4th Analytical Biosciences Early Career Researcher Meeting

15 March 2017 – 16 March 2017

University of Warwick

Through an outstanding talk programme and poster sessions, ECRM aims to bring together Early Career Researchers working in the field of Analytical Biosciences.

Keynote speakers

Mark Viant
University of Birmingham
Metabolomics

Matthew Baker
University of Strathclyde
Clinical spectroscopy

Peter O'Connor
University of Warwick
Mass spectrometry

Bronwyn Ormsby
Tate, London
Art conservation

Jonathan Aylott
University of Nottingham
Fluorescent bio-imaging

Kirsty Penkman
University of York
Biomolecular archaeology

Gillian Greenway
University of Hull
Sensors, environmental analysis

Lisa Hall
University of Cambridge
Molecular sensors

Christel Veyssier
MedImmune, Cambridge
Biological drug candidates

VRS Careers in Analytical Sciences Workshop
Industry recruitment trends, the key skills and experience required, opportunities available

warwick.ac.uk/ecrm2017

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twitter.com/RSC_ABG

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Programme

Wednesday 15th March 2017

Time	Event	Session Chair
10.00-10.30	Arrival and registration	
10.30-10.40	**Opening remarks** Alastair Dent, Chair of ABG	Session chair: **Lynne Dennany** *University of Strathclyde*
10.40-11.25	**Lisa Hall**, University of Cambridge *Bioinspired materials for Biosensors*	
11.25-11.40	**Husain A Naqi**, University of Bath *Pharmaceutical analysis of seized ethylone–ecstasy mixtures using quantitative NMR spectroscopy and ESI-MS*	
11.40-11.55	**Maria Lizio**, University of Manchester *Exploring peptide foldamer-membrane interactions using optical spectroscopic techniques*	
11.55-12.10	Break	
12.10-12.55	**Kirsty Penkman**, University of York *Through the looking glass, and what amino acids found there*	
12.55-13.10	**Neus Jornet Martinez**, University of Cambridge *Zein as sustainable alternative of petroleum-based materials in biokits and biosensors for in situ analysis*	
13.10-14.00	Lunch and posters	
14.00-14.45	**Jonathan Aylott**, University of Nottingham *Fluorescent nanosensors for imaging and quantifying biological systems*	Session chair: **Frankie Rawson** *University of Nottingham*
14.45-15.00	**Robert Johnson**, University of Utah *Base Flipping within a Nanopores Reveals the Identity of DNA Bases at the Single Molecule Level*	
15.00-15.15	Break	
15.15-16.00	**Bronwyn Ormsby**, Tate Britain *Analytical investigation and cultural heritage: preserving modern and contemporary art at Tate.*	
16.00-16.15	**Jerome Charmet**, University of Warwick *Practical considerations for micro/nanotechnology enabled biosensors*	
16.15-16.45	**VRS Career Workshop** Simon England, VRS Recruitment	
16.45 19.00	Wine and posters **Conference Dinner** *Woodland Grange Hotel*	

Thursday 16th March 2017

Time	Event	Session Chair
9.00-9.30	Arrival and coffee	
9.30-10.15	**Matt Baker**, University of Strathclyde *Developing Objective Spectroscopic Disease Diagnostics for the Clinical Environment*	Session chair: **Mark Seymour** *Syngenta*
10.15-11.00	**Christel Veyssier**, MedImmune *Higher Order Structure characterisation to support Biopharmaceutical R&D*	
11.00-11.15	Break	
11.15-12.00	**Mark Viant**, University of Birmingham *Exploiting the capabilities of non-targeted metabolomics to discover complex interactions between plants, animals and toxicants*	
12.00-12.15	**Mark Platt**, Loughborough University *Multi-Pores. Controlling and measuring the flow of charged species through tunable nanopores producing a rapid, multiplex assay.*	
12.15-13.15	Lunch and posters	
13.15-14.00	**Gillian Greenway**, University of Hull *A comparison of detection approaches for selective and sensitive biosensing*	Session chair: **Kirsty High** *University of York*
14.00-14.45	**Helen Yu-Ting Hsu**, Durham University *Small molecule biosensor development and alternative sensing material*	
14.45-15.00	Break	
15.00-15.45	**Pete O'Connor**, University of Warwick *2-Dimensional Mass Spectrometry: Proteomics in the Next Dimension*	
16.00	Closing remarks and the poster prize	

Conference sponsors:

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ROYAL SOCIETY OF CHEMISTRY | ANALYTICAL BIOSCIENCES GROUP

Analyst

Analytical Methods

Lab on a Chip

Analytical Biosciences Group (ABG) is one of the many Interest Groups within the Royal Society of Chemistry, bringing together members with an interest in the use of analytical chemistry techniques in "bio" applications, and promoting the development and propagation of such techniques.

History

The ABG came into being in 1945 as the Biological Methods Group when it first became obvious that the properties of complex new pharmaceuticals, such as penicillins, could not be adequately characterised by traditional analytical chemistry alone. Thus it acted as an early forum for the development of bioassays for drug potency.

The field of analytical biosciences has expanded massively from these beginnings and the ABG has evolved to promote the development and application of a wide range of bioanalytical techniques.

Purpose

The principal purpose of the ABG is to sponsor and organise events in the field of analytical biosciences in order to disseminate information and promote networking. The committee use RSC funds to sponsor conferences, seminars, training courses, etc. in relevant subject areas. Sometimes such events arise directly from ABG discussions but often the ABG will co-sponsor events already being planned by other parties providing financial support, publicity, and/or administrative input.

Keynote speakers:

Lisa Hall

Head of Cambridge Analytical Biotechnology and Director of Research in the Dept. Chemical Engineering & Biotechnology, University of Cambridge. Vice President of Queens' College, Cambridge.

Lisa was awarded the Gold Medal in Analytical Chemistry by Royal Society of Chemistry and the Alec Hough-Grassby Memorial Award by the Institute of Measurement & Control.
She was appointed Commander of the Order of the British Empire (CBE) in the Queen's 2015 Birthday Honours List.

She is co-founder of Cam^{bridge}Sens: a strategic vision to bridge sensor research activities across the University, (www.sensors.cam.ac.uk).

Her research is focussed on understanding how biology can be interfaced with electronic, mechanical and optical systems and new ways to answer fundamental and applied questions concerning new measurement regimes. She has been involved with successful spin-offs and has recently developed a special interest in approaches to diagnostics for resource poor areas. Her research links ***transduction technologies*** (electrochemistry, optics, ultrasound) with ***synthetic biology*** and ***nanomaterials*** to achieve **sensors** & **diagnostic systems**. The group has a world-class reputation for its lead in fundamental innovative research. It is directed towards medical environmental and industrial application and bridges fundamental investigation and application orientated collaboration with industry.

Kirsty Penkman
Department of Chemistry, University of York

My research focuses on the application of analytical chemistry to archaeological and geological questions. Fueled by the opportunity to work in the field of archaeological science during my 4th year MChem project at the University of Oxford, this interest took me to Newcastle for a PhD in geochemistry, and then to York with a postdoc and a Wellcome fellowship. Now a Senior Lecturer in Analytical Chemistry, my focus is on the analysis of proteins: their pathways of degradation, methods for their detection, and how these molecules can inform us of an organism's life and death history. I run the NERC-recognised amino acid dating facility, NEaar.

Jonathan Aylott
School of Pharmacy, University of Nottingham

Jonathan gained his degree and PhD in Chemistry from the University of East Anglia. He then undertook postdoctoral research at the University of Michigan with Professor Raoul Kopelman where he worked on developing miniaturised sensors for intracellular applications. He returned to the UK in 2000 to take up the position of Lecturer in Analytical Science at the University of Hull and moved to the School of Pharmacy, University of Nottingham in 2004, being promoted to Associate Professor in Analytical Bioscience in 2011. Jon is currently an EPSRC Foresight Fellow, investigating the integration of analytical science into manufacturing processes for new pharmaceuticals. Jon's research interests focus on the design, development and implementation of miniaturized analytical devices. Such devices can then be applied to measuring biological samples in-situ and in real-time, generating a better understanding of disease states. Additionally, collaborative projects with commercial partners are ongoing to develop diagnostic devices incorporating nanotechnology to detect drugs of abuse and to differentiate between bacterial and viral infections.

