MDHS Graduate Research Conference 2020

Biomedicine Booklet

https://mdhs.unimelb.edu.au/mdhs-graduate-research-conference-2020

mdhs-grconference@unimelb.edu.au

MESSAGE FROM THE CHAIRS

Dear Delegates,

Welcome to the virtual inaugural Medicine, Dentistry and Health Science Graduate Research Conference 2020 (MDHS GR Conference), a student conference for all biomedical graduate research students that are part of the MDHS Faculty of the University of Melbourne. The organising committee is made up of members from 11 different student society across the MDHS faculty campus. The conference schedule consists out of 12 parallel session covering a variety of interesting topics and accommodating our student talks as well as national and international keynote speakers, Science Communication workshop and a Career Panel Discussion. This event was only possible due to the generous support of the University of Melbourne and the Graduate Student Association (GSA).

We hope that MDHS GR Conference will provide you with opportunities to listen to national and international leaders talking about their ground-breaking research in different biomedical fields and communicate your research to a broad scientific audience. Despite the fact that this conference will be virtually it will give you a unique chance to meet and network with peers from different research fields engage in discussions. We hope that the MDHS GR Conference will inspire you with new possibilities for your future career by listening to our invited speakers from academia and industry.

We wish you all the best for your presentation and hope you enjoy the event and get novel project ideas, career opportunities and new connections out of it.

Martha Blank & Alexander Anderson

(Chair & Deputy-Chair of the Medicine, Dentistry and Health Science Graduate Research Conference 2020)

GENERAL PROGRAM

08.00 - 08.15 Conference Opening & Welcoming Address
Professor Alex Boussioutas and Martha Blank

08.15 - 10.00 Session 1

10.00 - 10.30 Break

10.30 - 12.30 Session 2

12.30 - 13.00 Break
Virtual Socialise

13.00 - 14.30 Science Communication Workshop
Dr. Shane Huntington

14.30 - 16.00 Break
Virtual Socialise | Networking | Games

16.00 - 17.00 Careers Panel Discussion
A/Prof. Nicholas Opie | Dr. Danijela Mirosa | Dr. Ashish Sethi
Dr. Maryam Hussain | Dr. Simranpreet Kaur

17.00 - 19.00 Session 3

19.00 - 20.00 Award Ceremony & Conference Closing
Martha Blank and Alexander Anderson

SCIENCE COMMUNICATION WORKSHOP

Dr. Shane Huntington

Dr. Shane Huntington has been providing consulting services in communication and strategy for over 20 years. As a successful broadcaster, business owner, academic and strategist he draws together experience from multiple sectors, offering clients a more detailed and analytical approach than competitors. Shane has trained thousands of people to communicate more effectively, especially in fields of research. His unique and engaging style has led to him delivering programs to some of Australia's most prestigious institutions.

CAREERS PANEL DISCUSSION

A/Professor Nicholas Opie

Synchron Founding Director and CTO
Co-Lab Head of the Vascular Bionics Laboratory, The University of Melbourne

Dr. Danijela Mirosa

Franchise Director of Oncology for the Oceanic Cluster
Takeda Pharmaceuticals

Dr. Ashish Sethi

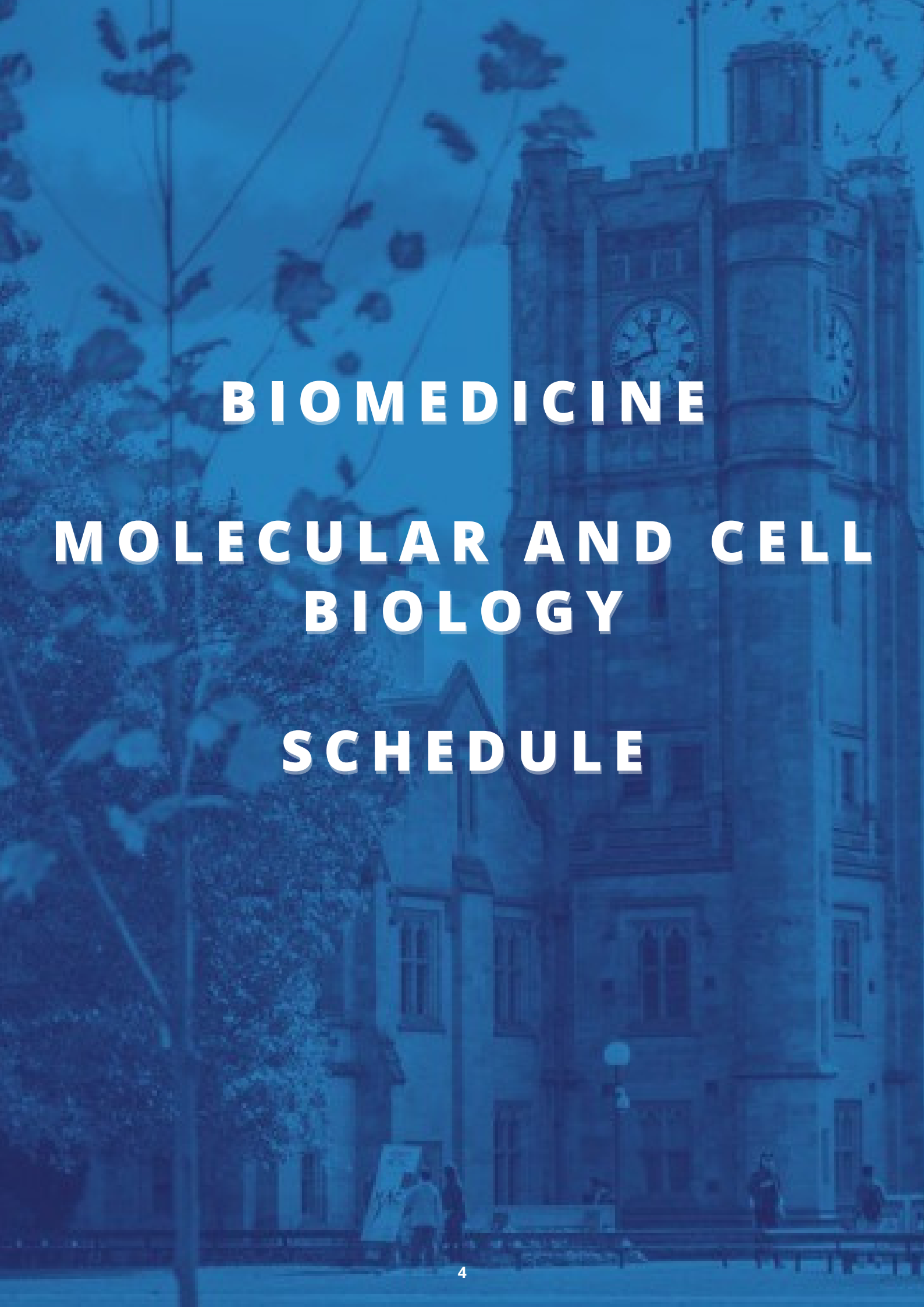
Postdoctoral Research Fellow
Department of Biochemistry & Molecular Biology, TheUniversity of Melbourne

Dr. Maryam Hussain

Medical Science Liaison
Boehringer Ingelheim

Dr. Simranpreet Kaur

Postdoctoral Researcher
MitoBrain Murdoch Children's Research Institute



BIOMEDICINE

MOLECULAR AND CELL BIOLOGY

SCHEDULE

BIOMEDICINE
Molecular and Cell Biology

SCHEDULE

SESSION 1		
08.15 – 08.45	**The role of mitochondrial metabolism in the tumor microenvironment** **Keynote Speaker:** Prof. Marcia Haigis	
08.45 – 09.00	**Expanding the peptide synthesis toolkit to produce bicyclic peptide mimetics for drug discovery** Qingqing Lin	9
09.00 – 09.15	**Characterising novel Coxiella burnetii effector protein interactions.** Cameron Oppy	10
09.15 – 09.30	**Bioinformatics analysis and virtual screening of the AAA+ ATPase p97 in protozoan parasites and Mycobacterium tuberculosis.** George Kobakhidze	11
09.30 – 09.45	**Ribosomal stalling on C9orf72 arginine-rich dipolypeptide repeats proteins** Viacheslav Kriachkov	12
09.45 – 10.00	**Malaria parasite trafficking defect: weird knobs, vesicle pile-up and failure to deliver a virulence factor** Olivia Carmo	13
SESSION 2		
10.30 – 11.00	**Ultra-high-throughput screening yields bromodomain inhibitors with record-breaking affinity and specificity** **Keynote Speaker:** Prof. Joel Mackay	
11.00 – 11.15	**Can the real kidney cell types please stand up?** Sean Wilson	14
11.15 – 11.30	**MmCSM-PPI: accurate estimation of the effects of multiple mutations in protein-protein binding affinity** Carlos Rodrigues	15
11.30 – 12.00	**Centrosomal mechanisms required for brain growth** **Keynote Speaker:** A/Prof. Dominic Ng	
12.00 – 12.15	**Targeting pathological mechanisms of the mitochondrial disease Sengers syndrome using human pluripotent stem cells** Yau Chung Low	16
12.15 – 12.30	**Investigating the role of Plasmodium falciparum exported proteins that bind the new permeability pathway protein RhopH2** Thorey Jonsdottir	17
SESSION 3		
17.00 – 17.30	**Small Molecule Binding to Disordered Proteins** **Keynote Speaker:** Dr. Gabriella Heller	
17.30 – 17.45	**The transmembrane organisation of the B cell antigen receptor** Samyuktha Ramesh	18
17.45 – 18.00	**Application of activity-based probes to interrogate the contribution of cathepsin X to dendritic cell function** Bangyan Xu	19
18.00 – 18.15	**The cell biology of the neonatal Fc receptor-albumin recycling system in immune cells** Xiao Peng Lin	20
18.15 – 18.30	**Discovery of the molecular machinery regulating antigen presentation of bacterial metabolites by MR1** Hui Lim	21
18.30 – 18.45	**The interaction of apoA-I with lipid monomers and implications for amyloid fibril formation** Phoebe Tou	22

BIOMEDICINE

Molecular and Cell Biology

Keynote Speakers

Professor Marcia Haigis
Dept. of Cell Biology
Harvard Medical School
Harvard University

Session 1 8.15 - 8.45 am

Marcia C. Haigis obtained her Ph.D. in Biochemistry from the University of Wisconsin in 2002. She performed postdoctoral studies at MIT studying mitochondrial sirtuins and metabolism. In 2006, Dr. Haigis joined the faculty of Harvard Medical School, where she i currently a Professor in the Department of Cell Biology. Dr. Haigis is an active member of the Paul F. Glenn Center for the Biology of Aging, a member of the Ludwig Center at Harvard Medical School, and was recently selected for the National Academy of Medicine Emerging Leaders in Health and Medicine Program.

