Honours projects on offer in the Charles Perkins Centre Hub 2021

**Level 3 East – Heart Research Institute projects**

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<table>
<thead>
<tr>
<th>Research group Leader</th>
<th>Department/School</th>
<th>Research Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr John O’Sullivan</td>
<td>Heart Research Institute, &amp; Central Clinical School, Sydney Medical School</td>
<td>Cardiometabolic Disease</td>
</tr>
<tr>
<td>Senior lecturer; Sydney Cardiovascular fellow (Sydney cardiovascular research consortium)</td>
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<td></td>
</tr>
<tr>
<td>Dr Melissa Farnham, <strong>working with</strong> Dr John O’Sullivan and Dr Kristina Cook (CPC)</td>
<td>Heart Research Institute, &amp; Central Clinical School, Sydney Medical School</td>
<td>Cardiovascular Neuroscience – sleep apnoea and hypertension</td>
</tr>
<tr>
<td>Dr Anna Waterhouse</td>
<td>Heart Research Institute, &amp; Central Clinical School, Sydney Medical School</td>
<td>Cardiovascular Medical Devices</td>
</tr>
<tr>
<td>Sydney Cardiovascular fellow (Sydney cardiovascular research consortium)</td>
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<tr>
<td>Dr Freda Passam</td>
<td>Heart Research Institute, &amp; Central Clinical School, Sydney Medical School</td>
<td>Haemostasis and thrombosis</td>
</tr>
<tr>
<td>Sydney Cardiovascular fellow (Sydney cardiovascular research consortium)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Professor Shaun Jackson</td>
<td>Heart Research Institute, &amp; Central Clinical School, Sydney Medical School</td>
<td>Atherothrombosis</td>
</tr>
<tr>
<td>NHMRC SPRF</td>
<td></td>
<td></td>
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<tr>
<td>Dr Ashish Misra</td>
<td>Heart Research Institute, &amp; Central Clinical School, Sydney Medical School</td>
<td>Impact of Diabetes of haematopoietic cells linked to atherosclerosis</td>
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<tr>
<td>Sydney Cardiovascular fellow (Sydney cardiovascular research consortium), <strong>working with</strong> Professor Shaun Jackson NHMRC SPRF</td>
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<td></td>
</tr>
<tr>
<td>Dr Xuyu (Johnny) Liu</td>
<td>Heart Research Institute, &amp; School of Chemistry</td>
<td>Cardiovascular signaling and drug discovery</td>
</tr>
<tr>
<td>Sydney Cardiovascular fellow (Sydney cardiovascular research consortium)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Honours project details

CARDIOMETABOLIC DISEASE
Dr John O’Sullivan

Research interest: Obesity-driven metabolic disease such as insulin resistance, diabetes, fatty liver disease, hyperlipidaemia, and hypertension are the major drivers of atherosclerotic cardiovascular disease in the modern era. Despite our best primary prevention efforts, this trend is continuing and expected to worsen. These complex diseases are the consequence of gene-environment interactions. Therefore, in order to tackle the cardiovascular consequences of the obesity epidemic, we must improve our understanding of the various levels of dysregulation. To do this, both genomic data and environmental data must be captured. The aim of our studies is to discover better diagnostic markers, predictors, and therapies for cardiometabolic disease.

Research projects:
We will probe carefully-phenotyped patient cohorts using genome scanning and metabolomic profiling to discover novel disease markers that may have clinical utility, e.g., by providing better diagnostic markers of disease, and allowing earlier intervention by predicting future disease. Furthermore, integration of genetic and metabolomic data allows delineation of disease pathways, which we then study in animal and cell models of disease. This allows us to determine disease-specific functional regulation, and potential for therapeutic intervention.

