



Annual Report 2010

ARC Centre of Excellence in
Structural and Functional
Microbial Genomics

ARC Centre of Excellence in

Structural & Functional



Microbial Genomics

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Introduction

Overview

The ARC Centre of Excellence in Structural and Functional Microbial Genomics brings together a team of internationally-recognised researchers from Monash University with complementary expertise in microbiology, molecular biology, biochemistry, structural biology, and vaccinology. The Centre conducts integrated research that elucidates key aspects of microbial pathogens and the hosts they infect, focussing on diseases of importance to Australian primary industry. At the core of the Centre's applied research program is a genomics-based development process, which utilises high throughput, robotics-facilitated protein production and analysis to identify and characterise lead candidates for novel vaccines or drug targets. Major projects include the development of vaccines against leptospirosis, fowl cholera, ovine footrot and avian necrotic enteritis. In 2010, fundamental research within the Centre into microbial genomics, pathogenesis and immunity was published in high quality scientific journals such as Nature and PLoS Pathogens among others.

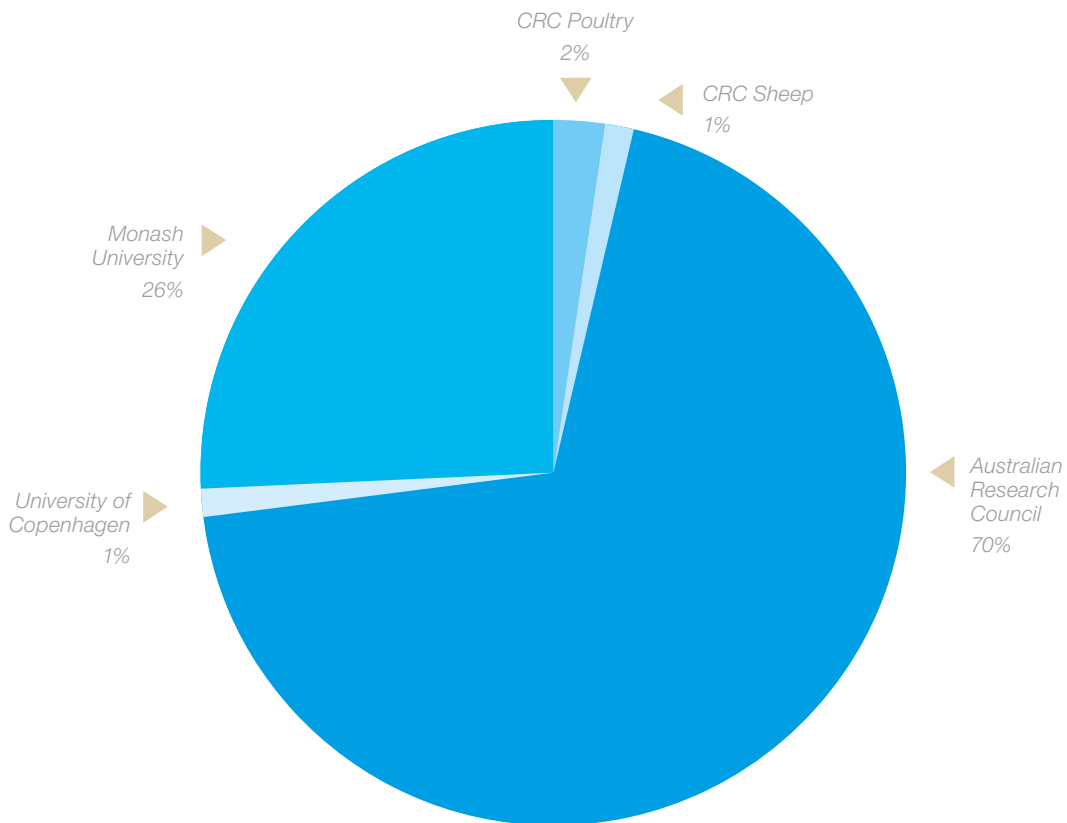
The Centre also works in partnership with scientists within Australia at The University of Sydney, The University of Queensland, CSIRO Livestock Industries, and the Victorian Bioinformatics Consortium, as well as with numerous collaborators in Europe, Asia and North America, as detailed in this report.



Image courtesy of Dr Terry Kwok

Financial support

The Centre's main sources of funding are the ARC Centre grant through the Centre of Excellence program and Monash University. The ARC provides approximately \$2 million per annum, with Monash University contributing \$699,000 per annum. In-kind contributions from Monash University, Centre partners, associates and collaborators amounted to approximately \$9 million in 2010. Additionally, the Centre obtained funding from collaborating partners such as the University of Copenhagen, The Australian Poultry CRC and The Australian Sheep CRC supported a student scholarship.



Income sources for 2010

Director's Foreword

It is a pleasure to introduce the Centre's annual report for 2010, a year which saw further enhancement of the Centre's international profile. In this regard, the key activity for the year was undoubtedly the Centre's organisation and hosting of the *First Prato Conference on the Pathogenesis of Bacterial Diseases of Animals*. Held at the superb Monash Prato Centre in Italy, the conference brought together for the first time scientists whose work focused on understanding how bacterial pathogens cause disease in production animals. With more than 130 participants from 31 countries, the conference was hailed enthusiastically as a resounding success. The generous support of major veterinary vaccine companies is hereby gratefully acknowledged. The scientific publishing company Elsevier will publish selected presentations from the conference, which have been subjected to full peer review, in a special issue of *Veterinary Microbiology*. Details of the conference appear elsewhere in this report. The success of the meeting was such that the second conference will be held at Prato in October 2012.

Planning for additional international activities over the coming two years is well advanced. In May, the Centre in conjunction with Queensland Health Scientific Services will hold a workshop on leptospiral diagnostics at the National Institute of Animal Health, Bangkok, Thailand.

The activity is funded by the Australian Government Department of Agriculture, Fisheries and Forestry together with the National Institute of Animal Health. In 2012 in conjunction with the European Molecular Biology Organisation (EMBO), the Centre will conduct an advanced summer school on pathogenesis of infectious diseases held on Spetses, Greece. The Centre will also organise and host the International Veterinary Vaccines Conference in Australia in 2012.

The year 2010 saw the retirement from the Centre of two foundation CIs, Phillip Nagley and John Davies. I would like to take this opportunity to publicly thank them and acknowledge their major contributions towards the Centre since its inception. Phillip served as Deputy Director from 2006 to 2009. We were delighted to welcome two new CIs to the Centre in mid-2010. Els Meeusen brings substantial expertise in veterinary immunology to the Centre, in particular as applied to the development of vaccines against helminth infections of production animals. Paul Hertzog is recognized internationally for his excellent work in the area of innate immunity. We look forward to working with them both.

Once again I would like to express my thanks to the Centre CIs, research staff and students, Centre associates, and administrative staff for their commitment during the year. As detailed in this report, the work within the Centre continues to be of the highest international standard. I am also grateful to the members of the Scientific Advisory Board who give so unselfishly of their time and extensive experience to assist in the continued success of the Centre. My special thanks and appreciation are extended to Graham Mitchell who stepped down from the Board in 2010 due to time commitments. A replacement for Graham is planned in 2011.



A handwritten signature in black ink, which appears to be 'B. Adler'.

Professor Ben Adler

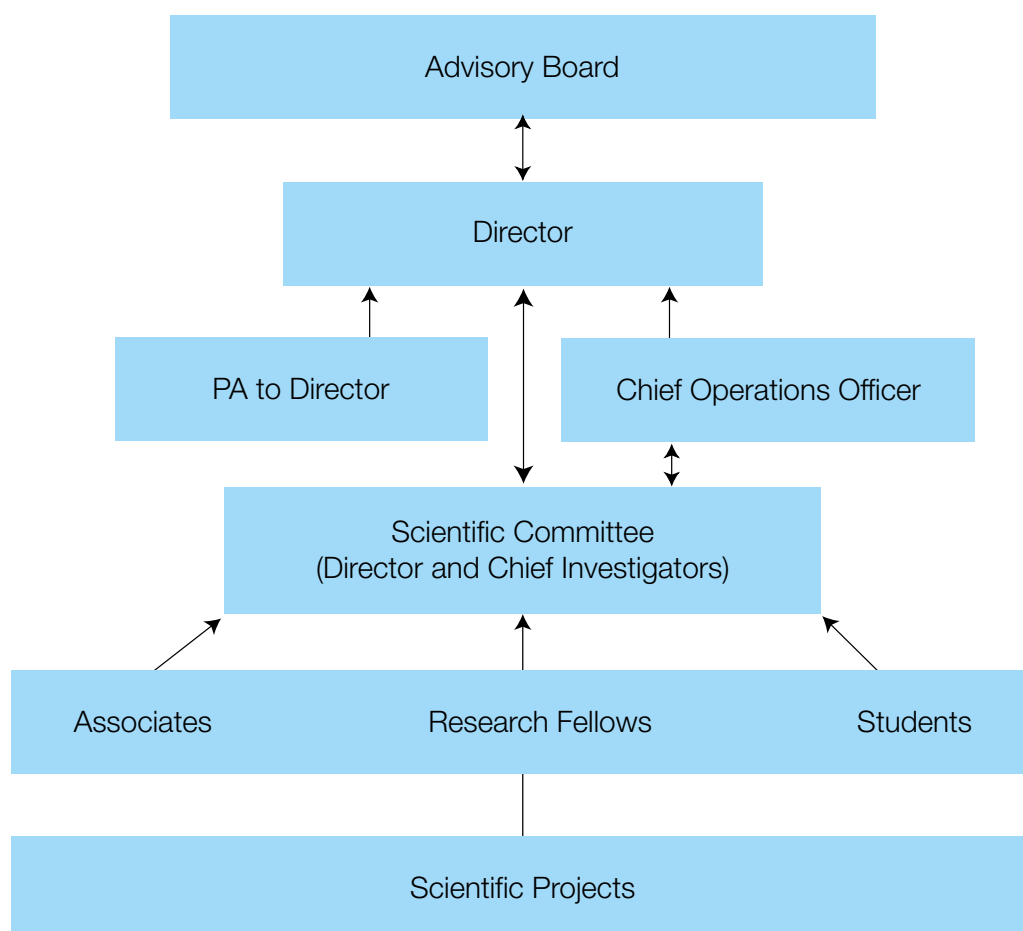
Director
ARC Centre of Excellence in Structural and
Functional Microbial Genomics

Organisation and Governance

Centre Governance and Management Structure

The Centre was formed in 2006 as a partnership of the participating institutions under a formal Centre agreement, with Monash University as the administering institution.

Organisational chart



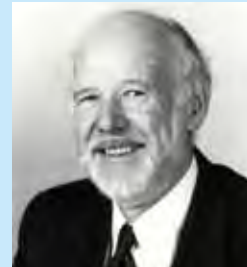
Scientific Advisory Board

The Scientific Advisory Board provides advice on research programs of the Centre, commercialisation opportunities and matters of strategic direction in research and other areas as may be relevant.

The Board meets twice a year or more frequently if necessary.

The Centre's Board members are:

Professor Jim Pittard AM FAA (Chair) is a microbial geneticist with a major interest in the regulation of gene expression, transport of small molecules across membranes and the molecular genetics of plasmids and their role in evolution. He is currently an Emeritus Professor at the University of Melbourne where for a number of years he alternated with Professor David White as Head of the Department of Microbiology (now Microbiology and Immunology).



Professor John Egerton is an Emeritus Professor of Animal Health at the University of Sydney, New South Wales. His research interests include lameness in sheep goats and cattle, treatment and vaccination against footrot in sheep, role of conventional and rDNA vaccines in eradication of footrot, and heritability of resistance to footrot in Merinos. His other research interests include anthrax in pigs, and necrotic enteritis (*Clostridium perfringens* Type C infection) in New Guinea highlanders.



Professor Graham Mitchell AO is a principal of Foursight Associates. He is veterinary graduate and University gold medallist of the University of Sydney and is recognised as one of Australia's leading biological scientists. His expertise extends over a wide range of science and technology and he has particular knowledge of the academia-industry interface. He is also an advisor on innovation to the Victorian Government. In 1993 he was appointed an Officer of the Order of Australia for services to science. (Jan – Mar)



Dr Nick Samaras is CEO of MuriGen Therapeutics. Nick has over 20 years' experience in the global life sciences industry and holds a PhD from the Walter and Eliza Hall Institute of Medical Research, University of Melbourne. He is also Chairman of Replikun Biotech Ltd., Teeleostin Ltd and Q-Gen Pty Ltd. and a member of the NHMRC Research Committee.





Dr Emanuela Handman is a parasitologist with an interest in intracellular pathogens and their interaction with the host at the molecular, cellular and organismal levels. Over a career spanning 30 years as Head of the *Leishmania* Laboratory at the Walter and Eliza Hall Institute of Medical Research she has focused on two main themes. On the parasite side, the elucidation of the structure, function and biochemistry of surface molecules involved in invasion of host cells and establishment of intracellular infection. On the host side, understanding the genetics of host responses to infection and their role in susceptibility or resistance to disease. More recently, using *Leishmania* functional genomics her group has made significant progress in the identification of novel targets for anti parasite drugs. She is a Fellow of the Australian Society for Parasitology and an Honorary Associate Professor in the Department of Microbiology at Monash University.

Professor Ben Adler, Centre Research Director (*ex officio*)

International Adjunct Board Members



Professor Joachim Frey is the Director of the Institute of Veterinary Bacteriology at the University of Bern, Switzerland since 2000. His research interests are the molecular mechanisms of pathogenic *Mycoplasma* species. He is Chairman of the Board of the International Organization for Mycoplasma (IOM) and is member of the international committee on systematics of prokaryotes, subcommittee on the taxonomy of Mollicutes.



Professor John F. Prescott is based in the Department of Pathobiology, Ontario Veterinary College, University of Guelph, Ontario, Canada. He is best known for work in the area of *Rhodococcus equi* pneumonia in foals, an area on which he has organized four international Workshops. He has been Co-Editor-in-Chief of *Veterinary Microbiology* (Elsevier Science), a member of the Canadian Veterinary Medical Association Council, and a Director of the Canadian Committee on Antibiotic Resistance. His current active research interests are in immunity and virulence in clostridial infections in animals.

Centre management and administration

Director

Professor Ben Adler, as Centre Research Director, is responsible for decisions affecting Centre scientific, financial, human and infrastructure resources. In addition, his role oversees the overall direction of the Centre Scientific projects. He also manages a joint research group co-headed by Centre Associate Dr John Boyce, numbering more than 24 staff and students in 2010.



Chief Operations Officer

Desmond Gul provides support to the Centre Director and Scientific Committee, by management of a wide range of Centre operational activities, particularly those associated with Centre funding bodies' reporting requirements, marketing and communications initiatives, and the identification of Centre business development opportunities.



Personal Assistant

Sherrie Barker provides administrative and secretarial support to the Centre Director. She is the minutes secretary for the Scientific Committee and Advisory Board meetings. Sherrie is also PA to Professor John Davies (Head of Department, Microbiology), and assists with postgraduate and undergraduate student administration matters for Microbiology department students.



Scientific committee

The Scientific Committee, which comprises the Centre Director and Chief Investigators, is responsible for the overall scientific direction of the Centre's fundamental and applied research programs. The Scientific Committee meets monthly, with meetings open to all Centre staff, students and associates.

The Centre's Scientific Committee members are:



Centre Director

Professor Ben Adler is a Professor of the Department of Microbiology at Monash University. In addition to his managerial role as Centre Director he is recognised internationally for his work on bacterial pathogens, especially *Leptospira* and other spirochaetes, *Pasteurella* and *Shigella*. His area of scientific expertise is in the application of genomics to elucidate molecular mechanisms of bacterial pathogenesis and in immunity to bacterial infection and vaccine development. He is, since 1986, a member of the Subcommittee on the Taxonomy of *Leptospira* of the International Union of Microbiological Societies and an executive member of the International Leptospirosis Society. He is also a member of the Executive of the Victorian Infection and Immunity Network.



Deputy Director

Professor Rod Devenish is Deputy Director of the Research Graduate School and Professor in the Department of Biochemistry and Molecular Biology at Monash University. He has an international reputation in yeast (*Saccharomyces cerevisiae*) molecular cell biology with research that has focused principally on aspects of mitochondrial biogenesis, in particular the structure and function of ATP synthase. More recently he has developed a new research interest in the area of autophagy. In collaboration with Centre colleagues he is investigating the interaction of bacterial pathogens with mammalian host cell autophagy. Other aspects of his work on autophagy concern the turnover of organelles, particularly mitochondria and the nucleus, in yeast.



Professor Ross Coppel is the Deputy Dean and Director of Research of the Faculty of Medicine, Nursing and Health Sciences at Monash University and a former Howard Hughes Medical Institute Infectious Diseases Fellow. He is an internationally recognised authority in molecular biology and genetic engineering as applied to infectious diseases and primary biliary cirrhosis. His work in bioinformatics led to the establishment of the Victorian Bioinformatics Consortium (VBC). Professor Coppel is also the VBC Director.



Professor John Davies is Head of the Department of Microbiology and Deputy Head of the School of Biomedical Sciences at Monash University. He is internationally recognised for his work on a variety of bacterial pathogens, especially *Neisseria* species. He has extensive experience in bacterial genomics and regulation of gene expression. Professor Davies retired as Chief Investigator in June 2010, but remains associated with the Centre of Excellence as a Research Associate.