Her research aims to: identify molecular mechanisms by which mitochondria respond to cellular stress and to elucidate how these cellular mechanisms contribute to aging and age-related diseases, such as cancer. The Haigis lab has made key contributions to our understanding of metabolic reprogramming in cancer, including identifying nodes of metabolic vulnerability in the control of fat oxidation in leukemia and dissecting the effect of diet on anti-tumor immunity.

PHD3 Loss Promotes Exercise Capacity and Fat Oxidation in Skeletal Muscle.
Yoon H, Spinelli JB, Zaganjor E, Wong SJ, German NJ, Randall EC, Dean A, Clermont A, Paulo JA, Garcia D, Li H, Rombold O, Agar NYR, Goodyear LJ, Shaw RJ, Gygi SP, Auwerx J, **Haigis MC**. Cell Metab. 2020 Jul 9:S1550-4131(20)30318-1. doi: 10.1016/j.cmet.2020.06.017

Metabolic recycling of ammonia via glutamate dehydrogenase supports breast cancer biomass.
Spinelli JB, Yoon H, Ringel AE, Jeanfavre S, Clish CB, **Haigis MC**. Science. 2017;358:941-946. PMID:29025995

Professor Joel Mackay
School of Life and Environmental Sciences
University of Sydney

Session 2 10.30- 11.00am

Joel Mackay trained as a chemist at the University of Auckland and a chemical biologist at The University of Cambridge before coming to Australia to postdoc with Glenn King at the University of Sydney. Over the course of the subsequent 25 years, he has succeeded in moving a total of about 20 metres up the corridor to where he runs a laboratory that is focused on the molecular mechanisms underlying mammalian gene regulation.

His main expertise is structural biology and the analysis of protein interactions, but that hasn't stopped the lab branching out into more areas than it probably should have. The work of his group has been recognized by several national scientific awards, including the Gottschalk medal from the Australian Academy of Science, the Roche medal and Labgear award from the ASBMB and the Prime Minister's Life Scientist of the Year award.

Cyclic peptides can engage a single binding pocket through highly divergent modes.
Karishma Patel, Louise J. Walport,....., **Joel P. Mackay**. Proceedings of the National Academy of Sciences Oct 2020, 117 (43) 26728-26738; DOI: 10.1073/pnas.2003086117

BIOMEDICINE

Molecular and Cell Biology

Keynote Speakers

A/Professor Dominic Ng
School of Biomedical Sciences
The University of Queensland

Session 2 11.30 am- 12.00pm

Dominic is an Associate Professor at the School of Biomedical Science, UQ where he leads a research program on the molecular control of tissue growth with a focus on the regulation and function of protein kinases. His PhD was awarded in 2004 followed by post-doctoral training overseas at the Institute of Molecular and Cell Biology, A*STAR, in Singapore where he worked on signalling regulation of microtubules in migrating cancer cells. He returned to Australia to the Bio21 Institute, University of Melbourne, on an NHMRC Peter Doherty Fellowship (2006-2010) followed by a Medical Faculty Trust Roper Fellowship (2011-2012) and an ARC Future Fellowship (2013-2016). In this time, he established an independent research program investigating complex signalling mechanisms in cancer, cardiovascular and neurological disease using approaches in optical imaging, proteomics, reverse genetics and diverse model systems.
In 2015, he relocated his group to the University of Queensland to take up a Senior Lectureship in Physiology and was promoted to Associated Professor in 2018. He is currently appointed to the Australian Academy of Science's National Committee for Cell and Developmental Biology, is National Secretary of the Australian Biochemical Society and associate editor for IUBMB Life, the flagship journal of the International Union of Biochemistry and Molecular Biology. His recent publications, and topic of this presentation, have described centrosome-associated signalling mechanisms required for stem cell fate specification and tissue morphogenesis.

Stat3 regulates microtubules by antagonizing the depolymerization activity of stathmin
DCH Ng, BH Lin, CP Lim, G Huang, T Zhang, V Poli, X Cao. T*he Journal of cell biology* 172 (2), 245-257

Dr. Gabriella Heller
Institute of Structural and Molecular Biology
University College London

Session 3 5.00 - 5.30pm

Gabriella Heller studied Chemistry and Mathematics at Pomona College in Claremont, California. She completed her MPhil degree in the Chemistry Department at the University of Cambridge in 2014 as a Churchill Scholar, and remained in that Department as a Gates Cambridge Scholar for her PhD. From 2019 to 2020 she was the Rosalind Franklin Research Fellow at Newnham College, Cambridge and is currently a Schmidt Science Fellow based in the Department of Structural and Molecular Biology at University College London.

Gabriella's research focuses on intrinsically disordered proteins, biomolecules lacking a single, rigid three-dimensional structure that instead exist as a dynamic equilibrium of conformationally distinct states. These proteins are highly prevalent in diseases such as dementia and cancer, yet are often considered therapeutically 'untargetable' as they lack traditional drug binding pockets. Using a combination of computational and experimental biophysical techniques, Gabriella has elucidated novel mechanisms by which small molecules can interact with disordered proteins.

Small molecule sequestration of amyloid-β as a drug discovery strategy for Alzheimer's disease.
Heller, Gabriella T., et al. Science Advances (2020). Vol. 6, no. 45, eabb5924.
DOI: 10.1126/sciadv.abb5924

ABSTRACTS

BIOMEDICINE
Molecular and Cell Biology

Expanding the peptide synthesis toolkit to produce bicyclic peptide mimetics for drug discovery

Qingqing Lin[1], Denham Hopper[1], Haoyue Zhang[1], Jordan Syris Qoon[1], Zihan Shen[1], John A. Karas[1], Richard A. Hughes[1] & Susan E. Northfield[1]

[1] Department of Pharmacology & Therapeutics, School of Biomedical Sciences, The University of Melbourne, Melbourne, VIC 3010, Australia

Introduction: The design and synthesis of cyclic peptides is a widely established practice in the field of peptide chemistry. This has been further expanded by the development of orthogonal chemical reactions allowing for production of more chemically complex peptides. We have recently reported a versatile method to produce bicyclic homodimer peptides that are selective mimetics of loops of large proteins, including neurotrophins.

Aims: Use 1,3-dichloroacetone (DCA) to selectively link free cysteine side-chains via an acetone bridge, producing bicyclic dimeric peptides.

Methods: Synthesised six backbone-cyclic peptides, each possessing a single cysteine residue, and created bicyclic dimeric peptides by linking two copies of the cyclic peptide together via an acetone linker using DCA. We systematically investigated a range of reaction conditions, including reaction stoichiometry, peptide concentration, pH and buffer composition.

Results: We were successfully able to identify the optimum conditions for peptide dimerisation for our six peptide sequences and have use these results to produce an overall guide for preparing acetone-linked bicyclic peptides. The peptides were subsequently analysed for proteolytic stability in human serum and were observed to still be fully intact after 48 hours.

Conclusion: This study provides valuable insights into the use of DCA as a tool in peptide synthesis. The non-reducible nature of the acetone linker between pairs of cysteine residues makes the DCA dimerisation reaction attractive compared to the better-known disulfide bond approach.

Characterising novel Coxiella burnetii effector protein interactions

Cameron C. Oppy[1,2], Yee-Foong Mok[1], Courtney O. Zlatic[1], Riley D. Metcalfe[1], Hayley J. Newton[2] & Michael D. W. Griffin[1]

[1] Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, Victoria, Australia
[2] Department of Microbiology and Immunology, Peter Doherty Institute, University of Melbourne, Parkville, Victoria, Australia

Introduction: Coxiella burnetii is an obligate intracellular bacterial pathogen that causes Q fever in humans and animals. Unlike other intracellular bacterial pathogens, C. burnetii occupies a unique autolysosomal niche termed the Coxiella containing vacuole (CCV). The establishment of the CCV is necessary for C. burnetii survival and replication within host cells. C. burnetii utilises an arsenal of over 130 type IV effector proteins (T4Es) some of which modulate host cell trafficking pathways allowing for the formation of the CCV. Cig57 (Cbu1751), Cbu1752, and Cbu1754 are three novel Coxiella T4Es implicated in CCV biogenesis through the subversion of host clathrin-mediated transport. Cig57 interacts with the host protein FCHO2, a nucleator of clathrin-mediated transport. A Cig57/FCHO2 interaction re-routes clathrin to the CCV with unknown effect, and is thought to be mediated by endocytic sorting motifs present on Cig57. Cbu1752 and Cbu1754 are hypothesised to act synergistically with Cig57, based on the ability of Cig57, Cbu1752, and Cbu1754 to co-immunoprecipitate. Although Cig57, Cbu1752, and Cbu1754 have been identified as T4Es implicated in CCV expansion, their mechanistic function and relationship with each other and the clathrin mediated transport system as a whole remains unknown.

Methods: Using a combination of analytical ultracentrifugation (AUC) and small angle x-ray scattering (SAXS) experiments, the basic structural characteristics of Cig57, Cbu1752, and Cbu1754 were determined. Effector interactions were probed with microscale thermophoresis (MST) and native polyacrylamide gel electrophoresis (native PAGE). Structures were solved with X-ray crystallography.

Results: Cig57 and Cbu1754 are monomeric and roughly globular in solution, whereas Cbu1752 adopted an extended conformation and displayed low levels of self-association. The crystal structure of the Cig57 central domain (residues 178-307) was solved with a resolution of 1.75 Å. The central domain of Cig57 adopts a 4-helix bundle and reveals two hypothetical endocytic sorting motifs form the hydrophobic core of Cig57, indicating that these previously described 'motifs' are highly unlikely to mediate interactions and instead serve to stabilize the Cig57 structure. Cig57 displays weak binding to the host protein FCHO2, whereas Cbu1752 appears to bind clathrin heavy chain.

Conclusion: This work is the first to structurally characterise any Coxiella protein, leading to a better understanding of novel effector protein function and clathrin mediated transport.