Project 1. Giving the failing heart the nutrients that it needs
The aim of this project is to determine the key cardiac substrates depleted in the failing heart, and to determine if replacing them can restore normal heart function. Heart failure (HF) associated with obesity and type 2 diabetes (leading to “stiff hearts”, termed “Heart Failure preserved Ejection Fraction”, or HFpEF), has exploded in prevalence, has no specific treatment, and is driven in large part by altered metabolism. Therefore, our specific aims are to:
1. Measure cardiac substrate changes in human HFpEF and determine association with heart function and clinical outcome;
2. Determine stages of metabolic alteration, and substrate turnover, during the natural history of HFpEF using model systems;
3. Investigate the effects, and underlying mechanism, of key substrate administration on cardiac function.

Project 2. Probing microbiome-metabolome-cardiovascular disease interactions
Recent research has shown significant health benefits deriving from high-dietary fiber/microbiome-accessible carbohydrate (MAC) consumption. Compared with native starch, dietary resistant starch is a high-MAC starch that significantly alters the gut microbiome. In recent studies, we determined the systemic metabolic effects in male C57BL/6 mice fed either a native- or resistant-starch diet for 18 weeks (n = 20/group). Metabolomic analyses revealed that the plasma levels of numerous metabolites were significantly different between these diets, many of which were microbiome-derived. Most strikingly, we observed
a 22-fold increase in the gut microbiome-derived tryptophan metabolite indole-3-propionate (IPA), which was positively correlated with several gut microbiota including Clostridiales, Allobaculum, Bifidobacterium and Prevotella. Major changes were also observed for metabolites solely or primarily metabolized in the gut, e.g., trimethylamine-N-oxide; metabolites that have a significant entero-hepatic circulation, i.e. bile acids; lipid metabolites, e.g. cholesterol sulfate; metabolites indicating increased energy turnover, e.g. tricarboxylic acid cycle (TCA) intermediates and ketone bodies; and increased anti-oxidants such as reduced glutathione. Our findings reveal potentially novel mediators of high MAC-derived health benefits. We will now extend this analysis and examine the role of IPA, and related indoles, as THE major mediators of the cardiometabolic benefits of high-fibre diets.

Project 3. Uncovering the Interaction of Obstructive Sleep Apnoea with Cardiac Metabolism, Function, and Disease

Working with Dr Melissa Farnham (Cardiovascular Neuroscience, HRI) and Dr Kristina Cook (CPC, USyd), we have recently uncovered that intermittent hypoxia (as seen in obstructive sleep apnoea) causes hallmarks of cardiac insulin resistance and changes in cardiac substrate utilisation such as upregulation of cardiac ketone bodies. We have developed a program that includes homes sleep studies in all our heart failure patients (with Prof Peter Cistulli, ResMed Chair in Sleep Medicine), a rat model of intermittent hypoxia (Dr Melissa Farnham, HRI), with expertise in the master regulator of hypoxia transcriptional change, HIF-1α (Dr Kristina Cook, Charles Perkins Centre).

We aim to do the following:

1. Determine the mechanisms of intermittent hypoxia-induced metabolic dysregulation via HIF-1α and other regulators.
2. Develop novel therapeutics to mitigate these effects.
3. Explore the cardiovascular perturbations consequent upon these changes.
4. Examine the metabolic and cardiovascular consequences of obstructive sleep apnoea clinically using our advanced echocardiography, vascular measurements, cardiac MRI, and sophisticated metabolic profiling.

**Outcomes:** 1. The first rigorous exploration of the protean cardiometabolic changes induced by obstructive sleep apnoea. 2. Novel management strategies and therapeutic agents to target these cardiometabolic complications
CARDIOVASCULAR MEDICAL DEVICES
Dr Anna Waterhouse

**Research interest:** Our research focuses on how medical devices – such as artificial hearts, stents and bypass machines – interact with the body. We apply cutting-edge bioengineering tools to develop new methodologies to assess and understand the interplay of events at the biointerface, where the devices interact with the patient, and manipulate this interplay to improve medical device function, create novel medical devices and diagnostics and both drug and non-drug-based avenues for therapies.

