



Poster Presentations

Name: **Agin Ravindran**

Theme: Cancer Biology

Poster Position: 1

Title: Dissecting the impacts of gene fusion-mediated translational dysregulation in B-cell lymphoblastic leukemia to highlight novel therapeutic targets

Authors: Agin Ravindran, Katrina Woodward, Nimeree Muntasir, Amna Choudhary, Shafi Mahmud, Madhu Kanchi, Stanton Tam, Alice Cleynen, Dipti Talaulikar, Eduardo Eyra, Nikolay Shirokikh**

Dysregulation of messenger (m)RNA translation into proteins (protein biosynthesis) is one of the hallmarks of malignant transformation. Translational dysregulation can originate in tumours from direct somatic mutations of the translation machinery, or through the alteration of epistasis during malignant transformation. B-cell acute lymphoblastic leukemia (B-ALL) is characterised by prevalent chromosomal translocations leading to the expression of gene fusions. Distinct B-ALL fusions are linked to responses to chemotherapy, proliferation, and malignant survival. Previously we uncovered two characteristic expression patterns in B-ALL patients with two most common fusion types, ETV6-RUNX1 and KMT2A-MLLT1. The KMT2A-MLLT1 fusion, almost invariably associated with bad prognosis, is associated with dysregulation of translational control components (such as IGF2BP2, CPEB2, EIF3B, EIF2AK3, CTIF). The ETV6-RUNX1 fusion, often associated with good prognosis, presented a transcriptome-wide redefinition of translation start sites and effects on translational control components opposing those of the KMT2A-type. We thus proposed that the ETV6-RUNX1 and KMT2A-MLLT1 fusions define a functionally distinct translational landscape and pathophysiology in B-ALL. Here, we set out to directly investigate translational landscape in REH (ETV6-RUNX1) and KOPN-8 (KMT2A-MLLT1) B-ALL cell lines and GM12878 non-malignant immortalised B-cell control. Using a combination of inhibitor-based translational complex stabilisation, polysome profiling and direct nanopore RNA sequencing, we investigated isoform-resolved translational patterns of mRNA across the cell models and identified fusion-specific epitranscriptomic landscapes. We observed differentially abundant and modified transcripts related to mitochondrial function, cell cycle regulation, DNA binding and repair in both malignant cell lines relative to the control, implying different survival and adaptation strategies. As B-ALL presents a general lack of mutations in gene regulators that commonly serve as therapeutic targets in other leukemias and solid tumours, we aim to highlight new fusion-specific strategies of drug resistance emergence and enable complementary therapeutic approaches for each B-ALL subtype.



Poster Presentations

Name: **Khadizatul Kubra**

Theme: Cancer Biology

Poster Position: 2

Title: Tp53 knockout zebrafish autonomously develops anaplastic sarcoma and hemangiosarcoma which are transplantable and metastatic in nature.

Authors: *Khadizatul Kubra, Faiza Basheer and Amardeep S. Dhillon*

Background

Tp53 plays a very significant role in maintaining the genomic integrity of multicellular organism by preventing mutation of the genome and oncogenic transformation under any stressful condition. More than 50% of all human cancers have disrupted tp53 function. However, to date the effect of tp53 mutation in developing cancers, their overall progression and metastatic nature of the cancers is not well studied. Therefore, to understand tp53 knockout effect in vivo, we have generated tp53 mutant zebrafish and characterized them.

Methods

CRISPR/CAS9 technique has been used to create tp53 mutation in zebrafish. Regular monitoring and histopathological test have been conducted to identify zebrafish cancer spectrum. Adult xenotransplantation has been performed to generate syngeneic zebrafish cancer model and observe the metastatic nature of the cancers.

Results

tp53 mutant (tp53 $-/-$) zebrafish autonomously developed cancers, mainly anaplastic sarcoma along with a good number of hemangiosarcoma and few rhabdomyosarcomas. By 4 months, the onset of tumour was observed while 66% of homozygous tp53 $-/-$ animals developed tumour by 27 weeks. Xenotransplantation of two largely developed cancer, anaplastic and hemangio-sarcomas in both immune competent and compromised zebrafish showed that both are transplantable and metastatic in nature where immune cells might play different role in metastasis and tumour engraftment.

Conclusion

tp53 knockout zebrafish allowed us to study two deadly cancers anaplastic sarcoma and hemangiosarcoma. The transplantable nature of the cancers has allowed to have the cancer model faster for further dissecting as well as to understand the role of immune cells in cancer microenvironment.



Poster Presentations

Name: **Chinkwo Kenneth Anye**

Theme: Cancer Biology

Poster Position: 3

Title: **Cancer preventive properties of cereals and pulses**

Authors: *Kenneth A Chinkwo, Nidhish Francis, Justin A Roby, Abishek B Santhakumar & Christopher L Blanchard*

Our studies offer insights into the anticancer properties of cereals and pulses. We delve into the mechanistic pathway triggering apoptosis and cell migration studies, which are critical in curbing cancer cell growth and invasion. Several biochemical assays were used to analyse phenolic extracts' effects of sorghum, rice, barley, oats and chickpea exposed to SW480 cells and HT29, shedding light on their potential mechanisms against cancer cell growth. Extracts from pigmented varieties of red rice, black sorghum and brown rice significantly reduce cancer cell proliferation and the expression of p53 and caspases. Also, HT29 cells when treated with either chickpea polyphenol extract (CPPE) or chickpea water (CPW) reduced HT-29 colon cancer cell viability at $\geq 50 \mu\text{g/mL}$ and at 250 and 500 $\mu\text{g/mL}$ concentrations by inducing apoptosis. CPW and CPPE treatments also decreased cell migration scratch areas by 34.42% and 15.27%, respectively, highlighting their potential to impede cancer cell migration. Overall, these studies underscore cereal bioactive compounds' potential health-promoting properties. Currently, we are conducting a pilot project in ovo engrafting tumours in chicken eggs with cancer cells to study the behaviour of these extracts.



Poster Presentations



Poster Presentations

Name: **Paige Taylor**

Theme: Cancer Biology

Poster Position: 4

Title: **Defining a New Binding Mechanism; The Structural and Functional Characterisation of KAT6A Nuclear Import.**

Authors: *Paige Taylor, Dr. Sofiya Tsimbalyuk, Dr. Brian McSharry, Dr. Martin Pal and Prof. Jade Forwood*

Lysine acetyltransferases (KATs) are a family of nuclear enzymes responsible for a wide range of regulatory functions, including metabolism, cell signalling, gene regulation, and apoptosis. The largest protein in this family is KAT6A (also known as MOZ and MYST3), which consists of 2,004 residues and various functional regions, including an acetyl-coenzyme A (Acyl-CoA) binding site, a histone acetyltransferase (HAT) domain, and PHD zinc finger domains. These components grant it the ability to interact with a wide range of proteins. Despite KAT6A being a nuclear protein, little is known about the pathways it utilizes to localize to the nucleus. Classical nuclear localization involves active transport into the nucleus for all proteins larger than 40kDa. The cargo protein must possess a functional nuclear localization signal (NLS) to interact with import receptors—a family of nuclear import proteins that attach to the NLSs displayed on proteins destined for the nucleus. This enables the transportation of these proteins into the cell nucleus by forming a tertiary complex with importin- β proteins before undergoing active transport through the nuclear pore. Structural elucidation of two predicted NLS regions identified a unique interaction interface between Importin- α (IMP α) proteins and KAT6A, shared with two previously characterized viral NLSs. Further investigation through alanine scanning for key residues within the bipartite binding region revealed, via the use of fluorescent polarization and electro-mobility shift assays, that this unique binding motif results in a high dependency on the functionality of the minor site binding region within the bipartite interaction.



Poster Presentations



Poster Presentations

Name: **Vihandha Wickramasinghe**

Theme: Cancer Biology

Poster Position: 5

Title: **mRNA export is a new therapeutic vulnerability in cancer**

Authors: *Vihandha Wickramasinghe*

Messenger RNA (or mRNA) is part of the central dogma of biology. RNA is enjoying a renaissance in both biology and medicine due to the efficacy of mRNA based therapeutics in treating COVID and holds tremendous promise for treating many diseases, including cancer. Export of mRNA from the nucleus to the cytoplasm is a critical step in gene expression. We have identified that mRNA export factors are systematically amplified in cancer. Our findings suggest that non-essential components of the mRNA export machinery are potential therapeutic targets. Furthermore, targeting mRNA export may increase the efficacy of transcription inhibitors. We will describe our work in developing nuclear mRNA targeting therapies to treat cancer. These have the potential to be an exciting new frontier in cancer therapeutics.



Poster Presentations



Poster Presentations

Name: **Adam Hagg**

Theme: Cell Biology & Signalling

Poster Position: 6

Title: **Activin A and Interleukin 6 act in unison to promote skeletal muscle atrophy**

Authors: *Adam Hagg, Roberta Sartori, Paul Gregorevic and Marco Sandri*

The Transforming Growth Factor β (TGF- β) superfamily comprises a network of over 30 structurally related signalling molecules, each with context specific regulatory roles in almost all mammalian cells. Over a period of more than 30 years, studies have continued to emphasize the TGF- β signalling network as a critical regulator of skeletal muscle size. Specifically, Activins and various Growth Differentiation Factors (GDFs) have been described as negative regulators of muscle mass owing mostly to their capacity to drive proteasomal degradation via activation of the intracellular SMAD2/3 signalling axis. In contrast, certain Bone Morphogenetic Proteins (BMPs) activate the SMAD1/5/8 signalling axis to promote skeletal muscle fibre size. Activins, in combination with the pro-inflammatory mediator, Interleukin-6 (IL-6), act to drive skeletal muscle atrophy. It is clear that these circulating factors play a central role in the pathogenesis associated with skeletal muscle wasting caused by cancer. However, the specific mechanisms underpinning Activin A and IL-6 mediated muscle atrophy remain unclear.

Using adeno-associated viral vectors, we aimed to further understand how Activin A and IL-6 act to regulate skeletal muscle mass in wildtype mice. We report an intricate interplay whereby Activin A and IL-6 act to suppress the anabolic capacity of the BMP pathway by transcriptional regulation of the BMP antagonist, Noggin. Noggin expression is sufficient to promote muscle fibre atrophy which is associated with dysfunction of the motor unit. Our work demonstrates that Activin A and IL-6 operate in a synergistic fashion to promote muscle atrophy. Our findings reveal new insights into the complex nature by which circulating growth factors and cytokines act to promote skeletal muscle atrophy.



Poster Presentations



Poster Presentations

Name: **Evie Hodgson**

Theme: Cell Biology & Signalling

Poster Position: 7

Title: **Elucidating a novel and essential mitochondrial targeting loop of the cytosolic iron-sulfur cluster synthesis protein, CIA1, in apicomplexan parasites**

Authors: *Evie R. Hodgson, Jenni A. Hayward, Giel G. van Dooren*

Apicomplexans are a large phylum of highly successful protozoan parasites. Understanding the key biological processes that are integral for parasite function, and how these differ from equivalent processes in animal hosts, is key for informing our approaches to disease eradication. Despite iron-sulfur (FeS) clusters being crucial co-factors in a range of fundamental cellular processes, their biosynthesis in apicomplexans remains poorly understood. Prior investigations from our group have demonstrated that, in contrast to their animal hosts, the synthesis of cytosolic FeS clusters operates along the outer face of the mitochondrion in the apicomplexan parasite, *Toxoplasma gondii*. Here we show that the CIA targeting complex, a heteromeric protein complex responsible for inserting FeS clusters into recipient cytosolic and nuclear FeS proteins, exhibits dual localisation to the cytosol and mitochondrion of *T. gondii*. We demonstrate that one member of the complex, TgCIA1, mediates the mitochondrial targeting of the two remaining complex proteins, TgCIA2 and TgMMS19. We show that a large insertion in the CIA1 protein that is found throughout apicomplexan parasites and their free-living relatives is both necessary and sufficient for mitochondrial targeting, and we show that TgCIA1 and its mitochondrial-targeting insertion are essential for parasite proliferation. Notably, we establish that a conserved, aromatic amino acid motif in this insertion is key for mitochondrial targeting. Our study elucidates a pivotal difference in an otherwise ancient and highly conserved biosynthetic pathway which may reflect a fitness advantage conferred on *T. gondii* parasites and related organisms, ultimately unveiling a unique aspect of apicomplexan biology.



Poster Presentations



Poster Presentations

Name: **Jennilee Davidson**

Theme: Cell Biology & Signalling

Poster Position: 8

Title: Amyotrophic lateral sclerosis-linked ubiquitin ligase SCF cyclin F dysregulates sequestosome-1/p62 solubility, foci formation and interaction network in disease pathogenesis

Authors: *Jennilee M Davidson, Sharlynn SL Wu, Michelle Newbery, Lezanne Ooi, Shu Yang, Roger S Chung, Albert Lee*

Protein aggregation is characteristic of amyotrophic lateral sclerosis (ALS) pathology. P62 is implicated in several types of ALS and protein clearance, however it is not known whether it is a culprit or bystander in disease pathogenesis [1]. We recently found that dysregulated p62 causes the hallmark TDP-43 aggregation and pathology [2], suggesting that p62 has an intermediary role leading to pathological protein aggregation. Emerging evidence indicates that the formation of sequestosome-1/p62 foci is a preceding step to protein clearance. In this study we identified an ubiquitin ligase that regulates p62, and how this protein interaction is dysregulated in ALS pathogenesis [3].

We demonstrated that p62 was an ubiquitylation substrate of the E3 ubiquitin ligase SCF cyclin F complex. We found that SCF cyclin F ubiquitylated p62 at lysine(K)281, and that K281 regulated the propensity of p62 to aggregate. Cyclin F expression promoted the aggregation of p62 into the insoluble fraction, which corresponded to an increased number of p62 foci. Notably, ALS-linked mutant cyclin F p.S621G aberrantly ubiquitylated p62, dysregulated p62 solubility in cells, patient-derived fibroblasts and induced pluripotent stem cells, and dysregulated p62 foci formation. Our data suggest that selective ubiquitylation of p62 facilitates biochemical properties required to regulate p62 solubility leading up to its function in protein clearance and implicates the differential ubiquitylation of p62 and p62 foci formation as an early pathogenic mechanism of ALS. In addition, the interaction network of p62 was markedly different in ALS mutant cells. Further investigation of the dysregulated downstream protein network will reveal potential key proteins fundamental to cellular proteostasis. Consistently, we found that motor neurons from post-mortem patient spinal cord tissue exhibited increased p62 ubiquitylation, suggesting that dysregulated ubiquitylation is a mechanism that persists through end stage of the disease.

References:

1. Davidson JM, Chung RS, Lee A. 2022 Neurobiol Dis. doi: 10.1016/j.nbd.2022.105653
2. Foster AD, Flynn LL, Cluning C, et al. 2021, Sci Rep. doi.org/10.1038/s41598-021-90822-2
3. Davidson JM, Wu SL, Rayner SL, et al. 2023 Mol Neurobiol. doi: 10.1007/s12035-023-03355-2

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Poster Presentations



Poster Presentations

Name: **Natasha Vassileff**

Theme: Cell Biology & Signalling

Poster Position: 9

Title: Nitric oxide signalling and altered protein nitrosylation in extracellular vesicles induced through microglial activation

Authors: Natasha Vassileff, Jereme G. Spiers, Sarah E. Bamford, Rohan G.T. Lowe, Keshava K. Datta, Paul J. Pigram & Andrew F. Hill¹

The early disease pathogenesis of neurodegenerative conditions is often characterised by neuroinflammation, which is predominantly initiated through microglial activation and can be induced through lipopolysaccharide (LPS) treatment. This activation culminates in the expression of the inducible form of nitric oxide synthase (iNOS), resulting in nitric oxide (NO) production. NO targets cysteine thiols resulting in post-translational S-nitrosylation, which may alter protein function. Furthermore, packaging of these NO-modified proteins into Extracellular Vesicles (EVs), a nano-sized lipid structure containing molecular cargo which acts as a form of cellular communication, further propagates the neuroinflammatory phenotype by allowing the exertion of NO signalling over long distances. While some EV molecular cargo has been well studied, the NO-modified proteome of activated microglial EVs has not been investigated. Therefore, this study aimed to identify protein post-translational modifications induced through NO signalling under neuroinflammatory conditions. EVs isolated from LPS-treated microglia underwent novel advanced surface imaging using time of flight-secondary ion mass spectrometry (ToF-SIMS), in addition to iodolabelling and comparative proteomic analysis to identify post-translation nitrosylation changes. ToF-SIMS imaging successfully identified NO modified cysteine thiol side chains in the EV proteins isolated from LPS treated microglia. Additionally, the EVs from LPS treated microglia were found to carry nitrosylated proteins indicative of neuroinflammation, as identified through the iodolabelling proteomic analysis. These included known NO-modified proteins and those associated with LPS-induced microglial activation, potentially imperative to neuroinflammatory communication. Together, these results show activated microglia, during neuroinflammation, are capable of exerting broad signalling changes through selective EV packaging which may have important implications for neurodegenerative diseases.



Poster Presentations



Poster Presentations

Name: **Sebastian Furness**

Theme: Cell Biology & Signalling

Poster Position: 10

Title: Constitutive activity of the ghrelin receptor provides a dominant second-messenger switch to re-code dopamine D2 receptor output required for co-ordination of voluntary defecation.

Authors: Sebastian GB Furness, Mitchell T Ringuet, Farhad Dehkhoda, Emily A Whitfield, Linda J Fothergill, Desye Misgenaw, John B Furness

The dopamine D2 (DRD2) and ghrelin (GHSR) receptors are co-expressed in preganglionic neurons of the spinal defecation centre. Pharmacological stimulation of either receptor results in colonic pressure waves, however ghrelin is absent from the central nervous system. In most neurons, activation of DRD2 results in neuronal inhibition via Gai/o and subsequent G protein-gated inwardly rectifying potassium channel activation. Contrastingly, DRD2 activation in defecation centre neurons results in depolarisation. This cellular output switch has been reported in hypothalamic neurons co-expressing GHSR with DRD2 and was previously attributed to heterodimerisation of these receptors.

In spinal defecation neurons, those exhibiting an excitatory response to dopamine also exhibited an excitatory response to GHSR agonists. Inverse agonists of GHSR blocked DRD2 dependent excitatory response, which was also dependent on intracellular calcium stores. This switch in DRD2 coupling to calcium mobilisation was also observed in recombinant cells, however this does not appear to be the result of a switch in the G protein preference of DRD2. Moreover, we were unable to detect allosterity between these receptors using ligand binding, nor did we observe them to be close enough to indicate dimerization using super-resolution microscopy. Instead, we observed a GHSR dependent priming of phospholipase C beta (PLC- β), that was dependent on GHSR's constitutive activity. In cells co-expressing a GHSR polymorph that lacks constitutive activity, coupling of DRD2 to calcium was restored by priming with a low concentration ghrelin. The requirement for PLC- β was also seen in spinal defecation neurons, where PLC- β inhibition reversed the response of these cells to dopamine from excitatory to inhibitory. Together this data indicates that dopamine mediated excitation is dependent on GHSR constitutive activity via a dominant second-messenger switch. This work has broad implications for determining metabotropic neurotransmitter responses via modulation through other G protein-coupled receptors.



Poster Presentations

Name: **Suyan Yee**

Theme: Cell Biology & Signalling

Poster Position: 11

Title: **Linking the biochemical specialisation of organelles to cell type-specific stress signalling in plants.**

Authors: *Suyan Yee, Riley Furbank, Atharva Valanju, Garima Bhatia, Robert Furbank & Kai Xun Chan.*

Abiotic stresses, such as heat, drought, and excess sunlight cost Australian agriculture upwards of \$1 billion in yield losses annually. These losses occur mainly due to the accumulation of reactive oxygen species (ROS) in chloroplasts under various environmental stresses, which disrupts and hinders photosynthesis. The over-production of ROS in plant chloroplasts leads to the production of stress signalling molecules that induce acclimatory gene expression changes. Thus, chloroplasts have been proposed to act as environmental stress sensors in plant cells. However, virtually nothing is known about how biochemical- and cell type-specialisation of chloroplasts can impact cellular signalling networks in response to environmental stress.

By integrating live-cell imaging, biochemical, metabolomic and single-cell transcriptomic approaches, this project aims to unravel the key stress signalling networks and components activated in specialised leaf cell types, especially the mesophyll (M) and bundle sheath cells (BS) of the C4 model species, *Setaria viridis*, which contain biochemically and structurally distinct chloroplasts. We observed time- and cell type-dependent differential patterns of ROS localisation and accumulation under heat and high light stress, which suggests cell type-specific activation and regulation of stress signalling networks. Correspondingly, we also observed certain chloroplast stress signals, such as 3'-phosphoadenosine 5'-phosphate (PAP), which are known to be critical to ROS responses, accumulate in a cell type-specific manner under stress.

To further investigate any correlations between the cell type-specific accumulation of these signalling metabolites (ROS and PAP) and gene expression, I developed and optimised a method to isolate M and BS cells from *S. viridis* for single cell RNA sequencing (scRNAseq). Preliminary analysis of the scRNAseq data suggests a cell type-specific modulation of chloroplast-to-nucleus signalling pathways during heat and high light stress, which correlates with specialised chloroplast architecture, as well as differential ROS and PAP levels in the C4 model plant, *S. viridis*.

Our results provide preliminary evidence that chloroplast architecture is a major contributor to the specialisation of stress signalling networks in distinct leaf cell types. This is significant as current crop bioengineering approaches that boost photosynthesis by altering chloroplast architecture in different cell types tend not to consider the importance of their associated stress signalling networks. Consequently, we propose that a deeper understanding of how chloroplast- and cell

type-specialisation intersect with stress signalling may provide novel strategies for enhancing crop productivity.



Poster Presentations



Poster Presentations

Name: **Victor Anggono**

Theme: Cell Biology & Signalling

Poster Position: 12

Title: **Copine-6 is a Ca²⁺ sensor for activity-induced AMPA receptor exocytosis**

Authors: *Jing Zhi Anson Tan¹, Se Eun Jang¹, Ana Batallas-Borja¹, Nishita Bhembre¹, Mintu Chandra², Lingrui Zhang¹, Huimin Guo¹, Mitchell T. Ringuet¹, Jocelyn Widagdo¹, Brett M. Collins², Victor Anggono¹*

¹Clem Jones Centre for Ageing Dementia Research, Queensland

The recruitment of synaptic α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors underlies the strengthening of neuronal connectivity during learning and memory. This process is triggered by N-methyl-D-aspartate (NMDA) receptor-dependent postsynaptic Ca²⁺ influx. Synaptotagmin (Syt)-1 and -7 have been proposed as Ca²⁺ sensors for AMPA receptor exocytosis, but are functionally redundant. Here we identify a cytosolic C2 domain-containing Ca²⁺-binding protein Copine-6 that forms a complex with AMPA receptors. Loss of Copine-6 expression impairs activity-induced exocytosis of AMPA receptors in primary neurons, which is rescued by wild-type Copine-6, but not Ca²⁺-binding mutants. In contrast, Copine-6 loss-of-function does not affect steady-state expression or tetrodotoxin-induced synaptic upscaling of surface AMPA receptors. Loss of Syt-1/-7 significantly reduces Copine-6 protein expression. Interestingly, overexpression of wild-type Copine-6, but not the Ca²⁺-binding mutants, restores activity-dependent exocytosis of AMPA receptors in Syt-1/-7 double-knockdown neurons. We conclude that Copine-6 is a postsynaptic Ca²⁺ sensor that mediates AMPA receptor exocytosis during synaptic potentiation.



Poster Presentations



Poster Presentations

Name: **Andrew Spiteri**

Theme: Cell Biology & Signalling

Poster Position: 13

Title: **The role of methylation in the regulation of ribosomal heterogeneity and mRNA selection**

Authors: *Andrew J Spiteri, Joshua J Hamey, Marc R Wilkins*

The ribosome is one of life's most important molecular machines that has historically been seen as a backstage participant in gene regulation, binding to mRNA and translating codons in a rote-like fashion. However, recent evidence suggests that core protein components of the ribosome can be regulated and diversified as a means to intricately manipulate the expression of the cellular proteome. Post-translational modifications of ribosome proteins is one likely means of regulation. Here we investigate the methylation sites present in the *Saccharomyces cerevisiae* ribosome, via ten methyltransferase knockouts analysed with a combination of polysome profiling experiments and quantitative mass spectrometry. Novel methods of purification and validation have been developed to characterise the ribosome and its post-translational modifications (PTMs) with a 2-dimensional size exclusion chromatography technique. This method successfully separated ribosomes from whole cell lysate with resolution at the polysome, monosome, and subunit level. It also allowed the relative quantity of methylation in different ribosomal fractions (for example, actively translating polysomes versus subunits unincorporated into a ribosome) to be evaluated. Mass spectrometry results demonstrated variations in the methylation state at six of the twelve confirmed sites on the yeast ribosome, which suggests that methylation may be present in PTM crosstalk or play a functional role in higher-order regulation of translation. Further research on heterogeneity in ribosomal methylation needs to be conducted to determine if PTMs are impacted by stimuli to the cellular environment and whether their presence leads to the selective translation of certain mRNAs. Our approach to the separation of ribosomes in yeast and downstream analysis of modifications provides a path to harmonise ribosomal heterogeneity and ribosome specialisation.