Bioinformatics analysis and virtual screening of the AAA+ ATPase p97 in protozoan parasites and Mycobacterium tuberculosis

George Kobakhidze[1], Ashish Sethi[1] & Isabelle Rouiller[1]

[1] Department of Biochemistry and Molecular Biology, Bio21 Institute, University of Melbourne, Parkville, Victoria 3010, Australia

Introduction: Protozoan parasites such as Plasmodium falciparum, Leishmania donovani, Trypanosoma brucei, Toxoplasma gondii, and the pathogenic bacteria Mycobacterium tuberculosis threaten many people worldwide. Currently, there are no effective vaccines and emerging resistant strains or superbugs pose a deep threat to humanity. One strategy to eliminate these deadly organisms is to compromise essential pathways. For this, we have turned our attention to the AAA+ ATPase enzyme, p97. A highly conserved and essential protein, p97 is a homohexameric unfoldase involved in various cellular activities such as endoplasmic reticulum-associated degradation (ERAD), autophagy, membrane fusion and more. Our approach is to inhibit this enzyme to cause proteotoxic stress to a cell, resulting in death of the pathogen. Here, we present a structural analysis of the homologues of p97 from five pathogenic organisms and virtual screening results for novel and specific inhibitors using bioinformatics tools.

Methods: Homologues of human p97 were discovered in each species using BlastP. Clustal Omega was used for multiple sequence alignment of the amino acid sequences to analyse conservation of specific regions in pathogenic organisms. SWISS-MODEL was used for homology modelling to generate 3D models. These models were used in docking experiments with known p97 inhibitors using AutoDock Vina. This led to virtual screening of a lead-like compound library (5921 ligands) obtained from ZINC15. A pocket between the D1 and D2 domains, where an allosteric site is located in human p97, was chosen for screening the library with FRED (OpenEye Suite).

Results: Homologues of human p97 were obtained with sequence identities ranging 36-75%. Multiple sequence alignment showed that nucleotide and cofactor-binding sites were highly conserved between species, but domain interfaces such as N-D1 and D1-D2 showed higher sequence variability. Inhibitor docking showed consistency in binding to the nucleotide-binding site but more variation for a potential allosteric site in the D1-D2 interface. Virtual screening predicted several compounds that would bind potently and specifically to this site for each of the p97 homologues.

Conclusion: The research aims to provide insight into the structure and function of p97 homologues in protozoan parasites and M. tuberculosis. The selective targeting of the p97 protein in these pathogens is a novel concept with potential for future studies in drug design. In the near future, in vitro assays will be developed to confirm the binding affinity of these compounds to their respective p97. This study will also be accompanied by experiments for structural determination and biochemical characterisation.

Ribosomal stalling on C9orf72 arginine-rich dipolypeptide repeats proteins

Viacheslav Kriachkov[1], Justine D Mintern [1] & Danny M Hatters[1]

[1] Department of Biochemistry and Molecular Biology. Bio21 Molecular Science and Biotechnology Institute. The University of Melbourne, Victoria 3010 Australia

Introduction: Long arginine-rich dipeptide repeat (Arg-rich DPR) proteins are the toxic species involved in the neurodegeneration associated with some forms of amyotrophic lateral sclerosis and frontotemporal dementia. The ability of these proteins to cause ribosomal stalling during translation is not well-studied and hypothesized to be one of the mechanisms underlying neurotoxicity in these diseases. Ribosomal stalling occurs as a result of defective translation and is known to be an important part of ribosome-associated quality control. It was initially proposed that the blockage of ribosome exit tunnel by aberrant peptides is the main event initiating ribosomal stalling on polybasic tracts such as arginine- or lysine-rich sequences. However, recent studies have shown that translational arrest on poly-Lys is codon-dependent and requires the presence of specific factors such as ZNF598 ubiquitin ligase. It remains unknown whether the same mechanism induces translational arrest in case of arginine-rich dipeptide repeats.

Methods: To identify the mechanisms that mediate stalling by long Arg-rich DPR and whether they are the same as that for poly-Lys, we implemented a dual-fluorescent reporter system that provides quantitative measurement of stalling efficiency in cells with elongation-stalled ribosomes. Using an essay with distinct phenotype tied to ribosomal stalling, we conducted a genome-wide CRISPR knockout screen to identify genes that modify translational arrest on poly(A) and long Arg-rich DPRs.

Results: After validation of top screen hits, a few novel genetic modifiers of ribosomal stalling were identified including initiation factor eIF6, depletion of which lead to more than 2.5-fold increase in poly(A) readthrough as well as a slight improvement of readthrough on poly-Pro-Arg staller. Interestingly, the knockout of known regulators of ribosome-quality control ZNF598 and RACK1 had the opposite effect between two types of stallers: it greatly alleviated stalling on poly(A) sequence but enhanced elongation arrest during translation of poly-Pro-Arg.

Conclusion: In this work, we generated a library of potential genes that either abrogate or enhance ribosomal translational arrest. Further comparison between two different types of stallers lead to hypothesis that elongation arrest caused by long Arg-rich DPR is much more durable and governed by a different mechanism comparing to ribosome stalling on poly(A) sequence.

BIOMEDICINE
Molecular and Cell Biology

Malaria parasite trafficking defect: weird knobs, vesicle pile-up and failure to deliver a virulence factor

Olivia M. S. Carmo[1], Emma McHugh[1], Boyin Liu[1], Gerald J. Shami[1], Adam Blanch[1], Snigdha Tiash[1], Eric Hanssen[2], Paul McMillan[3], Leann Tilley[1] & Matthew W.A. Dixon[1]

[1] Department of Biochemistry and Molecular Biology
[2] Melbourne Advance Microscopy Facility
[3] Biological Optical Microscopy Platform, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Melbourne, VIC 3052, Australia

The parasite Plasmodium falciparum is responsible for the most virulent form of malaria, killing ~500,000 people annually. The parasite's virulence arises during the asexual blood stage, in which the parasite invades red blood cells, remodels the host cell to survive and replicate, then egresses from the cell for subsequent cycles of invasion. The mature red blood cell is effectively a bag of haemoglobin, lacking any endogenous machinery for the parasite to co-opt. To adapt, the parasite exports ~500 proteins into the host cell to establish protein trafficking systems and allow for remodelling of the host cell membrane. This remodelling is important for the delivery of parasite antigens to the red blood cell surface and ultimately underlies pathogenicity of the parasite. Among the exported proteins, none bear semblance to canonical secretory machinery. Instead, more than a quarter of exported proteins contain extended, often repetitious regions. How these exported proteins coordinate to traffic parasite antigens to the surface and remodel the host cell is poorly understood.

In this work, we study one exported protein predicted to have an unstructured terminus, enriched in basic residues. Deletion of this protein results in an accumulation of parasite-derived vesicles in the infected red blood cell cytoplasm, in addition to ablation of surface-exposed parasite antigens and aberrant remodelling of the host cell membrane. In an environment void of canonical trafficking machinery, we hypothesize that our protein of interest contributes to a series of weak electrostatic interactions sufficient to traffic vesicles and proteinaceous complexes to the host cell periphery.

Can the real kidney cell types please stand up?

Sean B. Wilson[1,2,4], Jose Alquicira-Hernandez[4], Jessica M. Vanslambrouck[1,2], Sara E. Howden[1,2], Joseph E. Powell[4] & Melissa H. Little[1,2,3]

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[2] Department of Paediatrics, The University of Melbourne, Melbourne, Vic, Australia
[3] Department of Anatomy and Neuroscience, The University of Melbourne, Melbourne, Vic, Australia
[4] Garvan-Weizmann Centre for Cellular Genomics, Garvan Institute, Sydney

Introduction: Understanding the processes that drive kidney organogenesis has led to the development of protocols for differentiating pluripotent stem cells (PSCs) into kidney-like tissues in vitro. However, recent studies of kidney organoids have highlighted differences at both the transcriptional and morphological level between PSC-derived and bona fide kidney tissue. Single cell RNA sequencing is a powerful tool, however the snapshot of information can be limiting, particularly when considering kidney organoids are a dynamic population with cells at many stages of their differentiation trajectory. This makes categorizing cells into distinct identities difficult yet important as robust cell classification is essential to draw accurate conclusions from studies using organoid models such as disease modelling and drug screening. Using existing arbitrary methods for clustering cells makes it difficult to directly compare the growing number of datasets to each other or any novel datasets. To resolve this, the KidneyCC R package provides a complete cell classifier applicable to any single cell kidney organoid dataset for robust and reproducible cell classification.

Method: A composite human fetal kidney transcriptional reference generated from multiple published datasets was used to derive machine learning models for the supervised classification of cell type. Using a hierarchy of models enabled increased classification accuracy of similar cell identities. This classification method was then used on all available published datasets and a novel organoid sample to investigate direct comparisons between organoids.

Results: The application of KidneyCC was able to rapidly replicate the classification of existing kidney organoid datasets, while providing greater stringency with relation to stromal populations and nephron segments. Direct comparisons between multiple differentiation protocols and experimental batches reinforced predicted differences in nephron patterning, off target populations and relative maturation. We then used KidneyCC to classify and compare cells within a novel dataset showing that the addition of retinoic acid can cause depletion of nephron progenitor cells and a shift in patterning of the developing nephrons.

Conclusion: KidneyCC enables rapid, robust and quantitative identification of kidney cell identity on novel datasets while overcoming many challenges associated with current standard methods. We demonstrated how it can be used to gain further understanding of kidney organoid development from both existing and novel datasets. This provides a method for reliable comparisons between kidney organoids that will benefit future studies that utilise kidney organoid models.

MmCSM-PPI: accurate estimation of the effects of multiple mutations in protein-protein binding affinity

Carlos H. M. Rodrigues[1,2,3], Douglas E. V. Pires[1,2,3,4] & David B. Ascher[1,2,3]

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[2] Computational and Systems Biology, Bio21 Institute, University of Melbourne, 30 Flemington Rd, Parkville VIC 3052
[3] Computational Biology and Clinical Informatics, Baker Heart and Diabetes Institute, Melbourne VIC 3004
[4] School of Computing and Information Systems, University of Melbourne, Melbourne, VIC 301

Protein-protein interactions (PPIs) are known to coordinate vital biological processes within the cell. Missense mutations at protein-protein interfaces are known to directly contribute to function disruption and are enriched in many diseases. Understanding the underlying mechanisms by which missense mutations affect interactions is essential for understanding how to modulate these interactions and the development of therapeutics that target them. Despite significant effort in recent years to build computational approaches to predict the effects of missense mutations on PPIs, these have been shown to perform poorly on independent test sets and are usually limited to predicting effects of single-point mutations.