**Research projects:**

**Project 1. Developing models of biomaterial-device thrombosis.**
Advances in micro and nanotechnology have revolutionised bioengineering, allowing high precision manipulation of materials for modelling medical devices in the lab. Using bioengineering strategies, increasingly sophisticated devices are being constructed. However, protein and cellular interactions with materials are still poorly understood. One such example where this lack of understanding causes detrimental outcomes is blood-material interactions causing medical device failure. Blood is one of the most complex biological fluids containing multiple proteins and cell types. When blood contacts foreign materials in medical devices, it can cause fatal thrombosis (blood clots). The aim of this project is to develop novel bioengineering solutions to study how material properties and blood flow dynamics govern the initiation of biomaterial-induced thrombosis, with the ultimate aim of improving medical device function.

The majority of experimental systems to test biomaterial-induced thrombosis in vitro rely only on traditional in vitro clotting assays which are done in test tubes using solutions of individual enzymes, purified fibrinogen or platelet-free plasma. These systems do not account for the reaction dynamics of cellular components or physiological blood flow, both of which are integral to thrombosis. Microfluidic systems provide sophisticated, real-time analysis of proteins and blood components that drive thrombosis, combined with the ability to manipulate blood flow at physiologically relevant rates. Utilizing state-of-the-art facilities at Australian Institute of Nanoscale Science and Technology (AINST) (University Sydney), we aim to develop bioengineering solutions using microfluidics to investigate protein/cellular interactions at the biointerface. Different medical device materials will be assessed for their mechanism of thrombosis initiation. Furthermore, this platform system could be used to evaluate novel bioengineered surfaces, such as repellent, immobilized liquid surfaces or tissue engineered materials.

**Project 2. Investigating the mechanism by which super repellent surface coatings reduce thrombosis of medical devices**
Blood clots (Thrombosis) forming in medical devices can be costly and fatal for a number of reasons. First, these clots can cause failure of the device requiring costly replacement, or cessation of blood flow, which can be fatal. Second, embolism of thrombi formed in devices
can lodge in the lungs or brain, causing pulmonary embolism or stroke. Progress in this field has been achieved through the development of slippery, liquid immobilized surfaces, of Tethered-Liquid Perfluorocarbon (TLP) coatings, which have been demonstrated as effective to prevent thrombosis and biofouling by preventing surface adhesion of blood and pathogens.

The aim of this project is to determine the mechanism by which TLP coating can reduce the thrombogenicity of medical devices. Tethered-liquid perfluorocarbon (TLP) coatings reduce fibrin polymerization and platelet adhesion and activation in vitro under static and blood flow conditions. In vivo, an extracorporeal circuit consisting of TLP coated medically approved tubing and cannulae, remained patent for at least 8 hours at 15L/hr of blood flow in a swine arteriovenous shunt model without the use of any antithrombotic medication (Leslie et al., 2014). However, the mechanism by which proteins and cells are repelled by TLP remains poorly understood. Here we aim to explore how plasma proteins, such as fibrinogen, and blood cells interact with TLP surfaces. This will have implications for how thrombus propagation is induced on TLP surfaces. Utilizing this system, the contribution of adhesion and local accumulation of blood components vs. protein and cellular activation to thrombosis and prevention of thrombosis could be elucidated. Understanding the mechanism of the low-thrombogenic, repellent properties of TLP coatings will enable improved application to medical devices and provides insights for design improvements.


HAEMATOLOGY
Dr Freda Passam
Research Interest:
The Haematology Research Lab aims to discover novel pathways in blood clotting which can lead to the development of effective and safe drugs to treat thrombosis.

