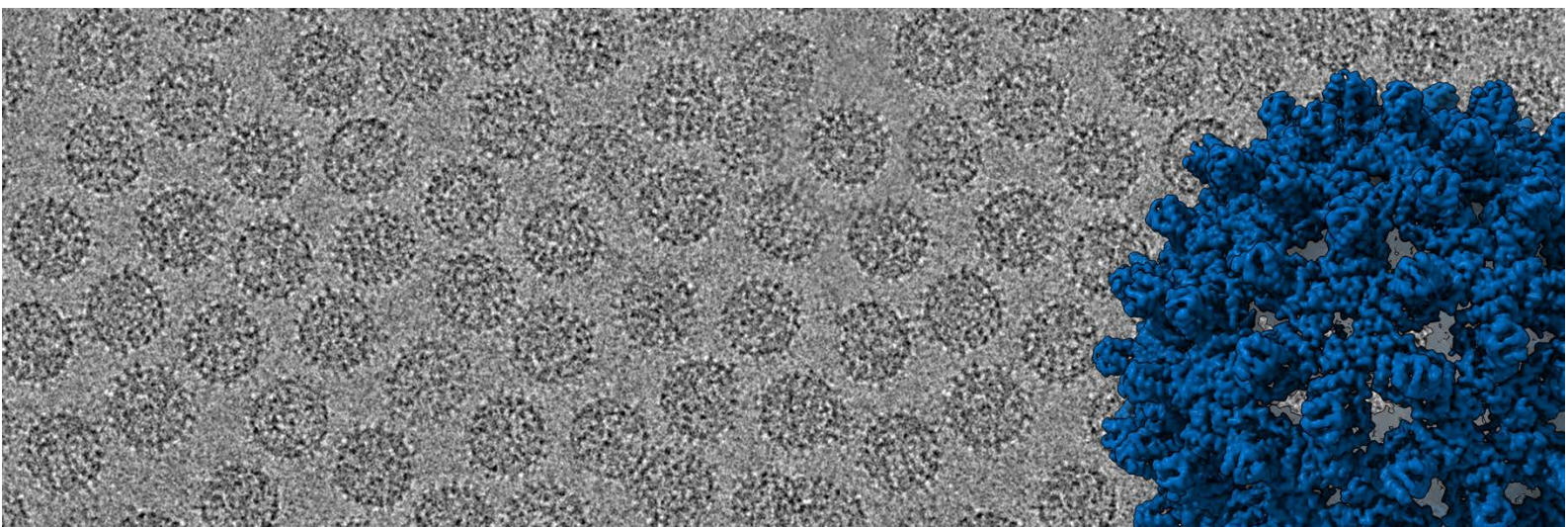
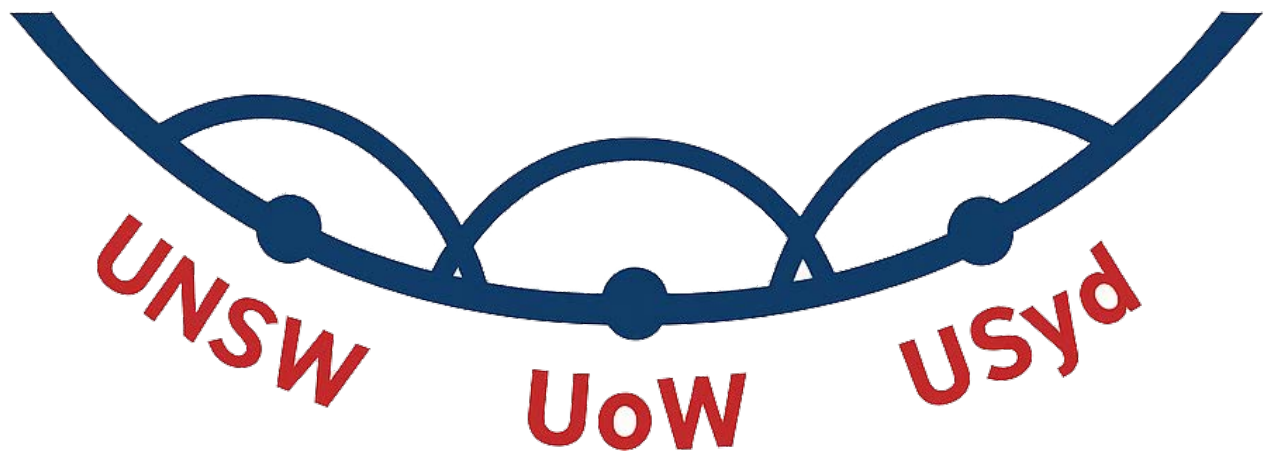




# CRYOZ 2025

**NOVEMBER 17–18, 2025**

UNIVERSITY OF WOLLONGONG, NSW





## **Conference organisers**

Dr. Daniel Luque (University of New South Wales)  
Dr. Jacob Lewis (University of Wollongong)  
Dr. Rachel North (University of Sydney)

## **Scientific committee**

Dr. Daniel Luque (University of New South Wales)  
Dr. Jacob Lewis (University of Wollongong)  
Dr. Rachel North (University of Sydney)  
Dr. Errin Johnson (University of Sydney)  
Dr. Bhanu Mantri (University of Sydney)  
Dr. Juanfang Ruan (University of New South Wales)  
A/Prof. Gökhan Tolun (University of Wollongong)  
Dr. James Bower (University of Wollongong)  
Dr. Anders Barlow (University of Melbourne)  
Dr. Nick Ariotti (University of Queensland)  
Prof. Georg Ramm (Monash University)



**CRYOZ  
2025**

## Platinum Sponsors

**ThermoFisher**  
SCIENTIFIC

**LINKAM**

**HSY**  
HINSCI

**M** MICROSCOPY  
AUSTRALIA

**in** instruct  
ES

 UNIVERSITY  
OF WOLLONGONG  
AUSTRALIA

 **UNSW**  
SYDNEY

CRYO  
CAP  
CELL

## Gold Sponsors

**G** GATAN  
AMETEK

**ZEISS**

Seeing beyond

*Leica*  
MICROSYSTEMS

**AXT**

**OXFORD**  
INSTRUMENTS

**JEOL**

**Coherent**  
SCIENTIFIC

## Silver Sponsors

MICROSCOPY SOLUTIONS

**AVS**  
Australian Vacuum Services

**HITACHI**

  
NewSpec

**CeMMP**



## **Day 1 | Monday 17 November 2025**

07:30 – 08:45 Registration and Arrival Coffee  
08:45 – 08:50 Welcome and Opening Remarks

### **Session 1 – Frontiers in Cryo-Electron Microscopy**

08:50 – 09:20 Keynote – Dr. Alessandro Costa (The Francis Crick Institute)  
09:20 – 09:35 Dr. Winnie Tan (Walter and Eliza Hall Institute)  
09:35 – 09:50 Dr. Nick Ariotti (University of Queensland)  
09:50 – 10:05 Dr. Hongyi Xu (Australian National University)  
10:05 – 10:20 Dr. Rhys Grinter (University of Melbourne)  
10:20 – 10:35 Dr. Nicholas Kirk (WEHI)

10:35 – 11:00 Morning Tea

### **Session 2 – Cryo-Electron Tomography**

11:00 – 11:30 Keynote – Dr. Wioleta Dudka (EMBL)  
11:30 – 11:45 Dr. Hamish Brown (University of Melbourne)  
11:45 – 12:00 Salakshmi Velamoor (Monash University)  
12:00 – 12:15 Jiwon Lee (Australian National University)  
12:15 – 12:30 Dr. Fabian Munder (Monash University)  
12:30 – 12:45 Dr. Matthew Johnson (University of Melbourne)

12:45 – 13:35 Lunch

### **Session 3 – Single Particle Cryo-Electron Microscopy #1**

13:35 – 14:05 Keynote – Dr. Alisa Glukhova (Walter and Eliza Hall Institute)  
14:05 – 14:20 A/Prof. Michael Landsberg (University of Queensland)  
14:20 – 14:35 Prof. Joel Mackay (University of Sydney)  
14:35 – 14:50 Sainath Polepalli (Indian Institute of Science)  
14:50 – 15:05 Lucy Fitschen (University of Wollongong)  
15:05 – 15:20 Riya Joseph (University of Melbourne)  
15:20 – 15:35 Crystall Swarbrick (Charles Sturt University)  
15:35 – 15:50 Raphael Caballes (University of New South Wales)

15:50 – 16:10 Afternoon Tea

### **Session 4 – Emerging Structural Biology Leaders #1**

16:10 – 16:25 Dr. Benjamin Gully (ONJCRI)  
16:25 – 16:40 Dr. Rachel North (University of Sydney)  
16:40 – 16:55 Dr. Jacob Lewis (University of Wollongong)  
16:55 – 17:10 Dr. Charles Bayly-Jones (Monash University)

### **Poster Session**

17:10 – 18:30 Poster Session and Refreshments

### **Dinner**

19:00 – 22:30 Conference Dinner (tickets must have been pre-purchased)



## **Day 2 | Tuesday 18 November 2025**

08:15 – 08:45 Coffee  
08:45 – 08:50 Opening Remarks

### **Session 5 – Advances in Cryo-EM and Cryo-ET Methods and Workflows**

08:50 – 09:20 Keynote – Dr. Jose Luis Vilas (CNB-CSIC)  
09:20 – 09:35 Mihin Perera (Walter and Eliza Hall Institute)  
09:35 – 09:50 Dr. Christy Ying (Monash University)  
09:50 – 10:05 Dr. Xavier Heiligenstein (CryoCapCell)  
10:05 – 10:20 Dr. Yufeng Zhao (University of Wollongong)

10:20 – 10:45 Morning Tea

### **Session 6 – Single Particle Cryo-Electron Microscopy #2**

10:45 – 11:15 Keynote – Prof. Gabriel Lander  
11:15 – 11:30 Dr. Lachlan Adamson (University of Sydney)  
11:30 – 11:45 Eric Chen (Thermo Fisher Scientific)  
11:45 – 12:00 Dr. Carus Lau (Victor Chang Cardiac Research Institute)  
12:00 – 12:15 Muhammad Zahir (University of Wollongong)  
12:15 – 12:30 Irene Antony (University of Canterbury)  
12:30 – 12:45 Xiaoqi Qian (University of Queensland)

12:45 – 14:00 Lunch  
13:00 – 14:00 Jeol lunchtime remote demonstration (optional)

### **Session – CryOZ Special Interest Group AGM**

14:00 – 14:30

### **Session 7 – Emerging Structural Biology Leaders #2**

14:30 – 14:45 Dr. Bradley Spicer (Monash University)  
14:45 – 15:00 Dr. Jennifer Baker (University of Wollongong)  
15:00 – 15:15 Dr. Emily Furlong (Australian National University)  
15:15 – 15:30 A/Prof. Gavin Knott (Monash University)  
15:30 – 15:45 Dr. Aidan Grosas (University of Wollongong)

15:45 – 16:10 Afternoon Tea

### **Session 8 – Facilities Discussion & Close.**

16:10 – 17:10 Facilities Discussion  
17:10 – 17:30 Awards and Symposium close

## Oral Presentations

### The structure and composition of native human nucleosomes

Xavier Reid<sup>\*</sup>,<sup>1</sup>, Meghna Sobti<sup>\*</sup>,<sup>2, 3</sup>, Yi Zeng<sup>2,3</sup>, Yichen Zhong<sup>1</sup>, Chandrika Deshpande<sup>1</sup>, Paul Young<sup>2</sup>, Simon Brown<sup>4</sup>, Jason Low<sup>#, 1</sup>, Alastair Stewart<sup>#, 2, 3</sup>, Joel Mackay<sup>#, 1</sup>

<sup>1</sup>*School of Life and Environmental Sciences, University of Sydney, NSW 2006 Australia*

<sup>2</sup>*Molecular, Structural and Computational Biology Division, The Victor Chang Cardiac Research Institute, Darlinghurst, NSW, Australia*

<sup>3</sup>*School of Clinical Medicine, Faculty of Medicine and Health, UNSW Sydney, Sydney, NSW, Australia*

<sup>4</sup>*School of Chemistry and Molecular Bioscience, Molecular Horizons, and Australian Research Council Centre for Cryo-electron Microscopy of Membrane Proteins, University of Wollongong, Wollongong, NSW, Australia*

Since the first high-resolution structures of recombinantly assembled nucleosomes, efforts have shifted towards understanding chromatin structure in a native context. Most of these efforts have focused on native-like, yet still recombinantly assembled, nucleosomes that contain native DNA sequences. To date, no high-resolution structures of native human nucleosomes have been reported. Here we report the high-resolution cryo-EM structure of native human nucleosomes isolated from HEK293 cells.

Surprisingly, despite the vast DNA sequence diversity of native nucleosomes, we observe conserved nucleotides that support the idea of a nucleosome positioning code. In addition to these striking features of nucleosomal DNA, we note alternate conformations of several DNA contacting histone residues that hint at dynamics in the HEK293-NCP. To complement the HEK293-NCP structure, we provide a mass spectrometry analysis of histone modifications and variants present in the sample, which demonstrates that a typical HEK293-NCP is composed of canonical histones with N-terminal tails that are methylated at K9, K27 and/or K36 of histone H3. Altogether these findings have implications for biological processes such as chromatin remodelling and transcription and improve our understanding of nucleosome and chromatin structure in a native context.

## **A potent protein antibiotic kills *Pseudomonas aeruginosa* by inhibiting the BAM complex**

Fabian Munder<sup>1,2,3</sup>, Matthew Johnson<sup>3</sup>, Imogen Samuels<sup>3</sup>, Chunxiao Wang<sup>3</sup>, Luis Jimenez, Jack Downard, Ashleigh Kropp<sup>1,2</sup>, Hari Venugopal<sup>6</sup>, Laura McCaughey<sup>9</sup>, Matthew Doyle<sup>5</sup>, Francesca Short<sup>1</sup>, Debnath Ghosal<sup>3</sup>, James Connolly<sup>8</sup>, Gavin Knott<sup>4</sup>, Rhys Grinter<sup>1,2,3</sup>

<sup>1</sup> Department of Microbiology, Biomedicine Discovery Institute, Monash University, Clayton 3800, Australia

<sup>2</sup> Centre for Electron Microscopy of Membrane Proteins, Monash Institute of Pharmaceutical Sciences, Parkville, 3052, Victoria, Australia

<sup>3</sup> Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Victoria 3010, Australia

<sup>4</sup> Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton 3800, Australia

<sup>5</sup> Faculty of Medicine and Health, The University of Sydney, Sydney 2006, Australia

<sup>6</sup> Ramaciotti Centre for Cryo-Electron Microscopy, Biomedicine Discovery Institute, Monash University, Clayton 3800, Australia

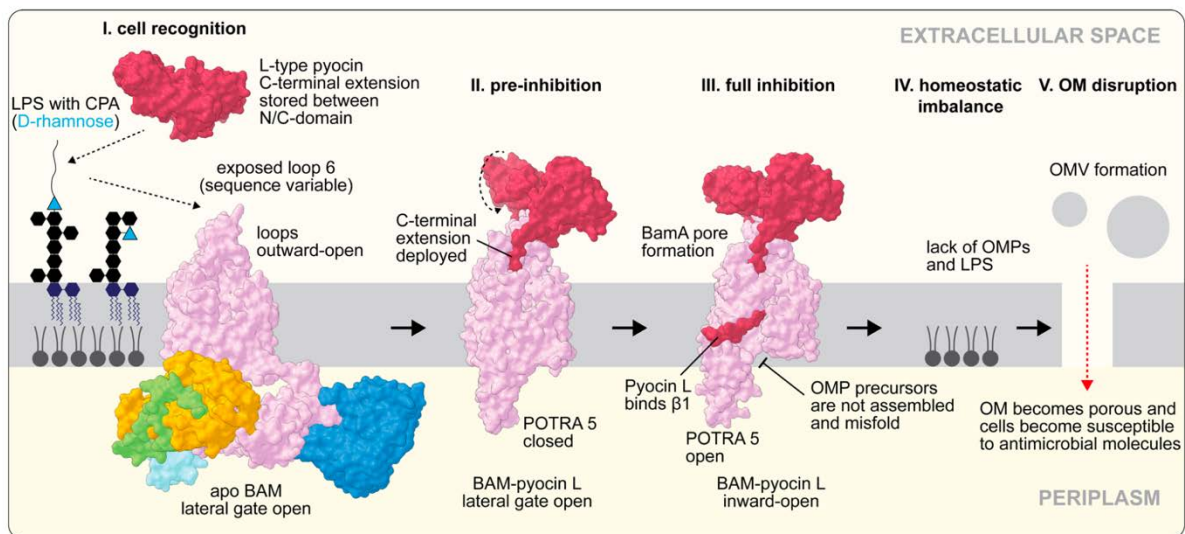
<sup>8</sup> Newcastle University Biosciences Institute, Newcastle University, Newcastle NE1 7RU, United Kingdom

<sup>9</sup> School of Infection and Immunity, University of Glasgow, Glasgow G12 8QQ, UK

The Gram-negative bacterium *Pseudomonas aeruginosa* is an opportunistic human pathogen that is resistant to many antibiotics at our disposal<sup>1</sup>. This is because of the outer membrane (OM) which is a permeability barrier for molecules >600 Da. L-type pyocins are large and potent protein antibiotics that can clear lethal infections of multidrug-resistant *P. aeruginosa* in mouse lungs<sup>2</sup>. However, their mode of action is almost entirely unknown<sup>3,4</sup>.

