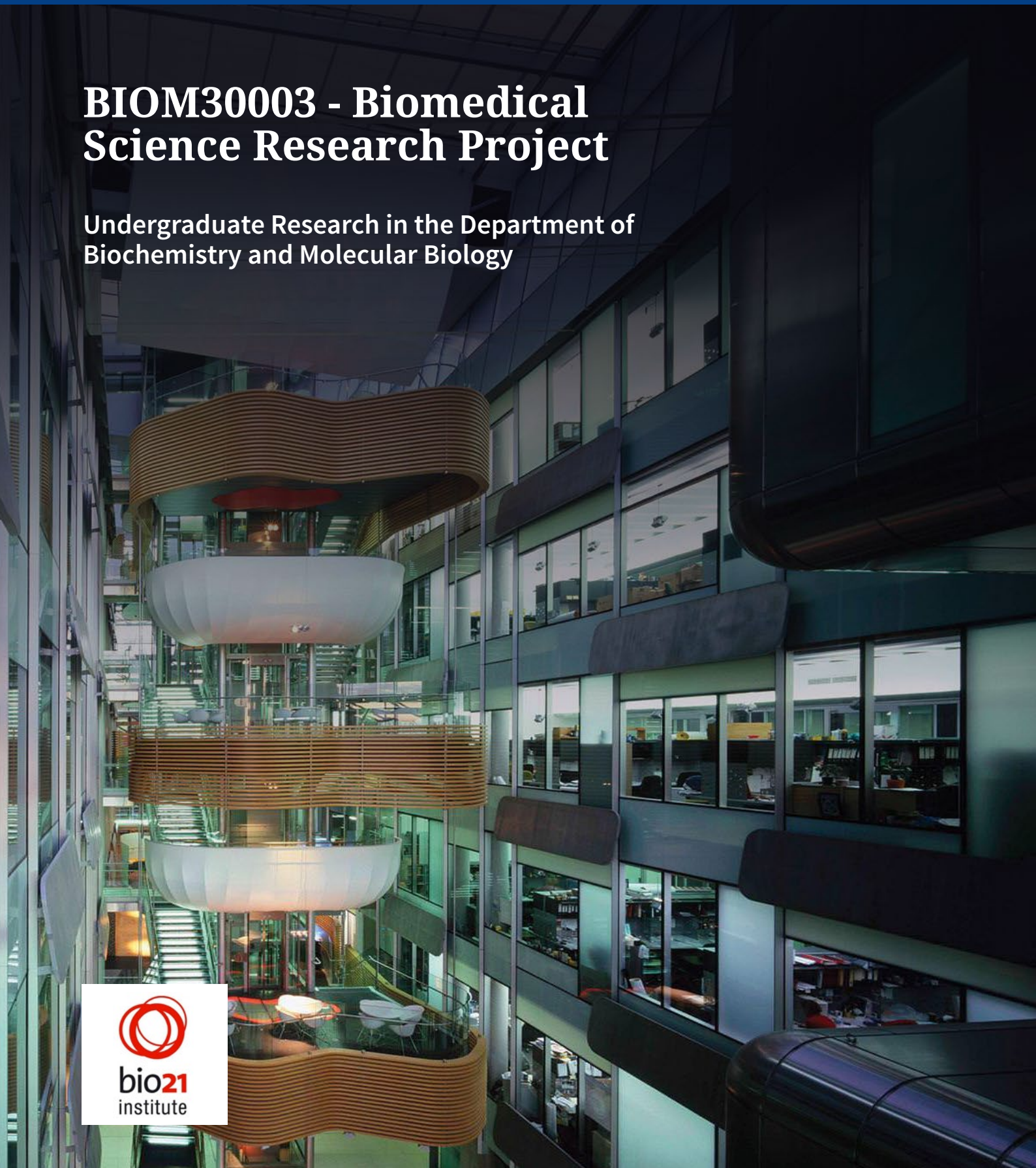
THE UNIVERSITY OF MELBOURNE

Faculty of Medicine, Dentistry and Health Sciences

School of Biomedical Sciences

BIOM30003 - Biomedical Science Research Project

Undergraduate Research in the Department of Biochemistry and Molecular Biology



bio21 institute

General Overview

What is BIOM30003?

BIOM30003 is your opportunity to see what a real research laboratory is like! Students spend a semester working with our internationally regarded researchers in the Department of Biochemistry and Molecular Biology. You will work on an exciting, research-based project, where you will learn a multitude of laboratory skills, access state-of-the-art technology, analyse data, think critically and communicate your research. This experience gives students a significant edge for future post-graduate research options in the Department, including Honours and Masters.

What are the entry requirements for BIOM30003?

1. An average score of 75 or better in relevant second and third year subjects.
2. Completing the equivalent of a major in Biochemistry and Molecular Biology.
3. You are expected to either be taking or have completed the third year Biochemistry and Molecular Biology practical subject 'Advanced Techniques in Molecular Science' (BCMB30010) or an equivalent practical subject in another department. This requirement is waived for students undertaking a computational project (i.e. not lab-based). Instead students must have skills in a discipline that is relevant to that project, e.g. coding, bioinformatics, maths.

Note: final selection of students into projects is at the discretion of the laboratory head.

When is BIOM30003 offered?

Either Semester 1, 2 or over the Summer break.

What are the time commitments for BIOM30003?

The projects have flexible arrangements based around 10 weeks of laboratory work with about 80-100 hours of contact in the laboratory (~8-10 hours per week). There are possibilities for more intensive laboratory work either in the vacation period before the start of the semester, during the 1 week mid-semester break during semesters or in Summer with intensive 3-4 week projects. It is expected that students will spend additional 80 hours in preparing for laboratory work, developing their presentation, and writing their final report for assessment.

How is BIOM30003 assessed?

You will work closely with your laboratory supervisor who will give you feedback early in the project to let you know your progress and give guidance on areas in which you can improve or consolidate your skills. You are expected to submit a 1000-word literature review to your supervisor for feedback at week 4. This is not formally assessed but will form the introduction for your final research report.

Formal assessment includes:

- A 3000-word scientific report structured as a scientific paper (60%) – marked by your supervisor and an academic outside the research group.
- A 15-minute presentation on your research project to the laboratory group (30%).
- Supervisor assessment of performance (10%).

Important information

What are the key dates?

Your time in the laboratory begins at the start of the semester. However, students and laboratories may find it useful if you start a week or two earlier to begin the process of learning experimental techniques and methods.

You will organize with your supervisor what times and days you will work in the laboratory;this may change regularly depending on the experiments you conduct.

Precise dates for submissions will be provided at the start of each semester.

	ASSESSMENT	DUE DATES
Formal start of the semester including: laboratory induction		Week 1
Submission of Literature review, informal feedback on progress		Week 4
Laboratory presentation	30%	Week 12
Laboratory performance	10%	End of semester
Research report: Draft (informal feedback) Final submission	60%	Friday of Week 12 Monday 11:30 pm of 2nd examination week (via Turnitin)

How do I find a project?

Find out about specific research projects on offer in this booklet. Discuss Research projects with staff members before applying. To meet staff members, contact them directly by email. You are free to approach different laboratories and supervisors to determine your preferred project but once you have reached an agreement to take a project you are obliged to continue in that laboratory. Considerable work and effort go into preparing projects and bench supervision. Agreements need to be honoured.

Where will my project be located?

Projects are supervised by departmental staff and their PhD students or senior scientists located in the Bio21 Molecular Sciences and Biotechnology Institute. The Bio21 institute has superb facilities and houses a large number of groups with strong interests in cellular, molecular, structural and chemical biology.

How do I apply?