Poster Presentations



Poster Presentations

Name: **Jessica Buchanan**

Theme: Cell Biology & Signalling

Poster Position: 14

Title: Assessing the viability of rapidly discovered cyclic peptides as specific binders to the RIPK3 RHIM functional amyloid

Authors: *Jessica A. Buchanan, Chi L. L. Pham, Toby Passioura, Huy T. Nguyen, Kat Harrison, Cheree Fitzgibbon, Richard Payne, James Murphy, Olivia Lavidis, Megan Steain, Margaret Sunde*

Organisms use multiple forms of programmed cell death to protect against invading pathogens. Necroptosis is a highly immunogenic form of lytic cell death. Dysregulation of necroptosis has been implicated in multiple disease states, including inflammatory bowel disease and ischaemic injury after stroke. Central to necroptosis is a functional amyloid complex referred to as the necrosome, which is comprised largely of the protein RIPK3. The necrosome forms when the ~18 residue RIP Homotypic Interaction Motif (RHIM) within RIPK3 adopts an amyloid cross- β structure and recruits additional RIPK3 monomers into a fibrillar form, whilst the N terminal kinase domain of RIPK3 remains exposed and available for phosphorylation. Functional amyloid formation by RIPK3 results in autophosphorylation of this kinase and subsequent phosphorylation of downstream effector protein, MLKL, leading to lytic cell death.

There are currently no small molecules available that selectively bind to the amyloidogenic RHIM region of RIPK3. Here, we have aimed to identify cyclic peptides that bind with high specificity to the RIPK3 RHIM region or to the RIPK3 kinase domain. The two RIPK3 regions were used as targets for screening against a cyclic peptide library in a selection known as random non-standard peptide integrated discovery (RaPID). Novel cyclic peptide binders to the RIPK3 RHIM amyloid fibril interface have the potential to be used as diagnostic tools to detect RIPK3 functional amyloid formation. Peptides that bind with high specificity to the RIPK3 kinase domain may allow future investigations of the distinct actions and interfaces of these two regions of the protein. Further characterisation has revealed that RIPK3 RHIM binding peptides independently form assemblies that display amyloid like properties. Investigation of peptide:RIPK3 RHIM binding and peptide:RIPK3 kinase domain binding is underway using multiple techniques, including fluorescent colocalization studies, transmission electron microscopy and immunofluorescence assays with cells that have undergone necroptosis.



Poster Presentations

Name: **Nadeeka Bandara**

Theme: Cell Biology & Signalling

Poster Position: 18

Title: **ZFP697 is required for adipocyte differentiation of stem cells.**

Authors: *Nadeeka Bandara, My Kvist, Ramin Shayan, Tara Karnezis*

Cellular gene switches that control cell differentiation can contribute to protection from obesity, but the molecular components of these switches remain to be largely explored.

We identified, ZFP697 was identified as a novel regulator for adipogenesis from a whole genome wide RNA-seq analysis which has been performed to identify novel adipogenic regulators. In this study, we investigated the requirement of ZFP697 on adipogenic differentiation by inhibiting ZFP697 mRNA levels in ASCs using siRNA gene knockdown strategy followed by an in vitro adipogenesis assay. Adipogenic differentiation was assessed by analysing adipocytes gene expression and protein expression by qPCR and western blot respectively and staining lipid droplets by Bodipy staining. We found that, adipocytes formation from stem cells were significantly reduced upon Zfp697 knockdown as evident by reduced mRNA levels of adipocyte specific Cebpa, Ppar α and Fabp4 and Fabp4 protein levels compared to control cells where Zfp697 is present. Furthermore, Bodipy positive adipocytes were significantly reduced in Zfp697 knock down cells compared control cells where Zfp697 is present. In conclusion, the results indicate that ZFP697 is required for adipogenesis. This may be a step on the way to changing the global obesity epidemic since a deeper understanding of the molecular mechanisms of adipogenesis may lead to the development new drugs or treatments.



Poster Presentations



Poster Presentations

Name: **Nicola Karakatsanis**

Theme: Cell Biology & Signalling

Poster Position: 19

Title: **The phospho-regulation of histone demethylases in *Saccharomyces cerevisiae*.**

Authors: *Nicola M Karakatsanis*

Joshua J Hamey

Marc R Wilkins

Histone methylation is a dynamic regulator of transcription that has been correlated with both gene activation and gene silencing. Dysregulation of the histone methylation system has been linked to the aetiology and progression of several diseases, including cancer and neurodegenerative disorders. In the model organism, *Saccharomyces cerevisiae*, histone methylation is mediated by a total of eight evolutionarily conserved methyltransferases and demethylases. There is emerging evidence that histone methylation itself is regulated by the kinase signalling network via phosphorylation. Despite this, the function of many phospho-sites on histone methylation enzymes – particularly demethylases – in *S. cerevisiae* is still unknown. Consequently, we seek to investigate the phospho-regulatory system of histone demethylases in *S. cerevisiae* and its role in modulating cellular response phenotypes. The histone H3K36 demethylase, Rph1p (human KDM4 orthologue), is of particular interest due to its number of phospho-sites and thus likelihood that it is an integrator of signalling information. To determine phospho-sites of regulatory potential we conducted liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis of purified Rph1p expressed under different growth stressors. Candidate sites were then mutated to create a library of phospho-nulls which will be tested for growth phenotypes under the same conditions. Our future work intends to link observed changes in Rph1p phosphorylation under specific conditions to changes in H3K36 methylation and the proteome profile. This will include using LC-MS/MS to measure global changes in H3K36 methylation states of Rph1p phospho-null strains under the same stressors, as well as using chromatin immunoprecipitation coupled to DNA sequencing to measure changes at specific genes/coding sequences. Moreover, LC-MS/MS-based techniques will be used to conduct differential expression analysis of Rph1p phospho-null strains under these conditions. Ultimately, our aim is to identify the kinases and upstream signalling pathways that act on the regulatory phospho-sites of Rph1p to induce specific changes in gene expression and cellular response phenotypes.



Poster Presentations

Name: **Sarah Garnish**

Theme: Cell Biology & Signalling

Poster Position: 20

Title: **Phosphorylation-dependent pseudokinase domain dimerization drives full-length MLKL oligomerization**

Authors: *Yanxiang Meng, Sarah E. Garnish, Katherine A. Davies, Katrina Black, Andrew Leis, Toby Dite, Cheree Fitzgibbon, Christopher R. Horne, Samuel N. Young, Arya Venkat, Natarajan Kannan, Akiko Koide, Shohei Koide, Alisa Glukhova, Peter E. Czabotar & James M.*

Necroptosis is a caspase-independent form of programmed cell death that is highly inflammatory in nature. The core signaling events of necroptosis are mediated by two serine/threonine protein kinases, RIPK1 and RIPK3, and the pseudokinase, MLKL. Our understanding of the precise mechanism of the terminal steps in the pathway, in which the RIPK3-targetted phosphorylation of MLKL triggers a conformation change and oligomerisation of the terminal pathway effector remains incomplete. Here, we show phosphorylated human MLKL pseudokinase domain adopts a closed conformation, that is the driver for human MLKL pseudokinase domain dimerization and further full-length human MLKL tetramerization. Using negative stain electron microscopy and modelling support, we predicted full-length human MLKL tetramerization to involve both the pseudokinase domain dimerization and assembly of the brace helices into a coiled coil. We subsequently validated this tetramer model by examining the contribution of interface residues to necroptotic function in HT29 cells. Mutations disrupting the interfaces between the four-helix bundle domains and within the brace region coiled coil abolished oligomer formation and necroptotic cell death. We showed mutation of R30 in human MLKL resulted in constitutive cell death, by releasing the four-helix bundle domain from the brace helix and enabling MLKL tetramer formation. Together our structural and in vitro data unifies the current understanding of human MLKL activation and asserts the importance of higher order assembly in driving the release and organization of the four-helix bundle domain to facilitate cell death.



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Poster Presentations

Name: **Emmanuel Osei-Frempong**

Theme: Click me

Poster Position: 21

Title: Determination of glycophorin C genotypes prevalence and association with Plasmodium falciparum density and diversity in malaria patients in Ghana

Authors: Emmanuel Osei-Frempong^{1,2}, Abena Busayo¹, Sherik-fa Anang¹, Elizabeth Cudjoe¹, Joseph Quacoe¹, Kwasi Akowuah Atweri¹, Acquah Kojo Festus¹, Nii Ayite Aryee², Linda Eva Amoah¹

Genetic diversity poses a barrier to success of vaccine development targeting Plasmodium species as evolutionarily favourable genes that have been chosen have significantly been impacted by it. Several malaria-protective polymorphisms have been linked to genes in the red blood cells that modify or impair their structure or activity. Identifying the genes involved and their impact on malaria risk is a potentially useful technique of examining the host-parasite relationship. The study investigated the genotypes of Glycophorin C (GYPC) protein and their effect on Plasmodium falciparum density and diversity in symptomatic malaria patients across Ghana.

The study used a cross-sectional design to examine the genotypes of the GYPC protein and determine their association with the diversity and density of Plasmodium falciparum infection in symptomatic malaria patients in Ghana. A total of 214 dry blood spot archived samples collected in 2021 from ten randomly chosen health facilities across Ghana's sixteen regions were used to characterize GYPC genotypes in the Ghanaian population into GYPC homozygous wild type, homozygous and heterozygous and GYPC exon-3 deletion using PCR and agarose gel electrophoresis and determine its association with P. falciparum density, diversity using PET-PCR and nested PCR respectively.

Out of the 214 samples with a history of febrile illness, 201(94%) had the GYPC exon-3 deletion with 13 (6%) of these being with the homozygous GYPC wild type and no record of the heterozygous GYPC wild type in the Ghanaian population. There was however no significant association between the GYPC genotypes and P falciparum density ($p=0.285$) and diversity ($p=0.805$).

This study serves as a baseline study to provide functional data on the impact of GYPC exon-3 deletion on Plasmodium falciparum infection in Ghana thus adding up to our current understanding of vector-host interactions from which further studies may bring bare the relationship between functional and structural diversity of RBC invasion concerning GYPC exon 3 deletions and P falciparum as well as knowledge on the possible development of transmission intervention tools and vaccines.



Poster Presentations

Name: **Seyedeh Sedigheh Abedini**

Theme: Computational Biology

Poster Position: 22

Title: **Deciphering Neurocognitive Landscapes: A Comprehensive Genomic Exploration of Copy Number Variants' Role in Neurodevelopmental Disorders**

Authors: *Seyedeh Sedigheh Abedini , Yuheng Liang, Hamid Alinejad-Rokny*

Neurodevelopmental disorders (NDDs), encompassing Autism Spectrum Disorder (ASD), Intellectual Disability (ID), and Schizophrenia (SCZ), are persistent conditions that pose a significant health challenge. Genetic factors account for approximately 50% to 80% of NDD etiologies, with Copy Number Variations (CNVs) being a predominant causative element. Establishing a clear connection between specific CNVs and disease phenotypes is paramount to delineating direct genotype-phenotype associations. The challenge lies in precisely associating specific loci with the corresponding phenotypes and distinguishing among them.

In our research, we analysed CNVs from 40,000 neurotypical individuals and 75,000 individuals presenting NDD phenotypes, further categorising them into groups: ASD, ASD with ID, ID, and SCZ. Through this analysis, we discerned 9 CNVs significantly associated with ASD, 69 with ASD coexisting with ID, 137 specific to ID, and 15 linked to SCZ. Notably, we pinpointed 15 previously unidentified CNV loci either linked to ASD with ID, ID or SCZ.

Subsequent gene ontology and pathway investigations revealed significant correlations between the 22q11.2 deletion syndrome and the Prader-Willi and Angelman syndromes across multiple phenotypic categories. Further analyses identified unique disease-associated regions correlating with specific pathways and gene ontology terms, including "Keratinization" for ID and "Beta defensins" for co-occurring ASD and ID. Utilising the FANTOM5 dataset, we also determined that likely candidate genes within these disease-associated regions predominantly manifest in the brain, immune system, reproductive system, and T-cells. Interestingly, our findings also unveiled that unique genes associated with specific diseases are notably prevalent in distinct cellular types.

Delving deeper using chromosome conformation capture data derived from brain cells, we established that approximately 60% of disease-associated non-coding CNV regions encompass at least one enhancer region predominantly expressed in the brain. These enhancer regions interact with the promoters of protein-coding genes with brain-enriched expression, suggesting potential disruption of enhancer-promoter interactions due to CNVs. Our research underscores the genetic heterogeneity underpinning NDDs and introduces novel genetic evidence supporting further subcategorisation within the broad spectrum of NDDs. The regions pinpointed in this study serve as a comprehensive resource for both researchers and clinicians to deepen their understanding of the aetiology of NDDs.



Poster Presentations

Name: **Kiflu Tesfamimicael**

Theme: DNA and Genes

Poster Position: 23

Title: **Genotyping platforms for pharmacogenomics: Evaluating accuracy and reliability using the CYP2D6 Gene as a Case Study**

Authors: *Kiflu G Tesfamimicael, David L Adelson, Michael Musker, Martin David Lewis*

Accurate genotyping is critical and the first step in implementing personalised medicine through pharmacogenetics (PGx). Clinical PGx, however, uses a limited predefined panel of variants for genotyping. This approach does not detect novel, rare, and complex structural variants. It is imperative to evaluate the accuracy and quality of variant panels / microarray genotyping in PGx testing. The aim of this study was to evaluate and compare the efficiency of array-based, short-read and long-read genotyping platforms for pharmacogenomic (PGx) testing, using the CYP2D6 gene as a case study. Full CYP2D6 gene of 20 Coriell Cell lines were genotyped using Global Screening Array (GSA), MiSeq (short-read) and Nanopore (long-read) sequencings. Genetic variants were called using gtc2vcf (bcftools +gtc2vcf), GATK and PEPPER-Margin-DeepVariant pipelines for microarray, MiSeq and Nanopore data, respectively. Long-range PCR (XL-PCR) was utilised to detect CYP2D6 gene deletion, duplication, and structural variants (CNV). CYP2D6 star allele calling and phenotyping of all platforms' data was conducted using the PyPGx program. A total of 21, 59 and 67 Single Nucleotide Variation (SNV) was detected by GSA, MiSeq and Nanopore, respectively. Only a small percentage (11%) of SNV were commonly identified by GSA and the other two sequence based (NGS and Nanopore) platforms. The PGx diploid and phenotype concordances between array-based and sequence-based platform was low. GSA and MiSeq failed to detect 79% and 14% of the CYP2D6-defining variants, respectively. More than 50% of individuals genotyped using GSA had different phenotypes compared to the Nanopore phenotype predictions. Conclusion: GSA-based genotyping was associated with a high rate of ambiguous genotyping. Nanopore showed more precise genotyping capabilities and could potentially become the gold standard for PGx testing.



Poster Presentations



Poster Presentations

Name: **Bini Zhou**

Theme: DNA and Genes

Poster Position: 25

Title: **Effects of univariate stiffness and degradation of DNA hydrogels on the transcriptomics of neural progenitor cells**

Authors: *Bini Zhou, Bo Yang, Yu Shao, Qian Liu, Songbai Gui, Dongsheng Liu*

Mechanical interactions between cells and extracellular matrix (ECM) are critical in stem cell fate decision. Synthetic models of ECM, such as hydrogels, can be used to precisely manipulate the mechanical properties of the cell niche and investigate how mechanical signals regulate cell behavior. However, it has long been a great challenge to tune the ECM-mimic hydrogel mechanical signals solely, since altering the mechanical properties of most materials is usually accompanied by chemical and topological changes. Here, we employ DNA and its enantiomers to prepare a series of hydrogels with univariate stiffness regulation, which enables a precise interpretation of the fate decision of neural progenitor cells (NPCs) in a three-dimensional environment. Using single-cell RNA sequencing technique, Monocle pseudotime trajectory and CellphoneDB analysis, we demonstrate that stiffness of the hydrogel alone does not influence the differentiation of NPCs, but the degradation of the hydrogel that enhances cell-cell interaction is possibly the main reason. We also find that ECM remodeling facilitates cells to sense mechanical stimuli.



Poster Presentations



Poster Presentations

Name: **Gurveer Kaur Gaddu**

Theme: DNA and Genes

Poster Position: 26

Title: **Characterising the role H2A.Z chromatin modifiers in zebrafish immunity and development.**

Authors: *Gurveer K Gaddu*

Amardeep S Dhillon

Faiza Basheer

DNA and proteins packaged in the cell nucleus are affected by histone acetylation, which affects chromatin organization and gene expression. The acetylation of core histones has been associated with chromatin opening and closing, gene transcription, DNA damage repair, and chromosome decondensation in mitosis and meiosis. Lysine residues are acetylated by tightly regulated histone lysine acetyltransferases (HATs: KATs) and deacetylases (HDACs). There is an increasing body of research indicating that dysregulation of KATs and aberrant lysine acetylation is linked to a spectrum of diseases including tumorigenesis, presenting an opportunity for finding new therapeutic targets in this area. Kat5 is a histone acetyltransferase family member and regulates DNA damage response by acetylating histones and chromatin remodelling.

My research is focused on investigating the effects of acute deletion of kat5 using CRISPR/Cas9 mediated deletion in zebrafish and its potential role in development and diseases. Zebrafish have been increasingly used as an animal model to better understand the genetics and biology of vertebrate development. I found that mutation of kat5 is lethal and associated with multiple developmental issues and diseases such as survival, lymphoma, and neurodegenerative disorders. Characterization of key mechanisms causing these defects and performing further studies will pave the way for a better understanding of the role of kat5 in zebrafish development and cancer studies.



Poster Presentations



Poster Presentations

Name: **Erick Tjhin**

Theme: Drugs, drug resistance and therapeutics

Poster Position: 27

Title: Investigating the antiplasmodial activity of spinosyns against Plasmodium falciparum

Authors: Erick T Tjhin, Dovile Anderson, Laura Shuttleworth, Shravan Divakarla, Evie Hodgson, Senwei Tan, Jonathon King, Yeping Cai, Aidan Whitfield, F Hafna Ahmed, Darren J Creek, Ian Cockburn, Christine Kritikou, Kevin J Saliba & Christina Spry.

Spinosyns are a family of compounds produced by the bacterium *Saccharopolyspora spinosa* during fermentation. Spinosad, a naturally occurring mixture of spinosyns A and D, is widely used as a broad-spectrum insecticide. Spinosyns and their synthetic analogues have been shown to exert their insecticidal activity by binding to the $\alpha 6$ subunit of insect nicotinic acetylcholine receptors. We have shown that this insecticidal activity includes the malaria vector *Anopheles stephensi*. Additionally, we have shown that spinosad possesses antiplasmodial activity. These properties place the spinosyns as potential transmission-blocking endectocides, sought-after tools in our effort to eradicate malaria. Spinosad is an effective systemic insecticide against *An. stephensi* when orally administered to mice, but it is unclear why its antiplasmodial activity is limited under the same conditions. Here, we present the results of experiments aimed at determining the antiplasmodial mechanism of action of spinosad, and at comparing the antiplasmodial activity of spinosyn analogues to inform downstream structure-activity optimisation. We show that spinosad is more effective against trophozoite-stage than ring-stage *P. falciparum*, inhibiting parasite proliferation rapidly and irreversibly (requiring just 4 hours of exposure). Furthermore, we show that spinosad reduces the intracellular ATP level and cytosolic pH of *P. falciparum* trophozoites, but not by inhibiting glucose uptake or lactate extrusion by the parasite. Metabolomic analysis of *P. falciparum* trophozoites treated with spinosad for 1 hour revealed a > 40-fold increase in orotate, an intermediate in the de novo pyrimidine synthesis pathway. Additional experiments are underway to determine if the build-up of orotate is caused by spinosad inhibiting orotate phosphoribosyl transferase (which catalyses the formation of orotidine 5'-monophosphate from orotate), and if this ultimately leads to parasite death. Currently, we have not been able to generate spinosad-resistant parasites through stepwise drug pressuring, although attempts are ongoing. Furthermore, we show that the in vitro antiplasmodial activity of spinosad is reduced when human serum is present in the culture medium in place of the serum substitute Albumax II, which potentially explains its reduced antiplasmodial activity in vivo. Encouragingly, our structure-activity relationship analysis has revealed spinosyn analogues that maintain their antiplasmodial activity in the presence of human serum.



Poster Presentations

Name: **John Tanner**

Theme: Drugs, drug resistance and therapeutics

Poster Position: 28

Title: The specter of Chloroquine: Evolutionary conflict at the molecular level in a Plasmodium falciparum multidrug resistance protein.

Authors: *John Tanner, Ben Corry*

Chloroquine was once the main frontline drug in the fight against Malaria. Mutations in the *P. falciparum* Chloroquine resistance transporter [PfCRT] conferred resistance to the parasite by transporting the drug away from its site of action. The resistance evolved multiple times and spread globally, leading to the end of its usage.

Artemisinin partner drug combination therapies are now used in place of Chloroquine to limit resistance evolution, but resistance to a number of the partner therapies (as well as artemisinin) is beginning to emerge. PfCRT again plays a role in modulating the susceptibility of five of the six available partner drugs. As fears of another drug failure like Chloroquine loom over the global health community and the millions of people at risk of malaria, interest in smarter ways of administering drugs is growing. Particularly, ways in which antimalarial resistance mechanisms can impose fitness costs in absence of drug pressure, causing a return of the drug's effectiveness. Or, might drive the evolution of the susceptibility to subsequent drugs.

As it happens, when Chloroquine usage subsides, resistance to the drug can also decline - with some populations returning to a drug susceptible state. The fitness cost of resistance appears to be because the ability to transport the drug disrupts PfCRT's native function of polyspecific peptide transport.

Given PfCRT's historical, contemporary, and likely future involvement in anti-malarial drug resistance, we sought to understand the molecular basis of substrate binding and the functional constraints that influence its evolution with Molecular Dynamics simulations. The simulations suggest binding sites for Chloroquine and a variety of peptides which share key mutation residues and align with expectations from experiments. These results aid in understanding how the molecular fundamentals of binding and protein accessibility might drive changes in the antimalarial drug susceptibility of populations.



Poster Presentations

Name: **Jordan Pederick**

Theme: Drugs, drug resistance and therapeutics

Poster Position: 29

Title: **Mimicking nature to disrupt *Staphylococcus aureus* cysteine metabolism**

Authors: *Jordan L. Pederick, Bethiney A. Vandborg, John B. Bruning*

Staphylococcus aureus remains a major human pathogen, being responsible for > 100 000 deaths per year worldwide. Furthermore, the ability of *S. aureus* to develop antibiotic resistance is of major concern. One avenue being explored to overcome this is to target central metabolic pathways that have been previously overlooked. In *S. aureus* there is mounting evidence that aspects of cysteine metabolism present as new targets for the development of antibiotics and antibiotic adjuvants. This is because cysteine is required for vital functions including i) protein and metabolite synthesis, ii) enzyme-catalysed reactions, and iii) maintenance of the redox environment. The primary pathway for cysteine acquisition in *S. aureus* is enzyme-mediated biosynthesis within the cytoplasm. This is primarily facilitated by cysteine synthase (CysK), which converts a serine derivative to cysteine. In addition to this activity, CysK also plays a key regulatory role in cysteine metabolism through forming two distinct complexes with other protein partners. The first, here termed Complex A, negatively regulates cysteine biosynthesis by inhibiting CysK and increasing the ability of the cell to produce the serine derivative. The second, here termed Complex B, acts as a transcriptional regulator to control expression of > 300 target genes involved in cysteine metabolism, stress resistance, and virulence. Therefore, simultaneous inhibition of CysK activity and Complex formation is an attractive approach to disrupting multiple aspects of this important pathway.

We have initiated a project aiming to mimic this natural protein-protein interaction and develop effective inhibitors of CysK. Both Complex A and Complex B are known to form through insertion of a C-terminal peptide motif into the active site of CysK; however, the nature of this interaction in *S. aureus* had not been characterised previously. To investigate this, we have used X-ray crystallography to determine high resolution structures of CysK alone, and in complex with short peptides derived from Complex A (Peptide A) and Complex B (Peptide B), revealing the molecular basis of these protein-protein interactions. Furthermore, we have successfully developed a surface plasmon resonance binding assay to measure the binding affinity of CysK for these peptides. While Peptide A displayed moderate affinity for CysK ($KD = 9 \pm 1 \mu M$), Peptide B possessed high affinity ($KD = 25 \pm 5 nM$), making it an excellent candidate for the development of CysK inhibitors. To begin this development phase a series of Peptide B derivatives were rationally designed to understand the structure-activity relationship for the CysK interaction. These derivatives incorporated a range of natural and non-proteinogenic amino acid substitutions at key positions within the Peptide B scaffold. Probing the binding of these Peptide B derivatives by surface plasmon resonance revealed that modification of the Peptide B scaffold generally resulted in reduced affinity. However, one position in the Peptide B scaffold was found to maintain the high

affinity interaction after substitution with a non-proteinogenic amino acid. Importantly, this provides critical information as to which interactions are responsible for high affinity binding of Peptide B and presents as a promising lead for development of next-generation CysK inhibitors.



