APG
Adelaide Protein Group

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2026 APG ANNUAL RESEARCH SYMPOSIUM

2ND JULY 2026 | 9:00 am - 5:30 pm | **AHMS Building**, Ground Floor, Adelaide University, City Campus West

KEYNOTE SPEAKERS

Adelaide University

A/Prof. Susan Woods

"Transforming Gut Cancer Detection And Treatment"

THE UNIVERSITY OF SYDNEY

A/Prof. Yu Heng Lau

"Double Or Nothing: Lessons Learned When Evolving A Multimeric Cage-Forming Protein"

MORNING SESSION: STUDENT SPEAKERS

Manjit Rana
Adelaide University

Sepideh Mehrpour Layeghi
Centre for Cancer Biology | Adelaide University

Jack Scanlan
Adelaide University

Katarina Amerl
SAiGENCI | Adelaide University

Xiaoying (Charlotte) Yu
Adelaide University

AFTERNOON SESSION: ECR SPEAKERS

Dr Jing Xu
SAiGENCI | Adelaide University

Dr Aelon Rahmani
Flinders University

Dr Sreshtha Malik
Adelaide University

Dr Michael De Ieso
Adelaide University

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APG Annual Research Symposium 2026 Program

Adelaide Health and Medical Sciences building, Ground Floor G030 Lecture Theatre
North Terrace, Adelaide University.

Thursday	9:00 am	Registration
2nd July 2026	9:30 am	**Keynote 1: A/Prof. Susan Woods**
		Morning tea
	10:50 am	**Student Talks**
	12:15 pm	**Platinum sponsor spotlight:** Solve Scientific
	12:30 pm	Lunch and posters
	2:00 pm	**Keynote 2: A/Prof. Yu Heng Lau**
		Afternoon tea
	3:00 pm	**ECR Talks**
	4:20 pm	Refreshments and networking
	5:00 pm	AGM and Award Presentations
		Including: • **Solve Scientific** Best ECR Talk • **Decode Science** Best Student Talk • **BMG Labtech** People's Choice Award • **SAGC** Best Poster Award (Student) • **Promega** Best Poster Award (Student) • **Metagene** Best Poster Award (ECR)
	From 7pm	Post-symposium dinner at the West Oak Hotel – please see a committee member if you are interested in joining us!

The Adelaide Protein Group acknowledges the Kaurna people as the traditional owners of the lands of the Adelaide Plains on which we gather for the 2026 Annual Research Symposium.

Contents

APG Committee 2026

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Keynote 1

9:30 am – 10.15 am

Transforming Gut Cancer Detection and Treatment

Associate Professor Susan Woods

SAHMRI, Adelaide University

Research in Susan's group aims to improve early detection methods for gut cancers, so that they may be identified at a point when most can be cured. This involves the application of synthetic biology approaches to engineer bacteria to act as disease detectors in the gut. The team is also developing engineered bacteria to deliver treatments directly to tumours, to reduce unwanted side-effects of treatment. Recent projects also investigate how the bacterial community in our gut is changed in cancer, and the role this plays in promoting this disease.

Biographical snapshot

A/Prof Susan Woods established her Gut Cancer research group at Adelaide University within the South Australian Health and Medical Research Institute in 2019. She trained both in Australia and in San Francisco, USA, with Nobel Laureate J. Michael Bishop, and is the Gastroenterology Society of Australia (GESA) Bushell Research Fellow. Her group is supported by Australian and US (NIH) Government funding, GESA and Hospital Research Foundation funding.

Student Talks

10:50 am – 12:15 pm

1.

Structural Basis Of Selective Androgen Receptor Modulation In AR-Positive/ER-Positive Breast Cancer

Manjit Rana [1], Wayne Tilley [2], John Bruning [1]

[1] Institute for Photonics and Advanced Sensing, School of Biological Sciences, Adelaide University, Adelaide, Australia
[2] Dame Roma Mitchell Cancer Research Laboratories, Adelaide Medical School, Adelaide University, Adelaide, Australia

The authors have requested the abstract not be included in the abstract booklet.

2.

Identifying Molecular Drivers of Neuroblastoma Through Human Stem Cell-Derived Sympathetic Neurogenesis Model

Sepideh Mehrpour Layeghi [1], Xiaochun Li [1], Andrew G. Bert [1], Aayushi Notra [1], Julie Bracken [1], Luciano Martelotto [2], Katherine A. Pillman [1], Quenten Schwarz [1], Yeesim Khew-Goodall [1,3], Gregory J. Goodall [1,3]

[1] Centre for Cancer Biology, Adelaide University and SA Pathology, Adelaide, SA, Australia
[2] Adelaide Centre for Epigenetics, Adelaide University, Adelaide, SA, Australia
[3] School of Medicine, Adelaide University, Adelaide, SA, Australia

Background:
Neuroblastoma (NB) is the most common extracranial solid tumour of childhood and remains a leading cause of paediatric cancer mortality. Unlike many cancers, NB is characterised by relatively few recurrent coding mutations but extensive transcriptional and epigenetic dysregulation, suggesting aberrant developmental programs contribute to tumour initiation and progression. As NB originates from neural crest-derived sympathetic progenitors, understanding the molecular mechanisms regulating sympathetic neuron differentiation may reveal key drivers of tumorigenesis.

Methods/Patients:
We utilised a hiPSC-derived in vitro sympathetic neurogenesis model to investigate transcriptional changes during neuroblast differentiation. Bulk and single-cell RNA sequencing datasets were generated to identify transcription factors (TFs) dynamically regulated throughout differentiation. Candidate TFs were prioritised based on differential expression patterns and associations with neuroblastoma patient survival. Functional studies using gain- and loss-of-function approaches are being performed to assess the effects of candidate TFs on neuronal differentiation and tumour-associated phenotypes.

Results:
Our analyses identified multiple TFs with dynamic expression during sympathetic neurogenesis and significant associations with neuroblastoma prognosis. Several candidates display expression patterns consistent with roles in maintaining undifferentiated neuroblast states or promoting neuronal maturation. Preliminary functional studies suggest dysregulation of these TFs alters differentiation trajectories and may contribute to neuroblastoma pathogenesis.

Conclusions:
This study identifies candidate transcriptional regulators involved in sympathetic neuron differentiation and neuroblastoma development. These findings provide insight into the developmental origins of NB and may support the identification of novel biomarkers and therapeutic targets for high-risk disease.

3.

Mining the ovarian cancer ascites proteome using nanoparticle enrichment to predict treatment response and map protease activity

Jack Scanlan[1], Parul Mittal[1], Noor A. Lokman[2], Martin K. Oehler[2,3], Peter Hoffmann[1], Manuela Klingler-Hoffmann[1]

[1] Mass Spectrometry and Proteomics Group, Centre for Pharmaceutical Innovation, College of Health, Adelaide University, Adelaide, SA 5000, Australia.
[2] Robinson Research Institute, Adelaide University, Adelaide, SA 5000, Australia.
[3] Department of Gynaecological Oncology, Royal Adelaide Hospital, Adelaide, SA 5000, Australia

Malignant ascites (MA) accumulates in the peritoneal cavity of >90% of advanced epithelial ovarian cancers, yet its composition and utility for personalised medicine remain poorly defined. We previously showed that MA is a rich source of tumour material, including multicellular spheroids that drive peritoneal metastasis and treatment resistance. However, its extreme dynamic range (~12 orders of magnitude) and resistance to depletion strategies have limited proteomic analysis due to ion suppression, restricting the discovery of clinically relevant protein markers.

Here, we evaluate strategies to enhance proteome coverage using ultra-sensitive mass spectrometry. Nanoparticle-based enrichment enabled the identification of >9,500 proteins across MA samples (n=25), representing a threefold increase in depth over current benchmarks. Given the highly proteolytic MA microenvironment, we developed a bioinformatics pipeline to detect endogenously cleaved peptides and infer protease-substrate relationships, providing insight into proteolytic networks that contribute to disease progression. This approach also enabled the quantification of >80 cytokines – an approximately tenfold increase over previous reports – with strong concordance to ELISA measurements, supporting its utility for profiling immune signaling in complex biofluids.

