MONASH University

MONASH BIOMEDICINE DISCOVERY INSTITUTE

Department of Microbiology

2024 Honours Programs in Microbiology

2024 Honours Coordinator

Professor John Boyce

Room: 215, 19 Innovation Walk
Phone: +61 3 9902 9179
Email: john.boyce@monash.edu

Deputy Coordinator

Professor Chris Greening

Room: 136, 19 Innovation Walk
Phone: +61 3 9905 1692
Email: Chris.Greening@monash.edu

GROUP OF EIGHT AUSTRALIA

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2024 Honours Programs in Microbiology

The Honours programs for both Bachelor of Biomedical Science (BBiomedSci) and Bachelor of Science (BSc) contain coursework and an independent research project. The objectives of these courses are to develop the laboratory skills required for research in microbiology and the ability to critically evaluate microbiological research. Students also achieve a detailed understanding of specialised topics in microbiology and enhance their communication skills in written and oral presentations.

The Department looks forward to welcoming you in 2024. We feel that our friendly, constructive and highly productive working environment provides an excellent opportunity for honours students to develop an understanding of the research process and to achieve their full research potential.

Formal Application Process

Application for Microbiology Honours entry involves a two part application process.

1. Formal application to the relevant faculty by

   B. Sc (Hons): November 17, 2023
   www.monash.edu/discovery-institute/honours/so-how-do-i-apply

   B. Biomed. Sci (Hons): November 17, 2023
   www.monash.edu/discovery-institute/honours/so-how-do-i-apply

2. Submission of project preferences to Professor John Boyce (no later than November 17, 2023).

Research Projects

The research project is the major component of both programs. All efforts are made to accommodate students in the laboratory of their choice, and to develop research projects that take into account the student's, as well as the supervisor's, interests. Brief outlines of the available projects for 2024 are in the following section.

Supervisor Interviews

Applicants are encouraged to discuss research projects with potential supervisors at any suitable time, by appointment. Following these discussions, students should apply online at the relevant faculty site and give Professor John Boyce a copy of their application forms: www.monash.edu/discovery-institute/honours/so-how-do-i-apply indicating their project preferences, and any additional documentation required. You do not need to wait until November 17th to hand in your preference forms, the earlier the better.

Projects Outside the Department

It is possible for students to complete their coursework within the Department of Microbiology at Clayton, and their research project off-campus. Under these circumstances, students must travel between locations when required. The thesis examination takes place at the same time for all students enrolled through Microbiology.

BBiomedSci and BSc Coursework

The honours year for both BSc and BBiomedSci students in the department of microbiology consists of short courses called colloquia, a statistics course and a seminar series. Coursework is usually completed, and students receive some feedback on their progress, by mid-year. The format of the colloquia will vary. Most involve reading recent research papers, an oral or poster presentation, and/or a written assignment.

Literature survey

During first semester the students must submit a literature survey on their research project. The literature survey which can be used as the basis for the introduction in the final report, allows the identification early in the year of those students who have problems with English expression so this can be addressed by directed English writing instruction. It also, of course, compels the students to become thoroughly conversant with their area of research.

Additional requirements

The programs will commence on February 19, 2024 (mid year begins July 15) with a series of introductory lectures, before the students start work on their research projects. These lectures contain information on the course, departmental facilities and laboratory safety. In the second half of the year students may be given specific training in the presentation of written reports, and in oral presentation of their work. It is compulsory for students to attend the introductory lecture course, all departmental seminars, and any short courses on written and oral presentations.

Assessment

Final assessment of the BSc Honours program follows the format:

Literature survey	7.5%
Research report/report review	60%
Seminar	7.5%
Coursework (including statistics)	25%

Final assessment of the BBiomedSci Honours program follows the format:

Literature survey	7.5%
Research report/report review	60%
Seminar	7.5%
Coursework (including statistics)	25%

Eligibility

Monash BSc Students

Entry to the course is restricted to those students who have qualified for the award of the pass degree of BSc (all subjects completed before enrolment), and have an average of at least 70% in 24 points of relevant level-three science units. This generally includes at least 18 points of Microbiology units. Students studying combined Science degrees must be eligible for the award of BSc.

BSc Graduates of Other Universities

As for Monash students, applicants are required to have a BSc and distinction grades in Microbiology or closely related subjects. **A certified copy of the applicant's academic record and a statement to the effect that they have qualified for a pass degree are required as soon as they are available.**

Monash BBiomedSci students

Students must have completed all requirements for the award of the pass degree of Bachelor of Biomedical Science offered at Monash University. They must also have an average of 70% or higher in at least 24 points at third year level, with 12 points from third year core units.

BBiomedSci graduates from other universities

Students applying for admission based on a qualification other than the pass degree of Bachelor of Biomedical Science offered at Monash University will need to demonstrate that they have achieved an appropriate standard in studies comparable to 24 points of BBiomedSci subjects as stipulated above.

Part-time study and mid-year entry

The department prefers students to study on a full-time basis. However, it may be possible under special circumstances to complete the Honours degree in two consecutive years by doing the coursework and research project in separate years. It is also possible to start the course mid-year. In both of these circumstances, the arrangements are made on an individual basis between applicants and supervisors.

Research Projects 2024

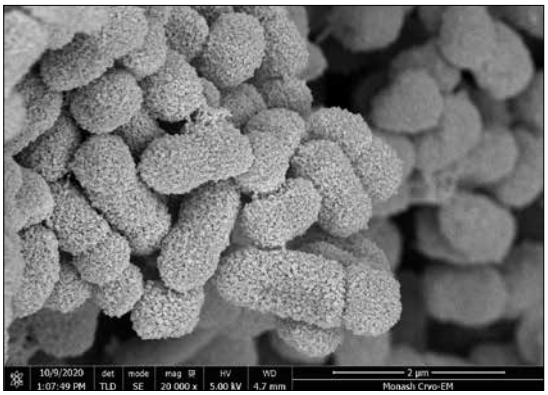
Professor John Boyce

EMAIL	john.boyce@monash.edu
TELEPHONE	+61 3 9902 9179
OFFICE	Room 215, 19 Innovation Walk (Building 76)
WEB	https://www.monash.edu/discovery-institute/boyce-lab

TWO VACANCIES

Professor John Boyce

Dr Marina Harper



Scanning electron microscope image of *Pasteurella multocida*

Understanding virulence gene regulation in *Acinetobacter baumannii*

John Boyce, Marina Harper, Amy Wright

Acinetobacter baumannii has been identified by the WHO as a priority 1 pathogen for which new intervention strategies are desperately required. *A. baumannii* is responsible for a large number of life-threatening infections, especially in hospital ICU settings, and the majority of strains are now resistant to multiple antibiotics.

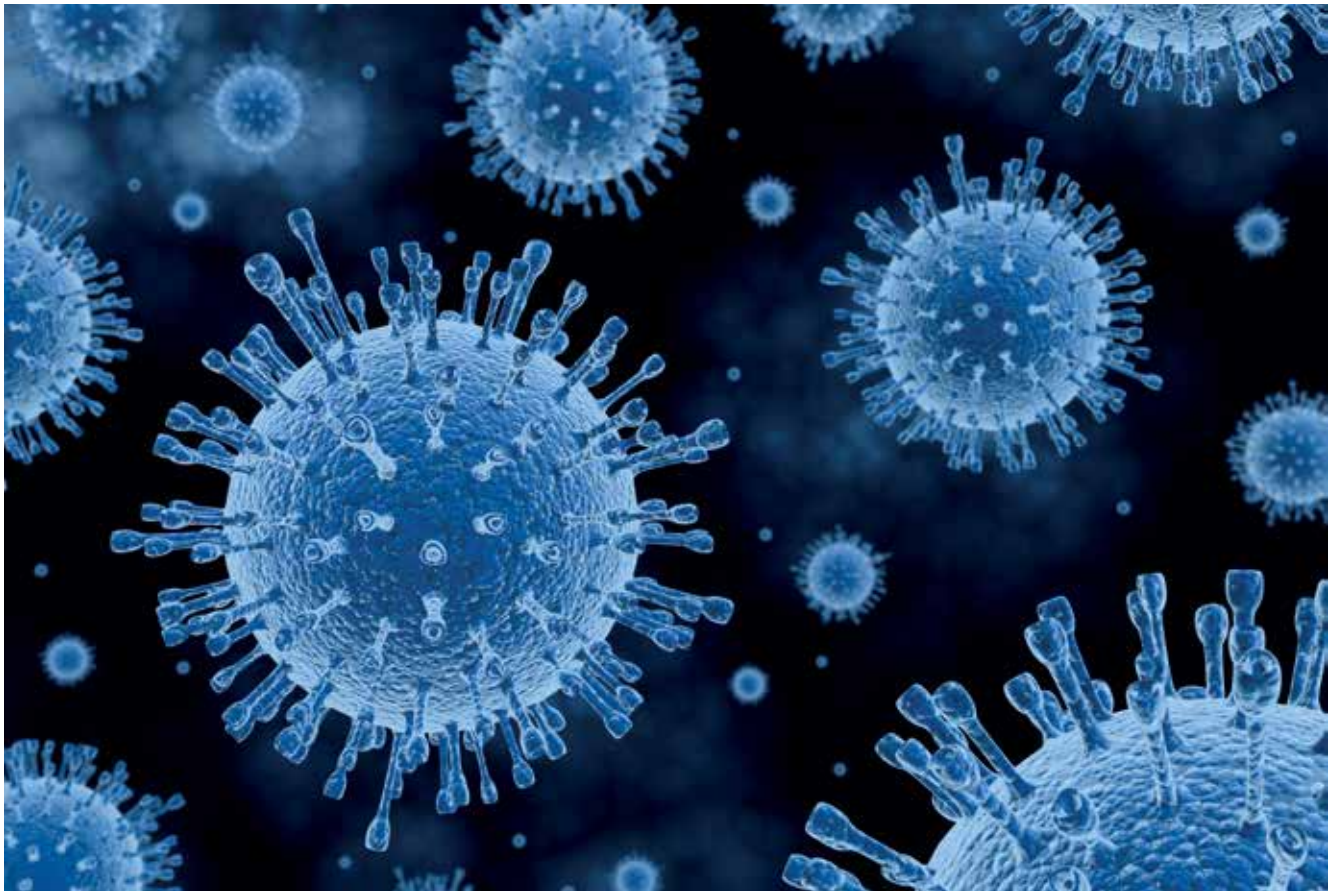
We aim to understand the molecular mechanisms of pathogenesis and virulence regulation in this organism. We have recently identified the protein Vfr as a global regulator of virulence. This protein is predicted to control virulence factor expression, carbon metabolism and the expression of other transcriptional and translational regulators, including small RNA molecules.

In this project you will use cutting-edge molecular biology, mutagenesis, high-throughput transcriptomics and proteomics methods, and a range of phenotypic assays including biofilm formation, antibiotic resistance and growth on different substrates, to define the regulon of Vfr and identify the specific virulence factors that it controls. These studies will lead to the identification of targets for future intervention strategies aimed at reducing the health burden of this problematic nosocomial pathogen.

Characterising new antibacterial toxic effector proteins delivered by the *Acinetobacter baumannii* type VI secretion system (T6SS)

Dr Marina Harper, Professor John Boyce

The *Acinetobacter baumannii* T6SS is a multiprotein nanomachine that is used as an antibacterial weapon via delivery of toxic effectors directly into competitor bacteria. We have shown that different *A. baumannii* strains deliver a diverse range of effectors that target essential cell structures such as peptidoglycan, DNA, RNA and bacterial membranes. We propose that many of these antibacterial effectors will have novel modes of action and that understanding their specific functions will allow us to re-purpose them as novel antibacterial molecules. In this project you will characterise novel toxic effectors using heterologous expression, protein purification, site-directed mutagenesis and utilise a range of phenotypic assays to identify the targets and modes of actions of these novel antibacterial molecules. You will assess how the proteins are delivered by the T6SS and test the activity of selected proteins against important Gram-positive, Gram-negative and fungal pathogens.



Defining the Mechanisms of *Pasteurella multocida* Pathogenesis and improving vaccines

Dr. Marina Harper, Dr. Thomas Smallman and Professor John Boyce

Pasteurella multocida is a Gram-negative bacterial pathogen that causes a range of diseases in humans, cattle, pigs and poultry. These diseases result in serious economic losses worldwide in multiple food production industries. We are interested in understanding the molecular mechanisms of pathogenesis in this bacterium with an aim to developing new, more effective and widely applicable vaccines or antimicrobial drugs.

Recent work in our lab, using comparative genomics and transposon insertion-site mutagenesis, has allowed us to comprehensively define the *P. multocida* genes essential for a range of virulence phenotypes, including growth in serum and production of the anti-phagocytic bacterial capsule. With this crucial data as a base, in this project we will use directed mutagenesis, complementation, whole-genome transcriptomic and proteomic techniques and established *in vitro* and *in vivo* phenotypic assays to define the molecular mechanisms by which this important pathogen avoids killing by the host immune system and causes disease. In particular, we will assess the role of quorum sensing in controlling the expression of virulence factors, including crucial surface structures such as capsule and LPS. Finally, we will test selected mutants as live-attenuated vaccine candidates in our chicken disease model.

Professor Mariapia Degli-Esposti

EMAIL	mariapia.degli-esposti@monash.edu
TELEPHONE	+61 3 9905 6162
OFFICE	Room 380, 15 Innovation Walk (Building 75)
WEB	https://research.monash.edu/en/persons/mariapia-degli-esposti

TWO VACANCIES

Professor Mariapia Degli-Esposti

Dr Iona Schuster

Dr Christopher Andoniou

MCMV, Immunity and Ageing

Professor Mariapia Degli-Esposti, Dr Christopher Andoniou and Dr Iona Schuster

With average life spans increasing we face novel challenges in managing age-associated health decline. A key factor in maintaining overall health is a well-functioning immune system. However, as we age the immune system becomes less functional with reduced production of T and B cells, as well as changes in the quality and composition of respective memory subsets. How immunological challenges such as viral infections impact and shape the aging immune system is not well understood. In this regard, we are particularly interested in cytomegalovirus (CMV), a virus that is never fully cleared and remains with its host life-long. CMV infection causes the gradual expansion of certain CD8+ T cell memory populations, a phenomenon that has been linked with both limiting and enhancing immune responses to other challenges. Using the well-established mouse model of murine CMV (MCMV) infection we aim to examine the impact of this in immune compartments during ageing. Approaches will include high-parameter multicolour flow cytometric analysis of immune cell subsets as well as bulk and single cell assays of immune functionality. The ultimate aim is to gain a better understanding of how CMV infection shapes the immune system over time and how this affects the aging immune system.

Viral Infection and Autoimmunity

Professor Mariapia Degli-Esposti and Dr Iona Schuster

Viral infections have long been suspected to play a role in autoimmunity, with members of the herpes virus family such as cytomegalovirus (CMV) specifically implicated. We use the model of murine CMV, a natural pathogen of the mouse with high similarity to its human counterpart, to investigate the mechanisms underlying the generation of protective antiviral responses and how these correlate with the onset of autoreactive responses. We have shown that a strong anti-viral T cell response generated in the absence of certain immune regulatory mechanisms improves viral control. However, once the virus is controlled, this strong anti-viral response leads to increased generation of auto-specific immune responses resulting in a loss of tissue function. The autoimmune disease generated represents the best available model of the second most common autoimmune disease of man, Sjogren's Syndrome, a condition that affects overall health by severely compromising exocrine gland function. Experimental approaches will include *in vitro* and *in vivo* techniques using wildtype as well as gene-targeted mouse strains. Techniques include the preparation of different tissues for histological analysis of tissue pathology, characterization of infiltrating cell types, and assessment of changes in tissue architecture. Furthermore, we use flow cytometry to characterize and quantify immune cell populations isolated from different tissues at various times post infection. The goal of this project is to further extend our understanding of the processes and mechanisms underlying the generation of autoreactive immune populations in the context of viral infection.

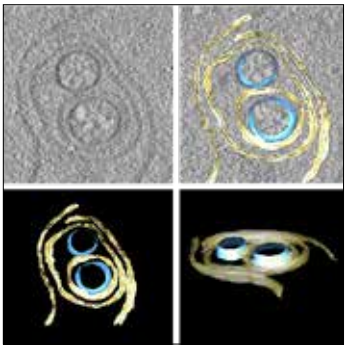
Dr. Johanna Fraser

EMAIL	johanna.fraser@monash.edu
TELEPHONE	+ 61 3 9902 0129
OFFICE	Building 53, 12 Innovation Walk

TWO VACANCIES

Dr. Johanna Fraser

Wolbachia-infected mosquitoes are being released in dengue-prone regions by Monash-based World Mosquito Program to reduce the burden of dengue in communities.



Wolbachia (blue) resides inside mosquito host cells and associates with ER membranes (yellow).

Defining the mechanisms of *Wolbachia*-mediated virus inhibition

Dr. Johanna Fraser

Wolbachia is an endosymbiotic bacterium found in nearly 60% of insect species. It is not naturally found in *Aedes aegypti*, the mosquito vector responsible for transmitting significant human pathogenic viruses such as dengue and Zika. Work over the last decade has shown that *Wolbachia* can be introduced into *Ae. aegypti*, and interestingly, it can impart a potent antiviral state in these mosquitoes. An overwhelming body of evidence has now demonstrated the efficacy of *Wolbachia* as a biocontrol tool, including significantly reducing dengue disease in regions where these mosquitoes are established in the field. However, the detailed mechanisms by which virus inhibition occurs are yet to be fully elucidated. This project will utilize a range of molecular techniques to examine hypotheses such as nutritional parasitism and host cell organelle disruption that may contribute to the antiviral impacts of *Wolbachia*. The project will make use of a unique set of *Wolbachia*-transinfected mosquito lines developed in-house and will be conducted in the lab alongside the World Mosquito Program - the pioneers and world-leading drivers of *Wolbachia*-based technologies.

Viral resistance towards *Wolbachia*

Dr. Johanna Fraser

As this *Wolbachia* biocontrol system ages in the field, there comes an increased risk of viral resistance developing towards *Wolbachia*. We have established a novel in *vivo* viral passaging technique in mosquitoes to study how virus populations evolve towards *Wolbachia*. This project aims to characterize the ability of *Wolbachia*-adapted viral variants to infect mosquito and human hosts by using molecular and classical virology methods.

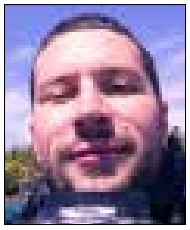
Professor Chris Greening

EMAIL	chris.greening@monash.edu
TELEPHONE	+61 3 990 51692
OFFICE	Room 136, 19 Innovation Walk (Building 76)
WEB	http://www.greeninglab.com

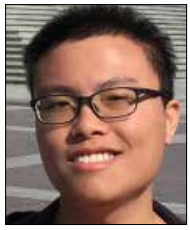
THREE VACANCIES


Professor Chris Greening


Dr Tom Watts


Dr Francesco Ricci


Dr Gaofeng Ni


Dr Pok Man Leung


Dr Sophie Holland


Dr Surbhi Jain

Carbon monoxide: poison or fuel for *Mycobacterium tuberculosis*?