Professor Paul Hertzog is the Director of the Centre for Innate Immunity and Infectious Disease at the Monash Institute of Medical Research (MIMR), Clayton, Deputy Director of the MIMR, and a Senior Principal Research Fellow of the National Health and Medical Research Council of Australia. Professor Hertzog is the Co-convenor of the Victorian Infection and Immunity Network. His research interests cover an integrated approach to understanding the molecular mechanisms of the innate immune response to infections. His group specialised in gene targeted mouse models of disease integrated with cellular, genomics and bioinformatics approaches to identify new immunoregulatory approaches.



Professor Els Meeusen heads the Biotechnology Research Laboratories (BRL) within the School of Biomedical Sciences at Monash University. The BRL has active research programs in parasite biology and vaccine development, innate immunity and vaccine adjuvants, allergy and asthma. The lab specialises in applied large animal immunology and aims to translate basic research findings into practical applications for animal and human health.



Professor Phillip Nagley is a Professor of the Department of Biochemistry and Molecular Biology at Monash University. His research field is biochemistry and molecular biology, and his interests extend to cell biology, genetics, infectious disease and neuroscience. The broad goal of his research is to understand the response of cells to stresses that may lead to death. The focus of his research in the Centre deals with the basic mechanisms of mammalian cell death and the responses in host cells after infection with disease-causing bacteria. Professor Nagley retired as Chief Investigator in June 2010, but remains associated with the Centre of Excellence as a Research Associate.



Professor Julian Rood has an international reputation for his extensive research on the genetics, regulation and pathogenesis of anaerobic bacteria, especially *Clostridium* and *Dichelobacter* species. His current Centre research, in the Department of Microbiology at Monash University is focused on the pathogenesis of ovine footrot and necrotic enteritis and understanding how bacteria transfer their virulence and antibiotic resistance genes.



Professor Jamie Rossjohn is an ARC Federation Fellow and a Professor of the Department of Biochemistry and Molecular Biology at Monash University. His group investigates the structural basis for defined events central to infection and cellular immunity. Specifically he has provided an understanding of receptor-recognition events at the immunological synapse as well as an understanding of processes central to bacterial physiology and host-pathogen interactions.





Professor Ian Smith is a protein biochemist and Professorial Fellow of the Department of Biochemistry and Molecular Biology at Monash University. He is also the Pro Vice-Chancellor (Research and Research Infrastructure) and Director of the Monash University's Biomedical Proteomics Facility. Professor Smith brings to the Centre internationally recognised expertise in protein purification and high throughput, high sensitivity proteomic analysis of complex protein mixtures.



Professor James Whisstock is a biochemist in the Department of Biochemistry and Molecular Biology at Monash University. He has particular expertise in structural biology and bioinformatics. His research focus includes proteases and their inhibitors as well as bacterial virulence factors. He was the recipient of the 2006 Science Minister's prize for Life Scientist of the year and the 2008 Commonwealth Health Minister's Award for Excellence in Health and Medical Research. In 2008 he was awarded an ARC Federation Fellowship and in 2010 the Australian Academy of Science Gottschalk Medal.

Centre associates

Dr Travis Beddoe is a National Health and Medical Research Council (NHMRC) Career Development fellow in the Department of Biochemistry and Molecular Biology at Monash University. He works on various proteins from a number of bacterial pathogens using X-ray crystallography and biophysical methods to determine their function.



Professor Stephen Bottomley is a National Health and Medical Research Council Senior Research Fellow in the Department of Biochemistry and Molecular Biology at Monash University. He is internationally recognised for his work on understanding protein misfolding and its links with disease. In collaboration with the ARC Centre he has established the Protein Production Unit (PPU), which is an automated protein production facility that enables researchers to express and purify their proteins in a high throughput manner.



Dr John Boyce has extensive experience in the identification and characterisation of virulence factors of bacterial pathogens, especially *Pasteurella multocida*. His work has focused on using whole-genome approaches such as DNA microarrays, signature-tagged mutagenesis, *in vivo* expression technology and proteomics to identify factors critical for the bacteria during the infectious process. This work has identified capsule and LPS as critical *P. multocida* virulence factors. He has recently used new generation sequencing technologies for the analysis of bacterial genomes and for the identification of the regulator of capsule expression. Dr Boyce also has extensive experience in targeted mutagenesis procedures in *P. multocida*. As a former senior Centre Research Fellow he is project manager for the Centre vaccine pipeline projects.



Associate Professor Brian Cooke is a National Health and Medical Research Council Senior Research Fellow in the Department of Microbiology at Monash University. Over the last 20 years, Brian's work has focussed on understanding the cellular and molecular basis by which parasites of red blood cells (particularly malaria and *Babesia*) cause disease and death in humans and animals. His research group is internationally recognized as playing a vital role in a worldwide consortium toward the functional analysis of novel genes identified in the recently sequenced genomes of malaria and *Babesia* parasites. Brian is an elected member of the International Advisory Editorial Boards for *Trends in Parasitology* and *Blood*.



Dr Stuart Cordwell is a graduate of the University of Sydney and was awarded his PhD in 1997. He was an author on the original manuscript that defined the term 'proteome' in 1995 and has been involved in proteomics research throughout his career. He was Senior Research Fellow at the Australian Proteome Analysis Facility from 1999–2003, and Director of Research and Development from 2003–2004. He returned to the University of Sydney in 2004 as Sesqui Senior Lecturer in Proteomics in the School of Molecular and Microbial Biosciences and the Department of Pathology. He is a member of the Bosch Institute and a Director of the University of Sydney Proteome Research Unit. He was awarded the Selby Research Award in 2006 and is a member of the Editorial Boards for the field-leading journals *Proteomics* and *Proteomics (Clinical Applications)*. His biological research interests lie in two major areas – bacterial pathogens and myocardial ischemia/reperfusion injury.





Dr Dena Lyras is a Senior Lecturer in the Department of Microbiology at Monash University and has played a major role in the development of methods of genetic analysis in *Clostridium* species. Her current research is focussed on the pathogenesis of infections caused by the emerging pathogens *Clostridium difficile* and *Clostridium sordellii*, particularly with respect to the role of toxins, adhesion factors and spores in disease. Her work has also investigated mobile DNA and antibiotic resistance mechanisms employed by *Clostridium* species, especially involving the analysis of mobilisable and conjugative transposons.



Professor Bruce McClane is a Professor in the Microbiology and Molecular Genetics Department of the University of Pittsburgh, Pennsylvania, USA. He is internationally known for his work on *Clostridium perfringens*, particularly the toxins and toxin-encoding plasmids of this bacterium.



Dr Ashley Mansell is a NHMRC R. D. Wright Fellow at the Monash Institute of Medical Research (MIMR), Monash Medical Centre. He is internationally recognized for his work in Toll-like receptor (TLR) signal transduction and negative regulation of these pathways. He initiated the formation and heads the Australian TLR research network and chairs the Australasian Society of Immunology Infection and Immunity special interest group.



Dr Rob Moore is the leader of the Gene Technologies group at the CSIRO Livestock Industries' Australian Animal Health Laboratories in Geelong, Victoria. He works on a number of bacterial pathogens including *Clostridium perfringens*, *Campylobacter jejuni*, and *Corynebacterium pseudotuberculosis*, and also studies the host response to pathogens. He has co-supervised several PhD students with Centre CIs.

Dr Ian Wilkie is a senior lecturer and pathologist at the School of Veterinary Science at The University of Queensland. His current area of research is in the pathogenesis of diseases caused by *Pasteurella*, especially avian cholera and haemorrhagic septicaemia. He has been collaborating with the Bacterial Pathogenesis group at Monash University for several years, and is currently involved in a Centre project to define virulence attributes of *P. multocida* type A for mice and chickens.



Professor Richard Whittington is a veterinary pathobiologist at the University of Sydney, Camden, New South Wales. He leads research on *Mycobacterium avium* subspecies Paratuberculosis, the causative agent of Johne's disease in ruminants, ovine footrot and infectious diseases of fish and wildlife. His major studies involve functional analysis and molecular studies of viruses and bacteria, immune responses, pathology and epidemiology in individual animals and animal populations.



Centre personnel and collaborators

Research fellows

Dr Trudi Bannam
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Dr Lan Gong
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University of Melbourne

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Dr Ruby Law
Monash University

Dr Sheena McGowan
Monash University

Dr Adrienne Paton
University of Adelaide

Dr Mathieu Picardeau
Institut Pasteur, France

Dr Corrine Porter
Monash University

Dr Torsten Seemann
VBC

Dr Michael de Veer
Monash University

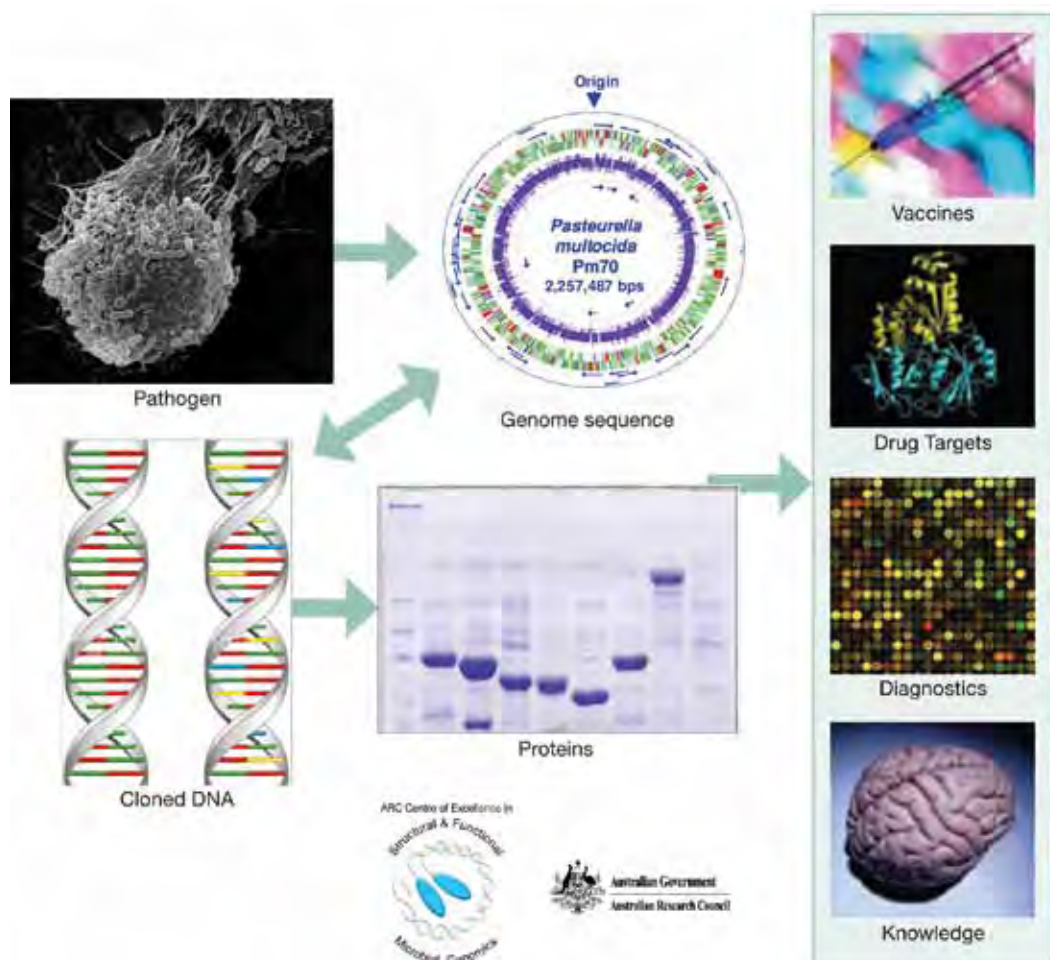
Dr Amanda Walmsley
Monash University

Mission

To conduct integrated research that will elucidate key aspects of microbial pathogens and the hosts they infect. The research will encompass genomic analyses, development of modern veterinary vaccines, identification of antimicrobial targets and development of antimicrobial agents.

Objectives

- To develop vaccines against microbial pathogens of importance to Australian primary Industry;
- To identify and validate genes essential for microbial survival and thus facilitate the development of novel antimicrobial agents;
- To characterise key host-pathogen interactions at the molecular and cellular levels in order to elucidate or control the processes whereby microbes evolve and cause disease; and
- To train a new generation of multi-skilled researchers based on the Centre's broad range of advanced technologies.



Infrastructure

The Centre host institution, Monash University provides access to research infrastructure and laboratory and office space through the School of Biomedical Sciences, Faculty of Medicine, Nursing and Health Sciences. Monash University also provides the Centre with priority access to state-of-the-art instrumentation, particularly the following facilities, housed in the School of Biomedical Sciences.

Biomedical Proteomics Facility

The Biomedical Proteomics Facility contains state of the art proteomics equipment, namely, nano-HPLC, MALDI target plate spotter, MALDI ToF ToF, ESI Q-Trap and ESI Q-ToF mass spectrometry, N-terminal sequencing and 1 and 2D gel analysis. In addition the facility ion trap mass spectrometer is equipped with ETD (electron transfer dissociation) capabilities. ETD is a method of peptide and protein sequencing, which allows mapping of the precise sites where proteins are phosphorylated.

We have also recently acquired a triple quad mass spectrometer which will allow the accurate quantitation of proteins. The facility thus has the capabilities to meet all current proteomic requirements of the Centre researchers as well as Monash researchers and also providing the state of Victoria and beyond with the qualified personnel necessary to support its growing biotechnology industry, especially in the area of proteomics.

Services provided by BPF include:

- Protein characterisation and identification;
- De novo and confirmatory protein sequencing;
- Post-translational modifications;
- Protein quantitation;
- Membrane protein identification (membrane shaving).

Under the successful awarding of the National Collaborative Infrastructure Scheme (NCRIS) funding in 2008, the BPF is now a member of Proteomics Australia, acting as the Victorian Node to offer a broad range of proteomic services to our collaborators and the wider scientific community at highly competitive service fees. This allows for more efficient and cost effective research and collaborations.

The ARC Centre supports Dr David Steer (facility manager) under the directorship of Professor Ian Smith. David, with help from other staff in the facility, provides proteomics support for Centre researchers.

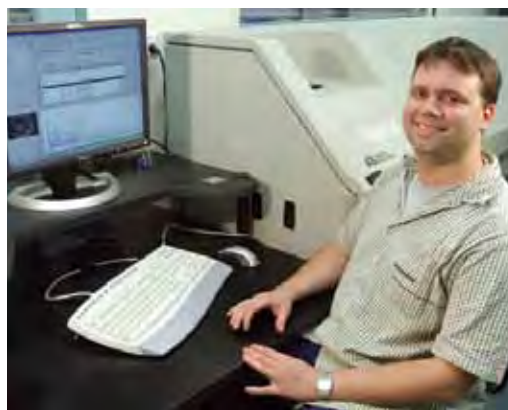
For more information see www.med.monash.edu.au/biochem/facilities/proteomics and/or contact Dr David Steer

Tel: +61 3 9902 9323 or

Email: david.steer@med.monash.edu



MALDI mass spectrometer



Dr David Steer, BPF facility manager

Protein Production Unit

The Protein Production Unit which operates as a Monash University practice was established in 2005. With support from the Centre, it has developed into a dedicated service platform to commercial and academic research facilities to alleviate the often time consuming task of protein production. In our custom built facility the Protein Production Unit offers a range of services that can enable rapid development of proteins required for various research projects. With the aid of a Tecan Freedom EVO liquid handling robot and ÄKTExpress™, the unit is able to offer an array of services including protein expression, screening of expression conditions, small scale and large scale protein purification, quality assurance and collaborative research. ÄKTExpress™ is a dedicated high throughput chromatography system for a multi-dimensional purification of His- and GST-tagged proteins. Specialized protocols can be adapted for those products that are sensitive to degradation. The unit has developed a number of different purification strategies that have been implemented with the high through-put pipeline of various Centre projects and is a fundamental part of the Centre's vaccine development and drug target identification processes.



PPU staff member with the ÄKTExpress™ equipment

In addition, one of the other major functions of the unit has been to use of the liquid handling robots to explore the expression space for a variety of different recombinant constructs. The ability to identify the conditions that show an improvement in the level of expression and quality of the recombinant proteins produced can be extremely beneficial for many pipeline projects. Using a range of equipment, protocols are currently being developed to analyse the effect of additives on the stability on numerous proteins in a high throughput manner. This will allow proteins to be concentrated or stored in various buffers that either increases the solubility or stability of the resulting proteins. These protocols are currently being adapted for inhibitor study trials with some promising early results.

A number of high throughput refolding screens have been developed within this unit to maximize the amount and quality of the protein being produced by refolding protocols.

To December 2010, over 450 proteins have been purified using the unit's novel purification strategies (Table 1).

This facility is presently operated by two Centre staff, Dr Noelene Quinsey and Mr Nik Sotirellis, under the direction of Centre Associate, Professor Steve Bottomley.