Finally, we integrated proteomic profiling of ascitic spheroids with functional drug response assays using clinician-compatible, drug-embedded plates. We show that proteomic signatures can predict first-line chemotherapy response prior to treatment within a clinically actionable timeframe, offering an alternative to the current trial-and-error approach. Collectively, these findings advance the molecular understanding of MA and support the implementation of mass spectrometryñguided personalised treatment strategies in epithelial ovarian cancer.

4.

p53 family proteins cooperate in a context-specific manner on the genome.

Katarina Amerl [1,2], Dr Daniel McDougal [3], Dr Katja Hummitzsch [1,2], Dr Luke Isbel [1,2]

[1] SAiGENCI ñ South Australian immunoGENomics Cancer Institute, Adelaide University, Adelaide, South Australia, Australia; [2] ACE ñ Adelaide Centre for Epigenetics, Adelaide University, Adelaide, South Australia, Australia; [3] Centre for Targeted Protein Degradation, School of Life Sciences, University of Dundee, Dundee, Scotland, UK.

The overwhelming majority of genomic regulatory factors, DNA-binding proteins called transcription factors, exist within evolutionarily related families. These protein families share highly similar structural elements, sometimes appearing almost identical. However, cell mechanisms governing the overlapping functions of homologous transcription factors at the genomic level, where they primarily function, remain largely unknown. Fundamentally, our inability to differentiate between the actions of related transcription factors hinders our capacity to exploit them as ìmaster regulatorsî of development and disease states.

To explore the dynamics of homologous transcription factors, we generated a tuneable stem cell expression system to assess the genomic binding of the evolutionarily related p53/p63/p73 family. Our system revealed p53 and p73 exhibited highly overlapping DNA specificity at the genomic level, where only a minority of binding sites were preferentially bound by either p53 (5%) or p73 (10%). Preferential binding appeared to depend on both DNA sequence and chromatin state; p53-preferred peaks had greater motif multiplicity, while p73-preferred peaks were enriched for active chromatin characteristics. Upon co-expression of p53 and p73, rather than competing for the same substrate (DNA) as expected, the binding of p73 enabled stronger binding of p53 to the genome. The presence of an N-terminal transactivation domain enabled cooperativity between the proteins, suggesting that chromatin regulates the cooperation of related transcription factors at a subset of DNA binding sites.

Together, our results support a model whereby multiple layers, including subtle DNA syntax and chromatin state, determine transcription factor cooperativity and specificity. These distinct preferences at the genomic level potentially explain the highly divergent function of p53 family proteins in development and cancer.

5.

The deubiquitylating enzyme USP9X controls the abundance of proteins involved in signalling and cytoskeleton dynamics

Xiaoying (Charlotte) Yu [1], Jozef Gecz [1] and Lachlan A. Jolly [1]

[1] University of Adelaide and Robinson Research Institute, Adelaide, 5005, SA, Australia

Ubiquitin-specific protease 9X (USP9X) is a deubiquitinating enzyme (DUB) that removes ubiquitin moieties from proteins. It can either protect substrates from proteasomal degradation or antagonise other ubiquitin-dependent events. The haploinsufficiency of USP9X causes neurodevelopmental disorders in females. We hypothesised that the deficiency of USP9X will directly impact the proteome. Hence, this project aims to identify the proteome-wide impact of USP9X deficiency and identify the direct USP9X interactors which may help understand the mechanisms through which USP9X controls development.

Fibroblasts derived from individuals with USP9X female syndrome were used to determine the proteomic consequences of reduced USP9X. Analysis of proteomes of four patient samples revealed differential abundance of 1,198 proteins compared to controls (Qvalue<0.05, unique peptides ≥2). Among these, 218 were decreased in abundance and therefore considered potential USP9X substrates.

To identify proteins that directly bind to USP9X in situ, a proximity-labelling strategy was employed using USP9X fused to APEX2 biotin ligase. Biotinylated proteins are therefore considered to include USP9X interactors and were isolated and subjected to mass-spec based identification and quantification. This approach identified 50 previously known USP9X interactors, and western blot analysis further confirmed the interaction with several signalling pathway regulators, including SMURF1, RAPTOR, and SMAD4. We also identified an interaction with 95 of the 218 proteins (44%) that were found to decrease in USP9X syndrome cells. Gene Ontology analysis of these 95 proteins suggests deregulation of cell adhesion, cytoskeletal dynamics and axon guidance.

Together, these data suggest USP9X may serve to integrate signal transduction events with cytoskeleton dynamics during development. Future studies will examine the USP9X interactome and regulated proteome in the context of cells of the developing brain.

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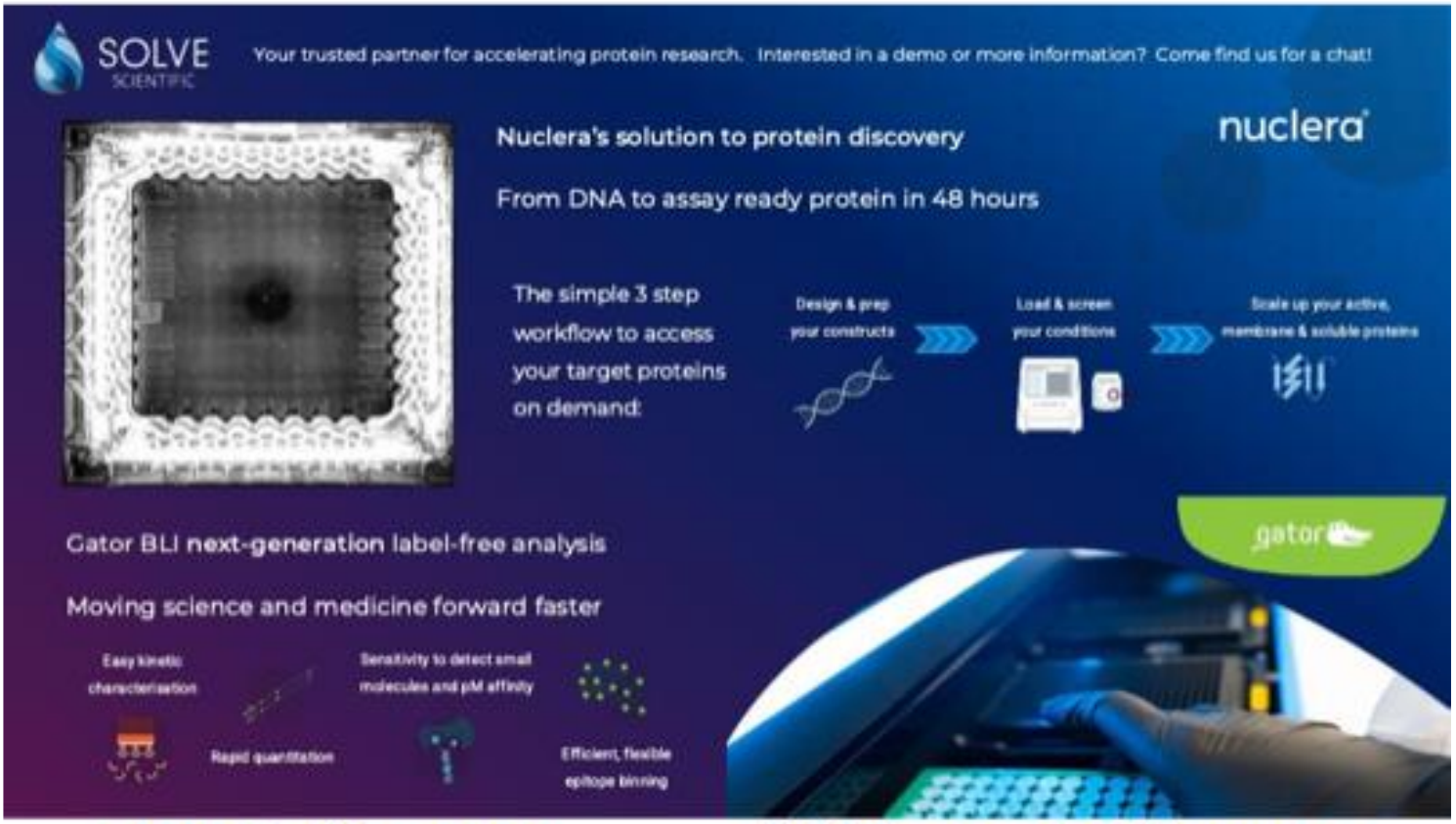

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Keynote 2

2:00 pm – 2:40 pm

Double or nothing: lessons learned when evolving a multimeric cage-forming protein

Associate Professor Yu Heng Lau

The University of Sydney, Australia

Directed evolution (DE) is a powerful technique for exploring protein sequence space, where new sequences with desirable traits can be selected for if they have superior fitness in an appropriately designed experiment.