Professor Chris Greening, Dr Tom Watts and Dr Thomas Naderer

Tuberculosis infects one third of the world's population and was the single biggest killer from infectious disease worldwide in 2019. Its causative agent, *Mycobacterium tuberculosis*, resides in patient's lung for decades in a latent state that evades immune defences and resists drug treatment. Our laboratory is investigating the mechanisms that allow this pathogen to stay energised during such infections. One overlooked energy source for this pathogen is carbon monoxide gas. During infection, macrophages produce this gas in large quantities through heme oxygenase-1 as a probable defence strategy. Moreover, the pathogen can be exposed to large amounts of carbon monoxide through cigarette smoking and air pollution. Alarmingly, we have uncovered evidence that mycobacteria not only tolerate this gas, but in fact use it as an energy source through the enzyme carbon monoxide dehydrogenase. In this project, you will investigate PC2 strains of *Mycobacterium tuberculosis* in both pure culture and macrophage infection models. You will derermine, through CRISPR interference, if carbon monoxide oxidation is mediated by carbon monoxide dehydrogenase, and test whether this enzyme and carbon monoxide supplementation enhances long-term survival. This project has potential to reshape our view of how the world's most successful pathogen lives: revealing how a host-derived inorganic defence molecule is exploited as an energy source.

Gas metabolism: a new frontier in the human microbiome revolution

Professor Chris Greening, and Dr Tom Watts

Within the human gastrointestinal tract, our microbiota constantly perform a range of metabolic processes that influence our health. The recent 'microbiome revolution' has revealed a range of metabolic by-products that have links with gut dysregulation and disease. Yet the mediators of gas metabolism, an important process performed by these gut microbes, are still largely unresolved. Bacterial fermentation within the human gut produces hydrogen gas (H_2), and other groups of microbes (such as sulfate, nitrate and fumarate reducers) are responsible for its subsequent disposal. A delicate balance of these processes must be achieved to prevent accumulation of H_2 and other potentially harmful related metabolic by-products, which have been linked to colorectal cancer, IBS and IBD. Furthermore, exploring these processes in the gut microbiota can aid in our understanding of how opportunistic gut pathogens, such as *Clostridium perfringens* and *Clostridioides difficile*, can utilise them in infection. In this study, you will use culture-dependent methods to investigate how these H_2-related metabolic processes occur within the human gut, who the key bacterial players are, and how these processes affect overall human gut health and disease. Depending on your interests, projects can either focus on beneficial bacteria, opportunistic pathogens, or potential carcinogens.

Novel survival strategies of archaea in extreme environments

Professor Chris Greening, Dr Pok Man Leung and Dr Sophie Holland

Discovered less than 50 years ago, archaea are the enigmatic third domain of life and our ancient ancestors. Diverse archaea reside in all ecosystems, spanning soils, waters, and our guts, and they dominate extreme environments such as hot springs and salt lakes. Yet little is known about how they survive limitation or variations in nutrient availability that occur in these environments. We have made the unprecedented discovery that most aerobic bacteria can 'live on air'; more precisely, they survive starvation for their preferred energy sources by scavenging two atmospheric trace gases, hydrogen and carbon monoxide. Aerobic archaea also encode the genes for trace gas consumption, though no study has yet tested whether they can live on air.

In this study, you will grow thermophilic archaea isolated from volcanic springs (*Sulfolobus* spp.) and salt lakes (*Haloplanus* spp.). You will use cutting-edge systems biology approaches (proteomics and transcriptomics) to gain a systematic understanding of how these organisms survive starvation. In addition, you will perform activity measurements to determine whether they can consume atmospheric hydrogen and carbon monoxide during growth and survival. This project is ideally suited for a BSc student keen to work on something exciting yet different.

Investigating the metabolism of deep-sea corals associated microbial communities

Professor Chris Greening and Dr Francesco Ricci

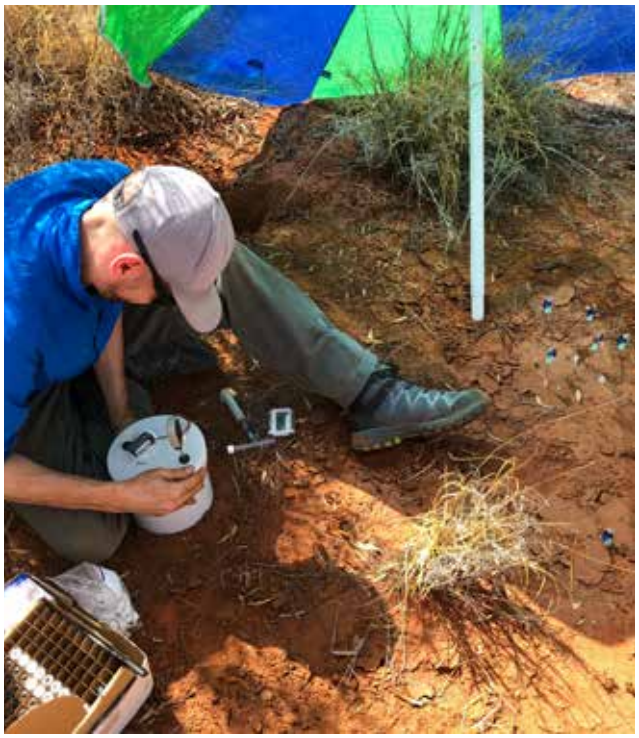
Deep sea corals colonize one of the most remote and unproductive environments on Earth, but unlike their shallow water relatives they lack symbiotic dinoflagellates which provide photosynthetic by-products supporting their survival and growth. Despite lacking photosynthetic symbionts, deep sea corals harbour diverse communities of microbes whose functions are still elusive. In this project we will investigate the involvement of the microbial community in the deep-sea corals metabolic budget and unravel how microbes aid host survival and growth in one of the most extreme environments on Earth.

We will use coral specimens from museum archives and investigate their microbiome through metagenome analysis. The prospective honours student tasks will involve DNA extraction, data analysis (e.g. data visualization, bioinformatic and statistical analysis) and preparation of a scientific manuscript.

Harnessing the Rumen Microbiome: Redirecting Methane Production to Combat Climate Change

Professor Chris Greening, Dr Gaofeng Ni and Dr Surbhi Jain

Livestock farming provides essential food and nutrients for the global population; however, methane emissions from enteric fermentation and manure account for 5% of global greenhouse gas emissions. These emissions originate from the activity of methanogenic archaea within the rumen microbiome. Through our extensive collaboration network within academia and industry, we have generated massive and comprehensive metagenomic and metatranscriptomic datasets from ruminant animals, which is a great asset for studying microbial metabolic pathways that regulate methane production in the rumen. In this challenging and exciting project, you will gain a comprehensive and systematic understanding of the rumen microbiome, specifically microbes involved in the hydrolysis of macromolecules, fermentation, methanogenesis, as well as nitrogen and sulfur metabolism. You will understand their genomes as well as the enzymes responsible for regulating key metabolite and electron flows. This knowledge is crucial for us to redirect methane emission into beneficial processes such as the production of short-chain fatty acids. You will be exposed to cutting-edge genome-resolved metagenomics and metatranscriptomics, and therefore computational and scripting experience is required. This project ultimately facilitates novel interventions to redirect methane emissions towards the production of short-chain fatty acids in rumens, which mitigates global warming and enhances nutrient and energy efficiency in livestock farming.



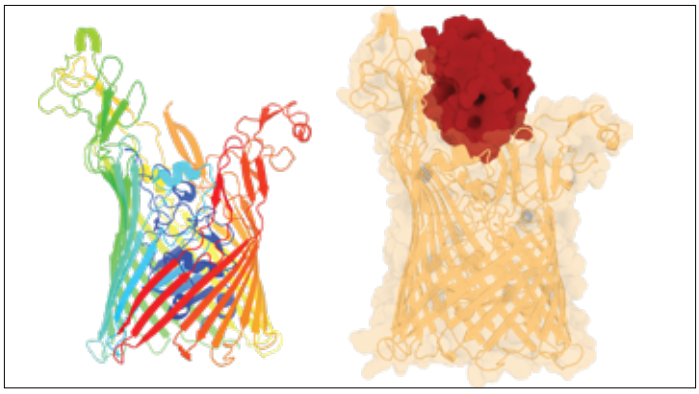
Dr Rhys Grinter

EMAIL	rhys.grinter@monash.edu
TELEPHONE	+61 3 9905 8924
OFFICE	Room 210, 23 Innovation Walk (Building 77)

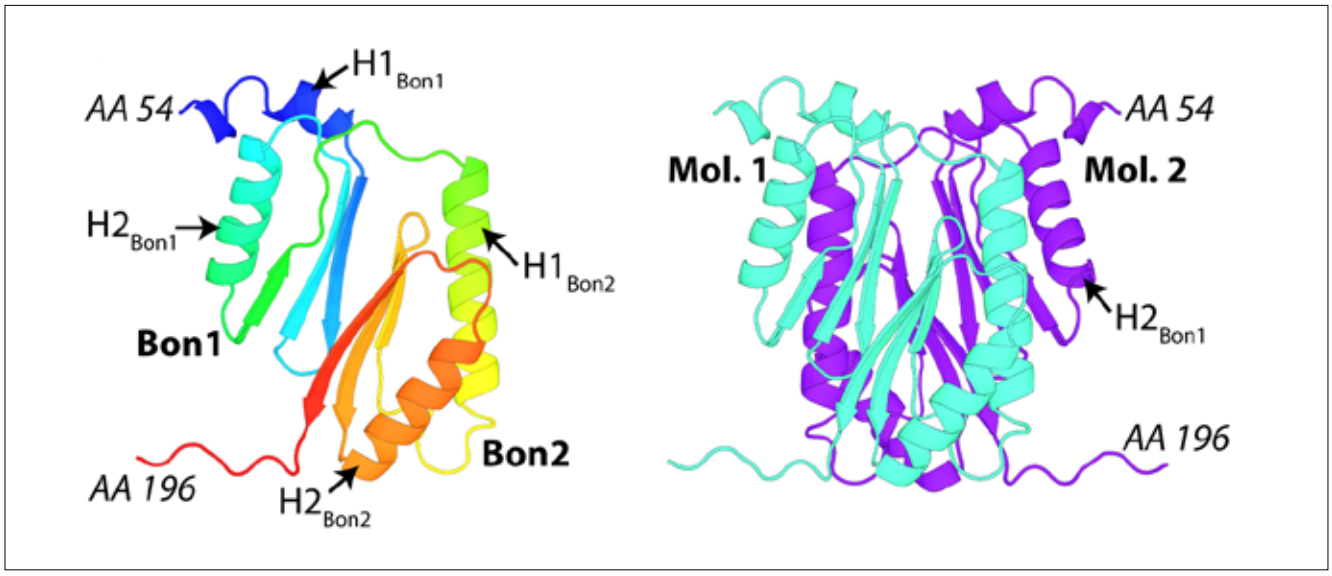
THREE VACANCIES

Dr Rhys Grinter

Dr. Simon Corrie



The structure of the outer-membrane transporter FusA and its substrate complex



The crystal structure of the outer-membrane protein BonA

Lab Biography

The Grinter lab studies how bacteria shape their physiology on a molecular level to infect their hosts or survive harsh environmental conditions, and how these adaptions can be targeted to develop novel antimicrobial therapies. Of key interest to the lab are how Gram-negative bacterial pathogens (including *Neisseria gonorrhoeae* and *Haemophilus influenzae*) obtain the essential nutrient iron from their host, by using specialised transporters that steal it directly from host proteins. We are also interested in how bacteria of the genus *Mycobacterium* have adapted to survive in harsh environments and the host to become some of the most successful pathogens of humans.

The Grinter lab utilises current and emerging technologies to understand how microbes work. It harnesses this understanding to prevent disease and to develop enzymes and natural products as tools for biotechnology. It utilises diverse techniques across genomics, bioinformatics, molecular microbiology, biochemistry and structural biology.

The lab is looking for up to three honours student to recruit to one of the projects outlined below.

Project 1: Understanding and isolating heme transporters from the outer membrane of *Haemophilus influenzae*

Dr. Rhys Grinter and Mr. Daniel Fox

Haemophilus influenzae is a Gram-negative bacterium that is an obligate inhabitant of the human respiratory tract. Most commonly it lives in our throats as a harmless commensal; however, it can cause serious disease. Before the introduction of the *H. influenzae* type b (Hib) vaccine in the 1980s *H. influenzae* infection was a serious cause of death due to meningitis and pneumonia, particularly in children under five. The Hib vaccine greatly reduced these deaths, however, *H. influenzae* strains that escape this vaccine and are resistant to antibiotics have emerged. These non-typeable *H. influenzae* (NTHi) are a major cause of ear and throat infections in children, and infection rates are rising.

One of the most important nutrients for *H. influenzae* during infection is the iron-containing cofactor heme. *H. influenzae* cannot synthesize heme and so obtains it from host haemoglobin using specialised transporters in its outer membrane. In the project, we will seek to understand how the expression of these haemoglobin transporters is regulated in response to an abundance or scarcity of heme. To achieve this, we will utilise biochemistry to isolate outer membranes from *H. influenzae* grown with different concentrations of heme. We will then utilise SDS-PAGE and mass spectrometry to determine how the abundance of outer membrane haemoglobin transporters changes in response to heme concentrations. Next, we will use this knowledge to isolate and purify haemoglobin transporters from *H. influenzae* membranes and use structural biology and biochemistry to determine how they bind haemoglobin and transport heme. Because *H. influenzae* requires exogenous heme to grow we can block its access to this nutrient as a means of preventing or treating infection. The understanding of haemoglobin transporters provided by this project will assist in the design of inhibitors that block heme-uptake.

Project 2: Discovering Lectin-Like Protein Antibiotics to treat bacterial infection

Dr. Rhys Grinter

Gram-negative bacteria are a major bacterial group that contains important plant, animal, and human pathogens. The impermeable outer membrane of these bacteria makes them highly resistant to many antibiotics. Furthermore, the efficacy of existing antibiotics that treat Gram-negative bacterial disease is rapidly diminishing due to the evolution and global spread of antibiotic resistance, a problem exacerbated by a near-empty antibiotic discovery pipeline. This makes the discovery and development of new antibiotics targeting Gram-negative bacteria of critical importance.

In this project, we aim to turn the greatest defence of Gram-negative bacteria against them by developing lectin-like bacteriocins (Llps), a class of antibiotic protein that targets outer-membrane protein assembly. Outer-membrane protein assembly is essential in Gram-negative bacteria and its disruption rapidly kills bacterial cells, making this process a promising new avenue for antibiotic development. To realize the potential of Llps as antibiotic agents we must first understand how they interact with their targets on the cell surface: the outer membrane assembly protein BamA and lipopolysaccharide (LPS).

Further, we need to understand the consequences of disrupting outer membrane protein assembly and why it is lethal to the bacterial cell. We will do this by investigating how Llps produced by Gram-negative *Pseudomonas* species select, bind to, and kill target cells. Members of the genus *Pseudomonas* are important pathogens of plants and animals and have a high inherent resistance to antibiotics, making the development of new antibiotics targeting them an important and timely goal

Project 3: Engineering herbicide degrading enzymes for biosensing and bioremediation

Dr. Rhys Grinter and A/Prof. Simon Corrie (Engineering)

2,4-dichlorophenoxyacetic acid (“2,4-D”) is one of the most frequently used herbicides worldwide to control weeds in agriculture, and despite clear benefits for global food production, it also contaminates waterways via leaching, runoff, and by drifting of sprayed droplets through the air (“spray drift”). This is a significant concern, because 2,4-D is a suspected human carcinogen, known to have serious effects on the endocrine and immune systems, and is toxic to a range of high value crops, animals and aquatic life. Microbes have evolved enzymes to degrade a range of herbicides and pesticides, and aryloxyalkanoate dioxygenases have been shown to catalyse the reduction of 2,4-D to 2,4-DCP.

In this project we will engineer these enzymes to improve their stability, catalytic efficiency and substrate specificity, along with investigating the structure/function relationships between enzyme and substrate. There is considerable commercial interest in developing aryloxyalkanoate dioxygenases for environmental biosensing and bioremediation to minimise off-target effects of potent herbicides and pesticides.

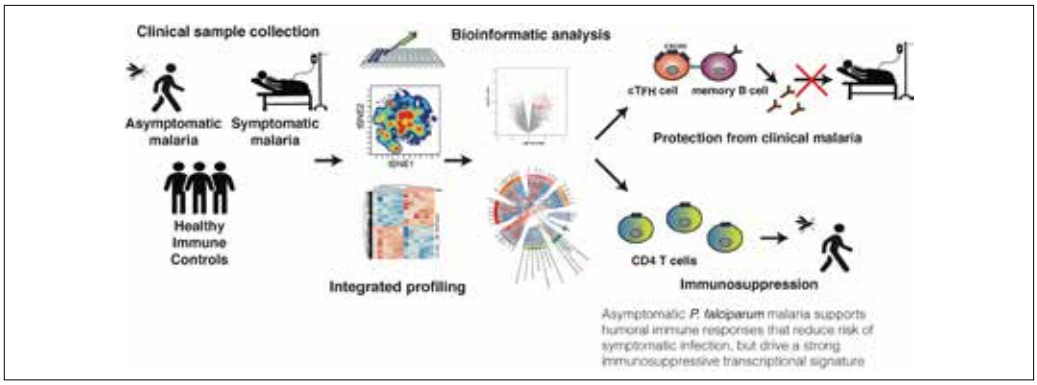
This project represents an excellent starting point for students interested in microbial enzymes, recombinant protein expression, computational/experimental enzyme engineering, and/or structural biology (we will tailor the specifics of the project to the student’s skill set and interests).

Prof Diana Hansen

EMAIL	diana.hansen@monash.edu
TELEPHONE	+61 3 990 53721
OFFICE	Room 114, 19 Innovation Walk (Building 76)

THREE VACANCIES

Professor Diana Hansen



Workflow for understanding pathogenesis and immunity to malaria and severe dengue fever

Mechanisms of pathogenesis and immunity to malaria and severe dengue fever

Our research focuses on finding solutions to tackle two devastating mosquito-borne infectious diseases: malaria and dengue. Together these account for 600 million clinical cases worldwide annually

Unlike other infections in which one single encounter with the pathogen is enough to induce long-lasting protection, immunity to malaria might take decades to develop in endemic areas. The Hansen lab investigates mechanisms by which *Plasmodium* infections prevent the acquisition of immunity. This work is undertaken in order to design therapeutic approaches to improve the induction immune responses to the *Plasmodium* parasite, including effective anti-malaria vaccines.

Clinical immunity to malaria is largely dependent on effective antibody responses. In collaboration with partners in malaria-endemic countries, our group investigates how the induction of antibody-mediated immunity is dysregulated during symptomatic a well as asymptomatic malaria and how constant exposure to *Plasmodium* parasites over time modulates the development of these responses.

There is no specific treatment for dengue and no validated way to predict which patients will progress to life-threatening manifestations of disease. The clinical course for dengue is difficult to predict mostly because the specific pathways influencing severity to disease are not understood. To address this challenge, our group pursues a comprehensive immunological and molecular approach to uncover key mechanisms involved in the development of severe dengue. Obtaining this information is of vital importance to design novel diagnostic tools for early detection of complicated cases, as well as therapeutic approaches to treat severe dengue.