1. Read the project descriptions in this book and arrange a meeting with the supervisor(s) you are interested in.
2. Obtain a provisional offer in the project by the supervisor.
3. Complete the online application form on the BCMB department website:

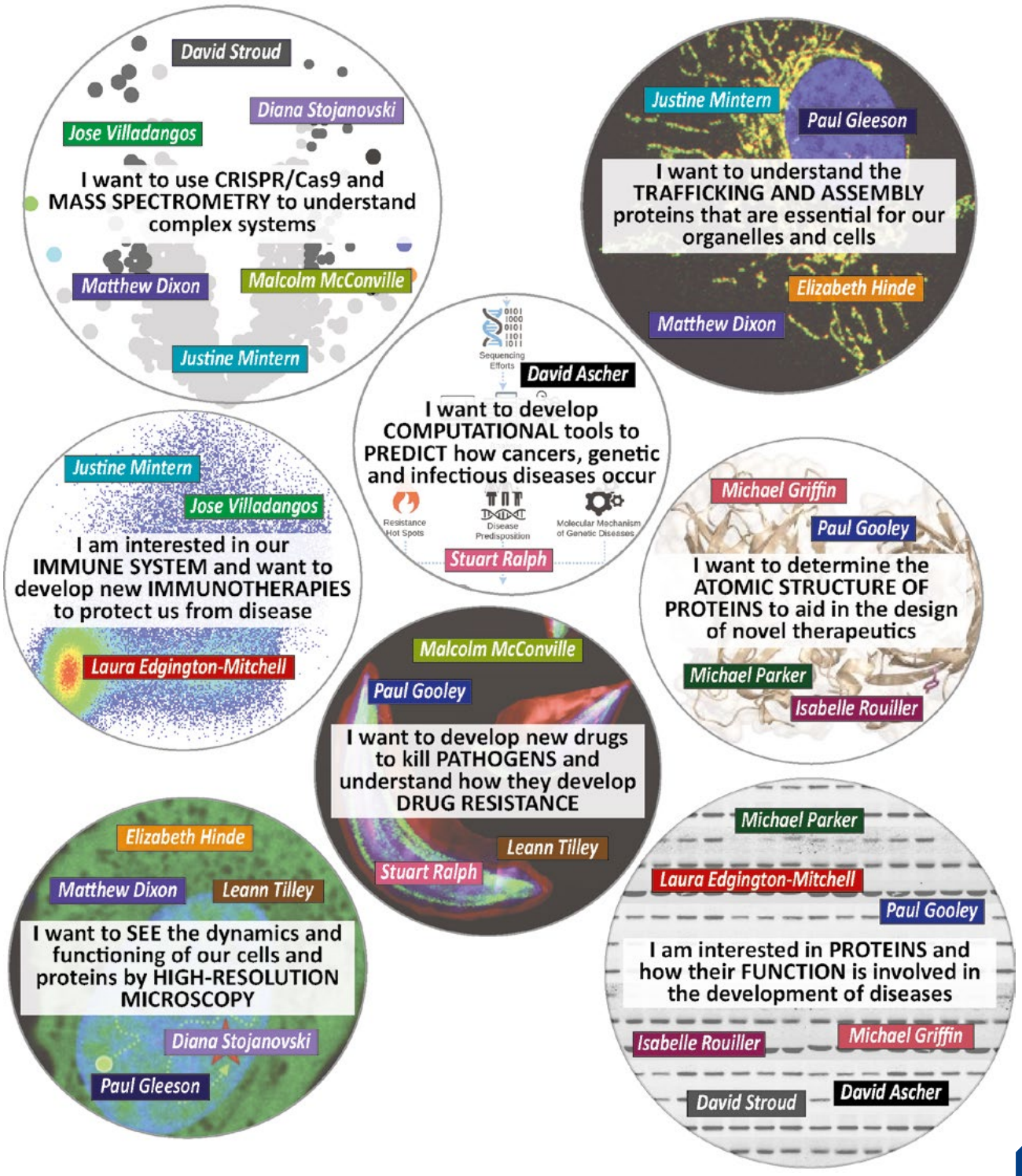
   https://biomedicalsciences.unimelb.edu.au/departments/biochemistry/study/undergraduate-research-training
4. Email the department coordinator, Leon Helfenbaum (**leonh@unimelb.edu.au**), to set up a meeting to arrange administrative enrollment procedures. Please do this as soon as you have completed the application form.

Who can I contact for general advice?

Students can obtain advice from Leon Helfenbaum (**leonh@unimelb.edu.au**), the departmental coordinator for BIOM30003 and coordinator of BCMB30010 'Advanced Techniques in Molecular Science'.

Guide to projects offered

Researchers in the Department of Biochemistry and Molecular Biology work on a large variety of exciting and important research topics. So how can you decide which project to do? We have prepared this visual guide to help you identify which projects you may wish to investigate in more detail. The general themes and techniques employed by our researchers are indicated in the circles below, and the names of respective researchers associated with each theme as indicated:



Projects offered

David Ascher – Treating the person not the disease

BENCH SUPERVISOR:
Dr David Ascher

OFFERED:
Semesters 2

Genomic sequencing is being more routinely used to diagnose patients with genetic diseases, including cancer, and optimise treatment strategies. In order to realise the power of genomic information in clinical settings, we need new tools to rapidly assess the functional impact of novel variants. We have developed a range of computational tools to deconvolute the molecular consequences of coding variants giving rise to different phenotypes and clinical outcomes. The same disease phenotype, in turn, may arise from many different mutations that alter a patient's outcome or how they may respond to a particular treatment. By analysing these mutations and predicting their effects on protein structure and function we are trying to revolutionise treatment strategies, an important step towards personalised medicine.

We are currently working on a range of diseases including genetic diseases (Alkaptonuria, Urea cycle disorders, VHL), cancer (renal carcinomas, gangliomas, prostate cancer), and drug/vaccine resistance (TB, cancer, malaria, HIV, influenza). These projects will use computational (bioinformatics)) approaches to unravel the molecular mechanisms driving these mutations and derive novel predictive methods to guide patient treatment. One of the ultimate goals of these projects will be the development of webservers enabling the rapid analysis of mutations to help guide clinical decisions.

This project will suit students with some familiarity with Linux operating systems and computer coding (Python).

Techniques used may include:

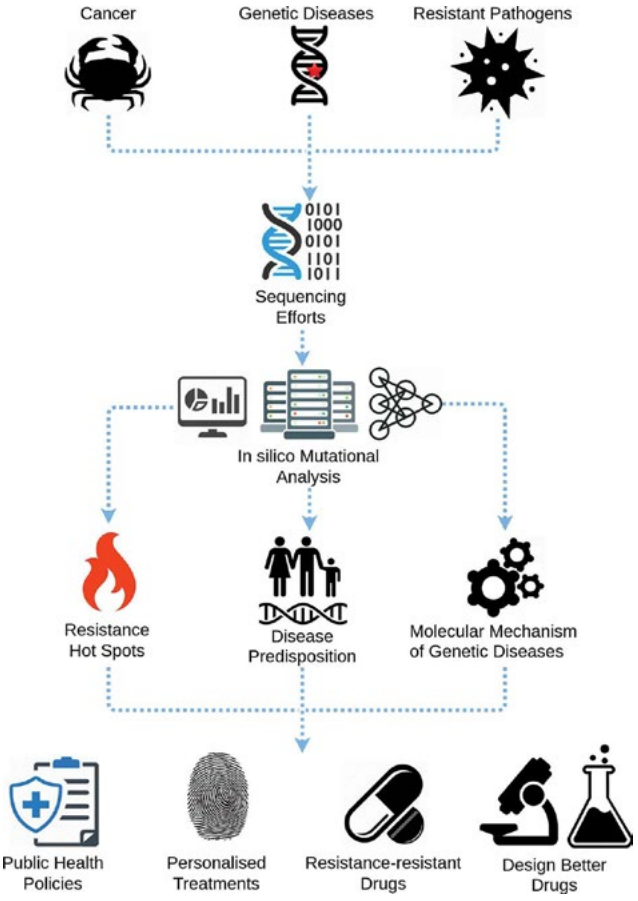
1. Protein structure analysis
2. In silico mutation analysis
3. Machine learning and neural networks
4. Webserver development

Recent papers from the lab:

Pandurangan AP et al., *Nucleic Acids Res* 2017

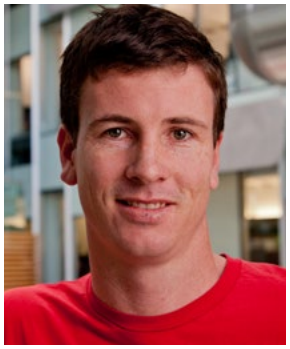
Pires DEV, Ascher DB. *Nucleic Acids Res* 2017

Andrews KA, et al., *J Med Genet* 2018



Contact:

Dr David Ascher to arrange an appointment
david.ascher@unimelb.edu.au



Matt Dixon – The Renovators Dream: Malaria host cell remodeling

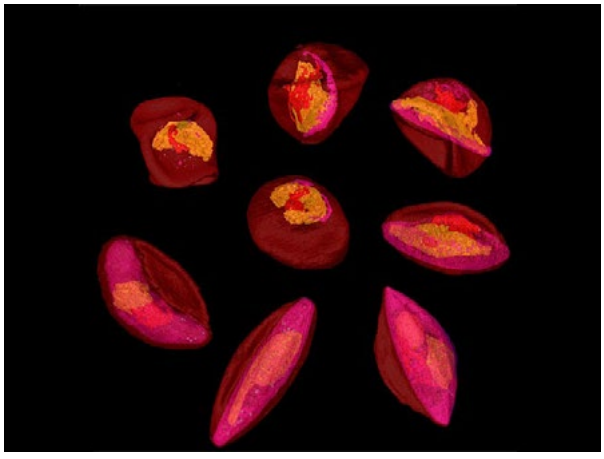
BENCH SUPERVISOR:
Olivia Carmo (PhD student) and Dr Matt Dixon

OFFERED:
Semester 2

Malaria is a significant global health problem, with more than 200 million clinical cases and up to 400,000 deaths annually. *Plasmodium falciparum* is the deadliest of the human malaria parasites and is responsible for the majority of the deaths. The lifecycle of the parasite is complex, including asexual replication within the red blood cells of the human host.