Bronwyn Ormsby
Tate, London

After beginnings in biochemistry and art conservation, Dr. Ormsby began working at Tate in 2003 after completing her doctorate. From 2003-2006, she held two post-doctoral positions where she investigated the effects of commonly used cleaning agents on 20th century acrylic emulsion paints. In 2007, Dr. Ormsby was employed as a Conservation Scientist, and went on to become Principal Conservation Scientist in 2016. Bronwyn now leads a team of 3 scientists and 2 preventive conservators and remains responsible for the spectroscopic and mass spectrometry analysis of works of art. Bronwyn has developed expertise in the characterisation of 20th century art materials and investigations into the effects of conservation treatments on 20th century painted works of art, resulting in over 80 papers, as well as CPD workshops for conservators. Bronwyn is currently leading Tate's contributions to two EU-funded projects - NanoRestArt (2015-2018) and the Cleaning of Modern Oil Paints (2015-2018) as well as co-supervising two PhD students, with two additional doctorates commencing in 2017-18.

Matthew J Baker
Department of Pure and Applied Chemistry, University of Strathclyde

Matt Baker gained his MChem from the University of Manchester Institute of Science and Technology (UMIST) in 2004 and his PhD from the Department of Chemical Engineering and Analytical Science, University of Manchester on "The Application of Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS)" under the supervision of Dr N. Lockyer and Prof. J Vickerman in 2007. He was awarded an EPSRC Life Science Interface Fellowship in 2007 on "Spectrometric and Spectroscopic Molecular Pathology and Diagnosis" taking him to Harvard Medical School and the Robert Koch Institute, Berlin.
Following that he was a project manager, researcher scholar and senior scientist for the Defence Science and Technology

Laboratory part of the Ministry of Defence where he performed research in the CBRN domain. He was appointed as Senior Lecturer in Chemistry at the University of Strathclyde in Oct 2014, following a period at UCLan. He has held visiting positions at 7 international institutions including Monash University, Australia, Royal Preston Hospital and the Walton Centre NHS Trust. MJB is Co-I and a Founder of the EPSRC Clinical Infrared and Raman Spectroscopy (CLIRSPEC) Network (@clirspec), Founder Director of the international Society for Clinical Spectroscopy and a Raman4Clinics COST Action member and was awarded the inaugural Emerging Leader in Molecular Spectroscopy 2016

Christel Veyssier
MedImmune Ltd,
Granta Park, Cambridge

Christel Veyssier is an Analytical Biochemist who obtained her MSc at University Joseph Fourier in Grenoble, France. She joined MedImmune in October 2010 after Mass Spectrometry and Chromatography Method development positions in different companies including Genzyme (now Sanofi Ltd) in Haverhill, Suffolk.
She works in the Analytical Sciences team which sits in the Biopharmaceutical development department. The team remit is to characterise MedImmune products, establishing quality of manufacture using a variety of analytical techniques including: Mass Spectrometry, Chromatography and Spectroscopy. The team also develops techniques to characterise novel molecule formats. The main area of focus for Christel is Higher Order Structure characterisation using a set of biophysical techniques including Light Scattering and Circular Dichroism and also a Chemical and Manufacturing Control representing her team for Novel molecules.

Mark Viant
School of Biosciences and Phenome Centre Birmingham, University of Birmingham

Mark Viant is a Professor of Metabolomics in the School of Biosciences at the University of Birmingham, UK, Executive Director of Phenome Centre Birmingham and Director of the NERC Biomolecular Analysis Facility, two of the UK's national metabolomics centres. As a passionate advocate for the growth of the metabolomics community, he leads training and outreach through the Birmingham Metabolomics Training Centre and as the ELIXIR-UK Sector Lead for metabolomics. Mark is also a past President of the International Metabolomics Society. His research programme and interests encompass the development of optimised analytical and computational workflows for metabolomics and the applications of these technologies to investigate the metabolic pathways underlying toxicity and disease, both in the context of human and environmental health. He is particularly fascinated by chemical and nanomaterial toxicology in 'non-animal' invertebrate model organisms, for example the waterflea *Daphnia*, and the translation of metabolomics based 'discovery' research into mechanistically based tools for chemical safety and environmental diagnostics. Mark has co-authored over 150 publications and his work has been recognised by the award of a 2015 Lifetime Honorary Fellowship of the International Metabolomics Society.

Gillian Greenway
University of Hull

Gillian carried out research for her Ph.D in the Department of Instrumentation and Analytical Science at the University of Manchester in the area of trace metal speciation by gas chromatography (GC)- microwave induced plasma (MIP) atomic spectrometry.

In 1985 she became a lecturer at Humberside University where she developed a research interest in chemical sensors. Then in 1988 she transferred to the Department of Chemistry at the University of Hull where she set up a research group working on flow detectors and sensors, on-line sample

preparation, separations and environmental trace metal. In 1996 she was awarded the Royal Society of Chemistry SAC Silver Medal for her research.

She is currently a Professor of Analytical Chemistry and Head of the School of Mathematics and Physical Sciences at the University. For the last fifteen years her research has mainly focussed on the area of sensors and microfluidics with the aim of developing point of care systems and portable environmental monitoring devices.

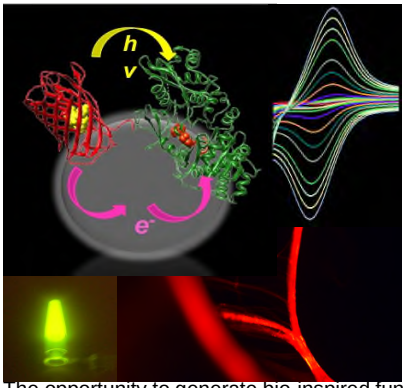
Peter B. O'Connor
University of Warwick, Department of Chemistry

Professor Peter O'Connor received his PhD from Cornell University under the supervision of Professor Fred McLafferty in 1995. He then did a Post-Doc at FOM-AMOLF in Amsterdam with Prof. Ron Heeren and worked in the mass spectrometry instrument development industry for 2 years before starting an academic career at Boston University School of Medicine. He was recruited to the University of Warwick in 2008. He has graduated 15 PhD students, has >130 publications, and an H-index of ~35.

Bioinspired materials for Biosensors

Hall, Elizabeth A H
Department of Chemical Engineering and Biotechnology, University of Cambridge

Embracing synthetic biology opens a door to structural and functional materials, inspired by biology but interfacing the biotic/abiotic and with potential to offer new biosensing materials. This presentation looks at emerging bioinspired materials. Structural engineered proteins (like silk) produced in bacteria provide a bulk manufacturing option for new materials. However, since there is no functional element, so alone they are only half the solution to achieving integrated sensing, therapy, defence or bioremediation, built entirely through assembly of protein constructs. The missing element for biosensing is the biorecognition component – a binding peptide or protein, an enzyme etc..



A ramification of synthetic biology is that nature provides a starting point for such materials, not a definitive source, so let's imagine luminescent silk-like fibre biosensors from polyQ or honeybee silk protein that can measure ATP, or think about new ways to use old proteins by engineering the interface between biology and a transduction system or where an electrode can be used to control enzyme activity and switch its oxidation state or the same system can be used as both a sensor and a drug delivery vehicle.

The opportunity to generate bio-inspired functional materials is opening up new opportunities to extend biosensors in new directions and this presentation will examine some examples that use electrochemistry or optics to turn a protein into a biosensor.

Through the looking glass, and what amino acids found there

Penkman, Kirsty
Department of Chemistry, University of York, York, YO10 5DD

Analytical chemistry can take you to strange places - for me it has been to the bottom of a quarry, being covered in dirt, excavating sesame-seed sized fossils that allow us to work out when prehistoric creatures such as mammoths and Neanderthals roamed Europe. Being able to identify (and isolate) a fraction of the protein contained within particular fossil shells which degrades at a predictable rate has allowed the development of a robust dating technique. This 'intra-crystalline' protein behaves as a closed system, and if this is targeted in a tiny calcite fossil (snail opercula, the little lids that close the aperture of snail shells) we can date back to at least 2.8 Ma. With LC and MS analyses of protein in fossil material ranging from Great Barrier Reef corals to South African ostrich eggshell and the ice cores from Greenland and Antarctica, collaborations with earth scientists and archaeologists have helped to push the analytical science forward, whilst advancing our understanding of our earth's history.