Poster Presentations



Poster Presentations

Name: **Rachel Leonard**

Theme: Drugs, drug resistance and therapeutics

Poster Position: 30

Title: Identification of a novel, highly potent inhibitor of the mitochondrial electron transport chain in the parasites that cause malaria and toxoplasmosis

Authors: *Rachel A. Leonard, Ruijia Liang, Adele M. Lehane, Giel G. van Dooren*

Apicomplexans are a large phylum of parasitic protozoans that impart considerable socioeconomic burdens on human societies. This diverse phylum includes the causative agents of cerebral malaria (*Plasmodium falciparum*) and toxoplasmosis (*Toxoplasma gondii*). Drugs that target the acute stages of apicomplexan infections are important for controlling the diseases caused by these organisms. However, the advent of parasite resistance to frontline drug treatments highlights the urgent need for new drugs targeting drug-resistant strains. The mitochondrial electron transport chain (ETC) is essential for apicomplexan survival, and is the target of drugs such as atovaquone and endochin-like quinolones (ELQs), although the emergence of parasite resistance to these drugs threaten their utility. To identify novel inhibitors of the ETC in apicomplexans, we used a medium-throughput Seahorse XFe96 flux analyser platform to screen the Medicines for Malaria Venture “Global Health Priority Box”, a collection of 240 compounds with activity against pathogens and their vectors, for inhibitors of mitochondrial respiration in *T. gondii* parasites. We identified several inhibitors, the most promising of which is termed MMV1794211. We demonstrate that MMV1794211 is a potent, selective and on-target inhibitor of Complex III of the ETC in *T. gondii*, inhibiting parasite proliferation with an EC₅₀ of 11 nM. We demonstrate that MMV1794211 is also an on-target inhibitor of the ETC in *P. falciparum*, and a particularly potent inhibitor of parasite proliferation, with an EC₅₀ of 19 pM. Notably, *T. gondii* parasite strains resistant to atovaquone and ELQs exhibited 3-to-7-fold hypersensitivity to MMV1794211. In sum, we have identified a novel, potent ETC inhibitor capable of targeting drug resistant strains of apicomplexan parasites. This compound therefore shows promise for future treatments of the diseases caused by *T. gondii*, *P. falciparum* and related apicomplexans.



Poster Presentations



Poster Presentations

Name: **Uyen Nguyen Phuong Le**

Theme: Drugs, drug resistance and therapeutics

Poster Position: 31

Title: **Bioactive Glycyrrhizic Acid Derivatives as 3CLpro Inhibitors Against SARS-CoV-2 and its Variants**

Authors: *Uyen Nguyen Phuong Le, Cheng-Wen Lin*

3CLpro of SARS-CoV-2 is highly desirable in the viral life cycle by processing viral polyprotein, thereby, 3CLpro is considered as a drug-promising target in COVID-19 therapy. Conjugating Glycyrrhizic Acid (GL) with activated ester and methyl ester of amino acid were examined their potential effects on 3CLpro inhibition in cis- and trans-cleavage FRET assay of 3CLpro in Wuhan-1 strain, Omicron, and drug-resistant variants. Regarding the tested compounds, including compounds 2, 6, 9, 15, and 18 exhibited the effects on 3CLpro strains activity on both FRET substrates in dose-dependent manners. Compounds 2 and 6 exerted antiviral effects against Wuhan-1-3CLpro, showing the IC₅₀ values of $0.84 \pm 1.29\mu\text{M}$ and $0.063 \pm 0.03\mu\text{M}$, respectively. In addition, compounds 2 and 6 are also probably against Omicron-3CLpro, showing the IC₅₀ values of $0.27 \pm 0.37\mu\text{M}$ and $0.27 \pm 0.26\mu\text{M}$, respectively. Computational molecular docking analysis showed the high binding affinity between our potential compounds and 3CLpro strains at amino-acid E166 and Q189 residues relating to Nirmatrelvir-resistant variants. According to the cell-based infection model, compounds 2 and 6 significantly improved the inhibited the cytopathic effect by SARS-CoV-2 single-round-infectious-particles (SARS-CoV-2 SRIP) and suppressed viral replication of SARS-CoV-2 SRIP in Vero E6 cells co-expressing S, E, and M proteins, with the EC₅₀ values of $0.28 \pm 0.39 \mu\text{M}$ and $0.71 \pm 1.20 \mu\text{M}$. Taken together, our study provides promising 3CLpro-targeted compounds for therapy against SARS-CoV-2, and its variants.



Poster Presentations



Poster Presentations

Name: **Amy Adair**

Theme: Drugs, drug resistance and therapeutics

Poster Position: 32

Title: A biparatopic nanobody with broad neutralisation against SARS CoV-2 variants of concern

Authors: Amy Adair, Li Lynn Tan, Srija Mukherjee, Purba Pahari, Francesca Mordant, Jaison D'Sa1, Kanta Subbarao, Stuart Turville, Dibyendu Kumar Das, Phillip Pymm and Wai-Hong Tham

The rapid emergence of the COVID-19 pandemic, and the SARS and MERS epidemics within the past two decades highlight coronaviruses as critically important human pathogenic viruses with pandemic potential. Our strategy is to use nanobodies to disrupt coronavirus entry. We show that nanobodies block SARS-CoV-2 entry into cells and suppress virus infection in mouse models. Using both cryo-EM and X-ray crystallography approaches, our findings reveal that the neutralizing nanobodies bind to distinct sites on SARS-CoV-2. Using these nanobodies, we have designed a biparatopic nanobody that is resilient against existing SARS-CoV-2 variants of concern. This biparatopic nanobody has increased affinity, potency and resilience to variants compared to the monovalent nanobody counterparts. Using virus fusion assays, single molecule FRET and structural approaches, we provide insight on the molecular mechanism of neutralization across a diverse collection of SARS-CoV-2 variants of concern including Omicron lineages.

To develop more potent neutralising nanobodies, we have immunised alpacas with the Omicron variant. The Omicron variants of SARS-CoV-2 have presented a dynamic and ever-changing mutational landscape resulting in immune evasion against COVID-19. Here we have completed several phage display campaigns against a panel of Omicron variants using nanobody libraries derived from alpacas immunised with recombinant Omicron spike and receptor-binding domains. Using next-generation sequencing we will identify collections of nanobodies that recognize multiple Omicron variants. The identified nanobodies will be further characterized for their ability to effectively inhibit viral entry across various SARS-CoV-2 lineages.



Poster Presentations



Poster Presentations

Name: **Asmita Deonath**

Theme: Drugs, drug resistance and therapeutics

Poster Position: 33

Title: Investigating PanD mutants that confer resistance to the tuberculosis drug pyrazinamide to uncover its mechanism of action

Authors: *Asmita Deonath, Xiaojun Yuan, Isabel Barter & Christina Spry.*

Tuberculosis is a deadly but treatable disease caused by the bacterium *Mycobacterium tuberculosis*. However, long treatment duration and emerging drug resistance necessitate the shortening of treatment and the development of new anti-tuberculosis drugs. Pyrazinamide is an important drug within the current first-line treatment. It is a prodrug that is converted into its active form, pyrazinoic acid (POA). The mechanism of action of POA remains unclear. Based on the discovery of POA resistant mutants containing mutations in the *panD* gene, a putative target of the drug is the *M. tuberculosis* aspartate decarboxylase PanD.

A prior study shows that POA competitively inhibits the enzymatic activity of PanD, while another demonstrates that POA upregulates the degradation of PanD by the Clp protease. Whether one or both ultimately result in death of *M. tuberculosis* remains unclear. PanD is currently best characterised in its tetrameric form, though we have discovered that it also forms an octamer and a dodecamer in solution. What role oligomerisation may play in sensitivity or resistance to POA remains to be determined. In this study, we aimed to investigate the mechanism of action of POA by determining the mechanism by which POA-resistant PanD mutants are resistant to POA. We used size exclusion chromatography and an enzyme-coupled assay to compare the oligomerisation and enzymatic activity, respectively, of the wild-type PanD with two different POA-resistant PanD mutants (C17R and M117I). We will report our kinetic characterisation of the tetrameric and octameric forms of wild-type PanD and the effect of POA on the activity on each of these forms. We will also show that one of the resistance-conferring mutations has an impact on the formation of higher order oligomers, and present the kinetic characterisation of the mutants in the presence and absence of POA. The implications of our results for understanding the mechanism of action of pyrazinamide will be discussed.



Poster Presentations



Poster Presentations

Name: **Lani Davies**

Theme: Drugs, drug resistance and therapeutics

Poster Position: 34

Title: **Fragment-Based Drug Design of Heparanase Inhibitors: A Multi-Faceted Approach**

Authors: *L. Davies, C. Whitefield, R. Frkic C. Jackson, C. Nitsche*

Human heparanase is an enzyme that has received considerable attention as drug target in recent years. Heparanase is the only mammalian enzyme that catalyses the hydrolysis of heparan sulfate in the extracellular matrix and its activity is an essential part of inflammation, angiogenesis and modification of the cell's environment [1-3]. It is therefore a promising drug target for the treatment of cancer, diabetes, inflammatory diseases, as well as COVID-19, where heparanase activity has been shown to contribute to pathogenesis [2,4]. However, no drugs or therapeutic treatment designed to specifically inhibit heparanase have successfully passed phase III clinical trials yet [5,6]. The majority of effective heparanase inhibitors have off-target effects and undesired high anticoagulant activity, such as oligo- and polysaccharide heparan sulfate mimetics. Similarly, small molecule inhibitors often fail clinical trials due to toxicity. In our research, we aim to use a combination of structural biology, high-throughput crystallography, computational methods and synthetic chemistry to screen fragment libraries against heparanase and elaborate hits into promising lead compounds. We have screened a small library of commercially available fragments, which has led to the discovery of 20 candidates that bind both at the active site cleft, and in shallow surface pockets. Of these 20 candidates, four were found to inhibit heparanase at the micromolar range. Moreover, five allosteric binding sites of heparanase were determined through in silico modelling, leading to an additional extensive high throughput virtual screening campaign. Four of the 20 fragments of interest selected from this study were found to bind heparanase, three of which were observed to inhibit at the micromolar range. Fragments that were found to bind and inhibit heparanase were then grown through information gained by using computational tools, leading to higher potency inhibitors. Altogether, this work has identified several fragments that have good affinity and inhibition of heparanase with the potential to be explored as potent small molecule inhibitors.



Poster Presentations



Poster Presentations

Name: **Lihua Yang**

Theme: Drugs, drug resistance and therapeutics

Poster Position: 35

Title: **Targeting the undruggable proteome using monobodies**

Authors: *Lihua Yang, Jason K.K. Low, Joel P. Mackay.*

Both fundamental and clinical medical research depend heavily on the capacity to utilise chemicals to selectively disrupt cellular function, yet the rate of discovery of small compounds that can do this is stagnating. Additionally, up to 85% of the proteome is considered undruggable. These proteins lack deep, easy-to-bind pockets to which small molecules can be targeted. Consequently, there is a strong unmet demand for reliable technologies that can be used to develop modulators of these undruggable targets.

Monobodies possess the same b-sandwich fold as nanobodies but do not require disulphide bonds for stability. As a result, they are insensitive to reductive environments and can be used to modulate the activity of target proteins in live cells. Being larger than small molecules, these 10-kDa domains include three antibody-like binding loops that can provide a larger interaction surface and can mediate selective and high-affinity interactions. On the other hand, their relatively small size compared to antibodies makes protein production simpler and more robust.

We have recently generated a monobody library and used the powerful RaPID (Random nonstandard Peptide Integrated Discovery) mRNA display technology to screen this library against a protein of therapeutic significance that is known to be largely unstructured under physiological conditions. We identified highly enriched monobody sequences and have used NMR spectroscopy to characterize the structure and dynamics of one of these monobodies as well as its mode of interaction with the protein target. Our work is a proof-of-concept that monobodies are a possible option when considering next-generation protein therapeutics for hard-to-target proteins.

Keywords: RaPID screen; monobody; protein structure; solution NMR.



Poster Presentations



Poster Presentations

Name: **Paremila Shanmuga Nathan**

Theme: Drugs, drug resistance and therapeutics

Poster Position: 36

Title: Elucidating the role of the C-terminal domain of an essential Mycobacterium tuberculosis enzyme, dephospho-coenzyme A kinase.

Authors: Paremila Shanmuga Nathan, Joe Kaczmarek, Stephen Fairweather and Christina Spry

Mycobacterium tuberculosis, the causative agent of tuberculosis, killed 1.5 million people in 2021. Although tuberculosis is treatable, the long-term therapies are associated with toxic side effects and poor adherence, and resistance to tuberculosis drugs is increasing. This highlights the need to identify new drugs, ideally against novel drug targets. The five-step biosynthesis pathway by which the essential enzyme cofactor coenzyme A is synthesised, has long been explored for exploitable drug targets. The final enzyme in the pathway, dephospho-coenzyme A kinase (DPCK), is essential for survival of M. tuberculosis under standard culture conditions, and no known salvage pathways allow for DPCK bypass. These properties make M. tuberculosis DPCK (MtDPCK) attractive for target-based drug discovery. Interestingly, MtDPCK possesses two domains, the N-terminal catalytic domain and a C-terminal domain of unknown function. This domain is absent from its human counterparts and could facilitate selective targeting of the enzyme. Furthermore, as various organisms across life encode homologues of this domain (PFAM ID: UPF0157), either expressed in isolation or fused to other proteins, elucidating its function could provide broader biological insights.

In this study, the role of the MtDPCK C-terminal domain was investigated through both computational and experimental approaches. We will present the results of sequence similarity analyses of UPF0157 domain-containing proteins across various kingdoms of life. As the crystal structure of the closely-related Mycobacterium paratuberculosis DPCK has been solved, we also investigated structural similarity. This was done to predict ligands that may bind the C-terminal domain. In our experimental approach, the MtDPCK C-terminal domain and Enterococcus faecalis GrpB – the only other UPF0157-containing protein for which a structure has been solved were expressed as His-tagged proteins in Escherichia coli. To explore ligand binding experimentally, we then performed a fluorescence-based thermal shift screen of metabolites against the purified proteins. Additionally, to explore a previously proposed hypothesis that the C-terminal domain of MtDPCK aids in the proper folding of the catalytic domain, we attempted to express the N-terminal domain alone. Our findings from both our computational and experimental analyses will be presented and their implications for the function of the MtDPCK domain, and UPF0157-domain containing proteins more broadly, discussed.



Poster Presentations

Name: **Rainbow Chan**

Theme: Drugs, drug resistance and therapeutics

Poster Position: 37

Title: **WEHI Nanobody Platform: identification of nanobodies against target antigens using phage display technology and next-generation sequencing pipelines**

Authors: *Rainbow Chan, Joshua Tong, Amy Adair, Kathleen Zeglinski, Quentin Gouil and Wai-Hong Tham*

Nanobodies are the smallest naturally occurring antibodies derived from members of the Camelidae family such as llamas, camels, and alpacas. They are smaller in size (~15 kDa), have higher stability across pH and temperature ranges, and are easily expressed in bacterial systems compared to conventional antibodies. They have a longer Complementarity Determining Region 3 (CDR3) loop, which is normally involved in binding the antigen. As such nanobodies are an interesting antibody modality as research tools facilitating crystallization chaperons and high-resolution molecular imaging approaches, as well as therapeutics and diagnostics.

The WEHI Nanobody Platform is a fee-for-service program for the identification of nanobodies against your target of interest. To generate a nanobody, we immunize alpacas and clone the nanobody repertoire from isolated plasma cells into our phage display vectors. Using the resulting phage display nanobody library, we will perform two rounds of phage display to obtain antigen-specific nanobodies. We use a variety of phage display panning approaches from display on magnetic beads, immobilised format or neutravidin-coated panning. Phage supernatants are screened using ELISA and positive nanobody binders are sequenced, followed by nanobody expression and purification using bacterial systems. The WEHI Nanobody Platform also adopts a next-generation sequencing pipeline with an open source bioinformatic program NanoLogix, which was developed at WEHI to analyse and identify enriched nanobody clones from phage display campaigns.



Poster Presentations



Poster Presentations

Name: **Vishnu Mini Sasi**

Theme: Drugs, drug resistance and therapeutics

Poster Position: 38

Title: **Predicting Antiviral Resistance Mutations in SARS-CoV-2 Main Protease with Computational and Experimental Screening**

Authors: *Vishnu M. Sasi, Sven Ullrich, Jennifer Ton, Sarah E. Fry, Jason Johansen-Leete, Richard J. Payne, Christoph Nitsche, and Colin J. Jackson**

The oral small-molecule drug nirmatrelvir (NTV)¹ is now widely distributed as combinational COVID-19 therapeutics (along with ritonavir) by the brand name Paxlovid, targeting one of the major enzymes responsible for SARS-CoV-2 viral replication, non-structural protein 5 (nsp5), also known as the main protease (Mpro). Paxlovid has shown positive therapeutic effects on patients suffering from mild-to-moderate COVID-19 with risk of progression to severe disease conditions. However, with increased administration (more than 1.7 million doses) of paxlovid across the globe, there is an alarming possibility of future SARS-CoV-2 lineages evading the inhibitor by acquiring resistant mutations in Mpro. Early prediction and evaluation of these resistance mutations is, therefore, critical in the development of newer and potent anti-viral drugs with activity against the resistant strains. In the present work, we have utilized *in silico* mutational scanning and substrate-inhibitor covalent docking against Mpro to identify potential resistance mutations². Mutational scanning was directed at residues, forming and neighbouring the active site, to generate 3 sets of mutant libraries, while covalent docking was performed using the substrate (SAVLQSGF) and inhibitor (NTV) successively to screen out models producing poor substrate and good inhibitor binding orientation. Subsequent *in vitro* experiments³ revealed mutations N142L, E166M, Q189E, Q189I, and Q192T reduced the potency of NTV and a previously identified potent macrocyclic peptide inhibitor of Mpro (Peptide I)⁴. The E166M mutant especially reduced the half-maximal inhibitory concentration (IC₅₀) of NTV by 24-fold and 118-fold for Peptide-1. Our findings inform the ongoing genomic surveillance of emerging SARS-CoV-2 lineages.



Poster Presentations



Poster Presentations

Name: **Nivea Prashant Kakde**

Theme: Drugs, drug resistance and therapeutics Poster Position: 39

Title: **Evaluating dephospho-coenzyme A kinase as a potential antimicrobial drug target**

Authors: *Nivea Prashant Kakde, Daniel Pavlich, Vrinda Gupta and Christina Spry*

Antibiotic resistance is increasing at an alarming rate. To address this issue, we need new antimicrobial drugs that operate through novel mechanisms of action. The pathway by which coenzyme A (CoA) – a fundamental enzyme cofactor - is synthesised, has attracted attention as a potential antimicrobial target due to its essentiality across life. However, recent genome wide studies are consistent with CoA biosynthesis enzymes not being “vulnerable” targets, meaning that inhibition of CoA biosynthesis gene expression is better tolerated than the inhibition of other genes is. This study aims to explore this finding in more detail, focusing more specifically on the last enzyme in the CoA biosynthesis pathway, called dephosphocoenzyme A kinase (DPCK). To do this, we are using clustered regularly interspaced short palindromic repeats interference (CRISPRi). We have designed and generated four different constructs for CRISPRi that express deactivated Cas9 (dCas9) and short guide RNAs to direct the dCas9 to *dpck* in *Escherichia coli*. We have transformed Top10 *E. coli* with these constructs, which should enable arabinose and anhydrotetracycline-inducible repression of *dpck* in the cells. We are currently measuring the effect of *dpck* repression on growth of *E. coli* and plan to correlate observed growth inhibition with DPCK mRNA and protein levels. Once we establish the “vulnerability” of DPCK for Top10 *E. coli*, we will investigate how this compares with pathogenic strains of *E. coli*. We expect the work to provide important insight to the potential of DPCK and the CoA pathway as targets for novel antibiotics.



Poster Presentations



Poster Presentations

Name: **Vrinda Gupta**

Theme: Drugs, drug resistance and therapeutics Poster Position: 40

Title: **Bacterial dephospho-coenzyme A kinase: The fragment hit-to-lead story**

Authors: *Vrinda Gupta, Barbara Schroeder, Junwen Zheng, Christoph Nitsche and Christina Spry*

Coenzyme A (CoA) is a ubiquitous enzyme cofactor and its biosynthesis has been highlighted as a promising target for antibacterial drug discovery. Of particular interest is the final step of CoA biosynthesis, in which dephospho-CoA kinase (DPCK) phosphorylates dephosphoCoA; this is an essential step of bacterial CoA biosynthesis, with bacteria possessing no known bypass mechanisms for it. Fundamental differences between the bacterial DPCK and its human counterpart should also allow for selective targeting.

A fragment-based drug design approach is being employed to identify inhibitors targeting the *Escherichia coli* DPCK. An initial fluorescence-based shift primary screen followed by an NMR-based secondary screen revealed >30 fragment hits. Subsequent structure-activity relationship (SAR) exploration of the highest affinity fragment ($K_D = 380 \mu\text{M}$) allowed for the identification of analogues which bound with up to 10-fold higher affinity to *E. coli* DPCK, including one which was co-crystallised with the enzyme.

To explore the binding site, and inform continued elaboration, a series of substituted fragment analogues were synthesised. The best of these elaborated compounds was found to inhibit *E. coli* DPCK activity with an IC_{50} of 2-4 μM . Additionally, testing of the elaborated compounds against the human DPCK counterpart was consistent with selectivity for bacterial DPCK. Unfortunately, antimicrobial activity of the compounds was not observed in whole cell bacterial screening carried out by the Community for Open Antimicrobial Drug Discovery (CO-ADD); at a concentration of 32 mg/L the compounds were without effect on key ESKAPE pathogens and two fungi tested. We are currently exploring the reason for the absence of whole cell activity to guide further compound elaboration



Poster Presentations



Poster Presentations

Name: **Andy Garcia**

Theme: Education

Poster Position: 41

Title: **Reinforcing “Researcher” Identity and Scientific Proficiency through Research Presentation Skills**

Authors: *Andres Garcia, Rachael Remington & Jennie Mallela*

Research School of Biology, College of Science

The Australian National University

Canberra ACT 2600

Incoming Masters of Sciences students at the Australian National University’s Research School of Biology take a post-graduate course in their first semester: BIOL8291 - Research Presentation Skills. The course develops reading, writing, and presentation skills critical for career development and advancement in science. It is taught as a series of workshops emphasizing the transmission of biological research through written and verbal communication aimed at different scientific audiences. Throughout the course, students investigate biological questions and research approaches that interest them personally. Peer-review and instructor feedback occur through interactive workshops in writing literature reviews, presenting journal club research and a poster symposium. This course encourages students to consider future research opportunities available at ANU with on-site academics. Instructional scaffolding is employed as students receive support throughout the process of class-based discussion about a given assessment, receiving peer feedback in workshops, and then incorporating post-submission feedback to improve future assessments. As a class, students brainstorm key similarities and differences of presenting research as a poster, an oral presentation, or a critical article commentary. Small discussion groups engage in peer feedback that is facilitated by demonstrators and conveners. Ultimately, students complete the course with outcomes of improved writing and oral presentation skills and a reinforced sense of self, not just as a proficient student, but also as a competent researcher with skill sets to be successful in the scientific community.



Poster Presentations



Poster Presentations

Name: **Daniel McDougal**

Theme: Engineering and Evolution of Protein and Peptides

Poster Position: 42

Title: **Exploring the effects of evolutionary variation on protein structure and dynamics in essential transcription factors**

Authors: *Daniel P. McDougal, Jordan L. Pederick & John B. Bruning*

Nature has a remarkable capacity to adapt to change using novel biochemical solutions. Over time, evolution has produced myriads of orthologous proteins with identical function(s) but vastly different primary amino acid sequences; the structural and dynamical consequences of this are ambiguous. The estrogen receptors α (ESR1; ER α) and β (ESR2; ER β) are physiologically essential transcription factor paralogues belonging to the ancient nuclear receptor superfamily (1). The transcriptional activities of ER α and ER β are initiated by estrogenic hormones, primarily 17 β -estradiol (E2), binding to an internal cavity in the ligand-binding domain (LBD) of apo receptor. This event allosterically activates the receptor leading to homodimerisation and recruitment of coactivators to the activation function 2 (AF2) interface of the LBD; these activities are isofunctional. In humans, essentiality is a double-edged sword as singular gain-of-function mutations in the ER α LBD are frequently enriched in most metastatic breast cancers (2). However, at least one ER is expressed in all vertebrates with broad differences in primary sequence identity, yet the LBD is functionally homologous in evolutionary distant species (3-5). If singular substitutions can decouple normal function, what mechanism(s) enables evolutionary changes to be tolerated? We aimed to understand the structural and dynamical consequences of long-term evolutionary variation in the ER LBD.

Here, we leverage comparative crystallographic and biophysical analyses to characterise distantly-related orthologues. To understand how evolutionary variation has shaped structure and vice versa we performed in-depth bioinformatics and statistical modelling to derive an epistatic map of the ER LBD. Our data reveal that the ER LBD uses surprisingly simple mechanisms to adapt to change, with the evolutionary constraints that govern this deeply encoded within structure. To investigate how change affects the conformational landscape of active ER LBD we performed extensive molecular dynamics simulations for human and evolutionary distant ERs, and analysed trajectories with machine learning and Markov modelling. We find that evolution rewires the conformational landscape, but exerts little effect on normal function of the ER LBD. Finally, we identify hundreds of population-level and disease-associated human missense variants and estimate their phenotypic likelihoods by cross-referencing to our data. We estimate that most missense variants are likely to be benign (no effect) and, furthermore, propose mechanistic basis for determinants of disease-causing variants. In summary, our work exposes principles that guide the evolution of essential transcription factors across sequence space and provides plausible mechanical basis for phenotypic determinants of human variants.

Word count: 393

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Poster Presentations



Poster Presentations

Name: **Edan Habel**

Theme: Engineering and Evolution of Protein and Peptides

Poster Position: 43

Title: In pursuit of brightness - designing more emissive lanthanide binding proteins with non-canonical amino acids and ML-based design tools

Authors: Edan I Habel, Haocheng Qianzhu, Elwy A Abdelkader, Gottfried Otting, Thomas Huber

The lanthanide binding tag is a well-established amino acid sequence that allows for the genetic incorporation of lanthanide binding properties into proteins. Trp7 of the motif acts as an antenna to sensitise a lanthanide to photons, which can then emit light as long-lasting luminescence. Recent advances in genetic incorporation of non-canonical amino acids has led to a rapid expansion of side chains that can be incorporated into a protein *in vivo*. Using this technology, more fluorescent sidechains can be incorporated at this Trp7 site to enhance the luminescence of the resulting complex. The lanthanide binding tag itself has remained relatively unchanged since its selection from a peptide library in 2005.