In this study, we show for the first time that L-type pyocins directly inhibit the 5-subunit  $\beta$ -barrel assembly machinery (BAM) of *P. aeruginosa* with two distinct binding states. Firstly, the pre-inhibition state, in which L-type pyocins bind to the extracellular loop 6 of the BamA subunit. Here, L-type pyocins undergo a dramatic conformational change by deploying their C-terminal extension into the transmembrane  $\beta$ -barrel of BamA. In the full inhibition state, the C-terminal extension binds the lateral gate of BamA, which stops the BAM complex from conformational cycling and prevents the assembly of nascent OM proteins (OMP).

We used transcriptomics and proteomics to show that BAM inhibition in *P. aeruginosa* has a range of downstream effects on the bacterium including the absence of OMPs. Then, we investigated the cellular effects of BAM inhibition by cryo-electron tomography and found an excessive formation of OM vesicles, which ultimately causes catastrophic disruption of the OM. Lastly, the treatment of *P. aeruginosa* with L-type pyocins makes the bacterium susceptible to existing antibiotics, that cannot overcome a healthy OM. This research opens avenues for future medical treatment of MDR *P. aeruginosa*.



**Figure 1: Schematic overview of the L-type Pyocin mode of action.** **I**, L-type pyocins (red) target *P. aeruginosa* cells by binding the D-rhamnose component (CPA) of lipopolysaccharides (LPS). **II**, L-type pyocins form a pre-inhibition complex with the essential  $\beta$ -barrel assembly machinery (BAM) by binding the extracellular loop 6 of BamA (pink) and deploying their C-terminal extension into the BamA  $\beta$ -barrel. **III**, the BAM complex is fully inhibited when the C-terminal extension binds the lateral gate of BamA at  $\beta$ -strand 1, which stops conformational changes of BAM and assembly of outer membrane proteins (OMP). **IV**, BAM inhibition leads to a range of downstream effects on the cell, including the absence of OMPs and a homeostatic imbalance of the OM. **V**, the homeostatic imbalance causes an excessive formation of outer membrane vesicles (OMV), which ultimately results in lethal outer membrane disruption. Additionally, the disruption of the outer membrane makes *P. aeruginosa* cells susceptible to otherwise ineffective antibiotics.

1. Miller, S. I. et al., 2016, mBio, Antibiotic Resistance and Regulation of the Gram-Negative Bacterial Outer Membrane Barrier by Host Innate Immune Molecules.
2. McCaughey, L. C. et al., 2016, Scientific Reports, Efficacy of species-specific protein antibiotics in a murine model of acute *Pseudomonas aeruginosa* lung infection.
3. McCaughey, L. C. et al., 2014, PLoS Pathogens, Lectin-Like Bacteriocins from *Pseudomonas* spp. Utilise D-Rhamnose Containing Lipopolysaccharide as a Cellular Receptor.
4. Ghequire, M. G. K. et al., 2018, mBio, Hitting with a BAM: Selective Killing by Lectin-Like Bacteriocins.

## Structure of human PINK1 at an endogenous TOM-VDAC2 complex

Nicholas S. Kirk<sup>1,2</sup>, Sylvie Callegari<sup>1,2</sup>, Zhong Yan Gan<sup>1,2</sup>, Alisa Glukhova<sup>1,2</sup>, David Komander<sup>1,2</sup>

<sup>1</sup>WEHI, Melbourne, Australia

<sup>2</sup>University of Melbourne, Melbourne, Australia

Damaged mitochondria must be recycled and replaced to maintain proper cell function. The ubiquitin kinase PINK1 plays a key role in this process by detecting depolarized mitochondria and starting a signaling pathway that marks these organelles for autophagy. We determined the structure of human PINK1 for the first time in complex with the translocase of the outer membrane (TOM) complex, which imports proteins into the mitochondria, and a pair of voltage-dependent anion channels. This structure revealed how stalling of PINK1 during import and oxidation of surface cysteine residues establishes a dimeric preactive complex that is prepared to initiate downstream signaling. It also revealed for the first time a bound TOM40 substrate at high resolution, the role of TOM20 in binding TOM40 substrates and stabilising PINK1 as well as the structural role of small TOM proteins TOM7 and TOM22 in guiding substrates through TOM40. The structure also revealed a previously unknown role for VDAC2, including an unpredicted disulfide bond, while resolving their human structures for the first time.

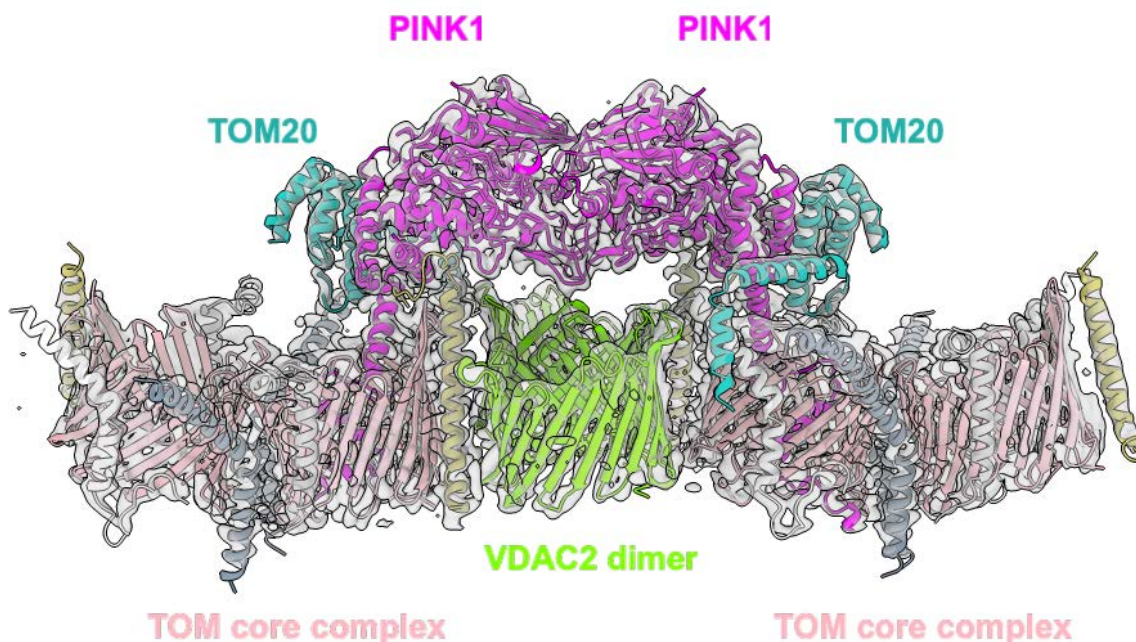


Figure 1. CryoEM density and model for human PINK1 bound to the endogenous TOM-VDAC2 complex

1. Callegari, S., Kirk, N.S., et al. Structure of human PINK1 at a mitochondrial TOM-VDAC array. *Science*, 388(6744): p303-310.

## Combining Real-time MicroED and Continuous SerialED for Structure-Based Drug Discovery

Lei Wang<sup>1</sup>, Emma Scaletti Hutchinson<sup>2</sup>, Pål Stenmark<sup>2</sup>, Yinlin Chen<sup>1</sup>, Gerhard Hofer<sup>1,\*</sup>, [Hongyi Xu<sup>1,3,\\*</sup>](#) and Xiaodong Zou<sup>1,\*</sup>

<sup>1</sup> Department of Chemistry, Stockholm University, 106 91 Stockholm, Sweden

<sup>2</sup> Department of Biochemistry and Biophysics, Stockholm University, 106 91 Stockholm, Sweden

<sup>3</sup> Research School of Chemistry, the Australian National University, Acton, Australia

Understanding protein–ligand interactions is a central step in structure-based drug discovery. While conventional single-crystal X-ray diffraction enables accurate and high-throughput structural analysis, it is constrained by the need for large, well-ordered crystals. Electron crystallography techniques, such as microcrystal electron diffraction (MicroED<sup>1,2</sup>) and serial electron diffraction (SerialED<sup>3,4</sup>), overcome this limitation by allowing structure determination from submicron protein crystals.

Here, we introduce an integrated workflow that utilizes real-time MicroED and continuous SerialED (c-SerialED) to enable rapid, reliable, and high-resolution analysis of protein–ligand interactions directly from microcrystals<sup>5</sup>. Real-time MicroED provides rapid structural feedback allowing us to quickly assess the presence and orientation of ligands in the binding pocket. Continuous SerialED is then employed to collect large snapshot diffraction datasets from a large number of microcrystals, enabling high-quality refinement and accurate determination of the binding mode at high resolution. To demonstrate the potential of this workflow, we investigated human MutT homolog 1 (MTH1), a cancer drug target involved in sanitizing oxidized nucleotides<sup>6,7</sup>. We performed soaking experiments with four small-molecule ligands spanning a range of binding affinities, each soaking step requiring only a few seconds. Ligand binding was confirmed by real-time MicroED within 25–50 minutes, and subsequent c-SerialED data collection yielded 1.7 Å resolution structures of the resulting protein–ligand complexes. Our results highlight electron crystallography as a versatile platform for accelerating structure-guided drug discovery.

1. Shi D. et al., 2013, eLife, 2: e01345.

2. Gemmi M. et al., 2019, ACS Central Science, 5: 1315-1329.

3. Smeets S. et al., 2018, Journal of Applied Crystallography, 51: 1262-1273

4. Bücker R. et al., 2020, Nature Communications, 11: 996.

5. Clabbers M. et al., 2020, Communications Biology, 3: 417.

6. Gad H. et al., 2014, Nature, 508: 215-221.

7. Svensson L. et al., 2011, FEBS Letters, 585: 2617-2621.

## Structure Illuminates Chemistry: A PLP Enzyme Inhibition Story

Sainath Polepalli<sup>1</sup>, Anupam Roy<sup>1</sup>, Bapan Mondal<sup>1</sup>, Prasenjit Naskar<sup>1</sup>, Amit Singh<sup>2</sup>, Somnath Dutta<sup>1</sup>

<sup>1</sup>Molecular Biophysics Unit, Indian Institute of Science, Bangalore, India.

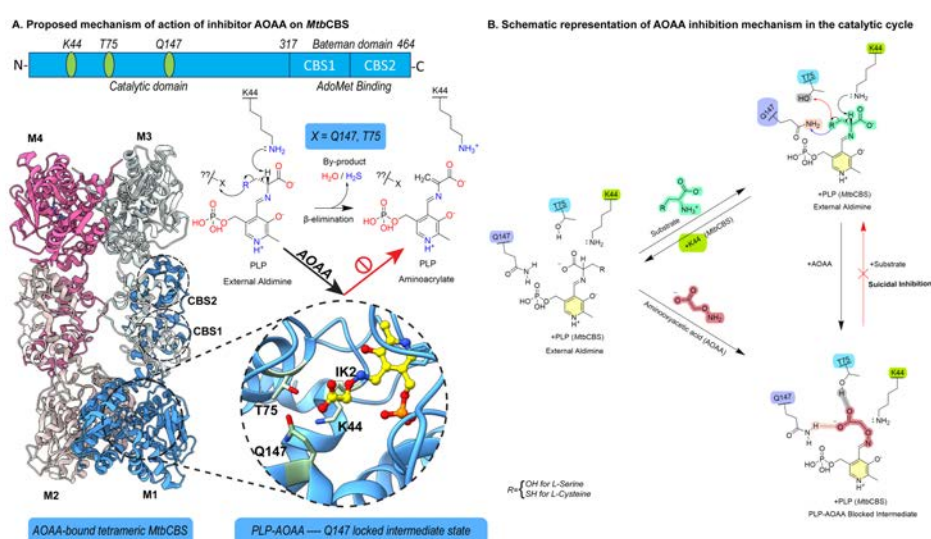
<sup>2</sup>Department of Microbiology and Cell Biology, Centre for Infectious Disease Research, Indian Institute of Science, Bangalore, India.

**Background:** Pyridoxal 5'-Phosphate (PLP) dependent enzymes catalyse a wide variety of chemical reactions, and alterations in the activity of PLP-dependent enzymes are associated with Down syndrome, cancer, and other neurological disorders. This story is about one such enzyme, Cystathionine  $\beta$ -Synthase (CBS) from *Mycobacterium tuberculosis*, crucial for sulfur metabolism and redox homeostasis. The commonly used inhibitor aminooxyacetic acid (AOAA) remains the best PLP-dependent enzyme inhibitor to date; however, the structural and mechanistic basis of inhibition is poorly understood.

**Methods:** To address this, we investigated using the model enzyme system full-length tetrameric MtbCBS (1), which can mimic the real physiologically active and allosterically modulated state, unlike truncated and mutant variants. We employed cryo-electron microscopy (cryo-EM), molecular dynamics simulations, quantum mechanics/molecular mechanics (QM/MM) calculations, structure-guided mutagenesis and biochemical inhibition assays to elucidate the AOAA interactions with MtbCBS.

**Key Results:** We report the first high-resolution cryo-EM structure of AOAA-bound tetrameric MtbCBS, revealing an unprecedented PLP-oxime adduct stabilised by active site residues (2). Our integrated approach uncovered the residue significance and, further, through cryo-EM, biophysical and computational studies utilising rational chemical mimics, the key electronic features enhancing adduct stability and inhibition were revealed, highlighting critical determinants of AOAA specificity.

**Conclusion:** Together, our results provide the first most detailed structural and mechanistic insights into CBS inhibition by AOAA (3). This work established a foundation for rational design of covalent CBS inhibitors and informs strategies for targeting PLP-dependent enzymes more broadly in future.



**Figure 1.** (A) Proposed mechanism of action of inhibitor AOAA on *MtbCBS*. (B) Schematic representation of AOAA inhibition mechanism in the catalytic cycle.

### References:

1. Bandyopadhyay P. et al., *Sci Adv.* 2022 Jun 24;8(25):eabo0097.
2. Polepalli S. et al., *Biophysical Journal.* 2024 Feb 8;123(3):134a–5a.
3. Polepalli S. et al., *BioRxiv.* Cold Spring Harbor Laboratory; 2025. <https://doi.org/10.1101/2025.07.03.662948>

## The Basis of Phospholipid Transport to the Bacterial Outer Membrane by the Translocation and Assembly Module

Lachlan S R Adamson<sup>1,2,3</sup>, Alanah G Eisenhuth<sup>1,2,3</sup>, Ghaeath S K Abbas<sup>1,2,4</sup>, Rachel A North<sup>1,2,3</sup>, Denisse L Leyton<sup>5</sup>, Constance B Bailey<sup>1,2,4</sup>, Alastair G Stewart<sup>6,7</sup>, Harris D Bernstein<sup>8</sup>, Aidan B Grosas<sup>9</sup>, Matthew T Doyle<sup>1,2,3</sup>

<sup>1</sup>Centre for Drug Discovery and Innovation, The University of Sydney, Darlington, NSW, Australia

<sup>2</sup>Sydney Infectious Diseases Institute, Faculty of Medicine and Health, The University of Sydney, Darlington, NSW, Australia

<sup>3</sup>School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Darlington, NSW, Australia

<sup>4</sup>School of Chemistry, Faculty of Science, The University of Sydney, Darlington, NSW, Australia

<sup>5</sup>Research School of Biology, The Australian National University, Canberra, ACT, Australia

<sup>6</sup>Molecular, Structural and Computational Biology Division, Victor Chang Cardiac Research Institute, Darlinghurst, NSW, Australia

<sup>7</sup>Faculty of Medicine, The University of New South Wales, Kensington, NSW, Australia

<sup>8</sup>Genetics and Biochemistry Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA

<sup>9</sup>School of Science, The University of Wollongong, Wollongong, NSW, Australia

The outer membrane of gram-negative bacteria is composed of phospholipids, proteins, and lipopolysaccharide. The balance of all these components is essential for outer membrane integrity and cell viability. The TAM complex has been reported to be involved both in the insertion of proteins into the outer membrane<sup>1,2</sup> and alternately as a transporter of phospholipids from the inner membrane to the outer membrane.<sup>3</sup> The complex consists of TamA, an outer-membrane embedded  $\beta$ -barrel; and TamB, a periplasm-spanning protein possessing a hydrophobic  $\beta$ -taco channel, and a C-terminal domain of unknown function. Knocking out of the TAM complex has been reported to disrupt the insertion of outer-membrane proteins.<sup>1</sup> The knocking out of TamB, in combination with related 'AsmA-like' proteins, has been shown to disrupt phospholipid transport to the outer membrane.<sup>3</sup> Structures of the individual components of the TAM complex have been reported,<sup>4,5</sup> but it is unknown how the two come into complex.