During the final stage of differentiation, the red blood cell (RBC) enucleates becoming a highly specialized sack of hemoglobin. *P. falciparum* infects RBCs and makes a home of the relatively desolate organelle-free cell. The renovation process includes (i) building an elaborate exomembrane system in the host cell cytoplasm, (ii) exporting hundreds of *P. falciparum* proteins into the host cell, and (iii) remodeling the host cell membrane skeleton in order to survive. A key organelle of the parasite's exomembrane system, Maurer's clefts, are populated by exported *P. falciparum* proteins and play an important role in both trafficking antigens to the surface of the red blood cell membrane and host cytoskeletal remodeling; however the details of these two pathways and how they converge at the Maurer's clefts are not fully understood.

We know that a host of *P. falciparum* proteins at the Maurer's clefts, or cleft resident proteins, are critical in the trafficking of other exported *P. falciparum* proteins. In this project students will be functionally characterizing novel cleft resident proteins to understand the role these proteins may play in trafficking other *P. falciparum* proteins to and from the clefts.



We will use CRISPR gene editing to create transgenic malaria parasites and characterize proteins by combining biochemical, proteomic, molecular and cellular biology techniques with super resolution microscopy to define the molecular players underpinning trafficking through the enigmatic Maurer's clefts.

Techniques used may include:

1. CRISPR gene editing of parasites to create gene knockouts, knockdowns, and epitope tagged cell lines
2. Biochemical protein characterization
3. Immunoprecipitation and proteomics
4. Widefield and confocal microscopy
5. Electron microscopy

Recent papers from the lab:

McHugh et al., 2020	DOI: 10.1128/mBio.03320-19
Looker et al., 2019	DOI: 10.1371/journal.ppat.1007761
Batinovic et al., 2017	DOI: 10.1038/ncomms16044

Contact:

Dr. Matt Dixon or Olivia Carmoto arrange an appointment
matthew.dixon@unimelb.edu.au or
ocarmo@student.unimelb.edu.au

Laura Edgington-Mitchell – Validating proteases as biomarkers and therapeutic targets for oral cancer

BENCH SUPERVISORS:
Dr. Laura Edgington-Mitchell and Beth Anderson (PhD student)

OFFERED:
Semester 2

Oral squamous cell carcinoma is the most common head and neck cancer. It is an extremely painful disease for which treatments are limited. Oral cancer often spreads to cervical lymph nodes, and once metastasis occurs, patient survival rates drop below 40%. Current methods to predict the spread of oral cancer are ineffective; thus, most patients undergo radical elective neck dissection to remove all cervical lymph nodes prior to the appearance of metastatic lesions.

Our laboratory is investigating the contribution of proteases to oral cancer pain and metastasis. Proteases are a large family of enzymes that function as tiny molecular scissors to cut proteins. This process facilitates protein degradation and turnover, but also contributes to many cellular signalling events that underlie the growth and metastasis of oral cancer.

This mini-project aims to evaluate the activity of key proteases in oral cancer cell lines and tissues from patients. We will evaluate the utility of protease activity as a biomarker for predicting metastasis and as a potential drug target for the treatment of this deadly disease.

Techniques used may include:

1. Protease activity assays
2. In vitro culture of oral cancer cells
3. Protein biochemistry (SDS-PAGE, western blotting)
4. Confocal microscopy
5. Histological evaluation of cancer tissue

Recent papers from the lab:

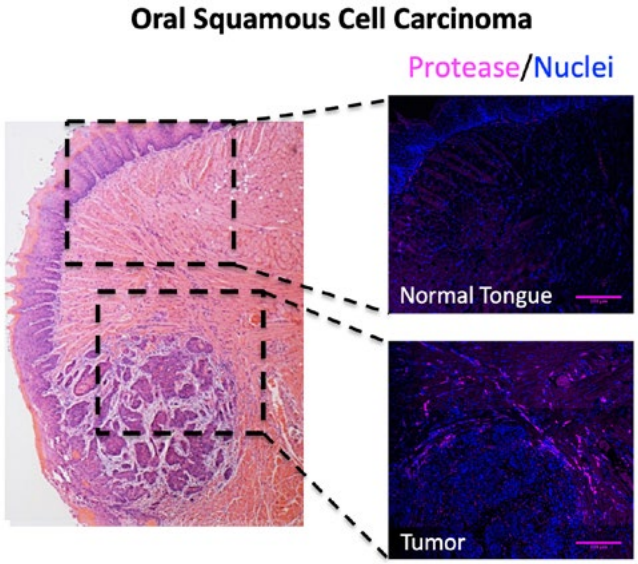
Edgington-Mitchell LE et al., *Oncotarget* 2015

Edgington-Mitchell LE et al., *Am J Physiol Gastrointest Liver Physiol* 2017

Edgington-Mitchell LE et al., *Bioord Med Chem Lett* 2017

Contact:

Dr. Laura Edgington-Mitchell to arrange an appointment
laura.edgingtonmitchell@unimelb.edu.au



Debnath Ghosal – Deciphering structures of bacterial and viral molecular machines that inject toxins into our cells

BENCH SUPERVISOR:
Debnath Ghosal

OFFERED:
Semester 2 and Summer 2021

Bacteria harbour at least nine different types of secretion systems to transfer macromolecules across cellular envelope. These are sophisticated multi-protein nanomachines that secrete myriads of substrates including proteins, nucleoprotein complexes and variety of small molecules and are central to pathogenesis of multiple human diseases. For example, many pathogenic bacteria utilize the Type III Secretion System (T3SS) to cause diseases such as dysentery (*Shigella*), typhoid (*Salmonella*), plague (*Yersinia*) etc. Other human pathogens employ the Type IV Secretion System (T4SS) to mediate gastric cancer (*Helicobacter*), brucellosis (*Brucella*), typhus and spotted fevers (*Rickettsia*), as well as Legionnaires' disease (*Legionella*). The T4SS is also associated with the spread of antibiotic resistance, which currently presents a major threat to public health. Therefore, these molecular machines are attractive targets for drug development to enrich our present repertoire of antibiotics. Structural studies with these molecular machines are extremely challenging due to their large number of components, flexibility and tight integration into the bacterial cell envelope.

Electron cryotomography (cryo-ET) has unrivalled power to visualize the native structure of macromolecules *in situ*. In recent years, improvement in software, detectors and subvolume averaging methods have allowed us to investigate macromolecules *in situ* at subnanometer resolution. We are harnessing this unique power of cryo-ET and combining it with correlative light and electron microscopy (CLEM), and Focused Ion Beam (FIB) milling to elucidate the structure and function of different bacterial injection modules at molecular resolution.