To fill this gap, here we introduce mmCSM-PPI, a scalable and effective predictive model for assessing changes in protein-protein binding affinity caused by multiple missense mutations. We expanded our well-established graph-based signatures which capture physicochemical and geometrical properties of the wild-type residue environment, and integrate them with substitution scores and dynamics terms from NMA. These were used as inputs to train a machine learning model. Given the unbalanced nature of our dataset comprising 1126 multiple mutations (897 increasing affinity, 224 decreasing affinity and 5 neutral), here we have also explored how the use of hypothetical reverse mutations can contribute towards more balanced predictions. mmCSM-PPI was able to achieve a Pearson correlation of 0.85 (RMSE = 1.30 kcal/mol) under 10 fold cross-validation and 0.83 (RMSE = 1.26 kcal/mol) on a non-redundant blind test, outperforming other methods. To further investigate potential biases in predictive performance, we assessed the robustness of our approach on leave-one-complex-out cross-validation. Here, mmCSM-PPI showed consistency and achieved a Pearson correlation of 0.78 (RMSE = 1.51 kcal/mol).

We anticipate mmCSM-PPI to be of great value for the study of how multiple mutations affect protein-protein binding affinity and to a variety of applications, ranging from protein functional analysis, optimisation of binding affinity and understanding the role of mutations in diseases. Our method is freely available as user-friendly and easy-to-use web servers at http://biosig.unimelb.edu.au/mmcsm_ppi and an API to assist users when integrating our predictions into their research pipelines.

BIOMEDICINE
Molecular and Cell Biology

Targeting pathological mechanisms of the mitochondrial disease Sengers syndrome using human pluripotent stem cells

Yau Chung Low[1] , Cameron McKnight[1] , Yilin Kang[2] , Thomas Jackson[2], Daniella Hock[2], David Stroud[2], David Elliot[1], Eric Hanssen[3], Michael Ryan[4], Diana Stojanovski[2], David Thorburn[1,5] & Ann Frazier[1]

[1] Murdoch Children's Research Institute, Parkville, VIC, Australia
[2] Department of Biochemistry and Molecular Biology and The Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, VIC, Australia
[3] Advanced Microscopy Facility, The Bio21 Molecular Science and Biotechnology Institute, Parkville, VIC, Australia
[4] Monash Biomedicine Discovery Institute, Monash University, Clayton, VIC, Australia
[5] Victorian Clinical Genetics Services, Royal Children's Hospital, Melbourne, VIC, Australia

Introduction: Sengers syndrome is a potentially fatal mitochondrial disease characterised by hypertrophic cardiomyopathy, congenital cataracts, lactic acidosis, and exercise intolerance. This disease is caused by mutations in the acylglycerol kinase (AGK) gene. The enzymatic activity of AGK functions in mitochondrial lipid homeostasis. Additionally, AGK is a structural subunit of the mitochondrial TIM22 protein import complex and facilitates mitochondrial carrier protein biogenesis. Like most mitochondrial diseases, the limited range of effective treatments for Sengers syndrome is due to factors including unidentified disease mechanisms, tissue specific clinical presentations, difficulty accessing patient tissues, constraints of animal models, and limited patient cohorts for clinical trials. This project seeks to overcome these challenges by investigating the molecular and cellular pathogenesis underlying Sengers syndrome using human embryonic stem cells (hESCs) differentiated to clinically relevant cardiomyocytes.

Methods: AGK-/- hESCs were generated using CRISPR/Cas9 gene editing technology, and validated for pluripotency and karyotype. Mutants were characterised by genetic (DNA and RNA) and immunoblot experiments. ATP synthesis assay was performed to determine the effects of these mutations on ATP production. Selected clones were differentiated into cardiomyocytes (hESC-CMs) and further functional analyses performed including calcium imaging and electron microscopy experiments.

Results: DNA sequencing and cDNA studies identified multiple hESC clones with AGK mutations causing a frameshift and premature stop codons, or splicing defects. As expected, RNAseq revealed that the AGK gene was the most significantly downregulated gene in the selected hESC mutant. Mutant hESCs were successfully differentiated into cardiomyocytes as confirmed through immunocytochemistry staining and FACS analysis. Immunoblot experiments revealed that TIM22 complex assembly is disrupted in AGK-/- cells. This affected the biogenesis of the ATP carrier and glutamate carrier, resulting in a significant defect in ATP synthesis. Additional preliminary investigations suggest that AGK-/- hESC-CMs display an irregular beating pattern and abnormal calcium handling compared to controls. Furthermore, samples analyzed by electron microscopy suggest that the AGK-/- hESC-CMs possessed disorganised myofibrils. Doxycycline inducible correction lines have been generated to rescue these defects observed.

Conclusion: The AGK-/- hESCs and differentiated cardiomyocytes have the potential to provide clinically relevant tissue samples for further investigations of disease pathomechanisms, with future experiments planned including proteomic and lipidomic analyses of mutants. Ultimately, these cells could be used to facilitate pre-clinical studies testing potential treatments for Sengers syndrome by targeting the affected pathways to improve mitochondrial function and cardiomyocyte physiology.

Investigating the role of Plasmodium falciparum exported proteins that bind the new permeability pathway protein RhopH2

Thorey Jonsdottir [1,2], Natalie Counihan [3], Benjamin Dickerman[1], Smitha Sudhakar [3], Joyanta Modak [3], Betty Kouskousis[1,4], Mikha Gabriela[1,3], Paul Sanders[1], Haley Bullen[1], Tania de Koning-Ward [3], Brendan Crabb [1,2,5] & Paul Gilson[1]

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Introduction: Every day over 1000 people die of malaria, most of who are children under the age of five. The emergence of drug resistance in Plasmodium falciparum, the causative agent of severe malaria, is alarming and we need new antimalarial drugs. A crucial part of this process is to understand the basic biology of the parasite and elucidate targetable proteins/pathways. The ability of P. falciparum to survive within the erythrocyte is dependent on the new permeability pathways (NPPs), which provide import of vital nutrients, making them an attractive drug target. The NPPs have been affiliated with the activity of three parasite proteins: RhopH1, RhopH2 and RhopH3. We previously showed that RhopH2 was associated with 30 additional proteins predicted to be exported into the erythrocyte. Protein export is a prerequisite for NPP activity and we therefore hypothesised that some of these exported proteins might be important for NPP function. Here we study 13 of the 30 proteins associating with RhopH2 and their potential role in NPP activity.

Methods: All 13 proteins we tagged with HA protein tag for detection and a glmS riboswitch to conditionally knockdown individual proteins for phenotypic analysis. An exported nanoluciferase reporter was introduced into each parasite line to observe changes in sorbitol lysis (NPP uptake) during protein knockdown. Immunoprecipitation assays were performed to confirm protein interaction and analysed via mass spectrometry and western blotting.

Results: Sorbitol lysis assays showed that none of the 13 proteins were individually required for NPP function. Reciprocal immunoprecipitation assays further showed that only six proteins were able to co-precipitate RhopH2. Majority of these six proteins are known cytoskeletal proteins and therefore likely interacting indirectly with RhopH2 due to cytoskeletal binding and not due to direct NPP function. We also identified potential new parasite complexes within the infected erythrocyte, interacting with both J-dots and Maurer's clefts structures, which are trafficking hubs of the parasite.

Conclusion: Although the 13 proteins studied do not appear to be essential for the NPPs, this study greatly expands our current knowledge of exported proteins. We reveal for the first time the location of five new proteins, hypothesised to be exported, as well as providing a clearer picture of protein-protein interaction within the P. falciparum infected erythrocyte.

The transmembrane organisation of the B cell antigen receptor

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I**ntroduction:** The antigen receptors on T and B cells, the T-cell receptor (TCR) and B-cell receptor (BCR), respectively, are the key initiators of adaptive immune responses against both internal and external threats to the body. Being single-pass membrane protein complexes, structural characterisation by traditional biophysical techniques had proven challenging in the past due to their hydrophobic transmembrane domains (TMDs). Recent advances in cryo-EM enabled the first high-resolution structure of an intact receptor of this class to be resolved: the octameric αβ TCR complex. The central αβ interface reflected in the TMD of this structure was previously identified by our lab using a combination of experimental and computational methods. We now employ a similar combination of techniques to map the organisation of the much less studied tetrameric BCR complex, composed of a homodimeric ligand-sensing membrane-bound immunoglobulin (mIg) protein and a signal-transducing CD79AB dimer.

Methods: We perform cysteine-crosslinking to identify points of close packing between the four helices of the BCR TMD in an in vitro translation and assembly system supplemented with endoplasmic reticulum microsomes to replicate physiological assembly. This data is then used as distance restraints for molecular dynamics (MD) simulations to generate models of how the TMDs would pack in a membrane. Additionally, interfacial residues were mutated to analyse their roles in the assembly.

Results: Crosslinks were successfully identified between the TMDs of the four BCR chains and used to restrain MD simulations. The mIg homodimer model thus generated shows that a polar network similar to an N-T-Y network stabilizing the TCRαβ TMD interface likely also exists in the BCR mIg, comprising S-Y-S-T residues. Mutations to remove the hydrogen bonding -OH groups from these residues reveal complex interactions between the mIg and CD79s. Modelling of the complete tetrameric TMD is currently under way.

Conclusion: We have thus provided novel structural details on the packing and interactions within the BCR TMD, and identified a structural motif shared with the TCR. We aim to better understand the evolution of antigen receptor structures and the structure-function relationship in these complex membrane protein assemblies.

Application of activity-based probes to interrogate the contribution of cathepsin X to dendritic cell function

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I**ntroduction:** Cathepsin X/Z/P (Cat X) is a lysosomal cysteine protease that exhibits unique monocarboxypeptidase activity. Increased Cat X expression is associated with cancer and inflammation, although its roles in normal physiology are still poorly understood. It is highly expressed by antigen-presenting cells such as dendritic cells (DCs). DCs undergo functional changes upon agonism of pattern recognition receptors (e.g., Toll-like receptors) by pathogen-associated molecular patterns such as CpG. Mature DCs can secrete cytokines, present antigens to T cells, etc. We hypothesise that Cat X contributes to DC function, but this has not been examined in detail until now.

Methods: We used activity-based probes, immunoblotting, and immunofluorescence imaging to measure active and total levels of Cat X in naïve DCs (DC1940) or those stimulated with agonists of TLR1/2, 2/6, 3, 4, 7/8, and 9 (Pam3, FSL-1, Poly I:C, LPS, R848, and CpG, respectively). To examine the impact of Cat X on DC function, we generated Cat X-deficient DC1940 cells using CRISPR-Cas9. DC maturation was examined using flow cytometry by detecting maturation-related surface markers. Cytokine secretion was quantified using a cytometric bead array, and antigen presentation was analysed by measuring DC-induced T cell expansion.