This work reports the cryoEM structure of TamA in complex with a truncated TamB bearing the domain of unknown function. We show that the C-terminus of TamB forms an unprecedented stable 'hybrid-barrel' with TamA by forming beta-sheets with the C-terminal strands of the TamA barrel. We use 3D variability analysis to identify a dynamic loop in TamB and use a combination of disulfide-crosslinking and phenotypic assay to show that the dynamicity of the loop is integral to outer membrane integrity. Combined these results provide insight into the function of the TAM complex and highlight its importance in the maintenance of the outer membrane.

(1) *Discovery of an archetypal protein transport system in bacterial outer membranes* | *Nature Structural & Molecular Biology*. <https://www.nature.com/articles/nsmb.2261#citeas> (accessed 2025-08-28).

(2) Wang, X.; Nyenhuis, S. B.; Bernstein, H. D. The Translocation Assembly Module (TAM) Catalyzes the Assembly of Bacterial Outer Membrane Proteins in Vitro. *Nat Commun* **2024**, *15* (1), 7246. <https://doi.org/10.1038/s41467-024-51628-8>.

(3) *Absence of YhdP, TamB, and YdbH leads to defects in glycerophospholipid transport and cell morphology in Gram-negative bacteria* | *PLOS Genetics*. <https://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1010096> (accessed 2025-08-28).

(4) Josts, I.; Stubenrauch, C. J.; Vadlamani, G.; Mosbahi, K.; Walker, D.; Lithgow, T.; Grinter, R. The Structure of a Conserved Domain of TamB Reveals a Hydrophobic  $\beta$  Taco Fold. *Structure* **2017**, *25* (12), 1898-1906.e5. <https://doi.org/10.1016/j.str.2017.10.002>.

(5) Gruss, F.; Zähringer, F.; Jakob, R. P.; Burmann, B. M.; Hiller, S.; Maier, T. The Structural Basis of Autotransporter Translocation by TamA. *Nat Struct Mol Biol* **2013**, *20* (11), 1318-1320. <https://doi.org/10.1038/nsmb.2689>.

## A Hyper-stable Glutamate-Binding Protein from a TAXI Transporter

Irene R. Antony<sup>1</sup>, Michael C. Newton-Vesty<sup>1,2</sup>, Kelsi R. Hall<sup>1,2</sup>, Zachary D. Tillett<sup>1</sup>, Michael J. Currie<sup>1</sup>, James S. Davies<sup>3</sup>, Timothy M. Allison<sup>1,4</sup>, Annmaree K. Warrender<sup>5</sup>, Santosh Panjekar<sup>5</sup> & Renwick C.J. Dobson<sup>1,6\*</sup>

<sup>1</sup> *Biomolecular Interaction Centre, MacDiarmid Institute for Advanced Materials and Nanotechnology, and School of Biological Sciences, University of Canterbury, Christchurch 8140, New Zealand*

<sup>2</sup> *Australian Research Council Centre for Cryo-electron Microscopy of Membrane Proteins, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, Victoria, Australia*

<sup>3</sup> *Victor Chang Cardiac Research Institute, Sydney, New South Wales 2010, Australia*

<sup>4</sup> *School of Chemical and Physical Sciences, University of Canterbury, Christchurch 8140, New Zealand*

<sup>5</sup> *Australian Synchrotron, Australian Nuclear Science and Technology Organisation (ANSTO), 800 Blackburn Road, Clayton, Victoria 3168, Australia*

<sup>6</sup> *Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, Victoria 3010, Australia*

Thermostable proteins provide valuable models for understanding molecular adaptations that enable life in extreme environments. Tripartite ATP-independent periplasmic (TRAP) transporters utilise a substrate-binding protein to deliver essential nutrients to a membrane component for transport into the cell. TRAP-associated extra-cytoplasmic immunogenic (TAXI) transporters are sub-group of TRAP transporters characterised by a unique substrate-binding protein.

Here, we present detailed biochemical and structural characterisation of *Tt*GluP, a TAXI glutamate-binding protein from *Thermus thermophilus* HB8, which displays extraordinary thermal stability and strong ligand retention. Under standard conditions, *Tt*GluP remains folded above 100 °C and is only destabilised in citrate buffer (100 mM, pH 4.5) at temperatures exceeding 88 °C; remarkably, it also remains folded after treatment with 8 M urea or 6 M guanidine hydrochloride. We determined the crystal structure of *Tt*GluP at 1.1 Å resolution, providing unprecedented atomic detail of the glutamate-binding site and overall protein architecture. The structure reveals water networks within the protein as well as several salt bridges, disulfide and hydrogen bonds, features likely contributing to thermostability and tight glutamate binding. Mutagenesis of the glutamate-binding site identified two key residues for stability, with Y32A (68.5 °C) and E111A (71.5 °C) mutants showing markedly reduced melting temperatures compared to the wild type. Y32's aromatic ring likely stabilizes the binding site through hydrogen bonding or stacking, while E111 appears to form a salt bridge with the glutamate's amino group.

Analysis of *Tt*GluP's exceptional stability provides insights into thermophile adaptation strategies, laying a foundation for rational engineering of highly stable binding proteins for biotechnology applications.

## **Novel interactions between the Human T-lymphotropic virus 1 HBZ protein and host nuclear import receptors**

Dr Crystall Bryan<sup>1</sup>, Alexia Street<sup>1</sup>, Dr Emily Cross<sup>1</sup>, Dr Justin Roby<sup>1</sup>, Prof Jade Forwood<sup>1</sup>

<sup>1</sup> *Gulbali Institute, Charles Sturt University, Wagga Wagga, Australia*

Human T-lymphotropic virus 1 (HTLV-1) is a human oncogenic retrovirus that causes lifelong infections associated with leukemia and myelopathy, as well as a range of chronic inflammatory conditions that promote secondary infections that reduce both the quality and length of life. There are currently no specific vaccines or antiviral therapies to treat HTLV-1 infection, and the pathogen remains a major burden to closing the gap in Indigenous Australian health and lifespan.

A characteristic of HTLV-1 infection is the suppressed transcription of most viral genes, with detectable protein expression only occurring during infrequent bursts. This transcriptional suppression is mediated by the viral basic leucine zipper protein HBZ. Constitutive low-level HBZ expression is correlated with HTLV-1 pathogenesis, as this factor enters the nucleus and silences proviral transcription from the 5' LTR. Low antigen expression is thought to help HTLV-1 escape immune detection and promote viral persistence.

Although nuclear translocation appears crucial for the inhibitory activity of HBZ, little is known regarding the precise mechanisms determining localization. Studies have revealed that 3 distinct basic regions of HBZ may act as nuclear localization signals (NLSs), and we have determined their precise interactions with host nuclear import receptors. Utilising a combined structural biology and biochemical approach we have determined the precise molecular interactions between HBZ and various nuclear import factor.

## Multiscale biological methods uncover human MORC2 as an ATP-dependent DNA compaction machine

Winnie Tan<sup>1,2,3</sup>, Jeongveen Park<sup>4</sup>, Hariprasad Venugopal<sup>5</sup>, Jieqiong Lou<sup>2</sup>, Prabavi Dias<sup>6</sup>, Pedro Baldoni<sup>1</sup>, Toby Dites<sup>1</sup>, Kyoung-Wook Moon<sup>4</sup>, Christine Keenan<sup>1</sup>, Alexandra Gurzau<sup>1</sup>, JoonYoung Lee<sup>4</sup>, Timothy Johanson<sup>1</sup>, Andrew Leis<sup>1</sup>, Jumana Yousef<sup>1</sup>, Vineet Vaibhav<sup>1</sup>, Laura Dagley<sup>1</sup>, Ching-Seng Ang<sup>2</sup>, Laura Corso<sup>1</sup>, Chen Davidovich<sup>5</sup>, Stephine Vervoort<sup>1</sup>, Gordon Smyth<sup>1</sup>, Marnie Blewitt<sup>1</sup>, Rhys Allan<sup>1</sup>, Elizabeth Hinde<sup>2</sup>, Sheena D'Arcy<sup>6</sup>, Je-Kyung Ryu<sup>4</sup>, Shabih Shakeel<sup>1,2,3</sup>

<sup>1</sup>Walter and Eliza Hall Institute of Medical Research, Parkville, Australia.

<sup>2</sup>University of Melbourne, Parkville, Australia.

<sup>3</sup>ARC Centre for Cryo-Electron Microscopy of Membrane Protein, Parkville, Australia.

<sup>4</sup>Seoul National University, Seoul, Republic of Korea.

<sup>5</sup>Monash University, Clayton, Australia.

<sup>6</sup>University of Texas at Dallas, Texas, United States of America.

The packaging of DNA into chromatin determines which regions of the genome are transcriptionally active or repressed. This process is tightly regulated by ATP-dependent chromatin remodelling enzymes. Microorchidia CW-Type Zinc Finger 2 (MORC2) is a recently identified chromatin remodeller implicated in transcriptional repression, yet its molecular mechanism remains poorly understood.

Here, we reveal that MORC2 activity is regulated by phosphorylation through the p21-activated kinase 1 (PAK1) kinase. Using recombinant full-length human proteins, we demonstrate that PAK1 directly phosphorylates MORC2 on six serine residues (Ser725, Ser730, Ser735, Ser743, Ser777 and Ser779). Unexpectedly, phosphodead MORC2 mutants retained DNA-binding capacity but exhibited markedly increased ATPase activity, suggesting that phosphorylation acts as a regulatory brake on MORC2's enzymatic function.

To investigate the structural basis of MORC2 activity, we employed cryo-electron microscopy, quantitative crosslinking, and hydrogen-deuterium exchange mass spectrometry. We determined that full-length MORC2 forms a symmetric dimer, engaging DNA through its coiled-coil (CC1) domain. *In vivo* live-cell single-molecule imaging and Chromatin Immunoprecipitation (ChIP) sequencing showed that MORC2 colocalises with DNA adjacent to chromatin. Finally, by applying high-speed atomic force microscopy (HS-AFM) and magnetic tweezers (MT), we provide the first experimental evidence that MORC2 directly compacts double-stranded DNA molecules in an ATP-dependent manner<sup>1</sup>.

Together, these findings establish MORC2 as a DNA compaction enzyme regulated by PAK1-mediated phosphorylation. This work uncovers a previously unrecognised mechanism of transcriptional repression, providing new insights into how ATP-dependent remodellers govern chromatin architecture.

### Reference:

1. Tan, W. *et al.* MORC2 is a phosphorylation-dependent DNA compaction machine. *Nat Commun* **16**, 5606 (2025).

## **An efficient eukaryotic cell-free expression and correlative cryo-electron tomography platform for structural biology of macromolecular complexes**

Vikas Tillu<sup>1</sup>, Dominic Hunter<sup>1</sup>, Yann Gambin<sup>2</sup> and Nicholas Ariotti<sup>1\*</sup>

<sup>1</sup>*Institute for Molecular Bioscience, UQ, Brisbane QLD, AUS*

<sup>2</sup>*UNSW, Sydney, NSW, AUS*

Cell-free expression using *Leishmania tarentolae* lysates allows rapid expression of eukaryotic proteins directly from DNA templates. Here, we develop a system that combines cell-free expression system with cryogenic fluorescence microscopy to target and resolve expressed protein complexes by cryo-electron tomography to high resolution that we term CC-FLEXCET (Correlative Cell-Free *Leishmania* EXpression and Cryo-Electron Tomography). We demonstrate the utility of this method by structurally characterising full-length Apoptosis-associated Speck like protein containing CARD (ASC) protein filaments. Cell-free expression of full-length ASC results in a polymeric structure characteristic of their cellular speck assembly, and sub-tomogram averaging allows us to resolve both the Pyrin Domain (PYD) to medium resolution, and show, for the first time, the arrangement of the flexibly linked caspase recruitment domain (CARD). CC-FLEXCET facilitates structural analysis of macromolecules and protein-lipid assemblies without need of purification, providing a pipeline from DNA template to protein expression to cryo-tilt series acquisition, within a single day.

## Comparison of electron diffraction data from crystallographic small molecules collected via different detectors and data collection software

Yufeng Zhao<sup>1,2\*</sup>, James Bouwer<sup>1,2</sup>, Mauro Gemmi<sup>3</sup>, Goulielmina Anyfanti<sup>3</sup>, David Waterman<sup>4</sup>, Hongyi Xu<sup>5</sup>, Sarfaraz Ali<sup>1</sup>, Paul A. Keller<sup>1</sup>, Michael Gardiner<sup>5</sup>

<sup>1</sup> School of Science, and Molecular Horizons Institute, University of Wollongong, Wollongong, Australia

<sup>2</sup> ARC Industrial Transformation Training Centre for Cryo-electron Microscopy of Membrane Proteins, University of Wollongong, Wollongong, Australia

<sup>3</sup> Center for Materials Interfaces, Italian Institute of Technology, Viale Rinaldo Piaggio 34, 56025 Pontedera, Italy

<sup>4</sup> Research Complex at Harwell, UKRI-STFC Rutherford Appleton Laboratory, Harwell, Didcot, Oxfordshire, United Kingdom

<sup>5</sup> Research School of Chemistry, Australian National University, Canberra, Australia

Followed by the workflow development of microcrystal electron diffraction (MicroED, also called 3D ED) (1), we further investigated the MicroED data quality collected via different detectors and data collection software. The *DIALS* package (2) was used for electron crystallographic data processing, and *SHELX* was used for direct phasing and structure refinement.

The below detectors and data collection software were used for acetaminophen (Table 1). The data collected by **settings III** showed the best quality, with  $cc1/2$  over 0.9 at the high-resolution shell while the cutoff threshold is 0.3.

Table 1. Acetaminophen electron diffraction data comparison

Instruments settings	Detector	Stage continuous rotating	Imaging	Completeness (%)	Structure resolution (Å)
I	Ceta camera	Delphi	TEM Imaging & Analysis (TIA)	100	0.8
II	Ceta camera	EPUD		97	0.7
III	Ceta-D camera	EPUD		80	0.6

Further experiments were carried out on acetaminophen treated via focused ion beam (FIB) milling. With the basic settings **I**, a high resolution of 0.6 Å was also achieved, but with a lower completeness of 80%.

The established MicroED workflow was then applied to study unknown lab compounds. The structure of one compound with good needle crystals was solved with **settings I** (Figure 1). A second compound with less ideal crystalline material demonstrated the challenge of MicroED. With settings **I**, a structure was achieved but with many disorders. However, X-ray single crystal diffraction yielded a structure with a good quality (Figure 2). The instrument **settings II** also yielded good quality of data that achieved 100% completeness at a resolution of 0.9 Å by merging 5 datasets (Figure 3), but it failed for direct phasing. A deeper data analysis vis PETS2 (3) was carried out, but it indicated the beam center was not stable. The reason wasn't clear. It could be related to the goniometer rotation axis operated via EPUD, or something related to the sample itself. Further experiments and investigations are being carried out now.

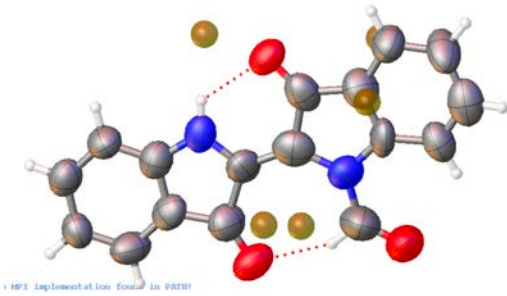


Figure 1. Lab compound sample #2 structure solved via MicroED.