Our laboratory became interested in DE as part of our program to engineer cage-forming proteins (encapsulins) as organelles for carbon fixation.[1] Encapsulins form icosahedral multimeric cages that typically have small pores at their vertices. As these pores can bottleneck the flow of enzyme substrates and products in and out of the cages,[2] we sought to engineer encapsulins with larger pores by DE.

In this talk, I will discuss how DE didn't work as planned, and how we eventually got it to work.[3] The key to unlocking a reliable DE workflow was to use gene duplication, a well-known phenomenon in evolutionary biology that had somehow never been used in the context of DE, which expands the accessible fitness landscape of sequence variants for multimeric proteins.

1. Szyszka et al., Nat. Commun. 2025, 16, 9493.
2. Adamson et al., Sci. Adv. 2022, 8, abl7346.
3. Siddiquee et al., bioRxiv, 2026.01. 12.698938.

Biographical snapshot

Assoc. Prof. Yu Heng Lau is a research group leader and NHMRC Investigator Fellow in the School of Chemistry at the University of Sydney. Yu Heng completed a PhD in organic chemistry at the University of Cambridge, then moved to Harvard Medical School as a Sir Henry Wellcome Postdoctoral Fellow in synthetic biology. He has previously developed new chemical methods for peptide cyclisation to target oncogenic protein-protein interactions, and genome-scale engineering methods for the reprogramming of synthetic microorganisms. His current research program spans two main themes: 1) Medicinal chemistry against novel telomeric cancer targets, and 2) Engineering cage-forming proteins as organelles for carbon fixation.

ECR Talks

3:00 pm – 4:20 pm

1.

Modification-aware AI enables terminal chemical modifications for peptide design and discovers potent antimicrobials

Jing Xu [1,2,3], Marcelo D. T. Torres [4,5,6,7], Chen Li [1,8], Jian Li [9], Fuyi Li [3], Jiangning Song [1,2], Cesar de la Fuente-Nunez [4,5,6,7]

[1] Biomedicine Discovery Institute and Department of Biochemistry and Molecular Biology, Monash University, Melbourne, VIC 3800, Australia.
[2] Monash AI Institute, Monash University, Melbourne, VIC 3800, Australia.
[3] South Australian immunoGENomics Cancer Institute (SAiGENCI), Faculty of Health and Medical Sciences, The University of Adelaide, Adelaide, SA 5005, Australia.
[4] Machine Biology Group, Departments of Psychiatry and Microbiology, Institute for Biomedical Informatics, Institute for Translational Medicine and Therapeutics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA.
[5] Departments of Bioengineering and Chemical and Biomolecular Engineering, School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, PA 19104, USA.
[6] Department of Chemistry, School of Arts and Sciences, University of Pennsylvania, Philadelphia, PA 19104, USA.
[7] Penn Institute for Computational Science, University of Pennsylvania, Philadelphia, PA 19104, USA.
[8] Department of Medicine, School of Clinical Sciences, Monash University, Clayton, VIC 3168, Australia.
[9] Monash Biomedicine Discovery Institute and Department of Microbiology, Monash University, Melbourne, VIC 3800, Australia.

Antimicrobial resistance (AMR) is an escalating global health threat, intensifying the urgent need for novel antibiotics. Here, we introduce Termini, a generative machine learning (ML) framework that integrates peptide generation, classification, and regression modules to systematically identify potent antimicrobial peptides (AMPs). A key feature of this pipeline is its explicit design of both N- and C-terminal modifications, combined with predictive modeling across 15 bacterial species. We experimentally validated the framework against 11 pathogenic species, representing the broadest spectrum of testing reported to date in such a study. We synthesized and tested 120 peptides (including 60 unique amino acid sequences) with and without terminal modifications. Remarkably, 111 of these peptides (92.5%) demonstrated antimicrobial activity in vitro. Comparative analyses demonstrated that terminal modifications substantially enhanced antimicrobial potency. Furthermore, the lead candidates identified through in vitro screening exhibited robust anti-infective efficacy in subsequent in vivo experiments, confirming their therapeutic potential. Collectively, these results highlight the high hit rate, biological relevance, and translational promise of this integrative, modification-aware AI framework for next-generation antimicrobial peptide development.

2.

Synaptically targeted proximity labelling in Caenorhabditis elegans to characterise subcellular-level proteome changes induced by memory formation in vivo

Aelon Rahmani [1], Michaela E Johnson [1], Rosemary A Coleman [1], Yee Lian Chew [1]

[1] Flinders Health and Medical Research Institute, College of Medicine and Public Health, Flinders University, Adelaide, Australia

The authors have requested the abstract not be included in the abstract booklet.

3.

Identifying the first herbicidal inhibitors of shikimate kinase using a repurposing strategy

Sreshtha Malik, Andrew S. Barrow, Peter Boutsalis, Tatiana P. Soares da Costa*
School of Agriculture, Food and Wine & Waite Research Institute, Adelaide University

Herbicides underpin modern agriculture as they are critical to circumvent environmental and economic losses caused by weeds. However, the increasing prevalence of herbicide resistant weeds poses a growing threat to global agriculture. The problem is further exacerbated by the lack of herbicides with novel targets introduced over the past four decades. Together, these issues underscore the urgent need to validate new herbicide targets. This study focuses on shikimate kinase (SK), an essential enzyme in the shikimate pathway, which is responsible for the biosynthesis of aromatic amino acids in plants, bacteria, and fungi. Despite the success of glyphosate, which targets another enzyme in this pathway, SK remains commercially unexplored. To address this gap, we employed a structure-guided repurposing strategy to evaluate whether bacterial SK inhibitors could be adapted as selective plant herbicides. Comparative sequence and structural analyses revealed strong conservation within the SK active site between bacteria and plants, supporting the feasibility of cross-species inhibition. A multi-parameter screening pipeline identified eight literature-reported bacterial SK inhibitors, which were synthesised and tested against recombinant plant SK, yielding a lead compound with in vitro and in planta activity. Building on this, 50 novel analogues were designed, several of which demonstrated improved inhibitory activity. These compounds showed robust post-emergence efficacy against soil-grown monocotyledonous and dicotyledonous weeds, while exhibiting no antimicrobial effects. This study has identified the first herbicidal inhibitors of plant SK and has provided evidence that a repurposing approach, combined with structure-guided synthesis, can generate highly specific and potent herbicide candidates, expediting the herbicide discovery process.

4.

Nuclear Receptor Ligand Analogue Screen Identifies Two Compounds with Anticancer Activity in Breast and Prostate Cancer

Michael De Ieso [1], Theresa Hickey [1], Wayne Tilley [1] & Amy Dwyer [1]
[1] Dame Roma Mitchell Cancer Research Laboratories, School of Medicine, College of Health, Adelaide University, Adelaide, SA, Australia.