For more information see <http://proteinproductionunit.med.monash.edu.au> and/or contact Dr Noelene Quinsey
Tel: +61 3 9902 0020 or
Email: noelene.quinsey@med.monash.edu

Project	Number of proteins purified
Fowl cholera vaccine	42
Footrot vaccine	60
Leptospirosis vaccine	350
Necrotic enteritis vaccine	45
Crystallisation projects	185
Other projects	84

Purification of proteins by the Protein Production Unit

Protein Crystallography Unit

The Protein Crystallography Unit was established at Monash University in 2002 by Prof Jamie Rossjohn. Protein crystallography is the major tool for solving the 3-D structure of proteins and as such provides detailed information on the structure and function of proteins, as well as a platform for rationally designing therapeutics. The X-ray crystallography laboratories include two Rigaku MicroMax-007 HF microfocus rotating anode generators and R-AXIS IV++ detectors. For more intense and wavelength-tuneable X-ray sources, there is ready access to the Australian Synchrotron. Further, the Protein Crystallography Unit, which now accommodates 10 independent laboratories, houses Australia's largest fully-automated crystallization facility within purpose built laboratories. The fully automated crystallization system ("CrystalMation") that includes liquid handling robots for screen preparation (Alchemist II) and crystal screen setup (Phoenix HT), imaging facility and storage systems (Minstrel HT and Gallery 700), are all inter-connected.

For more information contact Prof Matthew Wilce, Unit Head.

Tel: +61 3 9902 9244 or

Email: matthew.wilce@med.monash.edu



Fully automated crystallization system "CrystalMation"

Micromon

The Centre also has priority access to the Monash University Micromon DNA Sequencing Facility located in the Science and Technology Research Infrastructure Precinct (STRIP).

Micromon is the commercial services unit of the Department of Microbiology and was established in the mid 1980s as the Microbial Biotechnology and Diagnostic Unit to utilise the department's academic and technical expertise in a commercial service venture. The unit now specialises in high quality DNA technologies where long-read DNA sequencing is the core facility of the unit. Sequencing is supported by a small-scale oligonucleotide synthesis service, a microarray facility specialising in microbial arrays and quantitative PCR instrumentation.

High quality, capillary-based DNA sequencing technology is carried out using an applied Biosystems 3730S capillary sequencer that incorporates a 50 cm array capable of routinely generating read lengths in excess of 1000 bases. The unit in 2008 established a Genomic Sequencing facility using an Illumina Genomic DNA Analysis platform and also offers Bioinformatics support as a commercial service.

Micromon employs seven staff and is managed by Mr Mark Cauchi.

For more information, see www.micromon.monash.org/ and/or contact Mr Mark Cauchi.

Tel: +61 3 9905 4830 or

Email: mark.cauchi@med.monash.edu



Staff of Micromon



The High-throughput Microbial Pipeline

Leveraged as an entire system, the previously described infrastructure forms the Centre's High-throughput Microbial Pipeline.

The High-throughput Microbial Pipeline allows the Centre to adopt the reverse vaccinology or genomic approach to vaccine development and a rational drug design approach to antimicrobial drug target identification.

Independently each component can add value to specific projects as stand alone capability.

The Centre's High-throughput Microbial Pipeline



Key Features – Specialised Capability

<ul style="list-style-type: none"> Propriety sequence information ID of novel genes and gene products 	<ul style="list-style-type: none"> Specialised infrastructure Access to public and proprietary databases 	<ul style="list-style-type: none"> High throughput and output A variety of expression systems Rapid 	<ul style="list-style-type: none"> Synchrotron X-ray crystallography Mass Spectrometry 	<ul style="list-style-type: none"> Vaccines Drug targets Diagnostics Knowledge
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Research Project Achievements

Significant achievements and outcomes in 2010 included:

- Elucidation of the structure and genetic basis of LPS assembly in all 16 known serovars of *Pasteurella multocida*, the cause of fowl cholera.
- Characterisation of the mode of action of the Fis protein, a key regulator of virulence genes in *P. multocida*.
- Expression and purification of one of the largest mycobacterial proteins, the polyketide synthase Pks13 which is responsible for the condensation reaction of mycolic acid formation in *Mycobacterium spp.*
- Characterisation of the mycobacterial protein Rv0228 as an acyltransferase responsible for synthesising a previously undescribed mycolic acid intermediate, acyl trehalose monomycolate.
- Determining that the N-terminus of Mul1 projects into mitochondria, with the bulk of the protein lying within mitochondrial membranes.
- Determining that LC3-associated phagocytosis (LAP) is the primary response of mammalian cells to infection by *Burkholderia pseudomallei*.
- Determining that the loss of protein BPSS1394 can dramatically influence the infectivity and pathogenicity of *B. pseudomallei*.
- Development of new methods to analyse complex datasets measuring innate immune responses to infection.
- Completing our first Next Generation Sequencing experiment on the innate immune response.
- Determination of the structure of the key host defence protein perforin and the mechanism of pore formation.
- Determination of the structures of two important malarial virulence factors in complex with novel compounds.
- Refinement and improvement of current Standard Operating Procedures (SOPs) for protein quantitation using isobaric tagging methods as well as label free quantitation techniques
- Characterisation of post translational modifications and application of established literature methods for the enrichment of phosphorylated proteins.
- Determining basis of recognition of the T-cell receptor by natural killer T cells.
- Elucidating the structure of *Legionella* NTPDase which is structurally and catalytically related to eukaryotic NTPDases.
- Elucidating the structure of mycobacterial cell wall synthesising enzyme aldo-keto reductase.
- Understanding mechanisms of lipid-Ag presentation of the CD1 family.
- Determination of the crystal structure of clostridial NetB toxin.
- Demonstration that NetB toxin is encoded on a conjugative plasmid in a strain that carries three closely related conjugative plasmids
- Demonstration that TcpG is a functional peptidoglycan hydrolase that is required for efficient conjugative plasmid transfer in *Clostridium perfringens*.
- Discovery that the protease AprV5 is involved in the processing of three proteases from *Dichelobacter nodosus*.
- Identification of a potential vaccine for the control of ovine footrot.
- Demonstration of significant protection using a larval-specific antigen against *Haemonchus contortus* in sheep immunized on pasture.

Centre Research Projects

Identification and characterisation of antimicrobial drug targets

The identification, characterisation and structure determination of drug targets in *Mycobacterium* spp.

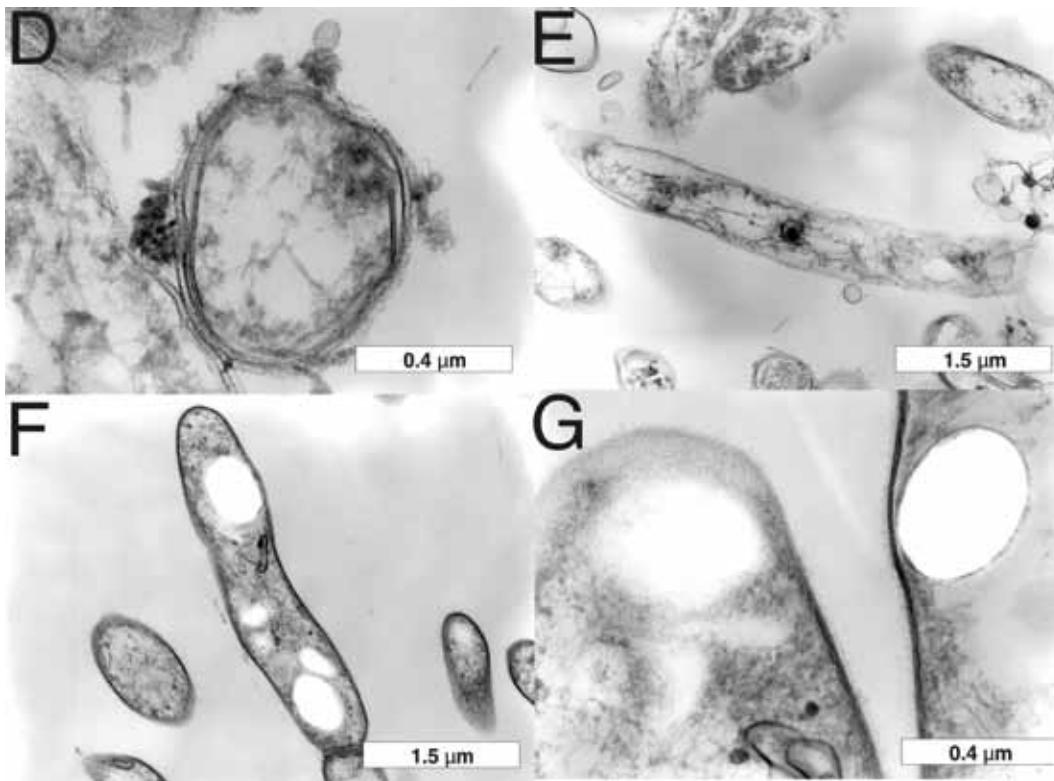
(Prof Ross Coppel, Prof Jamie Rossjohn, Dr Paul Crellin, Dr Travis Beddoe, Ms Rajini Brammananth, Mr Arek Rainczuk)

Mycobacterium spp. are the causative agents of serious diseases in animals and humans. To address the problem of resistance to existing antimicrobial agents, we are identifying and characterising essential mycobacterial enzymes involved in cell wall synthesis as potential targets for the design of specific antimicrobial drugs. These studies have two basic components: a) functional characterisation of essential mycobacterial enzymes using the closely related organism *Corynebacterium glutamicum* as a model species, and b) structural characterisation of essential mycobacterial enzymes.

In 2010, we have characterised *C. glutamicum* mutants with deletions in four genes: *Rv0224* (methyltransferase), *Rv0225* (glycosyltransferase), *Rv0228* (membrane acyltransferase) and *lpqW* (partially characterised lipoprotein essential for cell wall lipoarabinomannan (LAM) biosynthesis). The first three genes are of particular interest because they are essential for the growth of mycobacteria and are adjacent to a major cluster of cell wall biosynthesis genes in *C. glutamicum*, while the characterisation of *LpqW* is an ongoing Centre project. Surprisingly, the *Rv0224/5/8* mutants were found to have a common phenotype, despite the genes encoding completely different enzyme activities. All mutants have a slow rate of conversion of cell wall trehalose monomycolate (TMM) to trehalose dimycolate (TDM), due to lack of production of a newly identified mycolic acid intermediate which we have shown to be an acetylated TMM species (AcTMM). We have shown that *Rv0228* produces AcTMM from TMM, which is consistent with its proposed role as a membrane acyltransferase. We hypothesise that acetylation of TMM promotes membrane translocation to

the periplasm where it is deacylated and converted to TDM. The roles of *Rv0224* and *Rv0225* in the production of AcTMM are still to be determined and we are exploring the possibility of interactions between the three enzymes. The *lpqW* mutant has been extensively characterised, with studies showing that all periplasmic LAM intermediates are missing, but early cytoplasmic intermediates are unaffected. This striking cell wall phenotype strongly suggests a role for *LpqW* in the translocation of LAM intermediates across the cytoplasmic membrane or in “priming” reactions on the periplasmic side. For comparison, we have produced a new *ppm* mutant which lacks the mannose donor for periplasmic LAM biosynthesis; this strain has a phenotype indistinguishable from that of the *lpqW* knockout, further supporting the functions proposed for *LpqW*.

Structural work has focussed on several high profile targets. We have targeted the late steps of the mycolic acid biosynthetic pathway by cloning and expressing Pks13, a polyketide synthase responsible for the condensation reaction of mycolic acid formation, FadD32, a priming enzyme thought to interact with Pks13, and *CmrA*, the reductase responsible for the final step of mycolic acid biosynthesis and identified in previous projects within the Centre. All of these enzymes represent excellent drug targets and a 3D structure of Pks13, at 180kDa, would be the largest mycobacterial structure ever solved. We have also cloned and expressed *Emb* and *DprE1*, a high-profile drug target described in a *Science* paper in 2009. In a separate study, we have shown that *DrpE1* is essential in *M. smegmatis*. While insolubility is a common problem for mycobacterial proteins expressed in *E. coli*, we have developed a new expression system based on *M. smegmatis* which gives soluble expression of all these targets, even Pks13. Purification and concentration of these targets has proved to be challenging, but large scale crystal screens have been set up for Pks13.



Transmission electron microscopy of a *Mycobacterium smegmatis* (MSMEG_6394) conditional knock-out showing loss of cell wall integrity (D,E) or intact cells with large electron transparent zones (F, G).

Pathogenesis of bacterial infection

Mechanisms of pathogenesis in fowl cholera.

(Prof Ben Adler, Dr John Boyce, Dr Marina Harper, Dr Ian Wilkie, Dr Andrew Cox, Dr Xenia Gatsos, Ms Marietta John)

Characterisation of genes involved in the biosynthesis of LPS, an important immunogen and virulence factor of *P. multocida*.

We have elucidated the LPS structure and sequenced the LPS assembly loci in the *P. multocida* strains representing all 16 known serovars. Using this information we are determining the relatedness between serovars with respect to LPS structure and shared LPS biosynthesis genes. This detailed knowledge of *P. multocida* LPS will be used to develop effective diagnostic tools as well as improved vaccination strategies.

Recently, analysis of the LPS structure and corresponding LPS biosynthesis genes of serovar 14 and serovar 1 strains revealed that they shared the same LPS outer core locus with the exception of a deletion within the phosphocholine biosynthesis gene *pcgA* encoded by serovar 14 (Fig. 2). The lack of phosphocholine (PCho) on the LPS prevented the galactosyltransferase, GatA, from adding the 1,6-linked β -galactose, present on serovar 1 LPS (Fig. 2). Complementation of serovar 14 with an intact *pcgA* gene resulted in PCho decoration of serovar 14 LPS and the elaboration of the 1,6-linked β -galactose. Thus, the major difference in the LPS structures between serovar 14 and serovar 1 is due to a single inactivated PCho biosynthesis gene. This single genetic change results in altered LPS structure and the different serological classification of these strains.

The LPS structures produced by serovars 3 and 4 are related, but the serovar 4 LPS is shorter, terminating between the first and second galactose residues (Fig. 3). Data on the LPS biosynthesis genes of serovars 3 and 4 reveal that they share the same LPS assembly locus, but in serovar 4 there is a disruption of *pm1138* that encodes the transferase required for the addition of α -N-acetylgalactosamine in serovar 4 (Fig. 3). As the serovar 4 LPS structure ends before the addition of the second galactose, it was hypothesized that the α 1-4 galactosyltransferase required for this addition, predicted to be Pm1139, may be non-functional in serovar 4. Complementation of the serovar 4 strain with the serovar 3 *pm1139* gene resulted in the production of LPS that was extended by two sugars, indicating that the serovar 3 Pm1139 transferase provided *in trans* was functional and that the serovar 4 Pm1139 encoded within the LPS outer core biosynthesis locus was not. An alignment of serovar 4 Pm1139 with the Pm1139 protein sequences of two serovar 3 strains, Pm70 and P1059, revealed a number of non-conservative amino acid changes at the C terminus of the serovar 4 Pm1139 protein. Given the apparent inactivity of the serovar 4 *pm1139* transferase, we hypothesise that one or more of the identified amino acid substitutions has rendered this transferase inactive. Further investigations are underway.

Other LPS glycosyltransferases whose functions have been identified include a heptosyltransferase, HptG, specific for the addition of DD- α -heptose to the 6 position of β -glucose and GatC, a galactosyltransferase specific for the addition of β -galactose to the 4 position of LD- α -heptose.

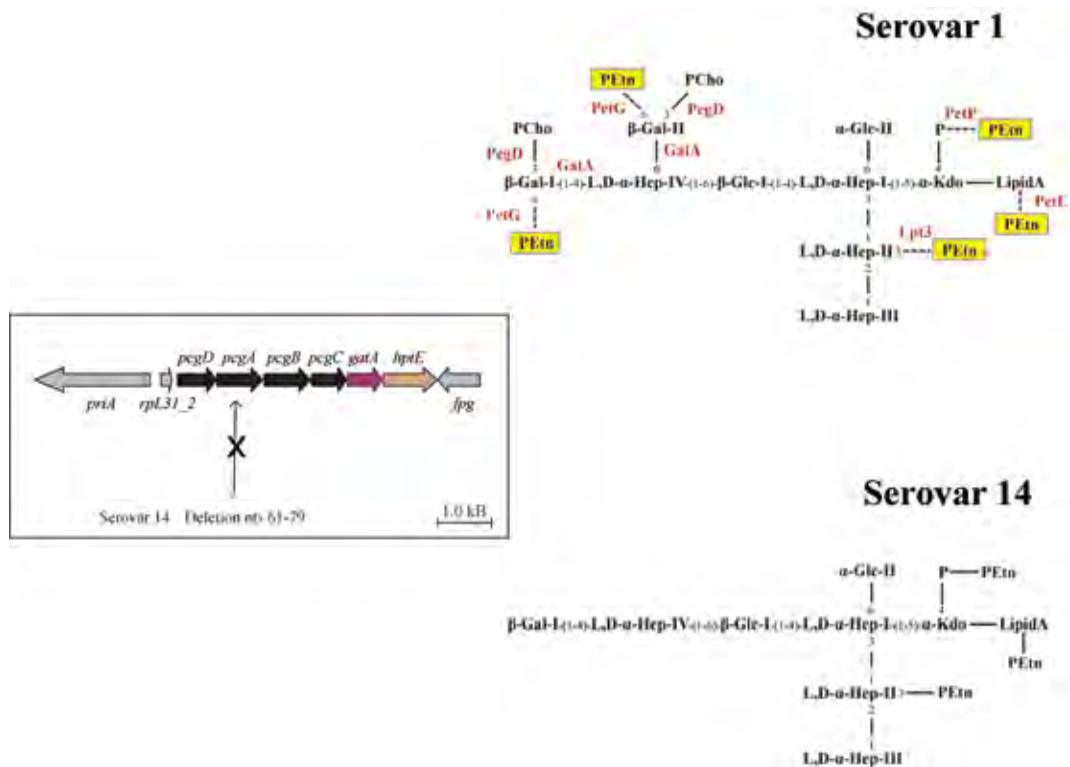


Figure 2: Schematic representation of LPS expressed by *P. multocida* serovars 1 (strains X73 and VP161) and 14 (strain P2225). The LPS outer core biosynthesis locus with the deletion leading to the inactivation of *pcgA* is shown in the inset.