Effect of acute and persistent malaria infection on host immunity to infection and vaccination

Prof Diana Hansen and Dr Rintis Noviyanti

Despite constant exposure to *Plasmodium* parasites, immunity to malaria takes many years to develop for individuals living in endemic areas. This form of protection is not sterilising but prevents clinical episodes by substantially reducing parasitaemia, with adults often experiencing non-febrile malaria infections. Naturally acquired immunity is known to require antibody responses. The acquisition of antibody-mediated immunity requires generation of high-affinity antibody-secreting cells and memory B cells, a process that is facilitated by T follicular helper cells in secondary lymphoid organs.

Using infection models as well as field studies in malaria endemic areas we are investigating the how *P. falciparum* and *P. vivax* infections influence these responses and the immunological and molecular factors that modulate the outcome to these processes.

We also investigate how chronic non-febrile asymptomatic infections modulate host immunity, thereby preventing full control of parasitemia and mounting efficient immune responses to vaccination.

Identification of cellular and molecular pathways predisposing to severe dengue fever

Prof Diana Hansen and Dr Tedjo Sasmono

Typical symptoms of dengue include sudden onset of fever accompanied by headache, muscle pains, rash, cough, vomiting and haemorrhagic manifestations. Hospitalisation may be required depending on signs of severity such as dehydration, bleeding or comorbidities.

There is no specific treatment for dengue, and care is mainly supportive. To date, there is no validated way of identifying which patients will progress to severe disease, meaning that in endemic areas, health facilities are often overwhelmed with patients admitted for inpatient observation, costing millions of dollars to health systems.

To address this issue, this project undertakes a comprehensive immunological and transcriptional analysis of individuals with mild versus severe dengue fever recruited at local hospitals in different regions of Indonesia.

The project will uncover key mechanisms involved in progression towards severe disease after initial patient presentation.

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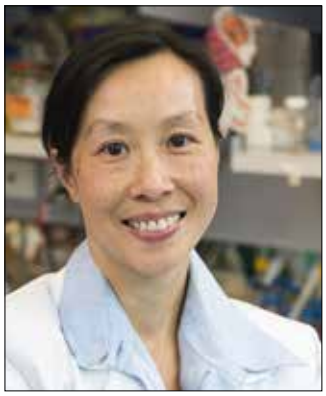
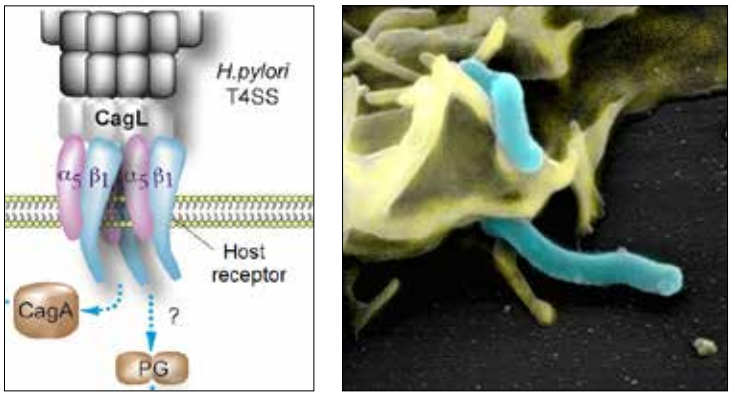
Dr Terry Kwok-Schuelein

EMAIL	terry.kwok@med.monash.edu.au
TELEPHONE	+61 3 9902 9216
OFFICE	Room 231, level 2, 19 Innovation Walk (Building 76)

TWO VACANCIES



Dr Terry Kwok-Schuelein



The oncogenic type IV secretion system (T4SS) of *H. pylori* (left) is activated upon *H. pylori*-host interaction (right).

The Molecular Mechanisms by Which *Helicobacter pylori* Causes Stomach Cancer

Helicobacter pylori is a Gram-negative gastric bacterium that has co-evolved with humans for more than 50,000 years. It colonises the stomach of over 50% of the world's population, making it one of the most prevalent human pathogens. It is a causative agent of severe gastric diseases including chronic gastritis, peptic ulcer and stomach cancer. *H. pylori* has been classified as a Group I (high-risk) carcinogen.

Highly virulent strains of *H. pylori* harbour a type IV secretion system (T4SS), a secretion machinery that functions as a "syringe" for injecting virulence proteins and peptidoglycan into the host cell. We discovered that CagL, a specialised adhesin present on the surface of the *H. pylori* T4SS, binds to the human integrin α5β1 receptor on stomach lining cells. This binding activates the T4SS and hence the secretion of virulence factors including the highly immunogenic and oncogenic protein, CagA, into stomach cells. 'Injected' CagA then interacts with host signalling molecules and triggers activation of a suite of host responses. Interestingly, our recent findings suggest that CagL can also directly modulate host cell functions. The precise mechanisms by which CagL functions both as a host-activated sensor of the *H. pylori* T4SS and as a direct activator of aberrant host responses remain to be fully understood.

Our team uses multi-disciplinary state-of-the-art approaches to study the molecular mechanism of *H. pylori* type IV secretion and *H. pylori*-host interactions. We aim to understand the molecular basis of how *H. pylori* induces stomach cancer, with the ultimate goal of providing knowledge for a better treatment and/or prevention of *H. pylori*-associated stomach diseases. Projects are available to address the following key questions:

- How does *H. pylori* trigger inflammation and carcinogenesis through the virulence functions of CagL and CagA?
- Can *cagL* and *cagA* genotypes predict gastric cancer risk and therefore help pinpoint cancer-prone patients for early treatment?
- How does CagL function as a host-activated sensor during type IV secretion?
- How do CagL and CagA modulate host cell signalling during chronic *H. pylori* infection?
- Can we utilise the type IV secretion system of *H. pylori* for delivery of therapeutic proteins?

The available honours projects will enable one to gain experience with the important techniques of molecular cloning and mutagenesis, bacterial culture, eukaryotic cell culture techniques, mouse infection models, CRISPR, RNAi, immunostaining, Western blotting, ELISA, confocal laser scanning microscopy, live cell imaging, etc. Someone who is enthusiastic in learning about the exciting secrets of bacteria-host interactions, infectious cancer biology and bacterial pathogenesis is strongly encouraged to apply.

Dr Rachael Lappan

EMAIL	rachael.lappan@monash.edu
TELEPHONE	+61 3 9905 5794
OFFICE	Room 135, 19 Innovation Walk (Building 76)
WEB	https://research.monash.edu/en/persons/rachael-lappan

ONE VACANCY

Dr Rachael Lappan

The airborne microbiome of healthcare settings and the risk of pathogen and antimicrobial resistance transmission

Dr Rachael Lappan, Professor Anton Peleg, Dr Xenia Kostoulias

Since the COVID-19 pandemic, there has been a surge in the recognition and mitigation of airborne pathogen risks. In hospitals and aged care facilities, recognised pathways of pathogen transmission include high-touch surfaces, shared patient rooms, bathrooms and staff contact, and these are generally well-monitored and controlled. Indoor air quality is regulated in some areas, but airborne microbial monitoring is not routine and represents a gap in our understanding of transmission pathways of a range of pathogens and antimicrobial resistance genes. In this project, the student will undertake and optimise air sampling in hospital and aged care locations, and develop an approach to analyse the air microbiome with metagenomic sequencing. The metagenome data will be interrogated for pathogen presence and abundance, including the antimicrobial resistance profile (the resistome), and compared with the findings from other indoor samples to examine the movement of organisms between patients or residents and their immediate environment. The project involves a combination of indoor sampling, molecular laboratory work and a significant bioinformatics component, so would suit a student with some experience in bioinformatics and an interest in computational microbiome work.

Antimicrobial resistance in Antarctica and other pristine ecosystems

Dr Rachael Lappan, Professor Chris Greening

The resistance of human pathogens to antibiotics is an increasingly important issue for human health, with many antimicrobial resistance genes readily passed between bacteria via plasmids and resistance selected for by the use (or overuse) of antimicrobials. However, antimicrobial resistance is not restricted to pathogens; it is likely an ancient strategy for microbial survival, enabling bacteria to compete and survive in a range of environments. Little is known about the prevalence and nature of antimicrobial resistance in ecosystems that are not dominated by humans, including Antarctica, other deserts, and cave systems. This project aims to evaluate the antimicrobial resistance profile (the resistome) and the array of plasmids (plasmidome) in such environments to understand how bacteria disseminate antimicrobial resistance, compete and survive. The project primarily involves the use of metagenomic data and bioinformatic analysis, so a student with some computational experience (e.g. through bioinformatics units) would be well suited to this project. There is also the opportunity for culture-based work and susceptibility testing on novel bacterial isolates from these environments, with the project tailorable to the student's interest in developing skills in both laboratory and computational areas.

Dr Simone Li

EMAIL	simone.li@monash.edu
TELEPHONE	+61 3 9902 9192
OFFICE	Room 223, 19 Innovation Walk (Building 76)

ONE VACANCY

Dr Simone Li

Cover of Nature Medicine featuring our recent gut microbiome study on faecal microbiota transplantation (September 2022)

Microbiome Systems Lab

We are a new research group in the Biomedicine Discovery Institute, with an interest in creating multi-disciplinary, data-oriented approaches to study and compare the evolution of microbial communities in natural environments, such as soil and the human gut microbiome.

New developments in DNA sequencing technologies have advanced the field of metagenomics, enabling the genomes of different microbes in a single ecosystem to be analysed simultaneously. This has revealed, for example, an intriguing dual role of the microbiome in maintaining homeostasis as well as disrupting health.

Our recent work on the gut microbiome revealed ecological patterns of engraftment in faecal microbiota transplantation (FMT) patients undergoing treatment for different diseases (Science 2016, Nature Medicine 2022). Here, we developed new computational methods and tools to accurately profile the 1000s of microbial species in the human gastrointestinal tract and identify and track donor strains over time (Genome Biology 2015, Bioinformatics 2016, Nucleic Acids Research 2017, eLife 2019). By integrating our results with clinical metadata, we discovered certain bacteria that are more likely to persist in patients and identified aspects of the medical procedure that would facilitate this phenomenon. These findings transformed our understanding of how FMT works and its lasting impact on the microbes in our gut. It also motivated the use of precision medicine approaches to increase the success of therapies that target the microbiome - a growing global market that is estimated to reach >US$1.5bil by 2027 (BCC Research, Feb 23).

By unravelling the complex layers of biology contained within the microbiome in its native context, we aim to discover novel approaches to prevent and treat disease, and introduce ways to improve and enhance current practices in clinical, biotechnology and bioremediation settings.

We use bioinformatics, machine learning and high-performance computing to test our biological hypotheses on new and existing data, generated by both established and emerging -omics technologies. Our team collaborates with experts around the world to develop, contextualise and build the biological picture around our findings and further our impact.

We are a growing lab and while our research is primarily data-driven, keen students with a good grasp of microbiology and genome analysis are welcome and encouraged to get in touch.

A data-driven investigation into antimicrobial resistance transmission by faecal microbiota transplantation

Faecal microbiota transplantation (FMT) involves the transfer of gut microbes from (the stool of a) healthy donor to patient. The medical procedure is well-known for its high success rates in treating recurrent C.difficile infection and has become a potential therapeutic option for an increasing number of chronic diseases. However, we still don't know how it works and there exists a risk of undesired side-effects. For example, drug-resistant infections have been described in some patients soon after they received FMT for unrelated illnesses - whether this was by pure coincidence or caused by FMT is unclear.

In this project, we will interrogate metagenomic and clinical data to look at antimicrobial resistance (AMR) genes in the gut microbiome of FMT patients and their donors. Do donors and patients already have AMR-possessing microbes in their gut? If so, what are their function and how prevalent are they? Are donor AMR genes and microbes being transferred to patients? Is this more likely to occur in some patients more than others? What are the driving factors behind this observation: could it be age, disease, treatment method?

Students will be able to tackle these questions and continue with their own, gaining experience in data science, statistics and antimicrobial resistance research in this exciting project at the nexus of discovery and clinical sciences. The project can be tailored according to your research experience and goals.

Professor Jian Li

EMAIL	jian.li@monash.edu
TELEPHONE	+61 3 9903 9702
OFFICE	Room 220, 19 Innovation Walk (Building 76)
WEB	https://research.monash.edu/en/persons/jian-li

THREE VACANCIES

Professor Jian Li

Dr Sue C. Nang

Dr Mei-Ling Han

Dr Jinxin Zhao

Laboratory of Antimicrobial Systems Pharmacology

My lab focuses on antimicrobial discovery and pharmacology against Gram-negative 'superbugs' (namely *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae*). There has been a marked decrease in the discovery of novel antibiotics over the last two decades. As no novel class of antibiotics will be available against Gram-negative 'superbugs' in the near future, it is crucial to optimise the clinical use of 'old' polymyxins using systems pharmacology and to develop new-generation antimicrobial lipopeptides and innovative therapeutics.

My major research programs include:

(1) optimising clinical use of antimicrobial and phage therapies using pharmacokinetics/pharmacodynamics/ toxicodynamics (PK/PD/TD) and systems pharmacology;

(2) elucidation of mechanisms of antibacterial activity, resistance, and toxicity of antimicrobials and phages; and

(3) discovery of new-generation antimicrobial lipopeptides and innovative therapeutics against multidrug-resistant (MDR) Gram-negative bacteria.

My lab is funded by the US National Institutes of Health (NIH) and Australian NHMRC.

Deciphering the Mechanisms of Antimicrobial Resistance and Phage Infection in Gram-negative bacteria Using Computational Biology

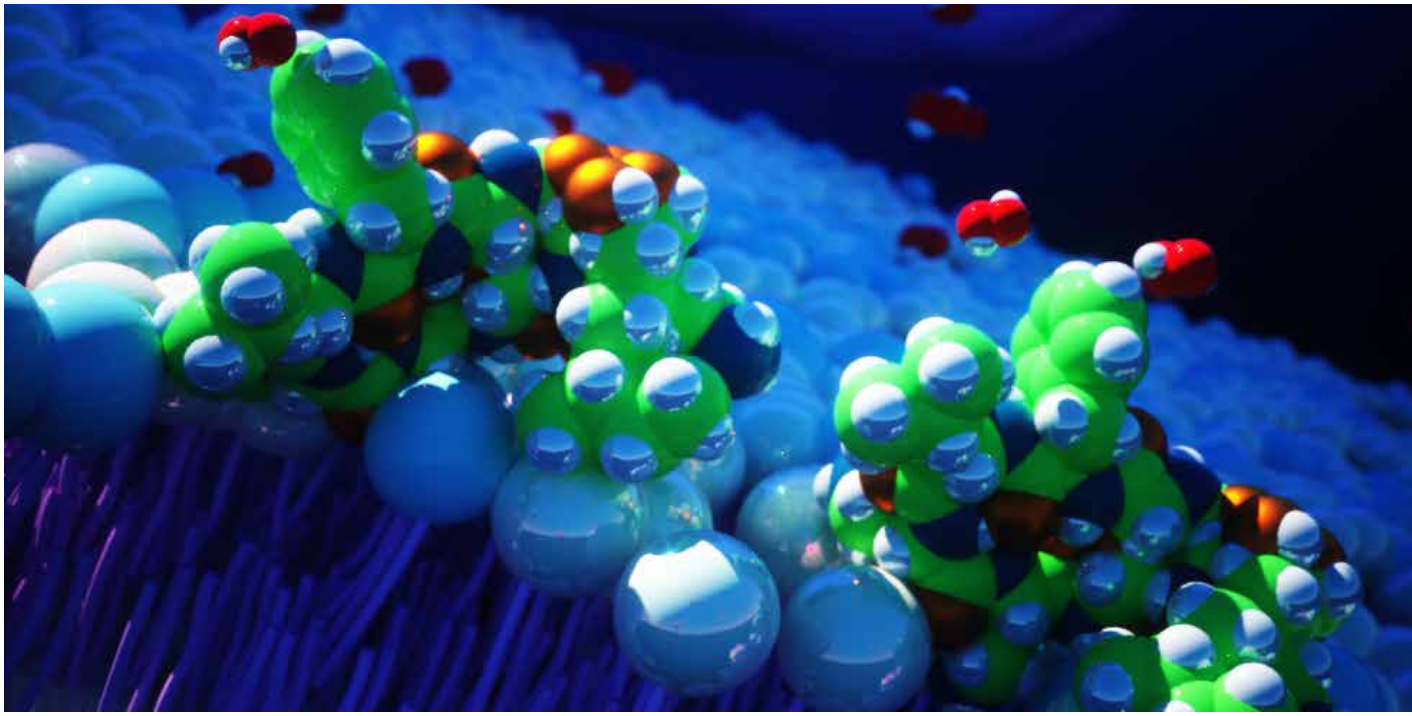
Dr Mei-Ling Han, Dr Jinxin Zhao and Professor Jian Li

Antimicrobial resistance is a critical threat to human health worldwide. Polymyxins are a group of last-line antibiotics against Gram-negative 'superbugs', including MDR. *Acinetobacter baumannii, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.*

We are integrating genomics, transcriptomics, proteomics, metabolomics, and lipidomics to systematically examine bacterial responses to antimicrobial treatment and phage infection.

This project aims to:

1. conduct comparative genomic analysis for phages to predict critical genetic determinants of infection;
2. construct genome-scale models for *A. baumannii*, *K. pneumoniae* and *P. aeruginosa* using multi-omics data;
3. employ the constructed genome-scale models to simulate bacterial responses to antimicrobials and phage infections;
4. predict key genes and pathways contributing to bacterial resistance to antibiotics and phages and validate their functions with our comprehensive mutant libraries.



This multidisciplinary project will characterise the complex interplay of signaling, regulation and metabolic pathways involved in resistance to antimicrobal killing and phage infection, thereby optimising antimicrobial combination and bacteriophage therapies in patients.

Phage-Antibiotic Therapy in the Postantibiotic Era

Dr Sue C. Nang and Professor Jian Li

Antimicrobial resistance has become one of the greatest global threats to human health and pandrug-resistant (PDR) *K. pneumoniae* has been identified by the WHO as one of the 3 top-priority pathogens urgently requiring novel therapeutics. These 'superbugs' cause life-threatening infections, particularly in the critically ill, and polymyxins are often used as the last option. Worryingly, increasing emergence of polymyxin resistance highlights the urgency to develop novel therapeutics to treat PDR *K. pneumoniae*. Bacteriophage (i.e. phage) have recently attracted substantial attention as a potential alternative against PDR bacterial infections; however, resistance to phage therapy (including cocktails) in *K. pneumoniae* can rapidly develop. Fortunately, phage resistance may restore bacterial susceptibility to certain antibiotics and therefore, optimal phage-antibiotic combination therapy provides a superior approach to fight against these superbugs. Contemporary antimicrobial pharmacology plays a critical role in optimising antibiotic dosage regimens, but lacks systems and mechanistic information. Importantly, antibiotic dosing strategies cannot be easily extrapolated into phage therapy, mainly due to the complex disposition, host specificity and self-replication of phages.