Techniques used may include:

1. Bacterial cell culture
2. Protein purification
3. Host-pathogen interaction
4. Electron cryomicroscopy
5. Electron cryotomography
6. Subtomogram averaging

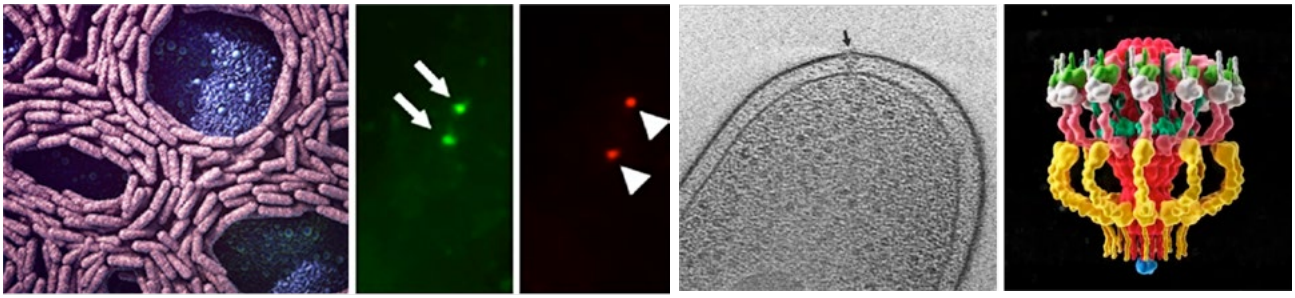
Recent papers from the lab:

Ghosal et al, *Nature Microbiology*, 2019a
doi: 10.1038/s41564-019-0603-6)

Ghosal et al *Nature Microbiology*, 2019b
doi: 10.1038/s41564-019-0427-4)

Contact:

Dr Debnath Ghosal to arrange an appointment
debnath.ghosal@unimelb.edu.au



Paul Gooley – How does the C-terminal domain of the rabies P-protein recognize STAT1

BENCH SUPERVISOR:
Dr Ashish Sethi

OFFERED:
Semester 2 and Summer 2021

While viruses are replicating in the cell they use their own proteins to hijack and subvert host cellular processes to avoid detection by the host. The rabies virus has only five genes/proteins to replicate itself and to control the cell. To carry out so many processes the rabies proteins must be highly multifunctional. The phospho-protein (P protein) of rabies has had over 20 functions ascribed to it. Due to leaky ribosomal scanning, the P protein is produced as five N-terminally truncated isoforms (P1 to P5), and importantly all isoforms do not have the same functions. The isoforms P2 to P5 lack an N-terminal binding site for the viral L protein, which is important for replication, and so compared to P1, P2 to P5 are not involved in replication – due to truncation causing a loss of function.

Remarkably, however, we have found that, truncation does not always result in loss-of-function but also in gain-of-function. For example, truncation of the first 53 residues produces the isoform P3 in which an N-terminal nuclear localization sequence and a C-terminal microtubule association sequence have been activated. These sequences are in P1, but are inactive.

We want to understand what are the structural differences between P1 and P3 that result in these functional differences.

Techniques used may include:

1. Bacterial overexpression of protein.
2. Protein purification.
3. Preparation of site-directed mutants.
4. Biophysical methods such as Isothermal Calorimetry, Circular Dichroism, Fluorescence and Nuclear Magnetic Resonance.

Recent papers from the lab:

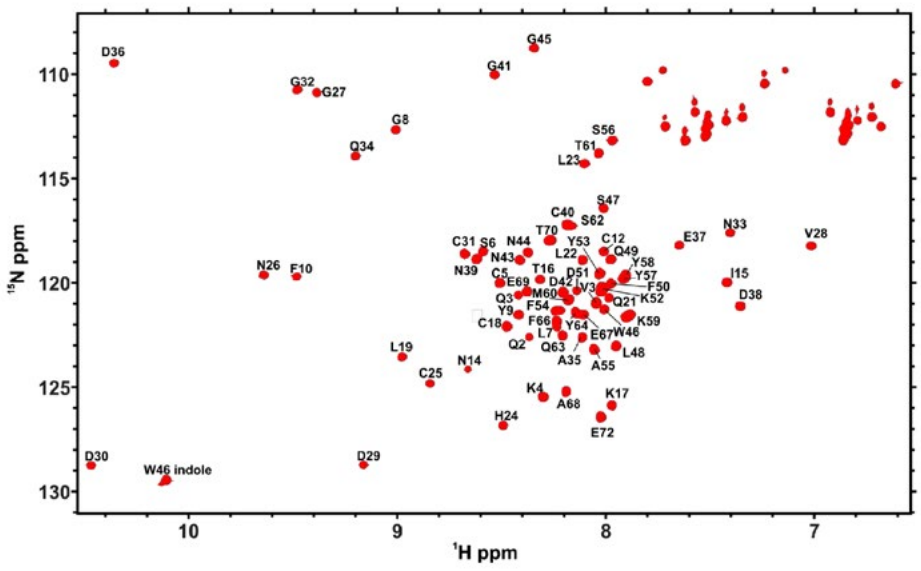
Ali N et al., *Sci Rep* 2016

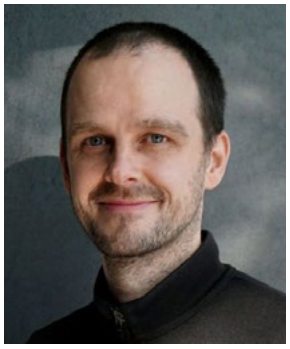
Sethi A et al., *Nat Commun* 2016

Mobbs JI et al., *Chembiochem* 2018

Contact:

Professor Paul Gooley to arrange an appointment
prg@unimelb.edu.au





Michael Griffin – Probing the chaperone mechanisms of small heat-shock proteins

BENCH SUPERVISOR:
Emily Selig (PhD student)

OFFERED:
Semester 2 and Summer 2021

Protein misfolding and amyloid fibril formation is implicated in debilitating diseases such as Alzheimer's disease, Parkinson's disease, dementia and type II diabetes. Our lab is interested in how small molecules and molecular chaperones modulate amyloid formation. Small heat-shock proteins (sHsps) are a class of molecular chaperones that are well known to inhibit amyloid formation. However, the mechanisms by which sHsps inhibit this process are poorly understood. Many existing studies have focused on investigating how sHsps interact with proteins prior to their aggregation, however sHsps have also been shown to bind to preformed aggregates. We have previously shown that this binding may induce the formation of larger inclusion body-like aggregates, which are thought to be less toxic. We have also recently discovered that sHsps inhibit the elongation and naturally occurring end-to-end joining of amyloid fibrils and that this may occur via a specific interaction between sHsps and the ends of amyloid fibrils.

We are therefore interested in determining how sHsps interact with amyloid fibrils. Specifically, this project aims to investigate how effectively various wild-type and mutant forms of sHsps interact with amyloid fibrils. Students will analyse the rates of amyloid fibril formation, and the sizes of amyloid fibrils formed and how these parameters are affected by wild-type and mutant forms of sHsps.

Techniques used may include:

1. Protein expression and purification
2. Protein biochemistry such as gel electrophoresis
3. Analysis of reaction rates
4. Analytical ultracentrifugation
5. Electron microscopy

Recent papers from the lab:

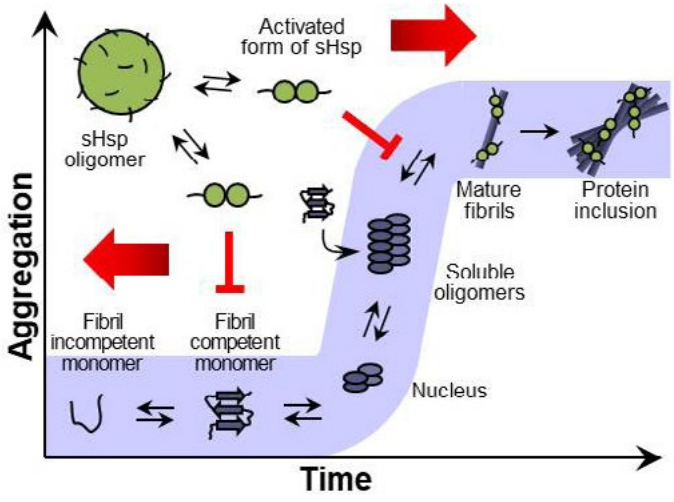
Cox D et al., *J Biol Chem* 2016

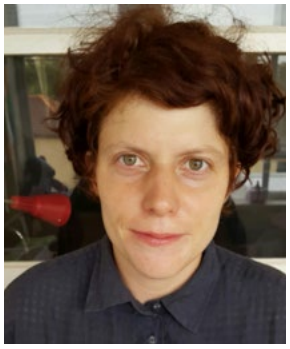
Todorova et al., *Biochemistry* 2017

Scott DJ et al., *Sci Rep* 2018

Contact:

Assoc Professor Michael Griffin to make an appointment
mgriffin@unimelb.edu.au





Elizabeth Hinde – Imaging transcription factor DNA target search in a living cell

BENCH SUPERVISOR:
Dr. Jieqiong Lou and Dr. Elizabeth Hinde

OFFERED:
Semester 2 and Summer 2021

Transcription factors have evolved DNA target search strategies that allow them to efficiently navigate the nuclear space and arrive at their specific DNA sequence. This target search strategy is underpinned by molecular diffusion, which in turn is controlled by the architectural organisation of the cell nucleus and oligomeric state of the transcription factor. Until recently no imaging approach could track the molecular mobility of protein oligomers within the nuclei of live cells. To address this research gap, we recently established a new microscopy method to image the transport and binding dynamics of different oligomeric species in live cells. The overall aim of this project is to use to use this technology to uncover how the spatial compartmentalisation of the cell nucleus regulates transcription factor complex formation and DNA target search in a living cell.