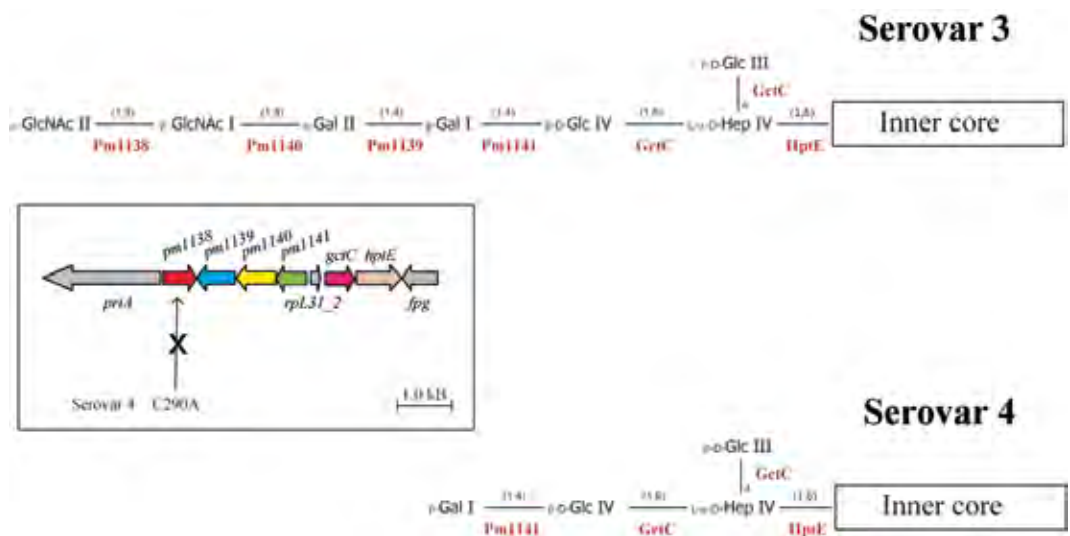


Figure 3: Schematic representation of the LPS expressed by *P. multocida* serovars 3 (strains Pm70 and P1059) and 4 (strain P1662). The LPS outer core biosynthesis locus with the mutation leading to inactivation of *pm1138* is shown in the inset.

Identification of the full cohort of LPS phosphoethanolamine (Petn) transferases in *P. multocida*.

Analysis of the LPS produced by *P. multocida* revealed that many of the LPS structures are decorated at various positions with phosphoethanolamine (PEtn), a positively charged residue that is also present on the LPS structures of bacteria such as *Neisseria meningitidis*, *Haemophilus influenzae* and *Campylobacter jejuni*. Addition of PEtn to LPS reduces the overall charge of the molecule, but decoration of the LPS structure with Petn can also affect the interaction of the LPS with innate host immune defences such as antimicrobial peptides and complement components.

Through bioinformatic analysis, site directed mutagenesis and complementation studies in *P. multocida*, we have identified four LPS-specific PEtn transferases. These are; PetL, required for the addition of PEtn to lipid A; PetP, required for the addition of PEtn to phosphorylated Kdo residue; Lpt3, required for the addition of PEtn to the 3 position of the second heptose; and PetG, predicted to be the transferase required for the addition of PEtn to the 3 position of both terminal galactose residues in strain X73 (Fig. 2). Homologues of PetL and Lpt3 have been characterized in *Neisseria meningitidis* and other bacteria, but both PetP (Lipid A PEtn addition) and PetG (terminal galactose PEtn addition) have not been identified in any other species. We have systematically inactivated all of the LPS-specific PEtn transferases and are currently assessing their role in the pathogenesis of fowl cholera.

Regulation of capsule expression in *Pasteurella multocida* by the global regulator Fis.

The capsule is an essential virulence factor in *P. multocida*. We previously identified a region required for Fis-regulated expression 70 to 90 nucleotides upstream from the transcript start site of *hyaE*, the first gene in one of two capsule biosynthesis operons. To determine whether transcription was the result of direct binding of Fis to the *hyaE* promoter region, electrophoretic mobility shift assays (EMSA) were performed and indicated a specific and direct interaction. Currently DNase footprinting using automated fluorescence capillary electrophoresis is being used to identify the exact binding sequence(s) within and upstream of *hyaE*. This method will also be used to identify Fis binding sequences in other Fis-regulated virulence genes, including a hemin binding receptor, the filamentous hemagglutinin operon and the *fis* operon itself.

An alignment and phylogenetic tree of the intergenic region of the capsule locus of currently sequenced *P. multocida* strains shows a high degree of sequence variation between the five capsule serogroups. Sequences of the capsule loci showed that the serogroups could be grouped into three distinct clusters; cluster 1 contains serogroups A and F, cluster 2 contains serogroups E and B, and cluster 3 contains serogroup D. This clustering leads to the question: Is capsule expression regulated in a different manner amongst the different serogroups? This warrants further investigation.

Thermoregulation of capsule.

The Group II capsule of *Escherichia coli* is controlled by thermoregulation, where there is undetectable expression of capsule during growth at 23°C but full expression at 37°C. Thermoregulation of capsule expression in *E. coli* requires a number of regulatory proteins, one of which is SlyA. As *P. multocida* also expresses a type II capsule, we investigated whether it too is thermoregulated. Using an acidic capsular detection assay, we were able to show that capsule was not detected when *P. multocida* was grown at 22.5°C, but was present at 37°C and at 42°C, the body temperature of chickens. Analysis of the *P. multocida* genome revealed that *P. multocida* VP161 does not encode a SlyA homologue, suggesting that a different repertoire of regulatory proteins may be involved in thermoregulation of capsule expression in *P. multocida*. Thermoregulation of capsule expression is currently being examined at the transcriptional level using quantitative RT-PCR assays.

Pathogenesis and virulence determinants of the ovine footrot pathogen, *Dichelobacter nodosus*.

(Prof Julian Rood, Dr Ruth Kennan, Dr Xiaoyan Han, Prof James Whisstock, Mr Wilson Wong, Dr Corrine Porter, Dr David Steer, Prof Ian Smith, Prof Richard Whittington, Dr Om Dhungyel, Dr Leo Calvo-Bado, Dr Elizabeth Wellington, Prof Laura Green)

The aim of this project is to develop a detailed understanding of how *Dichelobacter nodosus* is able to infect the sheep hoof and cause clinical footrot. We have shown that the AprV2 extracellular protease of *D. nodosus* is required for virulence and have focussed on structural analysis, which have led to the elucidation of the structures of several proteases, including AprV2, AprB2, BprV and BprB. To understand the role of the C-terminal extension of AprV5, we constructed a series of C-terminal-truncated deletions in *D. nodosus* by allelic exchange. Analysis of these strains showed that the C-terminal extension of AprV5 is required for the expression of normal wild-type levels of protease activity. We also demonstrated that AprV5 is involved in processing AprV2 and BprV to their mature forms and that the C-terminal extension of AprV5 is required for this function, but is not essential for protease secretion. These studies have increased our understanding of how these virulence-associated proteases interact and are secreted. In collaboration with colleagues at the University of Warwick, we have identified Pgr, a novel polymorphic repeat protein that may have value as an epidemiological marker for virulence studies.

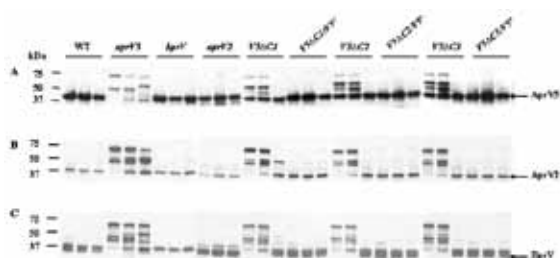


Figure 4: Western immunoblots provide evidence that AprV5 processes all three proteases

The cellular and molecular basis of host pathogen interactions

Effect of autophagic induction on host-pathogen interactions.

(Prof Rod Devenish, Dr Lan Gong, Dr Xuelei Li, Dr Mark Prescott, Ms Tanya D'Cruze, Ms Shu-lin Lai, Prof Ben Adler, Dr John Boyce, Dr Elizabeth Allwood, Ms June Treerat, Ms Priya Alwis)

We have continued our investigation of the role played by autophagy in response to *Burkholderia pseudomallei* infection, focussing on understanding which bacterial proteins are critical for evasion of autophagy.

Electron microscopy analysis of infected RAW 264.7 macrophage-like cells showed that the invading bacteria are either free in the cytosol, or sequestered in single-membrane phagosomes rather than double-membrane autophagosomes, suggesting that LC3 is recruited to *B. pseudomallei*-containing phagosomes. Complete or partial loss of function of type III secretion system cluster 3 (TTSS3) in mutants lacking the BopA (effector) or BipD (translocator) proteins respectively, results in delayed or no escape from phagosomes. Consistent with these observations, *bopA* and *bipD* mutants both show a higher level of co-localization with LC3 and the lysosomal marker LAMP-1, and impaired survival in RAW264.7 cells, suggesting enhanced killing in phagolysosomes. Thus, LC3 recruitment to phagosomes stimulates killing of *B. pseudomallei*. Furthermore, BopA plays an important role in efficient escape of bacteria from phagosomes.

We have assessed *B. pseudomallei* survival in RAW 264.7 cells transfected with a Beclin 1 (Atg6) siRNA construct. The Beclin 1 protein is involved in both the signaling pathway activating autophagy under starvation conditions and in the recruitment of LC3 to canonical phagosomes. The level of Beclin 1 expression is reduced by 80-90%. Both Beclin 1 knockdown cells and control cells when treated with rapamycin show similar levels of bacterial co-localisation with LC3 and intracellular survival, suggesting the rapamycin-induced phenotypes are independent of Beclin 1. Notably, when Beclin 1 knockdown cells are subjected to starvation there is little change in co-localisation or intracellular survival of the bacteria. By contrast, starvation of control cells shows increased co-localisation of bacteria with LC3 and decreased survival, thereby suggesting that starvation-induced suppression of *B. pseudomallei* survival is Beclin 1 dependent. These results indicate a differential involvement of Beclin 1 in the starvation- and rapamycin-induced responses to *B. pseudomallei* infection.

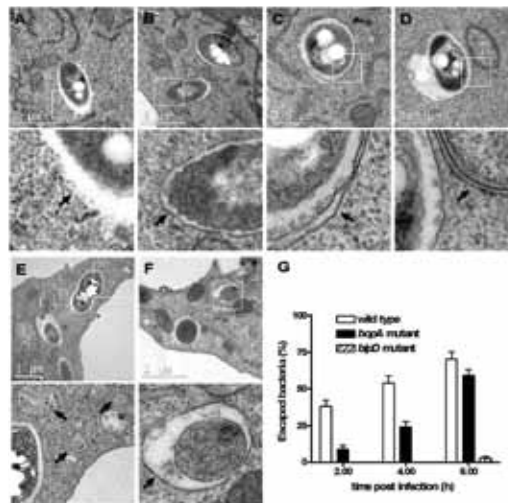


Figure 5: *B. pseudomallei* – containing vacuoles are bound by single membranes and TTSS3 is required for bacterial escape. (A-F) Arrows indicate detailed membrane ultrastructure. (G) The percentage of bacteria free in the cytosol of RAW 264.7 cells following infection with *B. pseudomallei*.

Pathogen effector protein screening in yeast (PEPSY).

(Prof Rod Devenish, Dr Mark Prescott, Ms Tanya D'Cruze, Prof Ben Adler and Dr John Boyce)

We have continued the characterisation of candidate *B. pseudomallei* pathogen effector proteins previously characterised by expression in yeast in terms of their effect on vacuolar morphology and autophagic processes. In particular, we have focussed on a mutant of *BPSS1394* which encodes a TTSS1 protein that shares significant homology with TTSS3 BsaS, a putative ATPase. The mutant displays attenuation for virulence in a mouse melioidosis model. Mutant bacteria escape from phagosomes at similar levels to wild-type bacteria, but exhibit increased co-localization with the autophagic marker protein GFP-LC3, and decreased intracellular survival. These data suggest that mutant bacteria are more susceptible to LC3-associated phagocytosis and intracellular killing. Interestingly, mutant bacteria also display non-actin based enhanced motility in comparison to wild-type bacteria, but the significance of this observation in relation to the change in virulence is presently unclear. Collectively, the data indicate that the loss of protein *BPSS1394* can dramatically influence the infectivity and pathogenicity of *B. pseudomallei*.

Mechanisms of innate immunity

Interaction of bacterial pathogens with the host innate immune system.

(Prof John Davies, Prof Phillip Nagley, Prof Paul Hertzog, Dr Ashley Mansell, Ms Heling Ng, Mr Tim Tra, Ms Jing-Jing Khoo)

This project is part of a collaborative initiative to investigate key aspects of how pathogenic microorganisms interact with host innate immunity. An extensive series of reporter constructs and assays has been successfully developed that allow the monitoring of various signal transduction pathways and the levels of pro-inflammatory transcription factors and cytokines.

We continued our work to investigate innate immune signalling in response to virus infection of mammalian cells, as a model for understanding cellular response to infectious agents. We have been working further on the recently identified mitochondrially localised protein Mul1, which we showed to interact closely with the MAVS antiviral signalling complex. Specifically, RIG-I-like helicases (RLH) initiate antiviral signalling by interaction with the mitochondrially localized adapter MAVS. We have characterised Mul1 as a negative regulator of RLH signalling. Depletion of Mul1 by RNA interference potentiated RLH-mediated signalling and increased inflammatory cytokine expression following viral challenge. Mul1 requires mitochondrial localization and is thought to negatively regulate innate immune antiviral signalling (Figure 6).

In order to investigate the specific topology of Mul1 with respect to the outer mitochondrial membrane, we have been working in association with Dr Kip Gabriel of the Department of Biochemistry and Molecular Biology at Monash University, to design novel protein constructs that will test out key propositions relating to the orientation of the N-terminal and C-terminal putative membrane-spanning domains of Mul1. We have shown that the N-terminus of Mul1 is buried inside the mitochondrial membranes. Current work is focussing on which domains in Mul1 interact with target proteins such as RIG-I and MAVS to effect binding and regulatory activities.

Technical approaches we have initiated include preparation of antibodies recognizing Mul1 and the development of mice affected in the expression of Mul1.

With the retirement from the Centre of Professor Phillip Nagley, this project will continue with the accession to the Centre of Professor Paul Hertzog as a CI from July 2010.

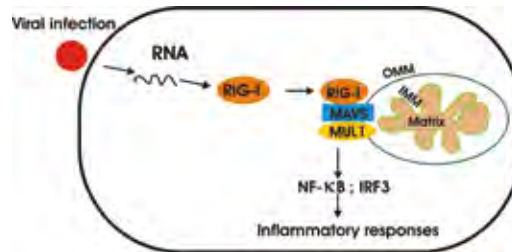


Figure 6: Schematic representation of innate immune signalling showing interactions of Mul1 with the MAVS complex on mitochondria, in response to viral infection of mammalian host cell.

Systems biology of innate immune signal transduction pathways.

(Prof Paul Hertzog, Dr Nicole de Weerd, Ms Layla Zaker-Tabrizi, Dr Laila Roisman, Dr Hugh Reid, Prof Jamie Rossjohn)

The type I interferons (IFNs) are integral components of the host response to bacterial and viral pathogens, modulating both the early innate immune response and sculpting ensuing adaptive immunity. They have practical applications, either as directly administered for anti-infectives, blocked in some cases to avoid excessive inflammatory disease, or induced or administered as an integral component of vaccine responses. This project aims to elucidate the structural details of how members of the IFN family (e.g. IFN α and IFN β) interact with their receptor components, Ifnar1 and Ifnar2 and thereby orchestrate complex cellular responses. Achievements to date include successful production and purification of biologically active ligands and receptors, formation and purification of ligand-receptor binary and ternary complexes and importantly crystals of IFN β –Ifnar1 complex that diffract to 2.7Å with complete data to 3.2Å (Figure 7).



Figure 7: Crystal of IFN β –Ifnar1 complex.

Structural aspects of innate immunity.