As the optimal phage-antibiotic combination and dosage regimens also depend on the dynamics of infection and host responses, innovative strategies incorporating systems pharmacology and host-pathogen-phage-antibiotic interactions have a significant potential in optimising phage-antibiotic combinations. This project will employ cutting-edge systems pharmacology to generate urgently needed information for rationally optimising novel phage-antibiotic combinations in patients.

Pulmonary Toxicity of Novel Polymyxin Combination Therapies

Professor Jian Li

Current dosing recommendations of parenteral polymyxins are suboptimal for treatment of respiratory tract infections due to poor drug exposure at the infection site. Moreover, nephrotoxicity is the dose-limiting factor and can occur in up to 60% of patients. Pulmonary delivery of polymyxins as monotherapy and in combination with other antibiotics has offered a great promise for bacterial eradication in the respiratory tract. However, we have shown that polymyxins localise in mitochondria of human lung epithelial cells and activate multiple apoptotic pathways. This multi-disciplinary project aims to investigate the effect of polymyxins and their synergistic combinations with other key classes of antibiotics on human lung epithelial cells, using fluorescence activated cell sorting (FACS), metabolomics, proteomics, transcriptomics and cutting-edge imaging techniques. This project will provide the much-needed pharmacological information for safer and more efficacious use of polymyxin inhalation therapy against life-threatening lung infections.

Professor Trevor Lithgow

EMAIL	trevor.lithgow@monash.edu
TELEPHONE	+61 3 9902 9217
OFFICE	Room 233; Room 252, 19 Innovation Walk
WEB	https://www.monash.edu/discovery-institute/lithgow-lab/home

ONE VACANCY

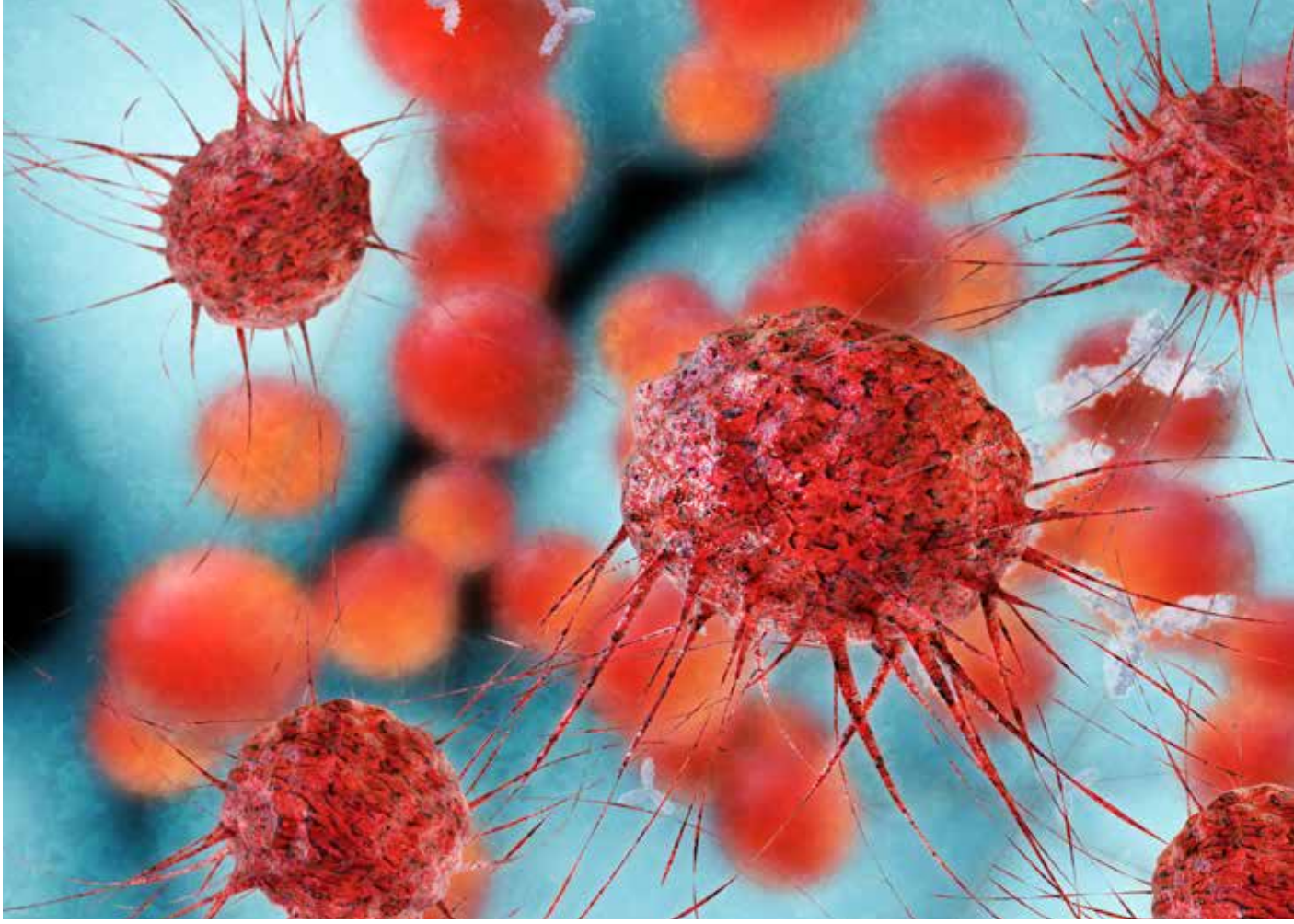
Lithgow lab group

Bacterial Cell Biology Laboratory

Bacterial cells were once dismissed as 'bags of enzymes' with minimal organization. Yet in the past 10 years it has become clear that bacteria segregate many cellular processes into subcellular structures (Greening & Lithgow **Nature Reviews Microbiology** 18:677). The Bacterial Cell Biology Lab uses all the tools of molecular cell biology to study and image bacteria at the sub-cellular level, and Honours, Masters and PhD students in this lab are trained how to manage their project work towards at least one first-author paper in a major journal. This is a critical outcome to ensure that graduates from the lab are offered exciting jobs (https://www.monash.edu/discovery-institute/lithgow-lab/members).

Examples of our recent PhD student publications include Eric Mandela's paper on spatial control that bacteria exert on their cell walls (Mandela et al. 2022. **eLife** 11:e73516); Manasa Bharathwaj's paper on how carbapenemases are secreted by *Klebsiella pneumoniae* (Bharathwaj et al. 2021. **mBio**12:e0130221), Von Torres' paper on how hypervirulent *K. pneumoniae* assemble their outer membranes (Torres et al. 2018. ***Mol Microbiol*** 109:584), Dilshan Gunasinghe's paper on the systems-level control of outer membrane protein assembly in *Escherichia coli* (Gunasinghe et al. 2018. ***Cell Reports*** 23:2782), Jiawei Wang's paper on using bioinformatics to predict effector proteins for type 6 secretion system with high accuracy (Wang et al. 2018. Bioinformatics 34:2546), and Chris Stubenrauch's paper on the mechanism behind how *E. coli* assembly fimbriae in urinary tract infections (Stubenrauch et al. 2016. ***Nature Microbiol*** 1:16064).

From a recent global assessment published in ***The Lancet***, we now know that human deaths associated with antimicrobial resistant (AMR) bacterial infections are much worse than we had feared. In 2019, 5 million people died with infections due to AMR bacteria making AMR the third biggest global killer of people, just behind ischemic heart disease and stroke. Across the world, six bacterial species contribute most to the burden of AMR deaths: *E. coli, Staphylococcus aureus, K. pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii and Pseudomonas aeruginosa*. In the Bacterial Cell Biology Lab, we have focussed our studies on three of these species: *E. coli, S. aureus* and *K. pneumoniae*, with many of the current projects addressing the mechanisms of AMR in *E. coli* and *K. pneumoniae*, and the search for new phages as therapies for infections caused by *S. aureus* and *K. pneumoniae*.



Predicting the evolution of AMR in *Escherichia coli* and *Klebsiella pneumoniae*.

Dr. Chris Stubenrauch and Professor Trevor Lithgow

Efflux pumps are membrane-spanning channels that use ATP hydrolysis to power the efflux of a range of molecules out of bacterial cells. Within this one readily defined group of proteins, there are pumps that are considered to be specialized for exporting heavy metals (silver, cadmium, mercury, etc.), small toxic molecules (polyamines, disinfectants, biocides in hand-soaps, etc) and antibiotic drugs. However, there is a growing awareness that efflux pumps are not always so substrate-specific and that some efflux pumps annotated as, for example “silver efflux” are also capable of exporting antibiotic drugs. This project would take multiple approaches for predicting the effects of efflux pumps on the evolution of AMR: in terms of their predicted structures using AlphaFold, their evolutionary relationships to one another using comparative genomics, and in terms of their distribution in the prevalent AMR superbugs, *E. coli* and *K. pneumoniae*, particularly their carbapenem-resistant forms (CRE). Using *E. coli* as a model superbug, a set of efflux pumps active for exporting biocides in hand-soaps will be tested for their activity in exporting antibiotic drugs.

The search for new phages as therapies for infections caused by *Staphylococcus aureus* (MRSA) and *Klebsiella pneumoniae*

Dr. Rhys Dunstan and Professor Trevor Lithgow

Bacteriophages (phages) are viruses that infect bacteria, and there is a current move to bring “phage therapy” to Australian hospitals in order to treat AMR infections. But where will these therapeutic phages come from? This project aims to assess phage diversity through a classical environmental microbiology approach: using water samples collected from diverse locations around the world, the phages therein will be concentrated and plated on a lawn of bacteria. Attention will be focused on phages that infect the pathogens *K. pneumoniae* or *S. aureus*, particularly on the carbapenem-resistant forms of *K. pneumoniae* (CRE) and the methicillin-resistant forms of *S. aureus* (MRSA). Using a combination of electron microscopy to assess virion morphology, and bioinformatics for comparative genomics and protein identification, the project would classify, catalogue and compare the various phage isolated. Finally, a systematic assessment of cocktails of the various phage will be undertaken to determine killing efficacy for future therapeutic work, driven by the Phage Australia Network (https://phageaustralia.org/).

Professor Dena Lyras

EMAIL	dena.lyras@monash.edu
TELEPHONE	+61 3 9902 9155
OFFICE	Room 152, 19 Innovation Walk (Building 76)

TWO VACANCIES


Professor Dena Lyras


Dr Yogitha Srikhanta


Dr Steven Mileto


Dr Milena Awad


Dr Galain Williams


Dr Melanie Hutton


Dr Sarah Revitt-Mills

Interrogating the effects of human host proteases on *Clostridioides difficile* infection

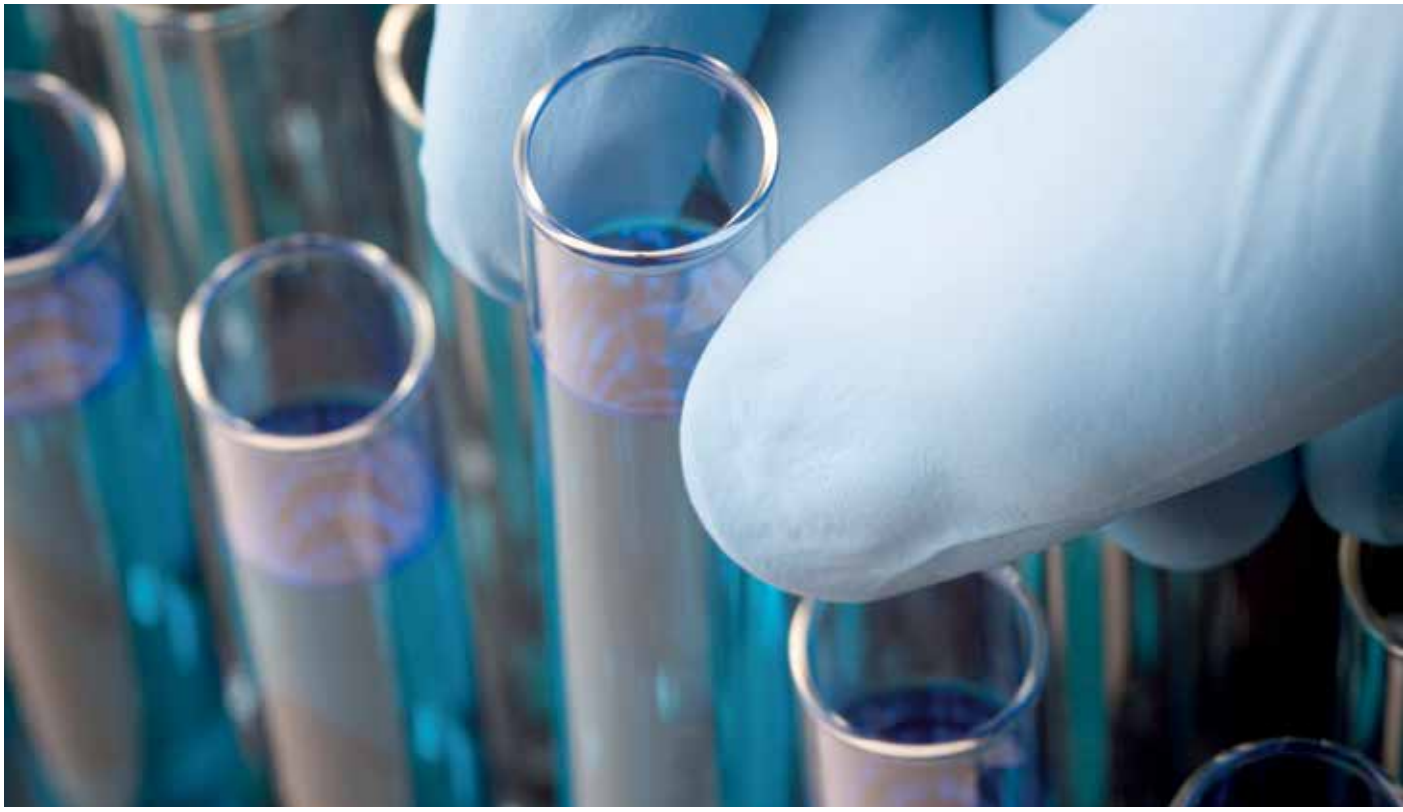
Professor Dena Lyras, Dr Melanie Hutton and Dr Steven Mileto

The human and financial cost of *Clostridioides difficile* global epidemics is substantial and alarming, with *C. difficile* listed as the number one antibiotic-resistant bacterial threat in the USA. A key driver of *C. difficile* infection (CDI) relates to the ability of this bacterium to form spores, an inert and highly robust cell type, which allows survival of the bacterium in hostile environments. Spores initiate and transmit disease, whereas the vegetative cell form of *C. difficile* colonises the gut and produces potent toxins that cause disease. We have found that the host protease plasminogen migrates to the gut following toxin mediated damage and that *C.difficile* spores recruit plasminogen to their surface leading to exacerbated disease. We have also shown that the proteolytic state of the gut changes during CDI, with some host proteases found in higher abundance in the gut during CDI, however the role of these proteases in disease, and the interactions between these proteases and *C. difficile* cells, has not been examined. To examine the contribution of these different host proteases to *C. difficile* disease, we have generated protease knock out mice. In this project, these mice will be characterised and compared to wildtype mice to assess the role of these enzymes during CDI, using in vivo, in vitro and cutting-edge imaging techniques. This project will provide new insights into how a bacterial pathogen interacts with host proteases during disease.

Determining the mechanism of toxin secretion in *Clostridioides difficile*

Professor Dena Lyras, Dr Yogitha Srikhanta and Dr Milena Awad

Clostridioides difficile (C. difficile) is of considerable medical interest due to the high disease burden and global challenge of managing the consequences of infection. A key driver of *C. difficile* infection relates to the ability of this bacterium to produce the large clostridal glucosylating toxins, TcdA and TcdB. These toxins are responsible for a range of diseases including mild diarrhoea, pseudomembranous colitis, toxic megacolon and can result in death. How these toxins are secreted into the host environment to cause disease remains elusive. In this project, we are interested in determining the mechanism of *C. difficile* toxin release. We have identified two possible gene targets, DL001 and DL002, that appear to influence toxin secretion. In this project we will further interrogate the role of these two targets in toxin release using a combination of different tools. This will include the use of toxin ELISAs, western blot analysis and cross-sectional TEM imaging. The outcome of this study will help us to better understand how these toxins are secreted from C. difficile, which may identify novel therapeutic targets and strategies to combat diseases caused by this important global pathogen.



Understanding the role of bacterial surface structures in horizontal gene transfer amongst enteric pathogens

Professor Dena Lyras, Dr Yogitha Srikhanta, Dr Sarah Revitt-Mills, and Dr Galain Williams

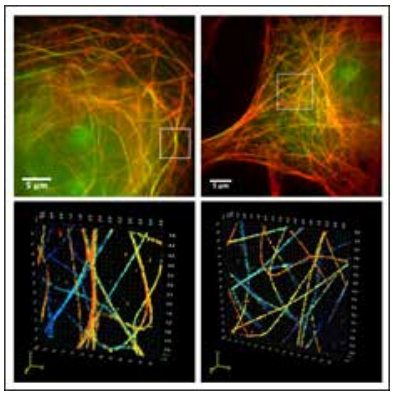
The widespread emergence of antimicrobial resistance amongst bacterial pathogens is a serious challenge to global healthcare systems. Of particular concern is the spread of antibiotic resistance genes through horizontal gene transfer, which allows resistance to develop at a rate much faster than would otherwise occur. One of the mechanisms underpinning this is bacterial conjugation, which involves the transfer of plasmid DNA between bacterial cells via conjugative pili. Alarmingly, conjugation occurs at high frequencies in the communities where bacteria naturally reside, such as in the human gut, yet our understanding of conjugation within these communities is limited. In this project, we will examine the factors that govern conjugation amongst Gram-negative enteric pathogens, with a specific focus on the role played by bacterial cell surface structures. You will develop skills in traditional microbiology and genetic techniques, and gain exposure to bioinformatics and live-cell microscopy. Students with a strong interest in bacteriology but who are not yet confident in molecular biology or genetics are still encouraged to apply. The findings from this project may lead to the identification of new therapeutic targets and strategies through which the spread of antibiotic resistance can be inhibited.

Associate Professor Greg Moseley

EMAIL	greg.moseley@monash.edu
TELEPHONE	+61 3 9905 1036
OFFICE	Room 136, 19 Innovation Walk (Building 76)

TWO VACANCIES

A/Professor Greg Moseley



Immunofluorescence microscopy (upper panel) and 3D dSTORM super-resolution imaging (lower panel) of the cellular microtubule cytoskeleton associated with viral protein reveals significant differences between proteins of lethal (left) and non-lethal (right) viral strains.

Viruses pose one of the grand challenges to human and animal health globally and within Australia. Viral disease progression is critically dependent on the formation of specific interaction networks between viral proteins and host cell factors, which enable viral subversion of important cellular processes such as antiviral immunity and cell survival.

We use advanced cellular/molecular biology approaches including quantitative proteomics, structural biology, functional genomics, immune signalling assays, and live-cell/super-resolution imaging to elucidate these interactions at the molecular and cellular level, and viral reverse genetics and *in vivo* infection models to define their functions in disease.

