UNIVERSITY OF SURREY

CUTTING EDGE PHYSICS WORKSHOP

EVENT PROGRAMME

22-23 June 2022

Cutting-edge methods in physics for studying intracellular bacterial pathogen interactions with host cells and small molecules

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UNDERSTANDING THE Physics of Life

The ambitious goal of this network is to bring biologists and physicists together to develop a unified framework for understanding biology that integrates the molecular and the system levels of thinking into a coherent, unified whole. The network is funded by the Engineering and Physical Sciences Research Council, the Biotechnology and Biological Sciences Research Council, the Medical Research Council and the Rosetrees Trust. It is managed by Durham University, The University of York and the University of Leeds.
physicsoflife.org.uk

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Professor Melanie Bailey,
Department of Chemistry

Dr Dany Beste,
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Professor Mark Chambers,
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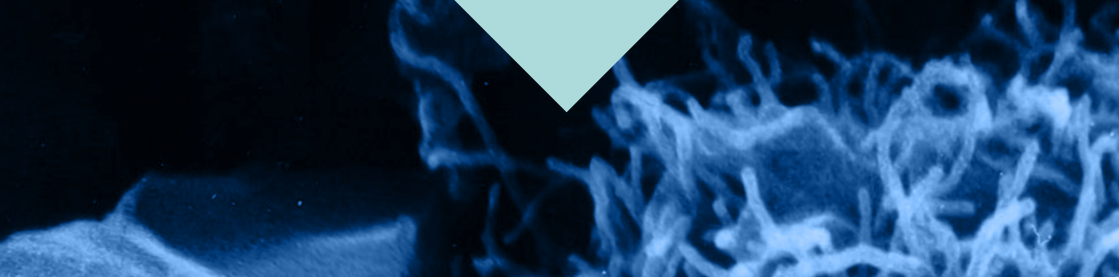
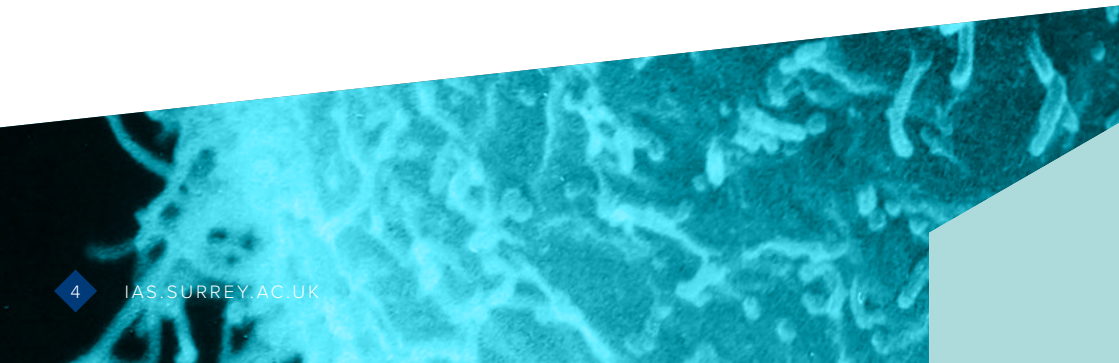
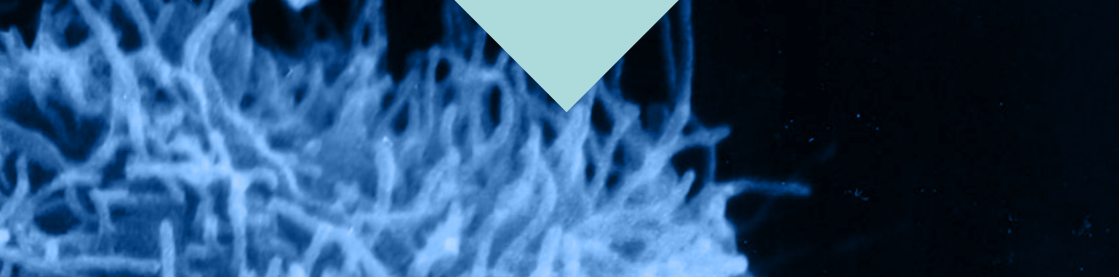


INTRODUCTION

Better understanding of host-intracellular pathogen interactions in living cells is vital to effectively develop diagnosis, preventive and treatment approaches against critical global infectious diseases caused by intracellular pathogens. Important global diseases caused by intracellular bacteria include brucellosis, listeriosis, chlamydia, salmonellosis and tuberculosis. According to the World Health Organisation (WHO), tuberculosis alone is responsible for around 10 million new cases and 1.4 million deaths worldwide each year. Drug resistance now occurs in around 0.5 million cases of tuberculosis each year and this reduces the rate of successful treatment from 85% to 57%. Tuberculosis imposes an immense burden of human suffering, specifically for poor and vulnerable people living in low- and middle-income countries. Thus, WHO has identified the importance of “Intensified Research and Innovation” as one of the three critical pillars for a global action framework for the end of the tuberculosis epidemic. For these reasons, tuberculosis will be the primary infectious disease focus of this workshop.