Techniques used may include:

1. Cell culture of the HeLa cell line.
2. Preparation of expression vectors, including GFP vectors.
3. Cell transfection.
4. Confocal laser scanning microscopy of transfected cells.
5. Fluorescence correlation analysis of molecular diffusion within microscopy data.

Recent papers from the lab:

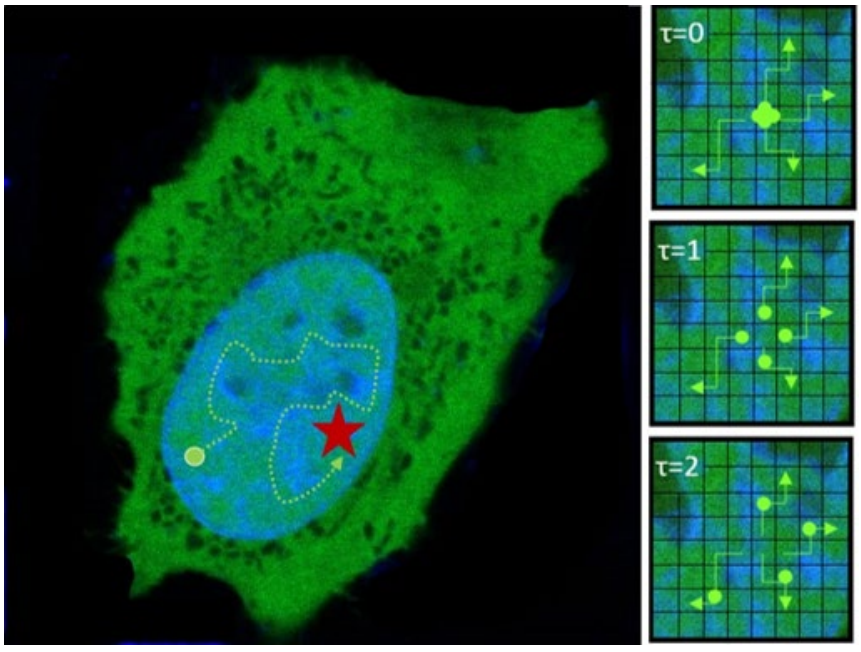
Hinde E et al., *Sci Rep* 2015

Hinde E et al., *Nat Comm* 2016

Hinde E et al., *Nat Nanotech* 2017

Contact:

Dr. Elizabeth Hinde to arrange an appointment
elizabeth.hinde@unimelb.edu.au



Malcolm McConville – Identifying new metabolic drug targets in parasitic protozoa

BENCH SUPERVISORS:
TBA

OFFERED:
Semester 2 and Summer 2021

Protozoan parasites cause a number of important human diseases including malaria, toxoplasmosis, and leishmaniasis, that collectively infect more than a third of the world's population. As current drug treatments for these diseases are inadequate or are being undermined by the emergence of resistant strains there is an urgent and ongoing need to identify new therapeutic targets. We have developed a duel approach for drug target identification. The first approach involves the systematic detection of all metabolic pathways that active in relevant parasite stages using mass spectrometry-based metabolite profiling and stable isotope labelling studies. Genetic studies (i.e CRISPR/Cas9) are then used to determine the role of novel or up-regulated metabolic pathways in pathogenic stages. In the second approach, we first use high through-put screening approaches to identify new compounds that kill relevant parasite stages and then define the mode of action of top hits using metabolomic approaches. These approaches have led to the identification of new metabolic pathways in all of these parasites, as well as potential lead inhibitors that will be further characterized in these projects.

Techniques used include

1. Cell culture (parasite and mammalian host cells)
2. Metabolomic profiling and stable isotope labelling approaches
3. CRISPR/Cas9 gene knock-out studies in Leishmania and Plasmodium
4. Enzyme assays on parasite cell extracts and recombinant proteins
5. Live-cell metabolic analysis (Seahorse XF platform)

Recent papers from the lab:

Sernee et al. *Cell Host Microbe* 2019

Saunders et al., *Molecular Microbiology* 2018

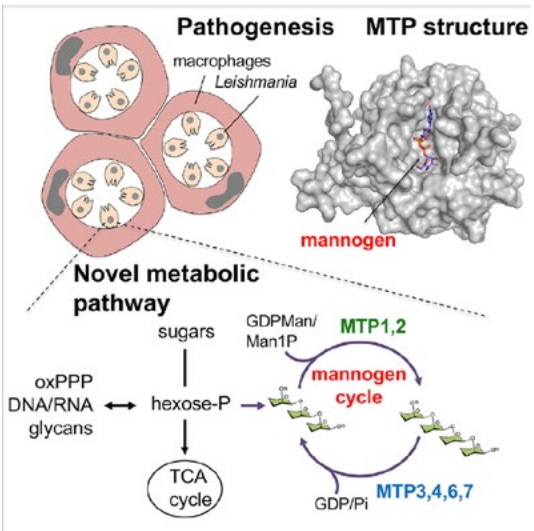
Kloehn J et al., *Curr Opin Microbiol* 2016

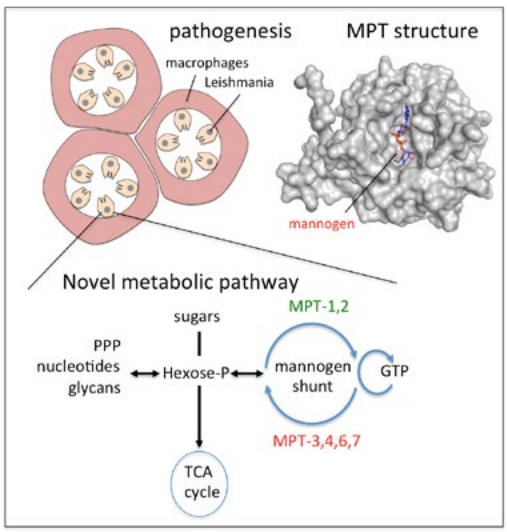
Uboldi et al., *Cell Host Microbe* 2015

Blume et al., *Cell Host Microbe* 2015

Contact:

Professor Malcolm McConville to arrange an appointment
malcolmm@unimelb.edu.au





Justine Mintern – Designing effective vaccines to fight infection and tumours

BENCH SUPERVISORS:
TBA

OFFERED:
Semester 2

Vaccination currently represents the most effective strategy for eliminating infectious disease. While many vaccines are in use worldwide, for several pathogens our current vaccines fail with ensuing uncontrolled disease. This is the case for HIV, malaria and tuberculosis resulting in disease and devastation worldwide. Vaccines also have the potential to prevent and/or treat cancer, however this is currently not a clinical reality. Therefore, vaccine design must be advanced, and to do so, we require a more comprehensive understanding of the cell biology involved. A key question in vaccine biology is how is the antigen cargo delivered to specialised compartments inside immune cells. This project will identify mechanisms of antigen trafficking for effective immunity.