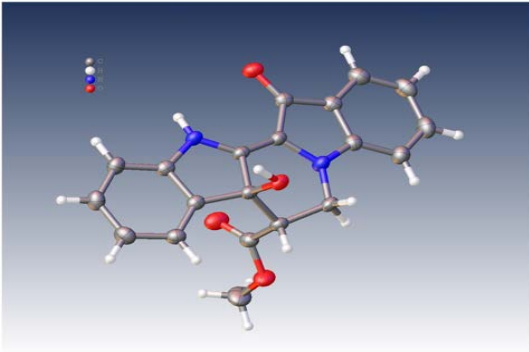


Figure 2. Lab compound sample #1 structure solved via X-ray single crystal diffraction.

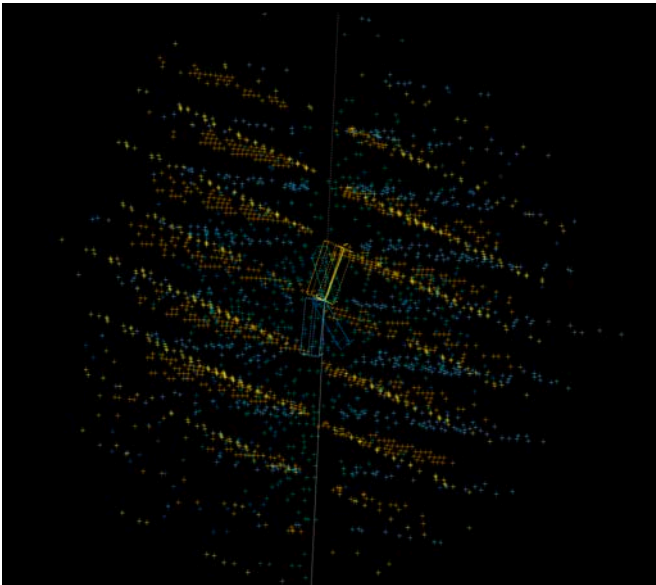


Figure 3. Reciprocal lattice merged from five datasets of sample #1, showing unit cells

1. Zhao Y, Oakley AJ, Waterman DG, Xu H, Bower JC, 2025, MicroED workflow development reveals the structure of a rare lysozyme polymorph and provides insights into MicroED applications. 13th Asia Pacific Microscopy Congress DOI: <https://doi.org/10.14293/APMC13-2025-0211>
2. Winter G, Waterman DG, Parkhurst JM, Brewster AS, Gildea RJ, Gerstel M, Fuentes-Montero L, Vollmar M, Michels-Clark T, Young ID, Sauter NK, Evans G, 2018, *Acta Crystallogr D Struct Biol* 74: 85-97.
3. Palatinus, L., Petříček, V., & Corrêa, C. A. (2015). Structure refinement using precession electron diffraction tomography and dynamical diffraction: theory and implementation. *Acta Crystallographica Section A: Foundations and Advances*, 71(Pt 2), 235-244.

## **CryoET of An Asgard archaeon from a modern analog of ancient microbial mats**

Matthew D Johnson<sup>1,2</sup>, Stephanie J Nobs<sup>3,4</sup>, Bindusmita Paul<sup>1,2</sup>, Doulin C Shepherd<sup>1,2</sup>, Brendan P Burns<sup>3,4</sup>, Debnath Ghosal<sup>1,2</sup>

<sup>1</sup>*Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, 3052, Australia*

<sup>2</sup>*ARC Centre for Cryo-electron Microscopy of Membrane Proteins, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, 3052, Australia*

<sup>3</sup>*School of Biotechnology and Biomolecular Sciences, The University of New South Wales, 2052, Australia*

<sup>4</sup>*Australian Centre for Astrobiology, The University of New South Wales, 2052, Australia*

The use of high-throughput sequencing and metagenomics has uncovered a new frontier of unculturable microorganisms. The biology of these organisms profoundly impacts our understanding of microbial interactions and evolution. However, many of these organisms are difficult to culture, genetically intractable, and rely on other microbes within their niche to proliferate.

CryoET is an ideal method to study genetically intractable microbial communities as it can determine high-resolution cellular details and macromolecular structures in situ without recombinant expression systems. Here we describe a highly enriched culture of a novel Asgard archaeon, *Nerearchaeum marumarumayae*, with a bacterium *Stromatodesulfovibrio nilemahensis* from a stromatolite-associated microbial mat (Fig 1A). Electron cryotomography revealed *N. marumarumayae* cells produce chains of budded envelope vesicles attached to the coccoid cell body by extracellular fibers, and intracellular tube- and cage-like structures. Furthermore, the two species were observed interacting via intercellular nanotubes assembled by the bacterium (Fig 1C and D). These characteristics and interactions may reflect an early step in the symbiotic evolution of eukaryotic cells.

Our work uncovers previously unseen interspecies interactions that occur in microbial mats and provides insights into the primordial nature of symbiosis and eukaryogenesis.

## Visualizing Mitochondrial Herniation in MEFs Using Cryo-FIB Milling and Cryo-ET

Sailakshmi Velamoor<sup>1</sup>, Sergey Gorelick<sup>2</sup>, Sylvain Trepout<sup>2</sup>, Patrick Cleeve<sup>1</sup>, Aseem Kashyap<sup>1</sup>, Kate McArthur<sup>1</sup>, Georg Ramm<sup>1,2</sup>

<sup>1</sup> Department of Biochemistry and Molecular Biology, Monash Biomedicine Discovery Institute, Monash University, Australia

<sup>2</sup> Ramaciotti Centre for Cryo - Electron Microscopy, Monash University, 15 Innovation Walk, Melbourne, 3800, VIC, Australia

Mitochondrial DNA are essential for cellular metabolism, ATP production, and signalling, but are highly susceptible to oxidative stress. When stressed, mtDNA released into the cytosol can trigger innate immune responses and has been associated with conditions including HIV and dengue infection, calcium overload, irradiation, or inflammatory diseases such as systemic lupus erythematosus or rheumatoid arthritis. During intrinsic pathway-mediated apoptosis, BAK/BAX induces mitochondrial permeabilisation, resulting in cytochrome c release and extensive remodelling of the mitochondrial network. Under conditions of prolonged permeabilisation and when normal apoptotic progression is inhibited, this remodelling can lead to mitochondrial herniation and the release of pro-inflammatory components [1]. Recent studies have identified a novel form of mitophagy initiated by the exposure of the inner mitochondrial membrane (IMM) to the cytoplasm. This process operates independently of the conventional PINK1/Parkin pathway and is driven by IMM ubiquitination [2]. While this pathway specifically targets IMM-mitophagy following BAK/BAX activation, alternative mechanisms also exist to sequester damaged mitochondria and mitigate inflammatory responses.

To investigate the mechanisms underlying mtDNA release, we employed advanced imaging techniques, including cryo-CLEM and cryo-electron tomography, to visualise mitochondrial herniation at nanoscopic resolution. Mcl-1-deficient mouse embryonic fibroblasts were stably transduced with TOMM20-HaloTag to label the outer mitochondrial membrane, and with TFAM-mNeonGreen to mark mitochondrial nucleoids. Cells were cultured on R2/2 carbon 200-mesh gold electron microscopy grids and treated with the caspase inhibitor QVD-oPH and the BH3 mimetic ABT-737 to induce apoptosis. Thin vitrified lamellae were prepared from plunge-frozen cells using focused ion beam milling, and cryo-electron tomograms were reconstructed to observe and analyse the herniating mitochondria. These findings reveal mitochondrial herniation as a key pathway for mtDNA release during apoptosis and highlight the importance of advanced imaging for dissecting mitochondrial biology.

### References:

1. McArthur, K., et al., BAK/BAX macropores facilitate mitochondrial herniation and mtDNA efflux during apoptosis. *Science*, 2018. 359(6378): p. eaao6047.
2. Saunders, T.L., et al., Exposure of the inner mitochondrial membrane triggers apoptotic mitophagy. *Cell Death Differ*, 2024. 31(3): p. 335-347.

## Cryo-EM Structures of the Herpes Simplex Virus 1 Annealase Protein ICP8 Reveal DNA Binding and Annealing Mechanisms

Lucy J. Fitschen<sup>1,2</sup>, Jodi L. Brewster<sup>1,3</sup>, Jordan J. Nicholls<sup>1,2</sup>, & Gökhan Tolun<sup>1,2</sup>

<sup>1</sup> School of Science, and Molecular Horizons, University of Wollongong, Wollongong, Australia

<sup>2</sup> ARC Industrial Transformation Training Centre for Cryo-electron Microscopy of Membrane Proteins (CCeMMP)

<sup>3</sup> Teva Pharmaceuticals, Sydney, Australia

The single strand annealing homologous recombination (SSA) pathway is one of many used for repairing dsDNA breaks. SSA is mediated by an Exonuclease-Annealase Two-component Recombinase (EATR) complex. The Herpes Simplex Virus 1 EATR complex is comprised of the exonuclease UL12 and the annealase ICP8. The currently available ICP8 structure (PDB ID=1URJ) was obtained using a C-terminally truncated version and lacks DNA. Therefore, molecular mechanistic details of how ICP8 forms homo-oligomers, binds to ssDNA strands, and anneals them, were still unknown. We present two ICP8 cryo-EM structures obtained using the full-length protein, one captured as a DNA-annealing intermediate.

Moreover, we employed a FRET (Fluorescence Resonance Energy Transfer) based EMSA (electromobility shift assay) for examining the DNA binding and annealing activities of ICP8.

We reconstructed a cryo-EM 3D density map of the ICP8 16-mer double ring bound to DNA, made up of 8 ICP8 dimers. The dimeric subunit of the ring was then further refined to a 2.2 Å resolution, showing the ICP8 dimer bound to DNA captured as an annealing intermediate (Figure 1, A.). Concurrently, using helical refinement, a DNA-free ICP8 filament was reconstructed to a resolution of 2.6 Å (Figure 1, B.). The asymmetric subunit from the filament also formed a dimer, showing an open confirmation that may allow DNA binding (Figure 1, C.). In addition, monomeric ICP8 was further refined using symmetry expansion, and resolved to a resolution of 2.0 Å, showing previously unmodeled loops.

Our results reveal molecular mechanistic details of how ICP8 annealase binds to and anneals DNA. The structures we determined and presented here have the potential to be used for future drug-development efforts.

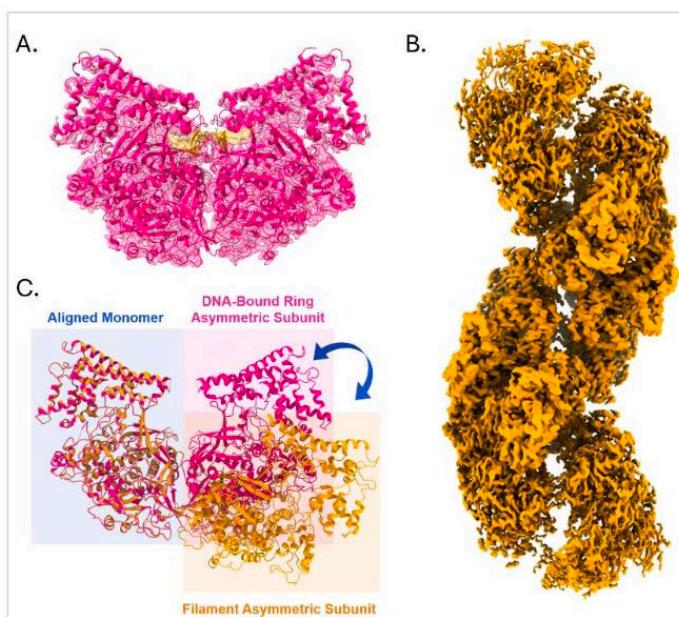


Figure 1. The oligomeric structures of ICP8. The dimeric form the ICP8 double ring map and structure (A.) showing protein (pink) and the annealing DNA (orange). The helical cryo-EM map (B.) of the DNA-free filament. A comparison between dimers (C.) from the DNA- bound ring (pink) and the DNA-free filament (orange).

## **A structural perspective on pore formation and regulation of *Bacteroides fragilis* toxins**

Riya Joseph<sup>1,2</sup>, Michelle P. Christie<sup>1,2</sup>, Bronte A. Johnstone<sup>1,3</sup>, Michael Gorman<sup>1</sup>, Craig J. Morton<sup>1,3</sup>, Rodney K. Tweten<sup>4</sup>, Michael W. Parker<sup>1,2,5</sup>

<sup>1</sup> Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, Victoria, Australia, <sup>2</sup> ARC Centre for Cryo-electron Microscopy of Membrane Proteins, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, Victoria, Australia, <sup>3</sup> CSIRO Biomedical Manufacturing Program, Clayton, Victoria, Australia, <sup>4</sup> Department of Microbiology and Immunology, University of Oklahoma, Health Sciences Center, Oklahoma City, Oklahoma, USA, <sup>5</sup> Australian Cancer Research Foundation Rational Drug Discovery Centre, St Vincent's Institute of Medical Research, Fitzroy, Victoria, Australia.

*Bacteroides fragilis* is a gut commensal that can become pathogenic under dysbiotic conditions. They utilise a novel family of pore-forming toxins, Cholesterol-Dependent Cytolysin-Like proteins (CDCLs), to outcompete neighbouring microbes (1,2). Despite their emerging significance, the mechanisms by which these bacteria form pores and avoid self-damage remain poorly understood (3).

This study focuses on the proteins involved in pore formation and regulation by *Bacteroides fragilis*, including two CDCLs, Bf long and Bf short, as well as BcdI, a surface lipoprotein that provides immunity to the producing strain. The amazing conformational changes of these proteins when they go from the monomer to the pore state are being studied using an integrated approach combining X-ray crystallography, SAXS, and cryo-EM.

I will be presenting SAXS solution structures of these proteins in their soluble monomeric state, the first-ever crystal structures of Bf short and BcdI, and negative-stain images and cryo-EM models of prepore-like and inserted pore states of these proteins. These structural studies will also be complemented by discussions on strategies to reconstitute CDCL pores on liposomes and visualise them by cryo-EM, as well as functional assays including liposome rupture and pull-down experiments. These findings provide new insight into the structural aspects of bacterial toxins and the elegant mechanism used by *B. fragilis* to deploy them while avoiding self-harm.

### References:

1. Wexler, H. M. (2007). *Bacteroides: The good, the bad, and the nitty-gritty*. *Clinical Microbiology Reviews*, 20(4), 593–621. <https://doi.org/10.1128/CMR.00008-07>
2. Johnstone, B. A., Joseph, R., Christie, M. P., Morton, C. J., McGuinness, C., Walsh, J. C., Böcking, T., Tweten, R. K., & Parker, M. W. (2022). *Cholesterol-dependent cytolysins: The outstanding questions*. *IUBMB Life*, 74(12), 1169–1179. <https://doi.org/10.1002/iub.2661>
3. Evans, J. C., Johnstone, B. A., Lawrence, S. L., Morton, C. J., Christie, M. P., Parker, M. W., & Tweten, R. K. (2020). *A key motif in the cholesterol-dependent cytolysins reveals a large family of related proteins*. *mBio*, 11(5), e02351-20. <https://doi.org/10.1128/mBio.02351-20>

## **Cryo-electron tomography to investigate the cellular architecture of *Plasmodium falciparum* female gametocytes**

Jiwon Lee<sup>1,2</sup>, Alexander Maier<sup>2</sup>, Melanie Rug<sup>1</sup>

<sup>1</sup>Centre for Advanced Microscopy, The Australian National University, Australia, <sup>2</sup>Research School of Biology, The Australian National University, Canberra ACT, Australia

*Plasmodium falciparum* is the causative agent of malaria, a disease that continues to impose a major global health burden. Despite significant progress in malaria control, efforts have since stalled, largely due to the emergence of resistance to all frontline drugs and the inability of most antimalarials to target the asymptomatic but transmissible sexual stages. To interrupt transmission and move toward elimination, there is an urgent need to better understand parasite biology and to identify novel intervention points.

Earlier electron microscopy studies provided critical insights into malaria parasite ultrastructure and laid the foundation for our understanding of sexual-stage biology. However, these approaches often required chemical fixation, heavy-metal staining and resin embedding, limiting preservation of native structures. Cryo-electron tomography (cryo-ET) now enables direct visualisation of vitrified parasites in their native state, offering three-dimensional information at nanometre resolution.