In this presentation we will share the design strategy novel luminescent complexes that have enhanced luminescence by characterising a fluorescent sidechain into this peptide sequence. We will share the surprising results where it was found that less fluorescent side chains produced more luminescent complexes. We will also present the exceptionally thermostable and highly expressing lanthanide binding protein that was designed using computation tools such as ProteinMPNN and RFDiffusion. This designed protein has enhanced brightness compared to the LBT and work is under way to combine the lessons from non-canonical amino acid incorporation and computational design to pursue an even brighter, genetically encoded, luminescent tag.



Poster Presentations



Poster Presentations

Name: **Haocheng Qianzhu**

Theme: Engineering and Evolution of Protein and Peptides

Poster Position: 44

Title: **Genetic encoding of fluoro-tryptophan analogues as site-specific NMR probes in proteins**

Authors: *Haocheng Qianzhu, Elwy H. Abdelkader, Gottfried Otting, Thomas Huber*

Tryptophan modified to contain a single fluorine atom in the six-membered indole ring is very similar in size and chemical properties to its parent canonical amino acid, tryptophan. Consequently, tryptophanyl-tRNA synthetases of prokaryotic and eukaryotic organisms accept fluoro-tryptophans (F-Trp) instead of tryptophan, and this has been used to uniformly install fluorine labels into proteins. Fluoro-tryptophans carry unique potential for spectroscopic analysis of proteins by nuclear magnetic resonance (NMR) spectroscopy and fluorescence measurements. While local fluorination of an amino acid has typically a negligible effect on the tertiary structure of proteins, the ^{19}F chemical shifts of F-Trp residues are exceptionally sensitive to their chemical environments. The global substitution approaches lack the capability of site-selectively installing single F-Trp residues in proteins containing multiple natural tryptophan residues. Here we present our developments to genetically encode four fluoro-tryptophan analogues individually for the efficient and site-specific incorporation into proteins. We identified mutant aminoacyl-tRNA synthetases by high throughput library selection method based on fluorescence-activated cell sorting (FACS). We show how our technique allows production of proteins with only a single hydrogen atom replaced by a fluorine atom. We highlight applications of ^{19}F NMR where (1) precise and minimum fluorine labelling of flavivirus NS2B-NS3 proteases is beneficial and necessary for measuring conformational equilibria; (2) the embedding of different fluoro-tryptophan analogues into cyclohexadienyl dehydratase (CDT) enables sensitive detection and identification of ligand binding.



Poster Presentations



Poster Presentations

Name: **Ibrahim Javed**

Theme: Engineering and Evolution of Protein and Peptides

Poster Position: 45

Title: **MICROBIAL PROGENITORS OF PROTEIN AGGREGATION DISEASES AND INFECTIOUS AETIOLOGY OF DEMENTIA**

Authors: *Syed Aoun Ali, Thomas Paul Davis, Ibrahim Javed**

The gut microbiota has recently been discovered for its role in extra-intestinal diseases such as neurodegeneration. Alzheimer's disease (AD) is a leading form of dementia where the presence of extra-neuronal plaques of Amyloid- β ($A\beta$) are a pathological hallmark. Initially considered as a functionless peptide, the physiological role of $A\beta$ remains unclear. Understanding this, can help in designing the future therapies of for dementia. My research that pivoted on the (1) clinical evidence and (2) our observations that $A\beta$ can dissolve and disintegrate microbial biofilms, is concluding towards the infectious aetiology of Dementia. Specifically, our results suggest that $A\beta$ monomers can target and disintegrate microbial amyloids of FapC and CsgA formed by opportunistic gut-pathogens, *Pseudomonas aeruginosa* and *Escherichia coli*, explaining a potential role of $A\beta$ in gut-brain axis. Employing a zebrafish based transparent in-vivo system and whole-mount live-imaging, $A\beta$ was observed to diffuse into the vasculature and localise with FapC or CsgA fibrils that were injected into the tail muscles of the fish. FapC fragments, produced after $A\beta$ treatment ($Fa\beta$), were toxic to SH-SY5Y neuronal cells whilst the intestinal Caco-2 cells were shown to phagocytose $Fa\beta$ in a non-toxic cellular process. Microbial fibrils also lost their native function of cell adhesion with intestinal Caco-2 cells, further $A\beta$ dissolved and detached microbial fibrils already attached to the cell membrane. Taken together, this study indicates an anti-biofilm role for $A\beta$ monomers that can help aid in the future development of selective anti-Alzheimer's and anti-infective medicine.



Poster Presentations



Poster Presentations

Name: **Pietro Ridone**

Theme: Engineering and Evolution of Protein and Peptides

Poster Position: 47

Title: **Engineering Flagellar Motor Stator Subunits: Insights from Experimental Evolution**

Authors: *Pietro Ridone, Matthew AB Baker*

Bacterial flagella play a pivotal role in the locomotion and navigation of many bacteria, driven by the remarkable bacterial flagellar motor (BFM). At the heart of this motor are stator subunits that harness the ion motive force across membranes to generate mechanical torque, enabling flagellar rotation. Interestingly, these BFM stators share evolutionary connections with other protein systems that couple ion flow to various biochemical processes.

While structural models of stators and related systems are readily available, the intricate details of their gating cycles and torque generation processes remain subjects of ongoing study and speculation, mainly due to the dynamic nature of these nanomachines. This research endeavours to delve into the functional intricacies of BFM stator subunits and their counterparts, with the ultimate goal of harnessing this knowledge to design ion-powered biological rotary motors customizable for diverse biomechanical and biochemical applications in biotechnology and biosensing.

Our approach encompasses classic mutagenesis techniques, phylogenetics analysis, structure-informed rational design, and directed evolution, enabling rapid and cost-effective screening of functional stator variants. Stators, as indispensable components of the BFM, create a "functional bottleneck" where selective pressure drives the emergence of mutations capable of "rescuing" our engineered proteins. The predictable genetic loci for rescue mutations make transgenic stators an invaluable model system for dissecting gene-environment relationships.

This work reports our recent advancements in comprehending the stator gating cycle, its remarkable adaptive capacity, and its degree of orthogonality with homologous systems. By bridging the gap between evolutionary biology and synthetic biology, we gain insights not only into the function and adaptation of stators but also into the broader principles governing the evolution of molecular machines.



Poster Presentations

Name: **Yu Heng Lau**

Theme: Engineering and Evolution of Protein and Peptides

Poster Position: 48

Title: **Surprising structural and dynamic (mis)behaviour of engineered protein cages**

Authors: *Taylor N Szyszka, Eric N Jenner & Yu Heng Lau*

Many forms of life use proteins as building blocks for achieving compartmentalisation. Bacteria use proteinaceous cages as primitive organelles for sequestering key biosynthetic pathways, while viruses assemble capsids to protect their genomes and define their morphology. Within this context, encapsulins are a widespread family of protein cages in prokaryotes that structurally resemble viral capsids, but natively function as small organelles that encapsulate metabolic enzymes via sequence-specific interaction with a short targeting peptide motif.[1] The modularity and robustness of encapsulins has led to interest in engineering applications for improving biocatalysis and drug delivery.

We have used encapsulin protein cages as a platform for studying the fundamentals of protein self-assembly and compartmentalisation.[2,3] While encapsulins generally display a high tolerance for sequence modifications without compromising the integrity of cage assembly, we have discovered two types of sequence modifications that result in unexpected changes to encapsulin self-assembly behaviour. In one instance, a single point mutation results in a dramatic shift from icosahedral to an unprecedented tetrahedral geometry. In the other, different choices of fusion protein partner results in remarkably divergent self-assembly dynamics.

These aberrant engineered protein cages evoke fundamental questions regarding the viral origins and evolvability of protein cages, their pathways to self-assembly, and how dynamics provide an important distinction between viral capsids and proteinaceous bacterial organelles.

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Poster Presentations



Poster Presentations

Name: **Caitlin Johnston**

Theme: Engineering and Evolution of Protein and Peptides

Poster Position: 49

Title: **Adapting fungal strategies to deliver antifungal agents**

Authors: *Caitlin L Johnston, Kenya Fernandes, Dee Carter, Margaret Sunde*

Hydrophobins are small surface-active fungal proteins that self-assemble into monolayers at hydrophobic-hydrophilic interfaces. The amphipathic films formed by Class I hydrophobins are composed of laterally packed amyloid fibril structures called rodlets [1]. The amphipathic nature and self-assembly properties of hydrophobins render these proteins as ideal candidates to be used for encapsulation and solubilization of hydrophobic drugs [2]. Amphotericin (AmB) is a poorly soluble and aggregation-prone drug that is used to treat serious fungal infections. The insolubility of AmB in aqueous environments and its binding to cholesterol leads to its high toxicity and can cause severe side effects [3]. Hence there is a need for strategies to increase the solubility of AmB enabling decreased drug dosages, thereby reducing toxicity-related side effects. To assess if the addition of hydrophobins increases AmB solubility, the hydrophobin proteins EAS and DewY were recombinantly produced and tested each in combination with AmB. Characterization of the solubility of the AmB in the presence of the monomeric and fibril forms of the hydrophobins confirmed that both EAS and DewY increase the solubility of AmB and can stabilize the drug in an aqueous solution over 48 hrs. Testing of the combinations against model and pathogenic fungal species demonstrated that the addition of the hydrophobins reduced the amount of AmB required to inhibit growth of the fungus. DewY was found to be more synergistic with AmB than EAS, reducing the minimum inhibitory concentration (MIC) of AmB up to 32-fold against the fungal growth. In addition to the hydrophobins, α -lactalbumin, bovine serum albumin, ubiquitin and lactoferrin were also tested in combination with AmB as control proteins. Interestingly these proteins also showed synergistic activity with AmB against the fungal pathogens with lactoferrin showing a similar fold decrease in MIC to DewY. Overall, these data demonstrate that the potential of utilizing proteins such as the hydrophobins for the development of novel combination antifungal therapies.

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Poster Presentations



Poster Presentations

Name: **Kapil Patel**

Theme: Engineering and Evolution of Protein and Peptides

Poster Position: 51

Title: **3D bioprinting of double network hydrogels for tissue engineering**

Authors: *Kapil D Patel, Mark R Shannon, Adam W Perriman*

Sodium alginate (Alg) is a natural polymer, usually obtained from seaweed and gelatin methacryloyl (GelMA) is semi-synthetic polymer made of chemical modification of gelatin with methacrylamide and methacrylate groups. Lately, GelMA has been greatly utilized as biomaterials in different form such as microparticles, scaffolds, electrospun nanofibers, hydrogel, and three dimensional (3D) bioprinting for drug delivery, wound healing, and tissue regeneration applications¹. Here, we have fabricated the Alg-GelMA-based mechanically tunable, photo-crosslinked double network (DN) hydrogels with varying concentrations of GelMA and three dimensional (3D) bioprinted with mouse myoblast cell line C2C12. Three different compositions of Alg-GelMA (Alg:GelMA = 1:0, 1:0.25, and 1:0.50 w/w) were fabricated. As the GelMA amount increases, the DN hydrogel exhibited higher compressive strength (28 kPa to 5318 kPa), and tensile strength (3.36 kPa to 5.95 kPa). The Cryo-scanning electron microscope (CryoSEM) images of Alg, and Alg-GelMA hydrogels revealed uniform networks with varying pore sizes. Furthermore, 3D bioprinted hydrogels were investigated for cellular activities in terms of cell viability (LIVE/DEAD assay), proliferation (DAPI/Phalloidin staining), and metabolic activity (AlamarBlue assay) for C2C12. The LIVE/DEAD assay exhibited high cell viability (~80% to 90%), and DAPI/Phalloidin staining showed significantly higher cell proliferation in 3D bioprinted Alg-GelMA hydrogel compared to bioprinting Alg hydrogel. The *in vitro* studies using C2C12 could demonstrate the myogenic differentiation and promote the myotube formation. These findings highlight that the Alg-GelMA-based 3D bioprinted hydrogel exhibits potential as a living therapeutic biomaterial; it is highly effective in supporting live cell proliferation and can promote the myogenic differentiation of C2C12 cells, which will eventually orchestrate to accelerate the skeletal muscle regeneration process.



Poster Presentations



Poster Presentations

Name: **Sonia Henriques**

Theme: Engineering and Evolution of Protein and Peptides

Poster Position: 52

Title: Spider beta-hairpin peptide engineered to target protein:protein interactions, reach the cytosol, and kill cancer cells

Authors: *Ferran Nadal-Bufi¹, Lai Y. Chan², Hadi H. Mohammad^{3,4}, Jody M. Mason³, Carlos Salomon^{5,6}, Andrew Lai⁵, Erik W. Thompson¹, David J. Craik², Quentin Kaas², and Sónia T. Henriques¹*

1Queensland University of Technology, School of Biomedical Sciences and Trans

Peptides hold great potential as a novel therapeutic modality to inhibit intracellular protein:protein interactions (PPIs) involved in cancer. A major and common challenge with peptide-based drugs is ability to enter cells and reach the cytosol, where many cancer targets are located. Lactate dehydrogenase 5 (LDH5) is a cytosolic enzyme overexpressed in aggressive and metastatic tumors and is an attractive target for anticancer therapy. Combining rational and computer-based approaches, we designed a cyclic beta-hairpin peptide with high affinity towards a β -sheet region that is involved in PPIs required for the enzymatic activity of LDH5 [1]. In addition, we reengineered the lead LHD5-inhibitor peptide to be cell penetrating and non-membrane disruptive. Using bioassays and proteomics we confirmed that the lead peptide, [R/r]cGmC9, inhibits LDH5 in the cytosol, modulates cancer metabolism and kills cancer cells [2]. This is the first non-competitive peptide-based inhibitor of LDH5 with activity in cancer cells. These studies demonstrate the potential of using peptides as inhibitors of intracellular PPIs relevant for cancer pathways and shows that active peptides can be rationally designed to improve their cell permeation. The success of this approach is relevant not only because it modulates the activity of a very important cancer target, but also because it tackles two challenges in the development of peptide-based drugs: the modulation of PPIs involved in cancer, and the efficient delivery of peptides into the cytosol.



Poster Presentations



Poster Presentations

Name: **Gemma Hart**

Theme: Immunology

Poster Position: 53

Title: **A rare IRF5 variant as a potential driver of autoimmunity**

Authors: *Gemma Hart*

Vicki Athanasopoulos

Simon Jiang

Autoimmunity is a term that broadly refers to diseases caused by a failure of the immune system to distinguish “self” from “non-self”, wherein the body begins to attack itself. The process in place to prevent this dysregulation – referred to as immunological tolerance – is tightly controlled by signalling pathways, and thus the presence of gain- or loss-of-function missense mutations in signalling components can be pathogenic. To expand our current understanding of the genes and types of mutations relevant to autoimmune disease, we analysed the genomes of individuals from a family with highly penetrant autoimmunity and identified a rare missense variant in the autoimmune-associated gene interferon regulatory factor 5 (IRF5); a transcription factor that controls the expression of pro-inflammatory cytokines and type I interferon (TI-IFN). Despite heterogeneity in disease manifestations in this family, all affected family members are heterozygous for this IRF5 variant. We therefore propose that this IRF5 allele is a gain-of-function variant capable of enhancing pro-inflammatory responses, in turn driving the aberrant activation of auto-reactive B cells that mechanistically contribute to patient disease. Using promoter reporter assays, we show that the variant indeed acts as a gain-of-function driving high expression of TI-IFN, and use a variety of in vitro techniques to characterise the molecular mechanism/s by which it alters IRF5 protein function. Moreover, immunophenotyping of patient leukocytes and direct stimulation of patient B cells also indicate dysregulation of IRF5 related signalling pathways, thus allows us to further infer the contribution of this variant to disease. Our findings represent the first instance of a gain-of-function IRF5 missense variant both identified in humans and associated with autoimmunity, thus providing a clear avenue for precision medicine to aid in the treatment of autoimmune disease.



Poster Presentations



Poster Presentations

Name: **Anushka Date**

Theme: Immunology

Poster Position: 54

Title: **Defining interactions between IRAK3-myddosome complex and mitochondria.**

Authors: *Anushka Date, Cassandra Cianciarulo, Trang H. Nyugen, Ilona Turek, Rohan Lowe, Joseph Tucci and Helen Irving.*

Interleukin 1 receptor associated kinase 3 (IRAK3) is a cytoplasmic homeostatic checkpoint protein in the Toll-like receptor 4 (TLR4) pathway of inflammation. IRAK3 interacts with the myddosome complex (MyD88-IRAK1/IRAK4) and acts as a negative regulator of this pathway. Recently Liu et al. (2022) showed the presence of the IRAK3-myddosome complex in the mitochondria, upon stimulation with pro-inflammatory cytokine IL1 β . We hypothesize that IRAK3-myddosome complex translocation to the mitochondria is driven by MyD88 upon the initiation of inflammation and acts as a cell signaling mechanism contributing to the suppression of immune response. In this study, we use two challenges of lipopolysaccharide (LPS) to THP1 monocytes to upregulate IRAK3 expression and other members of the TLR4 pathway. We performed a mass spectrometry (MS) experiment to understand the differential expression of the mitochondrial proteins at 30 minutes and 2 hours, post the second LPS-treatment. MS analysis revealed the presence of 185 differentially expressed mitochondrial proteins between the two timepoints. Based on log-fold change, 174 mitochondrial proteins were found to be significantly upregulated at 2 hours, post LPS stimulation compared to 30 minutes. Gene ontology (GO) enrichment analysis showed the biological processes and molecular functions of these upregulated proteins and identified 29 proteins involved in mitochondrial transmembrane transport, including proteins of the TIM-TOM complex (TOM40, TOM70, TIM23, TIM50, TIM44). It also revealed the presence of several members of solute carrier superfamily (SLC) which facilitate the transport of substrates using changes in membrane potential including SLC25A1, a mitochondrial citrate carrier protein. This proteomic approach provides some insight into the interactions between inflammation and mitochondria and poses more questions regarding the function and method of the IRAK3-myddosome translocation.

Liu, W., Zhou, H., Wang, H., Zhang, Q., Zhang, R., Willard, B., Liu, C., Kang, Z., Li, X., & Li, X. (2022). IL-1R-IRAKM-Slc25a1 signaling axis reprograms lipogenesis in adipocytes to promote diet-induced obesity in mice. *Nature Communications*, 13(1), 2748. <https://doi.org/10.1038/s41467-022-30470-w>



Poster Presentations

Name: **Cassandra Cianciarulo**

Theme: Immunology

Poster Position: 55

Title: **Exploring the proteomic landscape of THP-1 monocytes through two-challenge LPS induction**

Authors: *Cassandra Cianciarulo, Ilona Turek, Keshava Datta, Rohan Lowe, Trang H Nguyen, Terri Meehan-Andrews, Joseph Tucci and Helen Irving*

Chronic systemic inflammation increases with age and accounts for initiation and progression of many diseases including cancer, cardiovascular and autoimmune disorders. Acute inflammation is a powerful protective response against infections and is essential for injury repair. Homeostatic processes normally limit this inflammatory response in the crucial process known as 'tolerance', as acute inflammation contributes to chronic inflammatory diseases, and overactive immune responses, leading to organ failure in severe cases.

To model acute and systematic inflammation in vitro, the toll-like receptor (TLR) 4 ligand lipopolysaccharide (LPS) is administered to innate immune cells to generate an acute, localized, and transient inflammatory reaction. Typically, one dose of LPS – or one challenge – is enough to elicit a suitable response to measure inflammatory markers and prognostic outcomes, though a two-challenge model better embodies the chronic progression of inflammation.

The proteomic landscape of innate immune cells was assessed in THP-1 monocytes double-challenged with either mock or LPS treatments by LC-MS/MS, extending upon prior one-challenge studies (Mulvey et al. 2021). Proteins PREX1 and MMP14 were identified by gene ontology analysis as being significantly upregulated in the biological immune system process in the two-challenged LPS monocytes when compared to the mock at both timepoints. The MMP14 protein has been identified as a positive regulator for macrophage migration, whilst PREX1 has been instigated as essential for neutrophil activation. Comparison of these proteins to data gathered from previous experiments suggests that these proteins may also be linked to a mediator of the TLR signaling pathway, interleukin-1 receptor associated kinase 3 (IRAK3). Our aim is to identify new putative interactors of IRAK3 in the TLR signaling pathway and to elucidate the moonlighting role it plays in cells.



Poster Presentations



Poster Presentations

Name: **Lawton Murdolo**

Theme: Immunology

Poster Position: 56

Title: **How some of us remain asymptomatic after COVID-19**

Authors: *Lawton D. Murdolo, Danillo G. Augusto, Demetra S. M. Chatzileontiadou, Jill A. Hollenbach & Stephanie Gras*

The emergence of SARS-CoV-2 and the ensuing pandemic shifted research towards severe cases, with little attention given to understanding why about 20% of infected individuals remain asymptomatic. Our study aimed to unravel the natural immunity that shields asymptomatic individuals from symptoms.

T lymphocyte activation is driven by Human Leukocyte Antigens (HLA) on the surface infected cells. These HLAs present viral peptides to T cells, which then recognize them as antigens. However, the polymorphic nature of HLA molecules leads to variations in the adaptive immune response due to different peptides bound to various HLA types. With the help from our collaborators, we discovered a link between asymptomatic infection and a specific HLA, HLA-B15:01, present in approximately 3% of the global population. We sought to understand how HLA-B15:01 provided protection against SARS-CoV-2.

We examined four SARS-CoV-2-derived epitopes that could be presented by HLA-B*15:01 using samples collected before SARS-CoV-2 exposure. These individuals were unexposed to the virus, not vaccinated, and not exposed to SARS-CoV-1. One epitope, NQK-Q8, derived from the spike protein, triggered a robust T cell response in unexposed individuals. Most of the NQK-Q8-specific T cells were memory T cells, indicating prior activation from a previous infection. We also found that a similar peptide, NQK-A8, from human seasonal coronaviruses, was recognized by the same T cells. We investigated whether a single mutation between these two epitopes affected the stability of the peptide-HLA-B15:01 complex. The results showed no difference in stability.

Using X-ray crystallography to observe peptide presentation and affinity measurements of the T cell receptors (TCRs), we found that TCRs from COVID-recovered, vaccinated, and unexposed HLA-B15:01+ individuals exhibited high and similar affinity for both peptides. This suggests that both epitopes efficiently activate T cells and facilitate viral recognition and protection in HLA-B*15:01+ individuals.

Our study published in Nature, underscores the critical role of CD8+ T cells, activated through a specific HLA molecule, in establishing an effective early defense against the virus. This discovery not only enhances our understanding of the immunological factors behind rapid viral clearance but also provides a foundation for developing therapeutics that can elicit a similar response.



Poster Presentations

Name: **SELVA KUMARI RAMASUBRAMANIAN**

Theme: Immunology

Poster Position: 57

Title: **Immune repertoire sequencing enables accurate clonality determination**

Authors: *Selva Ramasubramanian, Chen Song, Pingfang Liu, Andrew Barry, Jian Sun, Bradley W Langhorst, Fiona J Stewart, Salvatore Russello, Eileen T Dimalanta, and Theodore B Davis*

The study of complex immunological diseases and tumor microenvironments has advanced through recent developments in sequencing of the immune repertoire. Using this approach, the interrogation of disease progression is facilitated through analysis of millions of V(D)J combinations from B cell antibodies (BCRs) and T cell receptors (TCRs). One major challenge of immune repertoire sequencing is to accurately capture the structural and sequence complexities of antibodies and TCR genes during both library preparation and bioinformatic analysis. Here, we present a method for accurate sequencing of full-length immune gene repertoires of B cells and T cells.

RNA was extracted from tissues and peripheral blood mononuclear cells (PBMCs) and used for reverse transcription, during which unique molecular identifiers (UMIs) were added to discretely barcode each mRNA molecule. BCR- and TCR-specific PCR primers were used to enrich full-length BCR and TCR sequences. We have implemented a data analysis pipeline to assemble the full length BCR/TCR transcripts and to collapse PCR copies of each mRNA fragment into a single consensus sequence using UMIs. UMI incorporation enables the absolute quantification of input RNA molecules and accurate ranking of antibody/TCR clone abundance. Furthermore, this method facilitates detection of distinct and shared clones in tissue and blood samples, allowing identification of disease-specific clones to evaluate immunotherapy effects. Our method accurately and sensitively detects target TCR clones down to 0.01%, enabling minimal residual disease (MRD) assessment.

Our immune repertoire sequencing approach allows accurate clonal determination for both BCR and TCR. This technique is applicable for a variety of applications including design of antibody chains for in vitro synthesis, investigation of T cell infiltration of tumor microenvironments, and monitoring of minimal residual disease in cancer patients.



Poster Presentations



Poster Presentations

Name: **Vicki Athanasopoulos**

Theme: Immunology

Poster Position: 58

Title: **A variant in TNIP1 contributing to an autoimmune disorder with elevated IgG4**

Authors: *Arti Medhavy, Maurice Stanley, Katharine Bassett, Yuke He, Paula Gonzalez-Figueroa, Grant J. Brown, Padmaja Tummala, Tom Lea-Henry, Cynthia M. Turnbull, Qian Shen, Jonathan A. Roco, Jean Cappello, Gaetan Burgio, Phil Wu, Eun Cho, T. Daniel Andrews, Matt A*

Next generation sequencing (NGS) and CRISPR-Cas9 technologies have revolutionised genomic medicine. While whole genome sequencing has identified genetic variants relevant to human disease, the introduction of these variants into mice by CRISPR-Cas9 genomic engineering is a powerful tool to confirm disease causation.