Research projects:
Project 1. Thiol isomerases as novel antithrombotic targets
It has recently been discovered that thiol isomerases constitute a new clotting pathway. Thiol isomerases are a group of enzymes that regulate the function of blood cell receptors and clotting proteins by reacting with their disulphide bonds. We have identified a thiol isomerase, named ERp5, which is released into the circulation from activated platelets and promotes clot formation in vivo. The aim of this project is to discover alternative pathways in the clotting system that can be targeted to develop efficient and safer antithrombotic drugs. We will dissect the role of ERp5 in platelet function and clot formation by using mice with genetic deletion of ERp5 in their platelets. We will investigate how this thiol isomerase
regulates the interaction of platelets with clotting proteins (fibrinogen, von Willebrand factor) and vascular cells (endothelial cells and neutrophils). We will explore the potential of ERp5 inhibitors to prevent thrombus formation and become candidate antithrombotic drugs.

These studies will employ platelet function tests, cell perfusion assays, flow cytometry and confocal microscopy, and will provide the opportunity to learn the method of intravital microscopy for the study of clot formation in mice.

Fig1. In vivo thrombus formation in the cremaster artery of (A) a mouse injected with inactive control compound and (B) a mouse injected with an ERp5 inhibitor. Platelets are labelled in red and fibrin in green.

Relevant publications:

Project 2. Redox biomarkers in thrombotic disease
The redox balance (balance of reduction and oxidation reactions in our blood) is essential for a healthy circulation. Redox imbalance causes alterations of protein function contributing to the development of thrombosis. We are focused on redox modification of disulphide bonds in two proteins critical for thrombus formation: the platelet receptor integrin α2βb3 and the plasma protein von Willebrand factor. We have found that reduced forms of α2βb3 and vWF have decreased thrombotic activity and may therefore protect from thrombotic disease, such as venous clots. The aim of this project is to identify new biological markers that can be used in the monitoring and treatment of patients with thrombotic disease.

We have developed assays which measure the redox balance in blood including tests which measure the disulphide reducing activity of plasma and the production of reactive oxygen species by platelets. We will study the redox modifications of platelet α2βb3 and plasma vWF which occur in patients at high risk for thrombosis to identify those most likely to benefit from drugs which restore the normal redox balance.

This project will employ mass spectrometry to study the posttranslational modifications of clotting proteins using disulphide labels specifically developed for this purpose. It also involves plasma and platelet functional assays.

Figure 2. Alterations of disulphide bonds in clotting proteins which cause decreased clotting activity in (A) the platelet receptor a2bb3, bond Cys177-Cys184 and in (B) von Willebrand factor, bond Cys1669-Cys1670.

Project 3. Developing biochips for the study of haemostasis and thrombosis

Many patients with bleeding and clotting disorders go undetected by routine laboratory tests in part because the available assays do not reflect the conditions in the circulation. The Haematology Research Group uses biochips in a microfluidic system that allows blood to flow through passages under controlled conditions. The passages are designed to mimic blood vessels and include features e.g. stenosis, that simulate the circulation in stenosed vessels. The flow of blood through these biochips generates thrombi that can be visualized by real-time microscopy and quantified. The aim of this project is to develop microfluidic devices which can detect the thrombotic or bleeding tendency in patients with clotting problems.

This project will study blood cell adhesion and thrombus formation in the microfluidic devices to assess for persisting thrombotic tendency in patients with a history of venous clots, who have completed treatment. Samples from patients with bleeding disorders on treatment will be assessed for haemostatic potential. A range of parameters, which participate in clot formation, will be measured in the microfluidics system including platelets, fibrin, neutrophil extracellular traps, von Willebrand factor. This project will involve the preparation of microfluidic chips, microscopy and image analysis.