Here, we apply cryo-ET to examine the cellular organisation of *P. falciparum* female gametocytes. While both male and female gametocytes are essential for transmission, female gametocytes are metabolically distinct and provide the lipid resources required for parasite development in the mosquito. By comparing tomographic reconstructions with fluorescence microscopy and 3D FIB-SEM volume imaging, we aim to place ultrastructural observations into the context of whole-cell organisation. Ongoing segmentation and analysis of tomograms will provide new perspectives on gametocyte biology and establish a framework for applying cryo-ET to malaria parasites. Ultimately, these efforts will contribute to a deeper understanding of the structural adaptations that underpin transmission.

## **Cryo-EM Structure of Human Pregnancy Zone Protein (PZP) Reveals Dimerization Details**

Muhammad Zahir Siddiqui<sup>1</sup>, Jordan Cater<sup>1</sup>, Amy Wyatt<sup>2</sup>, Haibo Yu<sup>1</sup>, Gökhan Tolun<sup>1,3</sup>.

<sup>1</sup>*School of Chemistry and Molecular Bioscience, and Molecular Horizons, University of Wollongong, Wollongong, NSW, Australia*

<sup>2</sup>*Medical Biochemistry College of Medicine and Public Health, Flinders University, Adelaide, Australia*

<sup>3</sup>*ARC Industrial Transformation Training Centre for Cryo-electron Microscopy of Membrane Proteins, Wollongong, NSW, Australia*

Pregnancy Zone Protein (PZP) is a 180 kDa plasma glycoprotein belonging to the  $\alpha$ 2-globulin family. First identified in the late 1950s (Smithies, 1959), PZP gained attention due to its dramatic upregulation during pregnancy, where serum levels rise 100–1000-fold in the second and third trimesters (Beckman et al., 1970; Ekelund & Laurell, 1994). This surge suggested a pivotal role in shaping the maternal immune and proteolytic environments. Although less studied than other plasma proteins, growing evidence indicates that PZP contributes to immune tolerance, tissue remodelling, and metabolic regulation during gestation (Cater et al., 2019). It functions as an immunosuppressive antiprotease, while also acting independently as a carrier protein and has also been linked to macrophage modulation, weight maintenance, lipid metabolism, and tumorigenesis (Wu Y. et al., 2025).

Unlike other  $\alpha$ 2-globulin family members, structure of PZP remained unknown, leaving many of its properties inferred from homology models or low-resolution data, despite increasing interest in its functional roles (Wyatt et al., 2014; Wyatt et al., 2016). Structural characterization is crucial to understanding how its unique dimeric architecture influences protease trapping, immune regulation, and receptor-mediated clearance.

In this study, we employed cryo-electron microscopy to determine PZP structures in multiple conformational states: potentially bound to an endogenous ligand in a partially open and closed states, and in a protease-treated closed state. These structures provide the first high-resolution insights into PZP's molecular architecture, revealing how its dimeric interface governs conformational plasticity and ligand interactions. Our findings will establish a structural framework for deciphering PZP's physiological and pathological functions, particularly in the dynamic immunological and metabolic conditions of pregnancy.

## Probing Early Inactivation Events in hERG Using the Scorpion Toxin cnerg1

Carus Lau<sup>1,2</sup>, Mark Hunter<sup>1,2</sup>, Chai Ng<sup>1,2</sup>, James Bouwer<sup>3</sup>, Alastair Stewart<sup>1,2</sup>, Jamie Vandenberg<sup>1,2</sup>

<sup>1</sup>*The Victor Chang Cardiac Research Institute, Sydney, NSW, Australia*

<sup>2</sup>*St Vincent's Clinical School, University of NSW, Sydney, NSW, Australia*

<sup>3</sup>*University of Wollongong, Wollongong, NSW, Australia*

Inactivation of voltage-gated potassium channels is a critical gating process that modulates ion conduction through conformational changes at the selectivity filter. The human ether-à-go-go-related gene (hERG, Kv11.1) channel exhibits unusually rapid inactivation, yet the structural rearrangement underlying this transition remains incompletely understood. Here, we use the  $\gamma$ -KTx scorpion toxin CnErg1, derived from *Centruroides noxius*, as a molecular probe to capture early conformational changes during hERG inactivation.

CnErg1 binds hERG with nanomolar affinity. Notably, it incompletely blocks wild-type hERG current but fully occludes the inactivation impaired S631A mutant, suggesting a mechanistic coupling between inactivation and toxin binding. Using cryo-electron microscopy, we resolved structures of hERG in complex with CnErg1 in both wild-type and S631A backgrounds. These reveal distinct rearrangements at the selectivity filter, including displacement of the Gly628 carbonyl and vertical movement of the selectivity filter.

Our findings support a model in which CnErg1 stabilises the open state in the inactivation pathway, and demonstrate that selectivity filter rearrangements precede inactivation. This work establishes CnErg1 as a state-sensitive probe of hERG gating and provides structural insight into the extracellular conformational dynamics that govern inactivation.

## **A Practical Workflow for Cryo-ET of Mammalian Cells: Optimized patterned Gold Coating and Cell Suspension-Based Deposition**

Le (Christy) Ying<sup>1,2</sup>, Denis Korneev<sup>3</sup>, Sergey Gorelick<sup>3</sup>, Sylvain Trepout<sup>3</sup>, Georg Ramm<sup>3,4</sup>, Vivek Naranbhai<sup>1,2,5</sup>

<sup>1</sup>*Department of Medicine, School of Translational Medicine, Monash University, Melbourne, Australia*

<sup>2</sup>*ARC Centre for Cryo-electron Microscopy of Membrane Proteins, Monash Institute of Pharmaceutical Sciences, Melbourne, Australia*

<sup>3</sup>*Ramaciotti Centre for Cryo-Electron Microscopy, Monash University, Melbourne, Australia*

<sup>4</sup>*Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Melbourne, Australia*

<sup>5</sup>*The Alfred Hospital, Melbourne, Australia*

Cryo-electron tomography (cryo-ET) enables visualization of cellular ultrastructure at near-atomic resolution while preserving native biological context. While widely applied to microorganisms, extending cryo-ET to human cells has been difficult due to challenges in sample preparation. Seeding mammalian cells directly on grids often results in poor single-cell coverage, uneven distribution, and grid damage, which lower the success rate of cryo-focused ion beam (cryo-FIB) milling and reduce tomogram quality.

Here we describe an optimized workflow for preparing human lung cancer cells (NCI-H441) for cryo-ET. By testing different grid coatings, we discovered that a pattern gold support, improved cell retention compared to conventional carbon films. Instead of growing cells directly on grids, we created a single-cell suspension, which was then applied directly to a coated grid for plunge-freezing improving isolation of individual non-overlapping cells. By adjusting cell density, suspension volume, and incubation time before plunge-freezing, we achieved reproducible single-cell deposition with minimal grid damage. We further utilized cryo-FIB lamella preparation by introducing “fillets” to stabilize milling and improve the overall yield of ready-for-TEM lamellae by 33%.

This workflow enabled reliable visualization of intracellular organelles in human lung cancer cells while using fewer grids and shorter preparation time. The combination of patterned gold supports, cell suspension-to-grid deposition, and modified milling provides a practical strategy for preparing mammalian cells for cryo-ET and should be readily transferable to other cell types.

## **PartiNet is a dynamic adaptive neural network for high-performance particle picking in cryo-electron microscopy**

Mihin Perera<sup>1,2</sup>, Winnie Tan<sup>1,3,4,7</sup>, Edward Yang<sup>1,7</sup>, Ojasvi Jain<sup>1,8</sup>, Mansi Aggarwal<sup>1,8</sup>, Hariprasad Venugopal<sup>5</sup>, Julie Iskander<sup>1</sup>, Joseph D. Berry<sup>2</sup>, Andrew Leis<sup>1,3</sup>, Shabih Shakeel<sup>1,3,4,6</sup>

<sup>1</sup> WEHI, 1G Royal Parade, Parkville, VIC, 3052, Australia

<sup>2</sup> Department of Chemical Engineering, The University of Melbourne, Parkville, VIC, 3052, Australia

<sup>3</sup> Department of Medical Biology, The University of Melbourne, Parkville, VIC, 3052, Australia

<sup>4</sup> ARC Centre for Cryo-electron Microscopy of Membrane Proteins, The University of Melbourne, Parkville, VIC, 3052, Australia

<sup>5</sup> Monash University, Clayton, VIC 3168, Australia

<sup>6</sup> Department of Biochemistry and Pharmacology, The University of Melbourne, Parkville, VIC, 3052, Australia

<sup>7,8</sup> Equal contribution

Cryogenic electron microscopy (cryo-EM) is a widespread methodology for visualising protein molecules. Advanced image processing techniques allow for reconstructing atomic-resolution 3D maps of protein molecules from noisy 2D projection images captured in a transmission electron microscope. The accuracy and resolution of cryo-EM structures is heavily dependent on the effectiveness of identifying these protein particles in micrographs. Existing algorithms struggle with heterogeneous and complex datasets, leading to errors in particle identification, and ultimately leading to low resolution 3D reconstructions of proteins.

We introduce PartiNet, a next-generation AI-based particle picker that integrates a dynamic neural network with an adaptive routing mechanism into the single-particle cryo-EM pipeline. PartiNet adjusts its architecture in real time to match the complexity of individual micrographs, accelerating inference up to 7-fold over current AI methods without sacrificing accuracy, making it compatible with on-the-fly workflows. Trained on a comprehensive dataset of ~10,000 micrographs, PartiNet consistently improves particle yields, enhances sampling of rare orientations, and enables higher-resolution reconstructions. An integrated denoising strategy further enhances performance, allowing robust identification of rare particle views.

Evaluation on benchmark datasets and novel structures - including the chromatin remodeler MORC2, rabbit muscle aldolase, and TcdA1 - demonstrates that PartiNet surpasses existing AI-based pickers for quality of particles for reconstruction and sampling in the Euler sphere. Furthermore, PartiNet enables detection of heterogeneous protein species in a single step, as shown with our testing on the Ankyrin-1 and MlaC-MlaD complexes. Thus, PartiNet sets a new standard for speed, accuracy, and adaptability in particle picking in cryo-EM.

## **Cryo-EM structure of *C. neoformans* Ade2 reveals fungal-specific active site insertions in the purine biosynthesis**

XIAOQI QIAN<sup>1</sup>, TRISTAN VILLAMOR<sup>1</sup>, CHENDI YU<sup>1</sup>, JOBICHEN CHACKO<sup>1</sup>, WEIXI GU<sup>1</sup>, HARI VENUGOPAL<sup>2</sup>, JAMES FRASER<sup>1</sup>, BOSTJAN KOBE<sup>1</sup>

<sup>1</sup> *The University of Queensland, SCMB, Brisbane, Australia*

<sup>2</sup> *The University of Monash, Ramaciotti Centre for Cryo-Electron Microscopy, Melbourne, Australia*

*Cryptococcus neoformans*, a WHO-designated critical fungal pathogen, depends on de novo purine biosynthesis to sustain infection in the purine-depleted human central nervous system. *C. neoformans* is an opportunistic fungal pathogen that causes life-threatening infections in immunocompromised individuals. De novo purine biosynthesis is critical for its fungal survival and virulence. Despite its importance, the molecular basis of this pathway in *C. neoformans* remains limited. This study focuses on the structural and functional characterisation of Ade2, a unique bi-fusion enzyme catalysing the conversion of 5-aminoimidazole ribonucleotide (AIR) to 4-carboxy-5-aminoimidazole ribonucleotide (CAIR). Unlike bacteria and archaea, which express CAIRS and CAIRM-I as separate enzymes, and metazoans, which use CAIRM-II, fungi uniquely encode a fused CAIRS-CAIRM-I enzyme. The cryo-EM structure of Ade2 was determined at 2.15 Å, revealing an octameric assembly consistent with mass photometry. An X-ray crystal structure was also obtained at 3.2 Å. Structural comparison with human homologues identified a unique region in fungal Ade2 near the active sites, suggesting a potential site for antifungal intervention. Domain functionality was assessed by constructing *C. neoformans* strains with targeted deletions of either the CAIRS or CAIRM-I domain. Both deletion strains exhibited impaired growth, indicating that CAIRS and CAIRM-I are each essential for Ade2 function in vivo. This work presents the first structure of a fungal Ade2 fusion enzyme and defines its essential role in *C. neoformans* physiology. Our results show that Ade2 is a structurally distinct, fungus-specific target in the purine biosynthesis pathway and provides a foundation for developing selective antifungal therapeutics.

## Waffling an Autogrid to improve cryo EM workflow

Xavier Heiligenstein<sup>1</sup>, Yann Bret<sup>1</sup>, Chie Kodera<sup>1</sup>, Martin Belle<sup>1</sup>

<sup>1</sup>CryoCapCell, Paris, France

As Cryo-electron microscopy is expanding to an ever-growing number of laboratories, it is also pushing the boundaries of the sample to be observed. As plunge freezing reveals to be limited to isolated particles or extreme thin samples (below 5 $\mu$ m thin), the waffle technique relying on High Pressure Freezing<sup>1,2</sup>, opens the way to samples ranging from 20 $\mu$ m to 200 $\mu$ m.

We have developed some novel tools that facilitate the waffle technique<sup>3,4</sup> by High Pressure Freezing EM grid, pre-clipped in Autogrid. The approach speeds up the sample preparation and its reliability to focus on the biological question.

To further expand the technique, we are now exploring combining our method to CLEM approaches, relying on our well-established experience using the CryoCapsule.

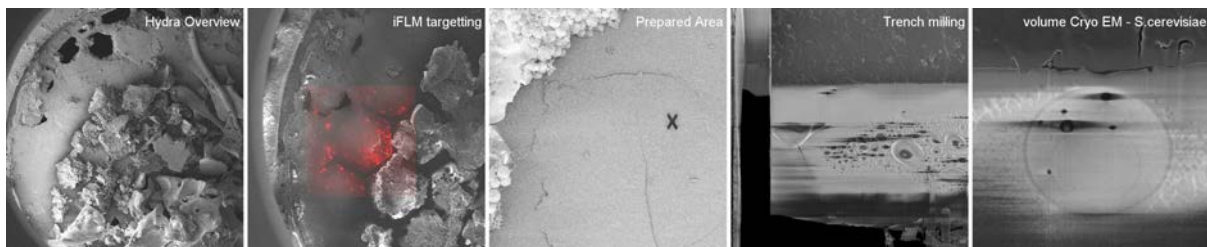


Figure 1. Cryo-FIB-SEM of a pre-clipped Autogrid, vitrified by High Pressure Freezing.

1. Heiligenstein, X. *et al.* Live-HPF CLEM using the HPM Live  $\mu$ : Finding back all needles in every haystack. The last frontier in CLEM. *bioRxiv* 2024.10.21.619370 (2024) doi:10.1101/2024.10.21.619370.
2. Heiligenstein, X. *et al.* HPM live  $\mu$  for a full CLEM workflow. *Methods Cell Biol* **162**, 115–149 (2021).
3. Kelley, K. *et al.* Waffle Method: A general and flexible approach for improving throughput in FIB-milling. *Nat Commun* **13**, 1857 (2022).
4. Schiøtz, O. H. *et al.* Serial Lift-Out: sampling the molecular anatomy of whole organisms. *Nature Methods* **2023** *21:9* **21**, 1684–1692 (2023).

## Montage cryo-tomography with square and rectangular beams

Hamish G. Brown<sup>1,2\*</sup>, Manasi Mudaliyar<sup>2,3</sup>, Matthew D. Johnson<sup>2,3</sup>, Bronte A. Johnstone<sup>2,3</sup>, Debnath Ghosal<sup>2,3,4</sup> and Eric Hanssen<sup>1,2,4</sup>

<sup>1</sup> *Ian Holmes Imaging Centre, The Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Victoria, Australia.*

<sup>2</sup> *ARC Industrial Training Centre for Cryo-electron Microscopy of Membrane Proteins, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Victoria, Australia*

<sup>3</sup> *The Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Victoria, Australia.*

<sup>4</sup> *Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Melbourne, VIC, Australia*

Cryo-electron tomography in transmission electron microscopy (TEM) enables high-resolution three-dimensional imaging of biological specimens preserved in vitreous ice by plunge or pressure freezing. Single tomograms typically capture small regions (areas of a few square micrometres) and larger fields of view such as eukaryotic cells and even tissue can be achieved without compromising resolution by montage. Montage involves collecting an array of overlapping high-resolution images that are computationally stitched (aligned and blended) into a single large image. In biological cryo-TEM samples this is challenging since the round lobes on standard TEM beams spread over the edges of the square or rectangular cameras and illuminate adjacent tiles in the montage array causing excess beam damage to the samples. We have recently developed square and rectangular condenser apertures that form corresponding square and rectangular beams in our electron microscope<sup>1</sup> that ameliorates this problem.