Results: Lysosomal and secreted Cat X levels were both significantly elevated in response to CpG treatment, and to a much lesser extent with other TLR agonists. Expression of surface markers indicating DC maturation was unaffected by Cat X deficiency. By contrast, we observed a reduction in the secretion of several chemokines and cytokines (CCL3, CCL5, CD14, IL-12 and TNF-α) upon CpG stimulation in Cat X-deficient cells. Cat X-deficient cells exhibited altered processing of cathepsin L, while the activities of other lysosomal proteases were unaffected. We also found that Cat X-deficient cells had impaired antigen presentation ability.

Conclusion: Collectively, these data indicate that Cat X is strongly upregulated by TLR-9 agonism in DCs. While it may not be essential for DC maturation, Cat X may regulate cytokine secretion, processing of related endolysosomal proteases, and antigen presentation. Re-expression of Cat X (wildtype, catalytically dead, or an integrin-binding mutant) will be used in the future to validate the phenotypes of Cat X-deficient DCs and further interrogate its molecular mechanism.

The cell biology of the neonatal Fc receptor-albumin recycling system in immune cells

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Introduction: In the adult, the neonatal Fc receptor (FcRn) is responsible for the intracellular recycling of endocytosed albumin and immunoglobulin G (IgG) and contributes to their long serum half-life. FcRn rescues endocytosed albumin and IgG from lysosomal degradation by capturing these ligands in acidic endosomes and recycling them back to the cell surface, thereby extending their lifetime within the circulation. As such, there is considerable interest in exploiting the biology of FcRn to increase the half-life of therapeutic proteins. However, many aspects of the cell biology of FcRn and the recycling of its ligands remain poorly defined, and the relative contribution of different cell types to FcRn-mediated recycling remains unclear. FcRn has shown to be expressed in immune cells such as monocytes, macrophages and dendritic cells. However, the function of FcRn in these cells and their contribution to albumin homeostasis in vivo is unclear. The aim of this project is to define the internalisation and trafficking itinerary of albumin in immortalised and primary dendritic cells and to determine the relative contribution of immune cells to albumin homeostasis in vivo.

Methods: Primary macrophages and dendritic cells were isolated from mice. Quantitative western blot was used to determine FcRn expression levels in different immune cells. Pulse-chase experiments, followed by high resolution fluorescence microscopy or FACS analysis, were performed to track the endocytosis and trafficking of fluorescently labelled albumin. Macropinocytosis was defined by the size of endocytic vesicles and by inhibition with macropinocytosis-specific inhibitors. Recycled albumin in dendritic cell culture medium was quantified by ELISA.

Results: FcRn is differentially expressed in different primary immune cells, including differences between the two splenic conventional dendritic cell subsets, cDC1 and cDC2. Albumin is mainly internalised by dendritic cells by macropinocytosis. Differences have been detected between the uptake of albumin by the macropinocytosis pathway in dendritic cells compared with the classical macropinocytosis pathway in macrophages. In dendritic cells, internalised albumin is trafficked to antigen-processing compartments and is slowly lost from the cells, likely due to a combination of degradation and recycling.

Conclusion: FcRn is expressed at different levels in different immune cells, which may reflect differential abilities to recycle albumin and IgG. Albumin is internalised by dendritic cells by a non-classical macropinocytosis pathway and is delivered to antigen-processing compartments. Internalised albumin is not rapidly degraded by the cells and a proportion is recycled, suggesting that dendritic cells may contribute to the prolonged half-life of albumin in vivo.

Discovery of the molecular machinery regulating antigen presentation of bacterial metabolites by MR1

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Major histocompatibility complex class I-related protein 1 molecule (MR1) presents bacteria-derived vitamin B metabolites that are synthesised by wide range of microbes. Antigen presentation of MR1 is critical for the establishment, development and activation of highly abundant innate-like T cells, mucosal associated invariant T (MAIT) cells. Activated MAIT cells secrete inflammatory cytokines and acquire cytotoxic activity to clear the infection. Recent study also showed that MR1 presents tumour associated antigen (TAA) that expressed by wide range of tumour cells and promotes tumour killing. Hence, understanding of the regulatory machinery MR1 antigen presentation is important for the development of potential therapy for bacterial infection as well as cancer.

MR1 is maintained intracellularly as an endoplasmic reticulum (ER)-resident pool in the absence of infection, but encounter of metabolic ligands induces the trafficking of MR1 to the cell surface. MR1-ligand complexes stay on the cell surface for several hours after which they are internalised and mostly degraded. Elimination of surface MR1 is a requirement to terminate MAIT cell responses, so the cellular machinery that controls the internalisation of MR1 plays a critical role in the regulation of the MR1-MAIT cell axis.

With genome-wide CRISPR-Cas9 library screen, we have identified adaptor protein complex 2 alpha subunit (AP2A1) as the regulator of MR1 internalisation. Interaction of AP2A1 and MR1 was observed via proximity ligation assay. When AP2A1 is depleted in the cells, MR1 is internalised at much lower rate and presents antigen for prolong time. MR1 cytoplasmic tail consists of tyrosine-based motif which is highly conserved across mammalian species. However, this tyrosine-based motif is not a canonical sorting motif. In conclusion, MR1 internalisation is regulated by AP2 using a novel recognition motif. These results open the possibility of manipulating the internalisation of MR1 without affecting the trafficking properties of most AP2-regulated membrane proteins

The interaction of apoA-I with lipid monomers and implications for amyloid fibril formation

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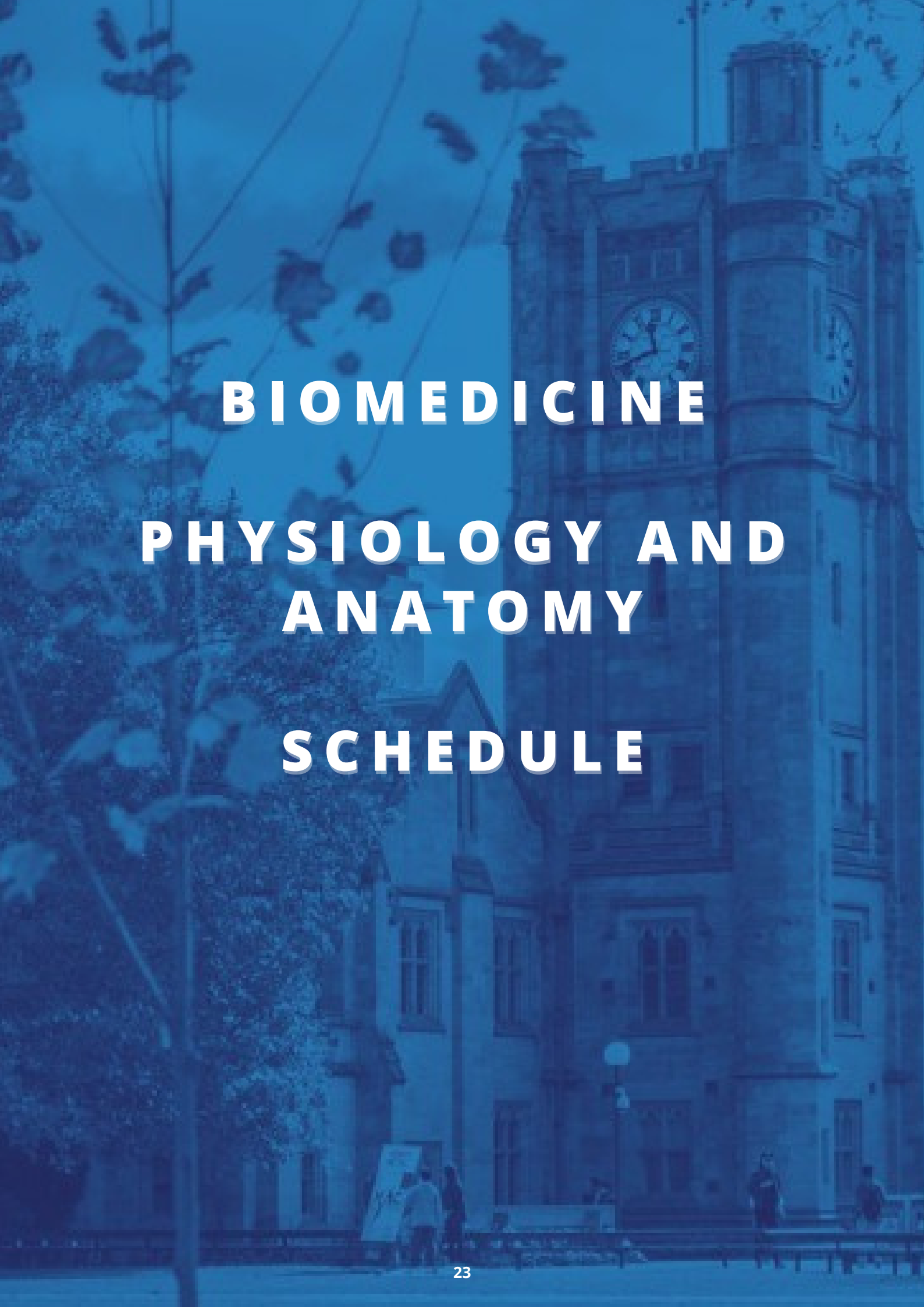
[1] Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Victoria 3010, Australia

Introduction: Apolipoprotein A-I (apoA-I) is known to be anti-atherogenic as it clears cholesterol and phospholipids from peripheral tissues to form high-density lipoprotein (HDL). Structurally, apoA-I is highly dynamic, making it well-adapted to bind various amounts of lipid molecules to form different HDL subspecies. However, this feature also contributes to its amyloidogenic propensity in atherosclerosis and hereditary amyloidosis. The majority of apoA-I exists in lipid-bound forms, known as discoidal and spherical HDL. However, there is also evidence suggesting the existence of lipid-free and lipid-poor forms. Herein, we used apoA-I interacting with various lipids at submicellar concentrations (ie. lipid monomers) as an experimental model to mimic the lipid-poor form. We aim to characterise this interaction using a range of biophysical techniques, and to determine the effects of lipid monomers and C-terminal truncation on fibril formation of methionine-oxidised (MetO) apoA-I, which is amyloidogenic itself.

Methods: His-tagged ApoA-I (WT and C-terminal truncated mutant [1-184]apoA-I) was expressed in E. coli BL21(DE3) strain, purified with HisTrap columns and subsequently size exclusion chromatography. Analytical ultracentrifugation (AUC) was performed to characterise the self-association states of apoA-I, where the sedimentation speed of proteins was monitored by measuring absorbance at 280 nm along the cell. The thermostability of apoA-I bound to lipids was probed by differential scanning fluorimetry (DSF). Fibrillogenic kinetics of MetO apoA-I incubated with submicellar lipids was monitored by measuring fluorescence emission of ThT dye at 480 nm continuously.