Intracellular replication of pathogens requires uptake of nutrients from the host cell, but little is known about the identify of host nutrients consumed by intracellular pathogens and their uptake mechanisms. Moreover, treatment of intracellular bacterial infections relies on the use of antibiotics, but the success of such treatment is dependent on effective concentrations of the antibiotic reaching the bacteria within host cells, often within favourable intracellular niches. However, little is known about the physical processes of antibiotics transport such as diffusion across cell membranes together with biologically regulated processes such as endocytosis. A key element of this workshop, therefore, will be to explore methodologies from physics and biology to study underpinning physical processes associated with the spatiotemporal dynamics of antibiotic transport. Additionally, fundamental challenges in physics on how to model antibiotic transport inside cells and across cellular membranes of both host and pathogen cells will be explored.



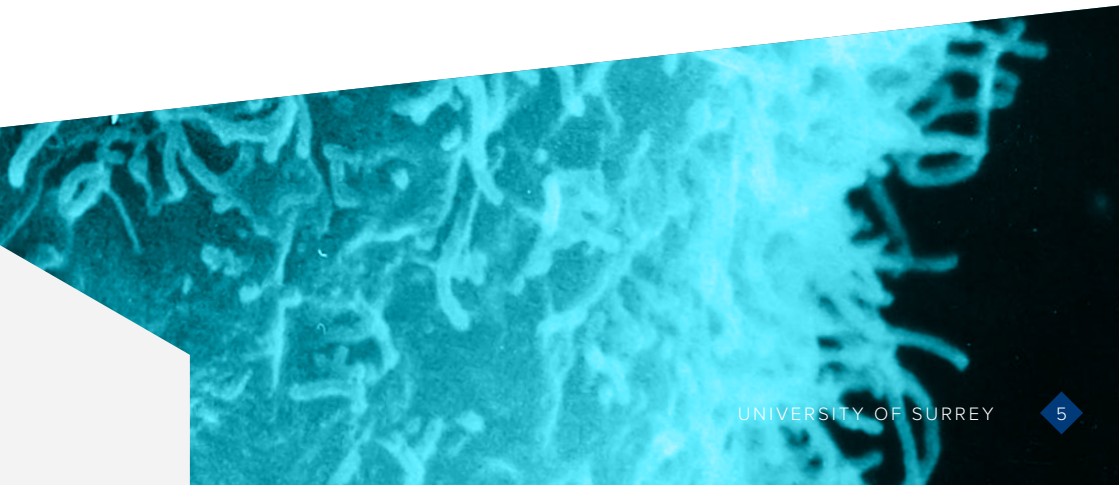


The following key questions will be addressed in the workshop.

- How to understand the transport of small molecules (i.e., antibiotics) inside a living cell?
- How to characterise heterogeneity in host-intracellular pathogen interactions?
- Define unmet challenges in understanding and monitoring host-intracellular pathogen interactions such as control of pathogen uptake, nutrient exchange between host and pathogen, intracellular trafficking, phagosome escape, cell death, antigen presentation and cell-to-cell spread of bacteria.
- What are the most appropriate physical methods to address the questions above and where do physical methods need further development for their study?

The workshop also features a showcase exhibition of new BioArt works by internationally renowned British artist Anna Dumitriu stemming from her artist residency at the University of Surrey and explores cutting edge scientific research being undertaken at the University, including quantum biology, carbon capture, vaccine research and tuberculosis. Dumitriu creates her artworks hands-on in the lab as well as the studio and uses the tools and techniques of science to create intricate artworks that reveal and explore strange histories and emerging futures. The residency is funded through an Institute of Advanced Studies Fellowship awarded to Professor Mark Chambers of the Faculty of Health and Medical Sciences.

Workshop Organising Committee



PROGRAMME

Day 1 Wednesday 22 June

LTB, Lecture Block

UK TIME	
09.00 – 09.20	Registration
09.20 – 09.30	Opening remark David Sampson, Pro-Vice-Chancellor, Research and Innovation, University of Surrey
09.30 – 11.00	**Session I – Studying Intracellular Bacterial Pathogens I** Chair: Graham Stewart (University of Surrey)
09.30 – 10.30	Keynote: Jost Enninga (Institute Pasteur, France) *Intracellular niche formation of entero-invasive bacterial pathogens*
10.30 – 11.00	Suzie Hingley-Wilson (University of Surrey) *Life and Death on the inside: Targeting mycobacterial survival mechanisms*
11.00 – 11.30	Break
11.30 – 13.00	**Session II – Optical Spectroscopy and Imaging I** Chair: Dany Beste (University of Surrey)
11.30 – 12.30	Hesper Rego (Yale University, USA) *Studying Mycobacterial Infections, One Cell at a Time*
12.30 – 13.00	Anna Dumitriu (University of Surrey) *The Role of Art in Exploring and Engaging Audiences in Cutting Edge Methods in Bioscience*
13.00 – 14.30	Lunch and Poster and BioArt Exhibition

14.30 – 17.40	**Session III – Mass Spectrometry** Chair: Melany Bailey (University of Surrey)
14.30 – 15.00	Melanie Bailey (University of Surrey) *Novel approaches for the spatially resolved analysis of tissues and single cells*
15.00 – 15.30	Yi Liu (Imperial College London) *Use of mass spectrometry approaches to decipher the role of transition metals in maintaining drug tolerance in Mycobacterium abscessus*
15.30 – 16.00	Andy West (GlaxoSmithKline Medicines Research Centre) *(GSK) - Single Live Cell Mass Spectrometry analysis in the context of drug discovery*
16.00 – 16.30	Break
16:30 – 16:45	Claire Davison (University of Surrey) How far can we expand the boundaries of laser ablation technology for biological sample analysis?
16:45 – 17:00	Kyle Saunders (University of Surrey) - Optimisation of Single Cell Lipidomics Methods Using Mass Spectrometry
17.00 – 17.40	Discussion
18.00	Networking Dinner at Lakeside Restaurant

