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Overview

The ARC Centre of Excellence in Structural and Functional Microbial Genomics brings together a team of internationally-renowned researchers with complementary expertise from the School of Biomedical Sciences at Monash University. The Centre conducts integrated research that elucidates key aspects of microbial pathogens and the hosts they infect, focusing 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, roboticised 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 collaboration with industry partners, Australian Wool Innovation, Pfizer Animal Health and the Australian Poultry CRC. In 2008, fundamental research within the Centre into microbial genomics, pathogenesis and immunity was published in high quality scientific journals such as *Nature, Immunity, Journal of Experimental Medicine,* and *PLoS Pathogens* among others.

The Centre also works in partnership with scientists 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 America.
Financial support

The Centre’s main sources of funding are the ARC Centre grant through the Centres of Excellence program, Monash University and the State Government of Victoria. 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 2008.

Additionally, the Centre continued an ARC Linkage program for vaccine development in leptospirosis (initiated in 2005), with Pfizer Animal Health as the industry partner, with a total income of $850,000 for the period 2006-2008, as well as the continuation of a Poultry CRC grant awarded in early 2006 for a joint Centre-CSIRO project on vaccine development in avian campylobacteriosis and necrotic enteritis.

Footrot Vaccine development within the Centre in collaboration with the University of Sydney is supported by the Australian Wool Innovation, with a total of $450,000.
2008 was an exciting and productive year for the Centre. Towards the end of the year most of the Centre’s scientists moved into new, purpose-built laboratories and facilities within the Monash University Science and Technology Research and Innovation Precinct (STRIP). Others will move early in 2009. The co-location in the new STRIP concept of combined and open plan laboratories will serve to enhance even further the interactions between Centre staff and students and other researchers in the Monash University School of Biomedical Sciences. We look forward with great anticipation to some exhilarating science in the years ahead.

As detailed elsewhere in this report, fundamental research in the Centre on mechanisms of pathogenesis and immunity continued to be published in highly ranked international scientific journals, whilst Centre CIs, staff and students were prominent globally as invited speakers at prestigious scientific conferences in Australia and abroad. On the applied aspects of the Centre’s research program, the ovine footrot vaccine development program proceeded at full swing, with very promising early results, while identified mycobacterial drug targets moved to small molecule screening, with several potential hits obtained. The Protein Production Unit and the Proteomics Facility continued to play key roles in these and other applied projects.

Again, I wish to thank sincerely the multitude of people who have made this year such a successful one. All Centre CIs, students, and scientific and administrative staff have contributed with enthusiasm to this success. I am grateful to members of the Scientific Advisory Board, who continued to give so generously of their time and immense experience and expertise. Their wisdom and sage advice are very much appreciated. Our Associates and Collaborating Partners have likewise been instrumental in many of the major Centre outcomes and I express my thanks to them sincerely.

Professor Ben Adler
Director, ARC Centre of Excellence in Structural and Functional Microbial Genomics
Organisation and governance

Centre Governance and Management Structure

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

Organisational chart

Scientific Advisory Board

Director

PA to Director

Operations Manager

Scientific Committee
Director and Chief Investigators

Protein analysis
sub-committee

Associates
Research fellows
Students

Scientific projects

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 an abiding 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 *Dichelobacter nodosus* infections, 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.

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., Teelecostin 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, the dissection of 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)
Centre management and administration

Research Director

Professor Ben Adler, as Centre Research Director, is responsible for decisions affecting the Centre’s financial, human and infrastructure resources. In addition, his role includes oversight of the overall direction of the Centre Scientific projects. He also manages his own research group, numbering more than 20 staff and students in 2008.

Operations Manager

Marianne Johnston 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.

Personal Assistant

Up until October 2008, Sheree Michael provided administrative and secretarial support to the Centre Director. She was the minutes secretary for the monthly Scientific Committee meetings, Advisory Board meetings and also the Microbiology department Finance Officer.
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.

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 co-convener (with Centre Associate Professor Paul Hertzog) of the Monash Infection and Immunity Network.

### Deputy Director(s)

**Professor Rod Devenish** is Deputy Director of the Research Graduate School and Professor in the Department of Biochemistry and Molecular Biology at Monash University. In 2008 he also became Deputy Director of the Centre taking over from Professor Phillip Nagley in October. 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 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 Ross Coppel** is a Howard Hughes Fellow and a Professor of the Department of Microbiology at Monash University. 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 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. He is head of Monash University's Protein Crystallography Unit and 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 Director of the Monash University's Biomedical Proteomics Facility and Deputy Dean (Research) for the Faculty of Medicine, Nursing and Health Sciences. 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 protein crystallographer and bioinformatician in the Department of Biochemistry and Molecular Biology at Monash University. He has particular expertise in 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.
Committee for the Automated, Multiplexed, High Resolution Protein Analysis Facility (AMHRPAF)

The committee for the AMHRPAF oversees the general operations of the Centre’s mass spectrometry facility.

The Protein Analysis Centre sub-committee consists of three Chief Investigators from the Centre (two from the Biochemistry Department and one from Microbiology), one member from an independent University faculty and a representative from Monash University’s Research Commercialisation Unit and several Centre Associates and/or Research Fellows. The sub-committee meets quarterly and operates under the umbrella governance of the Centre. It also serves as a sub-committee for the Protein Production Unit.

The Centre’s sub-committee comprises:

- Professor Ian Smith (Facility Director and sub-committee Chair)
- Professor Steve Bottomley (Protein Production Unit Head and sub-committee vice Chair)
- Professor Ben Adler (Centre Director)
- Dr John Boyce/Professor John Davies (Department of Microbiology)
- Dr Brian Cooke (Department of Microbiology)
- Professor Mike Hubbard (The University of Melbourne)
- Ms Marianne Johnston (Centre Operations Manager)
- Dr Noeline Quinsey (Manager – Protein Production Unit)
- Dr David Steer (Biomedical Proteomics Facility)
- Dr Tim Stinear (Department of Microbiology)
- Mr Michael Vovos (Monash University – Research Commercialisation Unit)
- Professor James Whisstock (Centre Chief Investigator)
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 or PCU, which is an automated protein production facility that enables researchers to express and purify their proteins in a high throughput manner.

**Dr John Boyce** is a Senior Lecturer in Microbiology and 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*. He is project manager for the Centre’s 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 recognised 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.
Professor Paul Hertzog is the Director of the Centre for Functional Genomics and Human Disease at the Monash Institute of Medical Research (MIMR), Clayton, an Associate Director of the MIMR, and a Principal Research Fellow of the National Health and Medical Research Council of Australia. Professor Hertzog is also a member of the Council of the International Society for Interferon and Cytokine Research and the Co-convenor (with Centre Director Professor Ben Adler) of the Monash Infection and Immunity Network. His research focuses on innate immune mechanisms.

Professor Bruce McClane is a Professor in the Molecular Genetics and Biochemistry 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 recognised 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.

Professor Els Meeusen is a Professor in the Department of Physiology at Monash University which she joined in 2006. Els' work aims to integrate a whole body approach to science into practical applications for animal and human health. Her particular area of expertise is helminth immunology, allergy and vaccine development.

Professor Christina Mitchell is the Head of the School of Biomedical Sciences of the Faculty of Medicine, Nursing and Health Sciences at Monash University. Professor Mitchell trained as a physician scientist and specialised in clinical haematology. She is recognised for her work in cellular signalling and is focussing on the signalling mechanisms in cancers cells and macrophages, and understanding how abnormal signalling can lead to immune diseases. In 2008 she was appointed the ‘Sir John Monash’ distinguished Professor at Monash University.
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 Tim Stinear directs a research group in the Department of Microbiology at Monash University. He studies a devastating human skin disease called Buruli ulcer, a major public health issue in Africa, caused by Mycobacterium ulcerans. Dr Stinear is an expert in the genomics of mycobacteria and his research has led to new diagnostics that have dramatically improved Buruli ulcer patient care, as well as showing how bacteria produce chemicals that suppress the human immune system. His research is helping combat Buruli ulcer and other important mycobacterial diseases such as tuberculosis and leprosy.

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.
Research fellows
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Dr Trudi Bannam
Dr Emma Byres
Dr Paul Crellin
Dr Honhua Ge
Dr Lan Gong
Dr Xiaoyan Han
Dr Marina Harper
Dr Kristy Horan
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Dr Noeline Quinsey
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Dr Judith Scoble
Dr Danielle Smith
Dr David Steer

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Pin Wang
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Professor James McCluskey,
The University of Melbourne
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Professor Peter Stuckey,
The University of Melbourne
Professor Geoff Webb,
Monash University

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Dr Andrew Brooks, The University of Melbourne
Dr Ashley Buckle, Monash University
Dr Meabh Cullinane, Monash University
Dr Om Dungyel, The University of Sydney
Dr Maria Garcia de la Banda, Monash University
Dr Paul Harrison, Victorian Bioinformatics Consortium (VBC)
Dr David Hoke, Monash University
Dr Ben Howden, Monash University
Dr Grant Jenkin, Monash University
Dr Anthony Keyburn, CSIRO
Dr Ruby Law, Monash University
Dr Miranda Lo, Monash University
Dr Sheena McGowan, Monash University
Dr Siew Siew Pang, Monash University
Dr Adrienne Paton, The University of Adelaide
Dr Assunta Pelosi, Monash University
Dr Corrine Porter, Monash University
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Katarzyna Rainczuk, Monash University
Shane Reeve, Monash University

PhD students
Sadia Deen, Monash University
Ben Wade, CSIRO Livestock Industries
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.
- To train a new generation of multi-skilled researchers based on the Centre’s broad range of advanced technologies.
The Centre’s 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 Monash 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 one and two-dimensional gel analysis. There is also an ion trap mass spectrometer with ETD (electron transfer dissociation) capabilities, a quadrupole time of mass spectrometer (Q-ToF) and a nano LC system which allows the very sensitive separation of tiny (sub picomole) amounts of proteins and peptides prior to mass spectrometry analysis.

The facility thus has the capabilities to meet all current proteomic requirements of the Centre’s researchers as well as providing the state of Victoria with the qualified personnel necessary to support its growing biotechnology industry, especially in the area of proteomics.

The facility is also the Victorian node of Proteomics Australia – an initiative of the National Collaborative Infrastructure Scheme (NCRIS).

Two ARC Centre staff, Dr David Steer and Ms Josie Lawrence work under the directorship of Centre CI Professor Ian Smith.
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 for commercial and academic research facilities to alleviate the often time-consuming task of protein production. Housing a Tecan Freedom EVO liquid handling robot and ÄKTAxpress™, 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. ÄKTAxpress™ is a dedicated high throughput chromatography system for a multi-dimensional purification of His- and GST-tagged proteins.

The unit has developed a number of different purification strategies that have been implemented with the high throughput pipeline of various Centre projects and is a fundamental part of the Centre’s vaccine development and drug target identification processes.

In addition, much of the protein production pipeline has required the development of novel informatic approaches to track and manage targets – these have continued development through 2008. One of the other major functions of the unit has been to develop the use of liquid handling robots to explore the expression space for a variety of different recombinant constructs. Protocols are currently being developed to analyse the effect of additives on the stability on numerous proteins in a high throughput manner. They will allow proteins to be concentrated or stored in various buffers that either increase the solubility or stability of the resulting proteins.

In 2008 over 480 proteins were purified using the unit’s novel purification strategies, – the majority of these proteins had not been expressed or purified before – (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.

<table>
<thead>
<tr>
<th>Project</th>
<th>Number of proteins purified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fowl cholera vaccine</td>
<td>20</td>
</tr>
<tr>
<td>Footrot vaccine</td>
<td>65</td>
</tr>
<tr>
<td>Leptospirosis vaccine</td>
<td>125</td>
</tr>
<tr>
<td>Swine dysentery vaccine</td>
<td>30</td>
</tr>
<tr>
<td>Necrotic enteritis vaccine</td>
<td>80</td>
</tr>
<tr>
<td>Crystallisation projects</td>
<td>130</td>
</tr>
</tbody>
</table>

Table 1. Purification of proteins by the Protein Production Unit.
Protein Crystallography Unit

The Protein Crystallography Unit was established at Monash University in 2002. Protein Crystallography is the major tool for solving the 3-dimensional 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 unit houses purpose built laboratories which contain a temperature-controlled crystallization laboratory and an X-ray diffraction laboratory. The temperature-controlled crystallization room contains three Leica stereomicroscopes, a PC-controlled digital camera and a Cartesian crystallization robot. In-house X-ray measurements are made using a Rikagu RU-3HBR rotating anode generator with OSMIC focussing mirrors as an X-ray source. Data are collected using an R-AXIS IV++ detector from flash frozen crystals. Purified proteins from the Protein Production Unit are crystallized and their structure determined in the Protein Crystallography Unit. It is a central component to the Centre’s high throughput protein production pipeline. The group also has access to synchrotron radiation sources within the USA and at Clayton, Australia.

The Protein Crystallography Unit comprises nine groups, led by Jamie Rossjohn, James Whisstock, Ashley Buckle, Craig Clements, Matthew Wilce, Jackie Wilce, Natalie Borg, Travis Beddoe and Fasseli Coulibaly.

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 offers high quality, capillary-based DNA sequencing technology using an Applied Biosystems 3730S capillary sequencer that incorporates a 50 cm array capable of routinely generating read lengths in excess of 1000 bases. Sequencing is supported by a small-scale Oligonucleotide Synthesis service and a Microarray facility specialising in whole genome microbial arrays. In 2008, the unit 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.
The High-throughput Microbial Pipeline

The Centre’s infrastructure, leveraged as an entire system, forms the 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.
Research projects

Significant achievements and outcomes in 2008 included:

- Identification of the essential enzyme responsible for addition of the critical second mannose residue during cell wall biosynthesis in pathogenic mycobacteria.
- Determination of the 3-dimensional structure of the first UDP-glucose specific glycosyltransferase from mycobacteria implicated in cell wall synthesis.
- Identification of molecules capable of binding PimA in a fragment-based drug screen as a first step to a new generation of anti-mycobacterial drugs.
- Identification of Fis as the regulator of capsule expression and at least 15 other virulence factors in Pasteurella multocida.
- Identification of critical residues for function in the LPS KDO kinase KdkA in P. multocida, important for LPS biosynthesis and virulence in fowl cholera.
- The production of a P. multocida strain which has switched from serotype 1 to serotype 2, identifying for the first time a serotype determinant.
- Determination that pm1730 encodes a D-methionine binding protein and that this gene is critical for the virulence of P. multocida in both chickens and mice.
- Elucidation of critical residues required for the elastase activity of the AprV2 and BprV proteases of Dichelobacter nodosus.
- Establishment of a method to label Burkholderia pseudomallei with fluorescent marker proteins facilitating their imaging under various conditions.
- Finding that a B. pseudomallei effector BopA mutant shows increased targeting to autophagosomes and decreased survival within infected mammalian cells.
- Consolidation of a high throughput TLR analysis system as a central facility for the Centre and other users.
- Identification of Mul1 (formerly known as Protein 20) as a component of the mitochondrial anti-viral signalling complex in mammalian cells.
- Elucidation of how the innate Natural Killer receptor, CD94-NKG2A, interacts with its ligand.
- Elucidation of the molecular basis of food poisoning caused by strains of E. coli producing the AB5 toxin.
- Biochemical characterisation of the mechanism of pore formation by a MACPF perforin protein.
- Determination of the structure of the cytoplasmic domains of the Gram positive conjugation protein TcpC.
- Determination that the TcpC, TcpD, TcpE and TcpG proteins are important in the conjugation process in Clostridium perfringens.
- Discovery that the gene encoding the novel pore-forming clostridial toxin NetB is located on a conjugative plasmid.
The identification, characterisation and structure determination of drug targets in *Mycobacterium* spp. (Prof Ross Coppel, Prof Jamie Rossjohn, Dr Paul Crelin, Dr Travis Beddoe, Dr Jerome Le Nours, Dr Versha Rai, Dr Judith Scoble, Dr Julian Vivian, Ms Rajini Brammananth, Dr Zara Fulton (nee Marland), 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. The oxidoreductase Rv2971, an enzyme involved in detoxification of reactive keto groups, has been implicated in drug resistance to isoniazid, a frontline anti-mycobacterial drug. We have crystallised and solved the structure of the *M. smegmatis* homologue (MSMEG_2407) with and without its cofactor NADPH; the structure revealed that NADPH is tightly bound by salt-bridges. However, the nicotinamide ring is very flexible due to glycine replacing bulky aromatic residues found in other oxidoreductases. In addition, we have performed enzyme kinetic assays and showed that MSMEG_2407 has very narrow substrate specificity compared to other oxidoreductases.

We have shown that isoniazid can inhibit enzyme activity of MSMEG_2407 in vitro and overexpression of Rv2971 in *M. smegmatis* has led to increase in resistance to isoniazid. Future work will be crystallising MSMEG_2407 with isoniazid and crystallising Rv2971. To date, we have been able to express soluble and active Rv2971 by changing expression systems.

The structure of Rv3802, a putative esterase, was solved and its closest structural homologue was a cutinase. Cutinase breaks down plant cell walls. However, as *Mycobacterium* does not infect plants, Rv3802 appears to have another functional role. We have developed several enzyme assays to test for the function of Rv3802 and it appears that it has lipase activity that would help in infection of the host. Due to the success of soluble expression of Rv2971, we have cloned several proteins of interest into pET45 vector via ligation-independent cloning. So far we have tested expression of Rv2509, a short-chain dehydrogenase involved in mycolic acid synthesis and it appears to be soluble.

Functional studies have focussed on three other essential enzymes. Gene deletion and other studies in *Corynebacterium glutamicum* have identified Rv2509 as the reductase CmrA responsible for the final step of mycolic acid biosynthesis and Rv2188c as the mannosyltransferase responsible for addition of the second mannose residue during biosynthesis of phosphatidylinositol mannoside (PIM) and lipoarabinomannan (LAM). Cell wall defects have been identified in Rv0224, Rv0225 and Rv0228 mutants and characterisation of these strains is continuing. Current data indicate that the genes encoding these proteins form a new mycolic acid biosynthesis cluster.

Alongside these fundamental advances, we have also been collaborating with researchers from the Faculty of Pharmacy and Pharmaceutical Sciences to develop first generation inhibitors against mycobacterial targets. Saturation transfer difference NMR screening of fragment libraries has revealed a set of small molecules targeting the essential mannosyltransferase PimA. These molecules share structural features with GDP-mannose, the sugar donor of PimA, indicating binding within the active site of the enzyme.
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 Keith Al-Hasani, Ms Marietta John, Mr Jason Steen, Mr Tamas Hatfaludi, Ms Jennifer Steen).

**Identification of a global regulator of virulence in *P. multocida***

Production of an extracellular polysaccharide capsule by *P. multocida* is critical for virulence, but there is no information on how capsule expression is regulated. We identified three spontaneous mutants that produced no capsular polysaccharide. Quantitative reverse transcription PCR showed that in these acapsular strains there was no transcription of the capsule biosynthetic locus. Nucleotide sequencing identified no mutations within the entire 16kb cap locus, suggesting that the transcriptional defect was the result of mutation of a trans-acting transcriptional regulator gene. Whole genome sequencing in-house of paired parent and acapsular mutant strains identified a point mutation in the acapsular mutant strain within the gene *fis*, a predicted transcriptional activator. Complementation of the acapsular strain with a wild-type copy of *fis* restored capsule production, thereby confirming that Fis is a positive regulator of capsule expression in *P. multocida*. DNA microarray analysis showed that expression of at least 15 genes not associated with capsule production was also down-regulated in a *P. multocida fis* mutant. These down regulated genes included another known *P. multocida* virulence gene (*pfhB2*) and the gene encoding a known protective surface antigen (PipE). Thus, Fis is critical for the regulation of virulence in *P. multocida*.

**Characterisation of LPS biosynthesis genes and their involvement in virulence***

*Pasteurella multocida* is classified into 16 serotypes (Heddleston strains 1-16) based on lipopolysaccharide (LPS), which is a critical virulence determinant. While there is a correlation between the serotype, host specificity and disease syndrome there are currently no data on the determinants of host specificity in *P. multocida*. We have, in collaboration with Dr. Andrew Cox (Institute for Biological Sciences, National Research Council, Canada), determined the LPS carbohydrate structure and identified the genes required for LPS assembly in each of the 16 *P. multocida* serotypes. *P. multocida* serotype 1 strains are the most common cause of fowl cholera in chickens and we have used directed mutagenesis to characterise the function, and determine the involvement in virulence, of all genes required for synthesis of the LPS in these strains. Heddleston 2 strains are the most common cause of haemorrhagic septicaemia in cattle; these strains express an LPS molecule which is structurally distinct from that expressed by type 1 strains and they can not cause disease in birds. We have cloned and expressed the genes required for synthesis of the type 2 LPS in a type 1 genetic background; this recombinant strain expressed LPS identical to the type 2 LPS as determined by structural analysis and antibody reactivity. Thus, we have changed the serotype of a fowl cholera-causing isolate and we are currently working to determine if this also changes the virulence or host specificity of this strain.

*P. multocida* produces two LPS glycoforms (A and B) simultaneously. These glycoforms differ in the structure of the LPS inner core region, with glycoform A containing a KDO-P structure and glycoform B containing a KDO-KDO structure. KDO kinase (KdkA) is essential for production of the glycoform A KDO-P structure. *P. multocida* strains which express truncated glycoform A LPS are highly attenuated for virulence, but strains which express no glycoform A (and only full length glycoform B) retain virulence. There are no structures available for this enzyme and no data on the residues essential for its function. While testing the virulence of mutants that expressed truncated glycoform A LPS, we recovered a number of strains with mutations in the KDO kinase gene. Analysis of these mutants identified two amino acids critical for the function of this important enzyme. We are currently extending this work to identify other residues critical for KdkA function and we are working to solve the structure of this key enzyme.

In similar work we are probing the residues critical for substrate specificity in the closely related transferases GatB and LosA. The amino acid sequences of these two transferases are highly conserved, but GatB adds galactose to LPS while LosA adds glucose. We have made a small number of site-directed mutants in GatB and these did not alter the activity or sugar substrate specificity. We are currently in the process of swapping gene segments between the *gatB* and *losA* genes to identify the sections of each protein which determine specificity.
Figure 1. Schematic illustration of the outer core LPS structure expressed by each of the P. multocida Heddleston serotypes and the genes responsible for outer core LPS assembly. Strains have been grouped based on genetic similarity. All strains express a conserved inner core which is not shown. Non-functional (pseudo) genes are indicated with an arrow and conserved non-LPS genes are shaded grey. Strain variation observed in the Heddleston 1 LPS structure is shown in red. Structural residues are Glc, glucose; Gal, galactose; Hex, Glucose or Galactose; Hep, Heptose; Rha, Rhamnose; GaINAc, N-acetyl-galactosamine; GlcNAc, N-acetyl-glucosamine; HexNAc, N-acetyl-hexosamine; Qui3NAc, quinovosamine; (1S)-GalaNAc, open chain N-acetyl-galactosamine; PCho, phosphocholine; PEtn, phosphoethanolamine; Ζ=glycerol-putrescine.
Functional characterisation of a *P. multocida* methionine binding protein

The gene *plpB* encodes a protein with similarity to D-methionine binding proteins in other bacterial species. A *P. multocida* *plpB* mutant was unable to cause disease in chickens or mice. PlpB represents the first virulence-associated MetQ homologue from bacteria. We cloned, expressed and purified the soluble recombinant protein from *E. coli*. Radioisotope binding assays showed that PlpB bound both D- and L-methionine but bound D-methionine at much higher efficiency.

Antibiotic efflux pumps of *P. multocida*

Two TolC homologues, PM0527 and PM1980, were identified for *P. multocida* and were shown to be involved in the removal of a range of antibiotics and toxic compounds from the bacterial cell. A *pm0527* mutant displayed increased susceptibility to a range of chemicals, including rifampin (512-fold) and acridine orange (128-fold). A *pm1980* mutant showed increased susceptibility to rifampin, cefazidime, and vancomycin. Neither mutant was attenuated for virulence.

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, Prof Richard Whittington, Dr Om Dhungyel).

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. Studies on the extracellular proteases of *D. nodosus* have focussed on structural analysis, which has led to the elucidation of the crystal structures of several of these proteases including AprV2, AprB2, BprV and BprB. Mutagenesis studies coupled with functional analysis of the resultant purified proteins have provided evidence that specific residues are associated with the ability of the AprV2 and BprV proteases to hydrolyse elastin. Preliminary studies indicate that the C-terminal domain of at least some of these proteases may be involved in the secretion process.
The cellular and molecular basis of host pathogen interactions

**Effect of autophagic induction on host-pathogen interactions**
(Professor Rod Devenish, Dr Lan Gong, Dr Xuelei Li, Dr Mark Prescott, Ms Tanya D’Cruze, Professor Ben Adler, Dr John Boyce, Dr Meabh Cullinane)

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. In the first instance we are taking a targeted approach and investigating proteins involved in actin-based motility (eg BimA) and those secreted by the Type Three Secretion System (eg. BopA).

We have characterised BopA and BimA mutants individually for effects on invasion of, and survival in, RAW macrophage cells. A *bopA* mutant showed delayed actin-tail formation and delayed multinuclear giant cell (MNGC) formation. In concert with previous observations of increased co-localisation of mutant bacteria with GFPLC3-labelled vesicles, these results suggest delayed escape from phagosomes and increased capture in an autophagic compartment. A *bimA* mutant showed no actin-tail formation and no MNGC formation in addition to very low co-localisation with GFPLC3-labelled vesicles. It remains to be established if these bacteria ever escape the phagosome.

Most interestingly, use of the bacterial two hybrid assay has indicated that BopA and BimA interact with each other.

We have established the use of siRNA to knockdown key host cell genes to test outcomes with respect to the autophagic response of cells to bacterial infection.

We have established the capability to label *B. pseudomallei* with fluorescent marker proteins, thus facilitating their imaging under various conditions.

**Pathogen effector protein screening in yeast (PEPSY)**
(Professor Rod Devenish, Dr Mark Prescott, Ms Tanya D’Cruze, Professor Ben Adler, Dr John Boyce, Dr Meabh Cullinane)

We have continued the characterisation of the expression of seven candidate *B. pseudomallei* pathogen effector proteins, previously characterised by expression in yeast, in terms of their effect on vacuolar morphology and autophagic processes. In order to determine the influence of the relevant *B. pseudomallei* genes in infectivity and pathogenicity, we have commenced mutagenesis studies to knock out expression of individual effector proteins. Currently, two knock-out mutants have been generated, for genes BPSS1394 and BPSL0670. Survival and invasion assays have been carried out on BPSS1394 and show that there is a decrease in survival following infection of RAW macrophage cells.

Confocal micrograph of RAW264.7 cells 6 h post infection with *Burkholderia pseudomallei* strain K96243 (magnification x600). Bacteria are stained red Staining of filamentous actin (green) shows the presence of actin tails associated with infecting bacteria (red). The cell nucleus is stained blue.
Mechanisms of autophagy; how cells turn over their organelles
(Professor Rod Devenish, Dr Mark Prescott, Ms Kristina Turcic)

We have continued our characterisation of the single gene deletion (YMR010W null mutant) yeast strain in which vacuolar uptake of mitochondria is delayed/decreased compared with wildtype cells.

We have probed for potential interaction of the YMR010W gene product with Uth1p (previously characterised as having involvement in mitochondrial turnover). A double knock-out strain was generated, but no significant difference in the level of mitochondrial turnover these cells compared with those lacking the expression of either Uth1p or the YMR010W gene product alone, was observed. Although the two proteins do not directly interact with one another, these observations support the idea that their genes are contributing to the same pathway.

YMR010W has five putative homologues in yeast: YOL092W, YBR147W, YDR352W, YDR090C and Ers1. Of these, the best characterised is Ers1 which has 17 per cent (%) amino acid sequence identity with YMR010W. (Ers1 is a homologue of human cystinosin, a protein required for cystine transport from lysosomes.) However mitochondrial turnover is normal in an Ers1 knockout strain indicating a lack of functional homology. YMR010W interacts with VPSS5 and VPS26 which together with VPS10, are involved in retrograde transport. Preliminary results suggest that both mitochondrial and cytoplasmic turnover are reduced in strains knocked-out for these proteins, suggesting a connection between retrograde transport and autophagy, which will be further investigated.
Mechanisms of innate immunity

Interaction of bacterial pathogens with the host innate immune system.

(Professor John Davies, Professor Phillip Nagley, Professor Paul Hertzog, Dr Ashley Mansell, Dr Danielle Smith, Dr Grant Jenkin, Dr Tim Stinear, Dr Ben Howden, Ms Katharine Goodall, Ms Pin Wang).

This project aims to investigate key aspects of how bacterial pathogens 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.

Modulation of interactions between bacteria and members of the Toll-like receptor (TLR) family of danger-associated molecular protein receptors may have important consequences for the progression and resolution of infection. Bacterial components or products of bacteria signal through particular members of the TLR family receptors on (or in) host cells. This has important implications for the detailed mechanism of activation of the innate immune response in infected tissues. Therefore we have set up a system for analysis of TLR signalling, which is capable of resolving the activation of various members of the TLR family using specially constructed reporter system in test cell lines. These systems rely on cells expressing individual TLR proteins, with luciferase-based reporter assays dependent upon activation of NF-κB expression as a result of TLR engagement.

We have begun to apply TLR analysis to study Mycobacterium ulcerans that causes Buruli ulcer, the third most prevalent mycobacteriosis in the world (after tuberculosis and leprosy). Significantly, M. ulcerans has been shown also to cause disease in several Australian animals, including possums and koalas. M. ulcerans produces mycolactone, an immunosuppressive and cytotoxic polyketide which is required for pathology, but not for in vivo survival of the bacterium. M. ulcerans infection produces an initially painless lesion which breaks down centrally after a period of time. Mycolactone appears to kill phagocytic cells and/or prevent their activation/recruitment to the disease site, resulting in an expanding area of subcutaneous fatty necrosis containing clumps of bacilli with little or no inflammation. Surrounding the necrotic core is a band of acute cellular infiltrate colonised with intracellular bacteria.

We investigated whether the immunosuppressive function of mycolactone previously observed was due to inhibition of TLR signalling and consequently a robust anti-mycobacterial immune response. We found that mycolactone has a broad immunosuppressive effect in a rapid, dose-dependent manner which includes but is not confined to, inhibition of TLR signalling. The cytotoxic effect of mycolactone was also shown to be cell-type specific, with macrophages being more sensitive than cell lines derived from murine connective tissue or human kidney epithelia.

We have extended this work to investigate innate immune signalling in response to virus infection of mammalian cells. We showed that the recently identified mitochondrially localised Protein 20 interacts closely with the MAVS antiviral signalling complex. Protein 20 was shown to have a ubiquitin E3 ligase-like domain and, consistent with current usage, this protein has been renamed Mu1. We showed that Mu1 has atypical targeting signals to drive this protein to become targeted to mitochondria and we identified a putative membrane-spanning domain near the N-terminus of the protein as being critical for correct localisation of Mu1 to mitochondria.
Protein targeting to mitochondria. Localisation of Mu1 protein to mitochondria requires the N-terminal putative transmembrane stem. Row A (from left to right) shows normal Mu1 protein (green) targeted to mitochondria (red) of human cells, with the overlay demonstrating concordance of the red and green images. Row B shows that when mutant Mu1 lacking the N-terminal transmembrane domain is expressed in human cells (green), the protein is seen to be diffused throughout the cytosol, but not associated with the mitochondria (red), as visualised more clearly in the overlay image.
Structural aspects of Innate Immunity
(Professor Jamie Rossjohn, Mr Kwok Wun, Mr Ruide Koh, Dr Siew Siew Pang, Dr Jobichen Chacko)

In collaboration with Professor James McCluskey, A/Prof. Dale Godfrey and Dr Andrew Brooks. The University of Melbourne) we have focussed on the CD1 family, which presents 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 2008, we explored the underlying energetic basis of the interaction between the human NKT TCR and the CD1d molecule. This revealed the minimal requirements underscoring the CD1d restriction of this innate interaction.

In addition, we have begun to explore how the innate receptors on Natural Killer (NK) cells interact with the monomorphic components of the immune system. Specifically, we have addressed how one NK receptor (termed CD94-NKG2A) can interact with the HLA-E molecule. This innate interaction was surprising, as the NK–mediated interactions were dominated by one subunit, namely CD94.

The role of phosphoinositide metabolism in host-pathogen interactions
(Professor Christina Mitchell, Professor Ross Coppel, Dr Kristy Horan, Dr Paul Crellin, Dr David Sheffield, Dr Assunta Pelosi)

Macrophages are important cells in the co-ordination of both the innate and adaptive immune responses to invading pathogens. Phosphoinositides are signalling lipids that play key roles in both the phagocytosis of invading pathogens and destruction of pathogens in the phago–lysosome. In addition, phosphoinositides regulate the secretion of both pro-inflammatory and anti-inflammatory cytokines, also regulating the adaptive immune response. Many pathogens have evolved strategies to evade killing during phagosome maturation in macrophages, including economically important Mycobacterium species. These pathogens disrupt both phagosome maturation and autophagy, thereby inhibiting their destruction.

Phosphoinositides can be phosphorylated at the D3, D4, and/or D5 position to generate mono-, bis- or trisphosphorylated phosphoinositides and PtdIns(3,4,5)P3 and PtdIns(3)P have been shown to regulate phagocytosis and phagosome maturation. Cellular phosphatases remove phosphates from these positions and thus terminate phosphoinositide signalling. We have previously demonstrated that the PtdIns(3,4,5)P3-5-phosphatases the 72-5ptase and SHIP1 regulate PtdIns(3,4,5)P3 accumulation at the phagocytic cup differentially during Fc©R-mediated and CR3–mediated phagocytosis, respectively.

The energetic footprint of the NKT T cell receptor – Cd 1 d interaction.
To further characterise the role of PtdIns(3,4,5)P₃ and the lipid-phosphatase SHIP1 in regulating phagocytosis of pathogenic bacteria, primary bone marrow macrophages (BMM) from SHIP1⁻/⁻ mice were infected with *M. avium* and cfu/ml determined at 2 h post-infection. Depletion of SHIP1 resulted in a significant increase in phagocytosis of *M. avium*. Downstream Akt phosphorylation and therefore activation are significantly enhanced in SHIP1⁻/⁻ BMM following stimulation with *M. avium*, compared to SHIP1⁺⁺ BMM. In addition, pre-treatment of both SHIP1⁻/⁻ and SHIP1⁺⁺ BMM with a pan Akt inhibitor demonstrated that Akt is a positive regulator of phagocytosis of *M. avium* in macrophages. These results also indicate that SHIP1 is an important negative regulator of Akt activation via its hydrolysis of PtdIns(3,4,5)P₃.

Efficient phagocytosis requires the expansion of the plasma membrane, by intracellular vesicles such as the recycling endosome compartment. Our studies have revealed that a novel PtdIns(3)P-3-phosphatase MTMR4 localises to recycling endosomes and regulates both the morphology of the recycling endosome compartment, and the trafficking of cargo through this compartment. MTMR4 hydrolyses both PtdIns(3)P and PtdIns(3,5)P₂. PtdIns(3)P has been implicated extensively in phagosome maturation. We hypothesised that MTMR4 may regulate phagocytosis via the regulation of focal exocytosis implying a potential role for PtdIns(3)P during phagocytosis. Immunofluorescence studies have demonstrated that endogenous and recombinant MTMR4 localises to the phagocytic cup and early phagosome, following phagocytosis of IgG opsonised particles and *M. marinum* (tuberculosis-like pathogenic mycobacteria). Over expression of MTMR4 led to an approximately 35 per cent (%) decrease in phagocytosis compared to vector-alone control. This is the first evidence for a role for a PtdIns(3)P-3-phosphatase in regulating early steps of phagocytosis.
Protemics analysis of bacterial pathogens

Proteomics studies of Mycobacterium spp
(Professor Ross Coppel, Professor Ian Smith, Dr Megan Rees)

This project commenced in 2008 and is aimed at applying advanced proteomics techniques to the elucidation of protein function in mycobacteria during growth and intracellular invasion of the macrophage. Initial studies were aimed at applying proteomics techniques to the unusually structured mycobacteria and show that such approaches are feasible in this organism and sensitive enough to detect proteins. Membrane-shaving protocols were used to identify proteins exposed on the mycobacterial surface that may be involved in interaction with the external environment and the host cell. Bacteria were exposed to varying concentrations of trypsin for varying times and the peptides released from mycobacteria analysed by mass spectrometry to identify released peptides. Several proteins were identified and the repertoire was compared with that released from protease-treated Staphylococcus. Experiments are now aimed at refining the digestion treatment and handling methods to minimise detection of internal proteins. Once the method is standardised, we will examine mutant bacteria that have altered cell wall glycolipids to determine how this perturbs exposure of protein and what alteration it makes to the set of detected proteins.

Molecular characterisation of bacterial outer surface proteins (Professor Ian Smith, Dr David Steer, Prof Julian Rood, Dr Ruth Kennan, Ms Paola Vaz)

This is an ongoing series of experiments where protein shaving experiments were carried out in an attempt to identify highly expressed surface antigens of Dichelobacter nodosus and Clostridium perfringens. The results from these experiments are now being used to prioritise the antigen selection process for subsequent vaccine trials.

Proteomic Analysis of Host Pathogen proteins (Prof Ian Smith, Dr David Steer)

During 2008 we have applied protein shaving technology to identify outer membrane 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 outer membrane pathogen surface proteins prior to a full structural analysis.
Structural and functional biology

Structural biology and drug target characterisation

(Professor Jamie Rossjohn, Dr Travis Beddoe, Dr Jerome Le Nours)

Biting off more than one can chew

We have continued a 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. We solved the structure of the pentameric B‑subunit of this toxin and revealed its specificity for glycoconjugates. Specifically, we demonstrated that the toxin has marked specificity towards the sugar Neu5Gc, a sugar not synthesised in humans. We demonstrated that humans become susceptible to this toxin via dietary intake, and the commonest source of this Neu5Gc sugar is red meat and dairy products, thereby providing a molecular basis for understanding the cause of so‑called ‘Hamburger disease’. We have also initiated a collaboration with Dr Martin Scanlon, Monash Institute of Pharmaceutical Sciences on essential enzymes from Neisseria, providing insight into the specificity of one target, DsbA.

Structural biology and bioinformatics

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

In 2008 we have built upon our discovery that Membrane Attack Complex/Peforin like (MACPF) proteins are distantly related to bacterial cholesterol dependent cytolysins. MACPF proteins play an important role in the innate immune response, for example, in complement‑mediated lysis of Gram negative bacteria and in the elimination of virally infected or transformed cells. We have begun to characterise the mechanism of membrane insertion and oligomerisation by MACPF proteins and, furthermore, determined the crystal structures of new members of the family. Together, these data give unprecedented insight into pore formation by both MACPF proteins and CDCs.

We have determined the crystal structure of four out of the five virulence‑associated proteases produced by Dichelobacter nodosus, the causative agent of ovine footrot. These data provide fascinating mechanistic insight into a novel group of enzymes that possess elastase‑like activity.

We have begun to structurally characterise the machinery associated with bacterial conjugation. The eventual goal of this project is to structurally characterise the complete conjugation complex.

As part of a new direction, in collaboration with Centre Associate Hertzog, we have started to build a new suite of algorithms aimed at understanding how genes encoding important immunity proteins are regulated. This work utilises data from publicly available resources, as well as in‑house microarray/knock–out data. Together we anticipate that important functional insight will be gleaned in regards to genes that perform poorly characterised roles.

Our work on the encapsulated genome alignment algorithm (EGA) has resulted in the development of a web server that lists pre‑computed alignments of selected microbial genomes. We are also developing approaches to model complex genome rearrangements. The project to develop an automated approach to build and compare topology diagrams is near completion and is providing important insight into identifying similarities between distantly related proteins.
Functional genomics of large clostridial plasmids (Prof Julian Rood, Dr Trudi Bannam, Prof Rod Devenish, Dr Sarah Teng, Ms Jennifer Parsons, Prof James Whisstock, Dr Corrine Porter, Ms Rachael Poon, Ms Radhika Bantwal, Ms Jessica Wisniewski, Prof Bruce McClane, Dr Francisco Uzal)

The overall objectives of this project are to understand how large clostridial plasmids are able to be transferred between strains of C. perfringens. Research has focussed on the Tcp conjugation locus from the paradigm conjugative tetracycline resistance plasmid, pCW3. The crystal structure of TcpC, without the transmembrane N-terminal domain has been determined and it has been shown to consist of a bilobed molecule that forms a trimer (Figure 3). Extensive site-directed mutagenesis studies have been carried out on TcpC and critical functional residues identified. Other studies have shown that TcpD and TcpE are essential for conjugative transfer, that TcpG is required for efficient transfer and that TcpI and TcpJ are not required. In all, 10 of the 11 genes in the Tcp locus have now been mutated and their involvement in the conjugation process determined.
Development of veterinary vaccines

**Vaccine development in fowl cholera and ovine footrot** (Professor Ben Adler, Dr John Boyce, Dr Keith Al-Hasani, Professor Julian Rood, Dr Marina Harper, Mr Tamas Hatfaludi, Professor Richard Whittington, Dr Om Dungyel, Professor Steve Bottomley, Dr Noelene Quinsey, Dr Amanda Walmsley, Mr Nik Sotirellis, Mr Ian McPherson, Ms Sadia Deen).

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

From the first 71 antigens of *P. multocida* that were tested in chickens, four proteins were selected for further investigation. No protection was observed with the two efflux pump proteins PM0527 and PM1980. In contrast to work published previously elsewhere, the methionine-binding lipoprotein, PlpB, did not confer protection against *P. multocida* infection in either chickens or mice. We identified previously an outer membrane lipoprotein (PlpE) which, when delivered as urea–solubilised protein, stimulated protective immunity against infection in both chickens and mice. The mechanism by which PlpE confers protection remains unknown. To explore the possibility of expressing PlpE in transgenic plants with the aim of developing a plant-based, PlpE vaccine against fowl cholera, a collaboration was established with the Plant Biotechnology group at the School of Biological Sciences, Monash University, headed by Dr Amanda Walmsley. The *plpE* gene was cloned into a plant expression vector (pTH210), and an EcoRI/HindIII fragment harbouring *plpE* was retrofitted into a 14 kb *Agrobacterium* plant binary vector, pGPTV. *Nicotiana benthamiana* leaves were co-infiltrated with mixed *Agrobacterium* suspensions containing PlpE, and a viral suppressor protein p19 for preventing post-translational gene silencing, to screen for transient expression of PlpE. Expression of PlpE in *N. benthamiana* was detected by Western blots analysis with chicken anti-PlpE sera.

There is a need to identify suitable antigens which can form the basis of vaccines against ovine footrot. Through bioinformatics analyses we identified 99 proteins that are likely to be surface-exposed or secreted by the bacteria, most of which were cloned and expressed in Gateway expression vectors. Fifty recombinant antigens of *D. nodosus* were purified for vaccine trials conducted in collaboration with Centre Associate Professor Richard Whittington, University of Sydney. The ability of 52 recombinant antigens to protect against ovine footrot was examined in four separate pen-based virulence trials. Groups of eight merino sheep were vaccinated twice subcutaneously in the neck region, with an interval of 14 days between the injections. Sheep were then subjected to experimental challenge with *D. nodosus*. The progression of disease on each foot was measured using a standard lesion scoring method at the start of the trial and then at weekly intervals. The total weighted foot score (TWFS) was used to provide an unambiguous overall score for the animal, a score that includes information from each of the four feet. Preliminary results indicate three antigens show evidence of protection.

**Host-pathogen interactions and 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 Paola Vaz, 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*. We have cloned the genes for some 90 surface associated or secreted proteins from *C. perfringens* and have tested many of them for their ability to protect chickens from disease challenge. These studies are made possible by funding from the Australian Poultry CRC. Studies on the *netB* gene, whose product we have shown to be essential for the pathogenesis of necrotic enteritis, have continued. We are in the process of testing the vaccine potential of this toxin. In addition, genetic studies have shown that the *netB* gene is encoded on a large conjugative plasmid, which has significant implications for the epidemiology of this important disease.
Identification of protective antigens and vaccine development in leptospirosis
(Professor Ben Adler, Dr Miranda Lo, Ms Katarzyna Rainczuk, Ms Deanna Deveson, Dr Noelene Quinsey, Mr Nik Sotirellis)

*Leptospira* is the causative agent of leptospirosis in cattle, which can result in reproductive failure and large production losses. Utilising the Centre’s vaccine development process, genes from *Leptospira borgpetersenii* serovar Hardjo-bovis encoding proteins likely to have vaccine potential were identified.

260 genes predicted to encode lipoproteins or outer membrane proteins were selected, and 240 have been expressed using the Gateway™ system. Following confirmation of the expression of the recombinant proteins, they were purified on a scale sufficient for use in subsequent antigen evaluation screening. 223 proteins have been purified and grouped into pools of four or five for vaccine screening in hamsters. Hamsters were vaccinated and boosted with preparations of proteins in adjuvant using standard protocols. Protection was evaluated by challenge with live *Leptospira* and assessment of renal colonisation. None of the purified recombinant proteins tested to date was able to stimulate protective immunity.

A cell-mediated response has been shown to be important in protective immunity against leptospirosis in cattle. Fifteen cattle were vaccinated with Spirovac™, which stimulates good cellular immunity, and then screened with the BOVIGAM™ assay which measures the release of IFN-γ by peripheral blood mononuclear cells (PBMCs). Good responses were seen against leptospiral serovar Hardjo as well as against one pool of recombinant proteins. Further testing is currently underway to confirm these results. Conditions for quantitative RT–PCR have been optimised; this will also be used to investigate whether the recombinant proteins can stimulate expression of other cytokines in bovine PBMCs. Western blot analysis using convalescent bovine sera against the purified recombinant proteins has commenced and has identified the major lipoprotein LipL32 as a key target of the bovine antibody response.
Key performance indicators

Research findings

Quality of publications
Centre researchers continue to perform well in peer-reviewed publications, with a total of 26 papers published in scholarly refereed journals in 2008. Of these, papers 77 per cent (%) appeared in journals with impact factor (IF) of ≥ 4.0. Details of publications are shown in Appendix 1: Publications.

Patents
In 2008 the following patent was filed: McGowan S, Porter C, Whisstock, JC, Lowther J, Stack C, Donnelly and Dalton J. (2008) Crystal structure of the malarial aminopeptidase or M1 malaria proteins. PCT 200890700, filed 14 February 2008.
Invitations to address and participate in international conferences

**Professor Ben Adler**
- Functional Genomics of Microorganisms. Institut Pasteur, Paris, France.
- Eighth Gordon Research Conference: Biology of Spirochetes. Ventura, USA.

**Professor Ross Coppel**
- Malaria Vaccine Initiative Tenth Anniversary Symposium, Brussels, Belgium.
- Children’s Rational Design of Malaria Vaccines: Washington DC, USA.
- Wellcome Trust Expert Input Forum For The Design of A Tropical Vaccine Institute, London, UK.
- Molecular Approaches To Malaria, Lorne Australia.

**Professor Rod Devenish**
- Gordon Research Conference on ‘Autophagy in Stress, Development and Disease’, Santa Barbara, USA.
- 33rd FEBS Congress and 11th IUBMB Conference, Athens, Greece

**Professor Julian Rood**
- Marie Curie Workshop on Clostridium perfringens, Torquay, UK.
- International Symposium on Plasmid Biology, Gdansk, Poland.
- 12th International Symposium on Microbial Ecology, Cairns, Australia.

**Professor Jamie Rossjohn**
- Cantoblanco Workshops on Biology, Initiation of Antigen Receptor signalling. Madrid, Spain.
- 1st International Singapore Symposium of Immunology, Singapore.

**Professor Ian Smith**
- American Association of Clinical Chemists Annual Meeting, Seattle, USA.
- Tenth International Chinese Peptide Symposium, Xian, China.

**Professor James Whisstock**
- Gordon Research Conference: Proteolytic Enzymes and their Inhibitors, New London, USA.
- The Fifth International Symposium on Serpin Biology, Structure and Function. Leuven, Belgium.
- Thirteenth Conference on Proteases and Inhibitors in Pathophysiology and Therapeutics Osaka, Japan.
Invitations to visit leading international laboratories

**Professor Ben Adler**
- Institut Pedro Kouri, Havana, Cuba.
- Institut Pasteur, Paris, France.

**Professor Rod Devenish**
- Max Perutz Laboratories, Department of Genetics, University of Vienna, Campus Vienna Biocenter, Wien, Austria.

**Professor Julian Rood**
- Tufts University Medical School, Boston, USA.
- University of Michigan, Ann Arbor, Michigan, USA.
- Hines VA Medical Center, Chicago, USA.
- Loyola University, Chicago, USA.
- Imperial College, London, UK.
- University of Exeter, Exeter, UK.
- Bristol University, Bristol, UK.
- University of Gent, Gent, Belgium.
- University of Freiburg, Freiburg, Germany.

**Professor Jamie Rossjohn**
- Immunology Division, National University of Singapore, Singapore.

**Professor Ian Smith**
- Sichuan University and West China Hospital, Chengdu, China.
- SynThesis Labs, Shanghai, China.
Commentaries about the Centre’s achievements

A number of media commentaries about the Centre, and its Chief Investigator achievements and activities, were published in 2008, including the following:

Publications

‘Human diet gives pathogens something to eat’, Monash Newsline, 30 October 2008


‘Monash researchers recognised with Federation Fellowships’, Monash Memo, 30 April 2008


‘Supporting Australia’s rural industries’, Discovery, Summer 2007-08
Postgraduate student education continued to be a critical component of the Centre’s activities in 2008. A primary focus is the training of students in advanced technologies. In 2008 the Centre funded three full scholarships and five ‘top up’ scholarships as well as supporting nine PhD scholarships funded either by Monash University (8) and Australian Pork Limited (1). In addition to project-based training, Centre HDR students have access to professional development provided by the Monash Research Graduate School (MRGS), the Monash Postgraduate Association (MPA) and the School of Biomedical Sciences Graduate School (SOBSGS).

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 participate in an annual young investigator symposium as part of the Monash Infection and Immunity Network. Financial support is also provided for students to attend national and international research conferences.

Professional development

MIIN Young Investigators Symposium

On July 3, the Monash Infection and Immunity Network (MIIN) hosted the Young Investigators Symposium. The Symposium talks included presenters from the Monash University Faculties of Medicine, Science and Pharmacy, the Monash Institute for Medical Research (MIMR), the Burnet Institute and the Centre. Professor Adler summed up the meeting in the closing session by commenting, “Each year the Young Investigator talks always exceed expectations.” The Centre sponsors the Best Poster Prize.

Centre Director Professor Ben Adler and Centre Associate, Professor Paul Hertzog are co-convenors of MIIN.

Recognition of teaching excellence

In 2008 Centre Deputy Director Professor Rod Devenish was the recipient of the MPA Postgraduate Supervisor of the Year Award. Rod is also the Deputy Director of the Monash Research Graduate School. This award is bestowed in recognition of the outstanding contribution a Monash staff member makes in the area of post graduate supervision and the respect and gratitude of their students. Professor Devenish currently supervises two Centre PhD students.
Centre postgraduate recruitments

**Jing Khoo (PhD)**
The role of Mitochondrial Ubiquitin Ligase 1 (Mul1) in the initiation of inflammation and apoptosis in response to viral invasion.

**Ben Wade (PhD)**
Virulence factors in necrotic enteritis strains of Clostridium perfringens.

**Jessica Wisniewski (PhD)**
The role of tcp genes in conjugative transfer of the clostridial plasmid pCW3.

Honours students

**Jason Fan (BMedSci Hons)**
Investigation of Burkholderia pseudomallei mediated evasion of autophagy.

**Jessica Wisniewski (BSc Hons)**
The role of tcp genes in conjugative transfer of the clostridial plasmid pCW3.

Centre postgraduate course completions

**Anthony Keyburn (PhD)**
The role of Clostridium perfringens toxins in the pathogenesis of avian necrotic enteritis.

**Zara Marland (PhD)**
A structural investigation into Mycobacterial Cell Wall Biosynthesis Proteins.

**Dalibor Mijaljica (PhD)**
Autophagic degradation of organelles in the yeast Saccharomyces cerevisiae: investigating the requirement of autophagy-related genes.
Undergraduate teaching

In 2008 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 3041 – Medical Microbiology
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

Professor John Davies
MIC3011 – Molecular Microbiology
MOL2022 – Molecular Biology

Professor Rod Devenish
BCH3031 – Advanced Molecular Biology: Modern concepts and applications
BMS3021 – Molecular Medicine and Biotechnology
MED1011 – Medicine 1

Professor Phillip Nagley
BMS3021 – Molecular Medicine and Biotechnology (convenor)
BMS2021 – Biochemistry in human function
CEL2012 – Cell Biology

Professor Julian Rood
BMS1062 – Molecular Biology
MED2031 – Medicine 3
GMA1011 – Medicine 1
MIC3032 – Pathogenesis of Bacterial Infectious Diseases
MIC4100 – Microbiology research project (Honours – convenor)
MIC4200 – Advanced Studies in Microbiology (Honours – convenor)

Professor James Whisstock
BCH3042 – Cell signal transduction: role in cancer and human disease
BMS2062 – Introduction to bioinformatics
Other research training and professional education

**Australian Crystallography School**
Chief Investigator and Federation Fellow Professor Jamie Rossjohn was part of the organising committee for the first Australian Crystallography School held in the Yarra Valley, Victoria in 2008. The workshop is designed for PhD students and early career researchers in either macromolecular or small-molecule crystallography and is open to Australians and New Zealanders. It aims to provide students with hands-on experience within a theoretical framework for the determination of molecular structures by single-crystal X-ray crystallography. The course consists of lectures and computer-based practical sessions. Chief Investigator and Federation Fellow Professor James Whisstock also delivered lectures in the seven day event.

**Postgraduate Lecture series on Biomedical Proteomics**
Professor Ian Smith lectured in Advanced Biochemical Techniques, an Honours course (BCH 4200), in proteomics.
Centre staff participation in the Annual Micromon Recombinant DNA Techniques Short Course was again the professional development training event for the Centre in 2008. This year Centre Chief Investigator Professor Julian Rood and Centre Associates Dr John Boyce and Dr Tim Stinear delivered expert lectures in the course. 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 enrols 40 participants, comprises ten 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. Micromon is the commercial services unit of the Monash University Department of Microbiology.

International, national and regional links and networks

**International visitors to the Centre**

- Professor Jamuna Vadivelu, University of Malaya, Malaysia.
- Associate Professor Kanitha Patarakul, Chulalongkorn University, Thailand.
- Ms Jirarat Songsri, Khon Kaen University, Thailand.
- Professor Stuart Hill, Northern Illinois University, USA.
- Professor Garry Taylor, University of St Andrews, Scotland, United Kingdom.
- Professor Mark Ellisman, University of California, San Diego, USA.
- Professor Mel Goodale, McGill University, Canada.
Income derived from other sources

**Professor Ben Adler**
Kuwait University, Purification and characterisation of cytotoxin from *Campylobacter jejuni* A$81,585 (Albert)

NHMRC Project Grant, Structural and functional analysis of leptospiral membrane proteins. $165,000 (Beddoe)

NHMRC Program Grant: The molecular basis of bacterial infectious diseases. ($280,000, Adler lab allocation only)

**Professor Philip 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. $242,000 (Horne)

**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 for complex glycolipid biosynthesis in Mycobacteria. $162,750 (Crellin)

ARC/NHRMC Research Network for Parasitology. $300,000

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

National Institute of Health (NIH), USA, Malaria and the red blood cell. US$130,000

**Professor Julian Rood**
NHMRC Program Grant, The molecular basis of bacterial infectious diseases. ($430,000. Rood lab allocation only)

NHMRC Project Grant, Functional biology of large serine recombinases from mobile antibiotic resistance elements ($139,900)( with M. Wilce and D. Lyras)

NIH/NIAD: *Clostridium difficile* toxin gene regulation. (USD $63,000 Rood lab allocation only)

Australian Poultry CRC: Identification of vaccine antigens from *Clostridium perfringens* and *Campylobacter jejuni*. (Moore, Boyce, Adler) $187,600

**Australian Poultry CRC: A new virulence factor in Clostridium perfringens causing necrotic enteritis in chickens – a route to vaccine development. (Keyburn) $112,355**

**Australian Wool Innovation: Vaccine potential of surface antigens of Dichelobacter nodosus. (Boyce, Whittington) $216,190.**

**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)

ARC Linkage Grant (Cytopia) Rational Drug design of protein tyrosine kinase inhibitors. $190,000 (Wilks)

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

**Professor Ian Smith**
NHMRC Control of proteases in infectious, degenerative and cardiovascular disease Program. $2,200,000

NCRIS Proteomics Australia (BPA). $750,000

**Professor James Whisstock**
ARC Federation Fellowship Structural and functional studies on MACPF proteins. $400,000

NHMRC Program $2.2 million (Pike, Bird, Bottomley, Buckle, Smith)

ARC Discovery Grant on Structural and functional studies on prokaryote serpins. $87,000 (Pike, Bird)

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

ARC LIEF on Australian high performance computational structural biology facility, $400,000 (Buckle, Wilce, Smith, Bottomley, Abramson, Webb, Garcia de la Banda, Appelbe, Coppel)

NHMRC Principal Research Fellowship. $127,000

US Air Force Grant $80,000. New approaches for predicting protein structure. (Webb)
Awards and recognition

**Professor Rod Devenish**
- 2008 Monash Postgraduate Association (MPA) Supervisor of the Year Award

**Professor James Whisstock**
- 2008 Commonwealth Health Minister’s Award for Excellence in Health and Medical Research
- 2008 ARC Federation Fellowship

Professor Rod Devenish with current and former students.

Chief Investigator and Federation Fellow Professor James Whisstock with Prime Minister Kevin Rudd and Professor Tanya Monro (The University of Adelaide) at the Federation Fellowship Award ceremony, Parliament House, Canberra, April 2008.
End–user links

Commercialisation activities

The Centre continued productive interaction with Pfizer Animal Health on the ARC Linkage bovine leptospirosis vaccine project. Reporting and planning meetings were held at six-weekly intervals throughout 2008.

In mid 2007, the Centre engaged the services of Dr Rocco Iannello from Monash University’s Research Commercialisation Unit to provide advice and guidance on Centre-related commercialisation issues and to lead Centre business development activities. Dr Iannello is a Business Development Manager with the Faculty of Medicine, Nursing and Health Sciences, and his association with the Centre continued through 2008.

A commercialisation agreement between Pfizer Animal Health and the Australian Poultry CRC has been entered into for the necrotic enteritis vaccine development project with the NetB protein as the lead candidate.

Government, industry and business briefings

Centre Associate, Professor Els Meeusen, Dr James Gilkerson, Professor Glen Browning and Chief Investigator Professor Julian Rood presented a session chaired by Centre Director Professor Ben Adler on ‘Modern approaches to the development of veterinary vaccines’ at Ausbiotech 2008 in Melbourne, Victoria in October.

Professor Ian Smith also presented a talk about the Centre at a business meeting with Pfizer Animal Health.
Public awareness activities

Public lecture 2008

In late 2008 the Centre hosted a public lecture on ‘Worms, Sheep and Biotechnology’.

The seminar, which covered topics such as vaccine development against worm infestation of sheep and the investigation of the relationship between dust mite allergy and asthma was presented by the Centre’s newest Associate, Professor Els Meeusen of the Department of Physiology at Monash University. Professor Meeusen's particular area of expertise is helminth immunology, allergy and vaccine development and aims to integrate a whole body approach to science into practical applications for animal and human health.
Other chief investigator professional activities

**Professor B Adler**
- Editorial Board, *Veterinary Microbiology*.
- Editorial Board, *Veterinary Sciences Tomorrow*.
- Deputy Chair: NHMRC Grant Review Panel.
- Co-convener, Monash Infection and Immunity Network.

**Professor RJ Devenish**
- Editorial Board, *Autophagy*.
- OZReader, ARC.

**Professor P Nagley**
- Member of the National Committee on Biomedical Sciences, Australian Academy of Science.
- Chair, School of Biomedical Science Education Committee, Monash University.

**Professor Julian Rood**
- Editor, *Plasmid*.
- Editorial Board, *BMC Microbiology*.
- Deputy Chair: National Health and Medical Research Council, Grant Review Panel.
- Ambassador for Australia, New Zealand and Oceania, American Society for Microbiology.
- Member, Australian Academy of Science National Committee for the Biomedical Sciences.

**Professor Jamie Rossjohn**
- Editorial Board, *Protein and Peptide Letters*.
- Editorial Board, *Essays in Biochemistry*.
- ARC Oz Reader.

**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*.
- Chairman NHMRC Grant Review Panel and Assessor Selection Committee.
- Deputy chairman Victorian Government Biotechnology Development Strategic Planning (BDSP) group.
- Member, Federal Government NCRIS Roadmap review and development ‘Maintaining and Promoting Good Health’ working group.

**Professor James Whisstock**
- Organising committee, 34th Lorne Conference on Protein structure and function.
Appendix 1: Publications

Journal articles

This list includes all publications published in 2008 by the Centre and Centre affiliated researchers. These publications have had varying levels of input from Centre facilities.


Conference abstracts

This list includes all conference presentations in 2008 by the Centre and Centre affiliated researchers. These presentations have had varying levels of input from Centre facilities.


Copple RL. (2008) Using the malaria genome sequence to design a malaria vaccine. European Malaria Vaccine initiative Tenth Anniversary Symposium Brussels, Belgium.


Nagley P. (2008) Engagement of undergraduate students with research can be much more than laboratory work*. Proceedings Australian Society for Biochemistry and Molecular Biology, 40, SYM-33-04, Canberra.


Rood JI, Kennan RM, Han X, Parker D, Dhungel O and Whittington RJ. (2008). Molecular pathogenesis of ovine footrot. 2nd Footrot Research Symposium, University of Sydney, Camden, NSW.


Rossjohn J. (2008), Antigen ligation leads to a conformational change within the constant domain of the T cell receptor. Cantoblanco Workshops on Biology, Initiation of Antigen Receptor signalling, Madrid, Spain.


## Appendix 2: Financial statement

<table>
<thead>
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<th>Income</th>
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<tr>
<td>ARC CoE Program</td>
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<tr>
<td>State Gov funds (Vic) DIIRD</td>
<td>326,000</td>
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<tr>
<td>Other ARC Centre grants Linkage (lepto vaccine)</td>
<td>92,029</td>
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<tr>
<td>Collaborating Institution Poultry CRC</td>
<td>204,142</td>
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<tr>
<td>Industry/Private Funds</td>
<td>216,190</td>
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<tr>
<td>Aust. Pork scholarship</td>
<td>16,560</td>
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<td>Linkage (Pfizer)*</td>
<td>110,000</td>
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<td>Monash University</td>
<td>699,000</td>
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<td>Carried forward from 2007 ARC funds</td>
<td>153,127</td>
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<td>Host Institution</td>
<td>1,362,951</td>
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<tr>
<td>Aust. Pork scholarship</td>
<td>1,718</td>
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<td>Total Income</td>
<td>5,347,109</td>
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<table>
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<th>Expenditure</th>
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<tr>
<td>Salaries</td>
<td>2,389,605</td>
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<td>Scholarships and Prizes</td>
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<td>Equipment</td>
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<td>Maintenance/Consumables</td>
<td>506,084</td>
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<td>Travel and Related Expenses</td>
<td>33,170</td>
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<td>Other Expenditure AWI footrot project</td>
<td>216,000</td>
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<tr>
<td>Poultry CRC project</td>
<td>204,142</td>
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<tr>
<td>Linkage (lepto vaccine)</td>
<td>202,029</td>
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<tr>
<td>Total expenditure</td>
<td>3,637,213</td>
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<tr>
<td>Funds carried forward to 2009**</td>
<td>1,709,896</td>
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*Total sum for entire grant ($330,000) received in 2006

** Incl AWI funds
### Appendix 3: Key result areas and performance measures table 2008

<table>
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<tr>
<th>Key Result Area</th>
<th>Performance Measure</th>
<th>Target</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Research findings</strong></td>
<td>Quality of publications</td>
<td>At least 75% on journals with an impact factor (IF) of ( \geq 4.0 )</td>
<td>77% of papers were published in journals with IF ( \geq 4.0 ).</td>
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<tr>
<td></td>
<td>Number of publications</td>
<td>20 refereed publications in international journals. 2 invited book chapters or reviews</td>
<td>26 refereed publications. 2 book chapters.</td>
</tr>
<tr>
<td></td>
<td>Number of patents</td>
<td>1 per year</td>
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<tr>
<td></td>
<td>Invitations to address and participate in international conferences</td>
<td>6 per year</td>
<td>19</td>
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<tr>
<td></td>
<td>Invitations to visit leading international laboratories</td>
<td>4 per year</td>
<td>16</td>
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<tr>
<td></td>
<td>Number and nature of commentaries about the Centre’s achievements</td>
<td>2 per year</td>
<td>5</td>
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<tr>
<td><strong>Research training and professional education</strong></td>
<td>Number of postgraduates recruited</td>
<td>4 per year</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Number of postgraduate completions</td>
<td>2 per year</td>
<td>3</td>
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<tr>
<td></td>
<td>Number of Honours students</td>
<td>6 per year</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Participation in professional courses</td>
<td>1 per year</td>
<td>1 Centre CI, &amp; 2 Associates lectured in Recombinant DNA Techniques Short Course at Monash University, Nov 2008</td>
</tr>
<tr>
<td></td>
<td>Number and level of undergraduate and high school courses in the priority area(s)</td>
<td>2 at 3\textsuperscript{rd} year level</td>
<td>BCH3031 – Advanced Molecular Biology&lt;br&gt;BMS2062 – Introduction to Bioinformatics&lt;br&gt;BMS3021 – Advanced Molecular Biology&lt;br&gt;MIC3011 – Molecular Microbiology&lt;br&gt;MIC3041 – Medical Microbiology&lt;br&gt;MIC3990 – Action in Microbiology&lt;br&gt;MOL2022 – Molecular Biology</td>
</tr>
<tr>
<td>Key Result Area</td>
<td>Performance Measure</td>
<td>Target</td>
<td>Outcome</td>
</tr>
<tr>
<td>--------------------------------------------</td>
<td>----------------------------------------------------------</td>
<td>-----------------</td>
<td>---------------------------------------------------</td>
</tr>
<tr>
<td><strong>International, national and regional links and networks</strong></td>
<td>Number of international visitors</td>
<td>2 per year</td>
<td>7</td>
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<tr>
<td></td>
<td>Number of national and international workshops</td>
<td>5 (if a network application is successful)</td>
<td>Nil. Network application not funded</td>
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<tr>
<td></td>
<td>Number of visits to overseas laboratories</td>
<td>4 per year</td>
<td>16</td>
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<tr>
<td><strong>End-user links</strong></td>
<td>Nature and number of commercialisation activities</td>
<td>3 partnerships involving cash</td>
<td>Australian Wool Innovation Australian Poultry CRC Pfizer Australia</td>
</tr>
<tr>
<td></td>
<td>Number of government, industry and business briefings</td>
<td>1 per year</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Number of Centre associates trained/ing in technology transfer and commercialisation</td>
<td>1 per year</td>
<td>1</td>
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<td></td>
<td>Number and nature of public awareness campaigns</td>
<td>1 per year</td>
<td>1</td>
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<tr>
<td><strong>Organisational support</strong></td>
<td>Annual in-kind contributions from Collaborating Institutions</td>
<td>$350,000 per year</td>
<td>~$620,000</td>
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<tr>
<td></td>
<td>Number of new organisations recruited to or involved in the Centre</td>
<td>1 per year</td>
<td>The Pasteur Institute, France</td>
</tr>
<tr>
<td></td>
<td>Level and quality of infrastructure provided to the Centre</td>
<td>$730,000 per year in equipment, $400,000 per year in personnel, and $330,000 per year cash</td>
<td>$699,000 cash contribution from Monash University. ~$2.4M in equipment infrastructure and ~$5.5M in Chief Investigator and other staff salaries (incl direct on-costs and overheads).</td>
</tr>
<tr>
<td><strong>Governance</strong></td>
<td>Breadth and experience of members of Advisory Board</td>
<td>6 Members specified</td>
<td>Dr Emanuela Handman recruited in 2008</td>
</tr>
<tr>
<td></td>
<td>Frequency and effectiveness of Advisory Board meetings</td>
<td>Twice per year</td>
<td>Met twice in 2008 on September 16 and November 24</td>
</tr>
<tr>
<td></td>
<td>Quality of Centre Strategic Plan</td>
<td>As outlined in submitted research plan</td>
<td>Changes outlined in Review submission.</td>
</tr>
<tr>
<td>Key Result Area</td>
<td>Performance Measure</td>
<td>Target</td>
<td>Outcome</td>
</tr>
<tr>
<td>-----------------</td>
<td>----------------------------------------------------------</td>
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<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>Effectiveness of arrangements to manage Centre nodes</td>
<td>Publication of cross-node papers</td>
<td>Centre is single node.</td>
</tr>
<tr>
<td></td>
<td>Adequacy of Centre Key Performance Indicators</td>
<td>Review by external assessor</td>
<td>ARC Review in October.</td>
</tr>
<tr>
<td>National benefit</td>
<td>Measure of expansion of Australia’s capability in the priority area(s)</td>
<td>Substantial increase in knowledge base. Progress in vaccine development and drug target identification</td>
<td>See list of publications. Vaccine development and drug target pipeline projects progressed satisfactorily in 2008. See progress reports.</td>
</tr>
<tr>
<td></td>
<td>Case studies of economic, social, cultural or environmental benefits</td>
<td>N/A</td>
<td>N/A</td>
</tr>
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</table>
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