In this presentation we discuss the development of a workflow, implemented in the freely available Serial-EM microscope automation software<sup>2</sup>, that allows rapid and routine collection of montage tomograms with rectangular beams. Users can indicate areas for collection by drawing polygons in the serial-EM interface covering the regions of interest. A script automatically calculates a montage scheme that efficiently covers that region of interest with montage tile overlap chosen to minimize beam damage distribute spread regions of excess dose as evenly as possible over the whole tomogram acquisition, see Figure 1. We demonstrate the approach on migrasomes—recently identified extracellular organelles formed during cell migration<sup>3</sup>—that extend several micrometres in length.

## Activation mechanism of an ABC toxin revealed by Cryo-EM

Solace Roche<sup>1</sup>, Yu Shang Low<sup>1</sup>, Nadezhda Aleksandrova<sup>1</sup>, Jasper Stone<sup>1</sup>, Michael Furlong<sup>2</sup>, Andrew Walker<sup>3</sup>, Evelyne Deplazes<sup>1</sup>, Mark RH Hurst<sup>4</sup> and Michael J. Landsberg<sup>1,3</sup>

<sup>1</sup> School of Chemistry and Molecular Biosciences, <sup>2</sup> School of the Environment, and <sup>3</sup> Institute for Molecular Biosciences, The University of Queensland, St. Lucia, QLD, Australia

<sup>4</sup> Resilient Agriculture, Bioeconomy Science Institute, Lincoln 7608, New Zealand

ABC toxins are mega-Dalton-sized, pore-forming proteins and virulence factors found in bacterial pathogens of insects and some vertebrates. They have two distinct components, the membrane binding and pore forming component (TcA), and the active component (TcBC). The complete ABC toxin pre-pore binds to specifically targeted host cell membranes in its pre-pore form, following which it has been hypothesised that receptor-mediated endocytosis and subsequent endosome acidification induces pore-formation. The toxin payload, encapsulated by TcBC, is then translocated through the TcA pore into the cell where it elicits its toxic effects. To study this process in more detail, we solved high resolution cryo-EM structures of the ABC toxin complex from *Yersinia entomophaga* (YenTc) in both the pre-pore and pore conformations under a variety of pH conditions designed to mimic different physiological environments. In doing so, we discovered a unique structural domain within YenTc not found in other ABC toxins characterised to date. By combining cryo-EM, biochemical assays, and molecular dynamics simulations, we establish that this domain plays an important role in stabilising the pre-pore configuration of YenTc. Moreover, we found that this domain is susceptible to proteolysis, and that proteolysis plays an important role in triggering YenTc pore formation. Treatment with enzymes found in the midgut of insects susceptible to YenTc produces a pattern of proteolysis that is sufficient to trigger pore formation under conditions of cryo-EM. Collectively our results suggest that host proteases found in the midgut of susceptible insects play a crucial role in the activation of YenTc *in vivo*.

## **Into the unknown: THV, a cryptic Class 4 RNA virus hiding in ancient archaea**

Raphael Caballes<sup>1</sup>, Juanfang Ruan<sup>2</sup>, Milad Ghafoori<sup>2</sup>, Daniel Luque<sup>2</sup>, and David Jacques<sup>1</sup>

<sup>1</sup> *Single Molecule Science, Lowy Cancer Research Centre, University of New South Wales Kensington Campus, Sydney, Australia*

<sup>2</sup> *Electron Microscope Unit, Mark Wainwright Analytical Centre, University of New South Wales Kensington Campus, Sydney, Australia*

The recent emergence of SARS-CoV2 highlighted the importance of understanding the structures of viral proteins for vaccine design. The pandemic has simultaneously raised fundamental questions regarding global preparedness for future health crises and the adequacy of existing biomedical research infrastructure. This project aims at establishing the capability of AlphaFold2 in aiding construct design with clear and defined domain boundaries of novel proteins from a putative virus. An uncharacterised archaeal virus (ThV), where the genetic material and not the virion was observed, had been studied in this project. Running the viral ORF through AlphaFold2 yielded intriguing models for an RNA-dependent RNA Polymerase (ThVP) and a viral capsid (ThVC). The resulting models were used to design the respective constructs for subsequent protein expression. The expression of the different proteins and their assembly state had been validated with mass photometry. Cryo-EM yielded electron density maps of the ThVC (2.85 Å) in its trimer state and the monomeric ThVP (4.00 Å) using single particle analysis, both closely resembling the AlphaFold2 predicted models. Furthermore, the ThVP was confirmed to be functional using a primed RNA extension assay. In conclusion, this study was able to fully demonstrate the competency of AlphaFold2 in elucidating the structural intricacies of novel proteins derived from an uncharacterised virus (ThV). The findings underscored the value of AlphaFold2 in improving our understanding of the structural landscape of viruses and potentially enhancing our preparedness for future pandemics.

# Emerging Leaders in Structural Biology

## The molecular triggers of $\gamma\delta$ T cell activation

Benjamin S. Gully

*Olivia Newton-John Cancer Research Institute, School of Cancer Medicine, La Trobe University, Vic 3084 Australia*

### Abstract

Here Ben will summarise the team's recent work on  $\gamma\delta$  T cell activation. Using advanced molecular imaging the team deciphered the precise molecular triggers of activation. This included the cryo-EM structure of the  $\gamma\delta$  T cell triggering complex, which revealed incredible receptor dynamism. Single molecule imaging revealed that altered T cell triggering stemmed from receptor dynamics, thereby modulating cellular activation. The pathways controlling  $\gamma\delta$  T cell activation are coveted due to the translational promise of  $\gamma\delta$  T cell therapies.

### Biography

Dr Benjamin Gully uses protein imaging to study the molecules that regulate immune cell activation. After completing his PhD at UWA, he was recruited to Monash University. Here he established a team researching a poorly understood adaptive immune cell type. Gamma delta T cells have homeostatic roles and detect aberrant cells in infections or cancer. In 2025 he founded the Immunosurveillance laboratory at the ONJCRI and La Trobe University School of Cancer Medicine to team study the pathways that these cells use to sense normality and dysregulation.



## From Bacteria to Humans: Structural Insights into Transport Mechanisms

Rachel North

*School of Medical Sciences, University of Sydney, Sydney, Australia*

### **Abstract**

Membrane transporters underpin cellular physiology across all domains of life, yet many remain poorly understood at the molecular level. My research program seeks to uncover how diverse transporter families recognise substrates, undergo conformational change, and contribute to human health and disease. My early work focused on bacterial sialometabolism and the structural and functional diversity of TRAP transport systems. Building on these foundations, my new laboratory at the University of Sydney integrates cryo-electron microscopy, structural biology, biochemistry, and computational approaches to explore two major themes: (1) deorphanising human SLC transporters, particularly atypical and brain-enriched members with poorly defined substrates or physiological roles; and (2) expanding our mechanistic understanding of bacterial TRAP transporters, a diverse and understudied family central to microbial physiology and nutrient acquisition. By combining biophysical screening, structure determination, and molecular modelling, our long-term vision is to illuminate how transporter dysfunction contributes to disease—and to lay the groundwork for targeting these systems therapeutically.

### **Biography**

Dr. Rachel North is a membrane protein biochemist and Group Leader in the School of Medical Sciences at the University of Sydney. Supported by an ARC DECRA Fellowship, her laboratory investigates the structure, function, and physiological roles of human solute carrier transporters—particularly atypical and understudied members relevant to brain biology and disease. Her group also continues to explore the mechanistic diversity of bacterial TRAP transport systems. Originally from Christchurch, New Zealand, Rachel completed her BSc and PhD in Biochemistry at the University of Canterbury, where she first developed her passion for membrane transporters. She undertook postdoctoral training with Professor Ren Dobson (University of Canterbury) and later with Professor David Drew (Stockholm University), gaining expertise in structural biology, cryo-electron microscopy, and transporter biochemistry.



## **Decoding genome instability through visual biochemistry**

Jacob Lewis

*University of Wollongong, Molecular Horizons, Wollongong, NSW, Australia*

### **Abstract**

DNA replication is one of the most fundamental yet intricate processes sustaining life, and its failure underlies diverse human diseases including cancer and mitochondrial disorders. Our lab at Molecular Horizons, University of Wollongong, investigates how replication machinery assembles, activates, and operates at the molecular level. We combine cryo-electron microscopy, single-molecule fluorescence imaging, and biochemical reconstitution to capture the dynamic architectures of human replication complexes in action. By integrating these approaches, we reveal how molecular machines coordinate DNA unwinding, synthesis, and quality control across nuclear and mitochondrial systems. Our long-term vision is to bridge structural and mechanistic understanding with biomedical insight, translating discoveries of replisome dynamics into new perspectives on genome maintenance and disease.

### **Biography**

Dr Jacob Lewis is a biochemist and Senior Lecturer at the University of Wollongong's Molecular Horizons research institute. His interdisciplinary research combines cryo-electron microscopy, single-molecule imaging, and biochemical reconstitution to uncover how genome replication operates and fails in human disease. After completing his PhD at UOW, he undertook postdoctoral training at the UOW and later at the Francis Crick Institute, supported by EMBO and Marie Skłodowska-Curie Actions fellowships. He now leads a dynamic team supported by an ARC DECRA and NHMRC Investigator Grant, exploring nuclear and mitochondrial DNA replication and genome instability.



## Structural biology of nutrient sensing in eukaryotes

Charles Bayly-Jones

*Monash University, Biomedicine Discovery Institute (BDI), Clayton, Australia*

### Abstract

Eukaryotic cells precisely coordinate growth and metabolism through nutrient-sensing pathways centered on mTORC1. These pathways are deeply conserved across the eukaryotic lineage (yeast to humans), coupling environmental and metabolic cues to control cell growth. Here, we combine cryo-EM, CRISPR–Cas9 genome editing, fluorescence microscopy, biosensors, and computational analyses to elucidate the molecular architecture and regulation of key mTORC1 pathway components. We determined high-resolution structures of TSC, LYCHOS, and lysosomal biogenesis machinery, revealing how nutrient and lipid cues are integrated to control mTORC1 activity. Our structures uncover the molecular basis of cholesterol sensing in mammals and provide mechanistic insight into TSC lysosomal docking to suppress mTORC1. Together, these findings establish a structural framework for understanding nutrient sensing and growth control in the eukaryotic branch of life — illuminating fundamental principles that underlie how complex cells interpret their metabolic environment.

### Biography

Charles is a recently appointed laboratory head and ARC DECRA fellow (2024-2027) who oversees an emerging team at Monash University. He completed his bachelor's degree (2016; BSc Science Scholar program) with specialisations in theoretical physics and biochemistry, before continuing his training within the ARC Centre of Excellence for Advanced Molecular Imaging to earn a PhD (2021). He was a post-doctoral researcher under the mentorship of ARC laureate fellow Professor James Whisstock (2021-2022), before joining the group of Professor Andrew Ellisdon (2022-2025). Charles uses integrative structural biology approaches (cryoEM, X-ray crystallography, biosensors) to study the mechanisms of nutrient sensing in eukaryotes with a focus on single-molecule methods to tackle protein conformational dynamics. Charles develops the software package "WIGGLE" and is currently building software and biotechnologies to accelerate discovery science in the mTORC1 field. In 2025, Charles was awarded the Victorian Premier's Award for Health and Medical research in the Basic Science category. He has successfully led projects at both the national and international level, bridging academia and industry. He has previously been supported by industry and NHMRC funding, he currently holds philanthropic funding through the Monash Medicine ECR Excellence Program and the Biomedicine Discovery Institute.



## Understanding and Harnessing Pore-Forming Proteins

Bradley Spicer<sup>1</sup>, Raymond Wirawan<sup>1</sup>, Bill Ho<sup>1</sup>, William Elliott<sup>1</sup>, Suzanne Wang<sup>1</sup>, David Jamieson<sup>2</sup>, Hannah Baird<sup>2</sup>, Christopher Lupton<sup>1</sup>, Charles Bayly-Jones<sup>1</sup>, Hari Venugopal<sup>1</sup>, Oliver Castell<sup>2</sup>, Colin Berry<sup>2</sup>, Michelle Dunstone<sup>1</sup>

<sup>1</sup> Monash University, Biomedicine Discovery Institute (BDI), Clayton, Australia

<sup>2</sup> Cardiff University, School of Biosciences, Cardiff, Wales

### Abstract

Pore-forming proteins (PFPs) are molecular machines that punch holes into biological membranes with remarkable specificity, serving as toxins, immune effectors, and key players in defence. Despite their shared outcome of membrane perforation, PFPs employ an incredible diversity with respect to mechanisms. These include the fundamental steps of pore-forming biology: targeting membranes or receptors, oligomerizing into higher-order assemblies, and ultimately inserting into the membrane to disrupt their targets.

Advances in cryo-electron microscopy have revealed many of these molecular details, ushering in a new era for understanding and engineering pores. Structural insights are now helping bridge biology and biotechnology, showing how PFPs can be adapted for applications from sensing to pest control. In this talk, I will highlight our lab's efforts towards characterising pore-forming proteins as biotechnological platforms and as environmentally relevant toxins with the goal of harnessing these proteins for practical use.

### Biography

Bradley is an ARC DECRA Fellow whose research explores the structural and mechanistic diversity of pore-forming proteins — molecular machines that remodel biological membranes. His interests include the evolutionary origins of these pore-forming systems and their potential applications in biotechnology and vector control. He has contributed to several academic and industry-led projects and aims to become a leader in harnessing structural biology to develop more sustainable agricultural solutions.



## **An interdisciplinary approach to anti-cancer drug design: From chemistry to structural biology**

Jennifer Baker

*University of Wollongong, Molecular Horizons, Wollongong, NSW, Australia*

### **Abstract**

Cancer remains one of the most complex diseases to treat, requiring innovative strategies that bridge multiple scientific disciplines. This work chronicles our journey from medicinal chemistry—focused on the rational design and synthesis of small molecules—to structural biology, where protein-ligand interactions are revealed at atomic resolution. By integrating *in silico* modeling, synthetic chemistry, bioassays, and cryogenic electron microscopy, we are developing and optimizing novel compounds that target critical oncogenic proteins. This talk will focus on the pathway I have taken from my home in Newcastle to San Diego, then on to Wollongong to establish my own research group, and the work we are doing to design small molecules to inhibit oncogenic targets.

### **Biography**

Dr Jennifer Baker completed her PhD at the University of Newcastle in 2020, focussed on the design, synthesis and biological analysis of small molecules targeting the Arylhydrocarbon Receptor for potential breast and pancreatic cancer treatments. In late 2020, she was part of a team that acquired an NHMRC Ideas Grant studying the impact of the inhibition of the histone lysine demethylase 4 (KDM4) in glioblastoma. Upon finalising the grant in 2023, she successfully obtained a Fulbright Postdoctoral Fellowship to work at the Scripps Research Institute from January-June 2024, learning cryo-EM for drug design. Upon her return to Australia, she began at the University of Wollongong as a Vice-Chancellor's Research Fellow



## Structural basis of bacterial nickel import by Type I ABC transporters

Sarah L. Mueller<sup>1</sup>, Simon H. J. Brown<sup>2</sup>, Emily J. Furlong<sup>1</sup>

<sup>1</sup> Division of Biomedical Science and Biochemistry, Research School of Biology, Australian National University, Canberra, ACT, Australia

### Abstract

Nickel is an essential cofactor for the function of at least nine fundamental bacterial enzyme families, including ureases, NiFe-hydrogenases and carbon monoxide dehydrogenases.<sup>1</sup> In many bacteria, nickel transport across the inner membrane is mediated by Type I ATP-binding cassette (ABC) transporter systems.<sup>1</sup> These systems are composed of five proteins: one periplasmic nickel binding protein, heterodimeric transmembrane domains and heterodimeric nucleotide binding domains. Here we present a 2.2 Å cryogenic electron microscopy structure of the primary nickel transporter, YntBCDE, from the uropathogen *Proteus mirabilis*. The structure is in an inward-open conformation, and our analysis highlights conserved key residues for nickel transport in the transmembrane domains of nickel ABC transporters and distinct features in the heterodimeric nucleotide binding domains.