PROGRAMME

DAY 2 Thursday 23 June

LTB, Lecture Block

UK TIME	
09:00 – 09:30	Registration
09:30 – 11:00	**Session IV – Studying Intracellular Bacterial Pathogens II** Chair: Suzie Hingley-Wilson (University of Surrey)
09:30 – 10:30	[Keynote] Digby Warner (University of Cape Town, South Africa) *Seeing is believing: combining imaging and molecular tools to elucidate cellular and genetic function in Mycobacterium tuberculosis*
10:30 – 11:00	Fernando Martinez Estrada (University of Surrey) *Foam cells in Tuberculosis*
11:00 – 11:30	Break
11:30 – 13:00	**Session V– Optical Spectroscopy and Imaging II** Chair: Youngchan Kim (University of Surrey)
11:30 – 12:00	Paul French (Imperial College London) *Multidimensional microscopy across the scales*
12:00 – 12:30	Sumeet Mahajan (University of Southampton) *Enhanced Raman Spectroscopy for Detection of Bacterial Strains and AMR*
12:30 – 13:00	Discussion

13:00 – 14:00	Lunch
14:00 – 15:30	**Session VI – Mathematical and Computational Analysis** Chair: Richard Sear (University of Surrey)
14:00 – 14:30	Richard Sear (University of Surrey, UK) *Molecular transport and drug delivery: Any antibiotic molecule not touching its target is wasted*
14:30 – 15:00	Katharina Nöh (Forschungszentrum Juelich, Germany) – *Computer Age 13C Metabolic Flux Analysis: Current Status and Future Directions*
15:00 – 15:30	Discussion
15:30 – 16:00	Discussion and Closing (led by Mark Chambers)

ABSTRACTS

Ordered according to the programme

22 JUNE 2022

Jost Enninga
Head of research structure
Institut Pasteur

Intracellular niche formation of entero-invasive bacterial pathogens

Enteroinvasive pathogens, such as Shigella and Salmonella, induce their uptake into non-phagocytic epithelial cells through the injection of effectors by the type-3-secretion system. The bacteria are ingested in tight bacterial-containing vacuoles (BCVs) that are surrounded by in situ formed infection-associated macropinosomes (IAMs). In contrast to previous reports, we have recently shown via novel 3D imaging techniques that macropinocytosis is not required for the entry of these bacterial pathogens, however the IAMs regulate their subsequent intracellular trafficking. In the case of Shigella, IAMs do not fuse with the BCV, and contact between these two compartments results in the destabilization of the BCV and membrane rupture. In the case of Salmonella two scenarios occur; IAMs either fuse with the BCV, which results in the generation of the Salmonella containing vacuole surrounded by Salmonella induced filaments (Sifs). Simple contact between IAMs and the BCV also promote vacuolar rupture in the case of Salmonella leading to cytoplasmic hyper-replication. Interestingly, BCV contacts with the surrounding compartments also dictates intravuolar bacterial growth or dormancy. We have performed ultra-structural studies, combined with dynamic imaging and proteomics of the involved compartments to identify the molecules that drive these complex interactions. This has shown a regulatoy network of Rab GTPases, the Exocyst complex, and SNAREs that is hijacked by injected bacterial effectors. I will describe how these factors determine the intracellular niche formation for both, Shigella and Salmonella.

Suzie Hingley-Wilson
Lecturer
University of Surrey

Life and Death on the inside: Targeting mycobacterial survival mechanisms

The causative agent of tuberculosis (TB), Mycobacterium tuberculosis (Mtb), is an intracellular pathogen infecting nearly 10 million people world-wide. Its cousin, Mycobacterium abscessus (MABC), is a major cause of death in immunocompromised individuals. However, despite the availability of multi-drug chemotherapy, the majority of deaths in both are due to drug-sensitive strains. This is often due to the presence of a phenotypically-resistant sub-population of bacteria termed antibiotic persisters in TB, which increase upon intracellular exposure, or antibiotic-tolerant biofilms within the lung airways for MABC. Determining antibiotic penetration and targeting persisters and biofilms can be challenging in highly heterogenous mycobacteria. We aim to use single cell techniques, such as microfluidics, nano-scale secondary ion mass spectrometry and atomic force microscopy, to evaluate antibiotic penetration and survival and to identify novel strategies to curtail these global health threats.

Hesper Rego
Assistant Professor
Yale University

Studying Mycobacterial Infections, One Cell at a Time

The ability of genetically identical cells to display different phenotypes is a significant obstacle for the treatment of many human diseases. This is especially true for tuberculosis (TB), a bacterial infection caused by Mycobacterium tuberculosis. Heterogeneity plays a key role in TB's continued presence as a global health threat, providing a huge reservoir of latent disease in the world and preventing cure of active disease. Genetic diversity of either host or pathogen does not, alone, explain all this heterogeneity. We hypothesize that non-genetic sources of heterogeneity in pathogen populations lead to variation in outcome. Testing this hypothesis with conventional methods is difficult as it requires studying pathogen cells as individuals, rather than populations, at timescales that can capture the dynamics of heterogeneity. In addition, developing therapeutics to target heterogeneity demands a deep understanding of the molecular mechanisms underlying this phenomenon. My lab uses a combination of fluorescent reporters, time-lapse microscopy, and bacterial genetics to understand the mechanisms and consequences of phenotypic heterogeneity in mycobacterial populations.