Anticancer screens typically use cytotoxicity as the primary endpoint to discover new cancer therapeutics, but cytotoxic drugs are associated with evolution of drug resistance. An alternative therapeutic strategy is to induce differentiation of cancer cells. Cellular morphology provides an under-utilised readout of cell differentiation in anticancer drug screening. Ligand-activated nuclear receptor (NR) proteins are transcription factors that regulate proliferation and differentiation in breast and prostate cancer cells. Hence, we performed a phenotypic screen of putative NR ligand analogues to identify compounds that induce morphological changes and inhibit growth.

We screened a chemical library of 3,056 NR ligand analogues (ChemBridge), select cognate NR ligands, and taxane controls in endocrine-sensitive and -resistant breast (n=3) and prostate (n=3) cancer cell lines. “Hits” were prioritised by induction of >40% growth inhibition and significant morphological change quantified by Mahalanobis distance, a measure incorporating nuclear, whole-cell and cytoskeletal parameters (Choo et al., SLAS Discovery, 2021). Candidate NR ligands that induced morphological changes similar to hormone deprivation therapies and cytotoxic taxanes were excluded. Two compounds (designated A and B) emerged as lead candidates after a medicinal chemistry review, and were tested for efficacy in patient-derived explants (PDEs) of primary human prostate tumour tissue cultured ex vivo. Cellular morphology, immunohistochemical quantification of NR protein expression, and Ki67 proliferative index were assessed as endpoints.

Compounds A and B reduced Ki67 in prostate cancer PDEs by 53% and 43%, respectively, relative to vehicle control ($p=0.03$ for both). The antiproliferative activity of both compounds exceeded that induced by the androgen receptor (AR) degrader ARV-110 (~24%; $p=0.009$). These findings identify compounds A and B as candidate NR ligands with anticancer activity.

Poster Abstracts

1.

Rhoptries, too small to see! How to overcome the diffraction limit using simple light microscopy.

Shamit Singla [1]

[1] Research Centre for Infectious Diseases, School of Biological Sciences, University of Adelaide, Adelaide, Australia, Institute for Photonics and Advanced Sensing (IPAS), University of Adelaide, Adelaide, Australia

Malaria is responsible for >250 million infections and ~600,000 deaths annually. To cause disease, malaria parasites must invade host cells using specialised secretory organelles called rhoptries. While we have a relatively good understanding of secreted rhoptry proteins, our knowledge of proteins that control rhoptry function is limited. Rhoptry proteins exposed to the cytoplasm are in the right place at the right time to facilitate rhoptry secretion due to their ability to mediate interactions with other parts of the cell. However, the rhoptries are too small to be observed using conventional light microscopy, making it challenging to determine rhoptry protein localisation or detect changes in rhoptry structure. We have applied a newly developed technology termed expansion microscopy that overcomes this problem by increasing the size of the parasite by ~4.5x. In conjunction with single-molecule localisation and advanced image analysis, we can create a 3D model of the parasite to visualise subtle changes in rhoptry structure and determine protein localisation to ~10 nm in resolution. Early results suggest that the essential rhoptry protein CERLI1 forms a ring around the rhoptry surface, potentially highlighting areas where key interactions occur. These findings will help us better define rhoptry biology and understand the role of this organelle during host cell invasion.

2.

Modelling the CNS-niche in vitro to investigate therapeutic vulnerabilities in acute lymphoblastic leukaemia

Luke Quinlan [1,2], David Yeung [1,2,4], Deborah White [1,2,3,4], Elyse Page [1,2]

[1] Blood Cancer Program, Precision Cancer Medicine Theme, South Australian Health & Medical Research Institute (SAHMRI), Adelaide, South Australia
[2] School of Medicine, Adelaide University, Adelaide, South Australia
[3] Australian and New Zealand Children's Oncology Group (ANZCHOG), Clayton, Victoria
[4] Australasian Leukaemia and Lymphoma Group (ALLG), Melbourne, Victoria

Background
Central nervous system (CNS) involvement in acute lymphoblastic leukaemia (ALL), termed CNS-ALL, is an adverse complication with a poor prognosis. The biology underpinning ALL persistence in the CNS remains poorly defined.

Aim
To explore therapeutic vulnerabilities for CNS-ALL through a novel in vitro ALL/leptomeningeal cell co-culture model using an integrated bulk RNA-sequencing and untargeted LC-MS proteomics approach.

Methods
RCH-ACV and Jurkat ALL cell lines were cultured alone (mono) or co-cultured (co) with human meningeal cells (HMCs) under norm (21% Oxygen, 10% FCS) or stress (3% Oxygen, 2% delipidated-FCS) conditions for 72 hours. Flow cytometry measured cell cycle by propidium iodide staining. RNA-seq and untargeted LC–MS proteomics quantified mRNA transcripts and protein abundance, enabling expression profiling and GSEA-mediated pathway enrichment analysis. Data represent n=3 replicates; statistics were performed using t-test and two-way ANOVA.

Results
In co-stress conditions, both cell lines demonstrate increased G0-G1 and decreased G2-M cell cycle proportions versus mono-norm (both $p<0.0001$), indicating reduced proliferation. The sensitivity of RCH-ACV and Jurkat cells to methotrexate was reduced in co-stress conditions (vs mono-norm $p=0.0297$ and $p=0.0055$, respectively). Co-stress conditions induce distinct transcriptional and proteomic states from mono norm in both cell lines, marked by enrichment of invasion and therapy resistance proteins such as LOX, ICAM1, and MGST1 (all $p<0.05$).

Conclusion
Using our first-of-its-kind in vitro model, we uncovered characteristics of ALL cells in the CNS associated with therapy resistance. Utilising an untargeted multi-omics approach, we defined a molecular signature of ALL cells in the CNS and identified actionable therapeutic vulnerabilities that may enhance ALL treatment responses in this site.

3.

Curing Parkinson's Disease with…Beer? Modulation of α-synuclein Aggregation Dynamics by Extracts of Brewer's Spent Grain

Rebekah Munro [1], David Nkurunziza [2], Helen Collins [2], Tara L Pukala [3], Blagojce Jovcevski [1]

[1] School of Biological Sciences, Adelaide University, Adelaide, Australia
[2] School of Agriculture, Food and Wine, Adelaide University, Adelaide, Australia
[3] School of Physics, Chemistry and Earth Sciences, Adelaide University, Adelaide, Australia

Protein misfolding diseases, such as Parkinson's Disease (PD), are among the fastest growing health concerns globally. At present, however, their increasing incidence has not been matched by advances in the development of targeted therapeutics. PD is characterised by the misfolding and aggregation of the 14 kDa protein α-synuclein, an intrinsically disordered protein involved in neuronal membrane dynamics and neurotransmission. Oligomeric and fibrillar aggregates produced during this process are highly cytotoxic, resulting in neuronal death and progressive neurodegeneration. As such, inhibition or modulation of this aggregation pathway is of great interest in the development of targeted therapeutics against PD. In this project, we have undertaken a natural products bioprospecting approach using brewer's spent grain (BSG), a major byproduct of the brewing industry, to uncover novel modulators of α-synuclein aggregation. Phenolic extracts obtained from BSG using conventional (NaOH) and eco-friendly deep eutectic solvents (choline chloride/maleic acid) were investigated, providing a structurally diverse screening library. BSG extracts demonstrated potent inhibition of α-synuclein fibrilisation in fluorescence kinetic assays and transmission electron microscopy, against both WT α-synuclein and PD-associated mutants. Extracts were observed to alter the kinetic profile of the aggregation pathway, with different extracts demonstrating interactions with distinct monomeric, pre-fibrillar and fibrillar α-synuclein species. Native ion mobility mass spectrometry also revealed conformational alterations induced in α-synuclein monomers in the presence of BSG extracts. Together, these findings demonstrate modulation of both fibrillar and pre-fibrillar α-synuclein species by BSG extracts, suggesting these extracts are a promising source of aggregation modulators for therapeutic development for PD.