Results: The AUC data revealed that a vast majority of lipids stabilised a smaller, more compact form of apoA-I. Interestingly, all submicellar lipids decreased the thermostability of apoA-I based on the DSF data. ThT assay showed that some lipids completely abolished fibrillogenesis of MetO apoA-I, while some lengthened the lag phase. This may reflect different lipids are inducing different conformations of apoA-I, and some are more protected from fibrillogenesis than the others. Comparing to the WT, [1-184]apoA-I was found to be monomeric and more thermodynamically stable (Tm of WT = 37.6±0.8oC, Tm of [1-184]apoA-I = 50.2±0.3oC). Accordingly, MetO [1-184]apoA-I formed fibrils at a slower rate (T1/2 = 50.6h) than the WT (T1/2 = 12.8h).

Conclusion: We have shown that lipid monomers induce conformations of apoA-I which differ in self-association states, thermostability and fibrillogenic propensity comparing to the lipid-free apoA-I. More studies are required to structurally distinguish the difference between lipid-poor apoA-I and apoA-I with higher level of lipidation, as well as the functional implications such difference.



BIOMEDICINE

PHYSIOLOGY AND ANATOMY

SCHEDULE

BIOMEDICINE
Physiology and Anatomy

SCHEDULE

SESSION 1

08.45 – 09.00	**Cholinergic transmission in the enteric nervous system increases during the dark phase of the circadian cycle** Anita Leembruggen	27
09.00 – 09.15	**Dopamine/ ghrelin receptor heteromers and their roles in lumbo-sacral defecation centres** Mitchell Ringuet	28
09.15 – 09.30	**Exercise responsive hepatokines and the modulation of glycaemic control** William De Nardo	29
09.30 – 10.00	**Cardiovascular System: How to address questions and understand its importance** **Keynote Speaker:** Prof. Eduardo Colombari	

SESSION 2

10.30 – 10.45	**Iron accumulation in skeletal muscles of old mice is associated with impaired regeneration after ischemia-reperfusion damage** Francesca Alves	30
10.45 – 11.00	**Use of a physiological reflex to standardize vagal nerve stimulation improves data reproducibility in a memory extinction protocol in rats** Andrew Butler	31
11.00 – 11.30	**Using families and fish to study titin-related heart disease** **Keynote Speaker:** Prof.Diane Fatkin	
11.30 – 11.45	**Trajectories of functional performance among geriatric rehabilitation inpatients and their clinical characteristics: an observational study** Cheng Hwee Soh	32
11.45 – 12.00	**Role of FANCM in fertility and meiosis in mice** Vanessa Tsui	33
12.00 – 12.30	**The new era of proteome-wide systems genetics** **Keynote Speaker:** Dr. Ben Parker	

SESSION 3

17.30 – 17.45	**Novel Mitochondrial Drp1 Inhibitors for Cardioprotection** Ayeshah Rosdah	34
17.45 – 18.00	**Nanopore direct RNA sequencing detects differential expression between human cell populations** Josie Gleeson	35
18.00 – 18.15	**Skeletal disease-in-a-dish: Using induced pluripotent stem cells to model human cartilage disorders** Jinia Lilianty	36
18.15 – 18.30	**Validation of a novel centroid method for estimating return time of reflected pressure waves in arteries** Avinash Kondiboyina	37

BIOMEDICINE

Physiology and Anatomy

Keynote Speakers

Professor Eduardo Colombari
Dept. of Physiology and Pathology
School of Dentistry of Araraquara
São Paulo State University
(UNESP)

Session 1 09.30 am- 10.00 am

The goal of research in Dr. Colombari's laboratory is to determine mechanisms of neuronal interaction between cardiovascular-respiratory control and body fluid homeostasis. In addition, possible dysfunction in CNS sympathetic and respiratory regulatory circuits in cardiovascular and metabolic diseases. Studies are carried out in animal models of disease. Major methodological approaches include in vivo single unit recording from individual CNS pre-sympathetic neurons, drug microinjections in CNS, neuroanatomical methods, whole-animal hemodynamic studies, and recording of cardiovascular function in conscious rats and mice. Additional techniques include immunohistochemical staining, water and salt intake, AAV- and Lentiviral gene transfer to site specifically alter gene expression in CNS neurons. Collectively these approaches are used to investigate sympathetic circuit dysfunction in models of arterial hypertension (SHR and Renal model) and in metabolic disorders (obesity & high salt diets) and sleep apnoea.

Lesions of the commissural nucleus of the solitary tract reduce arterial pressure in spontaneously hypertensive rats.
Akemi Sato, M., Vanderlei Menani, J., Ubríaco Lopes, O., **Colombari, E.,** *Hypertension,* 2001

Role of the medulla oblongata in hypertension.
Colombari, E., Sato, M.A., Cravo, S.L., Bergamaschi, C.T., Campos Jr., R.R., Lopes, O.U. *Hypertension* 2001

Professor Diane Fatkin
Victor Chang Cardiac
Research Institute
University of New South Wales

Session 2 11.00 am- 11.300 am

Professor Diane Fatkin trained in Clinical Cardiology and Molecular Genetics and is currently Head of the Sr Bernice Research Program in Inherited Heart Diseases, in the Molecular Cardiology Division of the Victor Chang Cardiac\ Research Institute, Sydney. She also holds appointments as Honorary Medical Officer in the Cardiology Department, St Vincent's Hospital, and Professor (conjoint) in the Faculty of Medicine, UNSW Sydney. Her research is focused on understanding the molecular genetic basis of inherited cardiomyopathies, with a specific interest in familial dilated cardiomyopathy and familial atrial fibrillation. This work spans from clinical and genetics studies in families with inherited cardiomyopathies, to functional genomics in cell, mouse, and zebrafish models. A major objective of the team's research is "bench-to-bedside" translation of new insights about disease mechanisms into personalised approaches to patient management.

Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction system disease.
Fatkin D, MacRae C, Sasaki T, Wolff MR, Porcu M, Frenneaux M, Atherton J, Muehle G, Vidaillet HJ, Spudich S, De Girolami U, Muntoni F, Johnson W, McDonough B, Seidman JG, Seidman CE. *N Engl J Med* 1999; 341:1715-1724.

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Physiology and Anatomy

Keynote Speakers

Dr. Ben Parker
Dept. of Physiology
The University of Melbourne

Session 2 12.00 - 12.30 pm

Dr. Benjamin Parker completed is PhD at the University of Southern Denmark and University of Sydney where he developed assays to quantify protein modifications by mass spectrometry. He performed post-doctoral research in the Diabetes Program at the Garvan Institute and then obtained an NHMRC ECF in the Metabolic Systems Biology Program at the Charles Perkins Centre. He is currently a group leader under the Driving Research Momentum scheme in the Department of Physiology at The University of Melbourne. His research team is focused on developing novel proteomic and computational tools to understand how genetic variants and signal transduction regulate metabolism with the goal of identifying new therapeutic targets to treat metabolic disease.

An integrative systems genetic analysis of mammalian lipid metabolism.
Parker B.L. et al. (2019). *Nature*, 567:187-193.

BIOMEDICINE
Physiology and Anatomy

Cholinergic transmission in the enteric nervous system increases during the dark phase of the circadian cycle

Anita J.L. Leembruggen[1], Marlene M. Hao[2] & Joel C. Bornstein[1]

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[2] Department of Anatomy and Neuroscience, University of Melbourne, Parkville, Victoria, Australia

Introduction: During the 24-hour circadian cycle, gastrointestinal function changes as it receives food, digests nutrients, and expels waste. In mice, colonic motility increases at night when mice are awake, and expression of clock genes and neuronal nitric oxide synthase (nNOS) in the intrinsic nervous system of the gut (known as the enteric nervous system, ENS) are known to fluctuate over the 24-hour cycle. However, changes in expression of excitatory neurotransmitters in the ENS remain uncharacterised.

Methods: Adult male Wnt1-Cre;GCaMP3 mice were maintained on a standard 12 hr day/ 12 hr night cycle and fed ad libitum. Proximal colon tissue from wildtype mice was collected at 6 hourly intervals (at Zeitgeber (ZT)1, ZT7, ZT13, and ZT19, where lights are on at ZT0 and off at ZT12), and RNA was extracted from the myenteric plexus and muscle, and analysed for expression of vesicular acetylcholine transporter (vAChT) using digital droplet PCR (ddPCR). Changes to myenteric neuron function in the presence of cholinergic agonist DMPP and antagonist Hexamethonium following single pulse (1P) or train (20P) electrical stimulation were assessed using live Ca2+ imaging on Wnt1-Cre;GCaMP3 positive mice from ZT1 and ZT13.

Results: Mice at ZT13 (1 hour after lights turn off) showed increased myenteric intracellular Ca2+ responses to DMPP compared to mice at ZT1 (1 hour after lights turn on) (ZT1: $\Delta F/F0 = 0.87 \pm 0.01$, n=566; ZT13: $\Delta F/F0 = 1.13 \pm 0.03$, n=482, $p<0.0001$). In the presence of Hexamethonium, myenteric neurons showed decreased intracellular Ca2+ responses following 1P electrical stimulation in both ZT1 mice (Control: $\Delta F/F0 = 1.00 \pm 0.03$, n=210; Hex: $\Delta F/F0 = 0.67 \pm 0.03$, n=111, $p<0.001$) and ZT13 mice (Control: $\Delta F/F0 = 1.13 \pm 0.06$, n=197; Hex: $\Delta F/F0 = 0.83 \pm 0.06$, n=127, $p<0.001$). A similar decrease in response was observed following 20P electrical stimulation in ZT1 (Control: $\Delta F/F0 = 0.99 \pm 0.01$, n=257; Hex: $\Delta F/F0 = 0.63 \pm 0.02$, n=258, $p<0.001$) and ZT13 mice (Control: $\Delta F/F0 = 0.95 \pm 0.01$, n=493; Hex: $\Delta F/F0 = 0.59 \pm 0.01$, n=490, $p<0.001$). However, ddPCR analysis revealed no significant change to vAChT expression over the 24-hour cycle ($p=0.07$).

Conclusion: Myenteric neurons have increased cholinergic transmission at night, when mice begin to feed and become active. This suggests that while expression of vAChT is unaltered, there may be gene regulatory pathways that modulate this change in excitatory neurotransmission in the ENS, in order to facilitate changes in gut activity.