Fluorescent nanosensors for imaging and quantifying biological systems

Aylott, Jonathan
School of Pharmacy, University of Nottingham, University Park, Nottingham, NG2 7RD.

Optical nanosensors are tools that utilise the sensitivity of fluorescence to enable the quantitative real-time measurement of biological systems. Nanosensor devices can be made in the size range from 50 nm to 1 μm diameter, dependent on the matrix they are synthesised from and the application requirements. The small size of the nanosensors allows them to make *in situ* measurements of biological systems, e.g., be delivered directly into cells, or be incorporated into sensing scaffolds upon which cellular systems can be grown. Nanosensors capable of measuring pH, oxygen, glucose, calcium, zinc and proteases have been demonstrated and work is ongoing to expand the range of analytes that can be detected using this technology.

An attractive feature of fluorescent nanosensors is that they can be imaged and quantified using standard fluorescent microscopies. We have used wide-field, confocal and super-resolution fluorescent microscopies to image and quantify a variety of biological systems ranging in complexity from single cells to whole organisms. In this presentation I will discuss how we have applied fluorescent nanosensors to image and quantify cellular uptake; make measurements in *C.elegans* nematodes; and develop sensing scaffolds for tissue engineering and regenerative medicine applications.

Analytical investigation and cultural heritage: preserving modern and contemporary art at Tate.

Dr. Bronwyn Ormsby
Principal Conservation Scientist
Tate Britain
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This presentation introduces audiences to some of the ways analytical science is being used to characterise materials used in works of art, and in particular, modern and contemporary painted works such as paintings and sculpture. The analytical focus will be on the use on spectroscopy and mass spectrometry techniques, presented within a wider investigative and cultural context, exploring artists' choices and the inherent challenges involved in preserving modern and contemporary art collections. Several case studies and research projects will be discussed, including research into acrylic paints, modern oil paints, new materials for art conservation, and how the research can be used to inform conservation practice. A particularly notable case study treatment will be discussed - Mark Rothko's *Untitled, Black on Maroon*, (Tate, T01170, 1958) which was the subject of an extended conservation treatment in 2012-14.

Developing Objective Spectroscopic Disease Diagnositcs for the Clinical Enivronment

Matthew J. Baker
University of Strathclyde, Department of Pure and Applied Chemistry

Spectroscopic analysis allows for the label-free objective classification of biological material on the molecular scale. This technique has been applied to histology, cytology and surgical pathology and can detect subtle changes in the proteome and metabolome. This new biochemical information has the potential to improve patient outcome through the identification of earlier stages of disease, drug resistance, disease states and high-risk populations.

Recently, IR serum analysis has been shown to be capable of rapid, specific and sensitive analysis of disease. Capable of diagnosing cancer severity as well as infectious disease. However, a full understanding of sample preparation effects and the effect of the coffee ring effect upon a spectrum is not fully understood which is standing in the way of clinical translation and development. In addition, the recent combination of broadly tunable laser sources (QCLs), refractive based high numerical aperture objectives and a large format detector system has enabled high-definition diffraction-limited resolution, and new opportunities for data collection including real-time and data collection for discrete frequency infrared (DFIR) imaging capable of speeding up our analysis into clinically relevant times.

This paper will discuss recent applications in the development and validation of FTIR and DFIR spectroscopic serum diagnostics with a focus on the development of liquid analysis, removing the variability inherent in the coffee ring effect. In particular, it will discuss a large patient study for cancer diagnostics based upon different methodologies to enable accurate analysis of liquid serum and results from an investigation on the impact of DFIR on the spectrum and the ability of spectral diagnostics to discriminate samples based upon discrete frequencies of the most salient information obtained from the dataset, including data analysis techniques to enable this.

Higher Order Structure characterisation to support Biopharmaceutical R&D

Veyssier, Christel, Me*dImmune Ltd, Granta Park, Cambridge, CB21 6GH*

An increasing proportion of new drug registrations are monoclonal antibodies or recombinant protein therapeutics. These molecules are dynamic structures in solution and can adopt a range of conformations. Furthermore, chemical modification of a protein e.g. cysteine oxidation, can lead to changes in higher order structure (HOS) properties which can impact efficacy or safety of the biotherapeutics.
This presentation will show two case studies demonstrating the importance of developing suitable analytical tools to characterise the HOS of proteins during drug development. Techniques such as Circular Dichroism, Hydrogen-Deuterium Exchange Mass Spectrometry and Size-Exclusion Chromatography coupled to Multiple Angle Light Scattering will be presented as part of the analytical strategy.

Cohen, E. S. *et al*. Oxidation of the alarmin IL-33 regulates ST2-dependent inflammation. *Nat. Commun.* 6:8327
doi: 10.1038/ncomms9327 (2015).

Exploiting the capabilities of non-targeted metabolomics to discover complex interactions between plants, animals and toxicants

Viant, Mark
School of Biosciences and Phenome Centre Birmingham, University of Birmingham, Birmingham, B15 2TT

Non-targeted metabolomics is now widely applied across fields from medicine and biotechnology to agriculture and ecology, with the goals to discover and help to characterise the molecular mechanisms associated with biological processes. The field of environmental metabolomics is uniquely challenging due to the vast metabolic diversity of the organisms being studied – from plants and animals to microbes – and due to the chemical complexity of the natural environment (the exposome). In this presentation I will first highlight some of the unique challenges of environmental metabolomics, introduce the analytical and computational workflows that we use, and then provide a case study in nanotoxicology. Specifically I will describe our investigations into the effects of one of the most widely used nanomaterials, zinc oxide nanoparticles (ZnO NPs), applied in consumer products from cosmetics to electronics. We exploited the non-targeted capabilities of metabolomics to investigate the molecular toxicity of waterborne ZnO NPs to the model organism *Daphnia magna*, the waterflea. Our discoveries took us down an unexpected path into chemical ecology and measurements of families of aliphatic sulfates and sulfamates that act as chemical messengers between waterfleas and their prey. Through the application of several bioanalytical methods we are now unravelling a complex NP-biota interaction with currently uncharacterised ecological consequences.

A comparison of detection approaches for selective and sensitive biosensing

Gillian Greenway
University if Hull

A range of different approaches for bioanalytical sensing will be discussed including:-

Chemiluminescence immunoassay detection with reproducible electrochemical immobilisation for hormones.

Label free optical biosensing using dye doped leaky waveguides.

Generic toxicity testing in microfluidic devices.

2-Dimensional Mass Spectrometry: Proteomics in the Next Dimension

Pui Yiu Yuko Lam, Maria van Agthoven, Christopher A. Wootton, Federico Floris, **Peter B. O'Connor**
University of Warwick, Department of Chemistry

2-Dimensional Mass Spectrometry (2D MS) is an old technique borrowing on concepts from 2-dimensional nuclear magnetic resonance. The technique was known in the 1980's, but was not viable until recent developments in data processing techniques and computational capacity caught up with the demands of the method. A typical 2D MS experiment will acquire 20-200 GBytes of data in a single multidimensional spectrum, which then needs to be 2D Fourier Transformed, calibrated, denoised, and peakpicked before the relevant information can be extracted, so the computational task is huge, and ongoing, but these spectra can now be processed with modern high performance computing.

Two dimensional mass spectrometry works by applying a modulation frequency to the mixture of precursor ions, periodically shifting them in/out of a fragmentation zone according to their native oscillation frequency. If this is done, then the fragments will be generated carrying that same modulation frequency, and a 2D-FT can extract both the native frequency and the modulation frequency which allows us to plot all fragments from all precursors simultaneously, without precursor ion isolation. The net effect, compared to traditional MS/MS, is an increase in data density, full correlation of all fragment ion information, an increase in signal/noise (due to fellgett signal averaging), and an increase in total spectrum acquisition time (if the sample is complex).

Thus, 2D MS is an ideal tool for very complex samples such as proteomics. We will show our current progress on using 2D MS for proteomics datasets.

Pharmaceutical analysis of seized ethylone–ecstasy mixtures using quantitative NMR spectroscopy and ESI-MS

Husain A. Naqi, Timothy J. Woodman, Stephen M. Husbands, and Ian S. Blagbrough
Department of Pharmacy and Pharmacology, University of Bath, Bath BA2 7AY, UK

Ethylone-ecstasy (MDMA) mixtures were obtained from different Bristol night clubs following Police seizures. NMR spectroscopic analysis allowed identification, structural elucidation, and quantification of these mixtures. Additional confirmation of the structures came from Electrospray Ionization Mass Spectrometry (ESI-MS). Quantitative NMR analysis, using maleic acid as the internal standard, revealed different ratios of the mixtures, e.g. 2:1, 1:2, and 1:3 ethylone:ecstasy.

The different chemical shifts of the methylenedioxy signals are diagnostic for both drugs in the mixture, ^{1}H δ 6.03 ppm in ethylone and 5.87 ppm in ecstasy. The fine splitting patterns of these methylenedioxy signals are another distinction. The ethylone methylenedioxy signal appeared as a very fine doublet, while the comparable signal in ecstasy was a sharp singlet. Maleic acid was used as a quantitative internal standard due to its high solubility and the non-overlapping peak at δ 6.30 ppm. In these mixtures, the majority of the NMR signals of ethylone and ecstasy are suitable candidates for quantification due to the absence of any peak overlap. The ESI-MS revealed both required molecular ions, *m/z* 194.1184 found for ecstasy and 222.1122 found for ethylone. Fragmentation of both $[M+H]^+$ provided further corroboration of the components in the mixture.

Such combinations of illicit drugs, e.g ecstasy with a Novel Psychoactive Substance (NPS), e.g. ethylone, might be aimed at altering the experience for the user. However, the presence of another pharmacologically active substance might result in serious harm, even lethality, especially as the biological activities of NPS are not well understood.

We gratefully acknowledge the Government of Kuwait for a studentship (to H.A.N.) and the Drug Expert Action Team (DEAT) of the Avon and Somerset Constabulary for the supply of seized samples.

Exploring peptide foldamer-membrane interactions using optical spectroscopic techniques

Dr Lizio, Maria Giovanna[1], Dr. De Poli,Matteo[1],Dr. Webb, Simon,J.[1]and Prof Blanch,Ewan[2].
[1] *School of Chemistry, Manchester Institute of Biotechnology, University of Manchester, 131 Princess Street, Manchester, M1 7DN, United Kingdom.*
[2] *School of Applied Science, School of Applied Sciences, RMIT University, GPO Box 2476, Melbourne, Victoria 3001, Australia.*

The evolution of multi-resistant microbial strains such as MRSA and *C. difficile* presents a serious threat to the provision of basic healthcare. The development of new and effective antibiotics is therefore of the utmost importance. Antimicrobial peptides (AMPs) represent a defensive mechanism against microbes produced by most organisms. Peptaibols are a class of AMPs produced by fungi *via* non-ribosomal synthesis and are characterized by a high content of non-proteinogenic residues, mostly α-aminoisobutyric acid (Aib; pept-**aib**-ol), which give them a high propensity to fold into helical structures, a C-terminal 1,2-amino alcohol (pept-aib-**ol**) and usually acetylated or acylated at the N-terminus[1]. Despite attracting significant attention because of their activity, their conformational behaviour is still not completely understood. The antimicrobial activity of peptaibols correlates strongly to the aggregation rate and conformational changes of the peptides in the membrane environment [2]. It is possible to design small synthetic peptides, known as foldamers, endowed with specific properties; in particular, Aib-rich foldamers are used as a model for the understanding of the folding and membrane interaction of the naturally occurring species. The aim is to investigate the conformational preference of monodisperse Aib-oligomers as well as understanding their interaction with bilayer membranes.

A large set of spectroscopic techniques have been used to establish the conformation of Aib-rich foldamers both in solution and when bound to membranes. In particular: Raman, Raman Optical Activity (ROA), Infrared (IR), Vibrational Circular Dichroism (VCD), Linear Dichroism (LD) and Neutron Scattering (NR) were employed to provide new structural insights.

These vibrational analysis (VA) and vibrational optical analysis (VOA) investigations in solution were focused on the identification of spectral features for 3_{10}-helix conformation, particularly with Raman and Raman Optical Activity spectroscopies. Spectroscopic markers for this conformation in the amide I region were successfully identified. Moreover, it is known that chiral Aib-rich peptides can show a right or left handed screw-preference based on the primary sequence. VOA studies successfully distinguished between peptides with opposite helicity.

VCD, ROA, LD and NR of Aib-foldamers bound to membranes were shown to be useful for identification of conformational preferences of the peptides within the membrane as well as for determining their orientation in the bilayer, and ultimately the effect of the peptides on the membrane structure.

[1] Chugh J. K. and Wallace B. A., *Peptaibols: models for ion channel*, Biochemical Society Transactions (2001) 29, part 4.
[2] Duval D., Ridell F. G., Rebuffat S., Platzer N., Bodo B., *Biochim. Biophys.* Acta., 1998, 1372, 370

Zein as sustainable alternative of petroleum-based materials in biokits and biosensors for in situ analysis

Neus, Jornet-Martínez, Sara, Bocanegra-Rodriguez, Pilar, Campíns-Falcó and E.A.H,.Hall
Departament de Química Analítica, Facultat de Química, Universitat of València, Dr.Moliner 50, 46100 Burjassot, Valencia, Spain.
Institute of Biotechnology,Department of Chemical Engineering and Biotechnology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QT, United Kingdom.

Plastics have replaced glass and metals in many areas of use due to their properties such as elasticity, biocompatibility, stability, low cost and easy manufacture. However, huge amounts of wastes are generated and have to be treated. Biodegradable and biocompatible materials extracted from renewable resources are are becoming progressively more important, since they offer less petroleum-dependent and are degradated naturally[1]. In this context, zein, which is a protein of maize, has been studied as a reagent delivery platform for the development of biokits and biosensors.
Nowadays, there is an increasing demand to develop *in situ* devices[2]. In order to find green alternatives for on site sensing, not only biodegradable materials are required, but also, biosensors have been explored, based on enzyme immobilization or using combinations of enzymes. The main mode of detection in this biosensor research has been electrochemical, linked with multi-enzyme systems that produce a better electroactive product or current amplification. Nevertheless, the same reagents can be used in an optical assay, by changing the enzyme's substrate, so that a fluorophore/colorimetric product is generated. This work takes the first steps to investigate whether devices, composed entirely of biodegradable materials, could be developed for optical biokits and biosensors.
The systems investigated here are based on alkaline phosphatase (ALP) activity/inhibition in the presence of phosphatase substrates, such as 3-O-methylfluorescein phosphate (OMFP) or p-nitrophenyl phosphate (p-NPP). The enzyme and substrate were packaged in a solid film of zein and delivered to the test solution, so all of them diffused from the zein disk into the solution. The potential applications presented here are: (1) inorganic phosphate (P_i) estimation, necessary to control nutrients and eutrophication (phosphate levels in water are regulated by the EU through the Urban Waste Treatment that underlines the maximum annual mean total phosphorous concentration of 1-2 mg/mL and the Water Framework Directive 2000/60/EC) , (2) the estimation of ALP in saliva, which allows distinction to be made between adult and child saliva and (3) the organophosphorus pesticide (OPs) estimation in control analysis of commercial formulations.
In conclusion, several options for biodegradable kit were reported with application in environmental and healthcare control, particularly in monitoring campaigns in situ and non-invasive diagnostic analysis. All of them, represent reliable and green alternatives to the conventional optical methods due to its sensitivity, simplicity and low cost.[3]

[1]D. Briassoulis and C. Dejean, J. Polym. Environ., 2010, 18, 384-400.
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Acknoweledgements: The authors thanks GV (PROMETO2016/109, NJ APOSTD2016/113 and SB predoctoral grant PROMETO2016/109)

Base Flipping within a Nanopores Reveals the Identity of DNA Bases at the Single Molecule Level

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Localized conformational changes at a single base-pair of double-stranded DNA are measured inside the protein nanopore alpha-hemolysin (αHL) and used to identify the presence of non-Watson-Crick (mismatched) base pairs. DNA is driven into the vestibule of αHL one duplex at-a-time, resulting in ion flux attenuation and a decrease in the measured current. Unique modulating current signatures are observed for duplexes containing mismatched base pairs when the mismatch site in the duplex is situated in proximity to narrowest part of the vestibule, the 2.6 nm latch constriction. The frequency and current amplitude of the modulation states are dependent on the mismatch type permitting discrimination of mismatches from one another, and from a fully complementary duplex that exhibits no modulation. The modulating current signatures are attributed to the biologically important process of base flipping. This talk will outline how base-flipping within a nanopore can be used to identify both a variety of mismatched base-pairs and important epigenetic modifications to cytosine.