4.

Don’t Stop at Red Lights: CRISPR Prime Integrase for Targeted Knock-out and Replacement without Plasmid Backbones in a Novel Traffic Light Assay

Jesse G Kennedy [1,2], Lachlan G Staker [1,2], Georgia R Tuck [1,2], Fatwa Adikusuma [1,2], Paul Q Thomas [1,2]

[1] School of Pharmacy and Biomedical Science, Adelaide University, Adelaide, SA, Australia; [2] South Australian Health and Medical Research Institute (SAHMRI), Adelaide, SA, Australia;

Easily programmable and highly efficient insertion of "gene sized” DNA fragments at specific genomic loci has been a longstanding but elusive goal of molecular genetics. Recent publications describe a two-step strategy that may make this a reality. Firstly, a 38bp attB BxbI integrase recognition site is incorporated into target DNA using CRISPR/Cas9 Prime Editing. This “second generation” editing system uses a Cas9-Reverse Transcriptase fusion enzyme to generate specific edits encoded by a dual purpose pegRNA which functions as a gRNA and repair template. Subsequently, the desired large DNA fragment is integrated at the attB “landing pad” using BxbI.

We aim to improve CRISPR Prime Integrase by using various Prime Editing technologies and a novel “traffic light assay”. Results include Next-Generation-Sequencing data of attB Prime Editing efficiencies in HEK293T cells for various gRNA and repair template lengths, pegRNA thermodynamics and nickase vs nuclease Prime Editors. Our most optimal Prime Editor achieved 88% attB insertion. Following this, we developed a “traffic light assay”, whereby insertion and expression of a target gene-RFP fusion simultaneously “knocks-out” GFP from a safe harbour target site in an engineered cell line, enabling red-green fluorescence quantification. FACS results include a 19% knock-out and 11kb full gene replacement efficiency that when re-directed to the native endogenous locus, achieved 45% and 8% integration in HEK293T and HeLa cells. With this assay, we demonstrated further optimisation, including evolved BxbI variants and contrasting knock-out profiles from different pegRNA combinations. We also demonstrated whole gene insertion from plasmid donors, without plasmid backbones, at 90% efficiency. Until now, this majorly limited Prime Integrase safety and thus enables non-minicircle dependent delivery. These advancements are progress towards making targeted, large DNA insertion a valuable addition to the genome editor’s toolbox.

5.

Integrative Mendelian Randomization and Bioinformatics Approaches Implicate Immune Dysregulation in Depression and Identify DUSP13 as a Causal protein Candidate

Endeshaw Abebe [1,2,3],, David Stacey [1,2], Iqbal Madakkatel [1,2], Anwar Mulugeta1[,2,4], Elina Hyppönen [1,2]*

[1]Australian Centre for Precision Health, School of Public Health, Adelaide University, Adelaide, South Australia, Australia.
[2]South Australian Health and Medical Research Institute, Adelaide, South Australia, Australia.
[3]Department of Biomedical Sciences, College of Health Sciences, Debre Tabor University, Debre Tabor, Ethiopia.
[4] Department of Pharmacology and Clinical Pharmacy, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia.

The authors have requested the abstract not be included in the abstract booklet.

6.

Inferring transporter specificity through language model-guided graph learning

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Membrane transporters orchestrate chemical exchange across cells, playing a central role in cellular metabolism and serving as prime targets for disease intervention. Yet, the specific substrates of most transport proteins remain unknown, largely because experimental characterization is hindered by the instability of membrane proteins in experimental assays. Existing computational tools are often limited to narrow substrate classes or fail to generalize to distant homologs. Here, we present TraSPIN, a geometric deep learning framework that jointly models paired representations of transporter proteins and small-molecule substrates. TraSPIN consistently outperforms existing predictors, demonstrating excellent generalization to both novel substrates and sequence-dissimilar transporters. The model moves beyond mere prediction to reveal the underlying structural mechanisms and motifs governing transporter-substrate recognition. Through large-scale inference across diverse species and successful de-orphanization of uncharacterized solute carrier proteins, we highlight TraSPIN's broad applicability. This powerful framework establishes a robust foundation for deciphering membrane transport, with immediate implications for drug discovery and systems medicine.

7.

DEVELOPING AN IN VITRO ASSAY TO EFFICIENTLY CHARACTERISE SMALL MOLECULE INHIBITORS TARGETING 2-OG-DEPENDENT HYDROXYLASES

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Fe(II)/2-oxoglutarate–dependent dioxygenases (2-OGDDs) hydroxylate the C-terminal transactivation domain of hypoxia-inducible transcription factors (HIFs), thereby regulating their transcriptional activity. More recently, 2-OGDDs have been recognised as key regulators of metabolism. Mouse studies have shown that deletion of specific 2-OGDDs increase metabolic rate, reduce weight gain, and enhance insulin sensitivity. These findings highlight 2-OGDDs as a promising therapeutic target class for metabolic disease. The aim of the present project is to develop an in vitro assay for enzyme activity amenable to kinetic characterisation and high throughput screening of small molecule inhibitors. A commercial bioluminescent succinate detection assay (Succinate-Glo™, Promega), originally designed for JumonjiC histone demethylases and other 2-OGDDs, was adapted for a specific 2-OGDD. This assay is less direct and less sensitive than the radiation-based CO_2 capture assay commonly used to measure 2-OGDD activity, with the luminescence output being much safer and simpler than scintillation counting. The Succinate-Glo assay was extensively optimised to establish a robust, reliable high-throughput screening platform in a 384-well plate format. Using this system, we show that known inhibitors, such as N-oxalylglycine (NOG), were competitive with 2-OG. Future work will focus on applying this platform to characterise known and novel 2-OG-dependent hydroxylase inhibitors. Collectively, this research establishes a robust in vitro system to accelerate the analysis of these inhibitors, advancing their potential applications in metabolic disease.

8.

Investigating the Functional Impact of Novel TMX2 p.Ile146Thr Variant

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Thioredoxin related transmembrane protein 2 (TMX2) encodes a protein that localizes to ER-mitochondria-contact sites with proposed functions in posttranslational protein modification and folding, calcium flux and cellular redox homeostasis. TMX2 variants have been previously shown to cause mitochondrial dysfunction, resulting in an autosomal recessive neurodevelopmental disorder characterised by microcephaly, cortical malformations, and spasticity (NEDMCMS). A novel homozygous genetic variant of uncertain significance (VUS) in TMX2 p.Ile146Thr was identified in a patient with spastic dystonic quadriplegic cerebral palsy. RNA-seq showed no transcript level impact in patient-derived cells, however TMX2 protein expression was reduced to 21% of wildtype (WT) levels by whole cell proteomics (SD 13.5%, control range 98-125%). We found no impact of this variant by either Extracellular Flux Asay or glucose-galactose growth assay, suggesting that mitochondrial dysfunction is not the disease mechanism. Cycloheximide chase showed increased turnover of the TMX2 p.Ile146Thr protein (half-life 3.8 variant v/s >10 hours control), however treatment with proteasome inhibitors MG132 or Lactacystin failed to restore the protein levels, suggesting that turnover was not regulated by the ubiquitin-proteasome degradation system. We further investigated the impact of this variant on protein stability by overexpression of WT and variant (p.Ile146Thr, p.Glu148Val, p.Ile146Arg, p.Ile146Leu, p.Leu150Arg) TMX2 in HEK293T cells. Both the patient p.Ile146Thr and p.Ile146Arg, p.Leu150Arg variants within the same helical region resulted in nearly complete loss of TMX2 protein, while p.Glu148Val and p.Ile146Leu were comparable to WT TMX2 protein. Together our data provided a genetic diagnosis for this patient. Further investigations of the impact of p.Ile146Thr on protein structure and function will clarify the disease mechanism and the role of TMX2 in brain development.

9.