Common variants in Tumour necrosis factor α (TNF- α)-induced protein 3-interacting protein 1 (TNIP1) have been associated with autoimmune disease in genome wide association studies, but to date no cases of human systemic autoimmunity have been attributed directly to pathogenic variants in TNIP1. TNIP1 represses the inflammatory NF κ B signalling pathway, in response to TLR, TNF, IL-1 and CD40 activation, by binding to polyubiquitin moieties on key signalling proteins and targeting them for proteasomal degradation. We identify and report the first human pathogenic mutation in TNIP1 in two unrelated patients with autoimmune disease and investigate the molecular mechanisms by which this variant impairs signalling pathways leading to the survival of self reactive B cells, production of antinuclear antibodies and autoimmunity.



Poster Presentations



Poster Presentations

Name: **Santosh RamaBhadra Rao Tata**

Theme: Membrane Transporters

Poster Position: 59

Title: **5-HT3 receptors are a moonlighting ion channel influencing mitochondrial function**

Authors: *Santosh T R B Rao, Ilona Turek, Julian Ratcliffe, Simone Beckham, Cassandra Cianciarulo, Siti S B M Y Adil, Christine Kettle, Donna R Whelan, Helen R Irving*

The 5-hydroxytryptamine 3 (5-HT₃) receptor is a member of the 'Cys-loop' family and the only pentameric ligand gated ion channel among the serotonin receptors. 5-HT₃ receptors play an important role in controlling growth, development, and behaviour in animals. Several 5-HT₃ receptor antagonists are used therapeutically principally in treating nausea and emesis but also for irritable bowel syndrome. Humans express five different subunits (A-E) enabling a variety of heteromeric receptors to form but all contain 5HT_{3A} subunits. 5-HT₃ receptors are well known components of the plasma membrane. However, bioinformatic analyses suggested that at least two subunits (A and E) contained mitochondrial localisation signals. We explored this idea by transiently transfecting HEK293T cells, that do not natively express the 5-HT₃ receptor, with epitope and fluorescent protein-tagged 5HT_{3A} and 5HT_{3E} subunits. Fluorescence microscopy and cell fractionation indicated that both A and E subunits localized to the mitochondria, while transmission electron microscopy revealed the location of the subunits on the mitochondrial inner membrane, where they could form heteromeric complexes. The presence of A and E subunits influenced changes in the membrane potential and mitochondrial oxygen consumption rates in whole cells and isolated mitochondria upon exposure to serotonin; this was inhibited by pre-treatment with ondansetron, a 5-HT₃ receptor antagonist. Therefore, it is likely that the 5-HT₃ receptors present on mitochondria directly impact mitochondrial function where they have a moonlighting job as possible inducible mitochondrial uncouplers.



Poster Presentations



Poster Presentations

Name: **jiansi long**

Theme: Membrane Transporters

Poster Position: 60

Title: Characterisation of the MlaC-MlaD interaction and phospholipid transport in bacterial pathogens

Authors: *Jiansi Long¹, Jing Zhang¹, Colin Jackson^{1,2}, Matthew D. Johnson¹, Denisse L. Leyton¹*

¹Research School of Biology, Australian National University, Canberra, ACT, 0200, Australia.

²Research School of Chemistry, Australian National University, Canberra, ACT,

The outer membrane of Gram-negative bacteria is an asymmetric phospholipid (PL) bilayer and serves as an antibiotic barrier. The maintenance of (phospho)lipid (PL) asymmetry (Mla) transporter was named based on its role in maintaining outer membrane PL asymmetry in *Escherichia coli* (Ec). The Mla transporter is an ATP-binding cassette transport system proposed to traffic PLs. It is composed of six core components, including the outer membrane lipoprotein, MlaA; the periplasmic PL chaperone, MlaC; the ATP-binding cassette, MlaE and MlaF; and two auxiliary proteins, MlaD and MlaB. Deletion of any *mla* gene in *Acinetobacter baumannii* (Ab) or *E. coli* largely reduced the minimum inhibitory concentration of all antibiotics tested, showing that the Mla transporter is essential for the maintenance of outer membrane integrity and is, therefore, necessary for bacterial survival. For these reasons, the Mla transporter is recognised as an attractive drug target.

MlaC and the periplasmic facing portion of MlaD play a key role in PL transport. MlaC transports PLs between the inner membrane and outer membrane, and MlaD receives or transfers PLs from or to MlaC. Although Mla components from *E. coli* and *A. baumannii* are structurally similar and both have substrates as PLs, AbMlaD has an additional region of 47-amino acids (aa), which is not found in any other Mla orthologues. In this study, PL-free MlaC was incubated with PL-bound MlaD (or vice versa), the Mla proteins were separated via size exclusion chromatography, and PLs were then extracted from the purified Mla proteins where thin layer chromatography was used to identify the bound PLs. We show that AbMlaD and EcMlaD can transfer PLs to AbMlaC and EcMlaC, respectively (but not vice versa), and that EcMlaD can transfer PLs to AbMlaC (but not vice versa). In contrast, PL transfer between AbMlaD and EcMlaC was observed in both directions. When the structures of AbMlaD and EcMlaD are superimposed, the 47-aa additional region of AbMlaD is found in a similar position to the periplasmic facing β 6- β 7 loop of EcMlaD that contains two hydrophobic residues previously shown to be required for the EcMlaC-EcMlaD interaction. Together these data suggest that the 47-aa additional region of AbMlaD is spatially positioned to interact with AbMlaC and EcMlaC via a larger hydrophobic surface and, therefore, hypothesize that this region may enhance the interaction between these proteins.



Poster Presentations

Name: **Jing Zhang**

Theme: Membrane Transporters

Poster Position: 62

Title: **Structural and functional characterisation of MlaD from *Acinetobacter baumannii***

Authors: *Jing Zhang, Jiansi Long, Xiaojun Yuan, Simon Williams, Colin Jackson, Matthew Johnson, Denisse Leyton*

Acinetobacter baumannii (Ab) is a clinically important pathogen that has rapidly developed resistance to many antimicrobials. The outer membrane (OM) of *A. baumannii* (and other Gram-negative pathogens) plays a crucial role in intrinsic antibiotic resistance by acting as a physical barrier that prevents drugs from reaching their targets inside the cell. The Maintenance of Lipid Asymmetry (Mla) transporter has recently emerged as a key player in maintaining the integrity of the OM in all Gram-negative bacteria through the transport of phospholipids (PLs). The importance of the OM in conferring intrinsic resistance to antibiotics and requirement of the Mla transporter in maintaining OM integrity renders this complex an attractive drug target.

The Mla transporter contains six components, including the inner membrane (IM) MlaFEDB complex, periplasmic MlaC, and MlaA which is located in the OM. MlaC is proposed to shuttle PLs between the OM and IM, through interactions with MlaA and MlaD, respectively. Through bioinformatic analysis, we revealed that MlaD from *A. baumannii* contains a novel mammalian cell entry (MCE) domain, with an additional 47-amino acid region not found in any MlaD orthologs. Upon commencement of this project, the structure and function of the additional region were unknown.

We successfully purified the soluble domain of AbMlaD in its hexameric form and solved the crystal structure of AbMlaD at 2.0 Å. AbMlaD forms a typical 7-stranded β -barrel MCE domain fold with a helical additional region. Although there is less than 35% sequence identity between the MCE domain of MlaD from *E. coli* and AbMlaD, the two structures are superimposable, with the exception of the 47-amino acid additional region in AbMlaD.

Through biochemical and biophysical approaches, we investigated the functional role of the additional region in AbMlaD, focusing on its impact on the AbMlaD-AbMlaC interaction. Our findings indicate that the additional region is essential for stability and oligomerisation of AbMlaD. It also influences the interaction between AbMlaD and AbMlaC, as well as the efficiency of PL transfer. Moreover, *A. baumannii* with a chromosomal deletion of the *mldD* gene showed a defect in growth in the presence of SDS, suggesting a role for AbMlaD in the maintenance of the OM integrity. In trans complementation of *A. baumannii* Δ *mldD* with *AbmldD* lacking the additional region restored the ability of the deletion mutant strain to grow on SDS agar, showing that the additional region is not directly responsible for maintaining OM integrity.

This study increases our understanding of the structure and function of the Mla transporter. The insights gained through this work pave the way for inhibition studies to ascertain the likelihood of being able to inhibit or modulate the Mla transporter with a small molecule drug.



Poster Presentations



Poster Presentations

Name: **Colin Cheng**

Theme: Pathogens

Poster Position: 63

Title: **Regulation of dengue non-structural protein 5 nuclear localisation is unique to each serotype**

Authors: *Colin Cheng, Alvin Tan, Kitti Chan, Wint Phoo, Jaclyn Wong, Milly Choy, Noelia Roman, Daniel Arnold, Amanda Bifani, Sean Kong, Babu Nath, Jade Forwood, Subhash Vasudevan*

Dengue virus non-structural protein 5 (DENV NS5), consisting of methyltransferase and RNA-dependent RNA polymerase (RdRp) domains, is critical for viral RNA synthesis within endoplasmic reticulum (ER)-derived replication complexes in the cytoplasm, however a significant proportion of NS5 is known to be localised to the nucleus of infected cells. The proportion of NS5 localised to the nucleus varies between each of the 4 serotypes of DENV. We still have an incomplete understanding of how DENV NS5 nuclear localisation is regulated. Within NS5, 2 putative nuclear localisation signals (NLS) have been identified; NLS_{Central}, residing in the palm of the RdRp domain, as well as the recently discovered NLS_{C-term}, residing in the flexible region at the C-terminal of the RdRp domain. We have previously shown that DENV2 NS5 nuclear localisation can be significantly reduced by single point mutations to the NLS_{C-term}. Here, we present biochemical, virological and structural data revealing that the importance of either NLS in NS5 nuclear localisation is unique to each of the 4 DENV serotypes; DENV1 NS5 subcellular localisation is regulated by a weak NLS_{Central}, DENV2 NS5 predominantly nuclear localisation is regulated exclusively by a strong NLS_{C-term}, while DENV3 NS5 can utilise either NLS for nuclear localisation. Lastly, in the case of DENV4, NS5 nuclear localisation occurs despite poor affinity of either NLS for IMP α , suggesting that nuclear import of DENV4 NS5 is dependent on either a post-translational modification, and/or is driven by an IMP α -independent pathway.



Poster Presentations



Poster Presentations

Name: **Crystall Swarbrick**

Theme: Pathogens

Poster Position: 64

Title: **Studies towards pan-flaviviral protease inhibitors**

Authors: *Crystall M.D. Swarbrick*^{1,2}, *Gerasimos Rassias*³, *Kitti W.K. Chan*¹, *Mark von Itzstein*⁴ & *Subhash G. Vasudevan*^{1,4}

¹Program in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore

²Gulbali Institute, Charles Sturt University, Australia

³Department o

The Flaviviridae family includes high profile viruses dengue and Zika for which there are currently no antiviral treatments available. Our research here focuses on a rational structure-chemistry approach to develop non-peptidic small molecules that can specifically inhibit the flaviviral protease. Starting with an inhibitor identified in a West Nile Virus in silico drug discovery campaign we undertook a scaffold-hopping exercise to discover new lead compounds. Following a structure-activity-relationship study, compound 17 was found to inhibit DENV2 and ZIKV protease at IC50 values of 1.16 and 0.52 μM respectively which are amongst the lowest reported values in the literature so far. In a second iteration of this work, we decided to test the inhibitory activity of “prodrugs” of the active compounds to examine their toxicity and potencies in cellulo. This led to the discovery of a “pro-drug” derivative of compound 17 that was efficacious and potent in cellulo achieving low micromolar EC50 against DENV2, thus promising a well-tolerated series of compounds targeting the flaviviral protease. The in cellulo mechanism-of-action of the lead compound was investigated using a time-of-addition assay which suggested that the compound interfered with the early stages of replication and specifically inhibited intramolecular cleavages in NS3 that have recently been shown to have a trans-dominant inhibitory effect on DENV replication.



Poster Presentations



Poster Presentations

Name: **Daniel Yu**

Theme: Pathogens

Poster Position: 65

Title: **The molecular basis of immune receptor recognition of FOLD effectors in the Fol-tomato pathosystem**

Authors: *Daniel S Yu, Ashley Smith, Daniel J Ericsson, David A Jones & Simon J Williams.*

Plant pathogens secrete proteins, known as effectors, that function in the apoplast and inside plant cells to promote virulence. Despite their importance, our understanding of fungal effector function and detection by immune receptors remains poor. Using protein x-ray crystallography, we identify a new structural class of effectors hidden within the secreted in xylem (SIX) effectors from *Fusarium oxysporum* f. sp. *lycopersici* (Fol). The recognised effectors, Avr1 (SIX4) and Avr3 (SIX1), represent the founding members of the Fol dual-domain (FOLD) effector class. We show that Avr1 and Avr3 are recognised by the tomato immune receptors, I and I-3, respectively using a protein-mediated phenotyping approach. Utilising structurally guided mutagenesis, we identify residues in Avr1 that are crucial for recognition by the I immune receptor. We also determine the recognition specificity of the I receptor is dictated by the island domain and rescue recognition of Avr1 in an allelic variant of I from a tomato cultivar which has no Fol resistance. Collectively, these data will aid future studies to understand the molecular basis of *F. oxysporum* effector function and recognition, and by extension, the design and engineering of immunity receptors with novel recognition specificities to help protect plants against *Fusarium* wilt disease.



Poster Presentations



Poster Presentations

Name: **Frankie Lyons**

Theme: Pathogens

Poster Position: 66

Title: **Nanobodies targeting malaria transmission-blocking candidate Pfs48/45**

Authors: *Frankie M. T. Lyons, Mikha Gabriela, Amy Adair, Li-Jin Chan, Melanie H. Dietrich and Wai-Hong Tham*

Malaria transmission occurs when a female *Anopheles* mosquito takes a blood meal from an infected host, and *Plasmodium* gametocytes are taken up into the mosquito midgut. Here they undergo sexual reproduction to produce sporozoites that migrate to the mosquito's salivary glands, ready to infect another host. The mosquito stages are a major bottleneck in the *Plasmodium* life cycle when parasite numbers are very low, representing a promising opportunity for intervention.

Malaria transmission can be blocked by inducing or administering antibodies that inhibit essential sexual stage antigens. A major target of transmission-blocking interventions is the 6-cysteine protein P48/45. The 6-cysteine protein family is a family of abundant, highly conserved and surface-exposed proteins that are expressed by malaria parasites throughout their life cycle and play critical roles in their development. P48/45 is expressed on the surface of gametocytes and gametes and is essential for male fertility, with knock-out males unable to attach to and fertilize female gametes. Recognition of P48/45 by human sera correlates with the ability of sera to block parasite transmission and antibodies against P48/45 have transmission-blocking activity. A recent trial of the most potent transmission-blocking antibody to date, TB31F, has demonstrated the potential of monoclonals as transmission-blocking prophylactics.

We have generated first collection of nanobodies against *Plasmodium falciparum* P48/45 and demonstrated specificity using western blotting, ELISA and bio-layer interferometry (BLI). We have isolated three high-affinity nanobodies that show transmission-reducing activity and are characterizing the transmission-blocking potency of our nanobodies in standard membrane feeding assays. Competition BLI has confirmed our nanobodies bind a different epitope of P48/45 to TB31F and we are further defining this inhibitory epitope using X-ray crystallography to provide insights into the domains of P48/45 involved in parasite transmission.



Poster Presentations



Poster Presentations

Name: **Seyed Mohammad Ghafoori**

Theme: Pathogens

Poster Position: 67

Title: Uncovering the binding preferences between host nuclear transport proteins and the key virulence factor ORF4b from MERS-CoV-related viruses

Authors: Seyed Mohammad Ghafoori, Thilini S. Munasinghe, Megan R. Edwards, Justin K. Foster, Loretta A. Bosence, Christopher F. Basler, Gayle F. Petersen, Justin A. Roby, Jade K. Forwood

The zoonotic threat of coronaviruses (CoVs) has been well established, particularly since the 2019 severe acute respiratory syndrome (SARS)-CoV-2 pandemic. Middle East respiratory syndrome (MERS)-CoV, a member of the Betacoronavirus genus, has an estimated mortality rate of 35%, with zoonotic transmission believed to occur between bats, camels, and humans. We recently demonstrated that the key MERS-CoV virulence factor ORF4b binds to the host nuclear transport protein importin alpha (IMP α) via a defined nuclear localisation sequence (NLS) and competitively inhibits NF- κ B-dependent innate immune responses. However, little is known about MERS-related viruses and their capacity to infect humans and challenge host immunity. As such, we aimed to investigate the binding of ORF4b from MERS-related viruses found in bats (HKU4, HKU5, and HKU25) and hedgehogs (HKU31) to various IMP α isoforms from humans and mice.

To determine the nature of these interactions, we performed biochemical protein-protein interaction techniques, such as bead binding assays and fluorescence polarisation (FP), complemented with X-ray crystallography. We also conducted an immunofluorescence assay to examine the nuclear shuttling of full-length ORF4b proteins within the cell.

Both bead binding assays and FP showed that ORF4b NLSs from MERS-related viruses HKU4, HKU5, and HKU25 could all bind to IMP α , with quantification revealing distinct IMP α isoform preferences. Conversely, HKU31 ORF4b NLS did not bind any of the tested IMP α isomers. We previously demonstrated that MERS-CoV ORF4b interacts with IMP α 2 and IMP α 3 in a bipartite manner, with “site1” and “site2” NLSs occupying the minor and major sites, respectively. High resolution structures of select MERS-related viruses revealed that unlike the bipartite NLS of MERS-CoV ORF4b, the HKU4 and HKU5 ORF4b NLSs were monopartite, using “site1” only, with FP analysis of “site1” mutants confirming the importance of this region. Immunofluorescence assays using the full-length ORF4b of MERS-related viruses revealed an intriguing pattern of nuclear localisation. HKU5 ORF4b displayed robust nuclear localisation, whereas HKU25 ORF4b showed comparatively weaker nuclear localisation. In contrast, HKU4 ORF4b exhibited limited nuclear localisation, likely due to the obvious low expression levels within the cells. Most interestingly, HKU31 ORF4b demonstrated nuclear localisation comparative to that of MERS ORF4b, which was unexpected based on the lack of IMP α binding detected in the biochemical assays.

Our study has revealed the preferential binding between ORF4b proteins from MERS-related viruses and specific IMP α isoforms. This may bring new insights into the potential zoonotic threat of MERS-related viruses.



Poster Presentations



Poster Presentations

Name: **Amina Mustafa**

Theme: Pathogens

Poster Position: 68

Title: Influence of proline-rich regions on the structure and function of autotransporters from enteric pathogens

Authors: *Amina Mustafa¹, Xiaojun Yuan¹, Xiaoxu Yang¹, Kwun Ting Li¹, Daniel Yu¹, Jason J. Paxman², Begoña Heras², Denisse L. Leyton¹*

¹Research School of Biology, Australian National University, Canberra, ACT, 0200, Australia.

² Department of Biochemistry and Genet

Autotransporters (ATs) are virulence factors found in Gram-negative bacteria that are secreted to and/or beyond the bacterial cell surface by the Type V secretion system. ATs mediate virulence traits of pathogens, including biofilm formation, adhesion to host cells, cytotoxicity, bacterial aggregation, immune evasion, etc. ATs consist of three domains, including a N-terminal signal peptide, a central passenger domain, and a C-terminal β -barrel domain. Here, we used RpeA [Rabbit-specific enteropathogenic *E. coli* (REPEC) plasmid-encoded AT] as a model AT. RpeA was first identified in REPEC and shown to mediate colonization of the bacterium to the rabbit intestine. RpeA belongs to the serine protease ATs of Enterobacteriaceae (SPATEs) subfamily and contains a characteristic serine protease motif (GDSGSP) located near the N-terminus of the passenger domain. Moreover, RpeA contains a proline-rich region (PRR) in the form of proline-rich tandem repeats of eight amino acids [PPE(S/T)EKPV] located towards the C-terminus of the passenger domain. RpeA, unlike other SPATEs, lacks a conserved autocatalytic cleavage site comprised of twin asparagine residues (NN) within the linker (a region located between the passenger and β -barrel domains), which suggested that RpeA is a non-secreted SPATE.

Through immunoblotting of cellular fractions and immunofluorescence microscopy, we show that, unlike other SPATEs, the RpeA passenger domain is not cleaved from its β -barrel domain and is exposed on the bacterial cell surface. Furthermore, protease activity assays using purified RpeA passenger domain revealed that RpeA has serine protease activity. The protease activity of RpeA could be inhibited by serine protease inhibitors and through mutation of the catalytic serine in the serine protease motif. Through qualitative and quantitative functional assays comparing *E. coli* TOP10 cells expressing wild-type RpeA, RpeA Δ PRR or empty vector, we show that RpeA does not mediate bacterial aggregation, yet does mediate biofilm formation although the PRR has no role in biofilm production. However, the PRR was necessary for the adhesion of RpeA to epithelial cells.

Through in vivo pulse-chase assays and biophysical analysis on refolded wild-type RpeA and RpeA Δ PRR, we show that the PRR has no role in the folding of the RpeA passenger domain. Furthermore, sequence analysis of ~1435 putative ATs revealed that 36% of ATs have PRRs where

66% of these are, like in RpeA, located towards the C-terminus of the passenger domain. Therefore, our findings have the potential to be applied more broadly.

These findings increase our knowledge about the biological role of RpeA and the contribution of its PRR to RpeA structure and function. Furthermore, our work suggests that PRRs in ATs could be exploited as a therapeutic target to reduce the virulence levels of bacteria expressing AT adhesins that harbor a PRR in the C-terminus of the passenger domain.



Poster Presentations



Poster Presentations

Name: **Jackson Feng**

Theme: Pathogens

Poster Position: 69

Title: **Neutralising nanobodies against HCoV-OC43**

Authors: *Jackson Feng, Amy Adair, Li Lynn Tan, Jason Girkin, Nathan Bryant, Mingyang Wang, Francesca Mordant, Li-Jin Chan, Nathan W. Bartlett, Kanta Subbarao, Phillip Pymm, Wai-Hong Tham*

In the past 20 years, human-infective coronaviruses (HCoVs) have been responsible for two epidemics (SARS-CoV and MERS-CoV) as well as the COVID-19 pandemic (SARS-CoV-2), highlighting coronaviruses as a critical human pathogen. Aside from these highly pathogenic coronaviruses, there are four endemic human coronaviruses: HCoV-229E, HCoV-NL63, HCoV-HKU1 and HCoV-OC43 which typically cause mild symptoms, and in rare cases resulted in mortality in vulnerable populations. To date, there are currently no targeted therapeutic treatments for HCoVs outside of SARS-CoV-2. Nanobodies, derived from camelid heavy-chain only antibodies, have shown promise as therapeutic candidates for respiratory diseases including SARS-CoV-2. We aimed to generate nanobodies against HCoV-OC43 that are high affinity and able to block virus entry. Here, we identified nanobodies by screening a phage display library generated from an alpaca immunised with recombinant HCoV-OC43 S1B which is the theorised receptor binding domain for HCoV-OC43. Characterisation of over 40 nanobodies showed specificity for the HCoV-OC43 S1B domain with binding affinities KD ranging from 1.55 to 149 nM. Epitope binning of the top 10 highest affinity nanobody binders shows that these nanobodies bound to two distinct sites on recombinant OC43. In addition, two of these nanobodies, WNb 293 and WNb 294 neutralized virus at 0.21 nM and 1.79 nM respectively. Both intranasal and intraperitoneal delivery of WNb 293 fused to a Fc domain significantly reduced nasal viral load in a mouse model of OC43 infection. Using structural approaches, we observed that WNb293 bound to an epitope on the HCoV-OC43 S1B domain that is independent of the glycan binding site. Our work provides the first description of HCoV-OC43 nanobodies able to block virus entry and reduce viral loads in vivo.



Poster Presentations



Poster Presentations

Name: **Jaison D Sa**

Theme: Pathogens

Poster Position: 70

Title: **Nanobodies against malaria parasite adhesins that block invasion**

Authors: *Jaison D Sa, Kathleen Zeglinski, Amy Adair, Li Jin Chan, Li Lynn Tan, Quentin Gouil, Phillip Pymm and Wai-Hong Tham*

Malaria is one of the most life-threatening parasitic diseases known to humanity, leading to hundreds of thousands of deaths. Malaria parasites must invade red blood cells to survive within the human host. A major contributing factor for host specificity is the interaction between parasite adhesins and their receptors on the red blood cell surface. Two homologous family of proteins, *P. falciparum* reticulocyte binding protein homolog (PfRh) and *P. vivax* reticulocyte binding proteins (PvRBP) are among the major parasite adhesins involved in red blood cell invasion and are important targets for vaccine development. Among these protein families, it is known that PfRh5 binds to Basigin, PfRh4 to Complement Receptor 1 and PvRBP2b to Transferrin Receptor 1. Intriguingly, a small subset of mouse monoclonal antibodies against these parasite adhesins do not block receptor engagement but nevertheless inhibit parasite invasion.