1. Waldron, K.J. and N.J. Robinson, Nat Rev Microbiol, 2009.

### Biography

Dr Emily Furlong earned her BSc and Honours at the University of Queensland before completing a PhD at the Institute for Molecular Bioscience, where she uncovered the structure and function of a previously uncharacterised class of copper resistance proteins in the uropathogen *Proteus mirabilis*. During her PhD, Emily trained in cryo-electron microscopy (cryo-EM) at the University of Oxford. This formative experience led her back to Oxford in 2019 as a postdoctoral researcher. There, she spent two years advancing her cryo-EM expertise while investigating the architecture of the bacterial flagellar motor. In 2021, she returned to Australia to join the Victor Chang Cardiac Research Institute, where she applied cryo-EM to study bacterial ATP synthases. In 2023, Emily was appointed Lecturer at the Australian National University's Research School of Biology, where she established her own lab focused on the structural biology of bacterial virulence and defence systems with the support of an ANU Futures Award.



**Gavin Knott**

*Monash University, Biomedicine Discovery Institute (BDI), Clayton, Australia*

**Abstract**

Bacteria and their bacteriophages have antagonistically co-evolved for billions of years, driving the evolution of diverse immune strategies and elaborate countermeasures. Critical to both microbial innate and adaptive immunity is the ability to differentiate self from non-self nucleic acids. Here I will describe our research program that explores microbial genomes for novel bacterial DNA or RNA targeting defence systems. Our team combines bioinformatics, microbiology, biochemistry, structural biology, and protein engineering to understand how these incredibly diverse microbial defence systems function across life. As with restriction enzymes and CRISPR-Cas systems, we aim to leverage our understanding of microbial defence systems to develop enabling technologies that can be applied to basic nucleic acid research, diagnostics, or in therapeutic applications.

**Biography**

A/Prof. Gavin Knott is a Snow Medical Fellow, Scientific Director of the AI Protein Design Program, and Lab Head in the Biomedicine Discovery Institute at Monash University. Gavin obtained his PhD from the University of Western Australia in 2016 where he trained as an X-ray crystallographer. In 2016, he trained as a postdoctoral fellow and cryo electron microscopist at UC Berkeley supervised by Prof. Jennifer Doudna and supported by an American Australian Association Fellowship. In 2021, he established his lab in the Biomedicine Discovery Institute at Monash University supported by an NHMRC Investigator Grant (EL1). Supported by a Snow Medical Fellowship, the Knott Lab now explores microbial innate and adaptive immune systems to develop enabling technologies for nucleic acid research, diagnostics, and therapeutics development.



## Folds to Fibrils: Protein Structure in Health and Disease

Aidan Grosas

*University of Wollongong, Molecular Horizons, Wollongong, NSW, Australia*

### Abstract

My lab uses cryo-electron microscopy including single-particle analysis and helical reconstruction routines. These are integrated with protein chemistry, biophysical assays and other structural biology techniques to understand how protein structure underpins function and failure. We focus on two connected themes. First, we study amyloid fibrils, from functional assemblies such as peptide hormones to pathological aggregates including A $\beta$ , tau,  $\alpha$ -synuclein and patient-derived fibrils. By resolving their architectures and ligand-binding sites, we ask how distinct fibril conformations encode either function or toxicity and use this to identify potential therapeutic opportunity. Second, we investigate membrane protein complexes, particularly bacterial and mammalian efflux pumps and peptide transporters that drive antimicrobial resistance and cell signalling. Across both areas, our aim is to link high-resolution structural insights into mechanism, tracing how proteins move from native folds to higher-order assemblies that shape health and disease.

### Biography

Dr Aidan Grosas is a Lecturer and Group Leader within the Molecular Horizons Research Institute at the University of Wollongong. Aidan obtained his PhD from the Australian National University in 2020, working on projects related to protein folding, unfolding, and aggregation. He then undertook a short postdoctoral position at ANU, and in 2022, commenced as a Postdoctoral Research Fellow at the UOW node of the Australian Research Council's Centre for Cryo-electron Microscopy of Membrane Proteins (CCeMMP). At the end of 2024, he established his lab at UOW in a continuing Teaching and Research position. He has published eight research articles and one book chapter, has won several internal grants to support his research, and in 2022 was awarded the Alvira and Venkat Reddy Award from the National Foundation for Eye Research for his work on eye lens crystallin proteins.



## Long-range quinone transport couples NADH oxidation to the respiratory chain in *Bacillota*

Ashleigh Kropp<sup>1,2,3</sup>, Jamie Stapleton<sup>4</sup>, Oleksii Zdorevskiy<sup>5</sup>, Pok Man Leung<sup>3</sup>, Rachel Darnell<sup>6</sup>, Chris Barlow<sup>7,8</sup>, Ben Hartmann<sup>9</sup>, Nick Yates<sup>3</sup>, Luka Simšič<sup>4</sup>, Kazem Asadollahi<sup>1</sup>, Henrik Strahl<sup>6</sup>, Chris Greening<sup>5</sup>, Vivek Sharma<sup>4,9</sup>, Jamie Blaza<sup>10</sup>, Alison Parkin<sup>3</sup>, Rhys Grinter<sup>1,2,3</sup>

<sup>1</sup> Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Victoria 3010, Australia

<sup>2</sup> Centre for Electron Microscopy of Membrane Proteins, Monash Institute of Pharmaceutical Sciences, Parkville, Victoria, Australia

<sup>3</sup> Department of Microbiology, Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia

<sup>4</sup> Department of Chemistry, University of York, York, YO10 5DD, UK

<sup>5</sup> Department of Physics, University of Helsinki, Helsinki, Finland

<sup>6</sup> Newcastle University Biosciences Institute, Newcastle University, Newcastle-upon-Tyne, United Kingdom

<sup>7</sup> Department of Biochemistry, Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia

<sup>8</sup> Monash Proteomics & Metabolomics Platform, Monash Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia

<sup>9</sup> HiLIFE Institute of Biotechnology, University of Helsinki, Helsinki, Finland

<sup>10</sup> York Structural Biology Laboratory, Department of Chemistry, University of York, York YO10 5DD, UK

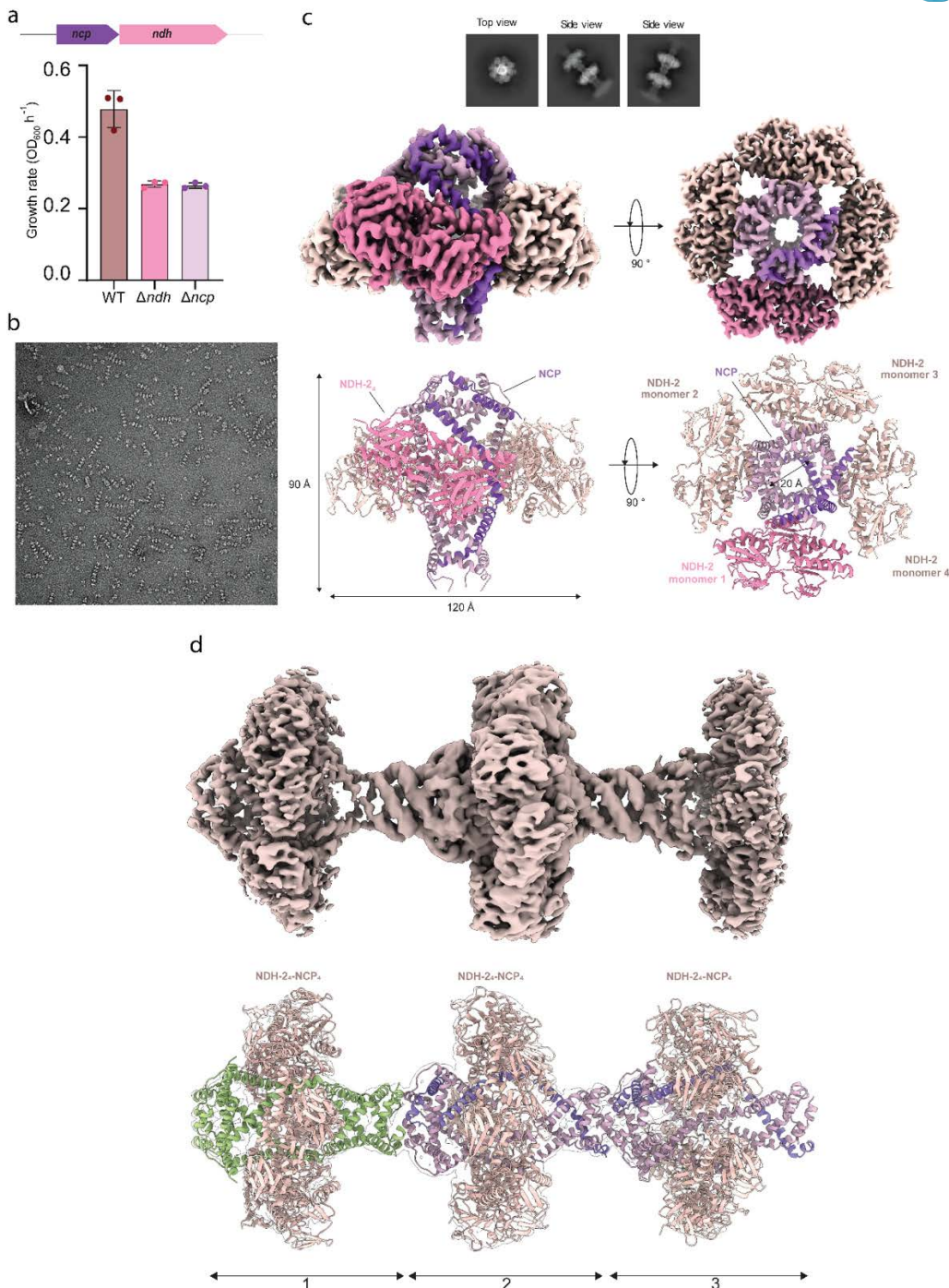
### Abstract

Respiratory chains are essential for energy generation in all life, coupling redox chemistry to proton translocation and ATP synthesis. Central to this process are hydrophobic quinones, which shuttle electrons within the membrane. However, the mechanisms that enable soluble enzymes to interact with these membrane-bound carriers remain poorly understood. We recently uncovered a novel mechanism of **long-range quinone transport**, where specialised protein complexes extract quinones from the membrane and shuttle them to soluble redox enzymes<sup>1,2</sup>.

Here, we present the structural and functional characterisation of a complex from *Bacillus subtilis*, composed of the NADH dehydrogenase NDH-2 and a membrane coupling partner from the DUF1641 family, which we term the NDH-2 Coupling Protein (NCP). Using negative stain and high-resolution cryo-electron microscopy, we show that NCP forms a tetrameric scaffold for four NDH-2 subunits, structurally reminiscent of the hydrogenase Huc complex<sup>2</sup>. These complexes assemble into extended filaments containing a central, lipid-lined hydrophobic chamber that sequesters respiratory quinones.

Biochemical assays, lipidomics, electrochemistry, and molecular dynamics simulations demonstrate that this internal chamber supports quinone mobility outside the membrane. Electrons are transferred from NADH to FAD in NDH-2, then passed to quinone within the chamber, with reduced quinone returning to the membrane to fuel the respiratory chain. This architecture enables NADH oxidation without the need for a membrane-integrated electron relay or permanent membrane association.

Our findings establish the NCP/DUF1641 family as a widespread solution for respiratory chain coupling in *Bacillota* and reveal a structurally elegant strategy for bridging the membrane–soluble phase divide via protein-mediated quinone transport.



**Figure 1. The Cryo-EM structure of the NDH2-NCP complex from *B. subtilis*.** (a) NADH dehydrogenase (*ndh-2*) from *B. subtilis* forms an operon with the gene *ncp*. Deletion of either gene leads to a comparable growth rate defect, indicating they form a functional unit. (b) Negative stain of the purified NDH-2 and NCP complex indicates that it forms filaments. (c) The cryoEM structure of NDH-2-NCP complex shows that it has a 4:4 stoichiometry with the NDH-2 subunits attaching to a central NCP scaffold. (d) Helical reconstruction of the NDH-2-NCP shows head-to-tail interactions drive filament formation.

- 1 Kropp, A. *et al.* Quinone extraction drives atmospheric carbon monoxide oxidation in bacteria. *Nature Chemical Biology* (2025).
- 2 Grinter, R. *et al.* Structural basis for bacterial energy extraction from atmospheric hydrogen. *Nature* **615**, 541-547 (2023).

### **Biography**

Dr Rhys Grinter is the Grimwade Research Fellow and runs the Molecular Physiology of Microbial Pathogens Lab in the Department of Biochemistry and Pharmacology at the University of Melbourne. He received his PhD from the University of Glasgow in 2015. In 2021, he founded his lab and was awarded an NHMRC Investigator Grant to continue his work on iron piracy by bacterial pathogens. He utilises current and emerging technologies to understand how microbes work, to prevent disease and to develop enzymes and natural products as tools for biotechnology. He utilises diverse techniques across genomics, molecular microbiology, biochemistry, and structural biology.



## Poster Presentations

Poster Number	Name
1	Bronte Carroll
2	Noah Graves
3	Hasti Iranmanesh
4	Seyed Mohammad Ghafoori
5	Jiahao Chen
6	Liam Turk
7	Joshua Hardy
8	Kashmira Raghu
9	Luca Troman
10	Jaanaky Vigneswaran
11	Thomas Ficker
12	Jared Lapkovsky
13	Jhonnatan Reales-Gonzalez
14	Jordan Nicholls
15	Declan Fisher
16	Kamran Khajehpour
17	Natalie Hooper
18	Heather Berensmann
19	Sylvain Trépout
20	Yan Jiang
21	Gesa Gruening
22	Denis Korneev
23	Amy Cheng
24	Anne Nguyen
25	Jobichen Chacko
26	Nada Alenzi
27	Chung-Han Tsai
28	Juanfang Ruan
29	Sepideh Valimehr
30	Yan Li
31	Felipe Kremer
32	Evan Bissett
33	Hari Venugopal

## Poster #1

### Elucidating the molecular mechanisms of orphan SLC transporters

Bronte Carroll<sup>1</sup>, Renae Ryan<sup>1</sup>, Rachel North<sup>1</sup>

<sup>1</sup>*School of Medical Sciences, Faculty of Medicine and Health, University of Sydney, Australia*

All cells are surrounded by a membrane that separates their internal machinery from the external environment, however, they also rely on external molecules and nutrients for their survival. Cells have developed a tightly controlled process of moving these nutrients, also known as substrates, across the membrane via specialised transporter proteins. Solute carrier transporters (SLCs) constitute the largest family of these transporters in the human body and are responsible for nutrient uptake, waste removal, ion transport, and even transport of synthetic drugs. Given their widespread importance it's no wonder SLCs are linked to several pathologies, as well as transporting many important drugs into cells.

Despite their vital roles in normal and pathogenic human physiology, up to 40% of SLCs have no known function and remain uncharacterised, classified as "orphan" transporters. I aim to discover how we can identify the structure and functions of orphan SLC transporters. This involves answering questions such as *1. What substrates do they bind and transport? 2. What are the structures of these membrane transport proteins? 3. Do they transport their substrates and what mechanisms are used to do this?*

Using fusion proteins, we show how we can optimise protein preparations for structural studies by single-particle cryo-electron microscopy, and using GFP-thermal shift assays and in silico docking studies, we show predicted substrates for orphan SLCs. With the ultimate goal of elucidating the molecular mechanisms and deorphanising these transporters, the outcome of this work will yield novel insights of broad relevance to human physiology, pathophysiology, and drug development.

## Poster #2

### Structural insights into alpha-synuclein fibril architecture and heterogeneity

Noah J. Graves<sup>1</sup>, A/Prof Emma Sierrecki<sup>1</sup>, A/Prof Yann Gambin<sup>1</sup>, Dr. Daniel Luque<sup>1,2</sup>, Dr. Juanfang Ruan<sup>2</sup>

<sup>1</sup> School of Biomedical Sciences, Faculty of Medicine & Health, University of New South Wales, Sydney NSW 2052, Australia

<sup>2</sup> Electron Microscope Unit, Mark Wainwright Analytical Centre, The University of New South Wales, Sydney NSW 2052 Australia

#### Background

Alpha-synuclein aggregation into fibrillar assemblies is a hallmark of Parkinson's disease and related neurodegenerative disorders. Structural and morphological diversity among fibril strains presents a major challenge for understanding their pathological roles.