A PPAR-fect Match? Developing Novel Peroxisome Proliferator Activated Receptor γ Ligands with High Affinity and Low Transactivation

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The Peroxisome Proliferator Activated Receptor γ (PPARγ) is a nuclear receptor that acts as the master regulator of adipogenesis and lipid metabolism and is involved in type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), cancer and inflammation. Drugs that target PPARγ for treatment of T2DM improve insulin sensitivity, however they also cause severe side effects including weight gain, decreased bone density and increased risk of CVD. These effects are caused by supraphysiological activation of PPARγ beyond what endogenous ligands can achieve; hence ligands that induce a similar or decreased level of transactivation compared to endogenous ligands may present a novel treatment without adverse side effects. AI was used to identify novel compounds predicted to bind to PPARγ to regulate its activity. We selected the top 80 compounds and tested them in vitro to investigate binding affinity for PPARγ using fluorescence polarisation assays with a probe that binds competitively to PPARγ, allowing for detection of both agonists and antagonists. Hits were tested in cell-based luciferase reporter assays to determine their effects on the transcriptional activity of PPARγ. Several compounds showed high affinity and low transactivation for PPARγ.

To guide optimisation of hits from our drug screen, we investigated the structure activity relationship of PPARγ via generating several PPARγ constructs with a variety of point mutations including structure-based alanine scanning mutants focussed on ligand binding, and clinically relevant mutants associated with disease. The effects on ligand affinity and transactivation were investigated to correlate these changes in structure with the function of PPARγ.

These data will inform the optimisation of hits from the initial drug screen to increase the potency of our ligands while minimising interactions which lead to full agonism. Once further optimised, these compounds may present novel drug leads for the treatment of T2DM.

10.
Developing a Method for IgG1 Analysis from Human Serum using Mass Spectrometry

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Immunoglobulin G (IgG) is a specific class of immunoglobulin (Ig) that comprises 75-80% of all Igs in the body. IgGs play a crucial role in adaptive immunity and can provide insights into a patient's immune response. There are currently limited methods to analyse these complex mixtures of IgGs due to the difficulty of detecting low-abundant IgGs from 1000 patient collated serum samples. The objective of this study was to optimise a workflow for isolating the IgG1 fragment antigen-binding (Fab) regions and to enable analysis of IgG1s from human IgG-enriched serum and plasma samples, allowing assessment of the Fab region subclone distribution. In this study, IgGs isolated from human plasma were subjected to various sample preparation workflows, including digestion with IdeS and IgdE proteases, to determine which method most effectively digests IgGs, allowing for the best characterisation of the resulting Fab regions. Mass spectrometry analysis enabled high-resolution separation and identification of IgG subclones, enabling comparative analysis across different samples. The optimised method demonstrated high sensitivity and reproducibility in isolating and digesting IgG subclones.

11.

Characterisation of a zoonotic human malaria's merozoite surface protein 3 family members during host cell invasion

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Nearly half of the world's population is at risk of malaria, with an estimated 282 million cases and 610,000 deaths in 2024 alone. The disease is caused by Plasmodium spp. parasites, with all clinical symptoms occurring during blood-stage replication, where Plasmodium merozoites undertake repeated cycles of host red blood cell (RBC) invasion. Merozoite surface proteins have long been thought to play a role in the invasion process, but direct evidence for this has been limited. We investigated the role of Plasmodium knowlesi (Pk) MSP3 family members and demonstrated a 50% reduction in parasite growth when all four PkMSP3s are knocked out. Despite our understanding of the dynamic environment in which these parasites grow, field-standard assays used to measure merozoite invasion of RBCs in vitro are conducted under static and non-physiological conditions. To overcome this, we employed orbital shaking during growth at a critical speed corresponding to previously measured forces in the microvasculature. Surprisingly, this revealed that while there was an overall decrease in growth of the parasite lines under shaking conditions, treatment with rapalog to induce the knockout of the final MSP3 revealed no significant difference in expansion rates between static and shaking conditions. Initial screen of various antibody and chemical inhibitors of invasion were found to be more inhibitory with shaking of the culture; however, no obvious changes in their inhibitory potency were observed in the absence of MSP3. Together, these findings do not support a role for PkMSP3 in parasite invasion of the RBC, instead suggesting that PkMSP3 function is more likely linked to an upstream process such as rupture from the RBC.

12.

Interaction selectivity for homologous E3 ligase cofactors can drive transcription factor specificity across the genome

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Cellular identity is tightly controlled by epigenetic regulation, with DNA-binding proteins called transcription factors serving as key drivers. Yet, much of their function at the mechanistic level remains enigmatic. A major hurdle towards understanding is that transcription factors require specific cofactor proteins to navigate a highly dynamic chromatinized environment in cells, a challenging setting for experimental testing.

To address this, we have applied our cellular genomics pipelines in mouse stem cells to characterize transcription factor interactions across a homologous family of dimeric, chromatin-bound TRIM cofactors (Trim24/Trim28/Trim33), using ChIP-seq, ATAC-seq and RNA-seq. Cutting-edge protein tools, including degron-tag alleles, enable the rapid perturbation needed to identify direct target genes with confidence. We find an unexpected degree of divergence for these TRIM proteins at the genomic level, both in recruitment to chromatin and in functional effects on gene expression. Structural modelling and swapping domains between TRIM proteins suggest that a tunable interaction interface exists that drives transcription factor selectivity.

We demonstrate that homologous chromatin-bound TRIM cofactors display appreciable selectivity in transcription factor binding, that may underlie their evolutionary divergence. Our findings show that transcription factors navigate chromatin with a high degree of specificity, with distinct cofactor interactions providing flexibility in genome regulation.

13.

Developing Gut and Motor Neuronal Cell Models of Superoxide Dismutase 1 Aggregation in MND/ALS

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Amyotrophic Lateral Sclerosis (ALS), is the most common form of Motor Neuron Disease (MND) where approximately 2,750 Australians are currently battling with the condition. ALS can be characterised by the progressive loss of motor neurons caused by the formation of protein inclusions. Superoxide dismutase 1 (SOD1) is one of the hallmark proteins that has been determined pathologically relevant in the context of ALS. The involvement of the gut-brain axis in MND/ALS onset and progression is growing, where dysfunction in the gut causes protein destabilisation (i.e SOD1) leading to the trafficking of aggregates to the CNS. It has been observed in ALS patients that gut symptoms precede neurological ones, where additionally, ALS-associated protein inclusions have been found in biopsies of the colon prior to diagnosis. This project has developed gut and motor neuronal-like cell models to investigate SOD1 aggregation propensity and associated toxicity. Distinct aggregate morphology has been observed suggesting differing aggregation mechanisms between cell types. ALS-associated SOD1 inclusion morphology in gut-like cells is strongly suggestive of Liquid-liquid phase separation (LLPS). Inclusion toxicity has also been observed to differ in gut-like cells where SOD1 aggregates are more cytotoxic when compared with motor-neuronal cells. When treated with known inhibitors of SOD1 aggregation (Telbivudine, CuATSM & Ebselen), gut-like cells exhibit poor efficacy in reducing SOD1 inclusion formation and increasing cell viability in comparison to motor neuronal cells. These findings highlight the lack of understanding regarding ALS onset and disease progression. It has been identified that gut cells are more prone to inclusion formation and may therefore be an early driver of ALS pathogenesis, where further understanding may be highly beneficial in progressing early detection and treatment of disease.

14.