(Prof Jamie Rossjohn, Mr Kwok Wun, Mr Ruide Koh, Dr Jobichen Chacko)

The innate immunity project, an emerging interest of the Centre, was initiated in 2006. In collaboration with Prof James McCluskey, Prof Dale Godfrey & Dr. Andrew Brooks (The University of Melbourne), we have focussed on the CD1 family, which present lipids for recognition by the immune system. One such “immune sentinel” is the invariant NKT cell receptor that specifically recognises the CD1d family member. In 2010, we collaborated with Prof Christian Kurts’s group (University of Bonn, Germany) to unravel mechanisms of lipid-Ag presentation. Further, we collaborated with Assoc Prof Laurent Gapin (University of Colorado at Denver, USA) to provide an understanding of NKT TcR autoreactivity.

Proteomics analysis of bacterial pathogens

Purification and characterization of cytotoxin from *Campylobacter jejuni*.

(Prof Ian Smith, Dr David Steer, Prof John Albert, Prof Ben Adler, Dr Xenia Gatsos)

This project aims to purify the protein possessing cytotoxic activity for Chinese hamster ovary (CHO) cells from a cytotoxic *C. jejuni* strain, by biochemical techniques and determine the identity of this protein by mass spectrometry. We have currently isolated an active fraction from ion exchange chromatography in which we have identified around 50 proteins. This fraction is stable to freezing and lyophilisation and activity is lost after heat exposure and treatment to the proteolytic enzyme trypsin, thus confirming that the toxin is a protein. By a process of elimination, based on described functions in the literature of the identified proteins we aim to generate a list of candidate proteins which may be the toxin as targets for further mutagenesis work. The work will be in parallel to a further isolation and purification of the toxin by chromatographic and electrophoretic methods. Ultimately we aim to functionally characterise the purified protein using suitable in vitro tissue culture models for toxicity and an in vivo animal model for diarrhoeagenic activity.

Proteomic analysis of host pathogen proteins.

(Prof Ian Smith, Dr David Steer)

During 2010 we have applied protein shaving technology to identify surface proteins expressed on a number of different pathogens. We have applied both N-terminal sequencing and classic mass spectrometric based proteomics to fully characterise a number of critical pathogen surface proteins prior to a full structural analysis.

Identifying and characterising novel protein post translational modifications of host pathogen proteins.

(Prof Ian Smith, Dr David Steer)

We have applied a number of proteomic technologies of both gel based and LCMS based approaches to identify both cytosolic and membrane proteins. We have also used LCMS technologies to identify and characterise post translational modifications such as phosphorylation in these pathogen proteins.

Structural and functional biology

Structural biology and drug target characterisation.

(Prof Jamie Rossjohn, Dr Travis Beddoe, Dr Jerome Le Nours, Dr Kelly-Anne Twist)

We have continued our collaboration with Dr Adrienne Paton and Prof James Paton (University of Adelaide) to undertake structural studies on an AB5 toxin from pathogenic *Escherichia coli*. Previously we had shown that the catalytic A-subunit specifically inactivated an essential ER-resident chaperone, termed BiP and how the B-subunit enters the cell by binding a sugar, Neu5Gc, not synthesised in humans. We have now crystallized and determined the structure of the intact AB5 toxin and carried out associated mutagenesis studies to address the basis of toxin assembly. Further, we have initiated studies on related AB5 family members.



Figure 8: Overall architecture of the X-ray structure of *Legionella pneumophila* Lp1NTPDase.

Our findings on *Legionella* virulence factors, made in collaboration with Dr Elizabeth Hartland (University of Melbourne), showed how *Legionella* has hijacked the scaffold of eukaryotic NTPdases for its pathogenesis.

In collaboration with Prof Ben Adler, we have continued our investigation into *Leptospiral* antigens, solving the structure of leptospiral heme oxygenase, shown by us to be an important virulence factor.

Structural biology and bioinformatics.

(Prof James Whisstock, Mr Carlos Rosado, Mr Gordon Lloyd, Prof Geoff Webb, Dr Ashley Buckle, Dr Corrine Porter, Mr Wilson Wong, Mr Khalid Mahmood, Dr Sheena McGowan, Ms Wan Ting Kan, Dr Ruby Law, Mr Cyril Reboul, Prof Julian Rood, Assoc Prof Peter Stuckey)

In 2010 we have further built upon our discovery that Membrane Attack Complex / Perforin like (MACPF) proteins are distantly related to bacterial cholesterol dependent cytolysins (CDCs) and elucidated the mechanism of pore formation by mammalian perforin. Perforin is critical for the elimination of virally infected or transformed cells. We have determined the X-ray crystal structure of perforin as well as the cryo-EM structure of the perforin pore (in collaboration with Helen Saibil, Birkbeck College London and Joe Trapani from the Peter MacCallum Cancer Centre). Our data revealed the unexpected finding that perforin forms pores with the monomer orientated “inside-out” relative to the bacterial CDCs. The structure also reveals how perforin deploys a C2 domain to facilitate initial interactions with the membrane.

Related to the above, we have begun to work on a number of important bacterial pore forming proteins that are essential for virulence. We are currently completing the structure of Listeriolysin O, a major virulence factor produced by *Listeria* spp. Furthermore, with CI Rood we have determined the structure of NetB, a haemolysin-like toxin important for avian necrotic enteritis.

We have continued our work on malaria drug targets, and determined the X-ray crystal structure of two important proteases that are essential virulence factors in malaria (M1 and M17) in complex with a variety of inhibitors.

We have also published a number of major bioinformatic approaches for the study of whole genomes and protein families. Our work on encapsulated gene-by-gene matching (EGM) has recently been published in *Bioinformatics*. MUSTANG-MR and MR-GRID (both are novel approaches for helping to determine crystal structures) were published in PLOS One and CASCLEAVE (an approach for predicting protease cleavage sites) was published in *Bioinformatics*.

Functional genomics of large clostridial plasmids.

(Prof Julian Rood, Dr Trudi Bannam, Dr Vicki Adams, Prof James Whisstock, Dr Corrine Porter, Ms Radhika Bantwal, Ms Jessica Wisniewski, Ms Lakmini Weeramantri, Prof Bruce McClane, Dr Francisco Uzal)

The overall objectives of this project are to understand how large clostridial plasmids are transferred between strains of *C. perfringens*. Research has focussed on the *tcp* conjugation locus from the paradigm conjugative tetracycline resistance plasmid, pCW3. We carried out extensive genetic analysis of both the IntP and TcpG conjugation proteins. These studies have revealed that TcpG is a peptidoglycam hydrolase with two functional active sites and have led to the hypothesis that the recombinase IntP is involved in the DNA processing reactions that occur prior to DNA transfer. Studies on the large toxin plasmids of *C. perfringens* have led to the construction of a series of isogenic epsilon toxin mutants that are ready for testing in an animal model. In addition, we have developed new methods for the rapid isolation of multiple toxin mutants in the same strain. These studies are an essential prelude to determining whether all of the toxin plasmids are conjugative.



Figure 9: *TcpG*-mediated autoaggregation

Functional analysis of NetB-producing strains of *C. perfringens*.

(Prof Julian Rood, Dr Trudi Bannam, Dr Corrine Porter, Prof James Whisstock, Ms Xu-Xia Yan, Dr Anthony Keyburn, Mr Ben Wade and Dr Rob Moore)

NetB toxin is the primary toxin involved in the pathogenesis of necrotic enteritis in chickens. Genetic studies have shown that the *netB* gene is encoded on a large conjugative plasmid. We have now sequenced that plasmid and shown that it carries other putative virulence genes. Strains carrying the NetB plasmid also carry other closely related conjugative plasmids, which we have also sequenced. Our studies on the biochemistry of NetB have led to the determination of its crystal structure. Finally, both random and site-directed mutagenesis has been used to make several *netB* mutants that have single amino acid substitutions which have been subsequently purified and analysed.



Figure 10: Crystal structure of clostridial NetB toxin

Development of veterinary vaccines

Vaccine development in fowl cholera and ovine footrot.

(Prof Ben Adler, Dr John Boyce, Mr Mark Edmunds, Prof Julian Rood, Dr Marina Harper, Mr Tamas Hatfaludi, Prof Richard Whittington, Dr Om Dungyel, Prof Steve Bottomley, Dr Noelene Quinsey, Dr Amanda Walmsley, Mr Nik Sotirellis, Ms Sadia Deen)

Dichelobacter nodosus and *Pasteurella multocida* are the causative agents of ovine footrot and fowl cholera respectively. We utilised a range of bioinformatics analyses of the annotated *D. nodosus* and *P. multocida* genome sequences and previously published experimental data to select genes encoding proteins likely to have vaccine potential. The central premise of this work is that protective antigens are likely to be surface exposed or secreted by the bacteria and therefore accessible to the host immune response.

The ability of 63 recombinant antigens to protect against ovine footrot has now been examined in five separate pen-based virulence trials. In these trials sheep are immunized with our recombinant proteins and then challenged with a highly virulent *D. nodosus* infection. Analysis of quantitative data obtained from these trials has led to the identification of several potential vaccine candidates. Several of these candidates were retested both individually and in combination in a further vaccine trial. The results provided evidence that one combination of antigens may provide protection against disease. The ability of this potential vaccine to provide cross-protective immunity against different *D. nodosus* serogroups is currently being tested.

Due to problems with the stability of the *P. multocida* challenge strain, no work was performed on fowl cholera vaccine development in 2010. Further assessment of candidate vaccine antigens is planned in 2011.

Vaccine development in necrotic enteritis in chickens.

(Dr Robert Moore, Prof Julian Rood, Dr Anthony Keyburn, Dr John Boyce, Dr Trudi Bannam, Prof Ben Adler, Dr Noelene Quinsey, Ms Xu-Xia Yan)

The overall objective of this project is to develop new methods for the control of necrotic enteritis in chickens, which is caused by specific avian strains of *Clostridium perfringens*. A reverse vaccinology approach has been used to identify novel vaccine candidates, including NetB toxin, which was discovered by this research team. Vaccine studies using recombinant antigens, especially the NetB toxin, are on-going, and are supported by funding from the Australian Poultry CRC.

Development of a vaccine against *Haemonchus contortus* in sheep.

(Dr David Piedrafita, Prof Els Meeusen, Dr Mike de Veer, Ms Jayne Sherrard, Mr Troy Kraska, Mr Chris Hosking; In Collaboration with Pfizer VMRD)

Haemonchus contortus is the most pathogenic of the gastrointestinal nematode (GIN) parasites and a major problem for the sheep industry in Australia and worldwide. Due to the complexity of the organisms, there are at present no vaccines available against any of the GIN parasites of either human or animal importance. In collaboration with our commercial partner, we have evaluated an experimental vaccine developed by our group using different adjuvant systems under market conditions. In addition, we are further characterising the unusual structure of our vaccine antigen and evaluating its large scale production.

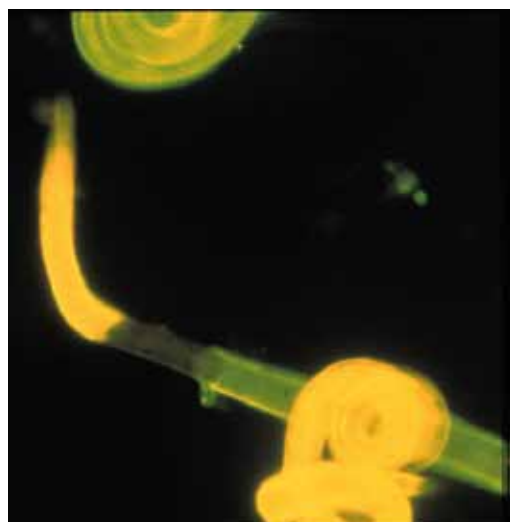


Figure 11: *Haemonchus contortus* L3 larvae during exsheathment, yellow fluorescence depicts the surface antigen exposed on the exsheathed L3 and used as a vaccine.

Key Performance Indicators

Research findings

Quality of publications

Centre researchers continued to perform well in peer-reviewed publications, with a total of 37 papers published in scholarly refereed journals in 2010. Of these papers, 84% appeared in journals with an ERA ranking of A* or A. Details of publications are shown in Appendix 1: Publications.

Patents

No patents were filed in 2010.

Since the Centre's inception in 2006, a total of 13 patents has been filed.

Centre hosted conferences and scientific meetings

First Prato Conference on Pathogenesis of Bacterial Diseases of Animals, Prato, Italy

The Centre established and hosted its first conference on veterinary bacterial pathogens from 6-9 October at the Monash University Centre, Prato, Italy. The conference was initiated primarily in response to a growing need for a conference that has a strong focus on veterinary pathogens and as such it was the first meeting of its kind. Over 130 delegates from over 30 countries attended the conference.

The organising committee of the conference was chaired by Centre CI, Prof Julian Rood. With 42 speakers and over 50 poster presentations, the conference was organised under the following themes: disease epidemiology, genomics, mechanisms of pathogenesis, extracellular pathogens and toxins, host-pathogen interactions, host responses and immunity and vaccines. The conference presentations delivered the latest exciting data and discoveries under these various themes and stimulated keen discussion and interest among the conference participants. The opening plenary session entitled *'The Problem of Relevance in Pathogenesis Research'* was delivered by Professor Emeritus Carlton Gyles from the University of Guelph, Canada.

The conference also received significant sponsorship from Intervet-Schering Plough Animal Health, CSIRO Livestock Industries, Monash University Faculty of Medicine, Nursing and Health Sciences, Pfizer Animal Health, BioX Diagnostics and Don Whitley Scientific. Travelling scholarships were awarded to four participants from less developed countries.

The conference was such a success and the enthusiastic responses from the participants prompted a second conference in the series to be planned for 2012.



Centre PhD student, Ben Wade with his poster at the poster session.



Conference participants



Organising Committee Chair, Centre CI Julian Rood (centre) at the conference dinner held at the Medici Villa 'La Ferdinanda' in Artimino.



Rudd Segers and Joachim Frey during lunch at the conference.



Centre Advisory Board Member Joachim Frey, Centre Director Ben Adler and Ruud Segers, Intervet Schering-Plough at post conference drinks on the 2nd day.

Centre hosted conferences and scientific meetings

Annual Scientific Meeting, Monash University Caulfield campus

For 2010, the Centre held its Annual Scientific Meeting on 29 November at the Monash University Caulfield campus, with over 45 Centre students and researchers in attendance.

The opening keynote address was given by Prof Charles Mackay from the Centre for Immunology and Inflammation at Monash University who presented his work on '*Diet, gut microbiota and regulation of inflammatory responses*'. The Centre was also fortunate to have Prof Henk Haagsman from Utrecht University, who was an invited speaker at the recent Prato Conference, present as a visiting scientist. He gave a talk on his research on host defence peptides and their use as therapeutic agents.

One of the aims of the meeting was to continue to foster a closer relationship among its students and staff and enable each one to find out more about others' research at the Centre. This was achieved in the last session of the meeting where every member of the Centre (students, research assistants and research fellows) presented a synopsis of their work in three minutes and using a single slide.

Invitations to address and participate in international conferences

Professor Ben Adler

- Tenth Gordon Research Conference: Biology of Spirochetes. Ventura, USA.
- First Prato Conference on the Pathogenesis of Bacterial Diseases of Animals. Prato, Italy.
- Spirochetes Havana 2010, Havana, Cuba.
- Tenth Awaji International Forum on Infection and Immunity, Awaji, Japan.
- Modern Veterinary Vaccines and Adjuvants, Budapest, Hungary.

Professor Rod Devenish

- Australian Health and Medical Research Congress, Melbourne, Australia.

Professor Paul Hertzog

- Australian Health and Medical Research Conference, Melbourne, Australia
- International Cytokine Society/International Society for Interferon and Cytokine Research 2010, Chicago, USA
- International Congress of Reproductive Biology 2010, Palm Cove, Australia
- Keystone Symposium on Innate Immunity: Mechanisms Linking with Adaptive Immunity, Dublin, Ireland

Professor Els Meeusen

- International Conference of Parasitology (ICOPA XII), Melbourne, Australia.
- Modern Veterinary Vaccines and Adjuvants, Budapest, Hungary.

Professor Julian Rood

- Combined Annual Scientific Meeting of New Zealand Microbiology Society and New Zealand Society for Biochemistry and Molecular Biology, Auckland, New Zealand.
- First Prato Conference on the Pathogenesis of Bacterial Diseases of Animals, Prato, Italy.
- 11th International Symposium on the Genetics of Industrial Microorganisms, Melbourne, Australia.
- International Footrot Workshop, Coventry, U.K.

Professor Jamie Rossjohn

- Malaysian Genome Institute Seminar, Kuala Lumpur, Malaysia.
- International Congress of Immunology, Kobe, Japan.
- Cell Press Exciting Biologies Meeting: The Biology of Recognition, Singapore.

Professor Ian Smith

- International Brain Research Organisation, Kuala Lumpur, Malaysia.
- The Biotech-Academic Interface, Peptide Symposium on Biomolecules for Diagnostics and Therapeutics, Prato, Italy.
- International HuPO meeting, Sydney, Australia.
- Euro-Bioimaging, 2nd Stakeholder Meeting, Vienna, Austria.
- International Peptide Symposium, Kyoto, Japan.
- PacifiChem2010 Congress, Hawaii, USA.