Techniques used may include:

1. CRISPR/Cas9 deletion of genes
2. Preparation of lentiviral vectors
3. Use of bioengineered nanoparticles
4. Isolation of primary cell types
5. Flow cytometry
6. Next generation sequencing
7. Immunoprecipitation, western blotting
8. Proteomics
9. Animal models of immunity and infection

Recent papers from the lab:

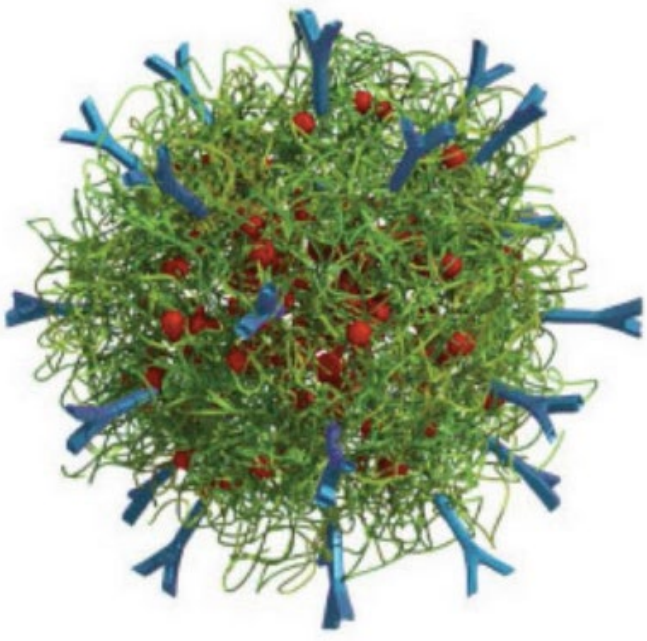
Liu H et al., *J Exp Med* 2016

Liu H et al., *Methods Mol Biol* 2016

Dumont C et al., *Traffic* 2017

Contact:

Assoc. Professor Justine Mintern to arrange an appointment
jmintern@unimelb.edu.au



Michael Parker – Overcoming cancer drug resistance

BENCH SUPERVISOR:
Dr. Craig Morton and Dr Claire Weekley

OFFERED:
Semester 2

Conventional cancer chemotherapy kills rapidly growing cells indiscriminately, causing significant side-effects and can lead to disease re-occurrence and resistance to the drugs. One of our interests is the Glutathione S-Transferase (GST) family of proteins that function by recognising foreign small molecule toxins in the body, causing them to be eliminated from the cell. Unfortunately, commonly used anti-cancer drugs are also recognised as toxic by GST, which is often overexpressed in cancer tissues and is associated with transformation to malignancy and the adaptive resistance to anti-cancer drugs. There is thus an urgent need for the design of new anti-cancer drugs that circumvent the development of GST-mediated resistance to treatment. Very recently, there has been an increasing interest in the development of metal-based drugs as effective and potent protein targeted chemotherapies. We are investigating, though structural and biochemical means, how a range of ruthenium, arsenic and osmium-based drugs and drug-like compounds interact with GSTs. Students will investigate how these compounds work, as well as any drug-like molecules we develop, using X-ray crystallography and a range of biophysical techniques.

Techniques used may include:

1. Protein expression.
2. Protein purification.
3. Protein characterisation (circular dichroism, differential scanning fluorimetry, dynamic light scattering, analytical ultracentrifugation, mass spectrometry).
4. 3D atomic structure determination (X-ray crystallography, cryo electron microscopy, synchrotron).
5. Protein-drug interactions (surface plasmon resonance, isothermal calorimetry, microscale thermophoresis, nuclear magnetic resonance spectroscopy, computational docking).
6. Structure-based drug discovery (virtual screening, fragment screening, computer-aided drug design).

Recent papers from the lab:

Baell JB et al., *Nature* 2018

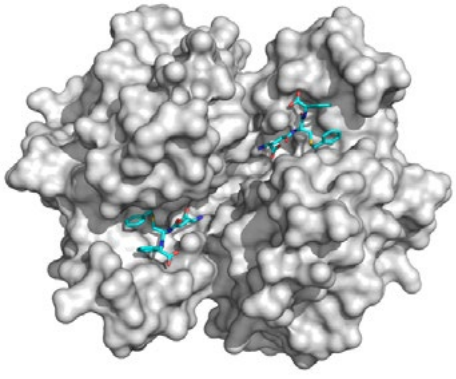
Broughton SE et al., *Nature Communs* 2018

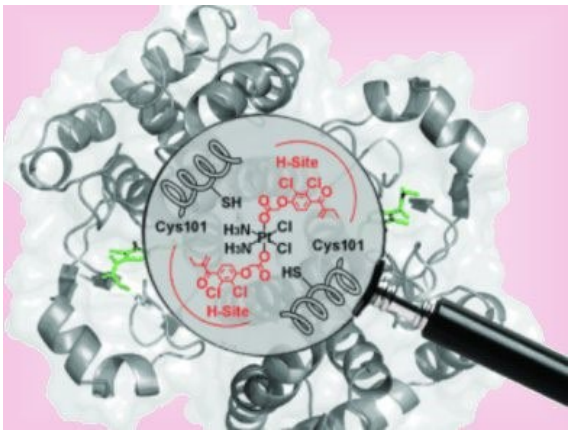
De Luca A et al., (2019) *Proc. Natl. Acad. Sci. USA* **116**, 13943-13951

Koach J et al., (2019) *Cancer Res*. **79**, 5652-5667.

Contact:

Professor Michael Parker to arrange an appointment.
mwp@unimelb.edu.au





Michael Parker – Understanding bone disease

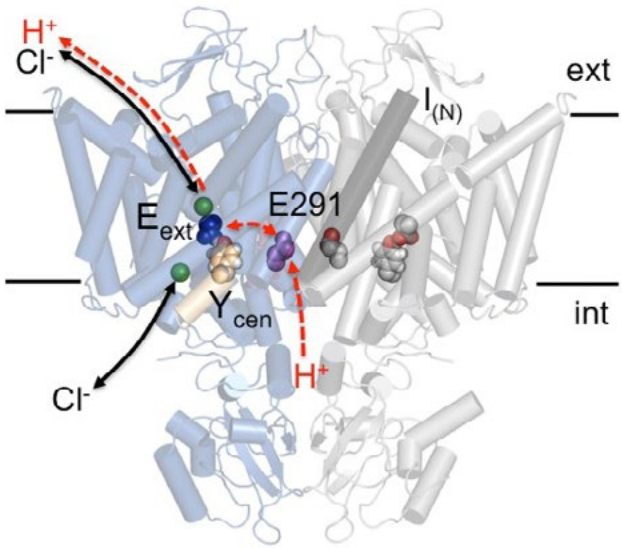
BENCH SUPERVISOR:
Dr Larissa Doherty and Dr. Craig Morton

OFFERED:
Semester 2

Bone diseases such as osteoporosis and osteosarcoma result in irreversible loss of bone material. Current treatments fail to regenerate bone material owing to functional coupling between cells that resorb bone (osteoclasts) and cells that deposit new bone material (osteoblasts). Conversely, in a rare heritable disease, osteopetrosis, bone remodelling is uncoupled due to a defect in the resorption machinery of osteoclasts; leading to accumulation of too much bone material. Osteopetrosis is caused by mutations in ClC-7.

Autosomal dominant osteopetrotic (ADO) mutations result in a mild phenotype that is usually identified later in life and typically limited to abnormally dense bones. The molecular mechanism of ClC-7 function is poorly understood, and it is unclear how ADO mutations affect ClC-7 function to cause osteopetrosis. CLC gating is strongly modulated by binding of nucleotides to intracellular cystathione-ß-synthase (CBS) domains. Our in silico modelling studies show that ClC-7 ADO mutations cluster in a protein region that is critical for coupling intracellular nucleotide binding to conformational rearrangements that regulate activity of ClC proteins.

These observations suggest that dominantly inherited osteopetrosis arises from defective regulation of CLC-7 exchangers and raises an important question: Are ClC-7 exchangers regulated by intracellular metabolite binding?



Aim:

Determine the functional effect of nucleotide binding to ClC-7 and ADO mutants.

Techniques used may include:

1. Bacterial expression of recombinant ClC 7 CBS wild-type and ADO mutants
2. Protein purification
3. Protein Characterisation (chromatography, SDS-PAGE, Western Blot, thermal melt assay, mass spectrometry)
4. Binding studies to characterise the binding of nucleotides to CLC 7 CBS and ADO mutants (Microscale Thermophoresis, Isothermal Titration Calorimetry)

Recent papers from the lab:

Bennetts, B et al., (2005) *J. Biol. Chem*. **280,** 32452-32458

Bennetts, B et al., (2007) J. *Biol. Chem*. **282**, 32780-32791

Bennetts, B et al., (2012) *J. Biol. Chem.* **287,** 25808-25820

Bennetts, B et al., (2013) *Nature Commun*. **4,** 2507

Contact:

Professor Michael Parker to arrange an appointment.
mwp@unimelb.edu.au

Stuart Ralph – Protein translation in human malaria parasites as targets for therapeutics

BENCH SUPERVISORS:
Stuart Ralph, Dr Emma McHugh, Emily Crisafulli, Madel Tutor, Vern Lee (PhD Students)

OFFERED:
Semester 2 and Summer 2021

Our laboratory is interested in the characterization of potential drug targets in the malaria parasite *Plasmodium falciparum*. Several anti-malarial drugs in clinical use act against the protein translation machinery, validating this as a target for therapeutic intervention. We are particularly interested in the aminoacyl tRNA synthetases (ARS) family of enzymes, which are responsible for attaching amino acids to their cognate tRNA.