#### Methods

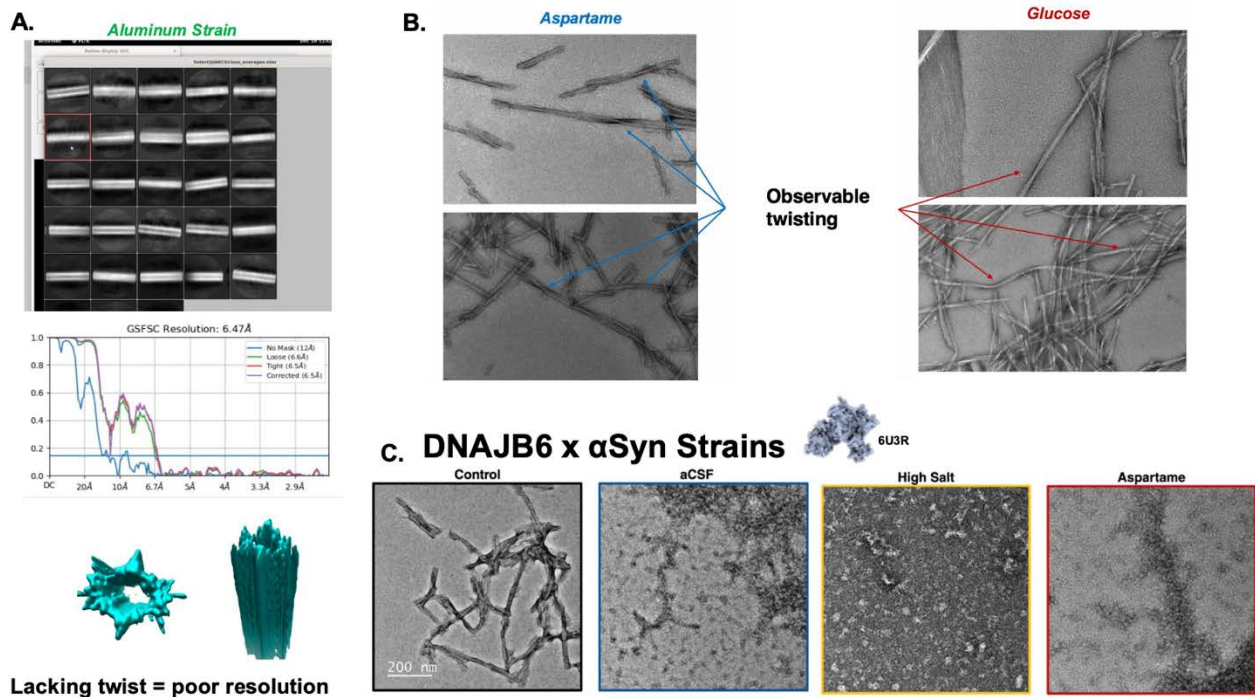
We combined CryoEM with complementary biochemical and single-molecule fluorescence assays to characterize distinct  $\alpha$ -syn fibril polymorphs generated under physiologically relevant conditions. Recombinant  $\alpha$ -syn was assembled into fibrils in the presence of varying metal ions and saccharide environments, then imaged using TEM and cryo-EM to assess filament morphology, crossover distance, and helical parameters. High-quality micrographs were acquired alluding to key variations in  $\alpha$ Syn strain structures.

#### Key Results

Preliminary two-dimensional and TEM classification reveals clear periodicity and cross-sectional features indicative of multiple fibril subtypes between samples. There are also indications of molecular chaperones binding to  $\alpha$ Syn strains in distinct manners. These morphologies correlate with differences in biochemical stability and binding profiles of molecular chaperones observed in parallel fluorescence-based coincidence assays.

#### Conclusions

Ongoing high-resolution reconstruction efforts aim to resolve the atomic architecture of selected fibril forms to enable comparative structural analyses. This integrated approach—linking EM-derived morphology, potential atomic structure, and biochemical reactivity—provides a framework for elucidating how environmental factors influence  $\alpha$ -syn fibril structure and, ultimately, pathogenic potential. Our results highlight cryo-EM as a powerful tool for visualising fibril heterogeneity while demonstrating the value of combining imaging with functional assays to capture the complexity of protein aggregation in neurodegenerative disease.



**Figure 1. Cryo-EM and TEM analysis of  $\alpha$ -synuclein fibril strains formed under distinct biochemical conditions and their interaction with molecular chaperones.** (A) Representative 2D class averages, Fourier shell correlation (FSC) curves, and 3D reconstructions of the aluminum-induced  $\alpha$ -synuclein strain, showing poor resolution attributed to minimal observable fibril twist. (B) TEM micrographs of fibrils formed in the presence of aspartame (left) and glucose (right) reveal distinct morphological differences, with aspartame-induced fibrils displaying clear helical twisting (blue arrows) compared to glucose-induced fibrils, which appear straighter (red arrows). (C) Negative-stain TEM images showing binding of DNAJB6 to  $\alpha$ -synuclein fibril strains assembled in different environments: control, artificial cerebrospinal fluid (aCSF), high salt, and aspartame. The inset model (PDB 6U3R)1 represents  $\alpha$ -synuclein fibril architecture for reference. Scale bars: 200 nm.

1. T.K. Karamanos, V. Tugarinov, & G.M. Clore, Unraveling the structure and dynamics of the human DNAJB6b chaperone by NMR reveals insights into Hsp40-mediated proteostasis, Proc. Natl. Acad. Sci. U.S.A. 116 (43) 21529-21538, <https://doi.org/10.1073/pnas.1914999116> (2019).

## Poster #3

### Visualizing ezrin's role in shaping cell membranes via Cryo-EM

Hasti Iranmanesh<sup>1</sup>, Katharine A. Michie<sup>2</sup>, Paul M. G. Curmi<sup>1</sup>

<sup>1</sup>*School of Physics and School of Biotechnology and Biomolecular Sciences, The University of New South Wales, Sydney, 2052, NSW, Australia*

<sup>2</sup>*Structural Biology Facility, Mark Wainwright Analytical Centre, The University of New South Wales, Sydney, 2052, NSW, Australia*

The plasma membrane is a dynamic interface between the intracellular and extracellular environments of a cell. Underlying this membrane in animal cells is the cortical actin cytoskeleton, a filamentous network that regulates cell shape, motility, and mechanical properties.<sup>1,2</sup> ERM (ezrin, radixin, moesin) proteins mediate the linkage between the plasma membrane and the actin cytoskeleton. Ezrin is known to act as a molecular switch, cycling between inactive and active conformations to regulate membrane-cytoskeleton interactions. Despite its importance, the structural transitions that enable ezrin to perform this function remain poorly understood. We use single particle cryo-electron microscopy (cryo-EM) and tomography to investigate the human ERM protein ezrin in multiple functional states. Single particle analysis of wild-type ezrin reveals a structure containing the FERM:CTD complex and parts of the central helical domain (~51 kDa). A phosphomimetic mutant (T567D) in the presence of membrane vesicles forms ordered assemblies between adjacent vesicles, consistent with domain-swapped homo-dimeric ezrin models. Cryo-EM images of ezrin with actin filaments confirm its ability to bind actin. Additionally, low-resolution maps of ezrin bound to Folch lipid:MSP1E3 nanodiscs demonstrate membrane association.

Together, these results show that cryo-EM can resolve distinct conformations of ezrin in its free, membrane-bound, and actin-bound states. Structural comparisons across these states provide insight into the conformational flexibility of ezrin and its role in force transmission between the membrane and cytoskeleton. This work lays the foundation for understanding how ERM proteins regulate cell mechanics through structural transitions.

1. Kusumi A. et al., 2012, *Annual Review of Cell and Developmental Biology*, 28: 215–250.
2. Rottner K., Faix J., et al., 2017, *Journal of Cell Science*, 130(20): 3427–3435.
3. Michie K.A., et al., 2019, *International Journal of Molecular Sciences*, 20(8): 1996.
4. Neisch A.L., et al., 2011, *Current Opinion in Cell Biology*, 23(4): 377–382.

## Poster #4

### New structural and functional insights on cJUN and cFOS nuclear localisation

Seyed Mohammad Ghafoori<sup>1,2</sup>, Silvia Pavan<sup>3</sup>, Trinh Xuan Duc<sup>3</sup>, Sepehr Nematollahzadeh<sup>3</sup>, Jeffrey Nanson<sup>2,4</sup>, Juanfang Ruan<sup>5</sup>, Daniel Luque<sup>5,6</sup>, Gualtiero Alvisi<sup>3</sup>, Jade K. Forwood<sup>2,4</sup>

<sup>1</sup>*School of Life and Environmental Sciences, University of Sydney, Sydney, NSW 2006, Australia*

<sup>2</sup>*School of Agricultural, Environmental and Veterinary Sciences, Faculty of Science and Health, Charles Sturt University, Wagga Wagga, Australia*

<sup>3</sup>*Department of Molecular Medicine, University of Padova, Padova, Italy*

<sup>4</sup>*Biosecurity Research Program and Training Centre, Gulbali Institute, Charles Sturt University, Wagga Wagga, Australia*

<sup>5</sup>*Electron Microscope Unit, Mark Wainwright Analytical Centre, University of New South Wales, Sydney, Australia*

<sup>6</sup>*School of Biomedical Sciences, University of New South Wales, Sydney, Australia*

#### Abstract

AP-1 transcription factors function as homo- or heterodimers of family members and share a basic leucine zipper (bZIP) domain responsible for dimerisation and DNA binding [1, 2]. These proteins regulate diverse biological processes including immune responses, bone development, angiogenesis, epidermal differentiation, and cell survival [3, 4]. While some aspects of their nuclear localisation have been studied, the structural basis for their import has remained unknown.

Here, we combined biochemical, structural, and cell-based approaches to investigate nuclear import of the c-Jun:c-Fos complex.

Beads pull-down assays showed that the c-Jun bZIP domain interacts with both importin- $\alpha$  isoforms and importin- $\beta$ 1, whereas c-Fos bZIP domain alone did not bind any tested importins. Mutational analysis indicated that disruption of either site-1 or site-2 basic motifs on cJUN was insufficient to abolish importin binding; only double mutation eliminated the interaction. X-ray crystallography revealed an atypical binding mode in which c-Jun site-1 engages the canonical major binding site on importin- $\alpha$ 2, while site-2 extends to residues outside the groove. A separate structure showed that a peptide containing only site-2 can also occupy both major and minor sites. Cryo-EM analysis suggested that the c-Jun:c-Fos heterodimer associates with importin- $\beta$ 1, potentially facilitating nuclear co-transport. Immunofluorescence assay confirmed that c-Jun enters the nucleus independently, whereas c-Fos relies on heterodimerisation with c-Jun for efficient nuclear localisation. In summary, we provide structural evidence for AP-1 nuclear import, uncovering an unusual interaction mode of c-Jun with importin- $\alpha$ 2 and defining a mechanism by which c-Jun:c-Fos heterodimers are transported into the nucleus.

1. Chinenov Y, Kerppola TK. Close encounters of many kinds: Fos-Jun interactions that mediate transcription regulatory specificity. *Oncogene*. 2001;20(19):2438-52.
2. Hess J, Angel P, Schorpp-Kistner M. AP-1 subunits: quarrel and harmony among siblings. *Journal of cell science*. 2004;117(25):5965-73.
3. Wagner EF, Eferl R. Fos/AP-1 proteins in bone and the immune system. *Immunological reviews*. 2005;208(1):126-40.
4. Yamashita J, McCauley LK. The activating protein-1 transcriptional complex: Essential and multifaceted roles in bone. *Clinical Reviews in Bone and Mineral Metabolism*. 2006;4(2):107-22.
5. Waldmann I, Waelde S, Kehlenbach RH. Nuclear import of c-Jun is mediated by multiple transport receptors. *Journal of Biological Chemistry*. 2007;282(38):27685-92.
6. Chida K, Nagamori S, Kuroki T. Nuclear translocation of Fos is stimulated by interaction with Jun through the leucine zipper. *Cellular and Molecular Life Sciences CMLS*. 1999;55(2):297-302.
7. Campos M, Kroon E, Gentz R, Ferreira P. Protein domains involved in nuclear transport of Fos. *Cell biology international*. 1999;23(1):81-8.
8. Arnold M, Nath A, Wohlwend D, Kehlenbach RH. Transportin is a major nuclear import receptor for c-Fos: a novel mode of cargo interaction. *Journal of Biological Chemistry*. 2006;281(9):5492-9.
9. Malnou CE, Salem T, Brockly F, Wodrich H, Piechaczyk M, Jariel-Encontre I. Heterodimerization with Jun family members regulates c-Fos nucleocytoplasmic traffic. *Journal of Biological Chemistry*. 2007;282(42):31046-59.
10. Forwood JK, Lam MH, Jans DA. Nuclear import of Creb and AP-1 transcription factors requires importin- $\beta$ 1 and Ran but is independent of importin- $\alpha$ . *Biochemistry*. 2001;40(17):5208-17.

## Poster #5

### Structural basis of erythromycin binding to hERG K<sup>+</sup> channels

Jiahao Chen<sup>1,2</sup>, Mark J. Hunter<sup>1,2</sup>, Carus H. Y. Lau<sup>1,2</sup>, Jamie I. Vandenberg<sup>1,2</sup>

<sup>1</sup> Mark Cowley Lidwill Research Program, Victor Chang Cardiac Research Institute, Darlinghurst, NSW, Australia

<sup>2</sup> School of Clinical Medicine, UNSW Sydney, Sydney, NSW, Australia

A wide range of structurally unrelated drugs can bind to *human ether-a-go-go related gene* (hERG) potassium channels and thereby increase the risk of acquired long QT syndrome and sudden cardiac arrest. Thus during pre-clinical drug development, industry spends billions of dollars screening for hERG binding and engaging in labour-intensive medicinal chemistry approaches to reduce affinity for hERG channel whilst maintaining affinity for the intended target of the lead compounds. Despite of many biophysical and computational investigations into hERG drug binding the primary question of 'how and why so many structurally unrelated drugs bind to hERG channels' remains uncertain. hERG channels can exist in closed (non-conducting), open (conducting), and inactivated (non-conducting) states (1). Previous studies have shown that most high affinity drugs preferentially bind to the inactivated state (2). Whilst prior structural studies have investigated drug binding to WT hERG channels no studies have specifically looked at the structural basis of state dependence of drug binding to hERG. To start to address this question, we expressed N588E hERG channels (enhances occupancy of the inactivated state) in HEK293 GnTI<sup>-</sup> cells and purified at 3 mM K<sup>+</sup>, to further enhance inactivation, in the presence of 30 μM erythromycin. CryoEM data were acquired using an FEI Titan Krios (3). A preliminary structure, determined using RELION-5.0.0 software, will be presented.

1. Vandenberg JI, Perry MD, Perrin MJ, Mann SA, Ke Y, Hill AP. hERG K(+) channels: structure, function, and clinical significance. *Physiol Rev.* 2012;92(3):1393-478.

2. Perrin MJ, Kuchel PW, Campbell TJ, Vandenberg JI. Drug binding to the inactivated state is necessary but not sufficient for high-affinity binding to human ether-a-go-go-related gene channels. *Mol Pharmacol.* 2008;74(5):1443-52.

3. Lau CHY, Flood E, Hunter MJ, Williams-Noonan BJ, Corbett KM, Ng CA, et al. Potassium dependent structural changes in the selectivity filter of HERG potassium channels. *Nat Commun.* 2024;15(1):7470.

## Poster #6

### **Analytical ultracentrifugation: classic biophysics, modern solutions**

Liam Turk<sup>1</sup>, Zac Tillett<sup>1</sup>, and Renwick Dobson<sup>1</sup>

<sup>1</sup>*Biomolecular Interaction Centre, University of Canterbury, Christchurch, New Zealand*

Analytical ultracentrifugation (AUC) is a first-principles biophysical technique that has been used for over a century to investigate the behavior of biomolecules. It enables the in-solution interrogation of macromolecular size, shape, and interactions. Recent improvements in both instrumentation and data analysis software have expanded the scope and accessibility of AUC.

In AUC experiments, macromolecules are subjected to extremely high centrifugal forces—up to 290,000 × *g* (60,000 rpm)—causing them to migrate through solution. Their movement is monitored via absorbance, interference, or fluorescence signals collected throughout the run. This allows for detailed characterization without the need for immobilization or labeling (unless using fluorescence).

We highlight key aspects of experimental design and showcase several modern applications of AUC, encouraging its integration with other structural biology techniques or its use when such methods fall short. Specific examples include:

1. Quantification of protein-bound detergent during membrane protein sample preparation,
2. Vaccine quality control through characterization of mRNA-loaded lipid nanoparticles, and
3. Confirmation of macromolecular complex formation and oligomerization.

## