2018/2019 Glycomics Research Projects

A list of selected Glycomics Research Projects is provided below. These projects are geared primarily to prospective Honours Students. However, they can also be further developed for PhD/MPhil candidates. For more information on these projects please contact the supervisors whose contact details are available in the Griffith Staff Directory or by contacting glycomics@griffith.edu.au.

Undergraduate students are also encouraged to look at the list of projects and contact the appropriate staff member highlighted in the projects list, to discuss third year projects and work experience opportunities.

Honour Scholarships

The Institute for Glycomics offers a number of prestigious honours scholarships to undertake glycomics research at Griffith University's Gold Coast Campus. There are Glycomics Circle Honours Scholarships worth $5,000 each, and one Sally & Warren von Bibra Honours Scholarship worth $6,000. Sally & Warren von Bibra are strong supporters of the Institute for Glycomics and have been generously sponsoring this honours scholarship scheme since 2003. The Glycomics Circle was established in 2015 and is a group of successful women in the local community who are driven to raising funds for Glycomics, in particular to support young women in science. One of their interests is to contribute funds to the Glycomics Honours Scholarship scheme.

Prospective students are requested to contact potential Institute supervisors to discuss suitable research projects, prior to submitting an application. Closing date for applications is on **Sunday 10 February 2019.**

Application guidelines and further information is available from the Griffith University Scholarships website:

Sally & Warren von Bibra Honours Scholarships: https://www2.griffith.edu.au/scholarships/scholarship-listings/sally-and-warren-von-bibra-honours-scholarship

Glycomics Circle Honours Scholarships: https://www2.griffith.edu.au/scholarships/scholarship-listings/glycomics-circle-honours-scholarship

Summer Scholarships

Every year the Institute for Glycomics offers Summer Scholarships worth $1000 each to Griffith University students to undertake research within the field of glycoscience. Prospective students are requested to contact potential Institute supervisors to discuss suitable research projects, prior to submitting an application. Closing date for applications is **Sunday 30 September 2018**.

Application guidelines and further information will be available on the Griffith University Scholarships website at https://bit.ly/2omdj0v

1. The design and synthesis of carbohydrate-based compounds as anti-malarial agents

Prof Mark von Itzstein, Dr Yun Shi & Dr Robin Thomson

Medicinal Chemistry

Malaria is the most serious protozoan disease in humans with an estimated 212 million cases of malaria, and over 400,000 deaths from malaria, in 2015. Resistance to commonly used anti-malarial drugs is now widespread among isolates of *Plasmodium falciparum*, the species responsible for the most severe disease and mortality.

The invasion of red blood cells (erythrocytes) by the merozoite form of the parasite is critical for the survival of the parasite, and a crucial stage in the development of disease. It is therefore a logical target for the development of interventions to control malaria. Interaction of the merozoites of *P. falciparum* with erythrocytes is mediated in part by the erythrocyte binding antigen (EBA-175) on the merozoite surface which binds to a sialylated glycoprotein on the erythrocyte surface, glycophorin A. This project will involve the synthesis of carbohydrate-based compounds to probe the interactions between the parasite and host cells, to further our understanding of this important stage of infection.

Techniques: Synthetic carbohydrate chemistry; Computational Chemistry including visualisation and molecular docking.

2. New approaches towards anti-Dengue virus agents

Prof Mark von Itzstein, Dr Chih-Wei Chang, Dr Yun Shi & Dr Robin Thomson

Medicinal Chemistry, Structural Biology

Dengue virus is a mosquito-borne flavivirus that causes both dengue fever and the potentially lethal complication dengue haemorrhagic fever. Dengue virus infection has become a major international public health concern with the disease now endemic in over 100 countries, an estimated 40% of the world's population at risk of infection, and 50 to 100 million cases of dengue infection worldwide each year. There is, however, no specific antiviral treatment for dengue fever and development of a suitable vaccine has proven difficult.

The surface of dengue virus is covered by a glycoprotein (the "Envelope" or "E" glycoprotein) that is essential in the life cycle of the virus, being involved in both binding to and fusion with target host cells. Stopping these critical initial stages of the infection process by blocking the interaction of E glycoprotein with the host cells is an attractive approach for therapeutic and/or preventative intervention against dengue virus infection. This multidisciplinary project can include work in one, or several, areas, including: synthesis of carbohydrate-based compounds as potential inhibitors of E-glycoprotein–cell interactions; structural biology using saturation transfer difference (STD) NMR to examine the interactions of the ligands with E-glycoprotein; computational chemistry to evaluate potential binding modes of carbohydrate ligands with E-glycoprotein.

Techniques: Synthetic carbohydrate chemistry; Computational Chemistry including visualisation and molecular docking; Advanced NMR techniques including STD-NMR.

3. Epitope binding investigations carbohydrate-recognising proteins by NMR spectroscopy

Assoc Prof Thomas Haselhorst, Dr Yun Shi & Prof Mark von Itzstein

Molecular Modelling, Structural Biology

Many carbohydrate-recognising proteins, eg Siglec 2 and *trans*-sialidases, have been implicated in clinically signfiicant diseases such as non-Hodgkin's lymphoma and trypanosomiasis, respectively. Recently, the method STD-NMR was developed to screen compound libraries against various protein targets. This method is suitable for determining an epitope map of a ligand within the protein's binding site as only regions of the ligand that are in contact with the protein's binding site are observed in the NMR spectrum.

Techniques: Computational Chemistry including visualisation and molecular docking; Advanced NMR techniques including STD-NMR; Protein purification

4. From structure to function – rational development of new sialidase inhibitors

Prof Mark von Itzstein, Dr Robin Thomson, Dr Mauro Pascolutti, Dr Andrea Maggioni, Dr Benjamin Bailly, Dr Yun Shi, Dr Thomas Ve & Dr Xing Yu (Hunan Normal University, China)

Molecular Modelling, Medicinal Chemistry, Biochemistry, Molecular Microbiology, Virology, Structural Biology

Sialidases are involved in the infective cycles of a range of viruses, bacteria, and parasites, such as the causative agents of influenza, cholera, and African sleeping sickness. The essential roles that the sialidases often play in the infection cycle make them interesting targets for drug design. In the case of influenza virus sialidase, development of potent and selective inhibitors of the enzyme, based on knowledge of the enzyme structure, led to a new drug class to treat influenza. In recent years, new structural and mechanistic characteristics of microbial sialidases have been discovered, presenting new opportunities for inhibitor design.

The von Itzstein group works on the development of new sialidase inhibitors against pathogenic organisms using a multidisciplinary approach that includes: computational chemistry and structure-based inhibitor design; synthetic chemistry, working on a range of inhibitor templates; expression and purification of recombinant enzymes; the use of whole virus particles or virus-like particles presenting the enzyme on a non-infectious particle; enzyme assays for evaluation of inhibitor affinity; cell-based evaluation of compounds, and; structural biology studies in solution phase (NMR) or through X-ray crystallography. A student working on this project may specialise in one particular aspect, or be involved with a number of the different interconnected aspects of the project.

Techniques: Computational Chemistry including visualisation and molecular docking; Synthetic carbohydrate chemistry; Purification including HPLC; Protein expression and purification; Virology; Enzyme Assays; Cell-based assays; Advanced NMR techniques including STD-NMR; X-Ray crystallography.

5. Carbohydrate-based biological probes for the investigation of microbial glycan biosynthesis

Prof Mark von Itzstein & Dr Robin Thomson

Medicinal Chemistry

Bacterial resistance to antibiotics is a growing problem, and is driving the search for novel antibacterial therapies. Importantly, bacterial cell membrane components often contain carbohydrate units and structural linkages that are not found in mammalian systems. The biosynthetic pathways to these structures are therefore attractive targets for the development of antimicrobial agents that affect the growth and integrity of, specifically, bacterial cell membranes. This project, as part of a continuing antimicrobial drug discovery programme, will involve the preparation of carbohydrate-based compounds for the investigation of bacterial cell wall biosynthetic enzymes, and their evaluation as inhibitors of bacterial growth.

Techniques: Synthetic carbohydrate chemistry; Bacterial cell growth assays.

6. Multivalent carbohydrate structures

Prof Mark von Itzstein, Dr Robin Thomson & Dr Chih-Wei Chang

Medicinal Chemistry

Interactions between cells, and between cells and microorganisms, are often based on simultaneous, multiple interactions between protein receptors and their carbohydrate ligands. Mimicking these interactions by the use of multivalent arrays of receptor ligands, for example dendritic structures terminated with biologically relevant molecules, has been successful for a number of carbohydrate-recognising proteins. This project involves the design and synthesis of small multivalent structures, carrying functionalised carbohydrate residues, to be used as biological probes in a number of biological systems, for example in cell-binding studies of human pathogenic viruses.

Techniques: Synthetic carbohydrate chemistry.

7. Chemoenzymatic synthesis of biologically active glycans

Prof Mark von Itzstein, Dr Chi-Hung Lin & Dr Robin Thomson

Medicinal Chemistry

Human cell-surface carbohydrates (glycans) on glycoproteins and glycolipids are involved in important cell–cell and cell–biomolecule interactions. They also often form the initial attachment point for invading pathogenic microorganisms. Studies in glycobiology often require the use of a natural, or specifically modified, glycan to characterise and increase understanding of a specific biological interaction. However, not all natural glycans are commercially or readily available. While methods of chemical glycan synthesis are advancing, there are significant advantages in the use of enzymes to construct both complex monosaccharides, and the linkages between monosaccharide units to form a glycan. Combining chemical manipulation of monosaccharide residues, or of a final glycan structure, with enzymatic linkage formation, it is possible to produce both natural and specifically modified complex glycan structures. This project will incorporate the use of both traditional carbohydrate chemistry techniques and the use of carbohydrate biosynthetic enzymes, to prepare glycans for use in a range of biological studies.

Techniques: Synthetic chemical and enzymatic carbohydrate chemistry.

8. Investigation of β-glucuronidases

Prof Mark von Itzstein, Dr Chih-Wei Chang, Dr Robin Thomson, Dr Yun Shi & Dr Xing Yu (Hunan Normal University, China)

Molecular Modelling, Medicinal Chemistry, Biochemistry, Structural Biology

β-Glucuronidases are essential mammalian enzymes, which play a major role in the normal structuring and turnover of components of the extracellular matrix. In addition to their roles in normal human biology, over-expression of, in particular, the endo-β-glucuronidase heparanase can facilitate tumour cell growth and spread. X-Ray structural information is now available for the important endo-β-glucuronidase human heparanase, as well as for a bacterial heparanase. This structural information can provide new insights into the catalytic mechanism of the enzymes, and offers opportunities for inhibitor development.

This project offers a number of avenues for the investigation of β-glucuronidases, which can be either undertaken separately or together; computational chemistry and molecular modelling studies with enzyme X-ray structures; the chemical synthesis of probes to explore enzyme function and activity; biological evaluation of probes and known

substrates or inhibitors using enzyme assay and NMR spectroscopy, and; investigation of enzyme–inhibitor complex formation by X-ray crystallography. Each of these aspects will lead to an improved understanding of this important class of enzyme.

Techniques: Computational Chemistry including visualisation and molecular docking; Synthetic Chemistry; Protein expression and purification; Enzyme assays; Advanced NMR techniques including STD-NMR; X-ray crystallography.

9. The discovery and characterisation of charged glycans as inhibitors of enterovirus 71 infection

Dr Chi-Wei Chang, Dr Benjamin Bailly, Dr Robin Thomson & Prof Mark von Itzstein

Medicinal Chemistry, Virology, Structural Biology

The picornavirus Enterovirus 71 (EV71) is a major cause of hand, foot and mouth disease in children less than 5 years old worldwide. While the disease usually presents mild symptoms, it can sometimes spread to the central nervous system and cause severe neurological infections such as flaccid-like paralysis or encephalitis. There are currently no treatments or vaccines against EV71 infection.

EV71 is thought to infect cells by binding to various cellular receptors including glycosaminoglycans (GAG) and sialylated glycans. While most efforts in anti-EV71 drug discovery are focussed on inhibiting the various viral proteases, we take advantage of the scaffold of naturally occurring glycan receptors to investigate the potential of functionalised glycans and GAG-mimetics to inhibit the virus binding to cells. This project therefore involves medicinal carbohydrate chemistry for the design and synthesis of glycans, virology techniques for the cell-based screening and evaluation of compounds, X-ray crystallography and STD-NMR technologies for the characterisation of virus/glycan binding events.

Techniques: Synthetic carbohydrate chemistry; Chemical characterisation including Proton and Carbon-13 NMR, Mass Spectrometry; Purification including HPLC; Virology; Cell biology; Crystallography; NMR techniques including STD-NMR;

10. The characterisation of enterovirus 71 binding specificity to host cell receptors

Dr Benjamin Bailly & Prof Mark von Itzstein

Virology, Structural Biology, Cell Biology

The hand, foot and mouth disease causing agent enterovirus 71 engages a variety of receptors on the surface of host-cells prior to entry. These receptors include the P-selectin glycoprotein ligand-1 (PSGL-1), the scavenger receptor class B member 2 (SCARB2), glycosaminoglycans (GAG) and sialylated glycans. The interplay between these receptors is still poorly understood. The types of GAGs and sialylated glycans the virus binds to have not been fully investigated, and we believe that given our progress with GAG-like binding inhibitors they may be more important than previously reported. Furthermore, in our experience different cell-types have different susceptibilities to glycan-based binding inhibitors, suggesting that cell binding events may be more complicated than previously characterised.

This multidisciplinary research project involves the differentiation of various cell types and subsequent functional assays to investigate virus-cell binding events, glycan-array experiments, cell-based chemical combination assays using glycans, competition STD-NMR experiments and crystallography using purified virus particles.

Techniques: Virology; Cell biology; Crystallography; NMR techniques including STD-NMR; Glycan-Array.

11. Structure Affinity Relationship (SAR) by NMR

Assoc Prof Thomas Haselhorst, Dr Andrea Maggioni & Prof Mark von Itzstein

Structural Biology, Biochemistry

Abbott laboratories have published a new NMR spectroscopic method called “SAR by NMR” to identify binding ligands and simultaneously to detect amino acids within the protein binding sites which play a key role in the binding event. This project will involve the expression and purification of ^{15}N labelled rotavirus VP8* protein in minimal media and the analysis of the purified labelled protein by means of high resolution NMR spectroscopy. $^{15}N/^{1}H$-HSQC experiments of the *apo* protein and complexed with potential binding ligands are acquired. For amino acids involved in the binding event a change in their chemical shifts is likely. This valuable information can then result in lead structures for the design of new anti-viral drugs.

Techniques: Chemical Characterisation including Proton and Carbon-13 NMR; Advanced NMR techniques including STD-NMR; Protein purification.

12. Parainfluenza virus glycan receptor characterisation and structure-based discovery of anti-parainfluenza viral agents

Prof Mark von Itzstein, Dr Patrice Guillon, Dr Ibrahim El-Deeb, Dr Mauro Pascolutti, Dr Larissa Dirr, Dr Thomas Ve, Dr Robin Thomson, & Dr Yun Shi

Molecular Modelling, Medicinal Chemistry, Molecular Biology, Biochemistry, Structural Biology

Human parainfluenza viruses (hPIV) are important respiratory tract pathogens, second only to respiratory syncytial virus. Infants, young children, the elderly and the immunocompromised are most severely infected, developing croup, pneumonia or bronchiolitis that may need patient hospitalisation. Currently there are neither vaccines nor specific antiviral therapy available to prevent or treat hPIV infections.

Among the hPIV proteins, the haemagglutinin-neuraminidase (HN) glycoprotein represents an ideal target for new antiviral drug discoveries. The HN protein is crucial in several steps of the virus life cycle. Firstly, HN recognises and binds sialic acid exposed at the surface of the host cell. Moreover, HN binding is necessary for the activation of the hPIV fusion (F) protein that allows fusion of the cell and virus membranes. HN has also an important action during the viral budding process because it cleaves sialic acid from glycoconjugates to prevent the accumulation of virions at the cell surface and their auto-agglutination. Sialic acid recognition is the trigger of all these HN activities and the research experiments of this project are focused on the development of high potency inhibitors of the HN–sialic acid interaction.

The X-ray crystal structures of the HN glycoprotein of hPIV types 3 and 5, and of Newcastle Disease Virus are available and can be used as homology models for the study of HN from other hPIV subtypes. While some characterisation of hPIV glycan receptor has been undertaken, a complete systematic study is yet to be done. Furthermore, the combination of molecular modelling, structure-based design, and synthetic chemistry, may provide new inhibitors of viral replication. Using biochemistry and structural biology techniques on whole virus and recombinant HN glycoprotein, the effect of these new inhibitors on the virus/glycan interaction can be investigated. A student working on this project may specialise in one particular aspect, or be involved with a number of the different aspects of the project.

Techniques: Computational Chemistry including visualisation and molecular docking; Synthetic Chemistry; Chemical Characterisation including Proton and Carbon-13 NMR, Mass Spectrometry; Protein expression and purification; Virology; Biological Assays; Advanced NMR techniques including STD-NMR, X-ray crystallography.

13. Structural characterisation and inhibition of Nipah and Hendra virus infections

Dr Thomas Ve, Dr Yun Shi, Dr Andrea Maggioni, Prof Johnson Mak & Prof Mark von Itzstein

Structural Biology, Biochemistry, Virology

Nipah virus (NiV) is a highly lethal (risk group 4) zoonotic paramyxovirus causing severe, rapidly progressive encephalitis in humans with the case fatality rate ranging from 40-70%. NiV is closely related to Hendra virus (HeV), another risk group 4 paramyxovirus that is native to Australia and infects both horses and humans. NiV is widely distributed in Southeast Asia, India, and Africa. WHO has earmarked NiV on a priority list of eight pathogens that is expected to cause severe outbreaks in the near future. While a one-health approach of vaccinating the intermediate host (horse in the case of Hendra) is able to block the transmission of Hendra virus, the limited uptake of the Hendra vaccine by horse owners could potentially make such approach ineffective. Furthermore, transmission of NiV to humans may occur after direct contact with infected bats, infected pigs, or from other NiV infected people making a one-health preventive approach not practical to NiV, due to the lack of reliance of an intermediate host.

The NiV envelope proteins, glycoprotein G and fusion protein F, are the determinants of viral entry. G and F achieve this via their recognition of the host- cell surface proteins Ephrin-B2 and -B3, and the glycosaminoglycan heparan sulfate (HS). Although detailed structural information is available for the G/Ephrin-B2/B3 interactions, the structural basis for how the G protein coordinates selective binding to specific glycans, such as HS is completely unknown. Furthermore, the mechanistic details of how any of the host-cell receptors trigger viral fusion are poorly defined.

This project will involve a combination of biophysical and cell-biology approaches and aims to define the molecular basis of NiV/HeV interaction with host-cell glycans (glycointeractome), define the molecular mechanisms underlying fusion activation and identify inhibitors targeting these processes. A student working on this project may specialise in one particular aspect, or be involved with a number of the different aspects of the project.

Techniques: X-ray crystallography, cryo electron microscopy (cryo-EM), saturation transfer difference nuclear magnetic resonance (STD NMR), library screening, surface plasmon resonance (SPR), glycan arrays, and viral infection assays using pseudotyped particles.

14. Investigating sialic acid metabolism

Prof Mark von Itzstein, Dr Andrea Maggioni, Dr Robin Thomson, Dr Yun Shi & Dr Xing Yu (Hunan Normal University, China)

Medicinal Chemistry, Molecular Biology, Biochemistry, Cell Biology, Structural Biology

Sialic acids are 9-carbon acidic amino-sugars, which are found predominantly at the ends of mammalian glycoproteins and glycolipids. The terminal location of the sialic acid residues on these cell-surface sialo-glycoconjugates results in their essential involvement in processes of cell–cell, cell–microorganism, and cell–biomolecule interactions. The amount of sialic acid expressed on a cell's surface, and sialic acid modifications such as *O*-acetylation, vary throughout development, and in diseases such as some cancers. A number of microorganisms also express sialic acids on their surface, in some cases mimicking human sialo-glycoconjugate structures which can help the microbe to avoid detection by the host immune system.

We have a number of projects that examine the steps in the sialic acid biosynthetic pathway, to give natural and modified sialo-glycoconjugates. Non-natural substrates for enzymes of this pathway, or the use of inhibitors, can be used to change the nature and/or level of sialic acid expressed at the cell surface, and so to alter subsequent biological interactions. In the case of pathogenic bacteria which express surface sialic acids, reduction in the level of sialic acid expression may leave the bacteria more vulnerable to attack by the immune system.

These projects cross a number of disciplines. Aspects of the projects, which can be undertaken either separately or together, include; computational chemistry and molecular modelling studies with enzyme X-ray structures; the chemical synthesis of enzyme probes and inhibitors; biological evaluation of probes and inhibitors using enzyme assay and/or NMR spectroscopy; study of changes of cell surface sialic acid and modifications; and investigation of enzyme–inhibitor complex formation by X-ray crystallography. Each of these aspects will help us gain to an improved understanding of the enzymes of sialic acid metabolism.

Techniques: Computational Chemistry including visualisation and molecular docking; Synthetic Chemistry; Chemical Characterisation including Proton and Carbon-13 NMR, Mass Spectrometry; Protein expression; Cell-based studies; NMR-based enzyme assays; Advanced NMR techniques including STD-NMR; X-ray crystallography.
Nipah virus (NiV) is a highly lethal (risk group 4) zoonotic paramyxovirus causing severe, rapidly progressive

15. Design and synthesis of a Glycosaminoglycans (GAGs) fragment library

Dr Chih-Wei Chang & Prof Mark von Itzstein

Medicinal Chemistry

Glycosaminoglycans (GAGs), found either on cell membranes or in the extracellular matrix, are the class of linear polysaccharides involving a wide range of physiological processes. The GAG family includes heparan sulfate (HS), chondroitin sulfate, and others. Most of their roles in interaction with proteins and modulating a host of diverse biological activities are still poorly understood. The synthesis of such complex glycosaminoglycans in a pure form for investigation of the biological interaction between GAG and GAG-binding proteins is not trivial.

In this project, we aim to develop new synthetic strategies to access a discrete heparan sulfate (HS) fragment library. These homogeneous HS fragments, that incorporate 2-O, 6-O and *N*-sulfate groups, in a defined manner will be used for the exploration of specific binding sequences between homogeneous GAGs fragments and proteins associated with a variety of diseases including cancer, virus infection and diabetes.

Techniques: Synthetic carbohydrate chemistry

16. Synthesis of novel glycosaminoglycans (GAGs) mimetics as GAGs alternatives

Dr Chih-Wei Chang & Prof Mark von Itzstein

Medicinal Chemistry

Glycosaminoglycans (GAGs) exist universally on the cell surface and have various functions that include sustaining the integrity of extracellular matrix (ECM) and acting as biological ligands for molecular binding recognition. In particular, their role as biological ligands has received significant attention in the fields of glycobiology and biomaterials. In terms of variation of length, sequence, sulfation degree and conformational flexibility, these polysaccharide chains give rise to a large number of complex sequences. Therefore, studying protein interactions with homogenous GAGs fragments is challenging because of the difficulties associated with their availability due to their scalable synthesis or reliable production for subsequent *in vivo* experiments can be a limiting factor.

In this project we aim to look for alternative GAGs-mimetic scaffolds that will replace native GAGs sequences. These new scaffolds will allow us to explore specific protein biological functions and opportunities to block their function.

We envision a long-term program to develop innovative approaches for creating new GAGs mimetic molecules that can potentially lead to translational research outcomes.

Techniques: Synthetic carbohydrate chemistry

17. Synthesis and biological evaluation of novel anti-cancer agents

Dr Ibraham El-Deeb, Dr Andrea Maggioni & Prof Mark von Itzstein

Medicinal Chemistry, Cell Biology

We have elucidated[1] a synthesis of a class of potent anti-cancer agents known as the duocarmycins. We are now using a further optimised synthesis of this class of compound to discover novel anti-cancer agents that contain varying extents of carbohydrates (glycoconjugates of duocarmycins). Our preliminary biological evaluation of some of these compounds, in cell-based assays, provides us with optimism that such compounds may have good anti-cancer activity. This project will look at the further development of these glycoconjugates as potential anti-cancer drugs.

1. El-Deeb IM *et al*, *Org Biomol Chem*. 12(24):4260-4 (2014). doi: 10.1039/c4ob00842a.

Techniques: Synthetic carbohydrate chemistry, Cell biology

18. Exosomes as cancer biomarkers and therapeutics

Dr Andrea Maggioni & Prof Mark von Itzstein

Cancer Biology, Biochemistry

Exosomes are vesicles that are secreted from cells and appear to have roles in the tumour microenvironment, including in metastasis. These vesicles are therefore thought to be invaluable in both a diagnosis setting as well as therapeutic targets. Little is known about the cell surface changes in glycans and glycan-recognising proteins. This project will explore these changes using a multidisciplinary approach that may identify potential biomarkers and therapeutic targets that could be used in diagnosis and drug discovery, respectively.

Techniques: Cell biology, Biochemistry, Glycoanalytics

19. Inhibition of TIR domain assembly formation in Toll-like receptor signalling

Dr Thomas Ve & Assoc Prof Haselhorst

Innate Immunity, Biochemistry, Structural Biology, Molecular Modelling

Toll-like receptors (TLRs) detect pathogens and endogenous danger, initiating innate immune responses that lead to the production of pro-inflammatory cytokines. At the same time, TLR- mediated inflammation is associated with a number of pathological states, including infectious, autoimmune, inflammatory, cardiovascular and cancer-related disorders. This dual role of the pathways in protecting against infection and contributing to pathological conditions has attracted widespread interest from pharmaceutical and biotechnology industries.

Cytoplasmic signaling by TLRs starts by their TIR (Toll/interleukin-1 receptor) domain interacting with TIR-containing adaptor proteins MyD88 (myeloid differentiation primary response gene 88), MAL (MyD88 adaptor-like/TIRAP), TRIF (TIR-containing adaptor inducing interferon-β/IFNβ), and TRAM (TRIF-related adaptor molecule) Combinatorial recruitment of these adaptors via TIR:TIR interactions orchestrates downstream signaling pathways, leading to induction of the pro-inflammatory genes. Although TLR pathways have been well characterized, molecular information on the signaling proteins is limited, impeding the development of therapeutic strategies and the understanding of the effects of polymorphic variants on human disease

This project aim to identify new inhibitors of TLR4 signalling, which will involve screening of small molecule compound libraries using an *in vitro* TIR domain assembly assay established for the TLR4 adaptor proteins MAL and MyD88. Compounds shown to inhibit assembly formation will be characterised in more detail for protein interaction using Saturation Transfer Difference (STD) NMR, surface plasmon/isothermal titration calorimetry, and X-ray Crystallography. This information will then be used with molecular modelling and structure analysis to generate more effective small-molecule inhibitors as potential leads for drugs.

Techniques: Production and purification of the TIR domains from the TLR adaptor proteins MAL and MyD88 using established protocols, screening of small molecule libraries using an established biochemical TIR domain assembly assay, STD-NMR, surface plasmon resonance or isothermal titration calorimetry, X-ray crystallography, *in silico* structure analysis and molecular modelling.

20. Sialic acid binding immunoglobulin-like lectins (Siglecs) and their interactions with sialosides

Assoc Prof Thomas Haselhorst & Prof Sörge Kelm (University of Bremen, Germany)

Structural Biology, Biochemistry, NMR spectroscopy, Molecular Modelling, Medicinal Chemistry

Siglecs (sialic acid-binding immunoglobulin like-lectins) form a sub-group of the I-type lectins and function as cell signaling co-receptors primarily expressed on leukocytes to mediate acquired and innate immune functions. They can be divided into two subsets: the first, evolutionary conserved group consists of Siglec 1, 2, and 4, which show selective binding properties: Siglec 1 (also known as sialoadhesin) and Siglec 4 (myelin-associated glycoprotein, MAG) preferentially bind α(2,3)-linked *N*-acetylneuraminic acid (Neu5Ac), whereas Siglec 2 (CD22) is highly specific for α(2,6)-linked Neu5Ac. In contrast, members of the second Siglec 3-related group (Siglec 3 and Siglecs 5–13) are more promiscuous in their binding, often recognizing more than one presentation of Neu5Ac.

We have cloned and expressed a number of myelin-associated glycoprotein (MAG) mutants with the prediction of a secondary binding site for sialic acid ligands. Protein-ligand investigations of these mutants can be carried-out using STD NMR spectroscopy. Molecular modeling techniques (docking studies) can also be used to interrogate the secondary binding site of MAG, and eventually other siglecs.

There is also great interest in developing synthetic inhibitors of Siglec interactions. We have developed sialic-acid based ligands which show good affinity for a number of Siglecs. The design and synthesis of next-generation ligands is now underway. These ligands can be assessed for Siglec binding in cell studies. Ligand–Siglec interactions can also be assessed through STD NMR and Surface Plasmon Resonance (SPR) studies.

Techniques: Cell culture; Protein expression, ELISA; Nuclear Magnetic Resonance Spectroscopy; SPR; Molecular modelling; Synthetic Carbohydrate Chemistry; Chemical Characterisation including Proton and Carbon-13 NMR, Mass Spectrometry; Purification including HPLC.

21. Carbohydrate-based compounds as potential anti-bacterial agents

Dr Milton J Kiefel & Dr Jennifer Wilson (School of Medical Science)

Medicinal Chemistry, Structural Biology

The prevalence of drug-resistant bacteria is becoming one of the major global health problems. Of particular concern is the emergence of multidrug-resistant Gram-negative bacteria, which are particularly difficult to treat with current therapeutics. In addition to their resistance to many common anti-bacterial agents, Gram-negative bacteria have evolved many virulence factors that are essential for them to infect host organisms. This project aims to develop carbohydrate-based inhibitors of enzymes directly involved in bacterial virulence. Significantly, many of the chemical messengers used by Gram-negative bacteria as virulence factors are unique to these organisms. This means that the compounds developed in this project have the potential to disrupt bacterial virulence without causing damage to the host. In addition to the synthetic chemistry component of this project, high field NMR spectroscopy will be used to undertake substrate-specificity studies with the enzymes of interest. Compounds prepared will also be evaluated for their antibacterial activity using standard assays. Students undertaking this project will learn modern synthetic chemistry methodology in state-of-the-art chemistry research laboratories, will gain "hands-on" experience with the use of high field NMR spectroscopy, and will have the opportunity to undertake antibacterial assays.

22. Investigations into bacterial virulence factors – potential drug targets?

Dr Milton J Kiefel & Dr Jennifer Wilson (School of Medical Science)

Molecular Biology, Structural Biology

The prevalence of drug-resistant bacteria is becoming a major worldwide health problem. Of particular concern is the emergence of multidrug-resistant Gram-negative bacteria, which have evolved many virulence factors that are essential for them to infect host organisms. This project aims to investigate specific proteins and enzymes that are known to play important roles in bacterial virulence, with a view to determining if these virulence factors are potential drug targets. Importantly, whilst these proteins and enzymes are known virulence factors, it is not yet known if blocking their function will result in disruption of bacterial virulence. This study aims to determine this by undertaking the expression and purification of proteins and enzymes associated with bacterial virulence. These biomolecules will then analysed using high field NMR spectroscopy to determine substrate-specificity profiles and provide an insight into the potential of developing small molecule inhibitors of these virulence factors. Students undertaking this project will learn modern protein expression and purification techniques, particularly those relating to the expression and purification of membrane associated proteins. Students will also gain "hands-on" experience with the use of high field NMR spectroscopy, and will have the opportunity to undertake some preliminary drug design techniques.

23. Small molecule probes for studies into quorum sensing

Dr Milton J Kiefel, in collaboration with Dr Gary Grant & Dr Shailendra Dukie (School of Pharmacy)

Medicinal Chemistry, Molecular Microbiology

Quorum sensing is a process whereby bacteria utilise small molecules to signal other bacteria, and use this communication process to coordinate certain types of behaviour based on local population density. The ability of certain pathogenic bacteria to effectively colonise a host has been directly linked to their ability to communicate via a quorum sensing mechanism. Since it is known that small molecules are the actual signalling mechanism, it seems reasonable that disruption to the biosynthesis or release of these signalling molecules could have a significant effect on the survival and virulence of certain pathogenic bacteria. To fully investigate this signalling mechanism, it is necessary to have access to appropriate small molecules. This project therefore involves the chemical synthesis of specific target compounds, and the analysis of these compounds for their ability to alter bacterial growth. Students undertaking this project will learn modern synthetic chemistry techniques, and will also have the opportunity to learn about analysing bacterial growth and viability using various fluorometric detection methods.

24. Investigations into synthesis of ulosonic acids using aldol condensations

Dr Milton J Kiefel

Medicinal Chemistry

Ulosonic acids are a family of higher order sugars the are intimately associated with a number of human diseases. Keto-deoxy octulosonic acids are key components of the outer membrane of Gram-negative bacteria, whilst nonulosonic acids include the sialic acids, pseudaminic acids, and legionaminic acids, all of which are known to be associated with human disease and bacterial virulence. Whilst much is known about the role these sugars play in disease, there remains much to be discovered. One of the current limitations in this field of research is the limited availability of synthetic derivatives and analogues of these important sugars. This project aims to expand on some of our preliminary work into developing a new highly efficient synthesis of ulosonic acids using an aldol condensation as the key step. Students undertaking this project will learn modern synthetic chemistry methodology in state-of-the-art chemistry research laboratories. They will also gain "hands-on" experience with the use of high field NMR spectroscopy, and will provide compounds that will potentially be used as biological probes.

25. Identification of specific amino acid residues responsible for interactions of chemosensory receptor Tlp1 with chemotaxis proteins CheW and CheV of *Campylobacter jejuni*

Prof Victoria Korolik & Dr Christopher J Day

Molecular Microbiology

The natural habitat of campylobacters is the intestine of warm-blooded animals, and therefore chemotactic motility is an important mechanism involved in the colonisation and pathogenicity of this microorganism. Bacterial motility is subject to sensory control mechanisms that introduce a bias into the swimming direction of the organism towards beneficial environments and away from unfavourable conditions. Although chemotaxis has been demonstrated for *Campylobacter* the chemical substrates, mechanisms involved in the sensory control of motility and the role of chemotaxis in disease, are poorly understood. We, therefore hypothesise that the chemosensory receptor proteins play a key role in chemotaxis and are involved in the pathogenicity of this organism as the first line of bacterial – host interaction and thus provide rational targets for the design of novel antimicrobial agents.

This project involves characterisation of interactions of the signaling domain of one of the chemoreceptors of *C. jejuni,* named Tlp1 with CheW and CheV chemotaxis proteins. The major aim of this project is to identify which amino acids in the signaling domain of Tlp1 are responsible for binding with CheW and CheV though systematic site-specific mutagenesis followed by analysis of the mutated proteins using yeast 2-hybrid protein-protein interaction system.

This project will further the studies to elucidate the role of Tlp1 chemoreceptor in chemotaxis and pathogenicity of *C. jejuni*, which can potentially provide a tremendous insight into the mechanisms of chemotaxis of this organism.

26. Identification of ligand specificities of chemosenory receptor Tlp7 of bacterial pathogen *Campylobacter jejuni*

Prof Victoria Korolik & Dr Bassam Elgamoudi

Molecular Microbiology

The natural habitat of campylobacters is the intestine of warm-blooded animals, and therefore chemotactic motility is an important mechanism involved in the colonisation and pathogenicity of this microorganism. Bacterial motility is subject to

sensory control mechanisms that introduce a bias into the swimming direction of the organism towards beneficial environments and away from unfavourable conditions. Although chemotaxis has been demonstrated for Campylobacter the chemical substrates, mechanisms involved in the sensory control of motility and the role of chemotaxis in disease, are poorly understood. We, therefore hypothesise that the chemosensory receptor proteins play a key role in chemotaxis and are involved in the pathogenicity of this organism as the first line of bacterial – host interaction and thus provide rational targets for the design of novel antimicrobial agents.

This project involves characterisation of interactions of one of the chemoreceptors of *C. jejuni*, named Tlp7 with environmental molecules. The major aim of this project is to identify which chemicals are responsible for triggering chemotactic responses governed by Tlp7. This will be determined using site-specific mutagenesis followed by analysis of the wild type and mutated proteins using small molecule and glycan arrays, chemotaxis assays and mammalian cell culture.

This project will further the studies to elucidate the role of Tlp7 chemoreceptor in chemotaxis and pathogenicity of *C. jejuni*, which can potentially provide a tremendous insight into the mechanisms of chemotaxis of this organism.

27. Regulation of cell surface sialylation by targeting the CMP-sialic acid transporter: Towards the development of anti-metastatic agents

Assoc Prof Joe Tiralongo & Assoc Prof Thomas Haselhorst

Biochemistry, Structural Biology

A correlation between increased cell surface sialylation and the metastatic potential of various cancers has been extensively reported [1]. Changes in the specific pattern and quantities of sialic acid (Sia) may account for the increased propensity of cancer cells, particularly colorectal cancer, to disseminate and survive in the circulation.

The sialylation of glycoproteins and glycolipids occurs in the Golgi apparatus, and a key step in this process involves the transport of CMP-Sia into the Golgi by the CMP-Sia transporter (CST). On the proposed model of metastasis, inhibition of the CST should result in a reduction in cancer cell surface sialylation and hence metastatic potential, and indeed this has been shown to be the case [2].

With the ultimate aim of developing novel anti-metastatic agents through the regulation/inhibition of cancer cell surface sialylation, we have now synthesised and screened a discrete library of glycosyl- and sialyl-nucleosides as potential inhibitors of the CST. A number of these compounds are showing promising inhibitory activity against the CST, and are now being assessed for their ability to inhibit cancer cell sialylation in a cell-based assay. However, for the development of additional potent inhibitors of the CST a better understanding of the protein-inhibitor interactions critical for CST inhibitor recognition will be critical. To this end, we have developed a novel method for interrogating the binding site of the CST with various ligands and putative inhibitors by Saturation Transfer Difference (STD) NMR spectroscopy [3]. Our methodology utilises Golgi-enriched fractions isolated from *Pichia pastoris* over-expressing recombinant CST that are directly applied to STD NMR spectroscopy. In this project we will use putative inhibitors and ligands to identify and map the binding epitope of CST ligands/inhibitors, information that will be employed to design more potent CST inhibitors.

1. Varki, N.M. & Varki, A., 2007, *Lab. Invest.*, **87**, 851-7.
2. Petrick, A.T., Meterissian, S., Steele, G., Jr.*, et al.*, 1994, *Clin. Exp. Metastasis*, **12**, 108-16.
3. Maggioni, A., von Itzstein, M., Tiralongo, J., *et al.*, *ChemBioChem.* **9**, 2784-6

28. CMP-sialic acid transporter structure elucidation: 3D crystallography

Assoc Prof Joe Tiralongo

Biochemistry, Structural Biology

A correlation between increased cell surface sialic acid (Sia) and the metastatic potential of various cancers has been extensively reported [1 and references therein]. The sialylation of glycoconjugates occurs in the Golgi apparatus, and a key step in this process involves the transport of CMP-Sia into the Golgi by the CMP-Sia transporter (CST). On the proposed model of metastasis, inhibition of the CST should result in a reduction in cancer cell surface sialylation and hence metastatic potential, and indeed this has been shown to be the case [2].

Even though the CST, a member of a highly conserved family of type III trans-membrane proteins collectively referred to as nucleotide sugar transporters (NST), has been well characterised at the biochemical level, the 3D structure of the CST is yet to be elucidated.

A rate-limiting step in the process of membrane protein structure elucidation is the quantitative production and purification of functional recombinant protein. We have now established an efficient procedure for the expression in

Pichia pastori and single-step purification of milligram quantities of functional CST that will be used in this project for 3D-crystallography trials.

1. Varki, N.M. & Varki, A., 2007, *Lab. Invest.*, **87**, 851-7.
2. Petrick, A.T., Meterissian, S., Steele, G., Jr.*, et al.*, 1994, *Clin. Exp. Metastasis*, **12**, 108-16.

29. Probing Microbe-Glycan and Primary Cell-Glycan interactions using Glycan Array Technology

Assoc Prof Joe Tiralongo & Dr Christopher J Day

Biochemistry, Molecular Microbiology

The identification and characterisation of carbohydrate binding proteins (or lectins) has been greatly enhanced through the development of glycan array technology. Binding specificities can be effectively interpreted by probing arrays, consisting of glycans (carbohydrates) of known structure covalently immobilised onto glass-slides. Typically, glycans are printed using robotic printing technology (similar to that used to prepare DNA microarrays) onto an appropriately functionalised glass-slide allowing covalent attachment to the glass surface. Specific interactions can then be visualised following interrogation of the glycan array with, for example, a fluorescent-labelled bacteria or cultured cells.

Glycan arrays are fast becoming the technique of choice for identifying and elucidating the specificity of lectins, and have been successfully used to identify and characterise the interactions of bacteria with their glycan receptors [1]. However, there are few reports of glycan arrays being used to study carbohydrate recognition by mammalian cultured cells.

1. Day, C.J., Tiralongo, J., Hartnell, R.D., *et al*., *PLoS One*, **4**, e4927

30. Establishment of a CellFrac microarray

Assoc Prof Joe Tiralongo & Dr Christopher J Day

Biochemistry

Adhesion to host cells is an essential virulence factor for the vast majority of microbial pathogens, with the specificity of these interactions being a major determinant of tissue tropism. Cultured mammalian cells have long provided valuable model systems for investigating host-pathogen interactions. However, different cultured cells possess distinct and diverse cell surface components to which pathogenic microbes are able to bind. Therefore, technologies that enable the interaction of pathogenic microbes with an array of cultured cells, or the corresponding cellular components isolated from those cells, to be evaluated in a high-throughput manner would be a valuable tool for studying tissue tropism. Therefore, our interest in better understanding host-pathogen interactions led us to establish a discrete CellFrac microarray comprising cytosolic and membrane fractions isolated from 15 diverse mammalian cell lines immobilized onto glass slides. This lab-on-a-chip technology has the potential to replace traditional cell-based binding assay systems for the study of not only host-pathogen but also protein-protein interactions.

In this project we will further validate our technology using antibodies and lectins of known specificity, as well as exploring host-pathogen interactions using a number of different microbes, including bacteria and fungal pathogens.

31. Isolation and characterisation of novel lectins from Australian macrofungi

Assoc Prof Joe Tiralongo & Assoc Prof Evelin Tiralongo (School of Pharmacy)

Biochemistry

Lectins are important sugar binding proteins that are ubiquitous in nature, occurring in plants, bacteria, viruses, fungi, animals and humans. Lectins can reversibly bind, but do not modify, free sugars, sugar residues of polysaccharides, glycoproteins or glycolipids leading to various physiological effect, including the ability to agglutinate cells. Lectin carbohydrate specificity varies widely and can be highly specific for certain glycan structures. Therefore, lectins can exhibit very specific affinity towards certain cell types depending on glycosylation patterns. Many lectins are quite toxic and are thought in plants to play a crucial role in the defense system; however, lectins from some food sources such as tomatoes, lentils, peas are non-toxic [1].

Lectins have been isolated and purified from plants, mushrooms, animals and microorganisms, and of the 60 or so commercially available, only that from *Agaricus bisporus* is of fungal origin. Australian fungi are greatly under-explored with a considerable number of un-described species of known chemical, genetic and biological profiles. Therefore, investigating fungal species may lead to the identification of novel lectins with unique glycan specificities that may prove to be useful for Glycomics and related biomedical and cancer research.

In this project protein extracts of Australian mushrooms will be generated and putative lectins isolated using well-established techniques including protein precipitation, size exclusion, ion exchange and affinity chromatography. Lectin purification will be monitored using a haemagglutination assay and assessed *via* SDS-PAGE. Isolated lectins will be biochemically characterised, specifically for lectin carbohydrate specificity.

1. Lehmann, F., Tiralongo, E., and Tiralongo, J. (2006) *Cell. Mol. Life Sci.* **63**, 1331-1354

32. Influence of the length of oligosaccharide on the biological activity of the lipooligosaccharide from *Moraxella catarrhalis*

Dr Darren Grice & Dr Jennifer Wilson (School of Medical Science)

Molecular Microbiology, Medicinal Chemistry

Almost all children suffer from middle ear infections (otitis media) at some point in their lives. In Australia, otitis media is particularly prevalent in Aboriginal children, and occurs very early in life. The bacteria most commonly associated with otitis media are *Streptococcus pneumoniae*, *Moraxella catarrhalis* (Mcat), and non-typeable *Haemophilus influenzae* (NTHi).

Gram negative bacteria such as Mcat have a layer at the outer surface that is predominantly made up of carbohydrates (oligosaccharide) attached via a membrane-embedded lipid (lipid A). The carbohydrate-lipidA molecule is known as lipo-oligosaccharide (LOS). The immune system of an infected person recognises and responds to the LOS of many bacteria, (including *M. catarrhalis*).

Previously we have structurally characterised Mcat oligosaccharides from mutant *M. catarrhalis* bacteria. Now we want to assess the biological activity of the lipooligosaccharide produced by the mutant bacteria and investigate whether there is a correlation between the structure of the oligosaccharide and its toxicity. Methods to evaluate the biological activity of the mutant LOS will include growth rate, toxicity and susceptibility to hydrophobic reagents as compared to wildtype.

33. Analysis of lipopolysaccharide structures from *Moraxella bovis*

Dr Darren Grice, Dr Ian Peak & Dr Jennifer Wilson (School of Medical Science)

Medicinal Chemistry, Molecular Microbiology

The aim of this project is to isolate carbohydrate components from the bacteria *Moraxella bovis* and determine the structures of these carbohydrate molecules. *Moraxella bovis* causes infectious bovine keratoconjunctivitis (IBK) in cattle, causing significant loss of weight and permanent blindness. Vaccine therapies have been unsuccessful in treating these infectious agents. Obtaining structural carbohydrate information will enable future studies to determine the role of these carbohydrates in disease and develop potential new vaccine strategies.

The project will require the development of knowledge and skills in the areas of cell culture, chemical and biochemical extraction and manipulation strategies, nuclear magnetic resonance (NMR) and mass spectrometry (MS) of isolated carbohydrate materials.

Identification of a capsular polysaccharide from *Moraxella bovis*. J.C. Wilson, P.G. Hitchen, M. Frank, I.R. Peak, P.M. Collins, H.R. Morris, A. Dell and I.D. Grice. *Carbohydr. Res.* 2005, 340(4), 765-769.

34. Analysis of surface carbohydrate structures from Gram-negative *Moraxellaceae* bacteria

Dr Darren Grice, Dr Ian Peak & Dr Jennifer Wilson (School of Medical Science)

Medicinal Chemistry, Molecular Microbiology

The aim of this project is to isolate surface carbohydrate components from bacteria in the *Moraxellacae* family, then determine the structures and biological significance of these carbohydrate molecules. Many bacteria in this family are commensals of the human upper respiratory tract and are important in protecting against disease. Obtaining structural carbohydrate information will enable us to determine the role of these carbohydrates, and potentially developing new strategies to promote upper-respiratory tract health.

The project will require the development of knowledge and skills in the areas of cell culture, chemical and biochemical extraction and manipulation strategies, nuclear magnetic resonance (NMR) and mass spectrometry (MS) of isolated carbohydrate materials.

1. De Castro, Grice, Daal, Peak, Molinaro, Wilson. *Carb. Res.* (2014) 388:81-86.
2. Wilson, Hitchen, Frank, Peak, Collins, Morris, Dell and Grice. *Carb. Res.* (2005) 340:4, 765-769.

35. Development of novel phthalic acid diesters as potential human parainfluenza virus therapeutics

Dr Darren Grice, Dr Andrew Pearson (School of Medical Science) & Assoc Prof Evelin Tiralongo (School of Pharmacy)

Medicinal Chemistry

Infectious diseases, such as Dengue (DENV), Chikungunya (CHIKV) and Human parainfluenza virus (hPiV3), are a major cause of avoidable mortality and morbidity, particularly for children in developing countries. However, due to the rapidly mutating nature of viruses, resistance to conventional drug therapies occurs. The proposed research project is ongoing and follows on from our previous work where we isolated a chemical compound from a Bangladeshi mangrove fern *Acrostichum aureum* that showed potent activity particularly against hPiV3, but also moderate activity against DENV2 and CHIKV. This project aims to synthesise the isolated compound and also synthesise analogues where chemical groups have been varied, then test these compounds for potency as potential anti-viral agents against hPiV3, DENV2 and CHIKV. It is hoped that this work will lead to the identification of a new agent with potent antiviral.

Uddin, Bettadapura, Guilon, Grice, Mahalingam, Tiralongo. *J. Antivir. Antiretrovir*. (2013) 5:6.

36. Design of Fluorescent Receptors for Carbohydrates and Related Biomolecules

Dr Todd A Houston & Dr Milton J Kiefel

Medicinal Chemistry

Bis(boronic acids) have been widely used in the development of chemosensors for sugars, most importantly glucose. We have recently reported that bisboronates have an even higher affinity for bis(□-hydroxycarboxylates) such as tartrate. We are now designing novel boronate-based receptors for hydroxyacids (sialic acid and KDO) and inositols, an important class of cell-signalling molecules. The latter type may have the biological impact of slowing cell growth, and thus may be coupled with other compounds in cancer therapy. Ultimately, these receptors will be developed into "boronolectins" that target specific cell types and may be used in drug targeting. They can identify targets complementary to those of nature's antibodies and lectins that normally survey cell surfaces.

37. Synthesis and Drug Targeting of Antitubercular Compounds

Dr Todd A Houston

Medicinal Chemistry

Tuberculosis is the most deadly infectious disease in the world today and there is a need for new, more effective, antitubercular compounds. Based on the affinity of *Mycobacterium tuberculosis* for cholesterol-rich membrane regions found in macrophages, we are synthesizing a number of steroid-based amines and aminoglycosidases for testing as inhibitors of mycobacterial growth. The biological testing will be carried out by collaborators at facilities that can deal with drug-resistant strains of the microorganism. This drug targeting protocol is being applied to compounds active against *Leishmania* protozoa, the causative agent of the widespread tropical disease, leishmaniasis.

38. Development of Novel Glycosidase Inhibitors as Potential Therapeutics

Dr Todd A Houston

Medicinal Chemistry

Glycosidase inhibitors have a wide range of biological activities including anticancer, antiviral (e.g. Relenza), and antidiabetic properties. Our group is involved in the synthesis and testing of novel glycosidase inhibitors. We have identified a unique b-galactosidase inhibitor by tethering a boronic acid onto a known amine inhibitor. The boronate should increase the affinity of this compound for cell-surfaces and serve as a method of drug targeting. Currently, we are applying this modification to other, more potent glycosidase inhibitors in order to increase the selectivity of these compounds.

39. Host immune responses to bacterial signaling molecules

Dr Ian Peak**,** Dr Christopher J Day & Prof Michael Jennings

Molecular Microbiology, Molecular Biology

The immune system responds to infections after it has recognised infectious agents. All bacteria secrete products , and some of these have profound effects on the host immune system, either acting as recognition molecules for immune attack, or by modifying the immune response to assist the microbe to survive. We are investigating how secreted molecules from pathogenic bacteria are detected. We are characterising host receptors for these secreted products, which will help understand diseases such as cholera, legionnaire's disease, as well as infections caused by *Pseudomonas* in burned, and cystic fibrosis patients.

The student will gain experience in a range of the following techniques:

Molecular genetics techniques, immunofluorescence microscopy, protein expression and purification, FACS analysis, cell culture and *in vitro* infections, *in vivo* infections using mouse models of disease, analyzing immune markers such as cytokine and chemokine responses of the host cell, small molecule purification and analysis by Mass Spectrometry and other techniques

40. Evolution of random gene switching mechanisms in bacterial pathogens

Prof Michael Jennings

Molecular Biology, Molecular Microbiology

Host adapted bacterial pathogens have evolved mechanisms to evade host immune responses. One of these mechanisms is phase variation (high frequency, reversible ON/OFF switching if gene expression). In most cases phase variation is mediated by hypermutagenic DNA repeat sequences in the coding sequence of the phase variable gene. Alternations in DNA repeat number lead to frame shift mutations. The DNA repeats that mediate these ON/OFF switching events are transcribed and translated and encode repeated peptide sequences in the resulting protein. These peptide sequences are not required for protein function, but must be compatible with the function of the protein in which they have evolved. For example, many phase variable genes encode glycosyltransferases that function to synthesize surface oligo- and polysaccharides. The constraints on the type of repeat sequences ans position in a coding sequence where it can evolve has not been addressed. The aim of this project is to use experimental evolution to observe the evolution of new high frequency switching mechanisms in bacterial glycosyltransferases. The project will involve construction of selectable/counter-selectable fusions with target genes, to cycle these constructs through multiple round of selection and counter-selection, to identify and characterize the resulting high frequency mechanisms that have evolved in this model system.

Moxon, R., Bayliss, C. & Hood, D. Bacterial contingency loci: the role of simple sequence DNA repeats in bacterial adaptation. *Annu Rev Genet* **40**, 307-33 (2006).

41. Investigation of glycan binding by the pore forming toxin perfringolysin O (PFO) from *Clostridium perfringens*

Dr Lucy Shewell & Prof Michael Jennings

Molecular Microbiology, Molecular Biology

Bacterial pathogens produce a range of virulence factors that contribute to disease outcome in the host. One of the major virulence factors produced by a number of Gram-positive bacterial pathogens is the cholesterol-dependent cytolysins (CDCs). The CDCs are pore-forming toxins that are able to form pores in cholesterol-containing membranes. Due to the requirement for cholesterol in target cell membranes in order for the CDCs to form pores, it was believed that cholesterol was the receptor for these toxins. However, a protein receptor was identified for one of these CDCs, intermedilysin (ILY). Recently in our lab we identified glycan receptors for two of the CDCs, pneumolysin (Ply) and streptolysin O (SLO), produced by the human pathogens *Streptococcus pneumoniae* and *S. pyogenes*, respectively **(1)**. This project aims to investigate the glycan binding properties of the CDC produced by the causative agent of gas gangrene *Clostridium perfringens*.

Techniques: glycan array, surface plasmon resonance (SPR), cell based assays, PCR, DNA cloning, protein expression and purification, western blot, flow cytometry, ELISA, site-directed mutagenesis.

(1) Shewell *et al*, 2014. *PNAS*. 111 (49): E5312-5320.

42. Investigation of glycan binding by the pore-forming toxin vaginolysin from *Gardenalla vaginalis*

Dr Lucy Shewell & Prof Michael Jennings

Molecular Microbiology, Molecular Biology

Bacterial pathogens produce a range of virulence factors that contribute to disease outcome in the host. One of the major virulence factors produced by a number of Gram-positive bacterial pathogens is the cholesterol-dependent

cytolysins (CDCs). The CDCs are pore-forming toxins that are able to form pores in cholesterol-containing membranes. Due to the requirement for cholesterol in target cell membranes in order for the CDCs to form pores, it was believed that cholesterol was the receptor for these toxins. However, a protein receptor was identified for one of these CDCs, intermedilysin (ILY). Recently in our lab we identified glycan receptors for two of the CDCs, pneumolysin (Ply) and streptolysin O (SLO), produced by the human pathogens *Streptococcus pneumoniae* and *S. pyogenes*, respectively **(1)**. This project aims to investigate the glycan binding properties of the CDC produced by the causative agent of bacterial vaginosis *Gardnerella vaginalis*.

Techniques: glycan array, surface plasmon resonance (SPR), cell based assays, PCR, DNA cloning, protein expression and purification, western blot, flow cytometry, ELISA, site-directed mutagenesis.

(1) Shewell *et al*, 2014. *PNAS*. 111 (49): E5312-5320.

43. Glycan-glycan interactions in host-pathogen adherence

Dr Christopher J Day & Prof Michael Jennings

Microbiology; cell assays; array technology; affinity and kinetics measurements

Recently we showed that pathogenic bacteria interact with host cell through direct contact of the carbohydrates expressed by both organisms (Day et al 2015 PNAS 112:E7266). Previously only three glycan-glycan interactions had been described (sea sponges, Lewis antigens and gangliosides) while our study extended this to over 60 new interactions. The role of glycan-glycan interactions in pathobiology and more widely throughout nature has not been fully elucidated. This project will investigate a wide range of bacterial polysaccharides for glycan binding and try to determine the minimal and sufficient structure required for these novel interactions. This study will utilise the glycomics arrays that we produce within the Institute for Glycomics as well as studies of affinity and kinetics using surface plamon resonance (GE Biacore T100) and micro isothermal calorimetry (TA Instruments nanoITC). Cell assays for bacterial adherence will also be performed.

44. Glycan-glycan interactions: Interactions in eukaryotic biology

Dr Christopher J Day & Prof Michael Jennings

Cell assays; array technology; affinity and kinetics measurements

Recently we showed that pathogenic bacteria interact with host cell through direct contact of the carbohydrates expressed by both organisms (Day et al 2015 PNAS 112:E7266). Previously only three glycan-glycan interactions had been described (sea sponges, Lewis antigens and gangliosides) while our study extended this to over 60 new interactions. The role of glycan-glycan interactions in pathobiology and more widely throughout nature has not been fully elucidated. In our studies we noted that bacteria that mimic human glycan structures were still capable of binding human glycan structures indicating that direct interactions between eukaryotic glycans are likely to occur. This project will investigate a range of eukaryotic glycans for their ability to recognise other eukaryotic glycans. This study will utilise the glycomics arrays that we produce within the Institute for Glycomics as well as studies of affinity and kinetics using surface plamon resonance (GE Biacore T100) and micro isothermal calorimetry (TA Instruments nanoITC). Cell assays to observed the binding of labelled glycans to appropriately glycosylated cells.

45. Identification of novel carbohydrate binding proteins

Dr Christopher J Day & Prof Michael Jennings

Array technology; affinity and kinetics measurements

Carbohydrate binding proteins (also known as lectins) are a broad range of proteins with a wide specificity for carbohydrate structures. Recently we have found that a large number of bacterial and eukaryotic proteins have the ability to bind to glycans that had not previously been appreciated. This project will investigate a range of proteins from bacterial and eukaryotic sources for their ability to interact with glycans. This study will utilise the glycomics arrays that we produce within the Institute for Glycomics as well as studies of affinity and kinetics using surface plamon resonance (GE Biacore T100) and micro isothermal calorimetry (TA Instruments nanoITC).

46. Investigating epigenetic gene regulation by phase-variable methyltransferases

Dr John Atack & Prof Michael Jennings

Molecular microbiology; bacterial pathogenesis; genetics

Many host adapted bacterial pathogens contain phase variable methyltransferases, encoded by *mod* genes, which control expression of multiple genes, and known as phasevarions (phase variable regulons). Non-typeable *Haemophilus influenzae* (NTHi) is a clinically significant otopathogen, and a major cause of otitis media (OM), or middle ear infection. There are currently twenty-one known *modA* alleles in NTHI, but only five of these alleles make up over two-thirds of alleles found in clinical isolates.

Our studies will investigate the mode of gene regulation through differential methylation by ModA proteins. We have identified a number of genes that are differentially expressed in several phasevarions: we will investigate if *modA* methylation recognition sequences localise within the promoters of any of these genes, thereby implicating that the addition of a methyl-group at this site by ModA alters regulation of these genes. Consequently, we will investigate if these genes are regulated in a *modA* dependent way through generation of reporter constructs, and investigate the effect of removing any *modA* recognition sequences from the promoters of these genes. This will be the first time direct regulation by ModA has been demonstrated at the promoter level, and has implications in NTHi treatment and vaccine development.

47. What is the mechanism of ModA gene regulation?

Dr John Atack & Prof Michael Jennings

Protein biochemistry; enzyme kinetics; gene regulation

The ModA proteins of NTHi are phasevariable methyltransferases, whose differential expression leads to global gene regulation differences – a system known as the phasevarion, for phase variable regulon. ModA proteins are highly conserved at their N and C terminal domains, but the central DNA regognition domain (DRD) is highly variable, and dictates the sequence at which ModA methylates DNA. Different ModA proteins contain different DRD's, methylate different sequences, and consequently control different phasevarions. Although the systems themselves have been studies for a long time, little is known about *how* ModA actually modulates gene expression. This project will investigate the method of action of ModA proteins through the use of protein over-expression and purification methods to allow us to study these methyltransferases *in vitro*. Biacore will be used to conduct kinetic measurements, and gel-shift assays (EMSA) will be used to study binding affinity and ability.

48. What is the role of novel phase-variable methyltransferases in bacterial pathogens?

Dr John Atack & Prof Michael Jennings

Molecular microbiology; bacterial pathogenesis; genetics

We recently characterised a system in the worlds foremost bacterial pathogen, *Streptococcus pneumoniae*, that contains a methyltransferase that is able to switch between six different specificities, and consequently results in six highly differentiated bacterial phenotypes within a population of pneumococcal cells. Analysis of several other important bacterial pathogens reveals that this system is extremely widespread amongst both gram positive and gram negative pathogens. This system has potential to impact gene expression differences cell-wide, and has implications in treatment, vaccine development and immune-evasion.This project will begin to analyse the nature of these systems in the highly relevant human pathogen *Neisseria gonorrhoea*, the etiological agent of gonorrhoeae, and the zoonotic pathogen *Streptococcus suis*, now the leading cause of bacterial meningitis in S.E. Asia. We will investigate the number of different specificities these systems can exist in in these bacteria, the sequences these enzymes methylate, and the gene expression differences that result from variable methylation of the genome.

49. A mutagenesis screen to identify key components of post-translational modification pathways bacterial pathogens

Dr Freda Jen & Prof Michael Jennings

Molecular Biology, Molecular Microbiology

Many pathogenic bacteria modify proteins after translation. Some of these modification are on proteins on the surface of the bacteria that are key in understanding host:pathogen interactions and in developing vaccines. Recent advances in *Neisseria meningitidis* have identified post-translation modification of virulence factors with glycans and phosphorylcholine. Some key post-translation modification pathway components have also been identified, but the picture is incomplete. The aim of this project will be to conduct transposon mutagenesis and screen for loss of key post-translation modifications. In this way novel post-translation modification pathway components will be identified and investigated.

50. The protein O-glycosylation pathway of pathogenic *Neisseria*

Dr Freda Jen & Prof Michael Jennings

Molecular Biology, Molecular Microbiology

Neisseria gonorrhoeae and *Neisseria meningitidis* are closely related *Neisseria* species and colonize only the human host. *N. gonorrhoeae* colonizes primarily the human genitourinary tract, causing the sexually transmitted disease, gonorrhea. In contrast, *N. meningitidis* is carried by 5 to 10% of healthy adults and can cause life threatening meningitis and sepsis when it crosses the epithelium and enter the blood. Pili of pathogenic *Neisseria* are major virulence factors, which protrude from the bacterial surface and have a crucial role in both colonisation of the host and adhesion to host cells. Pili of pathogenic *Neisseria* are subject to several different post-translational modifications, such as glycosylation. The aim of this study is to analyse the physiology of the glycosylation process to determine the roles of compartmentalisation and the features of the protein glycosylation interactome that facilitate efficient glycosylation.

51. Signal sequence non-optimal codons in protein structure

Dr Yaramah Zalucki & Prof Michael Jennings

Microbiology, protein purification and protein structure analysis

Codon usage is biased at the 5' end of secretory genes, with the highest percentage of non-optimal codons found compared to any region of the genome. The exact role for the observed bias is unknown. We have strong evidence that changing signal sequence non-optimal codons to the most optimal codon in the synonymous codon family results in structural changes in the mature region of the protein. However, detailed analysis on the exact nature of the structural change has not been done. In this project, we will alter the codon usage in the signal sequence of two small proteins (>20 kDa), purify them and determine any structural differences by NMR and other techniques. Determining any structural change from altering signal sequence codon usage will be a novel find, and important in the field structural biology and now proteins are targeted for protein export. The techniques used in this project will involve cloning, PCR, protein purification, NMR analysis, protein analysis (Western and coomassie staining techniques, DNA sequencing and phenotypic analysis of any mutants made.

52. Role of promoter mutations in the *mtrCDE* efflux pump in antibiotic resistance in *N. gonorrhoeae*

Dr Yaramah Zalucki & Prof Michael Jennings

Microbiology, molecular genetics and antibiotic resistance

Antibiotic resistance is in *N. gonorrhoeae* is a major public health concern. One of the major determinants of resistance is the MtrCDE efflux pump, which exports compounds from the inner membrane to the extracellular milieu. Expression of the efflux pump is controlled by a repressor, MtrR, and a conditional activator, MtrA. We have identified a number of novel promoter mutations in the *mtrCDE*, whose role in increasing expression of the efflux pump has not been characterised. In this project, we will place these mutations individually, and in conjunction with known promoter mutations, to measure their effect on antibiotic resistance in *N. gonorrhoeae*. These mutations will also be placed in the context of a promoter-less lacZ fusion, to measure their affect on the strength of the promoter. We will also look at how these mutations influence the binding of the two known regulators of the efflux pump, MtrA and MtrR to the promoter region. The techniques used in the project will involve cloning, PCR, MIC assays, RNA extraction and other general microbiology and molecular biology techniques.

53. How signal peptide changes influence NDM-1 antibiotic resistance

Dr Yaramah Zalucki & Prof Michael Jennings

Microbiology, evolutionary genetics and antibiotic resistance

New Dehli Metallo-β-Lactamase (NDM-1) is a type of β-lactamase resistance gene recently discovered from a patient with a *Klebsiella pneumoniae* infection in 2009. This gene is leads to resistance to nearly all forms of β-lactam type antibiotics, and can result in treatment failures. Due to the evolutionary history of the gene, NDM-1 has an unusual signal peptide, with a negative charge at the N-terminus, which results in inefficient export of NDM-1 to the periplasm. Using inverse PCR, we aim to select for higher resistance by altering the amino acid composition at the start of NDM-1. We will also investigate natural variants of NDM-1 signal peptide and see if these have improved the export and potentially increased the resistance level provided by NDM-1. These results will inform the community how signal peptide changes in NDM-1 could lead to higher resistance β-lactam type antibiotics. The techniques involved in this

project include cloning, inverse PCR, antibiotic resistance measurements by MIC, Western analysis and protein purification.

54. Rotavirus: Structure Based Drug design

Prof Mark von Itzstein, Dr Robin Thomson, Prof Helen Blanchard & Assoc Prof Thomas Haselhorst

Molecular Biology, Structural Biology, Medicinal Chemistry

Rotaviruses are double stranded RNA viruses that are the leading cause of infantile gastroenteritis globally. The resulting dehydrating diarrhoea following infection is responsible for 33% of all hospitalisation of infants.

The triple layered Rotavirus virion must be delivered across host cell membranes into cytoplasm in order to initiate viral gene expression. Cell-attachment and entry mechanisms are promising targets for therapeutic and preventative interventions against rotavirus diarrhoea.

Rotavirus outer capsids comprise a coat glycoprotein and a spike protein that mediate infection. VP8* is the 18 kDa protein fragment forming the spike tip and binds a cell-surface carbohydrate (sialic acid) during virus attachment to cells. The overall aim of this project is design of carbohydrate based drugs that could bind and block the active site of VP8* thus preventing the virus particle from attaching to the host cell and causing infection.

This project has the potential to combine the design and chemical synthesis of potential carbohydrate-based drugs with structure based modelling and X-ray crystallographic determination of the structures of VP8* in complex with these potential drugs.

Aims: Structure-based design of potential inhibitors of rotavirus and their synthesis.

Techniques: a) Expression and purification of wild-type and mutant VP8* protein from different strains b) Synthesis of compounds c) Assessment of ligand binding by Saturation Transfer Difference NMR d) Protein crystallisation of VP8* with sialic acid derivatives bound in the active site and elucidation of atomic structure by X-ray crystallography e) Computational Chemistry including visualisation and molecular docking

Effects on sialic acid recognition of amino acid mutations in the carbohydrate-binding cleft of the rotavirus spike protein. Kraschnefski, MJ., Bugarcic, A., Fleming FE., Yu, X., von Itzstein. M., Coulson, BS and Blanchard, H. (2009) *Glycobiology* 19(3):194-200

Sialic acid dependence in rotavirus host cell invasion. T. Haselhorst, F.E. Fleming, J.C. Dyason, R.D. Hartnell, X.Yu, G. Holloway, K. Santegoets, M.J. Kiefel, H. Blanchard, B.S. Coulson, M. von Itzstein. (2009) *Nat. Chem. Biol.* Feb;5(2):91-93

55. Three-Dimensional Atomic Structure Determination of galectin-inhibitor complexes by X-ray crystallography: Design of galectin-specific inhibitors

Prof Helen Blanchard & Prof Ulf Nilsson (Lund University, Sweden)

Structural Biology, Molecular Modelling

Focus is on elucidating atomic structure of the carbohydrate-binding proteins galectins that are involved in inflammation and cancer. Galectin-1 and Galectin-3 are involved in metastasis, where malignant cells migrate away from the initial tumour into the circulation then adhere to endothelial cells at distant sites where they proliferate forming new tumours. Galectin-1 and galectin-3 have functional activities involved in disease that are dependent upon β-galactoside binding, including apoptosis (cell suicide) of immune cells. These proteins are attractive targets for the development of new therapeutic strategies in oncology [1],[2].

This project aims to lead to the design of inhibitors of these galectins. X-ray crystallographic determination of the protein structure enables one to see exact atomic details as well as reveal the protein's interactions with inhibitors. This insight into the protein-ligand interactions is critical to, and drives, the design of more effective small-molecule inhibitors as potential leads for drugs.

Aims: a) Crystallographic structure determination of galectin-1 and galectin-3 inhibitor complexes. b) Structure analysis to input into the design of more effective inhibitors of these proteins.

Techniques: a) Protein expression and protein crystallisation, using established protocols b) X-ray crystallographic determination of the protein structures in complex with inhibitors.

[1] Protein subtype-targeting through ligand epimerization: Talose-selectivity of galectin-4 and galectin-8. Öberg, CT; Blanchard, H; Leffler, H and Nilsson, UJ. *Bioorg. Med. Chem. Lett* (2008) 3691-3694

[2] Taloside inhibitors of Galectin-1 and Galectin-3. Collins, PM; Öberg, CT; Leffler, H; Nilsson, UJ and Blanchard, H (2012) *Chem. Biol. Drug Des.* Mar;79(3):339-346.

56. Galectins: Design and Synthesis of Talose-based Galectin-3 specific Inhibitors

Prof Helen Blanchard & Dr Darren Grice

Medicinal Chemistry, Structural Biology, Molecular Modelling

This project has a chemical synthesis component along with options for computational chemistry and X-ray crystallographic structure determination that forms part of multi-disciplinary research program incorporating structure-based design of drugs targeting proteins with critical roles in cancer. Talose based compounds are one of the inhibitor frameworks under investigation.

Tumourigenesis is a complex multi-step process involving changes in cell proliferation and adhesion. To metastasise, malignant cells from the initial tumour migrate into the circulation and adhere to endothelial cells, proliferating to form new tumours. Altered galectin-expression significantly influences this process. Galectin-3 is important in human cancer. These carbohydrate-binding proteins recognise and bind β-galactosides. This project focuses on synthesis of specific inhibitors of galectin-3, including those based on the carbohydrate taloside framework, as part of an overall goal to develop lead compounds as novel anticancer agents.

Aims: a) Synthesis of carbohydrate based compounds as inhibitors of galectin-3 b) Computational design of potential inhibitors and assessment of protein-ligand interactions.

Techniques: a) Carbohydrate chemistry synthesis of compounds. b) Molecular modelling to assist directing our compound design. Compounds could also be assessed for binding to galectin-3 via Saturation Transfer Difference (STD) NMR. c) X-ray crystallography for investigation of the protein-ligand interactions and to aid in design of higher affinity inhibitors.

57. Zebrafish galectin: A potential model for Galectin-1 inhibitor design

Prof Helen Blanchard

Structural Biology, X-ray crystallography, Molecular Modelling, biacore.

Zebrafish are an important developmental and embryological model especially as the optical clarity of their embryos and larvae permit real-time viewing of developing pathologies. Recently, the use of these vertebrates to model a range of human diseases, including some cancers, has been indicated. Zebrafish Drgal1-L2 has been identified as homologous to mammalian galectin-1, a carbohydrate-binding protein that is over-expressed by many aggressive human cancers and involved in angiogenesis and metastasis.

We have determined the X-ray crystal structure of zebrafish Drgal1-L2 as part of our initial aims to understand atomic features of this protein and as a starting point for our studies in developing zebrafish as a model for assessing effectiveness of inhibitors geared toward targeting human galectin-1. Biacore will be used to assess binding affinities of inhibitors. The ultimate aim is progression of the design of inhibitors of human galectin-1 using structural information from both human galectin-1 and zebrafish Drgal1-L2 to design high affinity inhibitors.

Aims:

a) Structure analysis of human galectin-1 and zebrafish Drgal1-L2 atomic structures to input into the design of more effective inhibitors of these proteins.
b) Crystallographic structure determination of Drgal1-L2 in complex with inhibitors
c) Analysis of binding affinity of inhibitors by Biacore.

Techniques: a) Protein expression and protein crystallisation, using established protocols b) X-ray crystallographic determination of the protein structures in complex with small molecule carbohydrates c) *In silico* structure analysis and molecular modelling. d) Biacore analysis.

58. Galectin-14 : An Identified Drug Target in Inflammation

Prof Helen Blanchard

Molecular Biology, Biochemistry, Structural Biology, Molecular Modelling

Galectins are proteins involved in diverse physiological and pathological processes including immune and inflammatory responses, tumour progression and neural degeneration. Galectins are important therapeutic targets for a number of serious diseases. Galectin-14 is a recently identified carbohydrate-recognising protein implicated in allergic

inflammation due to its release into the lumen of the lung in a sheep asthma model. Galectin-14 likely regulates the activity of eosinophils (a type of white blood cell) during allergic responses.

Galectin-14 is under-explored. Its atomic structure and carbohydrate specificity are not yet characterised. This project focuses on gaining knowledge of galectin-14 structure and recognition of carbohydrate-based inhibitors, and subsequent application of this information to the design of inhibitors to regulate its activity.

Aims: a) Progress crystallographic structure determination and in parallel undertake molecular modelling to generate and analyse structures of galectin-14 b) Assessment of protein-inhibitor interactions for design of more effective inhibitors of these proteins.

Techniques: a) Galectin-14 protein expression and purification using established protocols. b) Protein crystallisation to generate crystals suitable for X-ray diffraction studies, c) X-ray crystallographic determination of the structure of galectin-14. d) Glycan array investigation to assess galectin-14 carbohydrate specificity. e) Saturation Transfer Difference (STD) NMR and ELISA methods for evaluation of the inhibitory ability of proposed inhibitors with galectin-14.

59. Fragment based design approach of inhibitors of lectins

Prof Helen Blanchard

Structural Biology, X-ray crystallography, Molecular Modelling

The aim is progression of the design of inhibitors of lectins that are carbohydrate recognising proteins that are involved in many biological processes as well as in disease progression. An example is the function of lectins to facilitate recognition and attachment of bacteria and viruses to host cells to enable infection.

There are significant challenges to designing inhibitors of lectins, in part due to the nature of the broad shallow binding site pocket on the protein surface that one needs to be targeted by an inhibitor that can bind with sufficient affinity in order to block pathogen-cell interaction. This project aims to design of inhibitors of lectins and the protein targets will be selected from a number of galectins (that recognise β-galactoside), and/or rotavirus and human metapneumovirus that recognise sialic acids.

A fragment based screening approach will be undertaken using primarily an experimental approach of crystallisation of proteins in the presence of a large range of small carbohydrate compounds. X-ray crystallographic determination of the protein structure will be done to reveal the protein's interactions with the carbohydrate fragments. The information will be used then with molecular modelling and structure analysis to modification to current inhibitor frameworks to generate more effective small-molecule inhibitors as potential leads for drugs.

Aims: a) Crystallographic structure determination of lectin small-molecule complexes b) Structure analysis to input into the design of more effective inhibitors of these proteins.

Techniques: a) Protein expression and protein crystallisation, using established protocols b) X-ray crystallographic determination of the protein structures in complex with small molecule carbohydrates c) *In silico* structure analysis and molecular modelling.

60. Development of a pre-clinical evaluation of a transmission blocking liposomal malaria vaccine

Prof Michael Good & Dr Danielle Stanisic

Parasitology, Immunology and Vaccinology

Malaria is a global public health problem with transmission still being reported in over 90 countries. It is an infectious disease caused by Plasmodium parasites which are transmitted by female Anopheline mosquitoes. Populations most at risk are children under 5 years of age living in Sub-Saharan Africa. Current control methods are becoming increasingly less effective, therefore the development of an effective vaccine is considered to be of critical importance.

The majority of malaria vaccine candidates are based on single malaria proteins, but many of these are highly variable and are not useful in inducing immune responses that will protect against multiple strains of the malaria parasite. An alternate approach currently being developed, involves using the whole malaria parasite – such a vaccine contains multiple parasite proteins including those that are conserved between different parasite strains. It has previously been shown in pre-clinical studies using rodent models of malaria, that a whole parasite asexual blood-stage vaccine approach is able to induce protective immune responses against different strains/species of the parasite and this vaccine approach is now being evaluated in clinical trials.

This study will involve the pre-clinical investigation of a *Plasmodium falciparum* transmission blocking liposomal vaccine. This vaccine type does not prevent an individual from being infected like an asexual blood-stage vaccine

aims to do, but rather stops an infected individual from transmitting malaria to other individuals. This is because it targets the parasite life-cycle stage that is infective to mosquitoes. It is thus seen as a community-based vaccine approach.

In this project, different vaccine candidates will be generated containing the *P. falciparum* gametocyte-stage parasite; this is the life-cycle stage that is found in the blood of malaria-infected individuals and is infective to mosquitoes. For some vaccine candidates, recombinant proteins/peptides derived from the gamete-stage of the parasite, which is the stage of the parasite within the mosquito, will also be included. Pre-clinical development of these vaccine candidates will include characterisation and optimisation of the vaccine formulations. Immunological and functional assays will also be undertaken to characterise the immunogenicity and transmission-blocking activity of the single and multi-component vaccine candidates ie whether the induced immune response impacts on parasite development and/or survival in the mosquito host.

61. Development and pre-clinical evaluation of a whole blood-stage parasite malaria vaccine using cationic liposomes

Prof Michael Good & Dr Danielle Stanisic

Parasitology, Immunology and Vaccinology

Infection with Plasmodium parasites continues to result in over 200 million cases of malaria and over 400,000 deaths each year, mostly amongst children < 5years. With existing control strategies for the mosquito vector (insecticides) and the parasite (anti-malarial drugs) becoming increasingly less efficacious, an effective malaria vaccine is urgently required. A number of sub-unit vaccine candidates have been tested in Phase II and III trials, but have demonstrated limited efficacy. An alternative vaccine strategy is to utilize the whole parasite which enables the inclusion of a broad antigen repertoire, thereby overcoming the major deficiency associated with sub-unit vaccines.

In rodent models of malaria, homologous and heterologous protection against parasite challenge has been observed following immunization with a low dose blood-stage infection/drug cure protocol, with low-dose, killed, adjuvanted blood-stage parasites and more recently with chemically attenuated blood-stage parasites. The chemically attenuated blood-stage vaccine approach has been progressed into a first-in-man study, where safety and immunogenicity was observed. For this vaccine approach, protective efficacy in rodent models of malaria relies on the delivery of parasites within intact red blood cells (RBCs). The inclusion of RBCs in the final product presents a small risk for the development of antibodies against RBC antigens. Thus, developing a whole blood-stage parasite vaccine formulation with an alternative targeting/ delivery system is prudent. As liposomes are lipid bilayered membrane vesicles, they may function as a RBC-mimetic; therefore an alternative strategy is the use of liposomes for the delivery of whole parasite material.

In this project, different vaccine candidates will be generated containing either rodent malaria parasites or the human malaria parasite, *P. falciparum* formulated with cationic liposomes. The cationic liposomes will include an immunomodulator, (trehalose 6,6-dibehenate (TDB), which is a synthetic analogue of a component of the mycobacterial cell wall and is thought to activate APCs via the macrophage inducible C-type lectin (Mincle). For some vaccine candidates, key recombinant proteins/peptides derived from the blood-stage of the parasite, will also be included. Pre-clinical development of these vaccine candidates will include characterisation and optimisation of the vaccine formulations. Immunological and functional assays will also be undertaken to characterise the immunogenicity and protective efficacy of the single and multi-component vaccine candidates and to examine immune mechanisms of protection. Results from this project will inform the transition of this vaccine approach into clinical studies.

62. Development and evaluation of controlled infection immunisation using slow release delayed death drug formulations

Prof Michael Good & Dr Danielle Stanisic

Parasitology, Immunology and Vaccinology

Malaria is a parasitic disease prevalent in many developing countries, with transmission reported in 90 countries. It is associated with extensive morbidity and mortality, mainly in pregnant women and young children. Currently available control strategies are becoming increasingly less effective; therefore the development of an effective vaccine is considered to be of critical importance.

Many researchers have focused on single parasite-derived proteins in their quest to develop a sub-unit vaccine against malaria. However, many of these proteins are highly variable, and are not useful in eliciting responses that can protect against multiple strains of the parasite. A vaccine approach that utilizes the whole malaria parasite

however, would contain multiple parasite antigens including antigens that are not altered by the parasite i.e. are therefore conserved between different parasite strains. Using rodent models of malaria, it has previously been shown that different whole parasite asexual blood-stage vaccine approaches are able to induce species and strain-transcending protective immune responses. One such approach is controlled infection immunization (CII). This involves administering a malaria infection at the same time as anti-malarial treatment with a "delayed death" drug (eg doxycycline and azithromycin) is commenced. These drugs specifically target the apicoplast of the malaria parasite and allow the progeny of the treated parasites to survive for an extra blood-stage cycle, thereby prolonging exposure to low levels of the parasite. Thus far, these immunization regimens have required either multiple days of anti-malarial treatment (which is not viable for a vaccine strategy) or a single large dose of drug that is not currently clinically indicated in humans and may not be tolerated. This may be overcome by the use of slow release drug formulations.

This project will involve further pre-clinical development and evaluation of the CII approach using slow release delayed death drug formulations. Using rodent models of malaria, pre-clinical development will initially involve characterising and optimising different slow release drug formulations. Their ability to control parasite growth will be examined. The optimal slow release drug formulation and parasite combinations will be evaluated for their ability to induce protection against subsequent challenge infection. Immunological and functional assays will be used to assess immunogenicity and to examine the immune mechanisms of protection. Results from this project will inform the transition of this vaccine approach into clinical studies.

63. Vaccine development for group A streptococcus and rheumatic heart disease

Dr Michael Batzloff & Prof Michael Good

Molecular Immunology and Vaccinology (MIV)

We have developed a prototype vaccine to protect against infection with group A streptococcus (GAS), the causative agent of rheumatic heart disease. The vaccine works by inducing an IgG antibody response to an epitope of the surface M protein. We are now exploring ways to induce an IgA response that will prevent colonisation of the throat with GAS. A vaccine that also induced an IgA response would be able to prevent transmission of the organism between different individuals as well as preventing disease in a colonised individual, and would thus have a significant public health benefit.

64. Development of an immunotherapy to treat systemic group A streptococcal infection

Prof Michael Good, Dr Michael Batzloff & Prof James McCarthy (QIMR)

Molecular Immunology and Vaccinology (MIV)

We have shown that antibodies to a conserved epitope on the surface M protein of group A streptococcus (GAS) can prevent infection with the organism and potentially prevent the post streptococcal complications of acute sepsis, rheumatic heart disease and renal disease. We now wish to test whether passive administration of antibodies to this epitope will be effective in treating acute infections, such as necrotising fasciitis. Initially we will develop an animal model and then plan to test humanised anti-streptococcal antibodies for in vitro efficacy prior to planning a human clinical trial

65. How respiratory viruses predisposes children to asthma

Prof Suresh Mahalingam

Virology, Immunology, Physiology

Asthma is the most common chronic disease of childhood. It is an inflammatory disease of the airways, characterized by episodes of inflammation and narrowing of the small airways in response to triggers that include allergens, viral infections and airway irritants. Human metapneumovirus (HMPV) is emerging as a major cause of morbidity and life-threatening lower respiratory tract disease in infants and young children. HMPV may also predispose people to develop asthma, exacerbate asthma and contribute to chronic or persistent disease. The objectives of this project are to determine the immune and physiological changes, which HMPV causes in lungs to exacerbate asthma and the immune and physiological changes which persistent HMPV infection causes in lungs to predispose an individual to asthma. The goal is to provide the necessary foundation for developing therapeutic interventions to ameliorate or control conditions that lead to the development or exacerbation of asthma associated with HMPV disease.

Techniques: Handling of primary human bronchial epithelial cells, mouse model of asthma, lung function studies, ELISA, real-time PCR, viral plaque assays, flow cytometry, histopathology, immunohistochemistry and western blotting.

66. Novel therapeutic intervention of human respiratory virus

Prof Suresh Mahalingam

Virology, Immunology, Physiology

Human metapneumovirus (HMPV), a paramyxovirus that causes respiratory tract disease in both children and adults has clarified the need for a HMPV therapeutic. HMPV is ubiquitous throughout the world and the global incidence ranges from 15% to 30%. At present there is no vaccine or antiviral drug against HMPV. The objective of this proposal is to develop novel drugs as a disease intervention strategy for HMPV. The strategy comprises short interfering RNAs (siRNAs), the molecules that induce RNA interference. RNAi technology offers the ability to rapidly and specifically design siRNA intervention strategies targeting any viral sequence, including genes of the viral replication machinery that have, up until now, been considered "undruggable". We have identified molecules that can inhibit the attachment protein G of HMPV. The objective of this project is to investigate whether siRNA silencing of the viral G gene will inhibit virus infection and replication, and reduce disease pathogenesis associated with inflammation and airway hyperresponsiveness. The outcome will be the development of prophylactic and therapeutic drugs that will be effective against a significant paediatric infectious agent.

Techniques: mouse model of HMPV disease, lung function studies, ELISA, real-time PCR, viral plaque assays, flow cytometry, histopathology, immunohistochemistry.

67. The role of macrophages in Rhinovirus infection

Prof Suresh Mahalingam

Virology, Immunology, Physiology, Cellular Biology

Rhinoviruses are the major cause of the common cold and result in the hospitalisation of infants, and are a major player in exacerbations of asthma and chronic obstructive pulmonary disease. Rhinovirus infections contribute to millions of dollars in healthcare costs, and the morbidity and mortality attributable to rhinovirus infections is substantial. At present there are no treatment or vaccine and progress has been hampered by the lack of small-animal models of infection. As a result understanding the mechanisms of disease caused by this infection has been slow. Recently, a mouse model of rhinovirus infection has been developed. The model is of major significance in determining important factors underlying rhinovirus-induced disease. Importantly, the mouse model will enable the identification of targets for the development of treatments for rhinovirus infection. We have identified that macrophages are the predominant cell type infiltrating the lung tissue following infection. The roles that these cells play in disease are not known. The objective of this project is to deplete macrophage infiltration and examine the effects on disease pathogenesis and viral replication. Macrophage will be depleted using a drug that specifically inhibits macrophage chemotactic factors. The findings may lead to the identification of macrophage chemotactic inhibitors as potential treatment for this condition.

Techniques: mouse model of rhinovirus disease, lung function studies, ELISA, real-time PCR, viral plaque assays, flow cytometry, histopathology, immunohistochemistry.

68. How viral infections predisposes individuals to arthritis and novel approaches to therapy

Dr Lara Herrero & Prof Suresh Mahalingam

Virology, Immunology, Cellular and Molecular Biology

Many viral infections can result in arthritis (e.g. HIV, influenza virus, cytomegalovirus, mosquito-borne viruses such as dengue and Ross River virus). However, the mechanisms contributing to disease are largely unknown. We have developed the first mouse model of viral-induced arthritis, which represents the only mouse model for studying the immunopathological processes that lead to musculoskeletal disease following infection. We have discovered that mannose binding lectin (MBL) (a member of the complement pathway) contributes to the development of severe disease. MBL binds to carbohydrates on certain micro-organisms and plays a role in their clearance and destruction by (i) activation of complement and (ii) by promoting opsonization by phagocytic cells. Complement activation is known to play an important role in autoimmune arthritis, appears to be important in gouty arthritis and may play a role in septic arthritis. However, little has been published on the role of complement in infectious arthritides. We have reported that mice deficient in MBL exhibited less severe rheumatic symptoms, with no significant differences in

viraemia. MBL appeared to be associated more with tissue destruction at the site of infection, rather than recruitment of inflammatory cells. The objective of this project is to investigate the anti-inflammatory properties of a novel inhibitor of MBL in mouse models of viral induced arthritis and whether the inhibitor reduced inflammation and tissue pathology. The outcome is the development of a new approach to target pathogenic pathways in infectious and autoimmune inflammatory conditions.

Techniques: mouse model of viral arthritis, clinical disease and joint hypersensitivity measurement, ELISA, real-time PCR, viral plaque assays, flow cytometry, histopathology, western blotting, immunohistochemistry.

69. How mosquito-borne viruses affect the innate immune system to cause disease

Prof Suresh Mahalingam

Virology, Immunology, Cell Biology

Arboviruses or mosquito-borne viruses include members of several virus families (Togaviridae, Flaviviridae, and Bunyaviridae) and are a significant cause of human diseases, ranging from hemorrhagic fever to encephalitis and arthritis. The early interactions between mosquito-derived arboviruses and the host cells they initially infect (dendritic cells (DCs) and macrophages) are likely to be crucial in determining whether the virus is able to successfully establish an infection. Understanding how viruses evade and suppress the innate immune response in these cells will significantly expand our knowledge of how arboviruses cause disease. Our preliminary data demonstrate that during early skin infection, viruses derived from mosquito cells (with high mannose N-linked glycans) infects higher percentage of cells, replicates to high titres and stimulates less IFN-α/β compared to viruses derived from mammalian cells (with complex carbohydrates). The objective of this project is to investigate the role of viral carbohydrates on early arboviral infection and host immune reactions. These investigations will add to our understanding of both arbovirus/host interactions and pathogenesis for this important group of human viral diseases, which may lead to improved vaccines or therapeutics for this emerging class of pathogens.

Techniques: Cell culture, ELISA, real-time PCR, viral plaque assays, flow cytometry, immunohistochemistry, intravital two-photon microscopy.

70. A glycomics approach towards the discovery of novel markers associated with viral inflammatory disease

Dr Penny Rudd & Dr Lara Herrero

Virology, Immunology, Cell Biology

Arthropod-borne arthritogenic alphaviruses such as chikungunya virus (CHIKV) and Ross River virus (RRV) cause large epidemics of severe musculoskeletal disease and have been progressively expanding their global distribution emerging in new regions of the world. The hallmark of alphavirus disease is crippling pain and joint arthritis, which often has an extended duration leaving patients bed-ridden and incapacitated. Interactions between virus and host determine the course of infection and are likely to be critical in understanding viral pathogenesis and control. Alphaviruses display N-linked glycosylated transmembrane glycoproteins with the exact composition of the glycosylation being dependent on the host species and cell type in which the virion was assembled. Our aim is to use glycan and lectin array technologies to discover interactions between virus and human cells to inform the rational design of therapeutics. The identification of the protein glycosylation status and glycan binding specificities of alphaviruses and human cells of the joints (chondrocytes, bone, fibroblasts, muscles) will provide an understanding of the virus-human host cell interaction and, consequently, potential novel insights into pathobiology.

Techniques: Handling of primary human bone cells, viral plaque assays, flow cytometry. Experiments will be undertaking in the state-of-the-art glycobioanalytical facility that has equipment to support array printing and analysis.

71. A glycomics approach towards the discovery of novel markers of virus transmission by mosquitoes

Dr Penny Rudd & Dr Lara Herrero

Virology, Viral-ecology, Cell Biology

The incidence of human and animal diseases caused by mosquito-borne pathogens has increased at an alarming rate globally. In nature, arboviruses are maintained in continuous transmission cycles between mosquito vectors and susceptible vertebrate hosts. The early interactions between the arbovirus and initially infected mosquitoes is likely to be a crucial step in determining whether the virus is able to successfully establish infection. Understanding how viruses infect these cells will significantly expand our knowledge of how arboviruses are transmitted and cause disease. This project utilises glyco-virological approaches, unique mosquito cells and an arbovirus model system to identify new markers associated with virus transmission by mosquitoes. Markers associated with transmission will be identified by

establishing global glycan and lectin profiles of the cells derived from a major mosquito species. The overall objective of this proposal are to explore the glycomics of mosquito cells and its role in arbovirus mosquito infection.

Techniques: Handling of primary mosquito cells, cell culture, viral plaque assays, flow cytometry, molecular biology. Experiments will be undertaking in the state-of-the-art glycobioanalytical facility.

72. Drug repurposing for the treatment of alphaviral infections

Dr Penny Rudd & Dr Lara Herrero

Virology, Virus-Host Interactions, Therapeutic Treatments

Ross River (RRV) and chikungunya (CHIKV) viruses are mosquito-borne viruses that cause severe joint and muscle pain in humans, which can last months or even years and may become a cause of chronic pain and disability. CHIKV and RRV can be found in over 100 countries across the globe. In 2015, over 700 000 cases of chikungunya were reported in the Americas alone to the Pan American Health Organization (PAHO) regional office and that same year, Queensland saw the largest epidemic of RRV in 20 years with over 4000 cases in the first quarter.

There are currently no specific treatments for these alphaviruses. Pain relief is prescribed to help alleviate symptoms. This project focuses on examining the repurposing of currently available drugs towards the treatment of viral induced arthritides. Discovering new uses for approved drugs provides the quickest possible transition from bench to bedside and may lead to novel treatments, which could prove beneficial towards pain management for hundreds of thousands of arthritic patients worldwide.

Towards this, we will 1) Examine if the drugs reduce joint swelling and ameliorate overall disease outcomes in RRV/CHIKV infected mice 2) Characterise the inflammatory cytokine/chemokine patterns during and after drug treatment and 3) Identify novel biomarkers which may serve as clinical markers for disease monitoring and outcomes.

The resolution of this project may lead to significant advancements for the identification of novel treatment strategies for patients suffering from CHIKV and RRV.

Techniques: mouse models of CHIKV and RRV infection, clinical disease monitoring, *in-vitro* assays, real-time PCR, cell-culture, viral plaque assays, immuno- histochemistry, bioplex assays.

73. Deciphering the mechanisms involved in Chikungunya virus (CHIKV) neuropathogenesis

Dr Penny Rudd & Dr Lara Herrero

Virology, Virus-Host Interactions, Therapeutic Treatments

Alphaviruses are a global health threat to humans and animals alike causing severe disease ranging from lethal encephalitis to debilitating long-lasting arthritis. Classically, the Old World alphaviruses like chikungunya (CHIKV) are primarily associated with painful and chronic arthritis. Yet, in recent years, neurological sequela has been consistently associated with CHIKV infection. Several thousand neuro-virulence cases have been reported especially in the young and elderly.

Very few studies have been undertaken to determine the mechanisms involved in CHIKV neuropathogenesis. We want to answer fundamental questions about the mechanisms involved in CHIKV neurovirulence using a combination of *in vitro*, *in vivo* and *ex vivo* approaches. Towards this, we will look at 1) How CHIKV enters CNS cells and disseminates throughout the brain structures 2) Determine which immune responses are elicited after CHIKV infects brain cells and 3) Assess how inadequate immune responses contribute to CHIKV neuropathologies.

Since there is no current treatment or vaccine, basic knowledge gained about CHIKV-host interactions will play a pivotal role in the discovery of new treatment strategies. These therapies will aim to reduce or circumvent neurological complications involved in CHIKV central nervous system (CNS) disease by preventing viral entry and spread or by counter-acting immune mediated pathology.

Techniques: mouse model of CHIKV infection, clinical disease monitoring, *in-vitro* assays, real-time PCR, cell-culture, viral plaque assays, immunohistochemistry, confocal microscopy.