Our aim is to identify specific or cross-specific nanobodies against these three major parasite adhesins, PfRh5, PfRh4 and PvRBP2b using phage display approaches, next-generation sequencing and a dedicated bioinformatic pipeline. We have immunised several alpacas with recombinant versions of these parasite adhesins. Phage display panning was performed against the three homologous malaria parasite adhesins using a) their single immunised library, b) their pooled immunised libraries (4 libraries) and c) the WEHI Library which contains 34 pooled libraries. Our phage display outputs were sequenced with next-generation sequencing using the NextSeq 2000 2 × 300 bp kit and the quality score for all sequences was > Q30. Using Nanologix, an open source bioinformatic software, we have identified the most promising leads specific to each adhesin and have identified nanobodies that are cross-specific. We will select the top fifty hits for characterisation of their affinities and epitope landscape using biolayer interferometry. We will examine the ability of these nanobodies to inhibit receptor engagement and parasite invasion of red blood cells using red blood cell binding assays, parasite growth assays and FRET-based receptor-adhesin interaction assays. Structural approaches will be used to determine the binding epitopes of these cross-specific nanobodies that inhibit parasite invasion against multiple *Plasmodium* species. This will help us identify novel inhibitory epitopes that can guide rational design strategies of future therapeutics against the blood stage parasite adhesins of *Plasmodium falciparum* and *Plasmodium vivax*.



Poster Presentations

Name: **Li Jin Chan**

Theme: Pathogens

Poster Position: 71

Title: **Nanobodies to inhibit malaria parasite fertilization and development in mosquitoes**

Authors: *Li-Jin Chan, Melanie H. Dietrich, Amy Adair, Mikha Gabriela, Frankie Lyons, Stephanie Trickey, Li Lynn Tan, Jaison D'Sa, Rainbow Chan, and Wai-Hong Tham*

Malaria remains a global devastating parasitic disease with 619,000 deaths annually, 80% of deaths being children under the age of five years. The first ever malaria vaccine will be delivered between 2023-2025, which has shown 56% efficacy and will decrease deaths caused by malaria. To further reduce the burden of malaria within a community we need to develop vaccines that halt parasite transmission. Transmission blocking vaccines are designed to prevent mosquitoes carrying malaria parasites from spreading them to humans. Blocking transmission of malaria parasites is crucial for malaria elimination, as you stop the malaria parasite from reinfection of communities.

Transmission blocking vaccines work by inducing antibodies against the sexual forms of the malaria parasites that infect mosquitoes. For malaria parasites, fertilisation occurs in the female *Anopheles* mosquito. Successful fertilisation results in the maturation of parasites within the mosquito, which are then transmitted to humans via the bite of an infected mosquito. By stopping parasite fertilisation, we can stop the transmission of the malaria parasites from mosquito to human. There are currently only three transmission blocking candidates in development and there is a need for the identification of novel vaccine candidates that block malaria parasite transmission.

Sexual reproduction and fertilisation are fundamental processes in eukaryotes to produce new offspring. For malaria parasites, only half a dozen proteins are known to be involved in fertilisation. Recently, we have identified new candidates for malaria parasite fertilisation by isolating core fertilisation complexes from the sexual stages of *P. falciparum*. We will investigate the importance of these new and known transmission blocking antigens in *P. falciparum* sexual stage development and transmission, including proteins from the 6-cysteine family, serpentine receptors and ancient fusogens. Using an immunized nanobody platform, next generation sequencing and a dedicated bioinformatic pipeline, we will generate the first comprehensive collection of nanobodies against these transmission blocking antigens. We will characterize nanobodies for their binding kinetics, epitope bins and potency in blocking transmission. High-resolution structures will be determined of fertilisation proteins and their complexes to understand how they interact and how transmission-blocking nanobodies function to inhibit transmission. We aim to elucidate the critical proteins involved in *P. falciparum* fertilization and development within the mosquito and to develop nanobodies as novel transmission blocking therapeutics.



Poster Presentations

Name: **Sophie Wacher**

Theme: Pathogens

Poster Position: 72

Title: **Structural characterization of the nuclear localisation signals of cytomegalovirus major immediate early proteins in complex with importin alpha**

Authors: *Sophie C. Wacher*^{1,2}, *Cecilia Moriarty*¹, *Martin Pal*¹, *Crystall M. D. Swarbrick*², *Jade K. Forwood*^{1,2} & *Brian P. McSharry*^{1,2}

Human cytomegalovirus (HCMV) is a ubiquitous β -herpesvirus that is associated with significant morbidity and mortality in immunocompromised and immunosuppressed individuals. The two major immediate early proteins of HCMV, immediate early protein 1 & 2 (IE1 & IE2), are both nuclear localized proteins that are key to regulating both host and viral gene expression during infection. IE1 and IE2 share a common N-terminal region of 85 amino acids with the C-termini of these proteins diverging due to alternative splicing. A predicted nuclear localisation signal (NLS) resides in the shared N-terminus of IE1 and IE2, with two additional NLS predicted to be encoded by exon 5 of IE2. However, the mechanistic basis regulating the nuclear localisation of these proteins remains to be elucidated. To identify the structural basis of binding of the NLS of IE1 & IE2 to nuclear import receptors (importins) each NLS was crystallised with importin α before the atomic structure of the complex was resolved. In addition, the specificity of the IE1/IE2 NLS for specific importin family members was determined by fluorescence polarization assay and electromobility shift assays. The crystal structure of the shared IE1/IE2 NLS in complex with an importin molecule defined that this NLS was bipartite in nature with key basic residues binding in both the major and minor binding site of the importin molecule. The NLS sequences unique to IE2 exhibited monopartite binding to the major site of importin alpha. Analysis of binding specificity for importin family members indicated that the tested NLS bound with nanomolar affinity to members of each importin α subfamily with enhanced affinity noted for importin alpha 3. This biochemical and structural analysis provides a platform for understanding viral-host structure function relationships during CMV infection as well as providing insights into the contribution of specific importin α isoforms to regulating the initiation of the viral gene expression cascade.



Poster Presentations



Poster Presentations

Name: **Xiaojun Yuan**

Theme: Pathogens

Poster Position: 73

Title: **Tale of the tail: the role of the C-terminal tail in the conformation and stability of PanD**

Authors: *Xiaojun Yuan, Jobichen Chacko, Isabel Barter and Christina Spry*

Mycobacterium tuberculosis is the bacterial pathogen causing the deadly disease tuberculosis (TB). The current treatment for TB typically takes more than 6 months and requires a combination of drugs. Pyrazinamide (PZA) is one of the critical drugs used in this treatment. After administration, PZA is hydrolysed to its active derivative pyrazinoic acid (POA), the mechanism of which is not fully understood. Since mutations in the Mycobacterium tuberculosis panD gene was found to confer resistance to PZA and POA, its translational product – the L-aspartate α -decarboxylase (PanD) protein has been proposed to be the target of POA. PanD converts aspartate to α -alanine in the pantothenate biosynthesis pathway, a pathway crucial for Mycobacterium tuberculosis virulence but absent from humans. Currently, our understanding of the molecular mechanism(s) underpinning POA's effect on PanD is incomplete.

In an aqueous environment, PanD predominantly exists in a well-characterised tetrameric form, the X-ray crystal structure of which has been resolved. However, we have also determined it exists as higher-order oligomers for which the structures are unknown. Furthermore, the C-terminal tail is missing from the available crystal structures, possibly due to its flexibility. This C-terminal tail carries particular significance for two reasons: (i) it bears the majority of the PZA/POA resistance-conferring mutations, and (ii) it has been shown to mediate recognition by the protease complex Clp (an interaction POA has been proposed to promote), leading to PanD degradation. Tail truncation mutants have also been shown to confer resistance to POA, reportedly by interfering with its binding to the active site of PanD. Another possible mechanism for resistance would be that tail truncation prevents recognition by Clp. In this study, we use a combination of size exclusion chromatography, AlphaFold2 and cryo-EM to analyse the structure of the higher-order oligomers of PanD, the C-terminal tail, and its role in oligomerisation. Our findings will be presented. We also show that the C-terminus plays a role in auto-processing of PanD into the functional form, and stability of the PanD tetramer. Our results improve our understanding of PanD and the mechanism of POA against PanD, which provides valuable information for discovery of novel anti-TB drugs to combat this lethal and persistent disease.



Poster Presentations

Name: **Carl McCombe**

Theme: Plant Biochemistry

Poster Position: 74

Title: **Hijacking Phosphate Homeostasis: How Pathogenic Fungi Exploit the Plant Phosphate Starvation Response**

Authors: *Carl L McCombe, Chenie S Zamora, Ely Oliveira-Garcia & Simon J Williams.*

Phosphorus is an essential and often limiting nutrient for crop production. The phosphate starvation response pathway is essential for maintaining phosphorus homeostasis and regulating interactions with beneficial microbes, such as phosphate-providing arbuscular mycorrhizal fungi. In this presentation, I will demonstrate how pathogenic fungi can manipulate plant phosphate sensing pathways by using infection-promoting secreted proteins (called effectors). We have identified a conserved family of predicted Nucleoside diphosphate linked to moiety-X (Nudix) hydrolase effectors in multiple pathogenic fungi, including the devastating *Magnaporthe oryzae* (the causal agent of rice blast disease). Through a combination of experimental and computational structural biology, in vitro enzymatic assays, and in planta analysis, we have established that Nudix hydrolase effectors from *M. oryzae* and other phytopathogenic fungi hydrolyse plant inositol pyrophosphate (PP-InsP) signalling molecules. Plants sense PP-InsP levels to control phosphate starvation responses, mostly via the activation of the phosphate response (PHR) transcription factor family. Accordingly, these effectors activate PHR transcription factors in plants. Phosphate starvation induction by the effectors coincides with the suppression of immune responses, consistent with previous phosphate deprivation studies which assess plant immunity. Importantly, when the expression of one inositol pyrophosphate hydrolysing effector from *M. oryzae* was reduced using RNAi, there was a significant decline in the pathogen's virulence on rice and an increase in plant immune responses. Therefore, the Nudix hydrolase effector is an ideal target for future efforts to improve rice blast disease resistance and strengthen global food security. Altogether, our research elucidates a novel molecular mechanism whereby various pathogenic fungi directly target a conserved signalling molecule involved in plant phosphate homeostasis to enhance disease progression.



Poster Presentations



Poster Presentations

Name: **Daniel Geiberras**

Theme: Plant Biochemistry

Poster Position: 75

Title: **Altering the performance of plant indole synthases (INS) via rational design.**

Authors: *Daniel Geiberras, Trevor W Stevenson, David M Stalker*

Blue indigo dye has been used by humans for thousands of years, historically derived from plants such as *Isatis tinctoria* and *Persicaria tinctoria*. These plants divert indole-3-glycerol phosphate from the tryptophan biosynthetic pathway towards indigo production via a specialised tryptophan synthase α (TNS- α) homologue, designated as indole synthase (INS). These plants accumulate vast quantities of indigoid metabolites as they develop. In this study, the activity of various native indigo producing plant INS enzymes was altered via a rational design process, using the extensively characterized INS from *Zea mays* as an initial comparative template. Activity of these synthetic INS enzymes was determined via a mutant *Escherichia coli* *trpA* complementation assay. INS amino acid residues that form loops 2 and 6, along with those thought to play a role in protein-protein interaction, were targeted for substitution. Substitutions to loop 6 of the *I. tinctoria* and *P. tinctoria* INS enzymes resulted in enhanced function as compared to the wild-type enzymes. Making equivalent but opposite changes to loop 6 of the *Z. mays* INS reduced its functionality. Substitutions made in loop 2 and residues thought to be involved in protein-protein interaction had lesser impact on INS activity. Clearly, the amino acid substitutions that occurred in loop 6 during the divergence of each INS from its parent TNS- α homologue are critical to INS gaining a specialised indole synthesizing function in plants.



Poster Presentations



Poster Presentations

Name: **Min Chen**

Theme: Plant Biochemistry

Poster Position: 76

Title: Biochemistry of photopigment and photopigment-binding protein complexes -- Heterodimer of allophycocyanin gives the most red-shifted absorption at 730 nm

Authors: *Min Chen*

Phycobilisomes, the main light-harvesting complexes in cyanobacteria, absorb light ranging from green to red, where chlorophylls have poor absorbance. Allophycocyanin (Apc) is located in the core of phycobilisomes and functions as the energy terminal emitters intermediating the energy of bulk phycobilisomes to the chlorophyll-binding protein complexes in the photosynthetic membranes. Here, we present a newly isolated phycobiliprotein complex that absorbs red and far-red light. According to proteomic analysis, this special red-shifted allophycocyanin is encoded by gene ApcD4, which has 4 cys residues, the potential chromophore binding sites. We confirmed phycocyanobilin (PCB) is the chromophores covalent bound in the red-shifted allophycocyanin at to the conserved Cys78. Site-directed mutagenesis revealed that Cys 61 and Cys78 are essential for binding chromophore, although only one chromophore bound at Cys78.

Additionally, the recombinant chromophylated monomer ApcB2 showed absorption of 615 nm and the chromophylated monomer ApcD4 has a red-shifted absorption of 688 nm. Interestingly, the heterodimer of ApcD4/ApcB2 demonstrated absorption of 730 nm and fluorescence emission peak at 742 nm. These red-shifted phycobiliprotein complexes were also isolated from the chlorophyll f-containing cyanobacterium, *Halomicronema hongdechloris*, grown under far-red light conditions. This observation indicated that heterodimer of ApcD4/B2 likely functions as energy terminal emitter for Chl f-binding protein complexes.

Spectral analysis of dissociated and denatured phycobiliprotein complexes show that the same bilin chromophore PCB is exclusively used in ApcB2 and ApcD4, suggesting that the red-shifted absorption features are due to altered states of this chromophore within the protein complexes. These new red-shifted phycobilisomes have implications for flexible strategies in chromatic acclimation, which would improve light-harvesting efficiency. The biochemical mechanism of red-shifted spectral feature will be discussed.



Poster Presentations



Poster Presentations

Name: **Joanna Melonek**

Theme: RNA Biology

Poster Position: 77

Title: **Discovery of a new clade within the mitochondrial transcription termination factor family implicated in fertility restoration in plants**

Authors: *Joanna Melonek & Ian Small*

In commercial hybrid breeding, cytoplasmic male sterility (CMS) is used to block self-pollination of plants. In turn, Restorer-of-fertility (Rf) genes are applied to suppress the CMS-trait and restore self-pollination.

Most restorer genes in crops encode RNA-binding pentatricopeptide repeat (PPR) proteins. Plants have hundreds of PPR proteins but only a few act as Restorers-of-fertility and these define a clade referred to as 'Restorer of fertility like' PPRs (RFL-PPRs). Recently, several candidates for restorer genes have been found to encode members of the mitochondrial transcription termination factor (mTERF) family in rye and barley. No sequence similarity between mTERF and PPR proteins exists, however, like PPRs, mTERFs function in RNA-associated processes and can directly bind to RNA. Currently, mTERFs remain a largely understudied group of organellar sequence-specific RNA-binding proteins in plants.

Our studies have shown that a group of mTERF genes share several genomic features with the RFL-PPR clade. We demonstrated that, as for RFL-PPRs, one clade of mTERF genes is highly expanded in plants, especially cereals and that they are organised into clusters adjacent to or intermixed with RFL-PPR clusters in the genome. Similarly to RFL-PPRs, clustered mTERFs showed extreme levels of copy-number and structural variation within and between species and illustrate the evolutionary mechanisms at work. Our analyses revealed that the location of mTERF clusters overlaps with mapped Rf loci in wheat and rye. Based on these discoveries we named this clade RFL-mTERFs.

Future studies will aim to characterise the newly discovered RFL-mTERF clade and understand its contribution to fertility restoration in plants.



Poster Presentations



Poster Presentations

Name: **Dayangku Nordiyana Binti Pengiran Hassanel**

Theme: RNA Biology

Poster Position: 78

Title: **Alternative Polymers for More Effective mRNA-LNP Vaccines**

Authors: *Dayangku Nordiyana B P Hassanel, Emily H Pilkington, Stephen J Kent, Nghia P Truong, Colin W Pouton*

Current mRNA vaccines are relatively safe and effective against COVID-19. However, they contain a widely used polymer – poly(ethylene glycol) (PEG) in their lipid nanoparticle (LNP) formulations, known to induce an antibody response resulting in rapid clearance of PEG-based therapeutic upon subsequent administrations. It is therefore highly desirable to find alternative polymers which can replace the PEG component in mRNA vaccines, while still maintaining the vaccines' efficacy. We employed reversible addition-fragmentation chain transfer (RAFT) polymerisation to synthesise five PEG alternatives that could stabilise LNPs encapsulating mRNA molecules. Importantly, the resultant RAFT polymer-LNPs exhibited analogous or higher in vivo transfection of a luciferase mRNA cargo in mouse muscle after 24 h of intramuscular injection, compared to the traditional PEG-based formulation. Additionally, the RAFT polymer-LNP formulation having the highest gene expression in the muscle also displayed higher ovalbumin-specific IgG antibody production compared to the conventional PEG-LNP formulation. This can be attributed to our synthesis strategy which allowed the introduction of positive charges along the polymer backbone, hence resulting in significantly improved in vivo gene expression and antibody production. This work expands the potential of RAFT polymers for use in clinical vaccines and offers an innovative strategy to develop more effective mRNA vaccines.



Poster Presentations



Poster Presentations

Name: **Ejiroghene Evivie**

Theme: RNA Biology

Poster Position: 79

Title: **What is the Role of the Ancient Plant miR159- GAMYB Pathway?**

Authors: *Ejiroghene Evivie, Yujun Sun, Luoixian Wang, Ke Lu, Leila Blackman, Julian Greenwood and Anthony Millar*

The highly conserved microR159 (miR159) has been widely studied in multiple, diverse plant species, however, no clear conserved functional role has been identified. It acts as a genetic switch, specifically silencing the expression of a family of regulatory GAMYB genes that encode R2R3 MYB domain transcription factors. Inhibiting miR159 in Arabidopsis, tobacco (*Nicotiana tabacum* and *Nicotiana benthamiana*) and *Oryza sativa*, through constitutive expression of a MIM159 target mimic transgene, resulted in the de-repression of GAMYB expression in all species. This had a strong negative impact on growth and development, resulting in stunted growth in all these species. In *Nicotiana tabacum*, RNA-seq analysis found that genes corresponding to a strong and broad defense response was strongly induced, and consistently these MIM159 *N. tabacum* plants appeared highly resistant to a major pathogen, *Phytophthora parasitica*. However, whether resistance was due to the upregulated defence pathways, or as a result of the dwarf stature needed resolving. Therefore, I am developing transgenic systems to inhibit miR159 transiently, via the expression of an inducible MIM159 transgene, or an inducible GAMYB gene that is resistant to miR159 silencing using the inducible XVE promoter which only allows expression in the presence of estradiol. I will report progress on my experiments.



Poster Presentations



Poster Presentations

Name: **Bernhard Lechtenberg**

Theme: Structural Biology

Poster Position: 80

Title: **Structural basis of the catalytic mechanism, specificity and regulation of the RBR E3 ubiquitin ligase family**

Authors: *Xiangyi S Wang, Thomas R Cotton, Simon A Cobbold & Bernhard C Lechtenberg*

Ubiquitination is a post-translational modification, best known as a marker for proteasomal degradation; however, the vast diversity of ubiquitination signals enables it to modify protein function in many other ways, including protein activity, interactions and subcellular localisation. This diversity allows the ubiquitination machinery to regulate nearly all processes inside the eukaryotic cell. The final step of the ubiquitination cascade is the transfer of the small protein ubiquitin from an E2 enzyme/ubiquitin conjugate to a target protein. This reaction is catalysed by the more than 700 E3 ubiquitin ligases that govern most of the selectivity and specificity of the system.

The 14 human members of the RING-between-RING (RBR) E3 ubiquitin ligase family are highly regulated enzymes with specific signalling functions, including in innate immunity and mitochondrial and ribosomal quality control. These specialised functions make the RBR ligases prime drug targets in diseases such as the neurodegenerative diseases Parkinson's disease (the RBR E3 ligase Parkin) and Gordon-Holmes Syndrome (RNF216) and inflammatory diseases (HOIP, HOIL-1). However, our limited understanding of the catalytic mechanism and regulation of the RBR ligases slows research into their physiological functions, roles in diseases and drug discovery.

To address these gaps in our knowledge, we combine biochemical, biophysical and structural techniques to characterise the regulation, catalytic mechanism and specificity of the RBR E3 ligase family. Using different techniques to stabilise transient enzyme complexes, we have determined the crystal structures of multiple RBR E3 ubiquitin ligases (HOIP, HOIL-1, RNF216) captured in different catalytic states in complexes with their E2/ubiquitin conjugate substrates and di-ubiquitin products. Our structures visualise the conformational flexibility of the RBR ligases throughout their catalytic cycle. We validate these structures using in vitro and cellular functional and biophysical assays. Our work identifies that and visualises how RBRs are allosterically activated by specific polyubiquitin chains in a feed-forward mechanism. Our work also identifies several structural alterations of the catalytic RING2 domain in different RBR enzymes. We show how these alterations determine RBR substrate and poly-ubiquitin chain specificity.

Together, our studies identify the defining catalytic and regulatory principles of the RBR E3 ubiquitin ligase family and highlight distinct adaptations of individual RBR family members to enable their specific cellular functions. Our work provides the foundation for subsequent functional

studies of RBR E3 ligase family members in cells and animals and will guide future drug discovery efforts targeting these enzymes in neurodegenerative or autoinflammatory diseases.



Poster Presentations



Poster Presentations

Name: **Clement Luong**

Theme: Structural Biology

Poster Position: 81

Title: **How do chromatin readers modulate the activity of chromatin remodellers?**

Authors: *Clement Luong, Lucien Lambrechts, Xavier Reid, Joel Mackay*

Eukaryotic life has evolved diverse methods to manipulate their genetic information. Many of these methods involve recognition and modulation of the DNA that transcends the genetic code itself. Chromatin remodelling enzymes are a prominent class of proteins that manipulate genetic material by sliding DNA against histone octamers to alter gene accessibility.

Chromodomain helicase DNA binding protein 4 (CHD4) is an essential ATP-dependent chromatin remodeller that operates broadly in complex animals. Despite its significance, the mechanisms that regulate CHD4 activity remain unclear. It is known, however, that CHD4 can interact directly with members of the highly conserved bromodomain and extra-terminal domain (BET) family of proteins. BET proteins 'read' acetyl-lysine on histones and other proteins and disruption of their normal function is a hallmark of several cancers.

We present work that begins to illuminate the mechanistic and functional connection between CHD4 and BET-family proteins. We show that the three ubiquitous BET proteins play functionally distinct roles in regulating CHD4 activity in lieu of their strong homology and make headway into understanding the mechanisms that govern transcriptional regulation.



Poster Presentations



Poster Presentations

Name: **Dalton Ngu**

Theme: Structural Biology

Poster Position: 82

Title: Characterisation of the solute-binding protein ModA from *Pseudomonas aeruginosa* and its role in molybdenum homeostasis

Authors: *Dalton H. Y. Ngu, Eve A. Maunders, Bryan, Y. J. Lim, Zhenyao Luo, Christopher A. McDevitt and Bostjan Kobe*

For the clinically significant and opportunistic Gram-negative bacterial pathogen *Pseudomonas aeruginosa*, the redox-active trace metal molybdenum is essential for energy production and respiration under anaerobic conditions. Acquisition of molybdenum occurs via the high-affinity ATP-binding cassette permease ModABC. Because solute-binding proteins (SBPs) are only present in bacteria, this makes them attractive drug targets. Therefore, this study aims to characterise the periplasmic SBP ModA from *P. aeruginosa* PAO1 and its role in molybdenum homeostasis.

Ligand-dependent protein mobility shift gel electrophoresis and nano-differential scanning fluorimetry suggest that ModA binds specifically to group 6 metal oxyanions (chromate, molybdate, tungstate). Despite their similar sizes, binding affinity for chromate is ~ 900-fold lower than molybdate and tungstate. The X-ray crystal structure of ModA shows a non-contiguous dual-hinged bilobal structure, with the ligand-binding pocket located at the interface between the domains. Each domain has five α -helices surrounding a central five-stranded mixed β -sheet. Upon ligand binding, ModA employs a "Venus' fly-trap" mechanism, resulting in a relative rotation of 22° of the domains and occluding the pocket from the bulk solvent, with the oxyanion coordinated by four residues contributing six hydrogen bonds. Phylogenetic analysis of 485 *Pseudomonas* ModA sequences shows that the ligand-binding residues and β -sheet structural elements are highly conserved. Comparison with orthologous bacterial ModA structures shows that ModA is highly conserved within the cluster D-IIIa SBPs. Despite chromate exposure causing dysregulation of molybdenum homeostasis, deletion of *modA* had no impact on chromate sensitivity and accumulation. Interestingly, this is the first study to directly compare ligand-free and ligand-bound ModA from the same organism and to show a unique oxyanion-binding chemistry with one less coordinating residue and hydrogen bond. Given the stability of ModA and high binding affinity for the environmentally toxic chromate oxyanion, future research would include protein engineering for bioremediation, besides development as a potential drug target.



Poster Presentations

Name: **Jeeun Shin**

Theme: Structural Biology

Poster Position: 83

Title: **Structurally investigating multi-drug resistance ABC transporter of *Candida albicans***

Authors: *Jeeun Shin, Jobichen Chacko, Chung-Han Tsai, Melanie Rug, Joseph Brock*

Candidiasis, caused by *Candida* yeast, is a fungal infection that can range in severity from mild to potentially fatal, depending on the type and severity of the infection. Common non-invasive forms of

candidiasis, such as oral or vaginal infections, can be effectively treated with medications. However,

invasive candidiasis affecting the bloodstream or organs can result in hospitalization and necessitate

intensive care.