Understanding the Interactions Between Small Heat-Shock Proteins and α-synuclein Mutations Associated with Juvenile Parkinson's Disease

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Parkinson's disease (PD) is the second most common and fastest growing neurodegenerative disease globally, however, disease pathogenesis and therapeutic targets are limited. Pathogenesis is linked to the aggregation of α-synuclein (αS) forming cytotoxic oligomers and amyloid fibrils that form large neuronal Lewy body inclusions. A recently discovered αS mutation, a 21-nucleotide duplication, resulting in a seven amino acid (MAAAEKT) insertion This mutation results in a severe and early-onset form of PD called juvenile-onset synucleinopathy (JOS) with unknown aggregation kinetics and structural conformations. Molecular chaperones, such as the small heat-shock proteins (sHsps), are cellular proteins involved in preventing protein misfolding and aggregation and thus were assessed as inhibitors of αS fibrilisation in vitro. Fibrilisation kinetic assays and TEM revealed that JOS αS aggregates slower than WT in pre-nucleated conditions. Additionally, the sHsps, Hsp20 WT and phosphorylation mimic (S16D/S59D) could only inhibit WT αS fibrilisation. Native cyclic IM-MS showed that JOS αS adopts a similar oligomeric state to that of WT but adopts more dynamic monomer conformations. Furthermore, native MS on the sHsps suggests that flexible and dimeric sHsps better recognise destabilised proteins to prevent aggregation. These results indicate that the toxicity and early-onset of JOS are linked to an increase in toxic oligomeric species and resistance to inhibition by sHsps. These mechanistic insights into αS aggregation is critical to guide therapeutic targeting and design against synucleinopathy and PD.

15.

INVESTIGATING THE IMPACT OF MITOCHONDRIAL FITNESS ON THE ANTI-TUMOUR EFFICACY OF CAR-T CELLS

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Cancer is one of the leading causes of mortality worldwide, accounting for nearly one in six deaths. Moreover, there is an alarming increase in the incidence of several solid cancers among young Australians. While chimeric antigen receptor (CAR)-T cell therapy represents a promising new cancer immunotherapy, the efficacy of CAR-T cells has been lacking against solid cancers. Previous studies have correlated metabolic fitness of CAR-T cells with their anti-tumour efficacy but differences in metabolic fitness between individual donors at the point of T cell isolation and the impact of this on CAR-T cell efficacy has not been comprehensively assessed. To address this, we assessed the mitochondrial membrane potential and mitochondrial mass of CD3+ T cells from a cohort of 23 healthy donors to generate a novel mitochondrial fitness (MF) score. Based on this scoring, T cells from donors with the highest and lowest levels of MF were manufactured into CAR-T cells. CAR-T cells were assessed in in vitro cytotoxicity assays and in vivo in a human colorectal cancer xenograft mouse model, demonstrating superior cytotoxic ability and anti-tumour efficacy of CAR-T cells derived from low MF T cells compared to the CAR-T cells derived from high MF T cells. Interestingly, CAR-T cells derived from low MF T cells displayed increased mitochondrial mass compared to the point of CD3+ T cell isolation and overall differences in MF between CAR-T cells derived from T cells with high and low MF were diminished following manufacturing. Thus, the results from this study can provide a rationale for screening donors for their mitochondrial fitness prior to CAR-T cell manufacture and stratify patients for whom metabolic reprogramming is essential to improve CAR-T cell anti-tumour efficacy.

16.

Establishing the interaction network of calcium and integrin binding proteins using ASAP-ID a novel proximity labelling approach

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The calcium and integrin binding protein (CIB) family is comprised of four evolutionarily conserved members (CIB1-4) with diverse cellular functions. Although some overlap exists between CIB family members, including the interaction with integrin αIIbβ3, they also have distinct biological roles. CIB1 is involved in the regulation of cell survival, proliferation, migration, and calcium signalling, whereas CIB2 plays a critical role in hearing and mechanoelectrical transduction. Notably, CIB1 and CIB2 can exert opposing effects in cancer, with CIB1 promoting pro-proliferative and pro-migratory signalling, while CIB2 suppresses neoplastic growth and migration. Given their broad expression and involvement in numerous signalling pathways, defining the interaction networks of CIB1 and CIB2 is essential for understanding their cellular functions.

To identify novel interacting proteins, we applied the proximity-dependent labelling approach, APEX2 and Small-tag Assembly of Proteins for Interaction Detection (ASAP-ID). An APEX2-nanobody fusion was expressed, purified, and applied to fixed cells overexpressing either CIB1 or CIB2, enabling proximity labelling of interacting proteins. Labelling was validated by immunofluorescence and western blotting, and biotinylated proteins were identified by proteomics (LC–MS/MS). A proximity ligation assay (PLA) was established to detect and validate CIB1 interactions. Furthermore, we investigated stimulus-dependent changes in CIB1 interactions under conditions known to alter its activity.

We aimed to leverage the novel ASAP-ID technique to further map CIB1 and CIB2 interactions beyond those identified using conventional approaches. Characterisation of these interactions will provide greater insight into the distinct and overlapping functions of CIB family members. In addition, defining the interactome of CIB1 will provide insight into its role in signalling pathways and allow validation of CIB1-directed therapeutics for cancer.

17.

Advancing Non-Invasive Staging of Endometrial Cancer Through Spatial Proteomic Profiling to Assess Metastatic Risk

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Current approaches to endometrial cancer management do not routinely incorporate preoperative prediction of lymph node metastasis (LNM), leading to unnecessary surgical procedures and associated complications. This project aims to address this gap by developing a protocol to uncover proteomic signatures driving metastatic spread, further translating and optimising it as a target approach for small tissue samples obtained during routine biopsies, and applying proteomic machine learning to identify molecular markers indicative of metastatic spread to lymph nodes. The resulting model is intended to be translated into clinical settings, where it will be utilised for decision-making. By integrating these methods, the study aims to improve patient stratification, reduce overtreatment, and advance understanding of the molecular mechanisms underlying endometrial cancer progression.

18.

ASSESSING FUNCTIONAL IMPACT OF VARIANTS IN COL4A1/COL4A2 IDENTIFIED IN THE AUSTRALIAN CEREBRAL PALSY BIOBANK (ACPB)

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COL4A1 and COL4A2 genes encode α1 and α2 chains of type IV collagen respectively, which together form a key structural component of basement membranes that support vasculature. Variants in COL4A1/COL4A2 cause multisystem disorders primarily affecting brain, eye, kidney and muscle. They are also a frequent finding in cerebral palsy, with pathogenic variants identified in ~2% of the Australian Cerebral Palsy Biobank (ACPB) cohort. A further ~2% of the ACPB cohort have a variant of uncertain significance (VUS) in COL4A1/COL4A2 requiring functional assessment. Since COL4A1/COL4A2 are not expressed in available patient cells (lymphoblastoid cell lines, LCLs), we developed transactivation tools using the CRISPR_dCas9_VPR system. COL4A1/COL4A2 genes were successfully transactivated in patient LCLs as confirmed by RT-qPCR. For variants impacting RNA processing, splicing was assessed by long-read amplicon sequencing. Using this approach, 2 out of 4 candidate splice variants tested were confirmed to result in pathogenic splicing defects. NM_001845.6 (COL4A1):c.615+4_615+9del caused intron retention, introducing a premature termination codon predicted to trigger nonsense-mediated mRNA decay.

NM_001846.3(COL4A2):c.4039+1del activated a cryptic splice donor site, resulting in an in-frame deletion in the collagenous triple-helical domain which is essential for collagen IV trimer assembly and secretion. Western blot of transactivated LCLs also demonstrates robust induction of COL4A1 protein expression following transactivation, supporting feasibility of downstream protein analyses. This approach is now be applied to patient samples enabling assessment of impact of loss-of-function and missense variants on protein expression, collagen IV assembly, secretion and cellular stress pathways. Overall, this study has developed scalable gene transactivation tools to assess COL4A1/COL4A2 variant pathogenicity and explore disease mechanisms in patient cells.

19.

Structural and functional analysis of Calcium and Integrin Binding Protein 1 (CIB1) cargo binding

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Calcium and Integrin Binding Protein 1 (CIB1) is a calcium regulated cargo-binding protein with partners that can contribute to oncogenic signalling such as cell survival, proliferation and migration. The interaction between CIB1 and its cargo protein sphingosine kinase 1 (SK1) promotes oncogenesis by inducing the translocation of SK1 from the cytoplasm to the plasma membrane, causing increased production and export of pro-proliferative sphingosine-1-phosphate (S1P). Previous studies have shown that the SK1-CIB1 interaction is key to oncogenesis in fibroblast neoplastic transformation assays. As the molecular interaction of CIB1 and SK1 is largely uncharacterised, information on the binding interfaces between CIB1 and SK1 will provide new strategies for designing new anti-cancer agents that block pro-oncogenic SK1 translocation. We employed biochemical and crosslinking techniques to probe the key interaction sites in the SK1-CIB1 complex. Mutations in the hydrophobic binding groove in CIB1 and the α8 helix in SK1 blocked the interaction revealing binding inferface of the complex. Using cross-linking and mass spectrometry (LC-MS/MS), we were able to identify two pairs of lysine residues that supported our mode of interaction and produce a structural model of the SK1-CIB1 interaction. Our data provides important insights towards the key residues of the CIB1-SK1 complex as well as the relative orientation of the complex. Characterising this complex facilitates our future studies on the development of novel anti-cancer therapeutics that target the SK1-CIB1 oncogenic axis.

20.

Altered expression of vesicular trafficking machinery in prostate cancer affects lysosomal dynamics and provides insight into the underlying biology and disease progression

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BACKGROUND: Prostate cancer is the second most diagnosed cancer in men worldwide and is a significant cause of cancer-related mortality, driven by metastasis and resistance to androgen-targeted therapies. Altered lysosome biology contributes to prostate cancer progression, but the mechanisms underlying its dysregulation remain unclear. Here, we investigate the role of lysosome trafficking in prostate cancer.

METHODS: Lysosomal motility was evaluated using confocal microscopy of LAMP1-transfected prostate cells and spot-tracking analysis. Expression of lysosomal trafficking machinery was evaluated in patient cohort databases and through immunohistochemistry on tumour samples. The roles of vesicular trafficking machinery were evaluated through over-expression and siRNA. The effects of androgen (R1881) treatment on lysosome vesicular trafficking were evaluated by RNA sequencing, protein quantification and confocal microscopy.

RESULTS: Altered regulation of lysosomal trafficking genes/proteins was observed in prostate cancer tissue, with significant correlations for co-expression of vesicular trafficking machinery in Gleason patterns. The expression of trafficking machinery was associated with poorer patient outcomes. R1881 treatment affected lysosome distribution, abundance, and trafficking machinery in hormone-sensitive cells. Manipulating trafficking genes altered lysosome positioning, cell phenotype, and migration, with distinct effects in non-malignant versus cancer cells.

CONCLUSIONS: These findings provide novel insights into the altered regulation and functional impact of lysosomal vesicular trafficking in prostate cancer pathogenesis.

21.
Characterisation of Dihydroceramide Desaturase (DES1) Regulation

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Sphingolipids play varying roles within cells of eukaryotic organisms and are integral components in cell membranes, vesicles and as signalling molecules. Within the de novo sphingolipid synthesis pathway, dihydroceramide desaturase (DES1) is a critical enzyme, inserting a double bond into its substrate dihydroceramide to produce ceramide, which is a precursor for complex sphingolipids and their derivatives. The irreversible DES1 desaturation reaction therefore controls the saturation of the cellular sphingolipidome, as many downstream enzymes are capable of using both the saturated and desaturated forms of ceramide. Saturated (dihydro) and non-saturated sphingolipids have distinct cellular functions due to differences in their rigidity and packing structure in lipid membranes2. Research into the role of saturated sphingolipids is in its early stages, however their potentially pathogenic roles in human disease are becoming increasingly apparent, with implications in cancer, metabolic and cardiovascular diseases. Hypomyelinating Leukodystrophy (HLD) is a rare neurogenerative disease caused by loss of myelination in white matter tissue with no current curative therapy. In recent years, three separate studies have linked HLD to loss-of-function mutations in dihydroceramide desaturase. To investigate the mechanisms by which DES1 is regulated and by which activity is lost, a series of expression constructs containing non-pathogenic DES1 variants, point mutations linked to HLD and post-translational modification sites were made. DES1 HLD and phosphorylation-null variants were assessed using enzyme assays, protein structural modelling and protein stability assays. Here we show for the first time the potential role of patient and phosphorylation mutants on DES1 enzyme activity, protein interactions and stabilisation. By uncovering new mechanisms of DES1 regulation we may find novel targets for the modulation of DES1 activity and the sphingolipid pathway in disease.

22.

Structural characterisation of the portal protein from a newly isolated Serratia bacteriophage

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The authors have requested the abstract not be included in the abstract booklet.

23.
Regulation of aromatic amino acid biosynthesis in annual ryegrass

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The use of herbicides for weed management is a crucial part of modern agricultural systems. An attractive herbicidal modality is the inhibition of enzymes in amino acid biosynthesis pathways, particularly those absent in animals, as this inherently reduces the likelihood of off-target effects. The shikimate pathway, responsible for the biosynthesis of the three aromatic amino acids (AAAs) – phenylalanine, tyrosine, and tryptophan – is one such pathway. Together with its high carbon flux (~30% in vascular plants) and consequent essentiality for survival, it has long been a target for herbicide discovery, with glyphosate being the most famous example. At the biochemical level, the shikimate pathway is known to be regulated through negative feedback inhibition by its products and downstream metabolites at the first step – the condensation of phosphoenolpyruvate (PEP) and erythrose-4-phosphate (E4P) by 3-deoxy-D-arabinoheptulosonate 7-phosphate synthase (DAHPS). This regulatory scheme is relatively complex, with isoform-dependent differential inhibition demonstrated in model plant species, but never in problem weeds.

Here, isoforms of DAHPS from annual ryegrass (Lolium rigidum) are identified, cloned for recombinant expression, and characterised in terms of Michaelis-Menten kinetics (real-time spectrophotometric assay) and inhibition (endpoint colorimetric assay) by metabolites identified in prior work as feedback regulators of DAHPS in other species. The determined kinetic parameters and inhibition profiles are in general agreement with those published for other grasses, with strong inhibition by shikimate, sigmoidal dependence of initial velocity on E4P concentration, and a tenfold affinity for PEP than for the other substrate; the novel enzymological property of substrate inhibition by PEP is also observed. This work suggests a conserved scheme for shikimate pathway regulation in grasses and opens a possibility for the development of novel grass-specific herbicides.

24.

Bassianolide, a fungal cyclic depsipeptide, potently inhibits mitotic progression in Plasmodium falciparum.

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With the spread of drug-resistant malaria parasites, natural products provide a rich source of structurally and biologically diverse scaffolds to address the growing need for new drugs with novel mechanisms of action. We previously identified a class of cyclodepsipeptides with activity against Plasmodium falciparum. Among these, bassianolide, an insecticidal cyclodepsipeptide isolated from pathogenic fungal species, emerged as the most potent compound and exhibited limited toxicity toward a human cell line. We characterised the anti-malarial activity and mechanism of action of bassianolide in asexual blood-stage parasites, where it exhibited potent nanomolar activity (IC50 = 50 nM), comparable to that of chloroquine.

Stage-of-activity assays demonstrated that bassianolide primarily acts during intraerythrocytic replication, blocking progression from single-nucleated trophozoites to multinucleated schizonts. Using ultrastructure expansion microscopy, we observed that bassianolide-treated parasites stalled not long after the onset of mitosis, forming mitotic spindles but failing to segregate their DNA into distinct daughter nuclei. These parasites accumulated approximately five genome copies inside a single ‘meganucleus’, indicating that DNA replication was initiated but nuclear division failed. To explore the molecular mediators driving this phenotype, we performed solvent-induced proteome profiling, which identified a putative RNA-binding protein as a high-confidence target candidate of bassianolide. Together, these data highlight bassianolide as a phenotypically unique inhibitor of nuclear division with promising potential to be developed as an anti-malarial.