Dopamine/ ghrelin receptor heteromers and their roles in lumbo-sacral defecation centres

Mitchell T Ringuet[1], Ruslan V Pustovit[1,2], Andrea Fanjul[3], Andrew Syder[3], Jie Huang[3], Lindsay Yoo[3], Sebastian GB Furness[4], Linda J Fothergill[1,2], Wendy Winchester[3], Paul Wade[3], Jill Wykosky[3] & John B Furness[1,2]

[1]Department of Anatomy and Neuroscience, University of Melbourne, Australia
[2]Florey Institute of Neuroscience and Mental Health, Australia
[3]Takeda Pharmaceuticals, Gastroenterology Drug Discovery Unit, San Diego, CA
[4]Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, Australia

Background and Objectives: Both dopamine and ghrelin activate the lumbo-sacral defecation centres and cause propulsive contractions of the colorectum. Dopamine, but not ghrelin, is found in nerve terminals in the defecation centres and dopamine could possibly be a neurotransmitter of the defecation pathways. There is evidence that dopamine D2 receptors (DRD2) and ghrelin receptors (GhrR) form heteromers in lipid membranes and transfected cells. Our studies were undertaken to determine whether dopamine, acting at DRD2/GhrR heteromers could have a physiological role in deletion.

Methods: Physiological studies were conducted in anaesthetized and conscious rats. DRD2 localization was investigated in reporter mice and by immunohistochemistry in rats and mice. GhrR was localized in reporter mice and by in situ hybridization histochemistry in rats. Dual expression was investigated in neurons of the mouse and human spinal cord.

Results: Ghrelin receptor agonists applied systemically or directly (intrathecally) to the defecation centres caused colorectal propulsion and defecation. Similar effects on colorectal function were observed in response to dopamine (intrathecal) or DRD2 agonists (IT or systemic). For both agonists, responses to IT application were prevented by cutting the nerve connections from the spinal cord to the colorectum. Behaviourally induced defecation in rats (water avoidance test) was reduced by both the DRD2 antagonist, sulpiride, and the GhrR antagonist, YIL781, suggesting the physiological involvement of both receptors. The effect of a DRD2 agonist, quinpirole, applied directly to the defecation centres of rats in vivo, was antagonized by systemic YIL781, indicating that activation of DRD2 directly or indirectly activates GhrR. GhrR was found in neurons of the defecation centres, but not in down-stream neurons. Neurons in the defecation centres of mice and human were revealed by in situ hybridization to express both receptors.

Conclusion: DRD2 and GhrR are in the same neurons of the defecation centres and are functionally involved in the defecation control pathways. Our pharmacological and localisation studies are consistent with these receptors forming heteromers and with dopamine being the physiological activator of the heteromeric DRD2/GhrR complex. The complex can be activated by both dopamine and ghrelin, but only dopamine has a physiological role.

Exercise responsive hepatokines and the modulation of glycaemic control

William De Nardo[1], Paula Miotto[1], Shuai Nie[2], Magdalene Montgomery[1] & Matthew Watt[1]

[1]Department of Physiology, The University of Melbourne, Melbourne, Vic, Australia
[2]Mass Spectrometry and Proteomics Facility, Bio21, Melbourne, Vic, Australia

Introduction: Exercise training has a multitude of beneficial metabolic effects in skeletal muscle, liver and other tissues. Alterations in muscle secreted proteins are well documented in response to exercise, however, little attention has been paid to adaptions within or secreted by the liver.

Methods: Mice were fed a high-fat diet for 6 weeks, endurance-trained (ET) or remained sedentary for a further 6 weeks. Hepatocytes were isolated 3 days after their last exercise bout and cultured in EXCELL protein-free media for 24 hours. Proteomics was performed on the isolated hepatocytes and conditioned medium to assess chronic adaptions within and secreted by the hepatocytes. Insulin-stimulated glucose uptake in the soleus muscle was assessed when cultured with ET or sedentary conditioned medium. Fatty acid metabolism was assessed in 300µm precision-cut mouse liver slices cultured with U-14C-Palmitic acid in ET or sedentary conditioned media.

Results: ET mice gained significantly less weight, reflected by a reduction in total inguinal and epididymal adipose tissue mass compared to sedentary mice ($p<0.05$). ET mice did not affect glucose tolerance or circulating insulin levels ($P=0.2$). Proteomic analysis 1590 hepatocyte-secreted proteins, of which, ET significantly altered 102 classically secreted proteins. ET hepatocyte secreted media enhanced insulin-stimulated glucose uptake in the soleus muscle ex vivo and increased fatty acid oxidation in murine liver slices. Proteomic analysis of the isolated hepatocytes detected 2657 proteins, of which, 137 proteins were significantly altered with ET.

Conclusion: Chronic adaptations in liver secreted proteins may provide an exciting novel therapeutic strategy for the treatment of obesity-related co-morbidities.

BIOMEDICINE
Physiology and Anatomy

Iron accumulation in skeletal muscles of old mice is associated with impaired regeneration after ischemia-reperfusion damage

Francesca M. Alves[1,3], Kai Kysenius[2], Marissa K. Caldow[1], Peter J. Crouch[2], Scott Ayton[3], Ashley I. Bush[3], Gordon S. Lynch[1] & René Koopman[1]

[1]Centre for Muscle Research, Department of Physiology, The University of Melbourne, Parkville Victoria, Australia
[2]Department of Pharmacology and Therapeutics, The University of Melbourne, Parkville, Victoria, Australia
[3]Melbourne Dementia Research Centre, The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, Victoria, Australia

Introduction: Oxidative stress is implicated in the insidious loss of muscle mass and strength that occurs with age. However, few studies have investigated the role of pro-oxidant iron, which elevates with age, in age-related muscle wasting and blunted repair after injury. We hypothesized that iron accumulation leads to membrane lipid peroxidation, muscle wasting, increased susceptibility to injury and impaired muscle regeneration.

Methods: To examine the role of iron in age-related muscle atrophy, we compared 3 month old 129SvEv FVBM with 22-24 month old mice. We assessed iron distribution and total elemental iron using LA-ICP-MS and Perls' stain on skeletal muscle tissue cross-sections. Aged mice underwent ischemic reperfusion (I-R) injury (90 minutes ischemia) and muscle regeneration was assessed 14 days post injury. Immunoblotting was used to determine lipid peroxidation (4HNE) and iron related proteins. To determine whether muscle iron content can be altered, aged mice were treated with deferiprone (DFP) in the drinking water and its effect on muscle regeneration after injury was assessed.

Results: We observed a significant increase in total elemental iron (+43 %, $P<0.05$) and lipid peroxidation (4HNE: +76 %, $P<0.05$) in tibialis anterior (TA) muscles of aged mice. Iron was further increased post injury (Adult: +81 %, Aged: +135 %, $P<0.05$) and associated with increased lipid peroxidation (4HNE: +41 %, $P<0.05$). Administration of DFP did not impact iron or measures of lipid peroxidation in skeletal muscle or modulate muscle mass. Increased muscle iron concentration and lipid peroxidation was associated with less efficient regeneration, evident from the smaller fibres in cross-sections of TA muscles (-24 %, $P<0.05$) and an increased percentage of fibres with centralized nuclei (+4124 %, $P<0.05$) in muscles of aged compared to adult mice. Administration of DFP did lower iron after IR injury but did not translate to histological improvements.

Conclusion: We have shown that muscles from aged mice have increased iron levels, which are associated with increased lipid peroxidation, increased susceptibility to I-R injury, and impaired muscle regeneration. Our results suggest that iron is necessary for effective muscle regeneration, highlighting the importance of iron homeostasis in muscle atrophy and regeneration.

BIOMEDICINE
Physiology and Anatomy

Use of a physiological reflex to standardize vagal nerve stimulation improves data reproducibility in a memory extinction protocol in rats

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Modulating brainstem activity, via electrical vagus nerve stimulation (VNS), influences cognitive functions, including memory. However, controlling for changes in stimulus efficacy during chronic studies, and response variability between subjects, is problematic. I hypothesized that recruitment of an autonomic reflex, the Hering-Breuer reflex, would provide robust confirmation of VNS efficacy. I compared this to measurement of electrode resistance over time. We also examined whether VNS modulates contextual memory extinction. Electrodes for VNS and diaphragm electromyography recording were implanted into anesthetized Sprague Dawley rats. When conscious, I measured the electrode resistance as well as the minimum VNS current required to evoke the Hering-Breuer reflex, before, and after, an inhibitory avoidance assay - a two chamber, dark/light model, where the dark compartment was paired with an aversive foot shock. The extinction of this contextual memory was assessed in sham and VNS treated rats, with VNS administered for 30 s at 1.5 times the Hering-Breuer reflex threshold during extinction memory formation. Assessment of VNS-evoked Hering-Breuer reflex successfully identified defective electrodes, where electrode resistance changes did not. VNS accelerated extinction memory and decreased multiple physiological metrics of fear expression. We observed an inverse relationship between memory extinction and respiratory rate during the behavioural protocol. Additionally, no current - response relationship between VNS and extinction memory formation was established. These data demonstrate that reliable, standardised experimental VNS studies can be produced by indexing stimulator efficacy to a physiological reflex.

Trajectories of functional performance among geriatric rehabilitation inpatients and their clinical characteristics: an observational study

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Introduction: More than 30% of hospitalized older adults experience a decline in functional performance, which is associated with poor quality of life and increased risk of institutionalisation. Geriatric rehabilitation aims to regain functional capacity of older patients. The aim of this study is to identify the trajectories of functional performance and the characteristics of geriatric rehabilitation inpatients.

Methods: REStORing health of acutely unwell adulTs (RESORT) is an ongoing observational, longitudinal inception cohort and consecutive patients admitted to geriatric rehabilitation wards were included. Latent class growth modelling was applied to identify the patients' trajectories of Activities of Daily Living (ADL) and Instrumental Activities of Daily Living (IADL) from two weeks prior acute hospitalisation to three months post-discharge from geriatric rehabilitation.

Results: Three distinct trajectories were identified for both ADL and IADL: poor baseline and no improvement (PB/NI: ADL=6.6%; IADL=42.1% of inpatients), good baseline and poor recovery (GB/PR: ADL=33.0%; IADL=23.0% of inpatients), and good baseline and good recovery (GB/GR: ADL=60.4%; IADL=34.9% of inpatients). The CFS and cognitive impairment were significantly associated with ADL PB/NI (OR=2.51, 95%CI: 1.64-3.84 and OR=6.33, 95%CI: 2.09-19.1 respectively) and ADL GB/PR (OR=1.76, 95%CI: 1.45-2.13 and OR=1.87, 95%CI: 1.24-2.82 respectively). The CFS was significantly associated with both IADL PB/NI (OR=1.64, 95%CI: 1.37-1.97) and IADL GB/PR (OR=1.63, 95%CI: 1.33-1.99). Cognitive impairment was associated with IADL PB/NI (OR=3.60, 95%CI: 2.31-5.61).