In recent times, there has been a concerning increase in the prevalence of multi-antifungal resistance in

species of *Candida* yeast. Notably, individuals with COVID-19 who are receiving medical care are particularly susceptible to invasive candidiasis and co-infection. The Centers for Disease Control and

Prevention (CDC) have emphasized the association between COVID-19 cases and increased mortality due

to invasive candidiasis.

Research on antifungal resistance in *Candida albicans* in particular has revealed that one of the primary

mechanisms involves the overexpression of multidrug efflux ABC transporters, such as the *Candida* drug

resistance 1 protein (CalbDR1 or Cdrp1). CalbCDR1 protein is involved in drug azole-type drug transport,

human steroids, rhodamine 6G and phospholipid translocation, thus making it a promiscuous transporter.

Investigating the structure of CalbCDR1 is crucial for advancing our understanding of its function and the

underlying mechanism of observed multi-drug resistance. In this study, we successfully expressed and

purified mTurquoise2 fusion CalbCDR1 using *Saccharomyces cerevisiae*. The expression level was assessed

through whole-cell and in-gel fluorescence analysis. Various detergents were screened using

Fluorescence-detection size-exclusion chromatography (FSEC) to identify the optimal conditions for large scale purification. Subsequently, we solved the structure of fluconazole bound CalbCDR1 using single

particle Cryo-EM of 3.4 Å, revealing inward-open conformation. The discovery of the azole type bound in

the TMD of CalbCDR1 offers a new avenue for advancing potent antifungal agents.



Poster Presentations



Poster Presentations

Name: **Lucien Lambrechts**

Theme: Structural Biology

Poster Position: 84

Title: **What is the role of the BET proteins and chromatin modifications in CHD4 chromatin remodelling?**

Authors: *Lucien Lambrechts, Joel Mackay*

How genes are switched on or off in a controlled manner remains a fundamental question in molecular biology. In eukaryotes, gene regulation involves physical restructuring of chromatin, the complex of DNA and histone proteins. Several important aspects of this process have already been identified, including reversible covalent modifications of histone proteins and enzyme catalysed movement of histones relative to DNA. The BET (Bromodomain Extra-Terminal domain) proteins bind to acetylated chromatin and represent major therapeutic targets due to their role in aberrant gene regulation. These proteins seem to recruit transcriptional regulators to regions of acetylated chromatin, which in turn mediate effects on gene transcription.

One such BET-associated transcriptional regulator is CHD4 (Chromodomain Helicase DNA-binding protein 4), a ubiquitous and essential – but poorly understood – ATP-dependent chromatin remodeller found at the promoters and enhancers of many active genes. We have used a range of tools including fluorescence remodelling assays, binding assays, and cryo-electron microscopy to probe the structural basis for this interaction and its functional consequences. Our data deepen our understanding of the mechanisms by which gene transcription is regulated and offer insight into two classes of proteins with prominent roles in a variety of human disorders.



Poster Presentations



Poster Presentations

Name: **Michael Landsberg**

Theme: Structural Biology

Poster Position: 85

Title: **RHS proteins: Containers for change**

Authors: *Yu Shang Low, Chandra Rodriguez, Jason N Busby, Minh-Duy Phan, Mark A Schembri, J Shaun Lott & Michael J Landsberg*

RHS (rearrangement hotspot) proteins are a family of proteins, ubiquitous throughout bacteria, characterised by the presence of multiple copies of a 15 amino acid consensus sequence known as the YD repeat. YD repeats are themselves found in a wider range of proteins distributed throughout bacteria and eukaryotes. The prototypical RHS-encoding genes were originally identified in *E. coli* and were proposed to play key roles in genomic rearrangement. In recent years however, bacterial RHS proteins have been increasingly implicated as virulence effectors associated with a variety of toxin delivery systems, including Type 5 and Type 6 Secretion Systems and the tripartite ABC or Tc toxin systems. Previously we solved the structure of the first example of an RHS protein – a heterodimeric complex of two proteins (YenB and YenC2) that associates with a larger, pore-forming protein complex from the insecticidal bacterium, *Yersinia entomophaga*. The YenB-C2 complex forms a protein cage that encapsulates a cytotoxic cargo which, upon association with the pore-forming component, delivers the cargo into targeted host cells via the pore lumen. The protein cage is primarily comprised of YD repeats, each of which adopts a beta-hairpin fold. A highly conserved domain which separates the C-terminal cytotoxic cargo from the N-terminal YD-repeat domain functions as an autoprotease, liberating the toxin from the protein cage. To investigate the extent to which this general mechanism of toxin packaging, assembly and release is conserved in RHS proteins found in other functional contexts, we have used cryo-EM to solve the structure at 2.9 Å of RhsA, a putative virulence effector that resembles T6SS-associated effectors, but for which the associated secretion system is currently unknown. We also used cryo-EM to solve the 3.6 Å structure of a bacterial homologue of eukaryotic teneurins. The latter are transcriptional regulators that promote neurite outgrowth and adhesion during development, and our analysis suggests that these mammalian signalling systems have evolved from ancient bacterial toxin systems. Collectively, our results demonstrate that RHS proteins have evolved to perform a variety of cellular functions that involve packaging of bioactive molecules. The underlying YD repeats can be used to assemble protein cages of various sizes, a finding that may have applications in protein engineering.



Poster Presentations

Name: **Mikayla Hoad**

Theme: Structural Biology

Poster Position: 86

Title: Adeno-associated viruses utilise the classical nuclear import pathway via interactions with host importin-alpha to perform their role as a gene therapy vector

Authors: *Mikayla Hoad, Jade K. Forwood, Justin A. Roby*

Adeno-associated viruses (AAVs) are small ssDNA viruses that do not cause phenotypic disease manifestations and have limited capacity to invoke the immune response. They are unable to replicate without a 'co-infection' helper virus, therefore there is no direct link to induction of illness. AAVs are the world's most studied viral vectors for gene therapy, thus many aspects of their cell transduction pathway have been revealed in detail. However, the specific mechanism/s of AAV nuclear localisation (an integral step for gene therapy) remain largely unknown. AAV capsid proteins VP1, VP2, and VP3 form the outer virion capsid shell and are responsible for directing AAVs through the cytoplasm and into the nucleus of the host cell, however, the manner in which AAVs translocate through the nuclear membrane has not been fully understood in detail previously outside of that it must move from cytoplasm to nucleus, that stretches of basic residues may be facilitating interactions with host proteins. As such, we aimed to reveal the biochemical and structural interactions between host nuclear transport proteins importin-alpha ($IMP\alpha$), including $IMP\alpha$'s different isoforms, and importin-beta ($IMP\beta$) and AAV serotypes 2 and 3. Fluorescence polarisation and electro-mobility shift assays were employed to determine $IMP\alpha$ isoform specificity and overall strength of binding between AAV VP1 and importin proteins. Our data shows that AAV capsid proteins preferentially use $IMP\alpha$ proteins (and not $IMP\beta$) for translocating into the nucleus, indicating use of the classical nuclear localisation pathway. Further, we found that AAV serotypes do have $IMP\alpha$ isoform specificity, which varies between AAV serotypes. In addition, high resolution structural data was obtained of AAV capsid protein VP1 in complex with $IMP\alpha$ proteins, $IMP\alpha 2$ and $IMP\alpha 3$, revealing direct binding interactions and key residues. Mutational studies of these key residues utilizing similar biochemical and structural analysis methods revealed the exact residues and regions necessary for $IMP\alpha$ binding. This data can have a significant impact on AAV capsid engineering and subsequent nuclear transduction for gene therapy purposes. For example, in the case of inefficient translocation into the nucleus by a particular AAV serotype, the sequence driving nuclear localisation can be modified to one shown to have a high affinity for a particular $IMP\alpha$ isoform that is more highly expressed within the targeted tissue.



Poster Presentations

Name: **Praveena Thirunavukkarasu**

Theme: Structural Biology

Poster Position: 87

Title: **Understanding the molecular recognition of *Bacteroides fragilis* glycosphingolipids by Natural Killer T-cell receptor**

Authors: *Praveena Thirunavukkarasu, Sungwhan F. Oh, Heebum Song, Ji-Sun Yoo, Da-Jung Jung, Deniz Erturk-Hasdemir, Yoon Soo Hwang, Changwon C. Lee, Jérôme Le Nours, Hyunsoo Kim, Jesang Lee, Richard S. Blumberg, Seung Bum Park, Dennis L. Kasper and Jamie Rossjohn*

Understanding the molecular recognition of *Bacteroides fragilis* glycosphingolipids by Natural Killer T-cell receptor

Praveena Thirunavukkarasu¹, Sungwhan F. Oh^{2,3}, Heebum Song⁴, Ji-Sun Yoo³, Da-Jung Jung³, Deniz Erturk-Hasdemir², Yoon Soo Hwang⁴, Changwon C. Lee², Jérôme Le Nours¹, Hyunsoo Kim⁴, Jesang Lee⁴, Richard S. Blumberg⁵, Seung Bum Park⁴, Dennis L. Kasper² and Jamie Rossjohn^{6,7}

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The human gut microbiota comprises more than 50% of *Bacteroides* species that produce small diffusible molecules like sphingolipids that play a key role in modulating the host's immune responses.

In particular, *Bacteroides fragilis* produces glycosphingolipids similar to α -galactosylceramides termed as 'BfaGCs' that can activate type I Natural Killer T (NKT) cells. While they share key

chemical similarities with the type I NKT cell marker antigen, α -galactosylceramide (KRN7000), they possess distinctive structural features including short sphinganine chains, branching and functional groups, implying a basis for their unique immunomodulatory properties. Our structural studies on two such CD1d-presented BfaGCs in complex with the type I NKT TCR revealed the TCR adopted a parallel docking topology atop the F'-pocket of CD1d in recognising the presented BfaGCs. Interestingly, the terminal sphinganine branching of the BfaGCs mediated unique interactions within the F'-pocket of CD1d, providing a mechanism for their differing agonistic properties. The NKT TCR recognised the CD1d presented stimulatory and non-stimulatory BfaGCs with nanomolar affinities. Thus, BfaGCs were demonstrated to be bonafide CD1d ligands that function as immunomodulatory mediators influencing the host's defence in the context of NKT cells. Together, this study sheds light on a better understanding of the existing symbiotic relationship between the microbes producing these endogenous lipids and the host. Furthermore, our observations provide clues for the design of new therapeutics for treating a wide range of human diseases.

References:

(* denotes co-first authors)

Sungwhan F. Oh*, Praveena T*, Hee Bum Song, Ji-Sun Yoo, Da-Jung Jung, Deniz Erturk-Hasdemir, Yoon Soo Hwang, Changwon C. Lee, Jérôme Le Nours, Hyunsoo Kim, Jesang Lee, Richard S. Blumberg, Jamie Rossjohn, Seung Bum Park, and Dennis L. Kasper. Host immunomodulatory lipids created by symbionts from dietary amino acids *Nature*. 2021 Dec;600(7888):302-307.



Poster Presentations



Poster Presentations

Name: **Sara Esmaeili**

Theme: Structural Biology

Poster Position: 88

Title: **Structural basis for nuclear import defects of mutant p53 in cancer**

Authors: *Sara Esmaeili, Crystall MD Swarbrick, Brian P McSharry, Jade K Forwood, Martin Pal*

TP53 is a tumour suppressor gene commonly mutated in cancer. As a transcription factor, the activity of p53 depends on its localisation into the nucleus. Previously, efforts have been made to characterise the nuclear localisation sequence (NLS) that enables translocation of p53 from the cytosol into the nucleus upon its activation. Surprisingly, to date there is still debate around the exact mechanism for the nuclear import of p53, including for its preference of nuclear import shuttle protein. Here, we describe a high-resolution protein crystal structure of the p53 NLS bound to Importin-alpha (IMPA), confirming that p53 exhibits a bona-fide bipartite NLS. Importantly, using p53 tumour databases we identified clinically relevant mutations in the NLS of p53 in a variety of cancers, potentially interfering with the binding of mutant p53 to IMPA. Confirming this, we performed biochemical binding assays *in vitro* and show that mutations in the NLS of p53 block binding to IMPA in both, the minor and major IMPA NLS-binding pocket. Since the majority of p53 mutations occur in the DNA-binding domain of the protein, targeting the nuclear entry of mutant p53 utilising structure-guided gene editing approaches presents a potential novel therapeutic strategy.



Poster Presentations



Poster Presentations

Name: **Xiaojing Huang**

Theme: Structural Biology

Poster Position: 89

Title: **Oligomeric remodelling by molecular glues revealed using native mass spectrometry and mass photometry**

Authors: *Xiaojing Huang, Hari Kamadurai, Piro Siuti, Ezaz Ahmed, Jack Bennett & William A. Donald*

Molecular glues stabilize interactions between E3 ligases and novel substrates to promote substrate degradation, thereby facilitating the inhibition of traditionally “undruggable” protein targets. However, most known molecular glues have been discovered fortuitously or are based on well-established chemical scaffolds. Efficient approaches for discovering and characterizing the effects of molecular glues on protein interactions are required to accelerate the discovery of novel agents. Here, we demonstrate that native mass spectrometry and mass photometry can provide unique insights into the physical mechanism of molecular glues, revealing previously unknown effects of such small molecules on the oligomeric organization of E3 ligases. When compared to well-established solution phase assays, native mass spectrometry provides accurate quantitative descriptions of molecular glue potency and efficacy while also enabling the binding specificity of E3 ligases to be determined in a single, rapid measurement. Such mechanistic insights should accelerate the rational development of molecular glues to afford powerful therapeutic agents.



Poster Presentations



Poster Presentations

Name: **Akhil Kumar**

Theme: Structural Biology

Poster Position: 90

Title: **Development of a Protein-based Gold Biosensor**

Authors: *Akhil Kumar, Dr. Dylan Peukert, Prof. William Skinner, Prof. Nigel Spooner, Dr. John Bruning*

The current method for determining the Au content in mining samples is the fire assay; it is a labour intensive and time-consuming method requiring samples to be transported to an off-site laboratory to determine the Au content. Fluorescent proteins are being investigated to create a biosensor that can provide real-time detection of Au in the μM or lower range. The availability of information on Au content in near-real time provides the potential to increase the efficiency of mining and mineral processing while also being low-cost and environmentally friendly. Previous research showed fluorescent proteins bind to elements like Cu, Ni, Zn and Co which reduced the fluorescent intensity. Testing with $500 \mu\text{M}$ of Au revealed these fluorescent proteins were getting quenched. The Apo structure of the fluorescent proteins was solved using X-ray crystallography allowing the identification of the Au binding site responsible for this quenching effect. Structural analysis will allow directed protein engineering to improve affinity and elemental specificity.



Poster Presentations



Poster Presentations

Name: **Andrea Daners**

Theme: Structural Biology

Poster Position: 91

Title: Investigating the structural basis for the interaction between chromatin remodeler CHD4, the nucleosome and the acetylation reader BRD4

Authors: *Andrea Daners, Joel Mackay*

Chromodomain helicase DNA-binding protein 4 (CHD4) is an essential chromatin remodelling enzyme that influences gene expression by regulating access to genes for transcription. Despite its critical role, the mechanisms that recruit and regulate CHD4 are only partially understood. To develop our understanding of how CHD4 is regulated and recruited, we have begun to investigate its interactions with its binding partners, specifically the BET-bromodomain proteins (BRD2/3/4/T).

BRD4 has been shown to increase the remodelling activity of CHD4 in vitro (unpublished data) however, the molecular mechanisms of this interaction remain unclear. We have attempted to image CHD4 and BRD4 in complex with a nucleosome by single cryo-electron microscopy as this would give insight into which regions and residues are important to this interaction. Here, we present progress towards this goal, which consists of a low-resolution map of the complex.



Poster Presentations



Poster Presentations

Name: **Emily Furlong**

Theme: Structural Biology

Poster Position: 92

Title: **Molecular structure of an axle-less F1-ATPase**

Authors: *Emily J Furlong, Yi C Zeng, Simon HJ Brown, Meghna Sobti & Alastair G Stewart*

F₁F_o ATP synthase is a molecular rotary motor that can generate ATP using a transmembrane proton motive force. Isolated F₁-ATPase catalytic cores can hydrolyse ATP, passing through a series of conformational states involving rotation of the central γ rotor subunit and the opening and closing of the catalytic β subunits. Cooperativity in F₁-ATPase has long thought to be conferred through the γ subunit, with three key interaction sites between the γ and β subunits being identified. Single molecule studies have demonstrated that the F₁ complexes lacking the γ axle still “rotate” and hydrolyse ATP, but with less efficiency. We solved the cryogenic electron microscopy structure of an axle-less *Bacillus* sp. PS3 F₁-ATPase. The structure suggests why the full-length axle is required for efficient ATP hydrolysis by F₁-ATPase and provides evidence for cooperativity between the α and β subunits in the absence of the γ axle.



Poster Presentations



Poster Presentations

Name: **Emily Wagon**

Theme: Structural Biology

Poster Position: 93

Title: Investigation of two newly emerged Henipaviruses: comparing how the Langya Virus and Angavokely virus matrix proteins interact with host cell nuclear import molecules.

Authors: *Emily Wagon*

Langya virus (LayV) and Angavokely virus (AngV) are two novel, emerging pathogens within the Paramyxoviridae family and Henipavirus genus. Currently there are five known henipaviruses, Hendra, Nipah, Cedar, Mojiang, and Ghanaian viruses, all of which produce pleomorphic, enveloped virions composed of a negative sense, single stranded RNA genome. Two zoonotic henipaviruses include the highly pathogenic Hendra and Nipah viruses. Hendra virus (HeV) was first isolated in the Brisbane suburb of Hendra, Queensland in 1994 causing a fatal respiratory illness in equines and staff within the equine industry. HeV has since resurfaced on more than 50 occasions, resulting in nearly 100 equine deaths and 4 human fatalities. In 1998, Nipah virus (NiV) emerged during an outbreak among porcine farmers in Malaysia causing encephalitis with a fatality rate of 40%. Outbreaks of NiV continue to occur annually in Bangladesh and India, with fatality rates reaching up to 75%. Both HeV and NiV infect a wide range of cell types, causing significant morbidity and mortality, and as there are no targeted antiviral therapies or FDA approved vaccines for humans, these pathogens are classified as Biosafety Level 4 (BSL4) agents. The aim of this project was to investigate two newly emerged henipaviruses, LayV and AngV, by comparing how the viral matrix proteins bind and interact with host cell nuclear import molecules. The process of trafficking viral matrix proteins into host cell nuclei has been demonstrated as a vital step in coordinating viral replication and budding. Translocation into host cell nuclei occurs due to nuclear localisation signal (NLS) sequences on the viral matrix protein binding to host importin alpha (IMPα) molecules. LayV and AngV were purported to contain two and three NLS sequences, respectively. Recombinant protein expression using genetically modified organisms was initially performed to produce five IMPα isoforms, and these were investigated in combination with synthetically produced LayV and AngV NLS peptides. Qualitative analysis was undertaken using electrophoretic mobility shift assays to determine which putative NLS sequences bind to each IMPα molecule. Quantitative analysis was performed using fluorescence polarisation and microscale thermophoresis assays to determine the strength of binding between the functional NLSs and each IMPα isoform. In parallel, IMPα isoforms were combined with LayV and AngV NLS synthetic peptides in protein crystallography screening. Crystals were sent to the Australian Synchrotron in Melbourne to undergo X-ray crystallography and results used to solve structures of protein-peptide interactions. Solved structures of the functional NLS sequences of LayV and AngV matrix proteins may provide targets for antiviral therapy or vaccine development.



Poster Presentations

Name: **Erekle Kobakhidze**

Theme: Structural Biology

Poster Position: 94

Title: **Investigating the role of histone variant H2A.Z in gene regulation**

Authors: *Erekle Kobakhidze, Joel Mackay*

Many essential biological processes, from fear conditioning to stem cell differentiation are governed at least in part by the dynamic control of genes. Despite this, the mechanisms that underlie the regulation of genes are still currently poorly understood. One aspect of gene regulation is the incorporation of post translational modifications onto the histone proteins that form chromatin. These modifications are thought to act as signals to recruit transcription factors and other transcriptional regulatory proteins to specific sites within the genome. These transcriptional regulators in turn modulate chromatin architecture, activating or repressing genes, as well as control the installation and removal of histone modifications. The diversity of histone modifications and the large number of transcriptional regulator proteins forms the basis of an immensely complex system of gene regulation required for complex life.

This project focuses on the histone H2A.Z, a variant to the canonical H2A histone. H2A.Z is associated with active genes, with suggested roles in heterochromatin boundary formation, stem cell differentiation and mitosis. However, how H2A.Z is involved in these processes is unclear. H2A.Z is of particular interest as it has a variety of unique binding partners, understanding the interactions between these proteins and H2A.Z may help us understand the role of H2A.Z.

In this project we look at two of these binding partners: BRD2 and PWWP2A. These proteins are also involved in similar processes to H2A.Z. These three proteins are also linked to histone acetylation control. Here we aimed to study the interactions between BRD2, PWWP2A and nucleosomes containing H2A.Z.

We hypothesise these interactions may be mediated by acetylation of H2A.Z. Thus, part of this project consisted of the semi-synthesis of acetylated H2A.Z containing distinct patterns of acetylation using native chemical ligation. We present the results of two biochemical assays – microscale thermophoresis and electrophoretic mobility shift assays – on the binding of BRD2 and PWWP2A to a range of modified nucleosomes. These data reveal unexpected potential mechanisms of binding which have identified many new avenues in this research.



Poster Presentations

Name: **Guan-Ru Liao**

Theme: Structural Biology

Poster Position: 95

Title: **CD1c presentation of a branched ganglioside, GD1a**

Authors: *Guan-Ru Liao, Tinh-Phat Cao, Adam Shahine & Jamie Rossjohn*

Antigen presentation is key in initiating T cell-mediated adaptive immunity in disease progression such as viral infection or autoimmune diseases. Well-known antigens, peptides, are presented to receptors on T cells (TCRs) via antigen presenting molecules known as major histocompatibility complex (MHC) proteins [1]. The CD1 family of proteins are MHC-like proteins that present lipid antigens to T cells and consists of 5 isoforms in humans. One member, CD1c, can present microbial lipid-based antigens from *Mycobacterium tuberculosis* (Mtb) in protective immunity [2], as well as self-phospholipids and acylglycerides that are recognised by autoreactive T cells [3].

Gangliosides are a class of sphingolipids enriched in the brain and nerve tissues, with studies showing that self-ganglioside presentation by CD1c to T cells play an aberrant role in mediating autoimmune disorders such as multiple sclerosis (MS) [4]. However, the molecular mechanisms that mediate this mode of activation in autoimmunity remain unknown. To answer this, we determined the crystal structure of CD1c in complex with a branched ganglioside, GD1a, by first producing CD1c recombinantly and loading GD1a into the CD1c antigen binding cleft in vitro. The two fatty acid tails of GD1a are anchored in F' pockets with the ganglioside head group protruding out of G' portal. Here, as opposed to standard lipid presentation by other CD1 members such as CD1d, where the branched headgroup is presented upwards towards the typical TCR recognition site, the ganglioside headgroup is presented uniquely sideways, representing a novel mechanism of antigen presentation. In summary we demonstrated, for the first time, the mechanisms of branched ganglioside presentation by CD1c. Future work will investigate CD1c-ganglioside autoreactive T cell receptor repertoire in healthy donor and patient with disease of interest.



Poster Presentations



Poster Presentations

Name: **Hien Thy Dao**

Theme: Structural Biology

Poster Position: 96

Title: **Molecular Basis for CD4+ T cell Recognition of Citrullinated Self-antigens in Rheumatoid Arthritis**

Authors: *Hien Thy Dao, Tiing Jen Loh, Jia Jia Lim, Ravi K. Sharma, Lars Klareskog, Vivianne Malmström, Hugh H. Reid, Jamie Rossjohn*

Rheumatoid arthritis (RA) is the most common form of chronic systematic autoimmune disease, affecting approximately 1% of the worldwide population. The exact cause of RA remains unknown, yet most evidence, derived from both clinical and animal studies, points to the strong association between RA development and certain Human Leukocyte Antigen (HLA) class II haplotypes. The association is accounted for by the HLA-DRB1 gene locus with certain alleles encoding what is known as the shared susceptibility epitope (SE). RA is also strongly associated with increased citrullination, a naturally occurring enzymatic post-translational modification (PTM) of proteins whereby arginine residues are deiminated to citrulline. Over 70% of patients develop anti-citrullinated protein antibodies (ACPA) in their sera. Hence, ACPA+ sera is a hallmark of RA and is a diagnostic indicator of disease. The presence of ACPA is also indicative of an HLA class II mediated T helper cell response. The basis for the association between SE+ HLA alleles and citrullination with RA susceptibility is considered to be due in part to the ability of polar/uncharged citrulline containing self-peptide antigens to be accommodated within the positively charged P4 pocket of SE+ HLA alleles, such as HLA-DR4, whereas a native arginine containing antigen cannot, due to its positive charge preventing the binding because of like charge repulsion. This in turn leads to the presentation of neo-antigens in the periphery that may be recognised by the T cell repertoire that has no central tolerance to the citrullinated peptide-antigens as they are not presented in the thymus.

Whilst the molecular basis for the interaction between citrullinated peptides and HLA-DR4 is reasonably well understood, structural studies examining the association between HLA molecules, citrullinated epitopes and the corresponding T cell repertoire, remains limited. Accordingly, our study has aimed to shed light at the structural level on the response of autoreactive T cells and to determine their specificity towards a panel of candidate citrullinated peptide antigens, starting with citrullinated tenascin (TNC) and cartilage intermediate layer protein (CILP) peptides, both of which have been shown to be abundantly expressed in the joint synovium of RA patients and are targets of ACPA. Importantly, our data shows that citrullinated peptide can be recognised by T cell receptors (TCRs) isolated from RA patient autoreactive CD4+ T cells restricted to HLA-DR4 presenting these PTM antigens. Additionally, using X-ray crystallography, we show that the citrulline residue not only has the ability to modulate binding of self-peptides to SE+ HLA allomorphs but can also modulate TCR specificity. TCR has been shown to be specific to only

citrullinated self-peptides, providing more evidence supporting the role of citrullination in the pathogenesis of RA.