Our laboratory uses biochemical, bioinformatic, molecular, and cell biological techniques to characterize *Plasmodium* enzymes as drug targets we need to be able to assay the activity of purified enzymes. To do this we will overexpress *Plasmodium* tRNA synthetases in *E. coli*, fused to a tag that facilitates their subsequent purification. We will perform kinetic assays for these enzymes, and microscopy to determine the subcellular localisation of tagged tRNA synthetases within parasites. We will also perform inhibitor assays to determine the growth response of parasites to inhibitors of tRNA synthetases.

Techniques used may include:

1. CRISPR/Cas9 manipulation of parasite genome
2. Bacterial protein overexpression and purification
3. Enzyme assay of malaria tRNA synthetases
4. Microscopy of *in-vitro* grown malaria parasites
5. Drug assays for *in-vitro* grown malaria parasites
6. Computational prediction of drug mode of action
7. Computational analysis of enzyme evolution
8. Bioinformatic prioritization of drug targets

Recent papers from the lab:

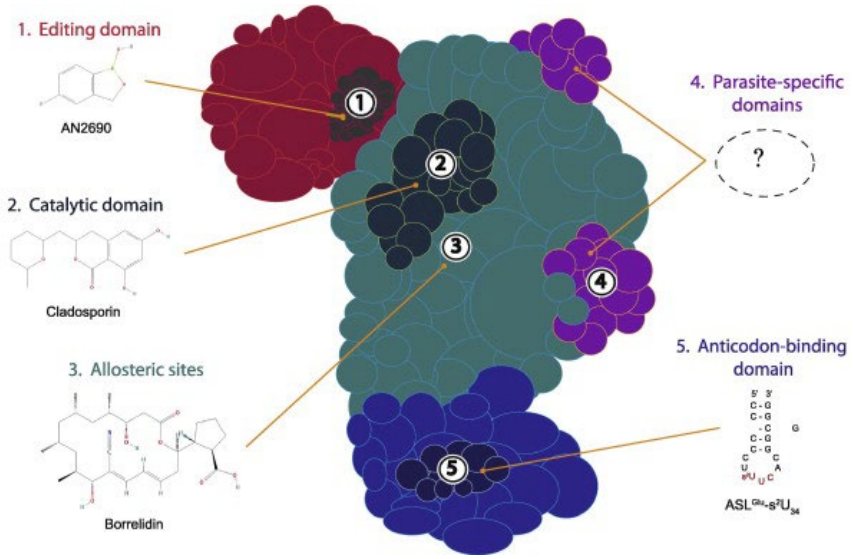
Goodman CD et al., *Trends Parisitol* 2016

Wong W et al., *Nat Microbiol* 2017

Yeoh LM et al., *BMC Genomics* 2017

Contact:

Assoc. Professor Stuart Ralph to arrange an appointment
saralph@unimelb.edu.au



Isabelle Rouiller – Understanding how the unfoldase protein p97 functions in health and disease.

BENCH SUPERVISORS:
Sepideh Valimehr, Nazanin Mohebali, Dr Ashish Sethi, and Dr Mohsen Kazemi

OFFERED:
Semesters 2 and Summer 2021

Cells need to efficiently discard unwanted proteins to stay alive and healthy. Improper protein degradation leads to numerous diseases including Alzheimer and Parkinson. In these neurodegenerative diseases, improperly folded proteins accumulate as aggregates in brain and muscle cells instead of being degraded. Inhibition of protein degradation is also a strategy for killing unwanted cells such as cancer cells, and invading bacteria and protozoa.

We are interested in the molecular mechanisms by which an abundant and essential protein, named p97, unfolds unwanted proteins. To do this, we use a combination of structural biology approaches (mainly cryo-EM) and biochemical assays. Students undertaking this project will take assess how p97 functions in human, mycobacteria or protozoa at the molecular level, using a combination of protein science, biochemical assays, cryo-EM and computational approaches.

Techniques used may include:

1. Single particle electron microscopy.
2. Protein structure analysis.
3. Protein expression and purification.
4. Biochemical and biophysical assays.
5. Proteomics.

Recent papers from the lab:

Makarkov et al., *Npj Vaccines* 2019

Alsahafi et al., *Cell Host & Microbe* 2019

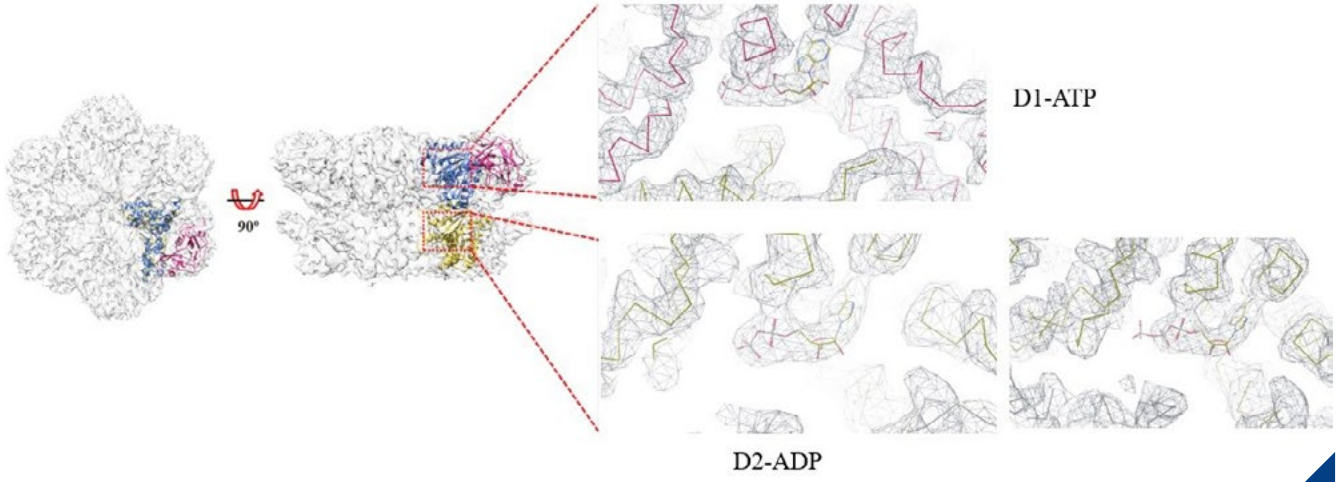
Carlson et al., *eLife* 2018

Lindsay et al., *Vaccine* 2018

Fabre et al., *J Biol Chem* 2017

Contact:

Assoc Professor Isabelle Rouiller to arrange an appointment
isabelle.rouiller@unimelb.edu.au



Diana Stojanovski – Mitochondrial biogenesis and protein quality control

BENCH SUPERVISORS:
Dr Catherine Palmer and Laura Fielden (PhD student)

OFFERED:
Summer 2021

Mitochondria are dynamic organelles that play a central role in diverse aspects of cell biology, including ATP production, regulation of metabolic processes and apoptosis. Mitochondrial dysfunction is associated with the ageing process and the onset of numerous neurological disorders, including Alzheimer's and Parkinson's disease. Mitochondrial function is dependent on the 1000-1500 proteins that reside within one of the four mitochondrial sub-compartments: the outer membrane, intermembrane space, inner membrane and matrix. The cell faces a major conundrum when it comes to the mitochondrial proteome since the majority of proteins localized within mitochondria are encoded in the nucleus. The life or "biogenesis" of these proteins is highly regulated and involves their ***synthesis*** on cytosolic ribosomes, ***targeting*** to mitochondria, ***import*** into mitochondria, ***folding*** into the correct 3D structure, ***assembly*** into complexes and, once the protein has fulfilled it role ***turnover*** for removal of the protein from the system. We are interested in how proteins a trafficked to and within mitochondria and how the mitochondrial proteome is regulated through the interplay of these processes. We used both fungal and mammalian cells systems in our analysis.