Anna Dumitriu
IAS Fellow/Artist in Residence
University of Surrey

[BioArt Exhibition] The Role of Art in Exploring and Engaging Audiences in Cutting Edge Methods in Bioscience

This presentation explores how the University of Surrey's first ever artist-in-residence Anna Dumitriu engages with cutting edge research methods in the health sciences to engage and inspire diverse audiences in new technologies that have the potential to affect all our lives.

Dumitriu works hands-on in the lab and the art studio to create sculptural or installation-based works that incorporate diverse materials such as altered historical objects, textiles, bacteria and DNA. Her high impact artworks draw threads across time, from the history of science and medicine to cutting edge fields such as synthetic biology and bacterial genomics and have been shown around the world in prestigious museums and galleries and featured across all forms of media and in numerous publications.

Since 2012 she has been developing artworks that explore tuberculosis, such as her 'Romantic Disease' and 'Susceptible' projects which have taken audiences on a journey from past superstitions about the disease to the application of cutting edge genomics techniques to combat issues of antibiotic resistance. In the past year she has been working with researchers at the University of Surrey to explore new research and techniques from looking for ancient bovine TB DNA in Iron Age bones, and the development of vaccines, to carbon capturing microbes, and quantum biology.

Her multi-layered artworks enable audiences to explore scientific ideas, as well as the ethical, cultural and societal impacts of new technologies through sensory and aesthetic approaches, inviting viewers to notice important things that have previously gone unnoticed and to think about them in different ways. She peels away layers of complexity and providing glimpses of 'weak signals' from the future, and always reflecting on the past.

Chelsea Davis
Post doctoral research associate
Aberystwyth University

[Poster] The uptake mechanisms of pathogen extracellular vesicles into host cells

Communication between the host and pathogen has historically been considered as the host recognising pathogen cell wall and secreted antigens. Recently, an alternative mechanism of communication involved in pathogenesis has emerged involving release of extracellular vesicles (EVs) from the cell surface of the pathogen. Currently, there is incomplete understanding of how EVs are formed, their molecular cargo and how they function in the host environment. Generally, EVs have been recognised as tools for communicating information between cells and organisms across many biological systems, whereby molecules and antigens are transported from pathogens to hosts to undertake functional properties involved in pathogenicity and pathogen survival

Research at Aberystwyth University (AU) has optimised isolation of EVs from numerous pathogens (Mycobacterium bovis BCG, Fasciola hepatica, Schistosoma mansoni, Anoplocephala perfoliata, Calicophoron daubneyi) and have characterised their morphology (transmission electron microscopy, dynamic light scattering, atomic force microscopy) and molecular content (mass spectrometry). Functional studies have identified that EVs can be internalised by cells (confocal microscopy and flow cytometry), sequester anthelmintic metabolites (mass spectrometry) and modulate the immune response (western blots and ELISAs).

Strong collaborations between physics and biological science departments at AU, have raised interest in investigating pathogen EV uptake mechanisms in host cells. We aim to use Mycobacterium bovis BCG as a model organism to discover how pathogen EVs and their cargo are transported across the host cell membrane and inside host cells using the facilities available in both departments. Additionally, we aim to investigate EV antibiotic transport and EV antigen delivery into host cells. Findings will provide evidence of the extent to which EVs are used for communication between hosts and pathogens and indicate their potential use as targets for diagnostics or novel vaccines.

Rachel Butler
Research Fellow
University of Surrey

[Poster] From replication to mutagenesis: the role of ADP-ribosylation in control of DNA metabolism in M. tuberculosis

The DarT/G toxin-antitoxin system encodes a pair of enzymes that mediate the addition of an ADP-ribose moiety onto thymidine in ssDNA in a reversible, sequence specific manner. Although originally characterised in Thermus aquaticus, the system is present in a number of important pathogens including all members of the mammal adapted M. tuberculosis complex, notably including human and bovine TB. Utilizing CRISPRi technology to silence DarG antitoxin expression, we have shown that DarT performs ADP-ribosylation of gDNA in cellulo in M. bovis BCG, leading to a rapid arrest of DNA replication and cell division, and that is ultimately toxic to the bacterium. In MTBC, darT and darG are transcriptionally linked to the dnaB gene, which encodes the replicative helicase that interacts with ssDNA at the chromosome origin (OriC) to initiate then drive DNA branch migration during replication. We demonstrate in vitro and in cellulo that MTBC DarT heavily ADP-ribosylates TTTW motifs in the AT-rich DnaB-loading region of OriC, suggesting that the DarTG system may work as a reversible regulator of replication. Furthermore, unregulated ADP-riboslyation by DarT induces the DNA damage SOS response, including the ImuA'ImuB/DnaE2 mutasome which has been implicated in DNA damage-induced mutagenesis and acquisition of resistance to antibiotics.

Immunopurification and NGS sequencing of ADP-riboslyated gDNA fragments has given further insight into the role of ADP-riboslyation in M. tuberculosis physiology, confirming ADP-ribosylation of OriC and demonstrating ADP-ribosylation at additional genomic loci, prominently including genes involved in the SOS response, DNA metabolism, and ribosomal proteins. This identifies the potential for ADP-ribosylation to act as a genome-wide epigenetic and cell signalling factor.