Much of the development of modern evidence-based medicine (EBM) stems from knowledge of the characteristics of molecular targets involved in disease processes. Hence, elucidating the molecular basis underlying citrullinated-peptide-HLA/TCR complex interactions may provide potential targets for the development of more affordable, higher efficacy therapeutic approaches to treat RA.



Poster Presentations



Poster Presentations

Name: **Jen Suh**

Theme: Structural Biology

Poster Position: 97

Title: **Safer, environmentally friendly pesticides targeting cattle tick hormone receptor**

Authors: *Jen Suh, Ingrid Macindoe, Hakimeh Moghaddas Sani, Nathan Lo, Emily Remnant, Ronald Hill, Joel Mackay*

The cattle tick is Australia's most serious external parasite of cattle and presents significant problems for the cattle industry. Current insecticides used against cattle ticks are nonselective, which presents issues for beneficial insects such as dung beetles.

Ecdysone receptor proteins regulate many major developmental processes in insects and vary subtly in three-dimensional structure between insect taxa. This variation provides an opportunity for the discovery and design of molecules that selectively bind to and interfere with pest ecdysone receptor proteins. We aim to discover lead compounds against the ecdysone receptor proteins that can be developed into commercial pesticides that are selectively toxic to cattle ticks but safe for dung beetles, humans, and other organisms in the environment.

The ecdysone receptor proteins are generally a heterodimer of the ecdysone receptor (EcR) subunit and the ultraspiracle (USP) subunit. We have cloned and expressed EcR with a histidine-tag and USP with a streptavidin-tag for cattle tick and dung beetle. To purify the proteins, we used affinity purification followed by size-exclusion chromatography. Our follow-up experiments include the use of a fluorescent polarisation assay to test the ligand-binding ability of the protein as well as circular dichroism to check the folding of the protein according to its predicted secondary structure. With these purified proteins in hand, we will next conduct chemical library screens to search for molecules that could selectively bind the cattle tick EcR while having lower affinity for the dung beetle homologue.



Poster Presentations



Poster Presentations

Name: **Jordan Nicholls**

Theme: Structural Biology

Poster Position: 98

Title: Unravelling the Details of Herpesvirus Single-Stranded DNA Annealing: The Structure of BALF2 by Cryo-EM

Authors: *Jordan Nicholls, Jodi Brewster, Gökhan Tolun*

Epstein-Barr Virus (EBV) is an extremely widespread dsDNA γ -herpesvirus. It is estimated that over 95% of the global population are infected with the disease, which causes life-long latent infection but can manifest as recurring symptomatic episodes. These active periods, termed lytic replication, are linked to the development of several different cancers, namely nasopharyngeal carcinomas, and Hodgkin's lymphoma. Herpesvirus lytic replication is strongly tied to high levels of homologous recombination. This occurs concurrently with the logarithmic generation of concatemeric replication intermediates during lytic replication and is thought to occur both for genomic maintenance and to increase genetic diversity. Studies focusing on the essential viral mechanisms involved are of great interest as potential antiviral targets.

BALF2 is an essential herpesvirus replication protein with dual roles as a non-specific single-stranded DNA-binding protein (SSB) and as a recombinase involved in the single-strand annealing (SSA) homologous recombination (HR) pathway (termed an annealase). As an SSB, it is involved in the formation of replication compartments (viral DNA factories), elimination of secondary structure at the replication fork, and protection of replication intermediates from nuclease digestion. As an annealase, ssDNA-binding is coupled to binding and then annealing homologous sequences of ssDNA. The latter role is a ubiquitously conserved process wherein HR is carried out in an ATP-independent manner by an Exonuclease-Annealase Two-Component Recombinase (EATR) Complex. In EBV, it is thought that the BGLF5 nuclease first exposes ssDNA which is then coated by the BALF2 annealase; this region of DNA is then annealed to a homologous region from an independently generated, and similarly processed, strand. This process is thought to occur during the generation of replication intermediates, and in the repair of spontaneous double-stranded DNA breaks.

Considering the essential roles BALF2 has both as an SSB and annealase, it is a very promising candidate for further study as an antiviral target. Unfortunately, very little is understood about its annealing mechanism. Determining the structure of BALF2 bound to DNA will provide invaluable information about its annealing mechanism. The lack of a structure is also a major roadblock in structure-based drug design.

In this study, BALF2 was cloned, expressed in insect cells, and purified via FPLC. Negative-staining electron microscopy was used to identify three distinct assemblies: monomeric BALF2, and two novel filamentous forms: irregular filaments which form in a reducing environment, and regular helical annealing intermediates. The latter of these is the functionally relevant form and was proceeded to cryogenic electron microscopy (cryo-EM) for structural determination. This revealed the filaments to be composed of two flexible BALF2-coated ssDNA assemblies arranged in a bipolar direction. A cryo-EM density map of BALF2+DNA in these filaments was reconstructed to 2.1 Å, into which an atomic model was built. This allowed for the characterization of an OB-fold, a zinc-binding loop, an active site of ssDNA-annealing, and it suggests a model for cooperative binding and oligomerization. This structure will strongly inform future studies on herpesvirus annealases, and has great potential as a starting point for structure-based drug design.



Poster Presentations



Poster Presentations

Name: **Lucy Fitschen**

Theme: Structural Biology

Poster Position: 99

Title: **Towards the Cryo-EM Structures of Viral Annealase Proteins**

Authors: *Lucy J. Fitschen, Jodi L. Brewster, Jordan J. Nicholls, Stefan H. Mueller & Gökhan Tolun.*

Double stranded DNA breaks are highly detrimental to organisms. The single strand annealing homologous recombination (SSA) pathway is one of many used for repairing these breaks. SSA is mediated by an Exonuclease-Annealase Two-component Recombinase (EATR) complex. EATRs are highly conserved throughout evolution, found in bacteriophages to humans. Two viral examples we study are the bacteriophage λ (lambda) EATR consisting of the exonuclease λ Exo and annealase Red β , and the Herpes Simplex Virus 1 (HSV-1) EATR composed of UL12 exonuclease and ICP8 annealase. Along with recombination and SSA, the bacterial genetic engineering method 'recombineering' is based on EATR proteins, specifically developed with the phage λ EATR. The HSV-1 EATR system is now seen as an appealing potential target for eukaryotic recombineering.

While there are structures available for the annealases of both phage λ Red β (1) and HSV-1 ICP8 (2), both structures are of truncated versions, and therefore, incomplete. While the structures have greatly aided our understanding of how the annealase proteins in EATRs function, the molecular mechanistic details of how these proteins process DNA and how they form an EATR complex with the partner exonucleases remain unknown. This work demonstrates the first structural investigations into the phage λ EATR complex to our knowledge and the continued structural investigations into the ICP8 complex with DNA.

The ICP8 crystal structure is not a co-crystal structure with a substrate DNA bound and is in a conformation that is possibly physiologically irrelevant. Therefore, our goal is to obtain a cryo-EM structure of ICP8 bound to DNA that reveals the detailed mechanism of annealing. Negative staining electron microscopy (NS-EM) is the prelude to cryo-EM and allows for a streamlined pipeline perfect for optimization purposes. Using NS-EM, we examined the multimeric ring and filament complexes formed by ICP8 with complementary ssDNAs.

How Red β interacts with its partner λ Exo to form an EATR and function as a complex is still unknown. We used NS-EM to examine the λ EATR. Our results show that the treatment of a dsDNA with λ Exo and Red β results in the formation of helical filaments.

Functional assays were also performed. After the successful purification of ICP8, Red β , and λ Exo, the activities of the annealase proteins were tested via a FRET (Fluorescence Resonance Energy Transfer) based EMSA (electromobility shift assay). The activity of purified λ Exo was also examined using a single-molecule characterization assay (3) determining the rate of digestion of dsDNA by λ Exo via TIRF (Total Internal Reflection Fluorescent) microscopy.

The results we present demonstrate the continued functional characterization of viral EATR proteins and show progress towards more complete structural data for the phage λ EATR and the ICP8+DNA complex via cryo-EM.

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2. M. Mapelli, S. Panjkar, P. A. Tucker, Journal of Biological Chemistry. 280, 2990–2997 (2005).
3. S. H. Mueller et al., Nucleic Acids Res. 1, e5 (2023).



Poster Presentations



Poster Presentations

Name: **Melanie Dietrich**

Theme: Structural Biology

Poster Position: 100

Title: **Blocking interactions of Plasmodium falciparum 6-cysteine proteins using inhibitory nanobodies**

Authors: *Melanie H. Dietrich, Li-Jin Chan, Frankie Lyons, Mikha Gabriela, Sash Lopaticki, Kitsanapong Reaksudsan, Matthew W. A Dixon, Amy Adair, Joshua Tong, Li Lynn Tan, Alan F. Cowman and Wai-Hong Tham*

The 6-cysteine protein family is one of the most abundant surface antigens of Plasmodium falciparum and expressed throughout the parasite life cycle. This protein family is conserved across Plasmodium species and play important roles in parasite fertilization and transmission, evasion of the host immune response and host cell invasion. Several 6-cysteine proteins are present on the parasite surface as hetero-complexes. To date it is not well understood how 6-cysteine proteins interact together or engage with host-cell proteins.

We seek to understand the function and structural mechanisms of 6-cysteine proteins during the malaria parasite life cycle for development of novel anti-malarial interventions. We have solved numerous 6-cysteine protein crystal structures as single or tandem domain proteins, as well as in complex with their binding partners. Using an immunised nanobody platform, we have characterised the first collection of nanobodies to different 6-cysteine proteins. In particular, we are able to show that our nanobodies can block the transmission of the malaria parasite from mosquito to human by blocking parasite fertilization in the mosquito mid-gut. In our recent work we investigate interactions of large family members using a combination of advanced structural biology techniques and functional parasite assays. Together with our large panels of nanobodies we aim to further dissect the roles of the 6-cysteine proteins to contribute to a better understanding of this important family of proteins and their critical function in the malaria parasite life cycle.



Poster Presentations



Poster Presentations

Name: **Sarah Mueller**

Theme: Structural Biology

Poster Position: 101

Title: **The orphan G protein-coupled receptor GPR55**

Authors: *Sarah L Mueller, Emily J Furlong, Tom S Peat & Sally-Ann Poulsen*

GPR55 is an orphan seven-transmembrane receptor that is abundant in brain and peripheral tissues and has been associated with diseases such as obesity and cancer, as well as neurodegenerative disorders including Parkinson's disease.

Although L- α -lysophosphatidylinositol, a bioactive lipid, is widely recognised as the endogenous ligand of GPR55, the receptor also shows high sensitivity to cannabinoid receptor ligands and can be modulated by endocannabinoids, phytocannabinoids, and synthetic cannabinoids. In addition, GPR55 is known to form heteromers with the cannabinoid receptors CB1 and CB2, resulting in alterations in signal transduction. Allosteric modulation, crosstalk with other receptors and the lack of GPR55-selective ligands has led to controversial observations and complicated the discovery of the pathophysiological role of GPR55.

The instability and low expression of the receptor have hampered the structural characterisation of GPR55, which is required to better understand its pharmacology.

We have investigated thermostabilising mutations and developed an expression and purification protocol to produce pure protein suitable for structure determination, ligand binding, and activity assays.



Poster Presentations



Poster Presentations

Name: **Xuefei Jing**

Theme: Structural Biology

Poster Position: 102

Title: **Preparing human P2X7 ion channel receptor for cryo EM structural determination**

Authors: *Xuefei Jing, Yi Zeng (Jack), Chandrika Deshpande, Alastair Stewart, Joel Mackay*

P2X ion channel receptors are abundant in the human body and play important roles in a variety of cell activities such as neurotransmission, inflammatory stimulus initiation, and afferent signalling by triggering selective ion transmembrane fluxes.

As such they have major clinical significance as therapeutic drug targets due to their critical position in the cellular signalling pathway and their exceptional drug accessibility.

Despite the undeniable importance of P2X receptors in a wide range of diseases, P2X receptor drug development has been slow over the last 30 years. Poor recombinant expression of these ion channel proteins, as well as the difficulty in purifying them, are two constraints that contribute to the slow pace of drug development.

We seek to fill the structural information gap in human P2X receptors by developing a method for producing stable native trimeric P2X7 that can be used for structural determination.

Using a baculovirus-mediated mammalian cell expression system, we were able to successfully express full-length human P2X7.

Different detergent conditions and purification procedures were optimised to obtain trimeric and monodisperse P2X7 protein that is suitable for cryo EM structural determination.

Images of negative staining from TEM revealed how detergent conditions may affect protein condition and aided in vitrification.

Preliminary cryo grid trials revealed challenges and potential optimisation directions for P2X7 vitrification.



Poster Presentations



Poster Presentations

Name: **Yanqiong Chen**

Theme: Structural Biology

Poster Position: 103

Title: **Mechanism of self-ganglioside presentation by the antigen presentation molecule CD1b**

Authors: *Yanqiong Chen, Jamie Rossjohn, Adam Shahine*

T cells play a critical role in adaptive immunity, mediated by receptors on the surface of T cell (TCR) that recognise antigens presented by antigen presenting molecules. Studies on T cell immunity are centred on classical major histocompatibility complex (MHC) that present antigenic peptides to TCRs. However, non-classical antigenic molecules such as lipids can also be presented by antigen presenting proteins CD1s, which in comparison to peptide-MHC, the mechanisms of CD1 proteins presenting antigenic lipids to TCRs remain largely unknown.

The human CD1 family are classified as Group 1 (CD1a, CD1b and CD1c) and Group 2 (CD1d) based on gene sequence and function. Compared to peptide-MHC, CD1 molecules in humans are non-polymorphic, as such, the mechanisms of T cell activation by CD1 proteins can be broadly applicable to all humans. CD1b, which is well-known for presenting lipids from the Mycobacterium tuberculosis cell wall (1), has recently been shown to play a role in autoimmunity, such as in multiple sclerosis, cellular stress, and cancer. However, the molecular mechanisms of lipid presentation by CD1b, and mechanisms of modulating autoimmunity, largely remain unexplored.

Significant structural studies into the presentation of self-phospholipids by CD1b have previously been conducted, however mechanisms of self-ganglioside presentation, which include ganglioside-monosialic acid (GM1) found to induce T cell mediated autoimmune responses in multiple sclerosis (2), remain unknown. To characterise this, a panel of gangliosides (CD1b -GM1, GM2, GM3 and sulfatide) previously identified as CD1 antigens were manually loaded into CD1b in vitro, and their structures were determined via x-ray crystallography. The ganglioside sphingosine and fatty acid tails are sequestered into the C' and A' portals of CD1b, respectively, while the ganglioside head group is positioned above the CD1b surface. CD1b presents gangliosides and phospholipids in a similar manner, however ganglioside head groups are distinctly larger and therefore protrude further above the CD1b surface than phospholipids. These findings provide novel molecular insights into the mechanism of antigenic lipid presentation by CD1b, and led to future understanding of their role in immune activation.



Poster Presentations

Name: **Zahra Falahati**

Theme: Structural Biology

Poster Position: 104

Title: The development of novel ecofriendly selective pesticides for the maintenance of managed honeybees

Authors: Zahra Falahati¹, Ingrid Macindoe¹, Hakimeh Sani¹, Emily Remnant², Ron Hill¹, Joel Mackay¹

The global decline in pollinator populations, particularly bees, threatens the essential role of animal-mediated pollination in fruit, vegetable, and nut crop development. Managed honeybees are critical pollinators for a wide range of crops, and their decline poses a significant risk to global agriculture, and impacts food security. Factors like bacteria, parasitic mites, fungi, protozoa, and viruses contribute to honeybee colony declines, with Small Hive Beetles (SHBs) causing significant damage in various ways. SHBs infest hives, disrupt hive materials, and create a slimy environment, leading to hive abandonment.

This research focuses on ecdysone, which is a crucial hormone involved in development in arthropods, including insects. Structural variation in the ecdysone-binding domain of ECRs among insect groups enables the design of pesticides that could specifically affect pests, while sparing beneficial organisms, including vertebrates which lack the ECRs entirely.

In our research, following successful cloning, expression, and purification of SHB ECR, we have conducted some preliminary characterization experiments, including Multi-Angle Laser Light Scattering (MALLS), 1D 1H Nuclear Magnetic Resonance (NMR), buffer screening, and fluorescence polarization ligand binding assays. These experiments lay the groundwork for our research, in which we aim to use Fragment Based Drug Discovery to identify lead compounds to develop a marketable pesticide exclusively harmful to SHBs while ensuring the safety of bees and humans. The success of this research will advance the development of safer, environmentally friendly pesticides for pest-related challenges in agriculture, paving the way for future innovations.



Poster Presentations



Poster Presentations

Name: **Jesus Ruiz Flores**

Theme: Synthetic Biology

Poster Position: 105

Title: **Establishing a novel synthetic nanobody yeast-surface display library for its application in structural and synthetic biology.**

Authors: *Jesus Ruiz Flores*

Joseph Brock

In the past, the production of antibodies for different purposes relied on the immunisation of animals, but much of the antibodies resulted to be unspecific and unreproducible for different applications they were meant to be used for and, since 2015, the use of animals was declared obsolete. A protocol for yeast surface display of a synthetic nanobody library was published in 2018 by the Kruse Lab, allowing the identification of high affinity nanobodies to a particular antigen. Nanobodies are a specific type of antibodies produced by camelids (llamas, camels, alpacas, etc.), that are composed solely by heavy chains instead of having two identical heavy (VH) and light chains (VL) polypeptides as the conventional ones. Such kind of antibodies bind to their target antigens through a single variable domain, termed VHH, which contains the entire antigen-binding surface. Their three-complementarity determining region (CDR) loops contain all the necessary biochemical features to achieve nano-molar binding affinity to a given antigen and have superior qualities for many applications relative to IgG's, due to their smaller size and stability. Nanobodies have been employed effectively to trap transient conformations of medically relevant proteins for structural biology, facilitate non-invasive diagnostic imaging, imaging of dynamic processes in the cell, super resolution imaging of protein complexes, point of care diagnostic biosensors and as next generation of cancer therapies and other diseases. Nowadays, there is the need for efficient methods to screen nanobodies for binding to specific antigens, and yeast-surface display is a powerful platform for the selection of protein binders. In this project, we aim to develop a new library and use yeast-surface display platform to screen nanobodies for binding to specific antigens, that later can be extended to other protein binding scaffolds, such as affibodies or sherpabodies, with modular antigen binding loop diversity generated by combinatorial golden gate cloning.



Poster Presentations



Poster Presentations

Name: **Andrew George**

Theme: Synthetic Biology

Poster Position: 106

Title: **Targeted mutagenesis and FACS selection system for genetic code expansion**

Authors: *Andrew George, Haocheng Qianzhu & Thomas Huber*

Targeted mutagenesis systems are able to introduce random mutations in vivo over a specified region of DNA, while preventing enhanced mutation rates in non-target genes. This is crucial during continuous evolution, where off-target mutations become quickly deleterious to the organism.

The eMutaT7transition targeted mutagenesis system uses a T7 RNA Polymerase which transcribes selectively between the T7 promoter and terminator. Fusion to cytidine and adenosine deaminases allows random introduction of C→T and A→G mutations. The addition of an inverted T7 promoter allows further mutations to be made simultaneously in the non-coding strand, making G→A and T→C coding mutations. The result is a targeted mutagenesis system able to randomly and evenly introduce all possible transition mutations across a strictly defined gene region.

Genetic code expansion involves the site-specific incorporation of a non-canonical amino acid by repurposing the amber stop codon. Incorporation of fluorinated non-canonical amino acids, such as trifluoroacetyl-L-lysine (TFAK), is particularly useful for probing in ¹⁹F NMR due to the highly sensitive chemical shift of fluorine. However, incorporation of each new ncAA requires re-engineering of the orthogonal aminoacyl-tRNA synthetase (RS) and results in varied RS efficiencies, evidenced by low incorporation activity of the TFAK RS.

Here, I present my studies in applying the eMutaT7transition targeted mutagenesis system with established FACS selection methods to evolve higher efficiency in the TFAK RS for use as a probe in ¹⁹F-NMR protein studies.



Poster Presentations



Poster Presentations

Name: **Joachim Larsen**

Theme: Synthetic Biology

Poster Position: 107

Title: **Paving the way for cyanobacterial neobiochemistry: Development of novel platform for fast and efficient genetic engineering**

Authors: *Joachim Larsen*

Evan Gibbs

Benjamin Matthews

Karl Hassan

Brett Neilan

Cyanobacteria ("blue-green algae") are photosynthetic organisms that use carbon dioxide and sunlight to sustain themselves. Because of this unique ability, cyanobacteria are increasingly being considered as a sustainable production platform for the future. Despite their potential, however, working with cyanobacteria can be challenging for a variety of reasons. One of the main issues is their slow growth rate, which can make the cultivation process time-consuming. Additionally, not many synthetic biology tools have been developed for cyanobacteria, making them difficult to engineer. Lastly, low transformation efficiencies pose another challenge, further complicating the use of cyanobacteria as a heterologous host. To overcome these challenges, we have developed a novel cyanobacterial platform for rapid and efficient genetic engineering. Our methodology allows for the integration of a foreign gene across all copies of the *Synechocystis* sp. PCC 6803 genome in a matter of weeks. This is far improved from the months required using traditional techniques. Utilising yellow fluorescent protein (YFP), we show the efficiency of our system compared to traditional engineering techniques. Further, we demonstrate the stability of the insertion over an extended period of time through the tracking of YFP. The versatility of our approach was demonstrated by both successfully converting the YFP engineered strain back to wildtype, as well as the successful deletion of endogenous genes. Our novel method outperforms traditional techniques and enables fast and easy introduction and engineering of novel genes in cyanobacteria, paving the way for the emerging field of cyanobacterial neobiochemistry within the ARC Centre of Excellence in Synthetic Biology.



Poster Presentations

Name: **John Chen**

Theme: Synthetic Biology

Poster Position: 108

Title: **Exploring the uncharacterized sequence space of periplasmic binding protein transcription regulators**

Authors: *John Z Chen, Colin J Jackson*

Transcription regulators (TRs) are a type of protein ubiquitous to all domains of life. Responsible for the temporal and spatial control of gene expression, TRs are vital to key cell processes including metabolism, cell division and response to internal and external stimuli.

Some of the largest TR families include the LysR and LacI families of TRs, which collectively encompass >1M sequences. Despite the large number of sequences, the vast majority of the sequence space is unexplored. We estimate that >95% of sequence clusters (groups of similar sequences) in this TR superfamily are 'orphans', with no closely related (>35% sequence identity) sequences of known function.

Hence, there is great potential to expand our understanding of these important biomolecules. The exploration of the various TRs in nature could reveal potential novel ligands and regulatory elements currently utilized by various organisms. Furthermore, the mechanistic understanding of TRs is can aid the study of numerous fundamental biological processes, such as protein-ligand interactions, induced conformational changes, DNA sequence recognition and gene regulation. The discovery and/or engineering of TRs with specific ligand binding and regulatory behaviors can also be harnessed for the creation of biosensors against a diverse set of ligands.

I aim to survey the TR superfamily through bioinformatics and experimental characterization of a large number of representative TRs, constructing a bioinformatics pipeline amenable for exploring large sequence datasets. Select representatives from the sequence space are synthesized and characterized for both ligand and response element binding. The functional data would then be used for machine learning to predict the ligand binding throughout the rest of the family and help design novel TRs for synthetic molecules or xenobiotics.



Poster Presentations



Poster Presentations

Name: Mark Shannon

Theme: Synthetic Biology

Poster Position: 109

Title: **3D bioprinted living bacterial microreactors degrade organophosphates**

Authors: *Mark R. Shannon, Graham J. Day, Hermes Bloomfield-Gadelha, Valeska P. Ting, Adam W. Perriman*

Engineered living materials (ELMs) are a rapidly emerging class of materials making use of both synthetic biology and advanced materials engineering. They demonstrate a wide range of functionalities, including responsive morphing, self-healing, and controllable bio-catalysis. 3D printable hydrogels have been widely used to fabricate high resolution, compartmentalised, and load-bearing structures suitable for hosting microbial metabolism, and accordingly represent an ideal environment for ELMs. The interactions between material frameworks, such as hydrogels, and encapsulated life are now beginning to be investigated. Herein, by 3D printing a hydrogel-encapsulated population of *Escherichia coli*, a chemically inducible and metabolically active microbial ELM was fabricated. The material micro-structure, cell behaviour in 3D, and the material's catalytic performance were characterised using a wide range of techniques, including fluorescence microscopy and cryogenic scanning electron microscopy. Organophosphorus compound (OPC) detoxification capabilities were conveyed to the living material by inducible expression of the enzyme *Agrobacterium radiobacter* phosphotriesterase (arPTE). A mass transfer limitation identified during OPC detoxification analysis was investigated using continuous fluorescence-imaging of the active ELM. Principal component analysis was then used to uncover spatial and temporal features within this data, with relevance for future optimisation of catalytic microbial ELMs. To further demonstrate the applicability of this 3D printable microbial ELM, the material was incorporated into an entirely 3D printed flow reactor, demonstrating robust cyclical detoxification of an OPC solution at high flow rates.



Poster Presentations