Techniques used may include:

1. Tissue culture of mammalian cells
2. Molecular Biology
3. Protein Techniques, including SDS-PAGE and Blue-Native PAGE
4. Mitochondrial Isolation
5. Yeast Genetics

Recent papers from the lab:

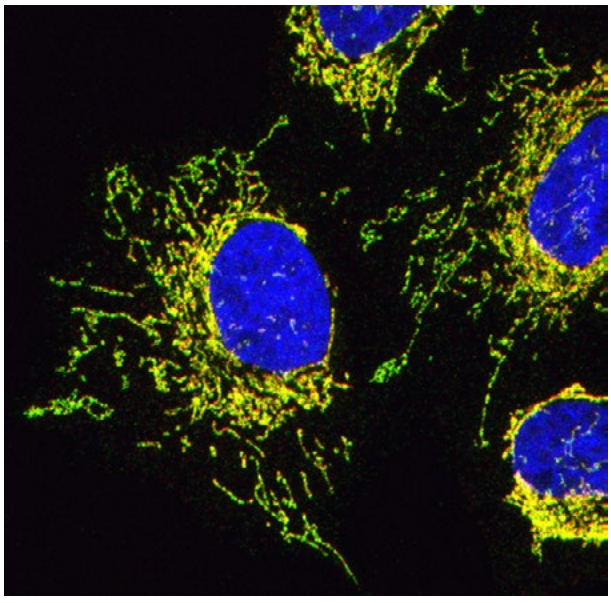
Kang Y et al., *elife* 2016

Kang Y et al., *Mol Cell* 2017

Kang Y et al., *Semin Cell Dev Biol* 2017

Contact:

Dr Diana Stojanovski to arrange an appointment
d.stojanovski@unimelb.edu.au



David Stroud – Understanding how mitochondrial machines are built and maintained

BENCH SUPERVISORS:
Dr. David Stroud

OFFERED:
Semester 2

Is has been estimated that, even at rest, our bodies turn over ~70kg of ATP each day. More than 90% of this is generated through mitochondrial oxidative phosphorylation, which occurs on the five membrane protein complexes comprising the respiratory chain. Mitochondria are comprised of ~1500 different proteins. Over 80 of these are subunits of respiratory chain complexes and >100 others are needed for their biogenesis and regulation. Several hundred more mitochondrial proteins support energy production indirectly. Surprisingly, we still don't know the functions of ~200 human mitochondrial proteins!

We are interested in nfunctionalize these proteins, and to do this we use state-of-art gene-editing (CRISPR/Cas9) and proteomics tools. Students undertaking this project will take charge of their very own nfunctionalized mitochondrial protein, use CRISPR-Cas9 to generate knockout human cell lines and use these to study the proteins role in mitochondrial function.

Techniques used may include:

1. Tissue culture of mammalian cells
2. Molecular biology including gene-editing with CRISPR/Cas9
3. Fluorescence activated cell sorting
4. Protein electrophoresis techniques and western blotting

Recent papers from the lab:

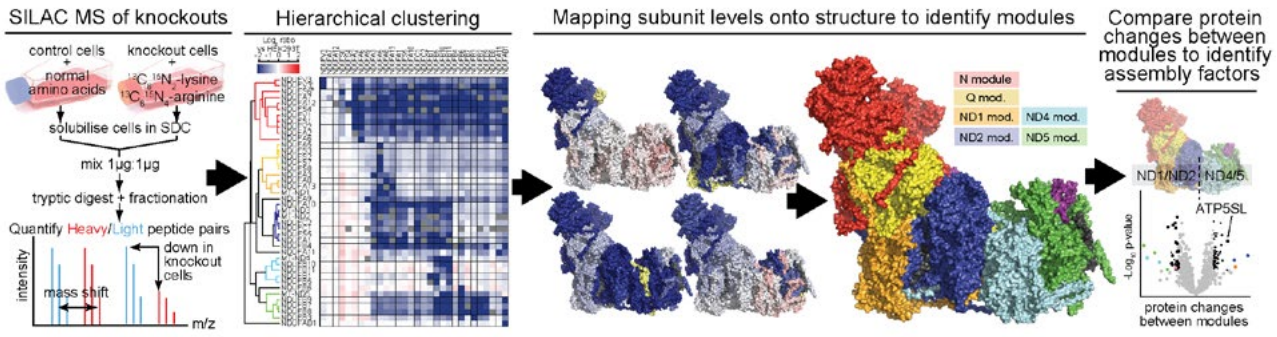
Stroud DA et al., *Nature* 2016

Dibley MG et al., *FEBS Lett* 2017

Formosa LA et al., *Semin Cell Dev Biol* 2017

Contact:

Dr David Stroud to arrange an appointment
david.stroud@unimelb.edu.au



Leann Tilley – A new target and novel drug class to fight Malaria infection

BENCH SUPERVISORS:
Dr Elyse Dunne

OFFERED:
Semester 2

Malaria is the leading cause of death in many tropical and sub-tropical areas and accounts for 2.6% of the world's total disease burden. Each year, 200 million people are infected with *P. falciparum*, the causative agent of malaria. Existing therapies have various pitfalls, such as drug resistance, toxicity and high cost. Up to 50% treatment failure is now observed, where the decreased efficacy of the frontline therapy, artemisinins, puts additional pressure on the limited selection of partner drugs. Renewed efforts to control *P. falciparum* infection have reduced prevalence by over half in the last 15 years, raising the prospect of eradication of malaria. Achievement of this goal requires identification of new drug targets and novel inhibitors to improve current therapies and combat resistant strains.

This project aims to investigate and kinetically characterise a novel drug target involved in protein synthesis, a key function for survival in all stages of the *P. falciparum* life cycle. Furthermore, the mechanism of action of potential inhibitors of this target will be characterised. The student will undertake techniques such as protein purification and the implementation of biochemical assays to assess protein activity.

Techniques used may include:

1. Protein purification.
2. Development of biochemical assays to measure protein activity.
3. Data analysis using kinetic principals.

Recent papers from the lab:

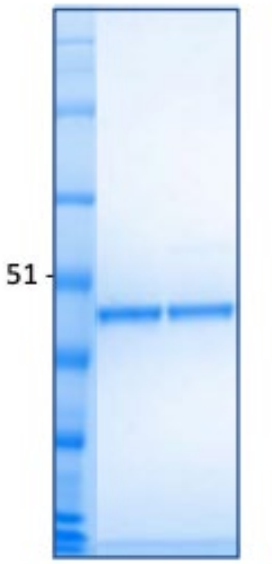
Batinovic S et al., *Nat Commun* 2017

Parkyn Schneider M et al., *PloS Pathog* 2017

Leliott PM et al., *Blood Adv* 2017

Contact:

Dr. Elyse Dunn to arrange an appointment
elyse.dunn@unimelb.edu.au



Jose Villadangos – Harnessing the cells and molecules that initiate adaptive immunity to fight infections, cancer, immunosuppression and autoimmunity

BENCH SUPERVISORS:
TBA

OFFERED:
Semester 2

The Villadangos Laboratory studies the cells and molecules responsible for *Antigen Presentation*. This process is central to adaptive immunity, underpinning the initiation, regulation, persistence and termination of every T cell response. Antigen (Ag) presentation entails intracellular processing of foreign, self or tumour components into ligands that are displayed, bound to Major Histocompatibility Complex molecules, on Ag presenting cells. When naïve T cells recognise these Ags they become activated and an immune response ensues. Activated T cells acquire effector functions that also depend on Ag presentation and recognition e.g. the capacity to stimulate or suppress the activity of other immune cells, or ability to kill tumour cells or cells infected with viruses. Ag presentation can also cause inactivation of naïve or effector T cells, a reaction that prevents autoimmunity but can be exploited by tumours to escape immune surveillance. Another outcome of Ag presentation is the formation of memory T cells that protect against re-infections or tumour recurrence.

Understanding antigen presentation will allow us to design new and better vaccines against infections or cancer, boost immunity in immunocompromised patients and inhibit autoimmune and allergic reactions. We use interdisciplinary research to achieve this goal, implementing a research program that covers from the whole organism through intercellular interactions to the molecular level.

Techniques used may include:

1. Cell isolation, culture
2. Flow cytometry
3. Transcriptomics
4. Gene editing (CRISPR/Cas9 technology) in cells and whole organisms
5. Protein chemistry
6. Microscopy
7. Animal models of infection and cancer

Recent papers from the lab:

McWilliam HE et al., *Nat Immunol* 2016

Roquilly et al., *Immunity* 2017

Liu H et al., *J Exp Med* 2017

Contact:

Professor Jose Villadangos to arrange an appointment
j.villadangos@unimelb.edu.au