We aim to further understand the role of DarTG in bacterial physiology including DNA replication, the DNA damage response, persistence and drug-resistance in Mycobacterium tuberculosis.

Belinda Hall
Senior Postdoctoral Researcher
University of Surrey

[Poster] Imaging the interaction between Mycobacterium ulcerans and macrophages

Mycobacterium ulcerans is the causative agent of the chronic skin infection Buruli ulcer. In contrast to most mycobacteria, M. ulcerans is predominantly found in the extracellular milieu in patient lesions. This is attributed to the production of a polyketide lactone, mycolactone, which inhibits innate immune responses and is cytotoxic to macrophages. Nevertheless, early in infection, intracellular bacteria are readily detectable and genetic evidence suggests that macrophages may play a role in controlling disease progression. In particular, polymorphisms in components of the autophagy pathway are reported to influence the severity of Buruli ulcer. However, little is known about the interaction between M. ulcerans and macrophages. We are currently investigating the cellular response to M. ulcerans by the autophagy pathway in macrophages. Early findings indicate that autophagy markers are upregulated in infected THP-1 macrophage-like cells, but these rarely colocalise with the bacteria.

George Mayson
MSci Student
University of Surrey

[Poster] NLRP3 Inflammasome Activation in Mycobacterium tuberculosis Infection: Lipid Accumulation on Canonical and Alternative Pathways within THP-1 derived Macrophages

Foam cells are lipid-laden macrophages that demonstrate resistance to cell death within Mtb exposure. Active NLRP3 inflammasomes induce fatal pyroptosis within infected macrophages. We investigated the effect of Linoleic-Oleic Acid lipid droplet accumulation on canonical and alternative NLRP3 inflammasome activity within THP-1 derived macrophages. Across most treatments, foam cells demonstrated significantly lower caspase 1 and caspase 8 activity as well as LDH release compared to THP-1 macrophages. Selective Ac-YVAD-CHO inhibition of caspase 1 identified apoptotic cross-reactive caspases and specific caspase 1 activity. Immunofluorescence consistently demonstrated higher abundance of intracellular lipid droplets with lower expression of NLRP3 in response to stimuli. The data suggest that accumulation of Linoleic-Oleic Acid lipid droplets within THP-1 macrophages decreases NLRP3 inflammasome and apoptotic caspase activity.

Khushboo Borah Slater
Research Fellow
University of Surrey

[Poster] Dissecting Host-Pathogen Interactions Using Systems-Based Omic Approaches

Metabolism of pathogens in infectious diseases is important for their survival, virulence and pathogenesis. Mycobacterial pathogens successfully scavenge multiple host nutrient sources in the intracellular niche. It is therefore important to identify the intracellular nutrient sources and their metabolic fates in these pathogens. Metabolic phenotype of an organism is defined by metabolic fluxes. We quantified in vivo fluxes of the pathogens and probed host-bacterial metabolic cross talks in tuberculosis (TB) and leprosy using systems-based strategies and techniques of isotopic labelling, metabolic modelling and metabolic flux analysis (MFA). We show that the TB pathogen metabolizes a number of carbon and nitrogen sources in human macrophages and identified vulnerable nodes such as glutamine and serine biosynthesis as potential drug targets. Mycobacterium leprae, the leprosy causing pathogen, uses host cell glucose in infected schwann cells and the enzyme, phoenolpyruvate carboxylase is a potential drug target. Our research provides an understanding of the intracellular diets and metabolism of these important human pathogens and identified vulnerable metabolic nodes that can be used for developing innovative chemotherapies in TB and leprosy

Holly-May Lewis
Research Fellow
University of Surrey

Single cell lipidomics and drug measurement using nanocapillary sampling coupled to liquid chromatography mass spectrometry

This work describes the development of a method to simultaneously detect lipids and measure drug levels in single living mammalian cells, selected microscopically. To identify isobaric species and improve measurement precision of drug analytes, nano capillary sampling is coupled to liquid chromatography. The efficiency of transferring analytes from the sampling capillary and to the LC column was explored and optimised in this work. The method was applied to 30 living cells, revealing cell-to-cell heterogeneity in the uptake of different antibiotics. Using this system, we could detect 14-158 lipids per cell, including ceramides, phospho- and sphingolipids, as well as the neutral triglycerides, diglycerides, monoglycerides. Bedaquiline uptake was associated with specific glyceride species.

Jordan Pascoe
PhD Student
University of Surrey

Metabolites tune the antimicrobial susceptibility of Mycobacterium tuberculosis

There is an urgent need for new approaches to treat tuberculosis (TB), however antibiotic development remains exceptionally costly and viable candidates for TB treatment remain scarce. An alternative approach which is attractive to TB pharmaceutical developers, is the production of adjuvant therapies that potentiate the activity of existing antibiotics and can prevent or even reverse resistance; thereby extending the life of current anti-TB drugs within the clinic. It is now well-established that the metabolic state of bacteria influences their susceptibility to antibiotics, and metabolite directed modulation of metabolism therefore has the potential to improve antibiotic efficacy and achieve this goal. Here we screened the effects of metabolites on anti-TB drug efficacy and identified metabolites that potentiate bactericidal activity, as well as those which have the opposite effect and enhance antibiotic tolerance. This provides us with important insight into the conditions which protect mycobacteria from drug treatment and enhance drug tolerance, largely considered a major impediment to TB treatment and a factor in the development of antibiotic resistance. For those metabolites which enhanced antibiotic killing we used a medicinal chemistry approach to synthesise drug like compounds which induce a similar antibiotic potentiating affect. Our work demonstrates that this is a viable approach for adjuvant TB therapeutics, and a potentially effective strategy to prolong the life of our current anti-TB drugs.