Professor James Whisstock

- Gordon Conference on Proteolytic Enzymes and their Inhibitors, Lucca, Italy.
- 24th Annual Symposium of the US Protein Society, San Diego, USA
- Lorne Conference on Protein Structure and Function, Lorne, Australia.
- First Prato Conference on the Pathogenesis of Bacterial Diseases of Animals, Prato, Italy.

Invitations to visit leading international laboratories

Professor Ben Adler

- Department of Medical Microbiology, University of Bologna, Bologna, Italy.
- Institut Pasteur de Nouvelle Calédonie, Noumea, New Caledonia.
- Schering-Plough Intervet, Boxmeer, The Netherlands.

Professor Rod Devenish

- Biotechnology Institute, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, China

Professor Paul Hertzog

- Dartmouth Medical School, New Hampshire, USA
- Pfizer, Boston, MA, USA
- University of Bonn, Bonn, Germany

Professor Els Meeusen

- Glaxo Smith Kline, Rixensart, Belgium
- Flemish Institute of Biotechnology, Gent, Belgium
- Department of Parasitology, Virology and Immunology, Faculty of Veterinary Sciences, Merelbeke, Belgium

Professor Julian Rood

- Norwegian Veterinary Institute, Oslo Norway.
- University of Warwick, Coventry, U.K.

Professor Jamie Rossjohn

- Malaysian Genome Institute, Malaysia

Professor Ian Smith

- EMBL Laboratories, Heidelberg, Germany
- Sichuan University and West China Hospital, Chengdu, China.
- Siemens Laboratories, Heidelberg Germany
- Varian Laboratories, Oxford, UK
- Biomedical Diagnostic Institute (BDI), Dublin, Ireland
- Trinity College, Dublin, Ireland



Commentaries about the Centre's achievements

Radio Interviews

Radio interview on Centre's vaccine research with Steve Price on Radio 3MTR on 27 April 2010.

Publications

'Destructive protein causes sheep disease', North Central News, 1 December 2010.

'Destructive protein causes debilitating sheep disease', Monash Media Release, 25 November 2010.

'Stopping the rot to save sheep', Science Alert, 29 November 2010.

The Centre was mentioned by Victoria DIIRD in *"Victoria – taking biotechnology from strength to strength"*, in Microbiology Australia May 2010 issue.

The Centre's Prato Conference was reported in *"Animal experts convene in Prato"*, in Monash Memo 1 December 2010.

The Centre's Prato Conference was reported in *"Animal Health Unit sponsor of first Prato Conference on the Pathogenesis of Bacterial Diseases of Animals"* in Intervet Schering-Plough Animal Health Unit's Animal Health Stories, Vol 3, 2010.

The Centre's work in leptospirosis was mentioned in *"Leptospirosis and Weil's syndrome: cause for concern"* by Kathryn Senior in *The Lancet Infectious Diseases*, Vol 10(12): 823-824, Dec 2010.

Research training and professional education

Post graduate student education continued to be a critical component of the Centre's activities in 2010. A primary focus is the training of students in advanced technologies. In 2010, the Centre recruited six new PhD students and one Masters student. This brings to a total number of 23 Centre PhD students currently working on Centre projects. In addition to project-based training, Centre postgraduate students have access to professional development provided by the Monash Research Graduate School (MRGS), the Monash Postgraduate Association (MPA) and the MBio Graduate School platform at the School of Biomedical Sciences. Students are encouraged to engage in teaching activity (demonstrating to undergraduate classes) for which training is provided by SOBSGS and their home departments. In particular, Centre PhD students are encouraged to participate in an annual young investigator symposium as part of the Victorian Infection and Immunity Network. Financial support was also provided for students to attend national and international research conferences as well as to visit overseas laboratories.

Commercialisation Seminar Series

The Centre organised a seminar series to assist its staff and students in developing research commercialisation and intellectual property management skills. The series aims to provide an understanding of the basic skills and processes involved in research commercialisation. Members from Monash Industry Engagement and Commercialisation (IEC) and Monash Solicitors contributed towards presenting the seminars. A total of four seminars were spread across the year as listed below:

- **9 March: Opportunity Evaluations**
Alastair Hick (IEC)
- **18 May: Alliance Dos and Don'ts**
John Morrison (IEC)
- **17 Aug: Legal Basics**
Mariette Chiodo (Monash Solicitors)
- **21 Sept: Patents for Dummies**
Darcelle Dixon (IEC)

Professional development

Victorian Infection and Immunity Network (VIIN) Student Symposium

Centre Director Professor Ben Adler and Centre Chief Investigator, Professor Paul Hertzog are members of the VIIN executive committee, with Professor Paul Herzog taking the role as co-convenor of VIIN.

The two flagship events for 2010 were the student symposium and the post-doc symposium held on 3 June and 30 September respectively. Centre students and research fellows participated in these events, presenting either oral or poster presentations. The Centre also sponsored \$150 cash prize each for the 2nd best oral and poster presentation as listed below:

Oral: Dr Claire McCoy, Monash Institute for Medical Research – The anti-inflammatory cytokine IL-10 inhibits miR-155 in response to Toll-like Receptor signalling.

Poster: Dr Kate Jones, Burnet Institute – A novel oligo-based fluorescent *in situ* hybridization (O-FISH) technique for the detection of single copy HIV-1 nucleic acids.

Visits to Overseas Laboratories

Centre PhD students are eligible for a grant of \$1000 from the Centre which they can use towards visiting laboratories overseas when they are presenting at international conferences. This creates opportunities for PhD students to learn from other labs, to present their research at lab seminars overseas and also to network and meet other international scientists. In 2010, Centre PhD student Ben Wade presented at the Prato Conference and took the opportunity while he was in Europe to visit the Sanger Wellcome Trust Centre, the John Radcliffe Hospital in Oxford, and Imperial College, UK.

*Ben Wade is an ARC PhD student in his third year. His project is to study the genomic basis of virulence in *Clostridium perfringens* induced necrotic enteritis in poultry and he is based at the CSIRO Animal Health Laboratory in Geelong. His supervisors are Prof. Julian Rood and Dr. Robert Moore.*

In October this year I was fortunate enough, thanks in part to the generosity of the ARC, to be able to make a number of visits to some prominent scientific facilities in the U.K. During my time in England I was lucky enough to visit laboratories based at the Sanger Wellcome Trust Centre, The John Radcliffe Hospital in Oxford and Imperial College London.

If someone didn't tell you where Imperial College was, you'd be excused for walking straight past it. Nestled snugly between the British Museum and the Royal Albert Hall, it's unassuming façade hides some truly impressive and modern lab complexes. Whilst at Imperial College I was kindly looked after by Professor Neil Fairweather, a member of the Centre for Molecular Microbiology and Infection (CMMI), who is well known for his work on the S-layer of *Clostridium difficile*. The work of the Fairweather lab on *Clostridial* pathogens, particularly those that cause enteric diseases, gave me an exciting opportunity to share my research with scientist with a very specific interest in my field of study.

Following this I travelled to Cambridge and the Wellcome Trust Sanger Centre. Most scientists will have heard of the Sanger centre and be well aware of its reputation, and a visit to the site does not disappoint! The grounds are impressive, the science exciting and the centre seems to be a rare thing in science indeed when I was told that the main limiting factor for getting work done was "a lack of people and not a lack of money". Many of us are used to the situation being the other way round! Dr. Trevor Lawley generously took time out of his busy schedule to show me his work and discuss the ups and downs of a career as a Post-Doc. Like Professor Fairweather, Dr. Trevor Lawley also works on *Clostridium difficile*, however Dr. Lawley's interest revolves around the genomics of clinical isolates, specifically doing some very interesting work of the evolution of strains in clinical settings.

The third of my visits was to the research group of Professor Rosalind Harding of Oxford University. Professor Harding has a varied research group looking at both veterinary and human bacterial pathogenesis. I unfortunately was unable to meet Professor Harding in person and instead was put onto Dr. Bernadette Young, a member of Professor Harding's research group based at the prestigious John Radcliffe research hospital. Here I was able to gain an eye opening insight into the use of next generation sequencing technology in a directly applicable clinical environment.

These valuable visits would not have been possible without the support of a number of organisations that supplied the funds for this trip; they include the ARC centre of excellence for structural and functional microbial genomics, Monash University and the CSIRO. A special thanks is extended to those kind individuals who took time out of what are often extremely busy schedules to show me their facilities and discuss their science as well as allowing me to discuss my own research.

- Ben Wade

Research training and professional education

Centre post graduate recruitments

Priyangi Alwis (PhD)

Virulence mechanisms in melioidosis

Amy King (PhD)

Protein interactions in *Leptospira*

Shu-lin (Alicia) Lai (M Biomed Sci)

Autophagy and infection of mammalian cells by *B. Pseudomallei*

Hamish McWilliams (PhD)

Novel approaches for the development of vaccines against blood flukes, *Schistosoma japonicum*

Sarah Preston (PhD)

Identification and modulation of cells and mediators in parasite resistant sheep

Adam Shahine (PhD)

Mycobacterial drug targets

Lakmini Weeramantri (PhD)

Role of conjugative plasmids of *Clostridium perfringens* in toxin production and virulence

Centre post graduate course completions

Gavin Higgins (PhD)

Diverse modes of cell death involving oxidative stress and mitochondria in primary cortical neurons

Heling Ng (PhD)

Mitochondrial gateway to cell death: characterisation of redistribution of mitochondrial proteins during apoptosis

Kristina Youngs (nee Turcic) (M Biomed Sci)

OTP1 – A novel open reading frame required for mitophagy in yeast

Honours students

Danielle Danastasio (B. Sc (Hons))

Type IV fimbrial biogenesis of *Dichelobacter nodosus*

Anindita Das (B. Sc (Hons))

Mechanism of plasmid replication in *Clostridium perfringens*

Sam Forster (B. BioMedSci (Hons))

Systems biology of interferon responses in innate immunity

Centre student achievements

Tanya D’Cruze

Awarded poster presentation prize at the Sixth World Melioidosis Conference, Townsville, Australia.

Sam Forster

Awarded the Deans award for top marks in BBioMedSci (Honours).

Hamish McWilliams

Awarded a NHMRC-Dora Lush Scholarship; Dean’s award scholarship; and the Australian Society for Parasitology travel award.

Heling Ng

Treasurer of the Local Organising Committee for the Young Scientist Forum at OzBio2010, 23 to 26 September 2010.

Sarah Preston

Awarded a scholarship from the Australian Sheep CRC.

Jessica Wisniewski

Awarded a travel fellowship by the International Society for Plasmid Biology to attend the International Plasmid Biology Conference in Bariloche, Argentina.

Undergraduate teaching

In 2010, Centre Chief Investigators delivered undergraduate lectures at first to fourth year levels in microbiology, biochemistry, bioinformatics, molecular biology, proteomics, pathogenesis, and structural biology at Monash University.

Professor Ben Adler

MIC 3990 – Microbiology in action research project (convenor)

Professor Ross Coppel

MED2031 – Medical Studies in Microbiology and Infectious Diseases

MED2042 – Medical Studies in Microbiology and Infectious Diseases

MIC3041 – Medical Microbiology

Professor Rod Devenish

BCH3031 – Advanced Molecular Biology: Modern concepts and applications (convenor)

MED1011 – Medicine 1

MIC3032 – Pathogenesis of bacterial infectious diseases

Professor Els Meeusen

BTH3012 – Biotechnology

Professor Phillip Nagley

BMS2021 – Biochemistry in human function

Professor Julian Rood

MIC3011 – Molecular Microbiology

MED1011 – Medicine 1

MED2031 – Medicine 2

GMA1011 – Medicine 1 (Gippsland)

MIC3032 – Pathogenesis of Bacterial Infectious Diseases

MIC4100 – Microbiology research project (Honours – convenor)

MIC4200 – Advanced Studies in Microbiology (Honours – convenor)

Other research training and professional education

Annual Micromon Recombinant DNA Techniques Short Course (November 2010)

Centre staff again participated in the Annual Micromon Recombinant DNA Techniques Short Course in 2010. Centre Associate Dr John Boyce was part of the teaching team that delivered expert lectures in the course. In addition, Centre PhD students were involved in the laboratory tuition. The Recombinant DNA Techniques course is an intensive, short course designed to teach essential skills to participants from all scientific disciplines that have had little or no experience in molecular biology. The course, which usually enrolls 40 participants, comprises 10 hours of theory and 30 hours of experimental laboratory work and tutorials ranging from topics on basic cloning requirements to reverse transcription and polymerase chain reaction (PCR). It is also an ideal workshop for those who wish to consolidate their current and basic-intermediate skill level and attracts participants from diverse backgrounds in private, government, scientific, clinical, educational and commercial organisations.

For information on future courses contact Mark Cauchi.

Email: Mark.Cauchi@monash.edu

Tel: +61 3 9905 4830.



Participants in the 2010 Micromon Recombinant DNA Techniques Short Course.

International, national and regional links and networks

International visitors to the Centre

Ms Ragnhild Bager,

University of Copenhagen, Denmark.

Professor Carlos Carmone,

Universidad de Montevideo, Uruguay.

Professor Tim Cawston

(Dean of Research), Newcastle University, UK.

Associate Professor Xiang-Dang Du,

Henan Agricultural University, Zhengzhou, P.R. China.

Dr. Jorge F. González,

Universidad de Las Palmas de Gran Canaria, Spain.

Professor Henk Haagsman,

University of Utrecht, The Netherlands.

Professor Dieter Imboden,

(CEO), Swiss National Science Foundation, Geneva, Switzerland

Dr Isabelle da Piedade,

University of Copenhagen, Denmark

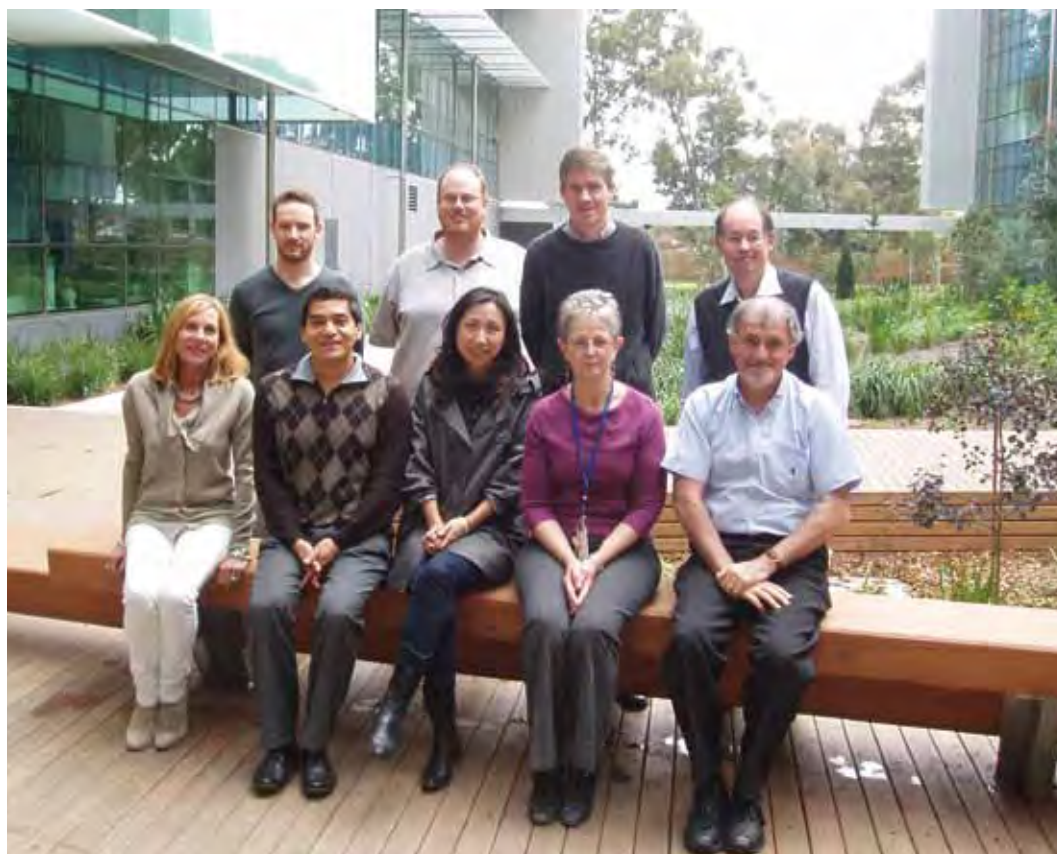
Professor Brian Stevenson,

University of Kentucky, USA.

Professor Elizabeth Wellington

and **Dr Leo Calvo-Bado,**
University of Warwick, U.K.

Delegation from Malaysian Genome Institute



Professor Elizabeth Wellington and Dr Leo Calvo-Bado from the University of Warwick, together with some members of the Centre.

Income derived from other sources

This list includes all active grants received by Centre Investigators in 2010. Monetary figures denote the amount allocated for 2010 and principal collaborators are indicated in parenthesis.