Figure 3. Microfluidic devices for measuring thrombotic and bleeding tendency. (A) Biochip containing channels for perfusion of blood (B) Schematic of a 90% stenosed channel for the study of thrombus formation (C) Blood sample with increased thrombus formation and (D) decreased thrombus formation at the
Relevant publications:

THROMBOSIS
Prof Shaun Jackson [Contact - A/Prof Simone Schoenwaelder]
Research interest:
The Thrombosis Research Group aims to understand the events leading to blood vessel occlusion of the macro- and micro-vasculature, precipitating thrombosis and ischaemia reperfusion injury (IR) in cardiovascular disease. Research carried out in the Thrombosis group focusses on the following themes:

- Cell death pathways regulating vascular dysfunction
- Mechanisms leading to microvascular dysfunction and poor cerebral perfusion in stroke and ischaemia/reperfusion injury
- Discovery/preclinical development of novel antiplatelet and/or anticoagulant treatments for stroke.
- Investigating mechanosensitive pathways regulating thrombus formation

Our approach to these research questions is to examine interactions between blood cells (platelets, leukocytes, erythrocytes) and injured blood vessels (primarily endothelial cells), in vitro and ex vivo, as well as in vivo using mouse models of thrombosis, ischaemic stroke and IR injury. We combine these approaches with cutting-edge technologies including: Advanced microscopy techniques (intravital imaging, confocal, TIRF, super resolution, 2-photon, tissue clearing); molecular mouse models and genome editing; Omic studies; Biomechanics/microfluidics, biomembrane force probe (BFP) studies.

Whilst our studies are primarily aimed at defining new mechanisms promoting thrombosis and inflammation (termed thromboinflammation), we also actively translate our research discoveries into new therapeutic approaches.

Project 1. Cell death pathways regulating vascular dysfunction
Acute myocardial infarction (AMI) and stroke are the major cause of disability and mortality globally. The primary focus of AMI and stroke therapy is to promptly re-open the blocked arteries to salvage the dying ischemic tissue. However, despite the re-opening of the culprit artery, blood perfusion in surrounding microvasculature supplying the tissue can remain poor, a common complication of ischemia reperfusion (IR) injury, known as microvascular obstruction (MVO). MVO occurs in 60% AMI patients and persistent MVO can lead to progressive worsening of heart function and infarction. Several pathogenic processes have been implicated in MVO, however targeted therapies have not been effective in improving microvascular perfusion. This is due in part to the lack of suitable animal models and technical difficulties associated with performing real-time imaging on the microvasculature. In order to gain a better understanding of the temporal and spatial events leading to MVO, thus affording
better insights into potential therapy options, we have established a mouse model of gut IR injury which allows access to the microvasculature in living animals during IR injury. Using this model, combined with cutting edge confocal microscopy, we have observed previously unappreciated in vivo changes within the microvasculature during IR injury. There is increasing evidence that genetically-regulated cell death pathways (necroptosis, apoptosis, pyroptosis, autophagy) play an important role in regulating the cardiovascular system in health and disease. We have recently uncovered new roles for apoptosis and necroptosis in regulating microvascular dysfunction during IR injury. Our ongoing studies aim to identify/characterise the cell death pathways promoting IR injury, and identify/test novel therapeutic targets which may reduce the impact of IR injury on end-organ function (heart, brain and gut). This is particularly important given that dysregulation of these pathways may also help explain the vascular problems experienced by COVID-19 patients.

Relevant publications from our group:

Project 2. Discovery and development of novel antithrombotics for the treatment of stroke

The development of a thrombosis or embolus in the cerebral circulation (ischaemic stroke [IS]) is the third most common cause of death and the most common cause of adult disability globally. Whilst considerable progress has been made in developing more effective treatments for coronary disease, progress in the management of stroke continues to be unsatisfactory. The central goal of acute stroke therapy is the prompt re-opening of occluded blood vessels to minimise tissue death. The delivery of fibrinolytic agents modelled on tissue-type plasminogen activator (t-PA) is the only clinically approved thrombolytic agent for IS therapy. However, thrombolytic therapy is not without its limitations, with lysis resistant blood clots, as well as haemorrhage presenting as major complications. One of the main factors delaying reperfusion and increasing the risk of re-occlusion of cerebral vessels is the presence of platelets in arterial thrombi, with numerous preclinical and clinical studies demonstrating the benefits of adjunctive anti-platelet therapy to enhance cerebral reperfusion and reduce re-occlusion following thrombolysis. Unfortunately, in IS patients, the benefits of combined antiplatelet/thrombolytic therapy are partially offset by the increased risk of life-threatening intracerebral bleeding, limiting the widespread use of this approach.

Our laboratory has a longstanding interest in identifying pathways in platelets that are important for arterial thrombus formation, but less critical for haemostasis. One of these pathways involves shear activation of platelets through activation of the p110β isoform of PI3-kinase (PI3Kβ). This project will examine the mechanisms by which PI3Kβ inhibitors
enhance reopening of the blood vessel, examine the impact of thrombus channel formation on blood flow, thrombus porosity and thrombus dissolution. Moreover, the impact of PI3Kβ inhibitors on end-organ damage, particularly in the stroke context, will also be examined. These studies will not only provide important insight into our understanding of blood clot formation but may also lead to new approaches to regulate the size and stability of blood clots forming in the body, providing major clinical benefit in the delivery of thrombolytic therapy (blood clot removal).

Studies involve the use of:

i. *in vivo models of thrombosis and thrombolysis*,

ii. *genetic mouse models*

iii. *state-of-the-art imaging systems* (tissue clearing techniques, confocal microscopy, intravital microscopy, laser doppler flowmetry and laser speckle contrast imaging)

iv. *behavioural assessment to determine cerebral damage following recovery from stroke*

Relevant publications from our group:

Project 3. Mechanosensitive pathways regulating thrombus formation - Multidisciplinary 4M’s approaches

To investigate platelet mechanobiology at cell-molecular scales, we have established the 4M’s approaches in Australia: Mechanics, Microscopy, Microfabrication & Molecular Mouse Models by combining the live-cell dynamic force spectroscopy BFP system with other complementary technologies including the TIRF/STORM super-resolution imaging, microfluidics, in vivo mouse models of thrombosis as summarised in the figure.

Relevant publications from our group:


Project 5 – Impact of Diabetes on haematopoietic cells linked to atherosclerosis working with Dr Ashish Misra [ATHEROSCLEROSIS AND VASCULAR REMODELLING]

Obesity and diabetes are major risk factors for a broad range of cardiovascular diseases. With three-times as many people in the world estimated to die from over-nutrition than from starvation or malnutrition in today’s society, the health implications of this "diabesity" epidemic are enormous. Based on current trends, this scenario will get worse, leading to a tsunami of cardiovascular diseases that could overwhelm a healthcare system already struggling to deal with an ageing population. Thus, there is an urgent need to uncover the fundamental mechanisms underlying the development of diabetes, including how cardiovascular risk factors affect atherosclerosis – in order to develop rationale strategies for minimizing the impact of these risk factors on our health and economy.

Bone-marrow derived stem cells (BMDSCs) and progenitor cells are integral to tissue homeostasis and repair and contribute to health through their ability to self-renew and
commit to specialized effector cells. Importantly, defects in a variety of progenitor cell populations have been described in both preclinical and human diabetes. The general perception is that diabetes drives defects in bone marrow derived stem cells (BMDSCs) which accrue damage over time, disrupting tissue homeostasis and increasing risk of morbidity. However, the mechanisms by which defective BMDSCs can influence the pathology of individual plaque cells in atherosclerosis, and the subsequent impact this has on diabetes and obesity remains unknown.

In this study, we will be characterizing effects of these BMDSCs on atherosclerotic plaque burden using state-of-the-art of the art transgenic mouse models and cardiovascular genetics. We will be using Cre-LoxP system, genetic knockouts, lineage tracing, clonal analysis, Single-cell RNA sequencing, bone marrow transplant and culturing BMDSCs, histology of mouse and human patient samples and general cell and molecular biology techniques.

**ATHEROSCLEROSIS AND VASCULAR REMODELLING**

*Dr Ashish Misra*

**Research Interests:**

Our main objective is to broaden understanding of the cellular and molecular mechanisms involved in blood vessel wall patterning, define the role of these pathways in vascular abnormalities and complications, and then link these insights to translational research to improve the prevention and treatment of human cardiovascular disease.

To this end, we employ a unique blending of lab models and cultured cells, as well as human samples, with the aim of unveiling the pathogenesis of cardiovascular diseases.

Our ultimate goal is to prevent and reverse vascular disease to prevent heart attack and stroke.

**CARDIOVASCULAR SIGNALLING AND DRUG DISCOVERY**

*Dr Xuyu (Johnny) Liu*

**Research interest:**

Despite the global burden of cardiovascular disease, the development of new cardiovascular drugs has stalled for over two decades. The primary attrition is the intolerance of drug-related side effects. Recently, there is considerable interest in the development of natural supplements for cardiovascular-protective therapeutics owing to the inherent safety profiles and the clinical evidence for ameliorating chemotherapy-induced cardiovascular complication. However, it remains a huge challenge to understand the cardiovascular-protective mechanisms at the molecular level, which impedes pharmacological optimisation of these bioactive agents for therapeutic use. Therefore, we aim to apply cutting-edge chemoproteomic-platforms to understand the intricate signalling interplay in cardiomyocytes.
in response to different natural products and construct a comprehensive chemotype database for cardiovascular drug discovery.

**Understand heart-healthy diets at the molecular level**
Sulforaphane and alliin are known to be the cardioprotective “ingredients” in broccoli and onion diets. They have been shown to promote cardiomyocyte survival against ischemic injury and exhibit potent anticancer activity by potentiating apoptosis. However, the protein target spectra of these small molecules in cell remain unclear. There is no unified model to explain the cell-type-dependent phenotypes observed in the treatment.

**Project 1:**
(1) Profile the cell-type-specific target spectra of sulforaphane and alliin and forge a molecular link between protein target engagement and phenotypic outcome;
(2) Engineer small-molecule transport proteins targeting specific organelles to enable protein target profiling of sulforaphane and alliin in a spatiotemporally controlled manner.

**Project 2:** In collaboration with the Payne research group (School of Chemistry, USYD),
(1) Optimise the efficacy and mitigate the cardiotoxicity of current chemotherapeutic strategies through conjugation with cardiovascular-protective natural products;
(2) Apply “click-and-release” chemistry to develop antibody-small-molecule conjugates enabling organ- and tissue-specific release of sulforaphane and alliin.

**Project 3:** Developing safer anti-clotting agents derived from “Mother Nature” for the treatment of stroke [In conjunction with the Thrombosis Research Group]
Thrombin is by far the most robust activator of blood clotting in both physiological haemostasis and pathological thrombotic response. Fibrin clots contain a large amount of thrombin, which is released into the circulation following administration of clot busting drugs to treat stroke (rtPA, thrombolysis). This pool of thrombin remains highly active and is responsible for the rethrombosis. While a strong rationale exists for the use of thrombin inhibitors as effective antithrombotic agents in enhancing clot lysis, all current thrombin inhibitors lead to severe bleeding complications, precluding their use in stroke, due to risk of intracerebral haemorrhage. We have identified novel anticlotting agents derived from naturally occurring proteins found in saliva of the bush tick. Our studies have demonstrated that these bug-derived proteins are able to dissolve blood clots in a disease model of thrombosis with fewer bleeding complications. This project will involve the synthesis of novel anti-clotting proteins and characterisation the mechanisms underpinning the safe antithrombotic mechanism, and testing whether administration of this novel drug in combination with rtPA provides for a safe approach to treatment of thromboembolic diseases such as stroke in the future. This project will be co-supervised by: Dr Xuyu Liu, A/Prof. Simone Schoenwaelder, Dr Jessica Maclean.