Our major focus is on highly lethal human viruses including rabies/lyssaviruses, Nipah, Hendra, SARS-CoV-2 and Ebola virus, as well as a number of agriculturally significant and potentially zoonotic animal viruses. The overarching aim of the research is to identify new strategies for the development of novel vaccines and therapeutics for currently incurable diseases. Beyond disease, we also research the biology of so-called “Giant Viruses” that straddle the boundary between living cells and “non-living” viruses, to better understand this potential fourth domain of life.

The research involves extensive collaborations within Monash University and other leading national (e.g. University of Melbourne, CSIRO-ACDP high-containment facility) and international institutes (e.g. Pasteur Institute, Paris; Gifu and Hokkaido Universities, Japan; IITB, Mumbai, India), enabling access to unique resources and technologies including novel and highly pathogenic viruses.

Elucidating the Rabies Virus P Protein Axis

A/Prof Greg Moseley, Dr Stephen Rawlinson and Ms Cassandra David

Rabies is a currently incurable disease that has the highest fatality rate of known infectious diseases. The etiological agents of rabies are lyssaviruses, such as rabies virus and Australian bat lyssavirus. Despite a very limited genomic capacity these viruses are able to mediate replication, assembly and budding, while simultaneously arresting potent control over the infected cell and host immunity. Central to this are multifunctional proteins including P protein, which resides at the core of the virus-host interface, forming a myriad of interactions with viral and host proteins. We showed that by inhibiting such interactions, we can prevent otherwise invariably lethal disease, identifying the P protein ‘axis’ as a therapeutic target. However, the molecular/structural mechanisms by which this small protein coordinates/regulates its diverse interactions remain unresolved, leaving major gaps in knowledge concerning fundamental processes in a lethal human disease.

The project will seek to define the specific molecular surfaces mediating key interactions of P protein, and to analyse their function using mutagenesis. This will contribute to the elucidation of the structural organisation and regulatory mechanisms of the virus-host interface and help to define novel mechanisms by which viruses efficiently co-regulate host cell subversion and replication. These findings have the potential to redefine our understanding of the virus-host relationship and to provide critical tools and data for the development of new vaccines and antivirals.

Viral Reprogramming of Host Cell Signalling

A/Prof Greg Moseley, Dr Stephen Rawlinson and Ms Cassandra David

Central to the spread of pathogenic viruses is their capacity to interfere with host immunity, in particular the antiviral system mediated by cytokines such as the interferons. It is well known that many viruses target interferon signalling to shut down the expression of antiviral genes. However, our recent work indicates that the interaction of viruses with cytokine signalling pathways is much more complex and intricate, involving interaction with multiple cytokine signalling pathways through a number of mechanisms including the remodelling of cellular structures of the cytoskeleton and nucleus. Importantly, mutagenic analysis and viral reverse genetics, indicates that altering viral targeting of these pathways profoundly inhibits pathogenesis *in vivo*, indicative of critical roles in disease. We are currently seeking to delineate the precise mechanisms by which viruses, such as rabies, SARS-CoV-2 and Ebola interfere with and modulate cellular pathways, not only to inhibit antiviral signalling, but also to reprogram specific signalling pathways toward 'pro-viral' responses, a novel concept in viral biology.

Can Rabies Cure Alzheimer's?

A/Prof Greg Moseley, Dr Stephen Rawlinson and Ms Cassandra David

Neuroinflammation is a major factor in human pathologies such as stroke, Alzheimer's disease (AD), and traumatic brain injury (TBI). Viruses such as rabies/lyssaviruses, paramyxoviruses Nipah, Hendra, measles, coronaviruses, and Ebola virus, have evolved powerful mechanisms to shut down inflammatory signalling for immune evasion. We aim to discover the molecular 'tricks' used by viruses to subvert host immunity, and to harness these mechanisms to develop new methods to prevent inappropriate responses underlying neuroinflammatory disorders and other diseases including cancers.

Are Giant Viruses alive, and how do they interact with other microbes?

A/Prof Greg Moseley and Ms Cassandra David

The recently discovered giant viruses inhabit a unique evolutionary space, somewhere between living biological organisms and non-living microbes. The evolutionary history and fundamental biological nature of giant viruses remain poorly defined, including such fundamental questions as whether giant viruses are 'alive' and what these viruses mean for our understanding of life. We recently initiated new studies of giant viruses in collaboration with the Indian Institute of Technology Bombay (IITB) using techniques including super-resolution imaging and proteomics to understand the life cycle of these viruses and their structure and to define whether giant viruses have evidence for a biochemical 'spark of life'. We are also examining how giant viruses interact with other microbes, and the implications for their biology and evolution.

Super-Resolution Analysis of the Virus-Host Interface

A/Prof Greg Moseley and Dr Toby Bell

Viruses are experts at remodelling the infected cell, and can fundamentally alter cellular biology to transform host cells into efficient virus factories. Although molecular/biochemical evidence indicates that certain viral proteins can functionally modify structures such as the mitochondria, cell membranes, the nucleus and cytoskeleton, understanding of the physical effects on these structures is limited due to the poor resolving power of standard cell imaging approaches. Using single molecule localization techniques to surpass the physical diffraction limit of visible light, we have developed methods to observe and quantify the effects of viral proteins on cellular structures at super-resolution, enabling us to directly measure viral remodelling of the subcellular environment. Using this approach, we demonstrated that virus protein targeting of the cytoskeleton correlates with the capacity to cause lethal disease *in vivo*. The project will apply state-of-the-art single molecule localization techniques such as 3D dSTORM to define viral effects on cellular structures in unprecedented detail; this will provide new insights into the ways that viruses co-opt cellular function to cause disease.

Why do Cytoplasmic RNA Viruses Target the Nucleolus?

A/Prof Greg Moseley and Dr Stephen Rawlinson

Many diverse viral proteins have evolved independently to target the nucleolus but this phenomenon had been largely overlooked, particularly for RNA viruses that replicate within the cytoplasm. Following the development of advanced 'systems-biology' approaches to analyse nucleolar biology, it has become clear that the nucleolus is complex, dynamic, and highly multifunctional machine that coordinates many critical cellular processes including immunity and cell survival. This has redefined our understanding of the nucleolus and suggests that the virus:nucleolar interface might represent a central hub for viral hijacking of cellular processes, important to viral replication and pathogenesis.

Using highly pathogenic RNA viruse, including rabies and Hendra virus, we are investigating the mechanisms by which viruses can reprogram the nucleolus to alter cellular biology. These studies are identifying for the first time specific nucleolar functions for RNA virus proteins. The project will utilize techniques including molecular biology, proteomics, super-resolution microscopy, virus replication and gene expression assays, and gene knockout approaches to delineate the precise events underlying cellular dysfunction caused by virus- nucleolus interaction.

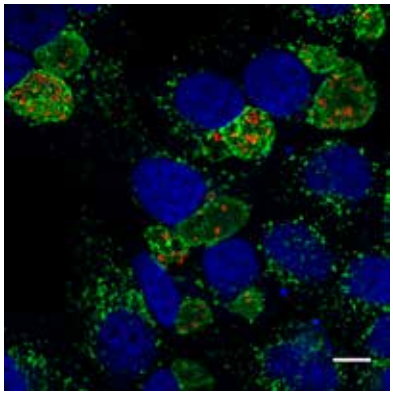
Professor Hayley Newton

EMAIL	hayley.newton@monash.edu
TELEPHONE	+61 3 9902 9159
OFFICE	Room 153, 19 Innovation Walk (Building 76)

TWO VACANCIES

Professor Hayley Newton

Dr David Thomas



Coxiella (red) replicates in a spacious lysosome-derived vacuole (green = lysosomal membrane protein).

Defining the mechanisms of lysosome survival by *Coxiella burnetii*

Professor Hayley Newton and Dr David Thomas

Our laboratory aims to create new knowledge in host-pathogen interactions driven by intracellular bacterial pathogens. Our research team has projects on *Coxiella burnetii, Legionella* species, *Salmonella enterica* and other intracellular bacterial pathogens. We are fascinated by *Coxiella burnetii*, the causative agent of Q fever, and *Legionella* species, the causative agents of Legionnaires' disease, which replicate inside human cells in a manner that requires the Dot/Icm type IV secretion system. This multi-protein apparatus delivers a huge number of novel effector proteins into human host cells, manipulating many aspects of human cell biology, to facilitate intracellular replication of the pathogen. However, very little is known about how individual effectors influence pathogen success.

The Dot/Icm type IV secretion system is central to the ability to establish a replicative niche within the human cell lysosome. However, even without this system *C. burnetii* can survive the degradative, hostile conditions of the lysosome. The key traits that make *C. burnetii* resistant to lysosomal killing remain unknown. This project will begin to examine the dynamics of *C. burnetii* lysosome resistance and develop a screening strategy for identifying bacterial factors that contribute to survival. Additionally, this project will examine the role of a specific *C. burnetii* protease that has been shown to be secreted into the *Coxiella*-containing vacuole. A mutant unable to make this protease will be examined in tissue culture models of infection and specialised mass spectrometry will be employed to identify the host targets of this pathogen protease. Techniques used in this project will include genetic manipulation of *C. burnetii*, cell culture infection and transfection, immunofluorescence confocal microscopy, protein biochemistry, mass spectrometry and molecular biology.

Characterisation of effector proteins that block human cell death

Professor Hayley Newton and Dr David Thomas

Coxiella burnetii employs a cohort of Dot/Icm effector proteins to block human host cell death allowing the pathogen to slowly replicate to large numbers intracellularly. We have preliminary data supporting a role in preventing host cell death for several novel *C. burnetii* effector proteins, but we do not yet understand their mechanism of action. This project will characterise one of these cell death blocking effectors by:

i) determining changes to infection dynamics associated with the absence of this effector

ii) examining the protection this protein offers human cells from different cell death stimuli

iii) identifying and characterising interactions between this effector and its host cell targets

This project will involve immunofluorescence confocal microscopy, protein biochemistry, mass spectrometry, molecular biology and the use of cell culture for infections, cell death assays and transfections.

Professor Anton Peleg

EMAIL	anton.peleg@monash.edu
TELEPHONE	+61 3 9902 9159
OFFICE	Room 153, 19 Innovation Walk (Building 76)

TWO VACANCIES


Professor Anton Peleg


Dr Margaret Lam


Dr Faye Morris


Dr Xenia Kostoulias


Dr Jhih-Hang Jiang

MECHANISMS OF PATHOGENESIS OF HOSPITAL-ACQUIRED ORGANISMS

Impact of Antibiotic Resistance on Immune Recognition of *Staphylococcus aureus*

Dr Jhih-Hang Jiang and Professor Anton Y. Peleg

S. aureus is one of the most common human bacterial pathogens, and is able to cause a wide range of life-threatening infections in the community and hospital setting. As a consequence of the rising rates of methicillin-resistant *S. aureus* (MRSA), agents such as vancomycin and daptomycin have been increasingly relied upon. Unfortunately, reduced susceptibility to these agents has now emerged. By using large-scale, whole-genome sequencing of clinical *S. aureus* isolates, whereby the first isolate is susceptible and the paired isolate is non-susceptible, we have been able to describe the genetic evolution of antibiotic resistance in patients. Interestingly, we have also identified, using both mammalian and non-mammalian model systems that these resistant strains have altered host-pathogen interactions, and appear to be more persistent.

Project 1

The aim of this project is to characterise the mechanisms of MRSA adaptation and evasion to antibiotic and host innate immune attack. The work will comprehensively identify genetic mutations that confer a survival advantage to MRSA under daptomycin pressure in the context of an immune response. This will be achieved by exposing clinically relevant MRSA strains to both antibiotic and host immune selection pressure, and apply a novel sequencing approach to characterise the full repertoire of mutations in specific phospholipid biosynthesis genes known to be important for antibiotic resistance. The significance of the identified mutations will be assessed by making independent mutants using our well-developed targeted mutagenesis system. The impact of individual mutations on antibiotic resistance, staphylococcal virulence, bacterial membrane biogenesis and host immune responses, will be assessed. This project will combine exciting bacterial genetic techniques and advanced biochemical approaches together with novel infection model systems.

Project 2

In collaboration with Professor Meredith O'Keeffe (Dept. of Biochemistry)

The aim of this project is to characterise the activation of pathogen recognition receptors by our paired susceptible and resistant clinical isolates. This will be achieved by studying one of the key first responders of our immune system; dendritic cells. Different dendritic cell types differ in their expression of pattern recognition receptors and hence the types of pathogens that they recognise. They also differ markedly in their subsequent innate response to pathogens, with discrete dendritic cell subsets specialised in the production of different cytokines and interferons. This project will focus on the differences in pathogen recognition and the subsequent immune activation between clinically important and drug-resistant *S. aureus* strains. Using established *S. aureus* mutants, we will also determine the impact of changes in bacterial surface characteristics on activation of pathogen recognition receptors.

Characterising Novel Virulence Mechanisms in the Emerging Hospital-Acquired Pathogen; *Acinetobacter baumannii*

Professor John Boyce, Dr Faye Morris, Dr Xenia Kostoulias and Professor Anton Peleg

Despite the significance of *Acinetobacter baumannii* in nosocomial environments, we currently have a very limited understanding of the connections between gene regulation, metabolic capacity and virulence. Our lab has previously identified numerous metabolic pathways, individual genes and small RNA (sRNA) molecules, important for in vivo fitness. We have generated an assortment of sRNA and individual gene mutants, and in this project we will characterise the genetic regulation, mechanisms of action and specific pathways through which these components contribute to the success of this pathogen.

By comparing our repertoire of strains (ie mutants, complements and overexpression strains) using high-throughput proteomics and RNA sequencing we will aim to identify and confirm the specific targets and pathways impacted, and validate these observations using a range of virulence-associated phenotypes (growth in human serum, biofilm formation, neutrophil chemotaxis and mouse infection models, etc). Where inactivation of particular pathways affects virulence, we will design and construct inhibitors and test these as novel antimicrobials.

Note: Working with animals is not compulsory for any of the advertised projects.

How does plasmid transmission drive antibacterial resistance and virulence in the clinic and the environment?

Dr Margaret Lam and Professor Anton Peleg

Plasmid transmission between bacteria of the same or different species is an important driver of genetic diversity, bacterial adaptation and evolution. In the clinical setting, the transmission of plasmids between hospital pathogens such as *K. pneumoniae* plays a critical role in the dissemination of virulence and antimicrobial resistance (AMR) genes that can subsequently imbue strains with the ability to cause invasive and untreatable infections. Outside of the clinical setting, bacterial samples from animal and environmental sources are also enriched with plasmids, and alongside the occasional detection of AMR genes, also encode for other lifestyle-enhancing traits such as heavy metal resistance or virulence.

Differences in plasmid distributions across the bacterial population and within different sampling niches highlight complexities in both the transmission dynamics of different plasmids and the varying ability of bacterial strains to uptake and maintain plasmids. For example, the AMR plasmids are detected at higher frequencies in the bacterial populations that circulate and cause outbreaks in hospitals. While comparative genomics and experimental evolution experiments have so far provided some insights into both bacterial and plasmid mechanisms responsible for this variation in transmission, many questions remain unanswered.

This project will use techniques within the comparative genomics space to examine how plasmid loads and types vary across *Klebsiella* strains from different sampling origins (i.e. clinical vs non-clinical) and genetically distinct lineages. These insights will be important for identifying 'high risk' species, lineages or plasmids that have higher plasmid transmission/maintenance rates, and therefore may be important targets in genomic surveillance or transmission intervention strategies.

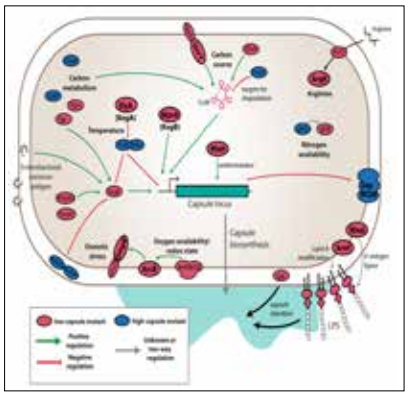
This project will largely utilise techniques in the genomics space including, but not limited to, *de novo* genome assembly and annotation, DNA sequence alignments, building phylogenetic trees and BLAST. The work is therefore suitable for students with an interest in computational biology and its application in studying bacterial pathogens.

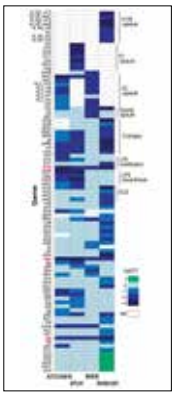
Dr Francesca Short

EMAIL	francesca.short@monash.edu
TELEPHONE	+61 3 9902 9282
OFFICE	Room 149, 19 Innovation Walk (Building 76)
WEB	https://research.monash.edu/en/persons/francesca-short

ONE VACANCY

Dr Francesca Short





(Left) Capsule regulation network of hypervirulent *Klebsiella pneumoniae*, constructed from high-throughput mutant profiling (Right) Strain-dependence of serum resistance in diverse *K. pneumoniae* isolates

Functional genomics to understand bacterial virulence control and adaptation

Dr Francesca Short and Dr Faye Morris

My research focuses on understanding virulence and antimicrobial adaptation in two Gram-negative 'superbugs' - *Acinetobacter baumannii* and *Klebsiella pneumoniae*. These bacteria require specific virulence factors such as protective capsule and iron-stealing siderophores for pathogenesis, but we have a very limited understanding of the molecular decision making that controls and coordinates these factors. My primary research focus is to address these knowledge gaps in order to improve prediction of phenotype from genotype in bacterial pathogens, and open the way for the development of new virulence-subverting drugs.