Professor Ben Adler

NHRMC Project Grant – Analysis and regulation of leptospiral virulence factors. \$153,000

NHMRC Project Grant – A functional and structural approach to understanding leptospiral host pathogen interactions. \$170,000 (Beddoe)

Professor Ross Coppel

NHMRC Program Grant – Malaria: from target identification to therapeutics. \$992,000 (Cooke, Plebanski, von Itzstein)

NHMRC Project Grant - Functional and structural studies of a glycosyltransferase essential or complex glycolipid biosynthesis in Mycobacteria. \$162,750 (Crellin)

National Institutes of Health (NIH), USA – Adherence of malaria-infected red cells. US\$116,000 (Cowman, Cooke)

National Institutes of Health (NIH), USA – Malaria and the red blood cell. US\$130,000 (Mohandas)

Professor Rod Devenish

ARC Discovery Grant – Autophagy and the nucleus. \$94,000

NHMRC Project Grant – How Burkholderia evades autophagy. \$152,000 (Boyce, Prescott)

Monash Faculty Strategic Grant – Studying autophagic events by correlative light electron microscopy. \$55,000 (Ramm, Prescott, Harper)

ARC LEIF Grant – Electron microscopy cryopreparation facility for biomedical research. \$250,000 (Harper, Lithgow, Mitchell, Lackmann, Ramm, Mak)

Professor Paul Hertzog

NHMRC Project Grant – Structural characterization function analyses of type 1 interferon-receptor. \$165,500 (Crack)

NHMRC Special Initiative – Development of a signature for early responses to influenza strains that determine/correlate with severity of infection, vaccine response and changes in virulence. (Visvanathan, Reading, Williams)

NHMRC Project Grant – New mechanisms of immunomodulation by interferon transsignaling. \$172,916

Cancer Council Victoria – Suppression of Type 1 interference defence pathways as a mechanism for breast cancer metastasis to bone. \$196,900. (Parker)

Victoria DIIRD and Provincial Government of Manitoba – Monman research program in murine models of cancer and inflammation. \$200,000 (Jenkins, O'Bryan, Hicks)

DEST and China – China-Australia Phenomics Program in Influenza. \$500,000 (Goodnow, Bertram, Hilton, Turner, Doherty)

ACRF Medical Genomics Facility for next generation sequencing. \$1,600,000 (Williams, Watkins, Jenkins, Gillespie, Fuller)

Professor Els Meeusen

NHMRC Development Grant – The Respire™ system: Portable pulmonary delivery platform for rapid, flexible and highly efficient treatment of elderly, paediatric and physically-compromised patients with chronic respiratory diseases. \$264,000 (Yeo, Friend, Morton, McIntosh)

Ministerio de Educación y Ciencia Spain National Grant – In vivo depletion of $\gamma\delta$ T-cells and eosinophilia and its effects on the resistance of the Canaria Hair Breed sheep against *Haemonchus contortus*. 33,000 €, (Gonzalez, Piedrafita, Molina, Rodriguez, Hernandez)

Seaweed Canaria, Spain – Bioactive compounds in seaweed. 20,000 € (Piedrafita)

ARC Linkage Grant – Characterisation and development of adjuvants for new generation veterinary and human vaccines. \$ 110,000

ARC Linkage Grant – Plant cells for improved oral delivery of vaccines. \$147,561 (Walmsley, Finnin, Hamill, Sanson, Webb)

ARC Linkage Grant – Development of a prototype vaccine against gastrointestinal nematode larvae. \$240,000

ARC Discovery Grant – Molecular determinants of an allergic response. \$110,000 (O'Hehir)

Professor Phillip Nagley

NHMRC Project Grant – Mitochondria: molecular and cellular insights into their diverse contributions to neuronal injury. \$177,750 (Beart)

NHMRC Project Grant – Secretion of alpha-synuclein: a diagnostic marker for Parkinson's Disease and a clue to its (patho)physiology. \$192,000 (Horne)

Professor Julian Rood

Australian Poultry CRC – Vaccine against *Clostridium perfringens* to protect birds from necrotic enteritis. \$36,411 Monash component only (Moore, Keyburn)

ARC LIEF – Establishing a small angle x-ray scattering facility \$500,000 (Beddoe, Bottomley, Cowieson, Hearn, Hertzog, Rossjohn, Whisstock, Wilce, Williams)

NHMRC Project Grant – Virulence mechanisms in hypervirulent epidemic strains of *Clostridium difficile*. \$110,000 (Lyras, Johnson, Gerding)

NHMRC Project Grant – Regulation of toxin production in *Clostridium difficile* \$151,133 (Lyras)

NHMRC Project Grant – Role of regulatory genes in the control of toxin production in *Clostridium perfringens*. \$177,000

NHMRC Project Grant – The pathogenesis of infections caused by *Clostridium sordellii* \$109,375 (Lyras, Aronoff)

ARC Discovery Grant – The role of virulence factors of *C. difficile* in food animals \$110,000 (Lyras, Riley, Songer)

Cancer Council Victoria Grant – *Clostridium*-directed enzyme prodrug therapy \$96,296 (Brown, Carter)

NIH/NIAID – *Clostridium perfringens* type B-D virulence plasmids US\$90,481 Rood Lab allocation only (McClane, Uzal)

Professor Jamie Rossjohn

ARC Discovery Grant – A structural investigation into the Peptide-loading complex molecular machine \$240,000

NHMRC Project Grant – An X-ray crystallographic investigation into the adaptive immune response to Epstein-Barr Virus. \$150,000 (Purcell)

NHMRC Program Grant – Antigen presentation, recognition and the immune response. \$510,000 (McCluskey, Carbone, Heath, Brooks, Shortman)

ARC Federation Fellowship – An investigation into infection, immunity and rational drug design. \$330,000

ARC Discovery Grant – A structural investigation into events within the immunological synapse. \$190,000 (McCluskey)

Cancer Council – A structural and functional investigation into tumor rejection by NKT cells \$100 (McCluskey)

Professor Ian Smith

NHRMC Program Grant – Control of proteases in infectious, degenerative and cardiovascular disease Program. \$2,200,000 (Whisstock, Bird, Bottomley, Buckle, Pike)

NCRIS – Proteomics Australia (BPA) \$750,000

NHMRC Project Grant – Melanocortin regulation of reproduction. \$210,000

ARC Discovery Grant – Structural and functional alteration of red blood cells by *Babesia* parasites \$130,000 (Cooke, McElwain, Narta)

Victoria Science Agenda (VSA) Strategic Project Fund – Victorian BioMedical Imaging Capability, \$8,500,000

Professor James Whisstock

ARC Federation Fellowship – Structural and functional studies on Membrane Attack Complex / Perforin-like proteins \$328,000

ARC Discovery Grant – Membrane Attack Complex / Perforin-like proteins \$273,000 (Dunstone)

ARC Discovery Grant – Structural and Functional Studies on Prokaryote Serpins \$87,000 (Bird, Pike)

NHMRC Project Grant – Structural and Functional Studies on Glutamate Decarboxylase \$160,000 (Rowley)

NHMRC Program Grant – Control of proteases in infectious, degenerative and cardiovascular diseases \$2.2 million (Bird, Bottomley, Buckle, Pike, Smith)

ARC LIEF – Establishing a small angle x-ray scattering facility \$500,000 (Beddoe, Bottomley, Cowieson, Hearn, Hertzog, Rood, Rossjohn, Wilce, Williams)

ARC SuperScience Fellowship – Pore forming proteins as delivery devices \$278,000 (Boyd, Friend, Houriggan, Porter)

Wellcome Trust Seeding Drug Discovery Award – Development of a novel small molecule treatment for cerebral malaria through inhibition of perforin \$400,000 (Denny, Engwerda, Spicer, Sullivan, Trapani)

NHMRC Project Grant – Structural and Functional studies on perforin \$210,000 (Trapani, Voskoboinik)

Awards and recognition

Dr Travis Beddoe

- Pfizer Fellowship commencing in 2010

Prof James Whisstock

- Australian Academy of Science Gottschalk Medal for 2010

End-user links

Government, industry and business briefings

Centre Business Breakfast Meeting

The Centre organised a business breakfast meeting on 29 April, held at the Park Hyatt Hotel in Melbourne. Centre Director Prof Ben Adler and Chief Investigators Profs Julian Rood, Ian Smith and Ross Coppel represented the Centre to present an important facet of the Centre's research program which has valuable applied and commercial value.

Over 40 people attended the meeting with significant representations from various biotech and pharmaceutical companies, investment firms and government organisations including:

Austrade, Avexa, Bayer Australia, Biota Holdings Ltd, Cellestis, CSIRO, CSL Ltd, GBS Ventures, Invetech, Murigen Therapeutics, Pfizer Animal Health, Rural Industries Research and Development Corporation, Starfish Ventures, Trans Tasman Fund Management, Victorian Department of Innovation, Industry and Regional Development

BioMelbourne Network Breakfast Meeting

Professor Paul Hertzog was invited to the BioMelbourne Network Breakfast on 13 April, where he networked with other representatives in Bioindustry. He also gave a short presentation 'Understanding Immune Responses to Influenza – a basis for diagnosis, vaccine development and public policy?' which addresses his research on innate immunity and how it affects efficacy of vaccinations.

Government Linkages

Professor Smith has made regular presentations to both state and federal government representatives regarding the research bioplatfroms that underpin the Centre's research activities. Furthermore, Professor Smith hosted a state government delegation on a visit to Monash University to see first hand the facilities available to the Centre researchers. In addition, many of the Centres core platform technologies are listed and promoted in the recently established Victorian Platform Technologies Network (VPTN) which is promoted on the Victorian Government "Bio-Portal".

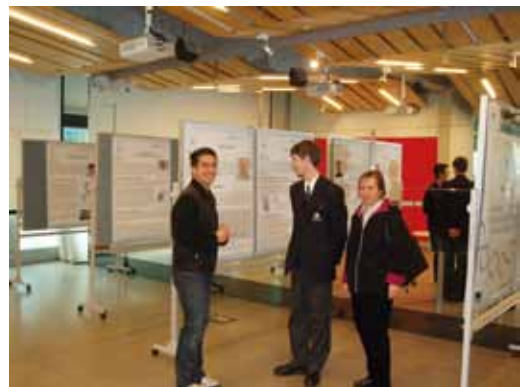
Public Awareness activities

As part of the National Science Week program and also the Monash University Research Matters week, the Centre held an open day on 20 August for the public to visit the Centre facilities and to present the research undertaken. A series of posters highlighting the Centre's chief investigators and their work was exhibited for a week at open space of the Centre building. These posters were created with a general audience in mind so that they could understand and appreciate the value and significance of the research that is being carried out in the Centre. Centre staff was also present at the open day to answer any questions from the public. In addition to the poster exhibition, the public was taken on a general tour of the Centre's facilities. Significantly, the open day impressed and inspired a number of high school students who were part of the public audience to further their studies in science.

With the aim of stimulating research awareness for high school students, three Year 10 students from the John Monash Science School, Damien Kaluarachchi, Lillian Leng and Mizna Shahbaz, were given the opportunity to spend a one week (21 – 25 June) attachment in the Centre's laboratories and work on some of the ongoing research projects. This opportunity provided them with the experience of working in a research-intensive environment in the life sciences which they thoroughly enjoyed.



Centre's publicity entry for National Science Week 2010



Centre Operations Officer, Desmond Gul explaining the research and activities of the Centre to students from John Monash Science School at the open day poster exhibition.

Other Chief Investigator professional activities

Professor Ben Adler

- Editorial Board, *Veterinary Microbiology*
- Editorial Board, *Veterinary Sciences Tomorrow*
- Organising Committee, *First Prato Conference on the Pathogenesis of Bacterial Infections of Animals*, Prato, Italy
- Organising Committee, *Modern Veterinary Vaccines and Adjuvants*, Budapest, Hungary
- Scientific Committee, *World Melioidosis Conference*, Townsville, Australia.
- Academic Editor, *PLoS One*
- Chief Guest Handling Editor, *Veterinary Microbiology Special Issue*
- Scientific Advisory Council, Institut Pasteur de Nouvell Calédonie
- Vice-Chair, 2012 Gordon Conference on *The Biology of Spirochetes*

Professor Ross Coppel

- Member of the NHMRC Academy

Professor Rod Devenish

- Editorial Board, *Autophagy*
- OZReader, ARC
- Organiser and co-chair of the Autophagy symposium at the 2010 Melbourne IUBMB/FAOBMB/ComBio Conference.
- Co-chair, Symposium on 'Autophagy and Phagocytosis in Infection and Inflammation' at the 2010 AH&MR Congress, Melbourne

Professor Els Meeusen

- Editor, *Parasite Immunology* Special Issue on Australian ImmunoParasitology Research.
- Editorial Board, *Parasite Immunology*
- Editorial Board, *ISRN Veterinary Science*
- Co-Chair, Organising Committee, *Modern Veterinary Vaccines and Adjuvants*, Budapest, Hungary

Professor Phillip Nagley

- Chair, School of Biomedical Science Education Committee, Monash University
- Co-Chair of OzBio2010 - 12th IUBMB – 21st FAOBMB-ComBio2010 Conference, "The molecules of life: from discovery to biotechnology", Melbourne, 2010
- Convenor of Young Scientist Forum at OzBio2010, September 2010

Professor Julian Rood

- Editor, *Plasmid*
- Editorial Board, *BMC Microbiology*, *Anaerobe*
- Chair, *First Prato Conference on the Pathogenesis of Bacterial Diseases of Animals*, Prato, Italy
- Ambassador for Australia, New Zealand and Oceania, American Society for Microbiology.

Professor Jamie Rossjohn

- Editorial Board, *Protein and Peptide Letters*
- Editorial Board, *Essays in Biochemistry*
- Chair, NHMRC Training Fellowship Panel

Professor Ian Smith

- Handling Editor, *Journal of Neurochemistry*,
- Editorial Board Member, *Journal of Molecular and Cellular Proteomics*,
- Editorial Board Member, *Protein and Peptide Letters*,
- Editorial Board Member, *International Journal of Peptide Research and Therapeutics*,
- Editorial Board Member, *Current Proteomics*,
- Editorial Board Member, *The Open Proteomics Journal*,
- Editorial Board Member, *Clinical Proteomics*,
- Board member NCRIS funded National Imaging Facility (NIF)
- Board member Victorian Life Science Computing Facility (VLSCI)
- Board member Monash Biomedical Imaging Laboratories (MBIL)
- Director, Victorian node Proteomics Australia
- Chairman, NHMRC RMGS User Reference Group (RURG)
- Member, The BioSciences Victoria Collaborative
- Chairman, Victorian BioMedical Imaging Consortium

Professor James Whisstock

- Organising committee, *34th Lorne Conference on Protein Structure and Function*
- Vice-Chair, *2012 Gordon Conference on Proteolytic Enzymes and Their Inhibitors*

Appendix 1: Publications

Journal articles

This list includes all publications published in 2010 by Centre affiliated researchers.

These publications have varying levels of input from Centre staff and students. Centre byline publications are indicated with an asterisk.

Abeynaike, L., **E.N.T. Meeusen**, and R.J. Bischof. (2010) An ovine tracheal explant culture model for allergic airway inflammation. *Journal of Inflammation*, 7: 46.

* **Adler, B.**, and A. de la Peña Moctezuma. (2010) *Leptospira* and leptospirosis. *Veterinary Microbiology*, 140(3-4): 287-296.

Ang D.K., C.V. Oates, R. Schuelein, M. Kelly, F.M. Sansom, D. Bourges, L. Boon, **P.J. Hertzog**, E.L. Hartland, and I.R. van Driel. (2010) Pulmonary *Legionella pneumophila* is controlled by plasmacytoid dendritic cells but not type I interferon. *The Journal of Immunology*, 184(10):5429-5433.

* **Beddoe, T.**, A. W. Paton, **J. Le Nours**, **J. Rossjohn**, and J. C. Paton. (2010) Structure, biological functions and applications of the AB5 toxins. *Trends in Biochemical Sciences*, 35(7): 411-418.

Burrows, S.R., Z. Chen, J.K. Archbold, F.E. Tynan, **T. Beddoe**, L. Kjer-Nielsen, J.J. Miles, R. Khanna, D.J. Moss, Y.C. Liu, S. Gras, L. Kostenko, R.M. Brennan, C.S. Clements, A.G. Brooks, A.W. Purcell, J. McCluskey, and **J. Rossjohn**. (2010) Hard wiring of T cell receptor specificity for the major histocompatibility complex is underpinned by TCR adaptability. *Proceedings of the National Academy of Sciences of the United States of America*, 107(23): 10608-10613.

Carter, G.P., **D. Lyras**, R. Poon, P.M. Howarth, and **J.I. Rood**. (2010) Methods for gene cloning and targeted mutagenesis. *Methods in Molecular Biology*, 646: 183-201.

Carter, G.P., **J.I. Rood**, and **D. Lyras**. (2010) The role of toxin A and toxin B in *Clostridium difficile*-associated disease: Past and present perspectives. *Gut Microbes*, 1(1): 58-64.

Chaisakul J, N. Konstantakopoulos N, **A.I. Smith**, and W.C. Hodgson. (2010) Isolation and characterisation of P-EPTX-Ap1a and P-EPTX-Ar1a: Pre-synaptic neurotoxins from the venom of the northern (*Acanthopis praelongus*) and Irian Jayan (*Acanthopis rugosus*) death adders. *Biochemical Pharmacology*, 80: 895-902.

* Cheung, J. K., **A.L. Keyburn**, G.P. Carter, A.L. Lanckriet, F. Van Immerseel, **R.J. Moore**, and **J.I. Rood**. (2010) The VirSR two-component signal transduction system regulates NetB toxin production in *Clostridium perfringens*. *Infection and Immunity*, 78(7): 3064-3072.

* **Crellin, P.K.**, J.P. Vivian, **J. Scoble**, F.M. Chow, N.P. West, **R. Brammananth**, N. I. Proellocks, A. Shahine, **J. Le Nours**, M.C. Wilce, W.J. Britton, **R.L. Coppel**, **J. Rossjohn**, and **T. Beddoe**. (2010) Tetrahydrolipstatin inhibition, functional analyses, and three-dimensional structure of a lipase essential for mycobacterial viability. *Journal of Biological Chemistry*, 285(39): 30050-30060.

de Veer, M., J. Kemp, J. Chatelier, M.J. Elhay, and **E.N. Meeusen**. (2010) The kinetics of soluble and particulate antigen trafficking in the afferent lymph, and its modulation by aluminum-based adjuvant. *Vaccine*, 28: 6597-6602.

Engwerda, C.R., and **E.N. Meeusen**. (2010) Parasites and the immune system: A perspective from down under. Editorial. *Parasite Immunology*, 32: 529-531.

Fox, A., J.F. Maddox, M.J. de Veer, and **E.N. Meeusen**. (2010) $\gamma\delta$ TCR+ cells of the pregnant ovine uterus express variable T cell receptors and contain granulysin. *Journal of Reproductive Immunology*, 84(1): 52-56.

* Godfrey, D.I., D.G. Pellicci, O. Patel, L. Kjer-Nielsen, J. McCluskey, and **J. Rossjohn**. (2010) Antigen recognition by CD1d-restricted NKT T-cell receptors. *Seminars in Immunology*, 22(2): 61-67.

Godfrey, D.I., **J. Rossjohn**, and J. McCluskey. (2010) Fighting infection with your MAITs. *Nature Immunology*, 11(8): 693-695.

Gough, D.J, N.L. Messina, L. Hii, J.A. Gould, K. Sabapathy, A.P.S Robertson, J.A. Trapani, D.E. Levy, **P.J. Hertzog**, C.J.P. Clarke, and R.W. Johnstone. (2010) Functional crosstalk between type I and II interferon through the regulated expression of STAT1. *PLoS Biology*, 8(4): e1000361.

Gras, S., L. Kedzierski, S.A. Valkenburg, K. Laurie, Y.C. Liu, J.T. Denholm, M.J. Richards, G.F. Rimmelzwaan, A. Kelso, P.C. Doherty, S.J. Turner, **J. Rossjohn**, and K. Kedzierska. (2010) Cross-reactive CD8+ T-cell immunity between the pandemic H1N1-2009 and H1N1-1918 influenza A viruses. *Proceedings of the National Academy of Sciences of the United States of America*, 107(28): 12599-12604.

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Grubman, A., M. Kaparakis, J. Viala, C. Allison, L. Badea, A. Karrar, I.G. Boneca, L. Le Bourhis, S. Reeve, **A.I. Smith**, E.L. Hartland, D.J. Philpott, and R.L. Ferrero. (2010) The innate immune molecule, NOD1, regulates direct killing of *Helicobacter pylori* by antimicrobial peptides. *Cellular Microbiology*, 12(5): 626-639.

* **Harper, M.**, A.D. Cox, F. St Michael, M. Ford, **I.W. Wilkie**, **B. Adler**, and **J.D. Boyce**. (2010) Natural selection in the chicken host identifies 3-deoxy-D-manno-octulosonic acid kinase residues essential for phosphorylation of *Pasteurella multocida* lipopolysaccharide. *Infection and Immunity*, 78(9): 3669-3677.

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Hein, W.R., A. Pernthaner, **D. Piedrafita**, and **E.N. Meeusen**. (2010) Immune mechanisms of resistance to gastrointestinal nematode infections in sheep. *Parasite Immunology*, 32: 541-548.

Higgins, G.C., P.M. Beart, Y.S. Shin, M.J. Chen, N.S. Cheung, and **P. Nagley**. (2010) Oxidative stress: Emerging mitochondrial and cellular themes and variations in neuronal injury. *Journal of Alzheimer's Disease*, 20(Suppl. 2): S453-S473.

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* **Kennan, R.M.**, **W. Wong**, O.P. Dhungyel, **X. Han**, D. Wong, D. Parker, **C.J. Rosado**, R.H.P. Law, **S. McGowan**, S.B. Reeve, V. Levina, G.A. Powers, R.N. Pike, **S.P. Bottomley**, **A.I. Smith**, I. Marsh, **R.J. Whittington**, **J.C. Whisstock**, **C.J. Porter**, and **J.I. Rood**. (2010) The subtilisin-like protease AprV2 is required for virulence and uses a novel disulphide-tethered exosite to bind substrates. *PLoS Pathogens*, 6(11): e1001210.

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- * Lo, M., G.L. Murray, C.A. Khoo, D.A. Haake, R.L. Zuerner, and **B. Adler.** (2010) Transcriptional response of *Leptospira interrogans* to iron limitation and characterization of a PerR homolog. *Infection and Immunity*, 78(11): 4850-4859.
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Book chapters

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Commentaries

* **Rood, J.I.** (2010) Infectious disease: Listeria does it again. *Nature*, 464(7292): 1138-1139.

Conference abstracts

This list includes all conference presentations in 2010 by Centre affiliated researchers. These presentations have had varying levels of input from Centre staff and students.

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Adler, B. (2010) Comparative analysis of *Leptospira* genomes targeting loci encoding virulence properties. Tenth Gordon Research Conference: Biology of Spirochetes, Ventura, USA.

Adler, B. (2010) Leptospiral genomics and vaccine development. Modern Veterinary Vaccines and Adjuvants, Budapest, Hungary

Adler, B. (2010) Leptospiral pathogenesis in the genomic era. Spirochetes Havana 2010, Havana, Cuba.

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Allwood, E., **L. Gong**, M. Cullinane, M. Prescott, **R. Devenish**, **B. Adler**, and **J. Boyce**. (2010) Elucidating the role of an intracellular motility protein, BimA, in avoidance of host autophagy by *Burkholderia pseudomallei*. Sixth World Melioidosis Conference, Townsville, Australia.

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Bannam, T., **X. Yan**, P. Harrison, T. Seemann, **A. Keyburn**, **R. Moore**, and **J. Rood**. (2010) NetB toxin is located on a conjugative plasmid in *Clostridium perfringens*. Australian Society for Microbiology Annual Scientific Meeting, Sydney, Australia.

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- Hertzog, P.J.** (2010) Regulation of mucosal immunity by a new type I interferon –epsilon in reproductive tract infections. Keystone Symposium on Innate Immunity: Mechanisms Linking with Adaptive Immunity, Dublin, Ireland.
- Hertzog, P.J.** (2010) Regulation of mucosal innate immunity by a new cytokine in reproductive tract infections. Australian Health and Medical Research Conference, Melbourne, Australia.
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- Higgins, G.C., R.J. Devenish, P.M. Beart, and P. Nagley.** (2010) Oxidative stress induces multiple caspase-independent cell death pathways in primary cortical neurons. OZBio 2010, Melbourne, Australia.
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- McClane, B.A., J.I. Rood, F.A. Uzal, S. Sayeed, D.J. Fisher, J. Vidal, A. Gurhar, J. Chen, M. Ma, J. Li, V. Adams, R. Poon, M. L. Hughes, T.L. Bannam, J. Saputo, and K. Miyamoto.** (2010) New insights into the Pathogenesis of *Clostridium perfringens* type B, C and D diseases. First Prato Conference on the Pathogenesis of Bacterial Diseases of Animals, Prato, Italy.
- Meeusen, E.N.** (2010) Mucosal immunity and mucosal adjuvants: facts or fiction? Modern Veterinary Vaccines and Adjuvants, Budapest, Hungary.
- Mijaljica, D., M. Prescott, and R.J. Devenish.** (2010) Vacuolar turnover of the nucleus in *Saccharomyces cerevisiae* by late nucleophagy. OZBio 2010, Melbourne, Australia.
- Rood, J.I., M.M. Awad, J.M. Brown, and G.P. Carter.** (2010) The Clostridia – Tumour Targeting Superbugs? Symposium Abstract, Australian Society for Microbiology Annual Scientific Meeting, Sydney, Australia.
- Rood, J.I.** (2010) *Dichelobacter nodosus*, the causative agent of ovine footrot: Pathogenesis, genomics and vaccine development. 11th International Symposium on the Genetics of Industrial Microorganisms, Melbourne, Australia.
- Rood, J.I., G.P. Carter, P.M. Howarth, J.R. O'Connor, S.P. Sambol, S. Johnson, D.N. Gerding, R. Govind, A. Antunes, B. Dupuy, J. Spencer, G.R. Douce, and D.Lyras.** (2010) The pathogenesis of *Clostridium difficile* infections. Joint meeting of the NZ Microbiological Society and NZ Society for Biochemistry & Molecular Biology, Auckland, New Zealand.
- Steen, J., X. Gatsos, P. Harrison, T. Seemann, M. Harper, I. Wilkie, B. Adler, and J. Boyce.** (2010) Fis is a critical regulator of virulence in *Pasteurella multocida*. First Prato Conference on the Pathogenesis of Bacterial Diseases of Animals. Prato, Italy.
- Smith, A.I.** (2010) Peptide acetylation in the brain: Impact on appetite and energy homeostasis. PacifiChem2010, Hawaii, USA.
- Smith, A.I.** (2010) Peptide acetylation in the brain: Impact on appetite and energy homeostasis. International Peptide Meeting, Kyoto, Japan.
- Smith, A.I.** (2010) Perspectives for research infrastructure in biomedical imaging (ESFRI). Euro-Bioimaging 2nd Stakeholder Meeting, Vienna, Austria.
- Smith, A.I.** (2010) New developments in biomedical imaging for neuroscientists. International Brain Research Organisation, Kuala Lumpur, Malaysia.
- Smith, A.I.** (2010) Peptidomic analysis of brain and pituitary tissues reveals a novel post-translational processing mechanism for regulating appetite and energy homeostasis. International HUPO Meeting, Sydney, Australia.
- Smith, A.I.** (2010) Technology platforms at Monash University – The biotech-academic interface. Peptide symposium on biomolecules for diagnostics and therapeutics, Prato, Italy.
- Treerat, P., P. Alwis, M. Prescott, R. Devenish, B. Adler, and J. Boyce.** (2010) Importance of the type III secretion system genes *bapA*, *bapB* and *bapC* in the virulence of *Burkholderia pseudomallei*. Sixth World Melioidosis Conference, Townsville, Australia.
- Uzal, F.A., B.A. McClane, J.I. Rood, S. Sayeed, D.J. Fisher, J. Vidal, A. Gurhar, J. Chen, M. Ma, V. Adams, R. Poon, M.L. Hughes, and J. Saputo.** (2010) Animal models to study the pathogenesis of *Clostridium perfringens* type D infections. Trans-RCE (NIH Regional Centers of Excellence) Workshop on Toxins, Bethesda, MD, U.S.A.
- de Veer, M.J., J. Van Gramberg, J. Chatelier, M. Elhay, and E. Meeusen.** (2010) Effects of different adjuvant formulations on cellular migration, maturation and antigen trafficking into ovine afferent lymphatics. Australian Society for Immunology, Perth, Australia.

Wade, B., A.L. Keyburn, M.E. Ford, J.I. Rood, and R.J. Moore. (2010) *Clostridium perfringens* genes with implications for both virulence and colonisation during necrotic enteritis. First Prato Conference on the Pathogenesis of Bacterial Diseases of Animals, Prato, Italy.

Wee, E.L.J., J. Singleton, **J.I. Rood**, G.P. Carter, and **D. Lyras.** (2010) Microscopic examination of *Clostridium difficile* spores and comparison to the other clostridial species spores. Australian Society for Microbiology Annual Scientific Meeting, Sydney, Australia.

Whisstock, J.C. (2010) Structural studies on Malarial proteases. Gordon Research Conference on Proteolytic Enzymes and their Inhibitors, Lucca, Italy.

Whisstock, J.C. (2010) The molecular function and dysfunction of membrane attack complex/perforin-like proteins. 24th Annual Symposium of the US Protein Society, San Diego, USA.

Whisstock, J.C. (2010) The molecular function and evolution of membrane attack complex/perforin-like (MACPF) proteins. First Prato Conference on the Pathogenesis of Bacterial Diseases of Animals, Prato, Italy.

Wisniewski, J.A., T.L. Bannam, W.L. Teng, and J.I. Rood. (2010) Role of the *tcp* genes in conjugative plasmid transfer in *C. perfringens*. Australian Society for Microbiology Annual Scientific Meeting, Sydney, Australia.

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Appendix 2: Financial statement

Financial statement for year ended 31 December 2010.

Income	\$
ARC	
CoE Program	1,908,607
Industry/Private Funds	
Sheep CRC	30,000
Poultry CRC	61,360
University of Copenhagen	15,000
Host Institution Support	699,000
Sale (Goods and Services)	56,362
Carried forward from 2008	
ARC funds	413,279
Host institution	1,464,825
Total Income	4,648,433
Expenditure	\$
Salaries	2,318,580
Scholarships, Prizes & Grants	46,171
Equipment & IT	14,172
Print and Media	7,882
Maintenance/Consumables	362,091
Travel and Related Expenses	6,546
Other Expenditure	
Poultry CRC project	61,360
University of Copenhagen collaborative project	15,000
Administrative Charges	2,012
Total expenditure	2,833,814

Appendix 3: Key result areas and performance measures 2010

Key Result Area	Performance Measure	Target	Outcome
Research findings	Quality of publications	At least 75% on journals with an impact factor (IF) of ≥ 4.0	68% of papers were published in journals with IF ≥ 4.0 . 84% of papers were published in journals with ERA ranking of A* or A.
	Number of publications	20 refereed publications in international journals. 2 invited book chapters or reviews	37 refereed publications, including 3 invited book chapters.
	Number of patents	1 per year	0. Since 2005, a total of 13 patents have been filed.
	Invitations to address and participate in international conferences	6 per year	16
	Invitations to visit leading international laboratories	4 per year	19
	Number and nature of commentaries about the Centre's achievements	2 per year	1 radio interview and 7 published commentaries
	Research training and professional education	Number of postgraduates recruited	4 per year
Number of postgraduate completions		2 per year	3
Number of Honours students		6 per year	4
Participation in professional courses		1 per year	Centre Associate and Centre PhD students taught in Recombinant DNA Techniques Short Course at Monash University, Nov 2010
Number and level of undergraduate and high school courses in the priority area(s)		2 at 3 rd year level	BCH3031 – Advanced Molecular Biology BMS2062 – Introduction to Bioinformatics BMS3021 – Molecular Medicine and Biotechnology MIC3011 – Molecular Microbiology MIC3032 – Pathogenesis of Bacterial Infectious Diseases MIC3041 – Medical Microbiology MIC3990 – Action in Microbiology MOL2022- Molecular Biology

Key Result Area	Performance Measure	Target	Outcome
International, national and regional links and networks	Number of international visitors	2 per year	11
	Number of visits to overseas laboratories	4 per year	19
End-user links	Nature and number of commercialisation activities	3 partnerships involving cash	Australian Poultry CRC Australian Sheep CRC Seaweed Canarias S.L.
	Number of government, industry and business briefings	1 per year	3
	Number of Centre associates trained/ing in technology transfer and commercialisation	1 per year	1
Organisational support	Number and nature of public awareness campaigns	1 per year	1
	Annual in-kind contributions from Collaborating Institutions	\$350,000 per year	~\$744,600
	Number of new organisations recruited to or involved in the Centre	1 per year	University of Warwick, UK University of Bonn, Germany
	Level and quality of infrastructure provided to the Centre	\$730,000 per year in equipment, \$400,000 per year in personnel, and \$330,000 per year cash	\$699,000 cash contribution from Monash University. ~\$2.4M in equipment infrastructure and ~\$2.1M in Chief Investigator and other staff salaries.
Governance	Breadth and experience of members of Advisory Board	6 Members specified	Existing board of 4 members and 2 international Adjunct Board Members.
	Frequency and effectiveness of Advisory Board meetings	Twice per year	Met twice in 2010 on March 11 and November 29.
	Quality of Centre Strategic Plan	As outlined in submitted research plan	Post-review changes incorporated into plan.

Key Result Area	Performance Measure	Target	Outcome
	Effectiveness of arrangements to manage Centre nodes	Publication of cross-node papers	Centre is single node
National benefit	Measure of expansion of Australia's capability in the priority area(s)	Substantial increase in knowledge base. Progress in vaccine development and drug target identification	See list of publications. Vaccine development and drug target pipeline projects progressed satisfactorily in 2010. See progress reports in Annual Report
	Case studies of economic, social, cultural or environmental benefits	N/A	N/A



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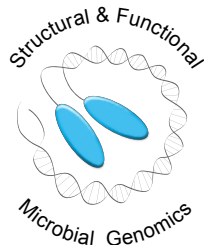
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