74. Identifying novel animal reservoirs of Ross River and Barmah Forest viruses

Dr Penny Rudd & Dr Lara Herrero

Virology, Epidemiology, Public Health

Ross River virus (RRV) is a serious disease with no specific treatment or vaccine. It affects thousands of Australians annually, in Queensland with an influenza-like disease and severe debilitating joint pain. It is the most common

mosquito-borne virus on our shores. The virus is a burden to Australia and surrounding islands in the South Pacific including Papua New Guinea and Fiji, putting a great number of people in hospital each year.

Most medically important arboviruses are transmitted to humans from other vertebrate species. To be an important reservoir for human infection, the reservoir host must be attractive for arthropod vectors i.e. they must be able to feed on these hosts. The hosts must also develop viraemia that is high enough to allow transmission to susceptible blood-feeding vectors. The ideal hosts must equally have low mortality to the infection and there must be low herd immunity. Serological studies and laboratory investigations have indicated that several domestic and wild animals serve as RRV reservoirs, including dogs, cats, possums, and horses. However, the primary reservoir hosts for RRV are marsupials with macropods playing a significant role.

In a recent publication, there is evidence for endemic circulation of Ross River virus in the Pacific Islands and the potential for emergence. This project aims at looking into what these potential reservoirs could be. Towards this we will 1) Examine susceptibility of various cell lines to RRV infection 2) Determine if Australian animals have antibodies against RRV 3) Assess if the target reservoirs identified and present in Samoa also have antibodies against RRV.

This project will have important ecological, clinical and public health outcomes. It will allow us to better understand disease ecology of RRV and help identify potential outbreaks and reduce the risk global spread.

Techniques: cell culture, *in-vitro* assays, real-time PCR, viral plaque assays, ELISA.

75. Super-resolution optical microscopy

Dr Erik W Streed

Biophysics, Microscopy

Microscopy is a crucial tool in biology. The resolution of optical microscopy is generally limited to ~200 nm by the wavelength of light. Many interesting biological processes occur at smaller length-scales and it is useful to optically image below the wavelength limit. Super-resolution (SR) imaging is a recently developed family of fluorescence microscopy techniques that leverage quantum physics to achieve higher resolutions, in some cases approaching the nanometer scale. Specific phenomena that enable SR include photon statistics, non-linear optics, wavelength-scale structuring of the illumination field. Structuring the pattern of illumination can thus selectively illuminate (or hide) parts of the sample with a resolution below the wavelength of light. The non-linear optical properties of fluorescent molecules can further change the apparent shape of the illumination.

Phase Fresnel lenses are micro-fabricated computer generated holograms of an ideal lens. These technology is used in the Centre for Quantum Dynamics to image single atomic ions [1] with record-setting wavelength scale resolution. The computer generated nature of phase Fresnel lens makes them ideally suited to creating complicated illumination patterns, reducing the cost and increasing the performance of super-resolution microscopes. This project will focus on demonstrating a super-resolution microscope based on Fresnel lens technology with the long-term goal of integrating it into the biomolecular ion trap experiment (See "Unfolding a single biomolecule").

Techniques: Optics, nonlinear optics, lasers, imaging, digital and analog electronics, nanotechnology, fluorescence microscopy, confocal microscopy. STED, GSD, STORM, dSTORM microscopy.

76. Unfolding a single biomolecule

Dr Erik W Streed

Molecular Biology, Structural Biology, Biophysics

The chemical functionality of large biomolecules is determined by both their molecular composition and their shape or "folding". Electrodynamic interactions such as hydrogen bonding, Coulomb force, and Van der Waals forces play a substantial role in the folding process. This project will develop a new method of investigating the folding of biomolecules through the combination of fluorescence microscopy and mass spectroscopy. Bimolecular ions will be generated using electrospray ionization and confined in a linear Paul ion trap mass spectrometer. The like charges of the ions repel each other, naturally separating them and greatly simplifying the process of isolating single ions. The biomolecules under investigation will be selected to have either intrinsic fluorescence (GFPs, tryptophan & tyrosine containing proteins) or those that can be tagged with commercial dye labels (DNA, RNA,etc.). The charge state of the ion can be changed a single electron at a time to drive unfolding through Coulomb repulsion. Changes in shape will be measured optically through fluorescence microscopy in large macromolecules (DNA) or fluorescent resonant energy transfer (FRET) in smaller molecules.

The initial stage of this project will focus on construction and characterization of an electrospray ionisation source for loading a Paul trap. The behavior of fluorescent biomolecular ions will be investigated under the rapidly changing

charge and solvation conditions of electrospray. This environment also offers the opportunity investigate reactions with mixing rates far exceeding those available in microfluidics experiments. Test ion candidates include green fluorescent proteins (GFPs), colloidal quantum dots, nucleic acids conjugated with commercial dye markers, and Lycopodium pollen spores. The long-term goal is to integrate this apparatus with the advanced fluorescence microscopy technology described in the "Super-resolution optical microscopy" project.

Techniques: Ion trapping, vacuum systems, mass spectrometry, electrospray, lasers, optics, imaging, high voltage electronics, digital and analog electronics, fluorescence microscopy, photon counting, FRET.

77. Vaccine development for *Neisseria gonorrhoeae*

Assoc Prof Kate Seib

Molecular Biology, Molecular Microbiology

Neisseria gonorrhoeae, the causative agent of gonorrhoea, is a significant health problem worldwide. The control of gonorrhoea depends on the development of a vaccine due to the continuing increase of antibiotic resistance and the staggering outcomes of infection, including infertility and increased transmission of HIV. This project aims to discover novel virulence factors based on identification of genes that are required for interaction with human cervical epithelial cells and that are upregulated during interactions with human cervical epithelial cells. The distribution of the identified vaccine candidates will be investigated in a diverse range of *N. gonorrhoeae* strains and the functions of proteins will be examined using a panel of antimicrobial stress assays. The vaccine potential of these candidates will also be assessed in a protection model to determine if antibodies raised against these proteins can inhibit association and invasion of cervical epithelia and as such offer protection against gonococcal colonization.

78. Role of meningococcal phase variable methyltransferases in disease

Assoc Prof Kate Seib

Molecular Biology, Molecular Microbiology

Neisseria meningitis is a major cause of septicemia and meningitis worldwide and meningococcal populations are comprised of distinct clonal complexes, several of which are considered "hypervirulent" due to the propensity of isolates to cause invasive disease. We have identified a new phase variable DNA methyltransferse that is associated with strains of the hypervirulent clonal complex 41/44 (cc41/44), isolates of which have been responsible for several recent meningococcal epidemics. Phase variation of this methyltransferse (ModD) differentiates the bacteria into two distinct cell types due to differential methylation of the genome in Mod-ON and Mod-OFF variants. This DNA methylation is involved in the regulation of numerous genes (a phasevarion), some of which are involved in meningococcal colonization and disease processes (1). This project aims to determine the set of genes regulated by ModD and the phenotypes of ModD-ON/OFF variants in different conditions that are relevant to meningococcal colonisation and disease. Determination of the role of ModD in virulence will advance our understanding of the basis of meningococcal disease, and may reveal key bacterial factors that enables meningococcal infection to progress from a state of harmless carriage to rapidly progressing, often fatal sepsis.

79. Characterising host-pathogen interactions using Glycan Arrays

Assoc Prof Kate Seib

Molecular Biology, Molecular Microbiology

Human mucosal surfaces, such as the airway, contain a range of carbohydrate structures (glycans) and many bacteria have evolved carbohydrate-binding proteins (lectins) that enable cell attachment, colonisation and invasion of host cells. Our aim is to identify glycans that host-adapted bacterial pathogens bind to during colonisayion and disease. This project will focus on bacteria including *Moraxella catarrhalis* (causes middles ear infections and exacerbations of chronic obstructuve pulmonay disease), *Neisseria gonorrhoeae* (causes gonorrhoea) and *Neisseria meningitidis* (causes sepsis and meningitis). We will probe Glycan Arrays (consisting of >300 glycans of known structure covalently immobilised onto glass-slides) using wild type bacteria and a series of mutant strains lacking key outer membrane structures. We will also use epithelial cell adherence and invasion assays to investigate the role of glycan interactions that are identified. These findings will contribute to understanding key bacterial and host factors involved in colonisation and disease, and may direct future vaccine development for these bacteria.

80. *Moraxella catarrhalis* phasevarions and impact on vaccine candidate expression

Asso Prof Kate Seib & Dr Ian Peak

Molecular Biology, Molecular Microbiology

Otitis media (OM), or middle ear infection, is a highly prevalent pediatric disease worldwide, ranking first in reasons why children make physician's office and emergency room visits, undergo surgery requiring general anesthesia, and experience hearing loss. OM is common and frequently severe in Australian Aboriginal children. In the worst affected areas, perforation of the tympanic membrane may affect more than 50% of children. *Moraxella catarrhalis* causes a significant proportion of OM and there is a pressing need to develop better methods to manage OM caused by *M. catarrhalis*, preferably via the development of vaccines. The most rational approach for the design of novel methods to prevent OM is one based on having a complete understanding of the molecular mechanisms that underlie the switch from commensal, to pathogen of the middle ear, and to identify those antigens that are variably expressed, so they can be ruled out. Via utilization of the 'phasevarion', a phase variable regulon, we propose that *M. catarrhalis* rapidly modifies expression of multiple genes important for adaptation to new niches or stresses. In every species studied to date, genes regulated by phasevarions include metabolic stress responses, virulence factors, vaccine candidates, or all of these. The aim of this project is to characterise the genes regulated by phasevarion methylases of *M. catarrhalis* and determine the importance of phase varions in models of colonisation, survival, and stress. These findings will contribute to vaccine development, and deeper understanding of the switch from commensal to otopathogen.

81. *Moraxella catarrhalis* phasevarions and impact on vaccine candidate expression

Assoc Prof Kate Seib

Molecular Biology, Molecular Microbiology

Moraxella catarrhalis is a microbial pathogen responsible for otitis media and exacerbations of chronic obstructive pulmonary disease (COPD). However, unlike similar pathogens *Streptococcus pneumoniae* and *Haemophilus influenzae*, there is no vaccine for *M. catarrhalis.* Part of the reason for this is that *M. catarrhalis* is an emerging pathogen and is less well characterised. This project aims to discover and characterise virulence factors and vaccine candidates from *M. catarrhalis* with regards to their distribution, expression and mechanism of function. Genes of interest will be be genetically mutated, and phenotyes assessed in assays including metabolic assays, airway epithelial cell adherence and invasion, and immune cell killing. These findings will lead to a greater comprehension of how this pathogen infects and initiates pathogenesis in the human host, which in turn will contribute to vaccine development against this important human pathogen.

82. Biologic drug engineering and design

Dr Jian Zhan & Prof Yaoqi Zhou

Molecular Biology, Protein Chemistry

Biological drugs such as proteins, nucleic acids, and sugars are medicinal products extracted from living systems. Unlike small molecular drugs, they are found to be more specific to the intended target, and more effective in interacting with multiple receptors. More importantly, developing biological drugs enjoys much higher success rate than small molecular drugs in every stage of drug development from preclinical to registration. It was estimated that 50% of top 100 selling drugs are biological drugs this year. This percentage is expected to increase further as the gap widens between the number of patents filed for biologics and the number filed for small molecules. However, unlike small molecules, biologic drugs are expensive to manufacture due to low yields and short in shelf-life due to their low stability. The objectives of our research are to improve drug production by codon optimisation, to increase drug stability by protein engineering, and to design peptides or proteins with specific therapeutic effects. We have successfully developed a new class of antibiotics and are ready to apply the same technique to other diseases. The summer and honour projects of students with molecular biology lab skills will be teamed with computational biologists to screen designed candidates for cancer and other diseases.

Techniques: Basic molecular biology

83. Novel bioinformatics methods for prioritizing disease-causing genetic variations

Prof Yaoqi Zhou

Bioinformatics, Computational Biology

One key element of personalized medicine is having accurate genetic tests that separate diseasing-causing/susceptible human genetic variants from neutral ones. Based on the Human Genome Mutation Database (HGMD), single nucleotide variations (SNVs), the largest class of genetic variations, are responsible for over 50% of known Mendelian diseases. This is followed by micro-insertions and deletions (INDELs, less than 20 nt), responsible for 24% known Mendelian diseases. So far, millions of SNVs and micro-INDELs have been observed in humans. Obviously, it is not practical to examine the biological functions of each variant. Thus, there is a critical need for effective tools for distinguishing the likely disease-causing variants from the ones that are functionally neutral. Our approach is to integrate sequence, structure and functional information of genes and its regulatory elements for more accurate discrimination of gene variations. Our long-term goal is to build an integrated tool for predicting susceptibility for specific disease/phenotype from all types of genetic variations.

Techniques: Computational algorithm development and bioinformatics database searching.

84. The development of a glycoenzyme (ZymeBank) data bank with supporting bioinformatics databases

Dr Matthew Campbell, Dr Chi-Hung Lin & Prof Mark von Itzstein

Bioinformatics and Biochemistry

Glycoenzymes are responsible for the biosynthesis of all glycans and glycoconjugates and as such provide a rich source of biocatalysts for industrial applications. Over 350,000 sequences (CAZY.com) have already been identified in genomic databases and the number is growing exponentially, but few glycoenzymes are readily available for the glycomics and biotechnology community. This project aims to develop an international glycoenzyme bank (called ZymeBank) that can be used to synthesis complex glycans for use in biological studies.

The project will focus on expressing and purifying human glycoenzymes and building a new database for storing this data along with a web-app interface. This will address a number of challenges: (i) identification of specific glycoenzyme activities for which there is a need in the community; (ii) establish screening protocols that are benchmarked against existing methods; and (iii) set standards and develop an open access databases for glycoenzyme activites and associated biological pathways.

Techniques: Bioinformatics; Databases; Protein Expression; Molecular Biology

85. Building a Melanoma Glycomics Database

Dr Matthew Campbell, Dr Jodie Abrahams, Assoc Prof Daniel Kolarich, Dr Andrea Maggioni & Prof Mark von Itzstein

Bioinformatics and Analytical Glycomics

Alteration of protein glycosylation is linked to the process of disease in a variety of ways. Notably, an important aspect of this relationship is the modification of the cell surface carbohydrate structures, which affect both adhesion properties and cell-cell communication. Despite the fact that changes in glycosylation accompanying malignant transformation of the cell have been known for several decades, we are only starting to understand the intricate mechanisms that give rise to specific glycan signatures.

This project is designed to take advantage of recent advances in analytical glycomics with an overarching goal to systematically explore novel glycan-based strategies for management of melanoma. By using a melanoma (tissue and cell line) data collection representative of different clinical stages, we will create a novel cancer knowledgebase that will integrate mass spectrometry and glycan structure profiles.

The outcome will be a unique research platform to assist the identification of molecular signatures, specifically related to the glycosylation pathway, that will improve our understanding of cellular glycodynamics implicated in cancer. It will also test the hypothesis that changes in the glycome can be used to monitor and predict melanoma progression.

Techniques: Bioinformatics; Databases; Statistics; Glycan Analaysis

86. Development of a live-attenuated Chikungunya virus vaccine candidate

Dr Adam Taylor & Prof Suresh Mahalingam

Virology, Immunology, Cellular and Molecular Biology

Chikungunya virus (CHIKV) is the causative pathogen of chikungunya fever, a mosquito borne viral disease causing highly debilitating arthralgia that can persist for months and progress to chronic arthritis. The disease occurs in periodic large-scale outbreaks. CHIKV and its mosquito vectors continue to expand their global distribution and cases

of the disease have now been reported on nearly all continents. There are no CHIKV antivirals, nor is there a licensed vaccine.

Our molecular investigation of the subcellular localisation of CHIKV capsid protein has identified a mutation that significantly attenuates CHIKV replication and virulence in vertebrate hosts. The attenuated mutant CHIKV thus functions as a live attenuated vaccine candidate; mice immunised with mutant CHIKV are protected from severe CHIKV disease when challenged with wild-type virus.

We now aim to further develop this vaccine candidate and investigate: 1. The mechanism of CHIKV attenuation. 2. The safety and efficacy of the CHIKV vaccine candidate. 3. Whether the CHIKV vaccine candidate affords crossprotection against other alphaviruses-induced disease such as Ross River virus.

Techniques: mouse models of viral arthritis, clinical disease and joint hypersensitivity measurement, ELISA, real-time PCR, viral plaque assays, flow cytometry, histopathology, western blotting, immunohistochemistry.