Conclusion: A great proportion of older geriatric rehabilitation inpatients do not regain their functional performance. Cognitive impairment and frailty are significantly associated with poor recovery of functional performance.

Role of FANCM in fertility and meiosis in mice

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Background: Fanconi anaemia (FA) is a rare genetic disease characterised by life-threatening anaemia and reduced fertility. The mechanism underlying reduced fertility in FA patients is not well understood, however it is known that meiosis is essential for fertility to ensure correct chromosome segregation. Reduced fertility is an important but an understudied phenotype relevant to individuals with FA patients. Case studies have identified individuals clinically diagnosed with FA, or having pathogenic mutations in both copies of FANCM, FANCA, or BRCA2 after presenting with non-obstructive azoospermia (NOA) or primary ovarian insufficiency (POI). FANCM is a translocase protein that recruits other FA proteins within the pathway to the site of DNA damage. Patients with homozygous mutations in FANCM have reduced fertility. Our aim is to understand the role of FANCM in fertility and reproductive processes in our Fancm mouse model.

Methods: 1) Male Fancm–/– mice of 6-10 weeks of age were sacrificed and the testes were harvested to perform a full fertility analysis. Testis and epididymis were weighed. Daily sperm production and sperm motility were assessed. 2) Female Fancm-/- mice of 6-12 weeks of age were sacrificed and the oocytes were harvested to perform oocyte counting and characterisation of chromosome segregation. 3) Meiotic characterisation of male chromosome spreads was performed and analysed by immunofluorescence of meiotic.

Results: Fancm-/- mice have normal blood counts and do not develop spontaneous or induced bone marrow failure. However, Fancm-/- mice have increased chromosomal instability and more micronuclei than controls. Fancm-/- mice have significantly lower testes and epididymis weight at 12 weeks of age in different mouse strains. Analysis of mature sperm showed defects in spermatogenesis including decreased sperm motility and reduced daily sperm production. Female Fancm-/- mice also show a reduction in oocyte numbers and litter sizes. Ongoing work in our laboratory is testing for the mechanisms that lead to the reduction in fertility in the Fancm-/- mice.

Conclusion & Significance: Our work and others represent a new opportunity for earlier detection of FA. Further, early detection will lead to improved treatment and follow ups, and also avoid chemotherapy-induced toxicity for cancer treatments. The link between reduced fertility and FA suggests that FA genes should potentially be included on gene panel screenings for NOA and POI patients.

Novel Mitochondrial Drp1 Inhibitors for Cardioprotection

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Introduction: Myocardial ischaemia-reperfusion injury (IRI) induces Drp1-mediated mitochondrial fission, leading to mitochondrial dysfunction and cardiomyocyte death. Inhibition of the mitochondrial fission GTPase protein, Drp1, have been shown to attenuate cell death. Mdivi-1, the only known small molecule reported to inhibit Drp1, is poorly water soluble, displays weak binding affinity for Drp1 and exhibits off-target effects. These bioavailability and specificity issues have been the main obstacles in the clinical development of cardioprotection targeting Drp1. This project aims to identify novel Drp1 inhibitors which are potent, selective and directly bind to human Drp1 for cardioprotection.

Methods: Computational screening was conducted to identify small molecules that could potentially bind to human Drp1. Surface plasmon resonance was employed to assess direct binding of candidate compounds, followed by a human Drp1 GTPase activity assay. The effect on mitochondrial morphology was assessed in Drp1 wild-type and knockout murine embryonic fibroblasts treated with hit compounds at 5-50 μM for 24 hours. The cytoprotective effect was examined in vitro (human fibroblasts) and in vivo (C57BL/6 mice subjected to acute myocardial IRI).

Results: We have identified three hit compounds (DRP1i1, DRP1i2 and DRP1i3) that bind directly to human Drp1, inhibit its GTPase activity ($p<0.05$), promote mitochondrial fusion in Drp1-dependent manner ($p<0.05$), and protect human fibroblasts against oxidative stress-induced cell death ($p<0.05$). Mice treated with a single bolus of DRP1i1 (1 mg/kg) at reperfusion significantly reduced myocardial infarct size compared with the vehicle control.

Conclusion: DRP1i1, DRP1i2 and DRP1i3 are drug-like small molecules that exhibit specific inhibition towards human Drp1 with cardioprotective properties.

Nanopore direct RNA sequencing detects differential expression between human cell populations

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Introduction: Accurately quantifying gene and isoform expression changes is essential to understanding cell functions, differentiation and disease. Therefore, a crucial requirement of RNA sequencing is identifying differential expression. The recent development of long-read direct RNA (dRNA) sequencing has the potential to overcome many limitations of short and long-read sequencing methods that require RNA fragmentation, cDNA synthesis or PCR. dRNA sequences native RNA and can encompass an entire RNA in a single read. However, its ability to identify differential gene and isoform expression in complex organisms is poorly characterised.

Methods: Using a mixture of synthetic controls and human SH-SY5Y cell differentiation into neuron-like cells, we show that dRNA sequencing accurately quantifies RNA expression and identifies differential expression of genes and isoforms. RNA was extracted from undifferentiated and differentiated SH-SY5Y cells and subsequently sequenced on an Oxford Nanopore MinION device.

Results: We generated ~4 million dRNA reads with a median length of 991 nt. On average, reads covered 74% of SH-SY5Y transcripts and 29% were full-length. Measurement of expression and fold changes between synthetic control RNAs confirmed accurate quantification of genes and isoforms. Differential expression of 231 genes, 291 isoforms, plus 27 isoform switches were detected between undifferentiated and differentiated SH-SY5Y cells and samples clustered by differentiation state at the gene and isoform level. Genes upregulated in neuron-like cells were associated with neurogenesis. We further identified >30,000 expressed transcripts including thousands of novel splice isoforms and transcriptional units.

Conclusion: Our results establish the ability of dRNA sequencing to identify biologically relevant differences in gene and isoform expression. Using spike-in controls we found that dRNA sequencing accurately quantified and identified fold changes between the synthetic RNA mixes. The discovery of thousands of novel transcripts indicates that the human transcriptome annotation is far from complete. Improving this annotation is a task in which dRNA sequencing will be highly valuable. In conclusion, dRNA is a promising method to decipher the complex expression and splicing patterns that characterise the transcriptome and to identify differentially expressed isoforms contributing to development and disease.

Skeletal disease-in-a-dish: Using induced pluripotent stem cells to model human cartilage disorders

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Introduction: Collagen II is the main structural collagen in cartilage, a tissue essential for normal bone development. Heterozygous mutations in the collagen II gene (COL2A1) cause a spectrum of cartilage and bone disorders, varying from mild to lethal. The pathogenic mechanisms remain unclear, and no drug therapies are available. We use induced pluripotent stem cells (iPSCs) and a new chondrocyte differentiation protocol to model collagen II cartilage disorders in vitro. The focus of this study is the COL2A1 p.R989C mutation which produces a severe cartilage disorder in patients.

Methods: Heterozygous and homozygous mutant iPSCs were generated using CRISPR/Cas9 gene editing. Mutant iPSCs and their isogenic control line were differentiated into cartilage using a protocol developed and optimised in our laboratory. Pellet cultures were grown in chondrogenic medium for 6-8 weeks to produce cartilage organoids. Organoids were analysed for cartilage maturity using histology, immunohistochemistry, EM, and mechanical testing. Proteoglycan content was quantitated with microCT, and gene expression was compared with bulk RNAseq.

Results: By day 48, the heterozygous mutant had deposited more extracellular collagen X than the control showed by immunohistochemistry. Since collagen X is a marker of mature hypertrophic chondrocytes, this suggests that the mutation leads to premature cartilage maturation. Collagen II immunohistochemistry and aggrecan histology showed reduction of these components in the homozygous mutant cartilage compared to the control and heterozygous mutant indicative of a severely compromised extracellular matrix. By day 55, the homozygous mutant had deposited collagen I. Collagen I is not normally found in cartilage, and this suggests that its expression could compensate for the collagen II loss.

Conclusion: Our current results demonstrated that this skeletal "disease-in-a-dish" model allows us to monitor chondrocyte maturity and explore the extracellular matrix and gene expression changes caused by mutations. Further analysis will include proteomics to examine the cartilage extracellular matrix structure and functional changes, and signalling pathway analysis to explore pathogenic pathways and identify drug targets.

Validation of a novel centroid method for estimating return time of reflected pressure waves in arteries

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Introduction: With every systolic contraction, the heart sends a forward pressure wave through the arterial network, which is partially reflected back towards the heart at various points along the vasculature. A prominent paradigm suggests that these pressure-increasing reflected waves return to the heart during diastole in youth but, due to increased arterial stiffening, this return shifts to systole with ageing, thereby increasing systolic blood pressure and ventricular afterload. The time taken by reflected waves to return to the heart, i.e. the reflection time (RT), is therefore an important metric of vascular health. However, current methods to estimate RT have limitations. The most widely used inflection-point method cannot detect an RT occurring in diastole and, as with the zero-crossover and foot methods for estimating RT, uses only a single feature point on pressure waveforms. Importantly, the accuracy of all these methods is uncertain because no ground truth RT (GTRT) has been available. We here introduce a novel centroid method to estimate RT that utilizes the entire pressure waveform, and assess its accuracy relative to other methods by comparison with a GTRT for the first time.

Methods: Using computational linear wave-tracking, we followed an impulse as it traversed through an anatomical model of the systemic arterial circulation and kept track of the amplitude and timing of every reflected wave produced. GTRT was calculated as the amplitude-weighted mean arrival time of reflected waves at the inlet. Linear convolution of the resulting impulse response with a realistic input waveform (flow waveform multiplied by characteristic impedance) produced a pressure waveform that was separated into forward and backward components. The time difference between the centroids of the backward pressure and input waveforms was taken as RT in the centroid method. We also conducted a parameter sweep (n=308) on the computational model to test the accuracy and precision of the various methods under different conditions.

Results: Compared to the inflection-point, zero-crossover, and foot methods, the centroid method estimated RT with the least mean difference to GTRT (107, 104, 171 vs. 8 ms; $p<0.001$) and least standard deviation (109, 34, 97 vs. 28 ms).

Conclusion: In this computational study, the centroid method substantially improved accuracy and precision for estimating RT compared with current methods. Future steps include further validation of this method under experimental conditions, and assessing changes in RT with ageing or in disease states by applying it to clinical datasets.

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