Multi-Pores. Controlling and measuring the flow of charged species through tunable nanopores producing a rapid, multiplex assay.

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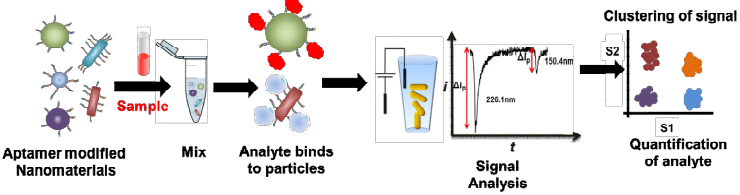
Background:

Nanopores have been created in a range of materials from graphene, polymers, silicon nitride and glass. The transport of the ions or analyte through these channels can by controlled by tuning the applied potential, pore wall charge, pore size, supporting electrolyte concentration and composition, with a further degree of selectivity by modifying the pore walls with selective ligands. If an analyte (protein, virus, bacteria) passes through the pore it occludes the ionic current causing a transient current decrease known as a "blockade event", see fig 1. The integration of aptamer technologies into nanopores in an emerging area. Where previously antibodies have been the capture probe of choice, aptamer technologies are gaining interest. Aptamers are powerful reagents that bind target ligands with affinities comparable to antibodies. Due to their comparable selectivity, stability and cost, over the last two decades, aptamers have started to challenge antibodies in their use on many technology platforms. They offer a cheaper, animal free alternative to antibodies, do not require refrigeration and as a result offer a simple, cost effective and robust sensor platform.

Measuring aptamers interactions to small molecules, proteins, cells, virus etc. is well suited to a nanopore platform, and offers a powerful combination of technologies. The group have been developing methods of characterising nanomaterials with nanopore technologies including working in serum (Analytical and Bioanalytical Chemisty, (2016) 408, 5757). Developing a range of detection strategies (**Faraday Discuss.**, 2016,**193**, 487 and **Nanoscale**, 2016,**8**, 19139), and pioneered the study of aptamer protein interactions on tunable pores (**Langmuir** 2016, 32, 1082, **Anal. Methods**, 2015, **7**, 8534, **Biosensors and Bioelectronics**, 2015, 68, 741, **Anal. Chem**., 2014, 86 1030, and **Small,** 2012, 8, 2436). Nanopore technologies used for the characterisation of nanomaterials offer many unique advantages over light scattering of fluorescent based technologies. The signal is not averaged as with DLS, and offers a particle-by-particle analysis, allowing particles of different sizes and shapes to be analysed and characterised individually. The technology can also work in turbid solutions without any dilution, making it ideal for whole blood or plasma.

Results:

Here we present some of our recent work developing multiplexed assays using aptamer modified nanomaterials and pores to compare the use resistive pulses or rectification ratios on a tunable pore platform. We compare their ability quantify the cancer biomarker Vascular Endothelial Growth Factor (VEGF). Secondly by tuning the ligands and the setup we then show how the translocation speed, conductive and resistive pulse magnitude, can be used to infer the surface charge of a nanoparticle, and act as a specific transduction signal for the binding of metal ions to ligands on the particles surface, used to extract and detect copper (II) ions (Cu2+) from solution. Other data will include the measurments of the protein corona around nanomaterials in serum, plasma and whole blood. Finally we show data from samples that contain bacteria and bacteriophage and strategies to quickly quantify them.



Practical considerations for micro/nanotechnology enabled biosensors

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The field of biosensors, driven among others by recent advances in micro and nanotechnologies, has seen the development of ultra-sensitive sensors. The progress has been such that the limit of detection of many micro/nano-sensors is low enough for the early diagnosis of a range of diseases[1,2]. The deployment of such sensors at the point of care would greatly improve patients' quality of life by providing rapid feedback and enabling swift, potentially life-saving procedures and treatments. However, the higher sensitivity of micro/nano-sensors has revealed a range of issues that need to be addressed before they can be deployed in clinical settings. For example, these sensors are now capable of detecting biological processes interfering with the measurement and that were not detected with older generation, lower sensitivity sensors[3]. Moreover, the decrease in the sensors capture area (linked to an increase in sensitivity for a range of sensors) puts a burden on the limit of detection of such sensors through a reduction of the number of captured analyte molecules. Here I will discuss how the measurements are influenced by the heterogeneity and stochastic nature of biological processes in the sensing environment[4]. In particular, I will examine the situation when analyte molecules are bound sparsely at random locations on the sensor interface. Such a configuration, that is common in many real-life situations, make the interpretation of the measurement particularly challenging for a range of micro/nano-sensors that have a location-dependent sensitivity (Fig. 1) such as mechanical sensors[3,4], localised plasmon sensors[5] and some electrochemical sensors[6,7] amongst others. I will also present a few strategies to overcome these issues and in particular, I will discuss how the quantification of the measurement variations due to stochastic processes can be used to define new statistical methods enabling high resolutions sensing without compromising the sensitivity of mechanical sensors[4]. The strategy presented can also be adapted to other sensor with location dependent sensitivity. I will also describe other strategies, including microfluidics-based approaches[8] to improve measurement resolution.

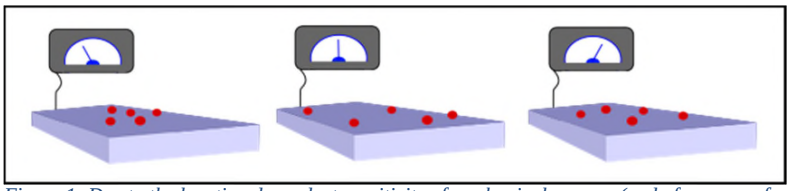

Figure 1: Due to the location dependent sensitivity of mechanical sensors (and of a range of other micro/nano-sensors), the same number of analyte molecules, binding at different location on the sensor, will result in different measurements.

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Small molecule biosensor development and alternative sensing material

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Surface plasmon resonance (SPR) biosensors have been extensively applied to immunoassays of large molecules. Small molecules such as progesterone required surface modification as well as high mass labels for binding signal enhancement in order to improve the detection sensitivity. This project focuses on progesterone sensor development using newly synthesised extended progesterone ligand P4-PEG-OVA (Figure 1) to enhance the sensitivity of both SPR and lateral flow immunoassay (LFIA) methods. The sensors have been developed and modified for bovine serum and milk analyses and validated by independent analysis. The results indicated the P4-PEG-OVA ligand allowed sensitive P4 detection in SPR sensing (0.29-1.94 ng/mL) and rapid, cost- effective strip sensor (LFIA) allowed bovine P4 cycle profiling.[1]
A biocompatible, biodegradable polyester polyhydroxyalkanoate (PHA) granule (Figure 2) has been investigated for the possibility of developing a new surface biosensor. The bifunctional PHAs 3GNZZPhaC are known to have high binding affinity to specific antibody and gold nanoparticles.[2] The preliminary studies of 3GNZZPhaC and ZZPhaC granules indicated the bifunctional PHAs have the potential applications for SPR and LFIA based sensor development.

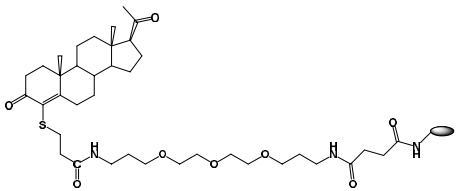

Figure 1. P4-PEG-OVA

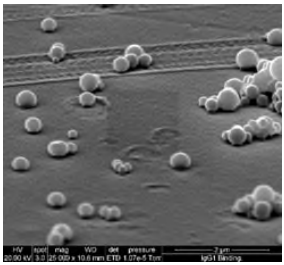

Figure 2. PHA granules on SPR sensor surface.