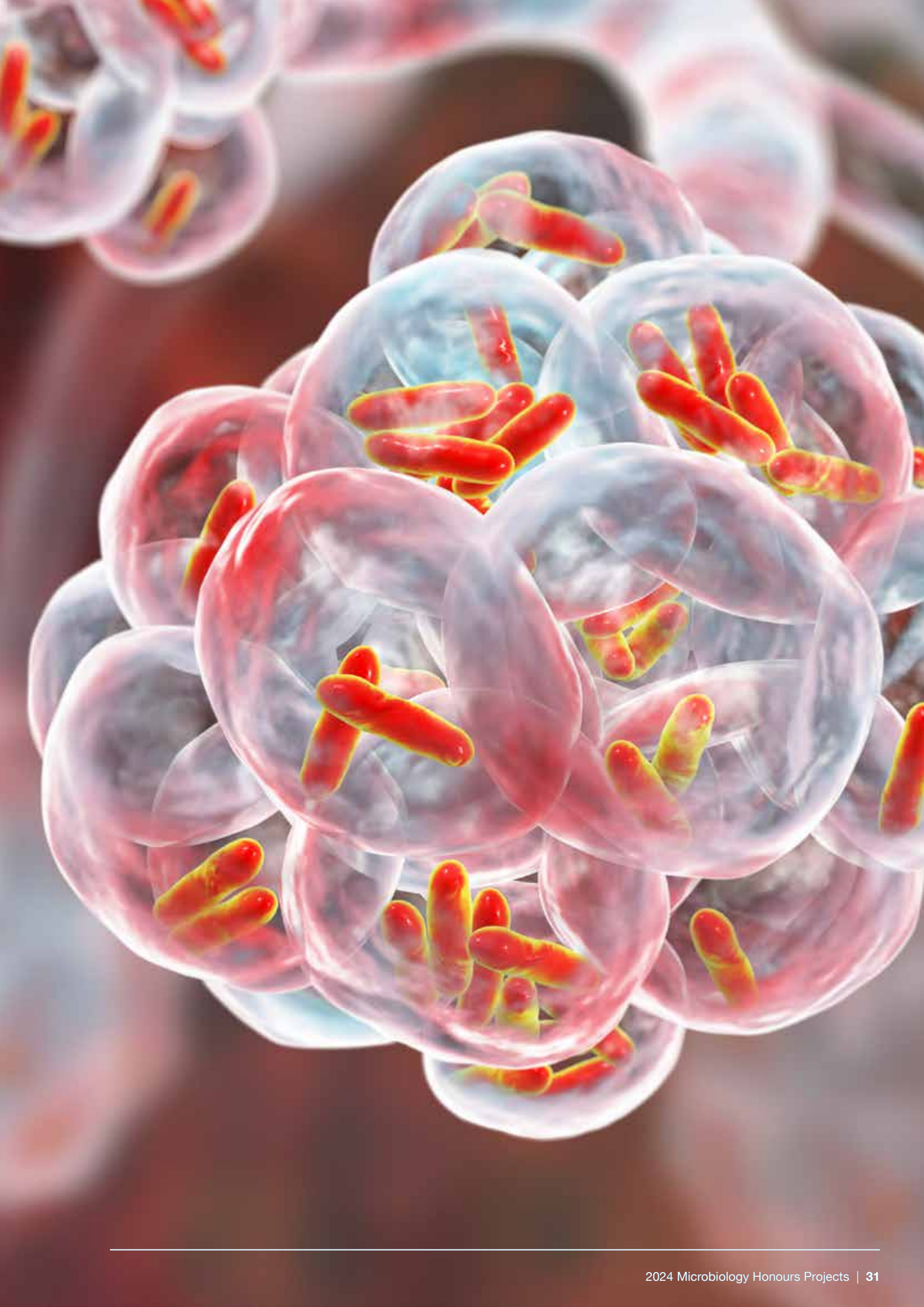
My second research interest focuses on the connections between disinfectant use and antimicrobial resistance. Disinfectants are essential for infection control but are poorly regulated, and there is increasing evidence that their use may be a hidden driver of AMR. I aim to understand the short- and long-term consequences of disinfectant exposure on target bacteria in order to prevent usage that compromises antibiotics. In all of my research I combine high-throughput genomic approaches with classic "wet-lab" microbiology to define the mechanisms underpinning bacterial behaviour. My research is funded by an ARC DECRA fellowship.

Characterisation of novel iron regulators in *K. pneumoniae*

K. pneumoniae is one of the most devastating pathogens of the antibiotic resistance crisis. This bacterium produces up to four distinct iron-stealing siderophores, which collectively determine the course and severity of infection. We have performed high-throughput functional genomic screens to identify novel transcriptional regulators contributing to iron acquisition in *K. pneumoniae*. This project will explore the function of one of these newly-identified regulators to find out what genes it controls, how it is distributed across the *K. pneumoniae* population, and how this regulator affects virulence and related phenotypes.

Understanding the collateral damage of benzalkonium chloride disinfectants

Recent research from our group and others has revealed that indiscriminate disinfectant use may be a hidden driver of antibiotic resistance for two reasons: 1) Adaptation to disinfectants often causes cross-resistance to certain antibiotics, and 2) Some disinfectants, when present, can directly antagonise antibiotic killing. This project will examine the effects of the widely used synthetic disinfectant - benzalkonium chloride - on the notorious hospital pathogen *Acinetobacter baumannii.* The trajectory of disinfectant adaptation will be defined under realistic exposure modes, and the consequences of these changes for antibiotic treatment will then be investigated. These long-term effects will then be matched with measures of direct benzalkonium chloride-antibiotic antagonism to build a complete picture of the interplay between this disinfectant, antibiotics, and the microbes they target.

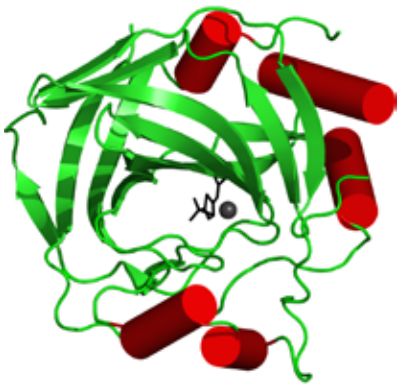


Associate Professor Anna Roujeinikova

EMAIL	Anna.Roujeinikova@monash.edu
TELEPHONE	+61 3 9902 9194
OFFICE	Room 151, 19 Innovation Walk (Building 76)

TWO VACANCIES

Associate Professor Anna Roujeinikova



H. pylori CA-inhibitor complex

Chemoreceptor sensory domain

Structural Studies of Virulence Factors of the Carcinogenic Bacterium *Helicobacter pylori*

Helicobacter pylori persistently colonize the epithelium of the stomach in roughly half of the world's population. It is a causative agent of gastric and duodenal ulcers, mucosa-associated B-cell lymphoma and gastric adenocarcinoma.

Although it is a definitive carcinogen, there is no effective vaccine against this bacterium. Standard *H. pylori* eradication therapy now fails in up to 30%-40% of patients, mainly due to an increase in clarithromycin resistance. There is a clear demand for new strategies to fight *H. pylori* infections, strategies that involve new or unconventional targets for drug design. A key to success with this lies in strong basic knowledge of the molecular basis of bacterial virulence and survival. Our laboratory focuses on the mechanisms of acid acclimation, damage to gastric epithelial cells and motility and chemotaxis. We use *in vitro* molecular biophysics and crystallography techniques to investigate structure and dynamics of biomolecules and formulate hypotheses about molecular mechanisms, which we then test *in vivo* using genetics, enzymology and cell biology methods.

Dissecting Architecture of High Torque Bacterial Motor

The bacterial flagellar motor is a remarkable nanoscale molecular engine. *H. pylori* evolved to be highly motile in the very viscous mucous layer of the stomach, and its flagellar motor is specialised for locomotion in viscous liquids – it produces a significantly higher torque (turning force) than, for example, enteric bacteria. Preliminary cryo-electron tomography reconstruction of this motor revealed a unique protein cage that supports a wider power-generating ring allowing it to sustain the larger torque. Our aim is to unravel the make-up of this cage and the structural basis for its ability to recruit more force-generating units.

How do Bacteria Sense Environmental Cues?

Many bacteria are motile. Chemotaxis, mediated by chemoreceptors, plays an important role in bacterial survival and virulence. In this project, we shall investigate what ligands such receptors recognize and why some molecules are attractants and some repellents, how binding to the receptor leads to signalling, how mutations in the sensor domain affect ligand specificity and, building on this, how bacterial chemoreceptors can be redesigned to recognize and respond to non-native ligands for innovative applications in biotechnology and bioengineering.

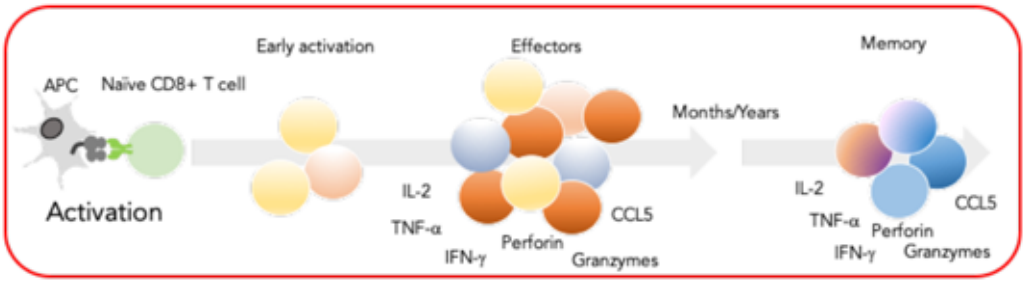
Applications are welcome from students with a strong interest in structural biology, X-ray crystallography, the biology of *H. pylori*, or protein biochemistry.

Professor Stephen Turner

EMAIL	stephen.j.turner@monash.edu
TELEPHONE	+61 3 9902 9138
OFFICE	19 Innovation Walk (Building 76)
WEB	https://www.monash.edu/discovery-institute/turner-lab

ONE VACANCY

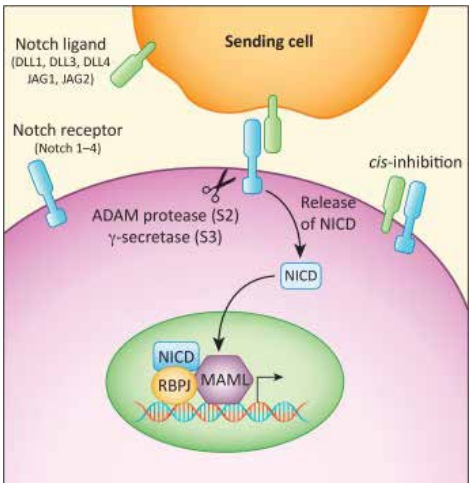
Professor Stephen Turner



Assessing the Role of NOTCH Signalling in Regulating Influenza-Specific Killer T Cell Immunity

Professor Stephen Turner

Virus infection triggers large-scale changes in the phenotype and function of CD8+ killer T cells and are critical for effective immune function; however, the precise gene regulatory mechanisms that control these changes are largely unknown. NOTCH is a cell surface receptor that is involved in regulating cell fate decisions in multiple systems. It mediates this via activation of a transcription factor called RBP-J. While NOTCH signaling is reported to be important for optimal virus-specific CD8+ T cell responses, the molecular mechanisms that underpin this regulation are not known. Our lab has recently shown that inhibition of NOTCH signaling can impact acquisition of CD8+ T cell function. This project aims to use mice where RBP-J deletion is limited to CD8+ T cells to assess the impact of RBP-J deficiency on influenza A virus-specific T cells responses. This project will use a combination of virology, cellular immunity, molecular biology and biochemistry to assess the impact of RBP-J deficiency on influenza A virus-specific killer T cell function and establishment of immunological memory.



Activation of NOTCH receptor by NOTCH ligands, such as delta-like ligand (DLL) family members, results in activation of the NOTCH signaling pathway. Upon activation, the NOTCH receptor is cleaved by a protease called γ-secretase, which releases the NOTCH Intracellular Cytoplasmic Domain (NCID). This is a transactivating protein that is transported to the cell nucleus where it pairs with RBP-J and another DNA binding protein called Mastermind-like (MAML). This complex then activates transcription of target genes. This project will examine the impact of RBP-J deficiency on CD8+ T cell activation after virus infection.(Figure adapted from Totaro et al., Trends in Cel Biology, 2018).

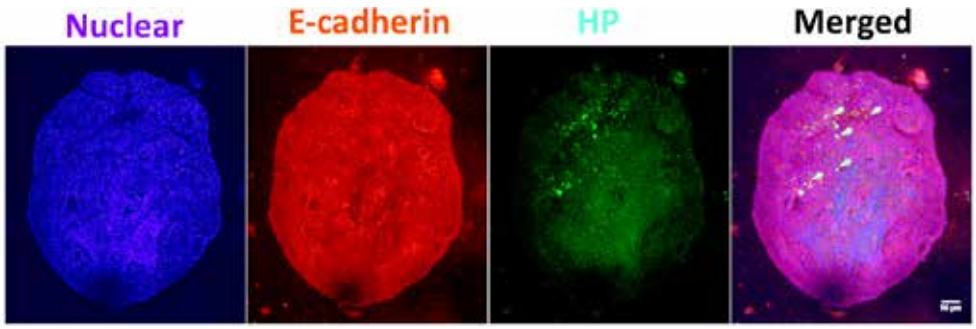
Projects Based at Affiliated Institutions

Professor Richard Ferrero

EMAIL	richard.ferrero@monash.edu or richard.ferrero@hudson.org.au
TELEPHONE	+61 3 9282 2111
OFFICE	Hudson Institute of Medical Research, 27-31 Wright St, Clayton
WEB	hudson.org.au/gastrointestinal-infection-and-inflammation/

TWO VACANCIES

Professor Richard Ferrero



H. pylori bacteria (green) within a gastric organoid. (Images courtesy of L. S. Tran and G. Kerr)

Defining the Role of a Novel NLR Protein in Stomach B Cell Lymphoma Associated with Chronic *Helicobacter* Infection

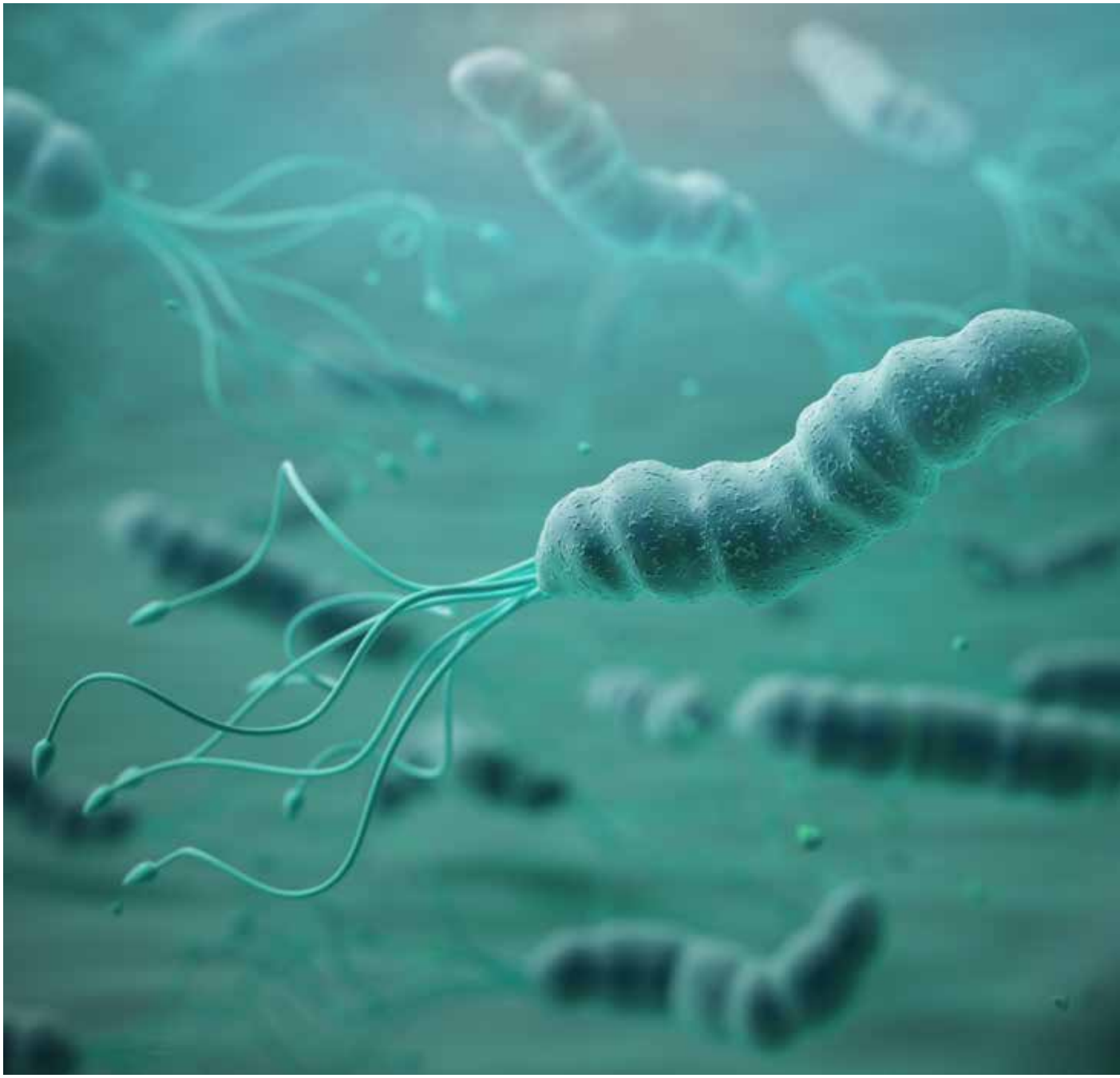
Professor Richard Ferrero and Dr Dongmei Tong

Our laboratory has identified a new NOD-like receptor (NLR) protein in the regulation of inflammation in responses to chronic *Helicobacter pylori* infection. Specifically, we have shown that conditional knockout mice lacking this NLR exhibit an accelerated formation of gastric B cell mucosa-associated lymphoid tissue (MALT), consistent with the early stages of MALT lymphoma, in response to chronic *Helicobacter* infection. We are now investigating how this novel NLR prevents B cell lymphomagenesis induced by chronic infection and whether this protein may play much broader functions in the host immune system. A particular focus of current studies is the role of NLRC5 in regulating anti-tumour immune responses. These studies will be undertaken using both *in vitro* and in *vivo* models, including conditional knockout mice. The project will involve various techniques, such as primary cell culture, mouse infection, immunohistochemistry, flow cytometry, cytokine ELISA and qPCR.

Characterisation of the Immunomodulatory and Oncogenic Properties of Bacterial Extracellular Vesicles

Professor Richard Ferrero and Dr Caroline Skene

The release of extracellular vesicles (EVs) is a property that has been conserved by both multi- and unicellular organisms during evolution. One of the major functions of these EVs is to facilitate intercellular communication and transport of molecules. The release of EVs by prokaryotes was first described over 50 years ago, yet the biological significance of these structures is only beginning to be appreciated. We have shown that bacterial EVs are potent modulators of host immune responses. Several projects are available to investigate how bacterial-derived EVs target the nucleus, interfere with host cell functions and even promote oncogenesis. These projects will involve cell culture and, potentially, mouse models to elucidate EV interactions with host cells and to characterise the responses induced by EVs. A variety of techniques will be used, including cell culture, mouse models, proteomics, molecular biology, fluorescence imaging, flow cytometry, cytokine ELISA and qPCR.



Development of a Vaccine to Prevent Stomach Cancer using Bacterial Vesicles

Professor Richard Ferrero and Dr Caroline Skene

It is estimated that half of the world's population have *Helicobacter pylori* infection. This bacterium lives in the stomach where it causes inflammation of the mucosa. Most individuals, however, do not know that they are infected. As a consequence; stomach cancer is often diagnosed once the disease is already advanced. Although antibiotic therapies are available to eliminate *H. pylori* infection, these are not always effective. Therefore, new approaches are needed to better manage the infection and associated disease.

A vaccine against *H. pylori* infection would be the most cost-effective means of preventing stomach cancer. Although vaccine trials in animals reported promising results, subsequent findings from clinical studies have generally been disappointing. The proposed project is directed at developing an entirely new type of vaccine based on bacterial vesicles. This project will involve a variety of techniques, such as molecular biology, bacterial mutagenesis, cell culture, mouse models, proteomics, fluorescence imaging, flow cytometry, cytokine ELISA and qPCR.