87. Oncolytic capacity of a live attenuated Chikungunya virus

Dr Adam Taylor & Prof Suresh Mahalingam

Virology, Immunology, Cellular and Molecular Biology

Oncolytic viruses are a novel form of cancer treatment. Many viral vectors in use today have been rendered safe by deletion of genes encoding viral structural proteins, thus making them unable to spread beyond the first infected cells. Hence, such replication-deficient constructs may lack efficacy.

Here, we aim to examine the oncolytic potential of the replication-competent vector CHIKV-NoLS, a live-attenuated mutant virus based on the parent chikungunya virus (CHIKV). CHIKV is the causative pathogen of chikungunya fever, a mosquito borne viral disease causing highly debilitating arthralgia. Compared to CHIKV, CHIKV-NoLS replication is attenuated in vitro and in vivo and shows no disease signs in a mouse model of CHIKV disease virulence. Despite it's avirulence in mice, CHIKV-NoLS has not lost its capacity to infect and kill cells.

This proposal aims to screen a panel of cancer cell lines to assess their susceptibility to infection and CHIKV-NoLS-mediated cell death. This study will investigate any mechanisms of CHIKV-NoLS tumour tropism including cell-virus attachment.

Techniques: glycomics, real-time PCR, viral plaque assays, flow cytometry, histopathology, confocal microscopy, immunohistochemistry.

88. Developing an alphaviral vector to deliver bioactive factors to bone: potential to treat bone disease

Dr Adam Taylor & Prof Suresh Mahalingam

Virology, Immunology, Cellular and Molecular Biology

Ross River virus (RRV) is the causative pathogen of Ross River virus disease, a mosquito borne viral disease causing highly debilitating arthralgia that can persist for months and progress to chronic arthritis. Bone remodelling is balanced by the actions of bone-forming osteoblast cells (OBs), bone-resorbing osteoclast cells (OCs) and 2 key soluble factors, RANKL and OPG. Increases in the RANKL/OPG ratio results in proosteoclastic conditions with active OCs causing bone loss leading to debilitating diseases such as osteoporosis or osteoarthritis. Ross River virus (RRV) replicates in these bone cells and activates OCs by disrupting the RANKL/OPG ratio, causing bone pathologies.

This project aims to examine the therapeutic use of exogenous OPG to suppress bone loss during RRV infection, with a view to use RRV as a gene therapy vector to deliver bioactive factors directly to bone.

Techniques: Animal models of infection, real-time PCR, viral plaque assays, flow cytometry, histopathology, confocal microscopy, immunohistochemistry.

89. Development of MALDI imaging mass spectrometry analysis of glycosphingolipids derived glycans

Dr Arun Everest-Dass & Prof Mark von Itzstein

Analytical Glycomics, Biochemistry

Given the universal presence of glycans on all cell surfaces, it is not surprising that several human diseases display changes in glycosylation of proteins and lipids. For example, cancer cells frequently display aberrant glycans than those observed on normal cells. Mass spectrometry (MS) based glycomic methodologies are now regularly used for the reliable profiling of glycans from clinical samples. Although, routine mass spectrometric glycan analysis is well established and reliable, the analysis of whole tissues destroys any information relating to the spatial distribution of

the analytes. Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) is an emerging technique that seeks to utilize the analytical advantages of mass spectrometry whilst preserving the spatial information of the biological molecule of interest inherent in the sample. The unambiguous correlation between histopathology and MALDI–MSI allows the mass measurement of glycans directly from tissue regions [1].

This project aims to develop novel MALDI MSI based imaging technology to characterise glycosphingolipid derived glycans directly from tissue sections.

Techniques: glycomics, MALDI imaging, histopathology.

[1] Everest-Dass AV *et al*, Mol Cell Proteomics. 2016 Sep;15(9):3003-16. doi: 10.1074/mcp.M116.059816.

90. Refining the development of HIV maturation inhibitors – a biochemical and biophysical approach

Dr Chandan Kishor, Assoc Prof Thomas Haselhorst & Prof Johnson Mak

Virology, Biochemistry, Molecular Biology

The emergence of drug resistant HIV (in particular in low- and middle-income countries) threatens the long-term effectiveness of patient care using existing anti-retroviral agents. Maturation inhibitors represent a highly effective class of antivirals, but the lack of structural details on the assembly and maturation process makes it difficult to refine this group of inhibitors for clinical application. The Gag protein of HIV drives the formation of virus particle. During HIV assembly, roughly 2500 Gag molecules come together for the assembly of immature particle, which is followed by a proteolytic processing maturation to generate infectious virus particles. The formation of virus particles and maturation process is highly regulated, and the smallest interference of these events can block the replication of HIV. Our lab has pioneered the production of full-length recombinant Gag for biochemical and biophysical analysis. Our works challenge many previously held assumptions, and have revealed novel insights of the viral assembly and maturation process. The objective of this proposal is to use a combination of protein biochemistry and biophysics tools to define the mechanism of HIV assembly for the development of novel anti-viral. More specifically, we will use surface plasma resonance and isothermal titration calorimetry to define the association constants and thermal dynamic regulation of virus formation. This information will enable much more potent inhibitor can be developed. We will use charge detecting mass spectrometry to define the kinetics of the olgomerization (therefore the assembly steps) HIV Gag proteins take for the formation of viral particles. These details will unearth fundamental parameters that govern the virus formation process. We will use Saturation-Transfer Difference (STD) NMR to determine the moiety of small molecule ligands (maturation inhibitor, lipids and nucleic acids) that bind to HIV Gag proteins to facilitate virus formation. We will use cryo-electron microscopy (in particular tomography and single particle analysis) to reveal the structural details of viral assembly by defining the assembly lattice and how maturation inhibitors can interfere with these structures. This project is primary a PC2 lab based research proposal, although any novel insight will be validated using the authentic infectious HIV assembly system to validate the importance of these findings *in vivo*.

Techniques: protein biochemistry, biophysics, molecular biology, molecular virology, structural biology, *in silico* molecular modelling of RNA-protein structure.

91. Pre-entry priming of HIV – redefining the entry process of the virus for the development of a novel HIV vaccine

Dr Belinda de Villers & Prof Johnson Mak

Virology, Biochemistry, Molecular Biology

Viruses are considered neither live nor dead, and are therefore thought to be inert until after entering the host cells. Using cryo-electron microscopy (cryo-EM) and single molecule fluorescence imaging, we have reported that HIV undergo a previously unknown pre-entry priming size expansion event upon receptor engagement, challenging the dogma of viral entry, more specifically, we showed that cell-free viruses are able to perform biological process as seen with live organism. These virus expansion events are induced by engagement of the receptor-binding site of the viral envelope (Env), and involves the re-modelling of multiple virus compartments. We have identified a number of candidate cellular proteins that are involved in this process. By using siRNA knock down (or ectopic expression of these cellular factors), we will evaluate the contribution of these cellular proteins in this process. This project will explore the potential of using pre-entry primed HIV as a novel vaccine candidate, and we will manipulate the level of pre-entry priming in virus-like-particles to determine their impacts on exposing vulnerable epitopes on the virion surface. This project has both PC2 and PC3 components, and they complement each other. The PC3 component is reserved to PhD students and post-doctoral fellows, in which live infectious HIV will be used. The PC2 component requires the use of synthetic virus like particles, and that is suitable for Hon students.

Techniques: virology, molecular biology, tissue culture, cell biology, data-mining of digital information and utilisation of super computer.

92. Preventing the transmission of HIV by targeting Glycan Molecules

Dr Christopher Day, Prof Michael Jennings & Prof Johnson Mak

Glycan Biology, Virology, Biochemistry, Molecular Biology

HIV-associated glycans are best known for their capacity to shield viral envelope (Env) from immune attack. As glycans cover most of the HIV- and host cell-surface, glycan molecules are likely to be the point of first physical contact between HIV and host cell prior to infection. Professor Jennings and Dr Day have recently made a novel observation that glycan-glycan interactions are critical for bacteria to infect their hosts, but the importance of glycan-glycan interactions as a general principle for other pathogens remains undetermined. Using one of the most comprehensive glycan arrays worldwide, we (Jennings, Day and Mak) have identified that HIV specifically binds to specific sugar molecules via glycan molecules on the virion surface. Suppression of these interactions, via homologous glycan competimers, results in reduction of virus infection, virus entry and delay in the HIV infection processes. This finding represents the first example of glycan-glycan interactions being of functional importance in virology. This work opens up possibility that many pathogens could share glycan-glycan interactions as a general principle to gain entry to their hosts. Thus, in addition to the established role of HIV-associated glycans as a 'glycan shield', HIV appears to also utilise these glycan-glycan interactions as a 'molecular Velcro' to attach onto the target cell and to facilitate subsequent viral entry. It is currently unknown: (i) what features of glycan molecules are responsible for this phenomenon; and (ii) whether the source of this virion-associated glycan is derived from HIV envelope protein or cellular glycoconjugates on the virion surface. It is important to determine whether the reliance of glycan-glycan interactions is conserved amongst different subtypes of HIV to mediate infection *in vivo.* As suppression of these glycan-glycan interactions is via homologous glycan competimers, we will explore the potential of using glycans to suppress HIV replication *in vivo*. This project has both PC2 and PC3 components, and they complement each other. The PC3 component is reserved to PhD students and post-doctoral fellows, in which live infectious HIV will be used. The PC2 component requires the use of non-infectious materials, and that is suitable for Hon students.

Techniques: glycan biology, biochemistry, virology, molecular biology, tissue culture, cell biology

93. Determining the internal architecture and the interactions amongst HIV particles associated proteins and nucleic acids for the development of novel HIV inhibitors

Prof Yaoqi Zhou & Prof Johnson Mak

Structural Biology, Drug Design, Virology, Biochemistry, Molecular Biology

Assembly and maturation of HIV is a highly-regulated process, and viral protease has to cleave precursor protein Gag in a sequential manner. Non-sequential proteolytic processing events or excess Gag processing intermediates, will render progeny HIV non-infectious. This vulnerability has made virus assembly and maturation an attractive antiviral target, and it has resulted in the development of two classes of HIV inhibitors aiming at both enzyme (protease inhibitors, PIs) and substrate (Gag - maturation inhibitors, MIs). The internal architectural arrangement of virion RNA and proteins, plus the interaction interfaces amongst virion associated components are excellent target for antiviral drug design. Using an in silico based approach and available atomic structural details of HIV proteins, we will explore novel peptide structures as potential antiviral peptides. Short-listed peptide structures will be evaluated for their capacity to repress assembly of HIV protein in vitro, which will be followed by their ability to block HIV particle formation in virus producing cell. Lead anti-HIV peptide candidates will then be tested for their suppressive activity against HIV in primary cells, as well as their potential to stop the propagation of known drug resistant HIV. In parallel with the drug development study, we will also utilize super-resolution microscopy analyses to define the internal architectural arrangement of HIV RNA and protein. Super-resolution microscopy (2014 Noble Prize of Chemistry) is a type of light microscopy technique that overcome the resolution limits imposed by the diffraction limit of light in optical physics. Given viruses are generally smaller than the 200nm diffraction limit of visible light, super-resolution microscopy is ideal for dissecting the internal structural arrangement of HIV particles for the design of novel antivirals. This project has both PC2 and PC3 components, and they complement each other. The PC3 component is reserved to PhD students and post-doctoral fellows, in which live infectious HIV will be used. The PC2 component requires the use of non-infectious materials, and that is suitable for Hon students.

Techniques: structural biology, drug design, biochemistry, virology, molecular biology, tissue culture, cell biology

94. Evolution glycomics – Deciphering the carbohydrate language changes in vertebrate-pathogen co-evolution

Dr Kathirvel Alagesan & Assoc Prof Daniel Kolarich

Glycomics, glycoproteomics, infection, evolution, zoonotic disease

Cell surface and body fluid proteins are extensively modified with species specific sugars. These glycans build a universal language used by cells but also abused by pathogens. Though eukaryotic organisms share one alphabet, evolution made them speak multiple different languages and dialects. We have developed glycomics & glycoproteomics tools to translate these languages and uncover how pathogens learned to speak and interpret glyco-languages between different species. Understanding this relationship is crucial as viral, bacterial and parasite pathogens have developed elaborate strategies to jump between hosts – and many of these strategies involve cell surface glycans. The influenza virus is just one fairly well understood example that uses this strategy. As part of this project several student projects are available that will characterise the plasma/serum glycome across several species of vertebrae and investigate the cross-species recognition of pathogen adhesins. The outcomes of this ARC Future Fellowship supported project will provide novel clues how infectious diseases can spread and uncover novel targets to stop their distribution. This project presents the first comprehensive glycan dictionary and long sought Rosetta stone translating glyco-languages of different species in the Chordata phylum, uncover their similarities and differences, and will show how many languages different pathogens can speak.

Techniques: mass spectrometry, glycomics, proteomics, microarray

95. Understanding the impact of glycosylation on stem-cell-factor and stem-cell-factor receptor signalling in health and cancer pathogenesis

Assoc Prof Daniel Kolarich

Glycoproteomics, biochemistry, signalling

Stem cell factor (SCF) is a cytokine that mediates its diverse cellular responses by binding to and activating the receptor tyrosine kinase KIT (also known as SCF receptor or CD117). KIT is a member of the type III family of receptor tyrosine kinases, which also includes PDGF-receptor-α and β, CSF-1 receptor and the FLT3 receptor, to name a few. A number of KIT mutations have been identified in various types of cancer, indicating that KIT plays a vital role in cancer pathogenesis. In addition, resistance against the drug imatinib, a KIT-specific inhibitor used in the treatment of gastrointestinal stromal tumours, occurs frequently and thus novel approaches are required to inhibit KIT. SCF and KIT are both glycoproteins, but to date there is hardly any data available on the individual glycosylation and how these are impacting their interaction and function. As part of a larger project a variety of student projects are available that include aspects of mass spectrometry applications (proteomics, glycomics and glycoproteomics) next to cell culture, Western Blot, electrophoresis and other standard biochemistry techniques. In combination these techniques are being employed to characterise and modulate the glycosylation of these important signalling molecules to understand how protein-specific glycosylation impacts protein function and cell signalling and to identify novel approaches to selectively inhibit KIT and KIT signalling.

Techniques: mass spectrometry, glycomics, proteomics, cell culture

96. Cancer glycomics/glyoproteomics

Dr Jodie Abrahams, Dr Kathirvel Alagesan, Dr Arun Everest-Dass & Assoc Prof Daniel Kolarich

Glycomics, cancer, cancer-biomarkers

Cell surface and body fluid proteins as well as plasma membrane lipids are extensively modified with specific sugar moieties, so called glycans. These glycans build the basis for a universal language (glycome) used between cells but are also abused by pathogens and cancer cells. Despite the fact that many functional aspects of the glycome are still awaiting discovery and explanation, it is well established that even small changes in the cellular glycosylation can severely affect the function and behaviour of cells. We have developed highly sensitive and selective glycan/glycoprotein sequencing tools to study cell surface glycoconjugates and their role in pathological processes. As part of the Australian Centre for Cancer Glycomics (A2CG) we are now systematically applying our glycan-sequencing technologies to sequence cancer glycomes in a variety of cancers (prostate, gastric, colon, breast, head and neck, melanoma etc). A number of student projects are available supporting this important endeavour that will result in a new generation of diagnostic and prognostic cancer markers.

Techniques: mass spectrometry, glycomics, proteomics,

97. Determining the glycosylation status of melanoma immunotherapy targets

Dr Jodie Abrahams & Assoc Prof Daniel Kolarich

Glycoproteomics, cancer biology

Australia has the highest incidence of melanoma in the world, and as a result melanoma is often referred to as Australia's national cancer. Glycosylated cell surface proteins are involved in the progression of melanoma and are targets for therapeutic intervention. Recent advances in immunotherapy strategies for the treatment of advanced stage disease has dramatically improved the prognosis of patients. However, there are still a high proportion of patients who do not respond or develop drug resistance. This project will investigate protein specific glycosylation of cell surface molecules involved in current immunotherapy pathways for the treatment of metastatic melanoma. The outcomes of this project will provide valuable insights into the role of glycosylation within the tumour environment.

Techniques: mass spectrometry, glycomics, proteomics, cell culture