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SOLID PHASE MICROEXTRACTION (SPME): *IN VIVO* DRUG DETECTION IN TISSUES AND ORGANS

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Introduction: Drug development requires preclinical testing whereby compounds are dosed in animal models to assess the tissue distribution of developmental compounds. Drug distribution analysis commonly requires animal sacrifice, organ homogenisation, and subsequent analysis; a sampling protocol that is inherently costly in terms of animal life. Solid Phase MicroExtraction (SPME) is an equilibrium technique for sampling drug molecules in complex matrices, which has been previously applied to quantitation of drug in blood. Since SPME is minimally-invasive, the technique allows for sampling *in vivo,* avoiding animal sacrifice. The aim of this project is to examine the potential of SPME for detection and quantification of drugs and metabolites in whole organ tissues.

Methods and Results: LC-UV and LC-MS methods were developed and subject to validation. The final method is as follows: 95% acidifed water/5% MeCN to 70% water over 3 min was employed (C18 column 2.1 mm x 1.8 mm, 1.7 μm, at 35˚C, flow rate 0.5 mL min^{-1}, 1 μL injection volume, monitoring at 223 nm or *m/z* transition *268 >> 116*). These were used in order to investigate extraction of a model drug (metoprolol) from a variety of matrices by SPME in comparison to existing extraction methods (Liquid-Liquid Extraction, Solid Phase Extraction). The influence of agitation methods on drug adsorption to and desorption from SPME fibres was examined. Subsequently, drug extraction from a variety of spiked aqueous and lipid matrices simulating organ tissues was performed to select the most suitable fibre for 'in-tissue' studies. For example, the extent of drug extraction from a near-saturated metoprolol phosphate buffered saline (PBS) solution as simulant of interstitial fluid was studied using fibres coated with C18, PolyDimethylSiloxane/DiVinylBenzene (PDMS/DVB), PolyDimethylSiloxane/Carboxen (CAR/PDMS), PolyDiMethylSiloxane/DiVinyl-Benzene/Carboxen (CAR/PDMS/DVB). Total drug extraction was broadly similar for all fibres, but inter-fibre variability was lowest for C18 SPME fibres; C18 extracted (4.3 μg ± 0.05 μg), PDMS/DVB (4.4 μg ± 0.33), CAR/PDMS/DVB (5.03 μg ± 0.25), and CAR/PDMS (5.2 μg ± 0.9). On the basis of the lipid and aqueous screens, the optimum fibres (C18), were then applied to the extraction of metoprolol from skin and liver tissue homogenates.

Conclusions and Future Work: Initial work suggested C18 as the optimal fibre type for metoprolol extraction from both model matrices (PBS, corn oil). This may be reduced as the technique is further developed by use of appropriate SPME-based calibration. Ongoing work will investigate extraction of metoprolol and additional model compounds from homogenised tissue matrices. Future work will employ unhomogenised tissue and later, sampling within organs *in vivo*; by use of perfused systems and live animals.

Acknowledgements and Funding: The authors would like to thank GSK for experimental assistance, and the NC3Rs for funding (Grant NC/L001683/1).

Tracking bacterial molecular footprints using surface mass spectrometry approaches

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The attachment of bacteria to medical devices and subsequent biofilm formation pose an enormous global healthcare challenge, especially in this era of increasing antimicrobial resistance. It is estimated that 80% of hospital-acquired infections are related to biofilms. In an effort to develop new materials that can resist bacterial attachment, novel polyacrylates have been discovered through a high throughput combinatorial discovery strategy employing polymer microarrays[1].

The reason(s) why bacteria do not form adherent biofilms on the 'hit' polymer surfaces is not well known. In this work, we aim to elucidate the bacterial surface sensing processes involved by exploiting surface analysis techniques to trace the footprints[2] (*i.e.* cell surface secreted molecules) deposited by the bacteria upon interacting with these chemically distinct surfaces. We are employing a suite of mass spectrometry (MS) approaches including liquid extraction surface analysis (LESA), matrix assisted laser desorption/ionisation (MALDI) and secondary ion MS (SIMS) for surface analysis.

It is acknowledged that each MS technique has inherent strengths and limitations. For instance, SIMS offers high spatial resolution MS imaging, but the samples have to be analysed under ultrahigh vacuum conditions and are normally extensively fragmented[3]. LESA on the other hand is a relatively new ambient ionisation technique, but limited in terms of spatial resolution[4]. MALDI can be used to analyse small and large biomolecules, but requires careful sample preparation[3]. Thus, by compiling the data obtained through this multi-interface approach, we are seeking to provide a molecular explanation for the anti-attachment properties of the polymers discovered.

In the first instance, we are targeting components of the extracellular polymeric matrix of the Gram-negative nosocomial pathogen - *Pseudomonas aeruginosa* that includes exopolysaccharides, proteins, nucleic acids, lipids, secondary metabolites, and quorum sensing signalling molecules[5] in our surface analysis to understand the detection limit for the target biomolecules under various MS techniques.

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Miniaturisation of an Electrochemical Peptide-Based Biosensor for the Detection of Trypsin

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The development of electrochemical peptide-based biosensors for the detection of proteases is attracting interest as proteolytic enzymes are known to be responsible for many physiological conditions[1]. We have previously established a macroelectrode sensor system for the detection of the protease trypsin[2]. Miniaturisation of such biosensing platforms is crucial to applications where implantable sensors are desirable. Furthermore, using microelectrodes could offer various advantages over macroelectrodes (e.g. decreased size, shorter response times, improved mass transport, higher signal-to-noise ratio giving higher sensitivity)[3]. In this context, we report the characterisation of an electrochemical biosensor for the detection of the protease trypsin employing self-assembled monolayer (SAM)-modified platinum microelectrodes of 25 µm diameter. The sensing probe is designed with methylene blue as the redox tag, a thiol-containing moiety as an anchor and a trypsin-specific peptide sequence (phenylalanine - arginine - arginine). Square wave voltammetry measurements have been carried out to monitor resulting changes in the peak current due to the specific proteolytic cleavage of these SAM-based redox-tagged peptides by trypsin. The construction of the sensing system has been optimised and the performance of the prepared sensors evaluated in terms of response to trypsin and assessment of non-specific binding. These microelectrode results will be compared to the previously obtained data using macroelectrodes.

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Acknowledgements

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Evaluation of maltodextrin as a lyoprotectant for the production of Taraba honey powder by freeze drying

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These investigations aimed to assess the suitability of maltodextrin (MD) as a lyoprotectant for the production of Taraba honey powder by freeze drying (FD). Honey is a sticky, viscous biomaterial composed of 80–85% carbohydrate (mainly glucose and fructose), 15–17% water, 0.1–0.4% protein, 0.2% ash and minor quantities of amino acids, vitamins, phenolic antioxidants, *etc*.[1,2]. Honey is of interest in the pharmaceutical and biomedical sciences because of its putative antimicrobial, antiviral, anti-inflammatory and antiulcer properties[3]. Using four different concentrations of honey and two of MD (Table 1), freeze dried powders were assessed for physicochemical interactions, stability and powder flow properties. Honey-MD formulations (forms) were prepared by homogenising (IKA® Ultra-Turrax® T8, Janke & Kunkel GmbH & Co. KG, Germany) aqueous mixtures for 5mins, before freezing at –5°C for 6hrs, then FD (Beta 1-8 LSC Plus, Martin Christ, Germany) at –20°C for 72hrs. Freeze dried powders were characterised for: 1) Morphology by SEM (Zeiss Evo®50, Carl Zeiss AG, Germany); 2) physical & chemical interactions by FTIR (Alpha, Bruker, Germany); 3) powder flow characteristics by bulk & tapped density (Tap Density 50-1100, Varian, USA), with calculation of Compressibility (Carr's) Index (CI)[4].

Table 1: Honey-MD Forms

		MD (%w/v)	
		5	**50**
Honey (%w/v)	**5**	A	B
	10	C	D
	25	E	F
	50	G	H

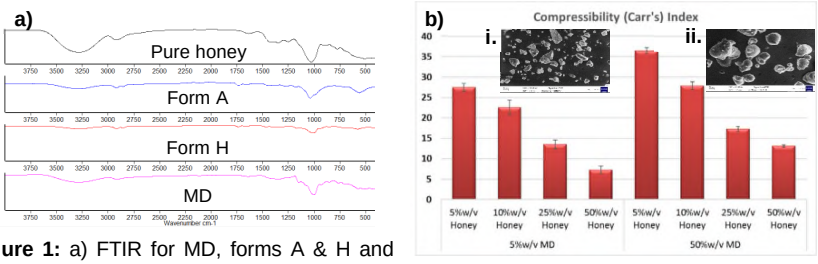

Figure 1: a) FTIR for MD, forms A & H and pure honey; b) CI and SEM (inset photomicrograph) of forms (i) A and (ii) H.

The freeze dried honey powders were white to yellow and fluffy to coarse in appearance with increasing honey concentration. FTIR spectra (Fig. 1a) for pure honey and MD showed peaks at 3312-3500cm^{-1} (-OH stretching). Regional peaks from 1500-750cm^{-1} is most likely major sugar and polysaccharide components in both pure and MD-honey powder forms. MD spectra also showed peaks at 2925cm^{-1} (-CH stretching) and 1646cm^{-1} (presence of C=O). Flow characteristics improved to good and excellent (CI≤15) with increasing honey concentration, probably due to the morphological shift towards more spherical particulates with less cohesive interactions (Fig. 1b). SEM indicated irregular (low, Fig. 1bi) and spherical (high, Fig. 1bii) particle morphology with increasing honey concentration, possibly due to crystallisation of honey's sugar component. In conclusion, MD appears to be an effective lyoprotectant for honey forms ≥25%w/v with good to excellent flow characteristics achieved.

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Method development for quantitative analysis of amino acid enantiomers from Pleistocene mammalian enamel

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Amino acid racemisation (AAR) data from a wide variety of calcium carbonate based biominerals has been a powerful tool for Pleistocene age estimation (0.01 – 2.5 million years). Accurately dating material of this age is fundamental to our understanding of past climate changes and their impact on the flora and fauna. However, as the technique is not applicable to bone, obtaining direct dates for mammalian remains older than ~50,000 years (the limit of radiocarbon dating) is extremely challenging. AAR geochronology in calcium carbonate biominerals targets protein entrapped within the crystal, providing a closed system for its decay, and tooth enamel (which is composed of a form of calcium phosphate) has the potential to provide an environment complementary to that found within the $CaCO_3$ subfossils.
However acquiring AAR data from enamel is not facile, due to the complex interactions between the inorganic phase and the separation technique used (liquid chromatography). A method for extracting amino acids from the inorganic ions via the formation of a gel has been developed, allowing for the robust and routine analysis by RP-HPLC of amino acid enantiomers in the enamel of mammalian remains spanning the Pleistocene. This enables us to test the viability of AAR geochronology on enamel and exploit the extraction method for a wider range of analyses.

A portable nucleic acid test for resource-poor settings

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Leptospirosis is a zoonotic disease caused by the bacterium *Leptospira* and is prevalent in low and middle income countries (LMICs). Some recent studies suggest that the burden of the disease has been underrepresented to date[1,2], as it presents with similar symptoms to other febrile illnesses and there is insufficient access to confirmatory diagnostic testing. A portable nucleic acid test would allow for early detection of the disease.
In order to achieve this aim, the functions of sample preparation, amplification, and detection should be combined into a small-scale system suitable for point-of-care use. Designing for local manufacture is a key consideration as it could overcome the cost of supply chains that span the globe, making the test affordable for LMICs while helping to grow the local economy.
Results to date demonstrate successful amplification with a polymerase construct designed for local manufacture, sonication as a promising lysis method, and first steps towards a colorimetric detection method. Future work will look to develop each of these parts further, with the end goal of integrating into a single device.

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Vibrational spectroscopy of blood plasma for the diagnosis of Alzheimer's disease and differentiation from dementia with Lewy bodies

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Background: Alzheimer's disease (AD) is currently diagnosed with a combination of tests such as measurement of amyloid-β (Aβ) in CSF, structural neuroimaging techniques or batteries of neuropsychological tests, which are either invasive or expensive and time-consuming. Although an early and accurate diagnosis of AD is crucial for appropriate palliative care, the sensitivity and specificity for its clinical diagnosis range from 70.9% to 87.3% and 44.3% to 70.8%, respectively[1]. Accurate differentiation of AD from dementia with Lewy bodies (DLB) is clinically important as their symptoms are similar but appropriate management can lead to improved outcomes. Vibrational spectroscopy, such as attenuated total reflection Fourier-transform infrared (ATR-FTIR), is an ideal technique for the investigation of biofluids and provides a 'spectral fingerprint' of all the molecules present within a biological sample, including lipids, proteins and carbohydrates. **Methods:** ATR-FTIR spectroscopy was used to analyse blood plasma samples (50μl per patient) from 164 individuals with AD, 34 with DLB and 202 age-matched healthy controls (HC). Pre-processing of the generated spectra and various multivariate analyses were then applied to extract the underlying biological information. Apolipoprotein E (*APOE*) genotype was also taken into account as ε4 allele is a major genetic risk for AD development. **Results:** Increased intensity at the Amide I region (indicative of protein secondary structure) of AD patients could be attributed to the formation of amyloid plaques and the deposition of amyloid-β peptide; the observed decrease in lipids could be caused by damaged cell membranes due to free radicals generated during oxidative stress[2]. AD was identified with ~70% sensitivity and specificity, which increased to 72.22% and 76.67% when individuals had no *APOE* ε4 alleles and 86% when individuals carried one or two alleles of *APOE* ε4. Segregation between AD and DLB was achieved with 90% sensitivity and specificity. **Conclusions:** Blood-based spectroscopy has been proven suitable for the differential diagnosis of AD and DLB with sensitivities and specificities equal (or even higher) than the ones obtained by current clinical/molecular methods. Its rapid, non-morbid, cost-effective and non-destructive nature, could easily make it a perfect candidate for clinical translation in the future.

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Upconversion Nanoparticle-Anthraquinone Sensors for Measuring pH

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The development of optical pH sensors for measuring intracellular pH is highly useful to studies of cellular biology[1] as well as applications in diagnostics[2] and therapeutics.[3] However, currently available pH-sensitive nanoprobes suffer from photobleaching and autofluorescence background in biological samples.[4] We designed a pH nanosensor that avoids these problems by taking advantage of near-infrared excitation with the use of upconversion nanoparticles (UCNPs) that emit visible light when excited with a 980 nm laser. This nanosensor is based on fluorescence resonance energy transfer between UCNPs and pH-dependent anthraquinone dyes. Due to the advantages of upconversion photoluminescence, the nanosensor has the potential to be used for detection of pH in *in vitro* and *in vivo* applications.

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Indicating/Quantifying Oxidative Stress Response to Exercise Stimuli

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Oxidative stress is imbalance between oxidant and antioxidant levels in living systems.[1] Human cells are protected from reactive oxygen species by endogenous enzymatic antioxidants.[2] Most of these compounds require particular redox metals in their structures as cofactors to allow them to scavenge the free radicals such as Cu, Zn-SOD, Mn-SOD and catalase (Fe). This study aimed to quantify these elements as oxidative stress biomarkers in vitro in skeletal muscle cells (C2C12) which were incubated under hypoxia/hyperoxia conditions generated by varying oxygen level from 1%-60% for 24 and 48 hours. ICP-MS was applied to quantify Zn, Cu, Fe and Mn in cell populations. Their concentration increased dramatically in cells grown at 25%-60% O_2, the most significant increase being 85% in Cu at 60%O_2. None showed any increase at 5%-15% O_2 indicating normoxia states. At 1%O_2, all elements except Fe showed a significant increase and the most remarkable growth was in Mn by 33%. Interestingly, increasing incubation to 48 hours had differing effects on the elements. Zn and Cu concentrations were unaffected by increasing incubation time except at 60%O_2 where they showed further growth. In contrast, Mn concentration grew sharply over oxygen levels of 30%-50% with no further effect at 1%, while Fe concentration decreased at 1%O_2 and grew steadily over oxygen levels of 5%-60%. It can be concluded that all four elements were significantly affected by stress conditions applied to cells, but at different rates. Further work comparing these studies with single cell analysis using laser ablation-ICP-MS will also be reported.

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Manuka honey or not Manuka? Using metabolomics to prevent food fraud

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Over recent years, sales of Manuka honey as a possible natural health remedy have soared due its beneficial antibacterial properties. Pure Manuka honey, however, is expensive, which means that honey producers, and therefore consumers, are under threat from food fraud. Although a few biomarkers have been identified, a more complex combination of markers is required to prevent adulterated honeys being deemed authentic.
Using LC-MS data, 357 samples of New Zealand honeys from various floral types, including Manuka, were analysed. The Unique Manuka Factor (UMF®) Honey Association has previously identified 20 stable variables of interest due to their presence in the nectar of the Manuka plant. These variables were used in Principal Component Analysis (PCA) to investigate any patterns in the data and Partial Least Squares Discriminant Analysis (PLS-DA) and stepwise logistic regression (LR) were used to produce models for the classification of Manuka/Non-Manuka honeys. Models produced achieved between 89-96% accuracy in classification, some of which did not require the previously identified biomarkers.
This approach could be used as a test of authenticity of “Manuka” honey in the future, thereby reducing the number of fraudulent honeys on sale.

Morphology Changes During the Growth of Bicalutamide Microcrystals

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Agricultural systemic fungicide formulations typically contain an active ingredient (AI) in aqueous media with surfactant. The AI is an organic crystalline molecule with poor aqueous solubility and the surfactant aids its uptake into the plant from the leaves[1]. Fungicides are normally delivered by a spray-dry process[2] where the formulation exhibits a variety of liquid-crystalline phases as water (anti-solvent) evaporates from the surface of the leaf. Evaporation of water increases the organic content of a droplet on the surface of the leaf and thus the solubility of the AI. The crystalline AI can then dissolve, becoming available for uptake. Furthermore, if it rains, the spray deposit will pick up water, causing the AI to become insoluble and crystallise on the leaf surface. Hence, dissolution and crystallisation of organic crystals is important in determining the efficacy of AIs with a view to improving the delivery process.

We present a model system for understanding the growth processes of organic microcrystals that occur in these fungicide spray deposits. Bicalutamide[3], a prostate cancer treatment, is used as a model drug. The morphology changes (Fig. 1) and growth kinetics of bicalutamide crystals are investigated in situ by focusing on single microcrystals. A combination of optical microscopy and finite element method modelling[4,5] allows the acquisition of a detailed quantitative picture of the growth kinetics of individual crystal faces. Real time in situ growth data is used to parameterise a finite element method model from which concentration profiles and fluxes at individual crystal faces are directly obtained. From the model, the growth process can be categorised as mass transport or surface kinetics controlled, or a mixture of both. The modelling data contributes to an understanding of why the morphology changes occur.

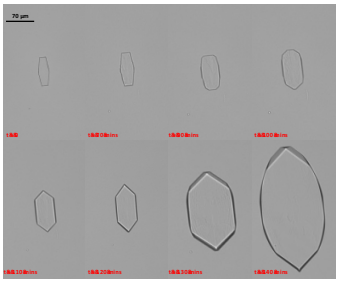

Fig. 1: *In situ* optical microscope images at 200x magnification of the growth of a single bicalutamide crystal in a saturated solution of bicalutamide in ethanol.

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Developing mass spectrometry based proteomic methods to identify and quantify protein carbonylation in plants

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Carbonylation modifications are seen as biomarkers of oxidative stress in animals and plants. Drought is one of the factors thought to cause these modifications. However, carbonylation is difficult to detect because of its low abundance, so the pathways affected by carbonylation modifications are largely unknown. There has been extensive research into how oxidative stress affects animals, but very little research has been done into plants. With global warming causing an increased lack of sufficient water for crops, the knowledge of how they are affected by drought is vital to continue to feed the earth's growing population. Aminoxy tandem mass tag (TMT) is a multi-fictional reagent which can be used for this. It is a derivative of the tandem mass spectrometry compatible TMT isobaric labeling reagent. AminoxyTMT contains a carbonyl reactive aminooxy group which allows reactive carbonyls to be tagged in proteins. Coupled with tandem mass spectrometry, it can therefore, be used to enrich the carbonyl content of a sample and give a better understanding of sights that are susceptible to oxidative damage.

Determinaton of trace impurites in formulated materials

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Trace organic impurities can have a large impact upon the stability and safety of pharmaceutical formulations as they can react with the active pharmaceutical ingredients (APIs) to form inactive or harmful compounds. Despite being present in sub-ppm quantities reactive impurities can be present at high stoichiometry to the API, particularly if they are distributed heterogeneously within the formulation. These reactive impurities can originate as a byproduct from the synthesis or as a result of degradation of excipients and APIs. Impurities can also be introduced through the packaging in which the formulation is kept. Formaldehyde and formic acid are typical examples of impurities introduced by excipient and packaging degradation (e.g. from PEG or PVPP).
In this project we are developing multimethod non-invasive *in-situ* approaches for investigating the distribution and dynamics of impurities in complex solid formulations. One of the methods, a surface-enhanced Raman spectroscopy (SERS) was successfully applied to detect HCHO in a complex matrix. SERS uses localized surface plasmons from noble metal nanoparticles to selectively enhance the Raman signal of a formaldehyde reporter molecule. Another approach uses electrochemical detection of formic acid and formaldehyde for use in electrochemical imaging. Our current work focuses upon developing and validating these two techniques for spatially and time resolved measurements in complex solid matrices.

Analysis of secondary structure of proteins by Fourier transform infrared spectroscopy and Self-organized maps

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Abstract

This project is aimed to examine the secondary structure content in proteins by vibrational spectroscopy with electronic spectroscopy. Although the main focus is on Raman spectroscopy, the first phase of the project has been to establish robust methods for infrared absorbance spectroscopy (IR) following our discovery that there was no accepted literature protocol for working with IR using aqueous solutions. Further, we have focused on using attenuated total reflectance spectroscopy as the sample handling is much easier than in small pathlength transmission measurements. The predictions were carried out by means of a self-organising map neural network approach, SSNN, which we originally developed for circular dirchoism spectroscopy (CD). We have compared the SSNN approach with band fitting and compared the results to those from CD.

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Development of an All-Diamond Conductivity Sensor with Simultaneous Temperature Monitoring for the Marine Environment

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Conductivity is a measure of total ion concentration, and these measurements are widely used in a variety of industrial and environmental applications, including determining water quality, quantifying total dissolved solids in solution, monitoring corrosion, and interface detection.[1] The popularity of conductivity measurements has arose from its many advantages, such as the speed and simplicity of the measurement, as well as the ability to be performed in situ, and the fact that no sample preparation is required or additional reagents need to be added.

Using all-diamond as the platform for conductivity sensing is advantageous for several reasons. Both boron doped diamond (BDD) and insulating diamond are extremely hard and possess excellent resistance to not only harsh chemical environments, but also high temperatures. Additionally, surface adsorption processes on diamond surfaces are significantly reduced compared to other materials. In this poster, we outline the development and fabrication route of this all-diamond conductivity sensor (Figure 1a),[2] as well as demonstrating that the sensor is capable of recording both two- and four-point probe conductivity measurements over six orders of magnitude (Figure 1b).[3]

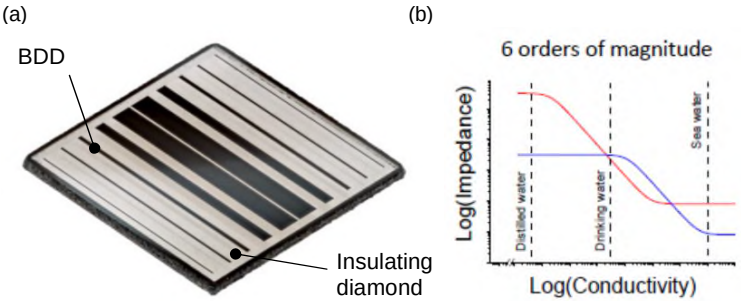

Figure 1. (a) Photograph of BDD conductivity sensors grown into an insulating diamond substrate. Size of the whole substrate is 11×11 mm. (b) Typical response of the conductivity sensor when operated at low (red line) and high (blue line) frequencies.[3]

For marine applications in-situ it is important to be able to measure accurately over the conductivity range 2–6 S m^{-1}. We show how we can tailor the geometry of the sensor specifically for this application. Furthermore, given the high thermal conductivity of diamond, temperature sensors can be incorporated into the diamond sensor, resulting in fast and accurate temperature-corrected conductivity measurements.

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