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Dr Sam Forster

EMAIL	sam.forster@monash.edu
TELEPHONE	+61 3 8572 2753
OFFICE	Room L2.52, Hudson Institute of Medical Research, 27-31 Wright St, Clayton
WEB	https://hudson.org.au/research-group/microbiota-systems-biology/

TWO VACANCIES

Dr Sam Forster

Dr Emily Gulliver

Dr Michelle Chonwerawong

Human Gastrointestinal Bacteria

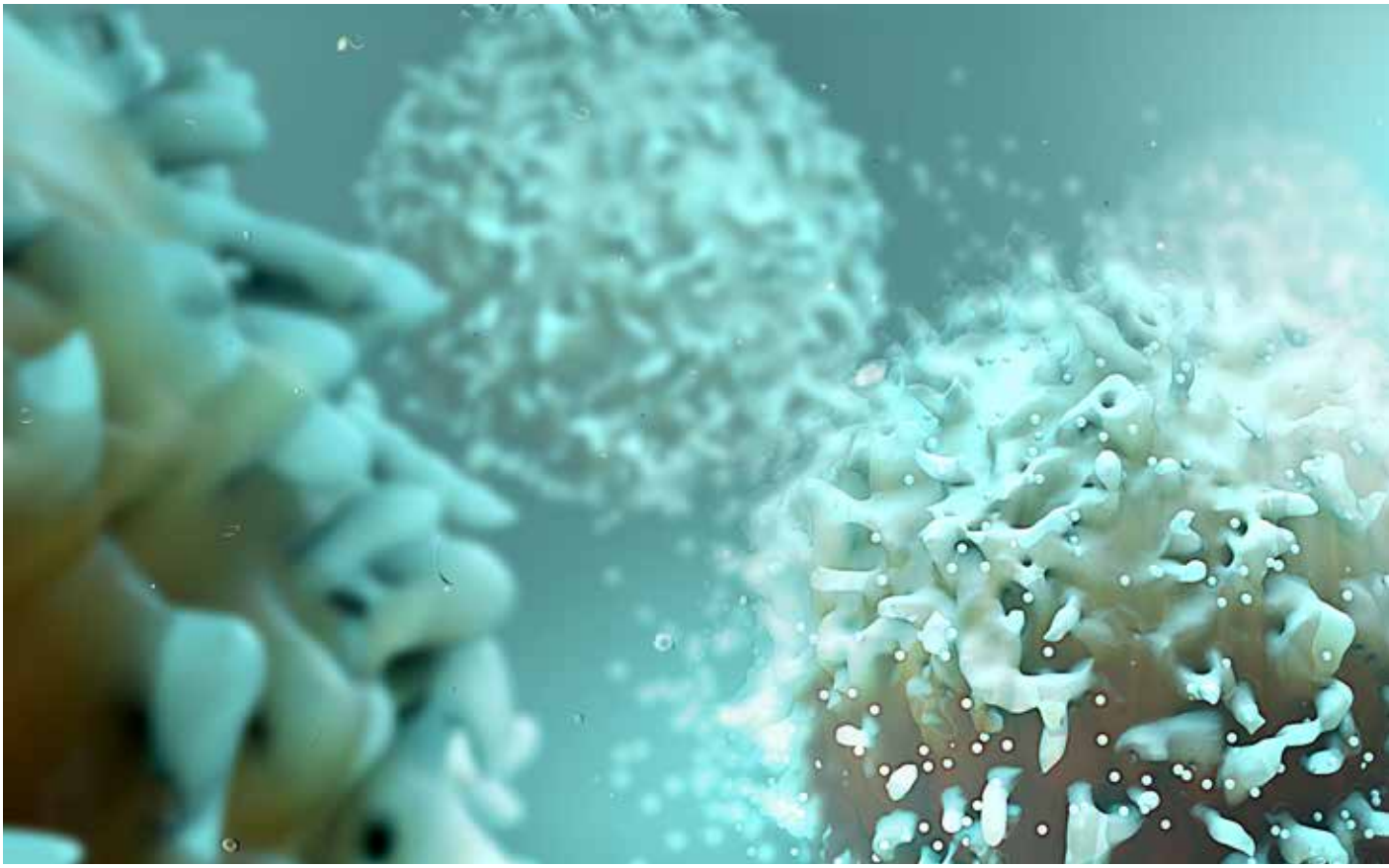
Understanding Microbiome Interactions with the Innate Immune System

Dr Sam Forster and Dr Michelle Chonwerawong

The innate immune system is capable of intricately detailed detection, differentiation and elimination of pathogenic bacteria. However, the vast majority of bacteria encountered by our innate immune system are beneficial to health. Indeed, over 500 species of these commensal bacteria, containing approximately 10,000-fold more genes than the human genome, exist in the human gastrointestinal tract alone.

Emerging research is demonstrating the importance of these bacterial communities in both maintaining health and causing or exacerbating disease. We have recently developed novel methods to grow the vast majority of bacteria from the gastrointestinal microbiota. This research has resulted in the discovery of hundreds of novel species which require further investigation. Combined with the established experimental and computational expertise in the analysis of innate immune signalling pathways, this project will include cutting-edge microbial culturing techniques, cell culture assays and advanced computational analysis to identify pro- and anti-inflammatory bacterial species.

Students interested in experimental or computational elements, will have the opportunity provided to develop skills in both areas.



Discovery of Antibiotic Resistance Gene Dispersal in the Human Microbiome

Dr Sam Forster and Dr Emily Gulliver

Antimicrobial resistance (AMR) is emerging at an alarming level, rendering some bacterial infections untreatable and increasing dependence on last-line antibiotics. There is an urgent need to provide clinicians with the data to inform antibiotic selection that will optimize treatment success, while minimizing the spread of resistance-containing species and dispersal of antibiotic resistance genes.

Despite the bacterial diversity within our microbiota, current understanding of the genetic factors that confer resistance is almost exclusively limited to pathogenic or opportunistically pathogenic organisms. For example, in the human gastrointestinal tract, there are 100 trillion bacteria, representing more than 500 species, which are exposed to selection for antibiotic resistance during oral antibiotic treatment. The resistance mechanisms in these commensal bacteria remain largely undefined, despite representing a significant, hidden source of antibiotic resistance genes that could be transferred to pathogenic or other commensal bacterial species.

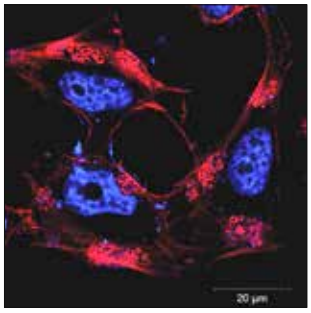
We have recently developed methods to culture the vast majority of the human gastrointestinal microbiota (Nature, 2016; Nature Biotechnology, 2019), which will provide an important resource to undertake these studies. This project will combine detailed genomic and metagenomic sequence analysis with *in vitro* microbiology to understand and monitor the diversity and distribution of antibiotic resistance within the human gastrointestinal microbiota. The opportunity also exists to focus the project to experimental or computational biology.

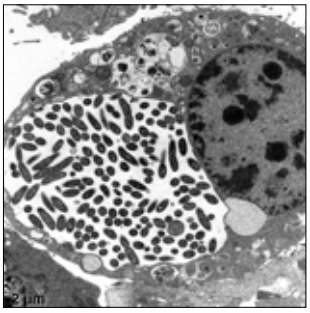
Professor Elizabeth Hartland

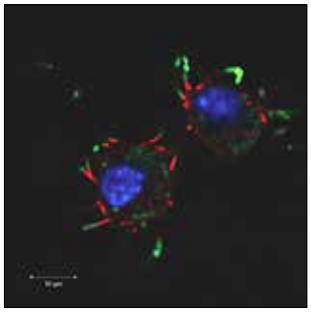
EMAIL	elizabeth.hartland@hudson.org.au
TELEPHONE	+61 3 8572 2800
OFFICE	Hudson Institute of Medical Research, 27-31 Wright St, Clayton
WEB	hudson.org.au/research-group/innate-immune-responses-infection/

TWO OR THREE VACANCIES

Professor Elizabeth Hartland







Left to right, Enteropathogenic *E. coli* adhering to epithelial cells and causing the rearrangement of actin (red) underneath the adherent bacteria. Cell nucleus (blue). Electron micrograph showing the replicative vacuole of the Legionnaire's pathogen, *Legionella pneumophila*. Actin tail formation (red) by the intracellular bacterium, *Burkholderia thailandensis* (green). Cell nucleus (blue).

Innate Reponses to Bacterial Infection

The subversion of host cell processes by microbial pathogens is an intrinsic part of the host-pathogen interaction. Many bacterial pathogens have the ability to transport virulence proteins, called effector proteins, into host cells via specialised protein secretion systems. We work on a range of virulence effectors from pathogenic *E. coli, Shigella, Legionella* and *Burkholderia* species that interfere with host innate immune responses. The aim of this work is to investigate the manipulation of host cell signalling and cell intrinsic immunity by effector protein families to understand their influence on host cell function, inflammatory signalling and the innate immune response. In this way effector proteins can be used as tools to understand the innate responses important for control of the pathogen.

The microvillus protein Eps8 as a target of EPEC infection

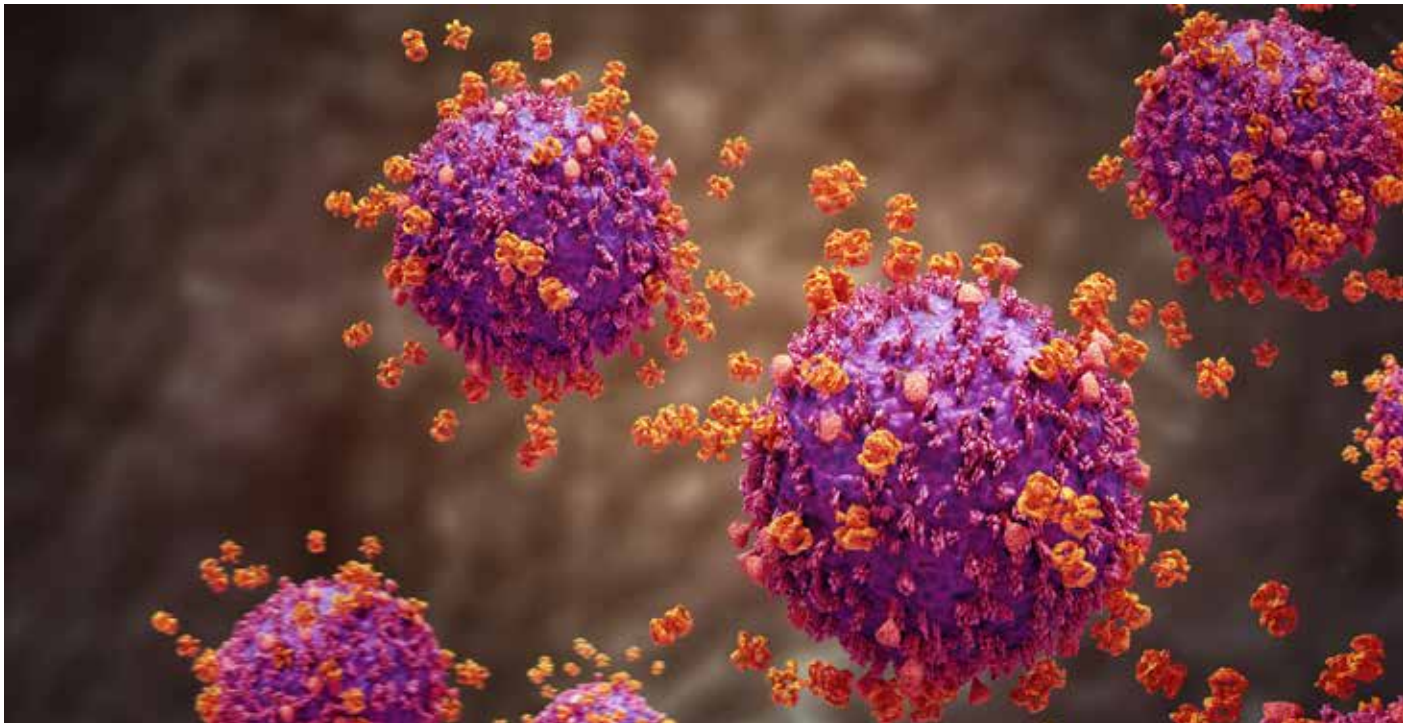
Professor E. Hartland, Dr C. Giogha, and Dr E. Chan

A hallmark of EPEC infection is the formation of attaching and effacing (A/E) lesions on the apical surface of host enterocytes, which are characterised by the destruction of microvilli and rearrangement of the host cell cytoskeleton at the site of bacterial attachment. We recently discovered that the EPEC T3SS effector kinases, NleH1 and NleH2 both specifically phosphorylate host EPS8, an actin bundling protein that is enriched at the tips of microvilli. Despite the important role of EPS8 in maintaining normal microvillus structure, the function of the protein and the basis of its localisation to the tips of microvilli is unknown. Since most studies of EPEC infection have not used cellular models that support microvilli, here we will use a polarized cell model of EPEC infection as well as human small intestinal organoids to determine the role of NleH1 and NleH2 in microvillus effacement during EPEC infection. This project will inform our understanding of A/E lesion formation in complex human tissue and help to elucidate the role of EPS8 in microvillus function. This project will employ techniques such as bacteriology, human organoid culture, bacterial infection, molecular biology, and high resolution microscopy.

Targetting of host mRNA by the intracellular pathogen, *Legionella pneumophila*

Professor E. Hartland, Dr K. McCaffrey and Dr R. Wibawa

Legionella pneumophila translocates more than 300 effector proteins into the infected macrophage that manipulate a range of host cell processes. We have discovered that one effector protein induces the degradation of mRNAs



encoding enzymes involved in glycolysis. We hypothesise that the effector is a novel RNA-binding protein and that this interference with host cell metabolism blocks the activation of alveolar macrophages, where the switch to a pro-inflammatory phenotype coincides with increased glycolysis. This project will define the mechanism of action of the anti-glycolytic effector protein and explore the impact of *L. pneumophila* infection on macrophage metabolism. We will also identify additional RNA-binding effectors from *Legionella* and other intracellular bacteria. This project will employ techniques such as bacteriology, protein biochemistry, molecular biology, confocal microscopy, live imaging and mammalian cell infection, mouse infection models and immunological analyses.

Understanding the role of T3SS effectors in *Burkholderia* infection

Professor E. Hartland, Dr G. Ng and Dr R. Wibawa

Melioidosis is a life threatening infection caused by the environmental bacterium, *Burkholderia pseudomallei*. *B. pseudomallei* has three T3SSs, one of which aids bacterial escape from the phagosome into the cytosol. Despite their significance in infection, relatively little is known about T3SS effector proteins and their role in melioidosis disease. This project aims to identify T3SS effector proteins of *B. pseudomallei* using a range of approaches, including bioinformatics to probe the *B. pseudomallei* genome for novel T3SS effector genes. We will develop assays and proteomics based methods to identify effectors secreted upon activation of the T3SS. Given our recent work on T3SS effector proteins that dampen the host immune response, we will prioritise screening for effectors that block inflammatory and cell death signalling. Effectors yielding interesting phenotypes will be functionally characterised during infection. Ultimately this will allow us to understand the molecular mechanisms by which *B. pseudomallei* causes disease. This project will employ techniques such as bacteriology, mass spectrometry, high-throughput screening, genetic modification of bacterial pathogens, cell culture, protein expression and purification, molecular biology, western blot, confocal microscopy and mouse models of infection.

Discovering the biochemical activity of NleA family T3SS effectors

Professor E. Hartland, Dr C. Giogha, Dr J. Gan and Dr E. Chan

The EPEC T3SS, NleA, is one of many effectors translocated into host enterocytes during EPEC infection. However, unlike other effectors, deletion of the gene encoding NleA strongly attenuates intestinal colonization by the pathogen. Several host binding partners of NleA have been described, including the inflammasome protein, NLRP3 and Golgi vesicle transport protein, Sec24. Despite this knowledge and the importance of NleA to EPEC virulence, the actual biochemical activity of NleA is unknown. Recent genome studies have also shown that NleA effectors can be found in *Shigella* and *Salmonella* species. Here we will apply advanced mass spectrometry techniques to identify possible post translational modifications of known binding partners of NleA. As nearly all experimental work to date on NleA has used non-intestinal and non-polarised epithelial cells, here we will perform additional unbiased pulldowns from infected intestinal organoids to and mass spectrometry identification to identify novel NleA binding partners. This project will employ techniques such as mass spectrometry, genetic modification of bacterial pathogens, cell culture, protein expression and purification, molecular biology, western blot, confocal microscopy, bacteriology and infection.

School of Public Health & Preventative Medicine and Department of Civil Engineering, Monash University

Dr Rebekah Henry

EMAIL	rebekah.henry@monash.edu
TELEPHONE	+61 3 9905 5068
OFFICE	Building 37, 17 Alliance Lane

ONE VACANCY

Dr Rebekah Henry

Dr Yussi Palacios Delgado

RISE Settlement Suva

The One Water Laboratory

The One Water Laboratory is located within the Civil Engineering Department at Monash University, Clayton, and has a specific focus on health-related urban water microbiology; some of our projects include: (1) understanding the pathways pathogens follow in informal urban environments, (2) development of passive sampling methods for use in wastewater based epidemiology, (3) understanding the human health risks caused by bacterial pathogens in water systems (reservoirs, rivers, ocean) and (4) understanding microbial community dynamics in complex environments.

Understanding Bacterial Contamination of Water Systems in Informal Settlements

Dr Rebekah Henry (School of Public Health), Dr Yussi Delgado (Dept. Civil Engineering) and Professor John Boyce (Dept. Microbiology)

Urban informal settlements, which concentrate more than a billion people living in poor and inadequate sanitation conditions, are hotspots of environmental contamination due to absent or inadequate access to sanitation services and infrastructure. High level of nutrient, changes in dissolved oxygen concentration and pH, are some of the factors that are reshaping microbial communities around the world and further impacting human health.

The Revitalising Informal Settlements and their Environment (RISE) program, which was established in 2018 in informal settlements of Indonesia and Fiji, is concurrently enabling us to study both anthropogenic and environmental factors that may impact nutrient levels, *E. coli* concentrations and physicochemical variations in water bodies. This is planned to help us disentangle key sources and processes that raise pollution in informal settlements of low- and middle-income countries.

Understanding sources and processes that lead to environmental pollution will be crucial to develop effective management and mitigation strategies to improve environmental health and decrease human exposure to pathogens in low-and middle-income countries. Honours students are welcome to join our team, which has a 5-years data collection of chemical, physicochemical data of water samples collected from 12 informal settlements of Fiji and Indonesia. Students will gain knowledge of current international standards in water quality assessment alongside analysis of genomic and targeted PCR analysis, which also includes an *E. coli* and coliform concentration database that is linked to rainfall data and a full pathogen and genomic data set for microbial community structure and metagenomic analysis.