Melanie J Bailey
Professor
University of Surrey

Novel approaches for the spatially resolved analysis of tissues and single cells

Under an EPSRC fellowship programme, our team are exploring the integration of elemental mapping, provided by an ion beam accelerator with “”omics””. We are developing new workflows for multimodal elemental and mass spectrometry imaging, and are applying these tools to various biological systems. Here we will show how these new tools may give new insight into the pathogenesis of tuberculosis. We hare also developing a nanocapillary sampling tool for the analysis of lipids and drugs in single, living cells. We will show how this approach has been used to explore the relationship between anti-TB drug uptake and lipid profiles in single cells.

Yi Liu
PhD student
Imperial College London

Use of Mass Spectrometry Approaches to Decipher Role of Transition Metals in Maintaining Drug Tolerance in Mycobacterium abscessus

Antimicrobial resistance (AMR) is one of the major challenges we are facing this century. Understanding the mechanism behind the rise of AMR is therefore crucial to tackle this global threat. This is the case for Mycobacterium abscessus (Mabs), a fast-growing non-tuberculous mycobacterium considered as a “clinical nightmare” due to difficulties in its treatment and its multiple resistance and tolerance mechanisms to antibiotics. Mabs is an environmental bacterium whose physiology is driven by environmental factors. Among those are transition metals such as copper, cobalt and nickel. How presence of these ions affects Mabs physiology and its drug susceptibility is unknown, and any research addressing this question will considerably increase our knowledge in AMR and help discovery of a potential drug target. During my PhD, I investigated the impact of transition metals on Mabs physiology and how it affects drug susceptibility, using microbiological and bioenergetic techniques in combination with targeted and untargeted metabolomics with liquid chromatography-mass spectrometry (LC-MS). Use of LC-MS flux analysis with stable isotope labelling identified how nickel (Ni^{2+}), a transition metal ion widely present in the water system, perturb Mabs central carbon metabolism and nitrogen metabolism. These results agree with RNA sequencing and bioenergetics studies using Seahorse analyser showing decreased activity of TCA cycle activity and changes in expression of the related enzymes. Mass spectrometry also enabled quantification of intracellular uptake of antibiotics by Mabs in presence of Ni^{2+} and efflux pump inhibitors, showing that increased uptake of antibiotics, such as clarithromycin upon Ni^{2+} treatment, could be linked to increased susceptibility of Mabs. In conclusion, this study demonstrates that transition metals in environment interfere with carbon and nitrogen metabolism, which in turn shapes its drug susceptibility. Targeting Mabs metabolic enzymes or the way Mabs senses and responds to trace elements could offer new solutions to tackle AMR.

Andy West
Scientific Director
GSK

Single Live Cell Mass Spectrometry analysis in the context of drug discovery

Understanding the distribution of a dosed drug within the body is a key part of the drug development process, both in terms of drug efficacy (does the drug reach the target area and at sufficient concentration to have a pharmacological effect?) and potential side effects (where else does the drug go and what does it do there?). As many current drug targets are intracellular the ability to visualise unlabelled compounds inside the cell at physiological dosages can offer valuable insight into the compound behaviour both on and off-target. Understanding the effect a compound has on the underlying biology and the reasons why cellular responses vary is vital to successful drug discovery.

This talk will discuss our work using mass spectrometry to image the distribution of dosed molecules in cells and their effect on endogenous molecules. Examples will include the use of use Secondary Ion Mass Spectrometry (SIMS) to image the 3-dimensional spatial distribution of unlabelled drugs and identify endogenous metabolites at cellular resolutions. Our use of the LiveCell MS technology, originally developed by Professor Masujima, using a semi-automated methodology that allows the collection of intracellular content with a modified CQ1 imaging system developed by Yokowaga will also be discussed. In this work, we show the applicability of the LiveCell MS technology to drug discovery and its ability to identify drug related material and endogenous metabolites when incubated in a mammalian cell at a therapeutic dose. We will also discuss the dynamic range of single cell analysis by direct comparison with data from a metabolomics assay using bulk samples.

Claire Davison
PhD Student
University of Surrey

How far can we expand the boundaries of laser ablation technology for biological sample analysis?

The presence of metals within biological systems has long been associated with physiological and metabolic processes of the body, including synthesis of complex biomolecules, energy production and intracellular signaling. The determination of elemental distributions in cells is therefore critical in advancing our understanding of disease pathogenesis. Of particular interest is the study of cellular responses to external stimuli, specifically for the development of novel drug treatments which minimize or eliminate undesirable side effects. For this purpose, it is necessary to assess how the metabolic state and variations in elemental distributions of a cellular population impact the effectiveness of drugs and susceptibility to infection. In order to carry out this type of research, sensitive instrumentation and accurate analysis methods are required, however current techniques often exhibit severe matrix effects, poor spatial resolution and high cost. Recent advances have focused on the use of Laser Ablation – Inductively Coupled Plasma – Mass Spectrometry (LA-ICP-MS) and Laser Induced Breakdown Spectroscopy (LIBS), with both techniques adapting the physical process of laser ablation in which a short pulsed laser beam is focused on the sample. The emission of light of a specific wavelength by the elements upon excitation by the laser and the removal of fine ablated particles from the surface of the sample are used for qualitative and quantitative analysis.

This project, funded by the Doctoral College at the University of Surrey and the Natural Environment Research Council (NERC/T009187/1), aims to open the door to new possibilities for cellular research by developing novel methods for biochemical elemental analysis. Investigation of the optimum sample preparation and substrate for the analysis of cells using LIBS has been explored, with issues surrounding sensitivity for intrinsic elemental analysis having been identified. Future work will focus on tackling the analysis of exogenous metals associated with Tuberculosis drugs incorporated into THP-1 cells.

Kyle Saunders
PhD Student
University of Surrey

Optimisation of Single Cell Lipidomics Methods Using Mass Spectrometry

The metabolic content of single cells has been a rapidly progressing area of research in the last decade of metabolomics. Single cell heterogeneity has been demonstrated in monoclonal cell populations due to differing epigenetic environments. Ergo, analysis at the single cell level could yield observations which are masked by bulk population analysis in myriad studies of cell populations, including infectious disease, immunology and ageing. Single cell metabolomics and lipidomics methods using mass spectrometry are still in the early stages of their development. Therefore, exploring changes to current methodology could yield significant improvements in the depth of information obtained from a single cell. The aim of this project is to optimise both selective single cell sampling approaches and liquid chromatography mass spectrometry (LC-MS) methods in order to maximise transfer efficiency, sensitivity and the coverage of lipid classes. The transfer efficiency of lipid standards has been improved from approximately 21.5 % to 70.0 % through the introduction of new apparatus and autosampler settings. Increases in the range of 35 % have been achieved at the single cell level using more targeted chromatography and enhanced cleaning procedures. Future work will aim to improve these measures even further through the optimisation of mass spectrometry methods. Once an optimal workflow has been achieved, this methodology will be used to explore the metabolic profile of cancer cell lines in response to various treatments (i.e. chemotherapy) and the intensity of fluorescence markers. Selective cell sampling techniques retain spatial information in addition to the lipidomic profile of cells. This information will also be used to investigate spatial correlations in lipids in response to stimuli.

23 JUNE 2022

Digby F. Warner
Molecular Mycobacteriology Research Unit, Department of Pathology and Institute of Infectious Disease & Molecular Medicine, Faculty of Health Sciences,
University of Cape Town, South Africa

Seeing is believing: combining imaging and molecular tools to elucidate cellular and genetic function in Mycobacterium tuberculosis

Understanding Mycobacterium tuberculosis physiology and evolution is critical to the development of novel interventions (including new antibiotics) for tuberculosis (TB), a leading cause of mortality owing to a single infectious agent and a major contributor to antimicrobial resistant deaths. This presentation will highlight our recent work combining molecular tools and microscopy to investigate cellular and genetic function in mycobacteria. Examples will include the application of CRISPR interference and quantitative, image-based analyses in developing a morphological profiling ("phenoprinting") platform to infer hypothetical gene function and to suggest drug mechanism-of-action. I will also describe ongoing efforts towards the identification of so-called "anti-evolution" compounds that might limit the capacity for resistance acquisition by targeting the mycobacterial mutasome, a mutagenic DNA repair system that has been implicated in DNA damage-induced drug resistance and host adaptation. Finally, I will touch on the development of technologies to investigate M. tuberculosis aerobiology given the importance of airborne transmission as a still very poorly understood stage in the TB disease cycle. In summary, I hope to highlight the utility of image-based analyses in elucidating cellular and genetic function in mycobacteria, and to amplify the call for continued development of novel tools to understand the biology – and potential vulnerabilities – of a pathogen exquisitely adapted to its obligate human host.

Fernando Martinez Estrada
Lecturer in Immunology
University of Surrey

Foam cells in Tuberculosis

Mycobacterium tuberculosis (Mtb) infects macrophages and macrophage-derived foam cells, a hallmark of granulomata in tuberculous lesions. We analyzed the effects of lipid accumulation in human primary macrophages and quantified strong triglyceride and phospholipid remodeling which depended on the dietary fatty acid used for the assay. The enrichment of >70% in triglyceride and phospholipids can alter cell membrane properties, signaling and phagocytosis in macrophages. In conventional macrophage cultures, cells are heterogeneous, small or large macrophages. In foam cells, a third population of 30% of cells with increased granularity can be detected. We found that foam cell formation is heterogenous and that lipid accumulation and foam cell formation reduces the phagocytosis of Mtb. Under the conditions tested, cell death was highly prevalent in macrophages, whereas foam cells were largely protected from this effect. Foam cells also supported slower Mtb replication, yet this had no discernible impact on the intracellular efficacy of four different antitubercular drugs. Foam cell formation had a significant impact in the inflammatory potential of the cells. TNF-α, IL-1β, and prototypical chemokines were increased. The ratio of inflammatory IL-1β, TNF-α, and IL-6 vs. anti-inflammatory IL-10 was significantly higher in response to Mtb vs. LPS, and was increased in foam cells compared to macrophages, suggestive of increased pro-inflammatory properties. Cytokine production correlated with NF-κB activation in our models. We conclude that foam cell formation reduces the host cell avidity for, and phagocytosis of, Mtb while protecting the cells from death. This protective effect is associated with enhanced inflammatory potential of foam cells and restricted intracellular growth of Mtb.

