driven microfluidic chips for highly miniaturized immunoassays. In this presentation, I will review how to program capillary flow and encode specific functions to form microfluidic elements that can easily be assembled into self-powered devices for immunoassays. An important part of our research here deals with the integration of reagents and receptors to chips. Reagents such as detection antibodies must be carefully integrated for efficient release with well-controlled concentration profiles. Concerning the integration of receptors, we have developed a chip fabrication method using mild steps, which do not compromise biological reagents. Further, recent work suggests that capture antibodies can be integrated to microfluidic chips by self-assembling functionalized microbeads into a flow path. I will also present how small peripherals can augment the functionality of microfluidic chips that have integrated electrodes for example for monitoring flow with sub-nanoliter precision and for providing connectivity to smartphones. This can be done using a low-cost chip peripheral and tracking flow using near-real time capacitance measurements of a liquid front gradually wetting a pair of electrodes. Finally, counterfeiting of point-of-care diagnostics is an issue, with sometimes dramatic consequences. Using capillary phenomena, we devised a method for producing in the chip a complex signal with a “time domain” for authentication of devices. All together, capillary-driven elements can bring extremely high control for manipulating sub-microliter volumes of samples and picogram quantities of reagents and may therefore extend the performances of microfluidic devices for point-of-care diagnostics to a next level of precision.

SHORT BIOGRAPHY

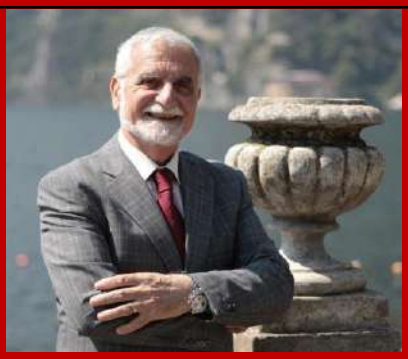
Dr. Delamarche is currently leading activities on Precision Diagnostics at IBM Research - Zurich with the goal of using expertise in micro/nanotechnology, physics and biochemistry for solving important problems in biology and medicine. His main projects deal with the development of portable and precise diagnostic devices using microfluidic concepts and smartphones, and with the development of a non-contact scanning microfluidic probe for analyzing tissue sections and studying biological interfaces. In

addition to his research, he is also a Lecturer at ETH Zurich and a contributor to scientific panels for grant agencies and governments. He published over 120 papers and is co-inventor on more than 70 patent families. He has received numerous awards from IBM, was named "Master Inventor" by IBM, and received the Werner prize of the Swiss Chemical Society in 2006.
Dr. Delamarche studied chemistry and received a degree in supramolecular chemistry in 1992 from the University Paul Sabatier of Toulouse in France. He received a Ph.D. in biochemistry in 1995 from the University of Zurich, for work done at IBM on the (photo)attachment of proteins on surfaces and was hired in 1997 by IBM as Research Staff Member.

QUESTIONS & ANSWERS:

1.- If you had to highlight major breakthroughs in your research field for the last 10 years, which would be your choice?
The major breakthroughs in the research field of point-of-care diagnostics actually come from consumer electronics and mobile phones. There has been so much progress on increasing the performances of devices and also on lowering their costs that a lot of electronic components ended up in research labs and made it to some diagnostic devices. For us, we are using off the shelves bipolar junction transistors for sensing, or smartphones for data acquisition and processing, or hobby electronic components with open source platforms making our devices more portable and "networked'.

2.- Could you envisage the major or most predictable and desirable outcomes within your area of work for the next 10 years?
I see two strongly desirable outcomes for point-of-care diagnostics for mobile health and they both relates to adjacent technologies. First, it would be great to provide solutions for monitoring therapeutic compliance. Many people do not comply with medication, which endangers their life but also create the ground for some pathogens to perpetuate. This is the case for tuberculosis where non-compliance during treatment is frequent. Technologies to assist or monitor compliance would be very useful and may help eliminate this disease – and chemists have a distinguished role to play! The second one relates to counterfeiting. There is time to time accounts of dramatic cases where counterfeited diagnostics are shipped in countries and used on a large scale. Counterfeiting is a booming activity and affects not just banknotes or luxurious goods but also drugs and diagnostics. There technologists can help by adding a number of security features to diagnostic devices. This is actually my new passion and a new part of our project at IBM.