Development of Rapid Field Sequencing Method

Dr Rebekah Henry (School of Public Health), Dr Yussi Delgado (Dept. Civil Engineering) and Professor John Boyce (Dept. Microbiology)

Monitoring of water bodies has an essential role in maintaining the public health of local populations. Catchment scale water quality monitoring facilitates sustainable management practices and provides essential information on potential drivers of change i.e. biological, chemical or environmental factors. To be informative the monitoring and quantification methods applied must have adequate sensitivity, and limits of detection, to not only alert governing bodies as to when quality has degraded, but long-term intensive data for a particular catchment. A problem arises; however, in pristine waterbodies, such closed catchment reservoirs, where contaminant concentrations are often below method detection limits. However, these waters still have a zoonotic disease risk from local wildlife populations.

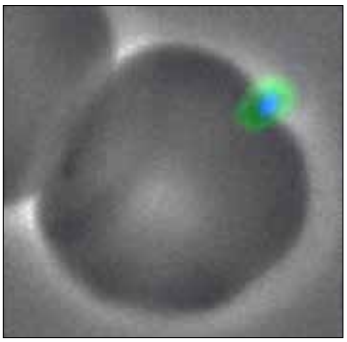
Improved method sensitivity and specificity on closed catchment water sources is required for informed mitigation, public health assessment and economic benefit. Rapid sequencing technologies (RST) enable submission, sequencing and reporting of a low number of samples (<1-5) within a 5-day period at significantly reduced cost. Representing a significant advantage over standard techniques, particularly in the water monitoring space. As part of this project students will sequence individual faecal DNA extracts and undertake RST methods to optimize and develop a library of catchment specific faecal sequences for comparison to existing libraries. Students will work alongside industry practitioners and gain an understanding of applied microbiological practice.

Professor James Beeson

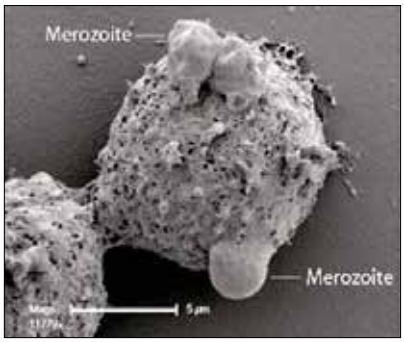
EMAIL	james.beeson@burnet.edu.au chrissie.collins@burnet.edu.au
TELEPHONE	+61 3 9282 2111
OFFICE	85 Commercial Road, Melbourne (AMREP Campus)

ONE VACANCY

Professor James Beeson



Immunofluorescence microscopy showing invasion of a human red blood cell by a malaria merozoite (green)



Phagocytosis of malaria merozoites by a human monocyte, an important mechanism of immunity, visualized using electron microscop

Developing Vaccines Against Malaria

Malaria is one of the world's leading causes of death and illness, particularly among young children. There remains a strong need for highly effective vaccines to reduce the burden of malaria and progress towards eventual malaria elimination. To date, most vaccines have achieved only modest levels of efficacy, emphasising the need for novel approaches in vaccine design that can induce potent immune responses.

This project will focus on identifying key antigens and specific epitopes that are targets of protective immunity against malaria and understanding the mechanisms mediating immunity, which includes antibodies and cell-mediated responses.

This knowledge is crucial for the development of effective vaccines against malaria. The project will also involve using knowledge of immunity to malaria for informing vaccine design, and the expression and testing of novel vaccine candidates. These studies will use novel approaches in molecular biology, cell biology and immunology to address these aims, and will build on recent major advances generated from our malaria vaccine program.

The project will primarily involve laboratory-based research, including western blotting, imaging, standard immunoassays, functional immunoassays (e.g neutralisation assays, cell-mediated immunity), flow cytometry, cell culture and protein expression. The project links with our malaria vaccine developoment work using mRNA, protein, and peptide-based vaccine platforms. The project could also include bioinformatics, structural modelling of vaccine antigens, or modelling vaccine impact depending on the student's interest. The specific activities and focus of the project will be refined to suit the interests and training background of the student and align with our research group's priorities. Interested students should contact Chrissie Collins, chrissie.collins@burnet.edu.au

Discovering the Mechanisms and Targets of Immunity Against Malaria

Antibodies are an important component of acquired immunity against malaria, as demonstrated in pivotal studies in which immunoglobulin G (IgG) from immune adults was transferred to malaria-infected children and resulted in clearance of infection. In recent studies, we have begun to uncover important roles for antibodies that can directly inhibit host-cell infection, interact with immune cells to kill and clear malaria, or recruit complement to neutralise infection.

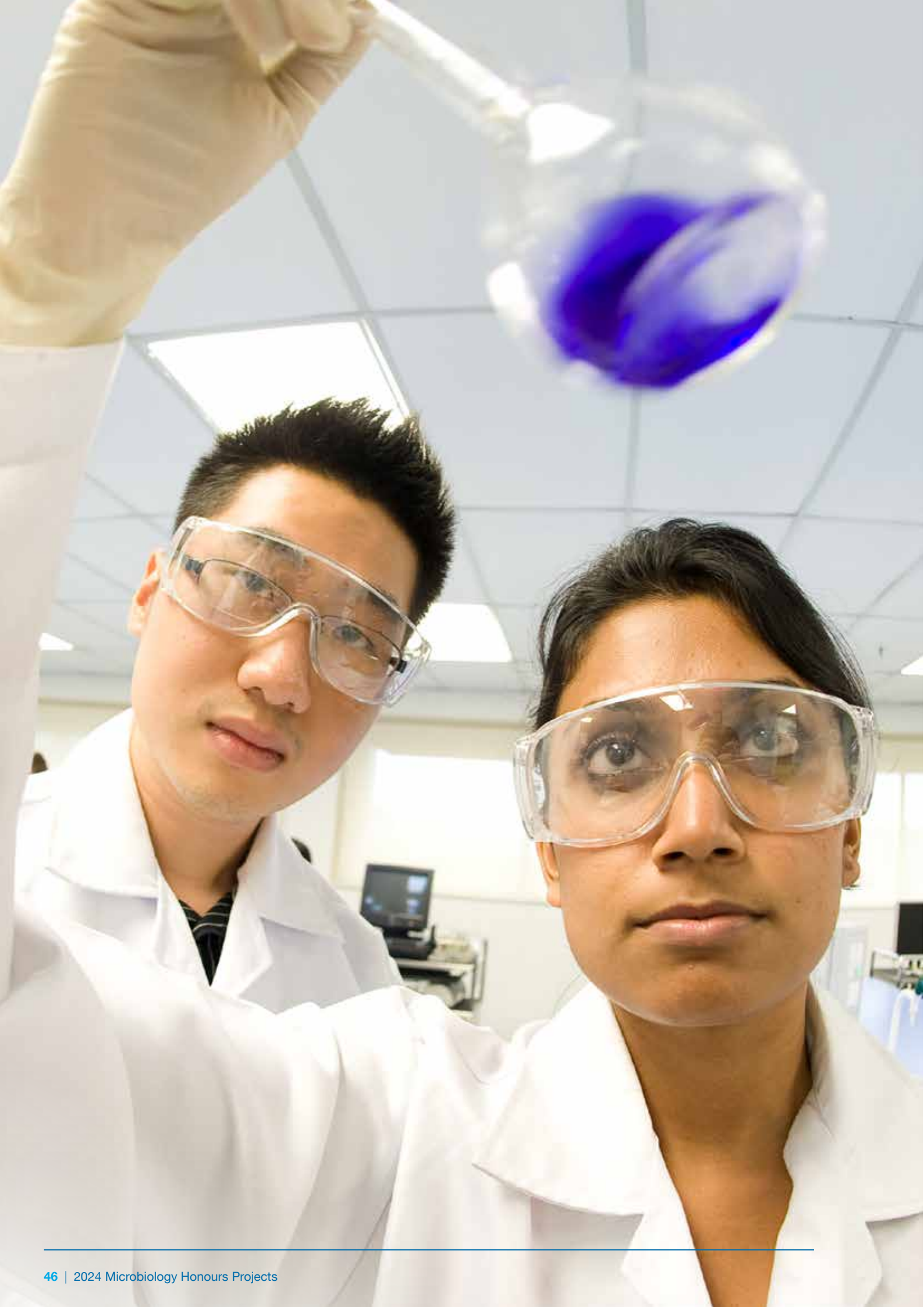
The aims of this project include identifying the key targets of specific mechanisms mediating immunity. The project may combine detailed studies of immune responses with clinical and population studies in Africa, Asia, and Papua New Guinea. It will examine how immune responses protect children from malaria or protect pregnant women and their developing babies from the devastating consequences of malaria in pregnancy.

The studies would particularly focus on using innovative approaches to understand how antibodies neutralise and clear malaria parasites in the blood, including interactions with monocytes/macrophages and dendritic cells, and identifying specific epitopes targeted by protective antibodies. Skills may involve assays of functional immunity, cell culture, isolation and analysis of immune cells, flow cytometry, western blotting, ELISA, and epitope mapping. The project will be tailored to best match the student's interests and training background and research priorities of the group. Interested students should contact Chrissie Collins, chrissie.collins@burnet.edu.au

Immunity to SARS-CoV-2 in Africa

Continuing epidemic waves of COVID-19 are anticipated in many countries because of emerging viral variants, the moderate efficacy of vaccines being implemented in many countries, waning immunity and incomplete vaccine coverage. The protective efficacy and longevity of immune responses induced by SARS-CoV-2 infection and vaccines vary markedly between individuals and populations. Knowledge on immunity to SARS-CoV-2 in African populations, and factors that impact on immunity, is limited but is important for understanding COVID-19 morbidity and prevention. Repeated exposure to malaria, intestinal parasites, and respiratory viruses, as well and specific nutritional deficiencies are common in sub-Saharan Africa, and can impact on innate and adaptive immunity to influence the magnitude and longevity of immunity.

As part of an international collaborative program, this project will investigate immunity in cohorts of naturally infected and vaccinated individuals in Malawi (central Africa) and address important knowledge gaps. The project will involve laboratory-based studies in Melbourne that assess the acquisition and longevity of immunity generated by SARS-CoV-2 infection and vaccination and determine how malaria and intestinal parasite infections, undernutrition, and anemia impact on immunity. We will use a comprehensive range of immunoassays including quantification of antibody magnitude (subclasses and isotypes), neutralizing antibodies to different variants, avidity and antibody-dependent cellular cytotoxicity. Additionally, studies will profile specific epitopes targeted by immunity and their relationship to immune escape by virus variants. The overall objective of this work is informing strategies to improve protection from COVID-19, and identifying those most at risk, by understanding key gaps in natural and vaccine induced immunity. Interested students should contact Chrissie Collins, chrissie.collins@burnet.edu.au



Victorian Infectious Diseases Reference Laboratory,
Peter Doherty Institute for Infection and Immunity.

Professor Peter Revill

EMAIL	peter.revill@mh.org.au
TELEPHONE	+61 3 9342 9604
OFFICE	Research and Molecular Development (Revill lab) VIDRL Peter Doherty Institute for Infection and Immunity, 792 Elizabeth St, Melbourne VIC 3000

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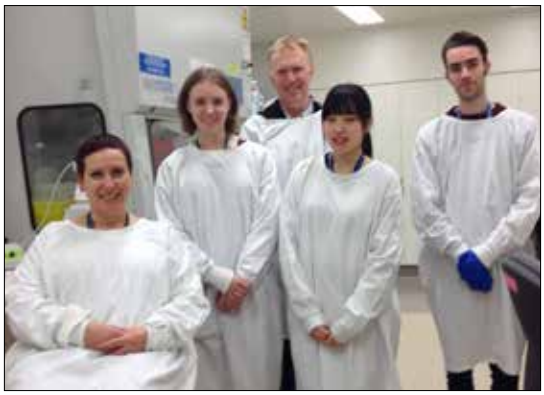
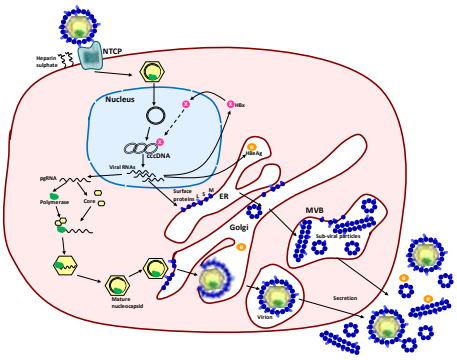


Revill group



The hepatitis B life cycle

Determining the importance of conserved residues identified throughout the HBV genome on viral replication and their potential as new therapeutic targets.

Professor Peter Revill and Dr Margaret Littlejohn

We have identified a number of residues throughout the HBV genome that are 100% conserved across all major HBV genotypes and phases of chronic HBV disease. This project will investigate which of these conserved sequences are most important for HBV replication and thus represent potential antiviral targets, using a range of *in vitro* and *in vivo* models. Techniques to be utilised include cell culture; HBV transfection and infection, DNA and RNA purification; northern, Southern and western blotting; quantitative serology; siRNA or CRISPR knockdown; PCR, droplet digital PCR and sequencing.

Professor Gilda Tachedjian

EMAIL	gilda.tachedjian@burnet.edu.au
TELEPHONE	+61 3 9282 2256
OFFICE	Retroviral Biology and Antivirals Laboratory (RBA) (Tachedjian Lab), Life Sciences Discipline Disease Elimination and Health Security Programs, Burnet Institute
WEB	www.burnet.edu.au/working_groups/21_tachedjian_laboratory

UP TO THREE VACANCIES

Top: Lactobacilli attached to a vaginal epithelial cell. Bottom: Cell infected with HIV (green dots). Right: An Australian black flying fox (Pteropus alecto).

Bat Antiviral Defenses Against Viruses

Professor Gilda Tachedjian, Dr Joshua Hayward and Dr Paula Ellenberg

Bats are a major reservoir of viruses such as Hendra virus, Ebola virus, and coronaviruses that are pathogenic in humans but not in bats. The reason why bats can coexist with these viral pathogens is unknown. However, one explanation is that the coevolution of bats and their viruses has resulted in a 'peace treaty', a biological equilibrium in which viral replication is regulated in such a way that both host and virus can co-exist without undue antagonism. The role of the host's immune system is to control infections and antiviral restriction factors are at the front line of the innate immune response that targets viruses. These antiviral restriction factors were originally discovered to restrict retroviruses from other mammalian species, such as HIV (Hayward *et al* 2018 Mol Biol Evol 35(7)), and some of these factors e.g. tetherin, can additionally restrict paramyxoviruses, coronaviruses and filoviruses. Bats have recently been found to be an important reservoir of retroviruses from the genus *Gammaretrovirus* and are involved in transmission of retroviruses between mammalian species (Hayward *et al* 2020 PNAS 117(17)). The genomic fossil record of endogenous retroviral seequences, which are present as a critical part of eukaryotic genomes, also indicates that bats have a long history of infection with gammaretroviruses and betaretroviruses indicating that retroviruses have circulated in bats throughout their evolutionary history. How the innate immune system of bats has adapted to tolerate or combat infection with retroviruses remains largely unexplored, and this project seeks to further characterise the interactions between bat restriction factors and the Hervey pteropid gammaretrovirus (HPG), the first extant retrovirus discovered in bats Hayward, Tachedjian et al. 2020 PNAS 117(17). This is a joint project between the Tachedjian Lab and CSIRO Australian Animal Health Laboratory.

Vaginal Microbiota and HIV Susceptibility

Professor Gilda Tachedjian, Dr Anna Hearps, Dr Lindi Masson, Dr Paula Ellenberg and Dr Joshua Hayward

The composition of the vaginal microbiota can influence the transmission of pathogens such as HIV. Women colonised with optimal vaginal bacterial communities, typically dominated by beneficial *Lactobacillus* spp., have a decreased risk of acquiring and transmitting HIV compared to women colonised with non-optimal microbiota. A non-optimal vaginal microbiota is characterised by a depletion of beneficial *Lactobacillus* spp. and high relative abundance of non-beneficial bacterial species, as exemplified by bacterial vaginosis (BV), which is a common form of vaginal dysbiosis in women of reproductive age that occurs in up to 55% of women in sub-Saharan Africa where HIV predominates (McKinnon *et al* 2019 AIDS Res Hum Retroviruses 25(3)). Non-optimal vaginal microbiota increase local pro-inflammatory cytokines, recruit activated HIV target cells and disrupt cervicovaginal epithelial barrier integrity that together drive increased risk of HIV acquisition. While studies have described the association between the vaginal microbiota and increased susceptibility to HIV and sexually transmitted infections (STIs), relatively little is known about how the microbiota and its metabolites act on the epithelium to mediate this effect.

Major distinguishing features of women colonised with optimal vaginal microbiota compared to women with BV is a dramatic increase in the levels of lactic acid and depletion of short chain fatty acids (SCFAs) suggesting a role for these metabolites as effector molecules of vaginal bacteria. Projects are available to determine the direct anti-HIV mechanism of vaginal microbiota metabolites, their immune modulatory and barrier promoting effects on cervicovaginal epithelial cells, and to examine the roles of key members of the vaginal bacterial community (Delgado-Diaz *et al* 2020 Front Cell Infect Microbiol 9:446; Chetwin *et al* 2019 Sci Rep 9(1917)). These studies are underpinning an exciting program at the Burnet to advance strategies to treat and prevent genital inflammation and consequently susceptibility to HIV, other STIs as well adverse reproductive health outcomes.

Discovery of a New Drug Class for HIV Treatment and Prevention

Professor Gilda Tachedjian, Dr Paula Ellenberg and Dr David Chalmers

There is a real threat that drug resistance, toxicity and intolerance will eventually lead to exhaustion of antiretroviral drug options for both HIV treatment and prevention, especially since there is little in the way of new drug classes in the pipeline. We have initiated a drug discovery program targeting HIV-1 reverse transcriptase (RT) to identify compounds that inhibit this essential enzyme. We are using an innovative and validated paradigm for drug discovery called fragment-based drug design (FBDD) that uses very small compounds called 'fragments' to find inhibitors with novel mechanisms of action against HIV-1 RT. Using FBDD means screening far fewer compounds than conventional drug screens since fragments are so small, and can be developed into more potent drugs. Two screens have identified promising fragments that inhibit HIV-1 RT. We have discovered fragments with mechanisms that are distinct from other drugs that inhibit HIV-1 RT in the clinic (La *et al* 2015 PNAS 112:6979) and some of these have been progressed to molecules that have low micromolar activity against wild-type and drug-resistant RT. The aim of this study is to progress fragment hits into more potent RT inhibitors or drug leads. Compounds that are structurally related to the fragment hit will be evaluated for their ability to bind and inhibit wild-type and drug-resistant RT, as well as inhibit HIV-1 replication. This study will identify leads for the development of a novel class of RT inhibitor for use in HIV treatment and prevention.

This is a joint project between the Tachedjian Lab and Monash Institute of Pharmaceutical Sciences.

Notes

Notes

Further information

Professor John Boyce
Honours Coordinator
Department of Microbiology
School of Biomedical Sciences

Room 215
19 Innovation Walk
Clayton Campus
Monash University, VIC 3800

Telephone: +61 3 9902 9179
Email: john.boyce@monash.edu

Monash Biomedicine Discovery Institute
23 Innovation Walk
Monash University
Wellington Road
Clayton, Victoria, 3800

Telephone: +61 3 9902 9400
Email: bdi-enquiries@monash.edu

www.med.monash.edu

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