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Multidimensional fluorescence imaging across the scales

We are developing open multidimensional fluorescence microscopy instrumentation, including endomicroscopy, high content analysis (HCA), super-resolved microscopy, and optical projection tomography (OPT). We have particularly focused on fluorescence lifetime imaging (FLIM) and Forster resonant energy transfer (FRET) to study molecular interactions and more recently on super-resolved microscopy using single molecule localisation microscopy (SMLM) to probe ultrastructure and molecular clustering. To provide a complementary label-free readout, we are developing semi-quantitative (single-shot) phase contrast imaging for cell segmentation, tracking and morphology quantification.

For our current and future fluorescence microscopy, we are developing a modular open-source microscopy platform based on openFrame, a low-cost, modular, open microscopy hardware platform to be used with open-source software tools, including MicroManager and FIJI, for instrument control, data acquisition, analysis and management, in order to make them practical in lower resource settings. We are particularly focussing on implementing our techniques in an open-source HCA platform for more robust cell biology studies.

For FLIM/FRET HCA, we have developed an automated multiwell plate FLIM platform utilising open-source software for data acquisition and analysis, which we have applied to assay protein interactions, including applications to viral disease processes. For super-resolved HCA, we are developing automated multiwell plate easySTORM, providing low-cost, large FOV SMLM together with accelerated open-source SMLM analysis parallelised on a high-performance computing cluster. We have applied easySTORM in studies of defective phagocytosis, cancer and kidney disease.

For clinical applications we are developing histoSTORM – an implementation of easySTORM with frozen or FFPE tissue sections and clinically-approved antibody labelling. Other open microscopy developments include a low-cost modular OPT platform that can image mm-cm scale samples, including live zebrafish, and can provide single-shot volumetric imaging.

Sumeet Mahajan
Professor of Molecular Biophotonics & Imaging, Head of Chemical Biology, Associate Director Institute for Life Sciences
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Enhanced Raman Spectroscopy for Detection of Bacterial Strains and AMR

In this talk I will describe recent work by myself, Niall Hanrahan, Callum J. Highmore and Jeremy S. Webb on multi-excitation Raman spectroscopy (MX-Raman) and multi-excitation SERS (MX-SERS), and the application of these two novel spectroscopy methods in robust and repeatable strain-level bacterial detection. We use the strong wavelength dependence of the scattering cross-sections to improve the information content of Raman spectra and thus increase specificity. The enhancement of specificity helps detect minute strain-level differences within bacterial species without additional reagents or sample preparation. Additionally the signals can be enhanced using nano-structured gold to enable highly sensitive detection at the single bacterium level, if needed. We have already shown species- and strain-level classification with accuracies up to 99.75% [1,2], enabling differentiation between antimicrobial resistant (AMR) and sensitive strains of the same pathogenic bacteria species within minutes. We have shown such high accuracies of detection for Cystic Fibrosis and WHO priority pathogens, and have performed our analysis directly in challenging and complex media such as sputum. Our findings present new capabilities in culture-free non-invasive bacterial detection. This could be hugely significant for real-time monitoring of infections and evolution of strains as well as for the rapid detection of AMR so that correct decisions regarding prescription of antibiotics can be taken.

Richard Sear
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Molecular transport and drug delivery:Any antibiotic molecule not touching its target is wasted

The talk will be an overview of the basic mechanisms of transport of molecules from A to B, in living organisms such as ourselves. This transport is crucial in any drug treatment, the drug is transported round our body via the blood, and then needs to move into the target cell by some combination of diffusion in aqueous solution, diffusion across membranes, through channels, etc. This is partially dictated by basic physics, it is generically true that transport of molecules over distances of a metre (size of a human body) is via flow, while over a micrometre (size of a bacterium) it is via diffusion. Transport can dictate drug efficacy, eg if drug cannot get inside pathogen, it is ineffective, while if a pathogen evolves to rapidly pump an antibiotic out of its cells, the drug concentration will be low there. This is problematic, even if a drug such as antibiotic is uniformly distributed inside the patient, this is horrifyingly inefficient - even then perhaps only one in a trillion antibiotic molecules is attacking the target bacteria. Drug delivery technologies are attempts to get around this problem, by concentrating the drug where it is needed, eg by preferential uptake by target cells.

Katharina Nöh
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Computer Age 13C Metabolic Flux Analysis: Current Status and Future Directions

Measuring metabolic fluxes in living cells by 13C metabolic flux analysis has become a key technology for improving our quantitative understanding of cellular metabolism. After two and a half decades of development, driven by diverse analytical and computational innovations, a rich set of tools has become available supporting all steps of the 13C MFA workflow, ranging from the assembly of high-fidelity models and their efficient simulation to convenient design of informative tracer compositions. For studying complex biological systems in less defined environments, such as pathogens residing in a host, however, many challenges remain. One critical step is the identification of a “useful” model formulation with which the fluxes are to be inferred from the data at hand. In the talk, I will present new directions in 13C MFA, powered by Bayesian statistics, to go about this hitherto neglected question and show first successful applications for Escherichia coli and Mycobacterium tuberculosis. Following this path keeps promise to strengthen the position of 13C MFA as an epistemic tool for explaining phenotypes and building models of metabolism.

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