SALVATORE FANALI

Institute of Chemical Methodologies. Italian National Research Council-C.N.R.
Area della Ricerca di Roma I, Via Salaria km 29,300 . 00015 Monterotondo (Rome)
Italy
salvatore.fanali@cnr.it
http://www.imc.cnr.it/salvatore-fanali/

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Nano-Liquid Chromatography: Main Features and Potentiality in Separation Science

The introduction of miniaturization in liquid chromatography took place about three decades ago, as documented by the work of Novotny and Knox's groups. Since that time the research has focused great attention on development of theory, instrumentation (pumping, detectors systems), columns and stationary phases (SP) etc. In nano-liquid chromatography (nano-LC) capillaries with narrow internal diameter (10-100 □m I.D.) containing selected SPs are currently employed, while in capillary liquid chromatography (CLC) 100-320 □m I.D. columns are currently used. Mobile phases are pumped at relative low flow-rates (nL/min and mL/min, respectively). The low flow-rate is particularly helpful in i) reducing the chromatographic dilution, ii) increasing the mass sensitivity, iii) reducing costs (solvents and waste) and easier coupling with mass spectrometry (MS) [1]. In the last decade some companies proposed new instrumentation dedicated to nano-LC, mainly applied in the proteomic field. In general these apparatuses offered the possibility to carry out a fast pre-concentration step into a short column of analyzed tryptic digest proteins and then analytical separation utilizing nano-flow. At the same time nano-LC was also studied and applied in other fields such as pharmaceutical, forensic, environment, food,

agrochemical etc. Although the good results achieved it is worth mentioning some drawbacks must be considered when deciding to use this miniaturized technique. First of all the extracolumn band broadening have to be minimized using, e.g., appropriate pumping system, detector at high frequency and low volume cell, tube connections and injection valve of low volumes (nL). In addition capillary columns containing SPs offering high efficiency and high selectivity are advised for successful sample analysis. To solve this last problem columns packed with particles with lower diameter (sub-2 m) or with core-shell material have been proposed and successfully applied.

In this communication, a general overview about the features of nano-LC, considering all the above mentioned remarks, will be proposed. Several examples to resolve problems coming by the use of miniaturized instrumentation will be discussed. In order to document the potentiality of this technique, some applications, reported in literature, in the field of pharmaceutical, food, agrochemical will also be presented.

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SHORT BIOGRAPHY

Dr. Salvatore Fanali is a Associate Senior Researcher at the Italian National Research Council (C.N.R.), Institute of Chemical Methodologies in Monterotondo, Italy. In 1974 he received the degree of Dr. in Chemistry at Rome University "La Sapienza" and later on the PhD in Analytical Chemistry at Comenius University – Bratislava, Slovakia. His research is focused on development of miniaturized techniques, e.g., nano-liquid chromatography/nano-LC, capillary zone electrophoresis/CZE, capillary electrochromatography /CEC. They were coupled with mass spectrometry. Studies on enantiomers separations, new stationary phases are carried out. Methods are applied to pharmaceutical, agrochemical, food, environmental, forensic analysis. He is author or co-author of about 320 publications in Journal (SCI) of international interest, chapters in books ($H_{index,\ scopus}$=44). He received awards, e.g., Bratislava University, University of Verona, Liberti Medal in Analytical Chemistry (Italian Chemical Society, 2009) and "G.Nota" for his contributions in capillary chromatography (2014). He is Editor of Journal of Chromatography A (Elsevier), he served as Editor-in-chief Journal of Separation Science and he is member of the advisory editorial board of 6 International Journals.

LOURDES RAMOS
Department of Instrumental Analysis and Environmental Chemistry . IQOG-CSIC,
Juan de la Cierva 3,
28006 Madrid, Spain
email: l.ramos@iqog.csic.es
http://www.iqog.csic.es/iqog/LourdesRamos/about

CONFERENCIA INVITADA

GCXGC-TOF-MS – A POWERFUL TECHNIQUE FOR ENVIRONMENTAL STUDIES

The chromatographic resolution offered by monodimensional gas chromatography (GC) has been demonstrated to be insufficient for the complete resolution of many complex environmental mixtures. In some cases, GC hyphenation with an appropriate mass spectrometric technique can contribute to solve the problem. However, in the case of trace components, appropriate and reliable identification and quantification is only achieved when both the sample preparation and the instrumental analytical conditions have been specifically developed and optimized for a certain type of sample and group of substances. This traditional *targeted* approach has been successfully used for several decades in many application studies, and it is the analytical strategy adopted in routine control programs at present. However, this approach has a significant drawback as it always will miss compounds which were not selected at the start of the analyses. Therefore, in general, all unknowns or other untargeted substances even in high concentrations or with severe toxic potential will be missed.

At present, most environmental monitoring programs are based on the Stockholm Convention on persistent organic pollutants (POPs), which focuses on 26 chemicals or groups of chemicals. In contrast, there are approximately 100,000 industrial chemicals or chemicals of commerce used currently [1] and environmental samples contain hundreds

of non-targeted compounds. Some of these untargeted compounds have recently been identified as novel bioaccumulative halogenated natural products, new isomers or metabolites of known contaminants [2], but also as non-previously described pollutants [3]. On the other hand, several screening computational studies have pointed out that, on the basis of their physico-chemical properties, many chemicals in use fulfill the Stockholm Convention criteria for POPs-like compounds. Among these bioaccumulative, persistent and long-range transport-capable chemicals, 98% are halogenated, and two-thirds are chlorinated, brominated and mixed halogenated compounds [1]. In other words, the number of potential POP-like compounds containing Cl^- and/or Br^- in their structure and reaching the environment is probably larger than the (rather limited) set of compounds monitored under the Stockholm Convention regulation.

This presentation discusses the potential of *non-targeted* comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC−ToF MS) to assess the organohalogen burden of complex environmental samples. The benefits derived from the enhanced separation and identification power provided by this multidimensional technique will be illustrated using tuna muscle samples subjected to a rather generic sample preparation procedure as case study. The potential of classifications and script tools for automatic data filtering on the basis of specific structural characteristics will be discussed. Examples of legacy and non-legacy POP-like families, and novel chemicals detected in these samples will be provided.

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SHORT BIOGRAPHY

Lourdes Ramos currently holds the position of Senior Scientific Researcher of the Spanish Scientific Research Council (CSIC, Madrid), at the Department of Instrumental Analysis and Environmental Chemistry of the Institute of Organic Chemistry. Her main research interests are the development of novel miniaturised sample preparation approaches for the fast and green determination of trace organic pollutants in environmental and food samples, and the evaluation of new chromatographic techniques, in particular hyphenated gas chromatography (GC) based approaches, for unravelling the composition of complex mixtures of organic microcontaminants. Member of the of the editorial board of various journals, including Journal of Chromatography A, invited editor of several special issues, she has co-authored over 80 peer-reviewed scientific papers, 12 book chapters, and has edited a multi-authored book on comprehensive two-dimensional gas chromatography.

Questions & Answers:

1.- If you had to highlight major breakthroughs in your research field for the last 10 years, which would be your choice?

As in many other analytical application areas, during the last decade, the miniaturization of the sample treatment has been consolidated as a clear trend in the environmental field. However, up to now, the degree of development and implementation of such technologies in routine laboratories is largely influenced by the physical stage of the sample: while fully hyphenated analytical systems are commercially available for the preconcentration and purification of liquid samples, the previous extraction step is limiting the development of equivalent set-ups for the treatment of solid samples. During the last years, research in this field has also been benefited from the development of novel sorbents with improved sorption and loading capability and with enhanced selectivity, which have also facilitate the developed of more simplified and integrated analytical procedures for sample treatment.

Regarding instrumental analysis, significant advances have derived from the development and commercialization of new mass spectrometric instruments improving the performance of existing couplings with liquid chromatography. But also in a well establish technique like gas chromatography has been advances. The commercialization of the ionic liquid-based stationary phases and the new vacuum ultraviolet detector could be mentioned as interesting examples.

2.- Could you envisage the major or most predictable and desirable outcomes within your area of work for the next 10 years?

In coming years, I expect to see an increasing use of novel engineered and nano-structured materials and solvents, whose improved and tailored properties will allow the development of more simplified, integrated faster and/or greener sample preparation procedures. I would also expect advances in comprehensive two-dimensional separation sciences and more powerful mass spectrometers both allowing and facilitating non-targeted analyses. These types of approaches, in combination with generic sample preparation processes, should make possible the identification of new and emerging toxicants. They will also allow a more systematic study of the effects derived from the combined exposure of organisms to multiple chemicals. However, advances in this research field are highly dependent on the desirable developed of more powerful tools for data processing and data mining.

ÁNGEL MAQUIEIRA

Instituto Interuniversitario de Investigación de Reconocimiento Molecular Molecular y Desarrollo Tecnológico (IDM)
Departamento de Química. E.T.S.I.M.N.
Universidad Politécnica de Valencia,
Camino de Vera s/n. 46022 Valencia
amaquieira@qim.upv.es http://www.uv.es/idm/

CONFERENCIA INVITADA

La Química Analítica frente a los retos de la globalización

Se presentan una serie de reflexiones sobre el papel de la Química Analítica en un contexto globalizado. Se expondrán los retos que representa moverse en un nuevo entorno, muy permeable, en el que además se vislumbra un cambio de época.

Se comentará el impacto y el significado que ello puede significar para la Química Analítica y para sus actores, desde diferentes ámbitos: formativo, académico, puramente científico y tecnológico.

La Química Analítica, sin duda, tiene por delante un periodo de gran protagonismo. En cualquier dirección que se mire existen importantes interrogantes a los que dar solución. Algunos ejemplos los encontramos en agroalimentación: autentificación de materias primas y productos procesados, nuevos productos, etc. En el ámbito de la energía también se vislumbran grandes cambios derivados de la "descarbonizacion de la economía". No digamos del cambio climático, de la movilidad de la población (aumento de infecciones, "fiebre del viajero" o aparición de alergias).

Otro ámbito de gran interés para la Química Analítica es el de Salud y Bienestar. Así, en el estudio de enfermedades como el cáncer existen muchos ensayos basados en secuenciación génica, que es la técnica más usada y quizás la mejor, pero otras alternativas más sencillas e *in situ* o que prescindan de la etapa de amplificación, tienen gran porvenir. Otra revolución, ya en marcha, será desarrollar ensayos de diagnóstico *in vitro* sustitutivos de los clásicos *in vivo*.

El envejecimiento de la población es un campo de trabajo sustancial en las sociedades más ricas, pero de crecimiento rápido en todo el mundo. La fármacogenética es otra área incipiente, donde la Química Analítica puede hacer grandes aportaciones, tanto en metodología como en interpretación de datos (quimiometría).

El seguimiento y control de poblaciones (*point of need*, asistencia telemática, etc.) basado en tecnologías de telecomunicación es un campo novedoso que presenta desafíos muy importantes.

Seguridad y contra-terrorismo también son áreas muy sensibles y con gran demanda de soluciones más prácticas que las actuales, aún poco efectivas. El transporte de mercancías también necesita de mejores soluciones, especialmente en aduanas, puertos y aeropuertos.

La industria está incorporando multitud de sensores, la mayoría físicos. Introducir sensores químicos con capacidad de analizar muestras mínimamente tratadas, es un tema complicado pero apasionante, y un gran reto para la Química Analítica.

A lo largo de la exposición se irán presentando, desde la apreciación personal del autor, ejemplos concretos, considerando los campos más interesantes y los enfoques más prometedores relacionados con el nuevo horizonte en el que la Química Analítica será un gran protagonista.

SHORT BIOGRAPHY

Ángel Maquieira Catalá, full professor at the Chemistry department of the Universitat Politècnica de Valencia, is specialized in bioanalytical chemistry. His research activity is designed with an interdisciplinary view, integrating concepts from biochemistry, organic, analytical and physical chemistry, mainly for developing immunoreagents, functionalize materials, anchoring probes and biointeractions study, all focused to biosensing based on new analytical concepts, using optical detection in micro and nano scales.

Questions & Answers:

1.- If you had to highlight major breakthroughs in your research field for the last 10 years, which would be your choice?

Without a doubt, the sequentiation of the human genome

2.- Could you envisage the major or most predictable and desirable outcomes within your area of work for the next 10 years?

Within the predictable outcomes, in terms of technologies, the introduction of intelligent robots in the analytical chemical field should change many things regarding methodologies, and analytical process.

If we think about the desirability, the development of chemical reactions and modes of detection that allow to

obtain direct genetic information, without the enzymatic amplification of the analyte/s (PCRs), can have great significance and value.
The development of implantable biosensors in humans and animals is another area of great analytical interest since, regardless of the detection technique; an effective analytical process is required and functioning under limiting physiological conditions.

KAREN FAULDS

Department of Pure and Applied Chemistry, University of Strathclyde,
Technology Innovation Centre,
99 George Street,
Glasgow, G1 1RD, UK.
Karen.faulds@strath.ac.uk
https://www.strath.ac.uk/staff/fauldskarendr/

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Multiplexed and Quantitative Bioanalysis using Surface Enhanced Raman Spectroscopy (SERS)

Surface enhanced Raman scattering (SERS) is an analytical technique with several advantages over competitive techniques in terms of improved sensitivity and multiplexing. We have made great progress in the development of SERS as a quantitative analytical method, in particular for the detection of DNA. However, the lack of quantitative data relating to real examples has prevented more widespread adoption of the technique. Detection of specific DNA sequences is central to modern molecular biology and also to molecular diagnostics where identification of a particular disease is based on nucleic acid identification. Many methods exist and fluorescence spectroscopy dominates the detection technologies employed with different assay formats. Another advantage of SERS over existing detection techniques is that of the ability to multiplex which is limited when using techniques such as fluorescence. We have clearly demonstrated the ability to identify the presence of a mixture of 6 analytes in solution using data analysis techniques.
Here we demonstrate the development of new molecular diagnostic assays based upon SERS which have been used successfully for the detection of DNA sequences from bacterial pathogens associated with meningitis using modified SERS active probes. The probes have been designed to give a specific SERS response resulting in discernible differences in the SERS which can be correlated to a specific DNA hybridisation event. The simultaneous detection and quantitation of 3 pathogens within a multiplex sample will be demonstrated for the first time.
We have also recently demonstrated the detection of pathogenic bacteria in food using functionalised nanoparticles combined with magnetic separation and SERS. Metallic nanoparticles have been functionalised with bio-recognition molecules (antibodies and lectins) which are specific for a bacterial strain and a Raman reporter to enable the SERS detection. A different Raman reporter was used for each strain of bacteria; therefore a SERS signal was only obtained when the SERS active nanoparticle binds specifically to its bacterial target. The aim is to develop a multiplexing assay where three bacterial strains can be simultaneously identified in a sample matrix.

SHORT BIOGRAPHY

Karen Faulds is a Professor in the Department of Pure and Applied Chemistry at the University of Strathclyde and an expert in the development of surface enhanced Raman scattering (SERS) and other spectroscopic techniques for novel analytical detection strategies and in particular multiplexed bioanalytical applications. She has published over 100 peer reviewed publications and has filed 5 patents. She has been awarded over £10M in funding as principal and co-investigator from EPSRC, charities, industry and governmental bodies. In 2009 she was presented with the *Nexxus Young Life Scientist of the Year award* and in 2011 was elected to the *Royal Society of Edinburgh Young Academy of Scotland,* the first such Academy amongst the national academies in the UK and was elected *Fellow of the Royal Society of Chemistry* in 2012. She was awarded the *2013 RSC Joseph Black Award*, the 2016 *Craver Award* from the Coblentz Society and was also recently named as one of the *Top 50 Women in Analytical Science* by The Analytical Scientist. She has given over 50 invited talks at national and international conferences. She is the Strathclyde Director of the Centre for Doctoral Training in Optical Medical Imaging, serves on the editorial board of *RSC Advances* and the editorial advisory board for *Analyst* and *Chemical Society Reviews* and is the current Chair of the UKs Infrared and Raman Discussion Group (IRDG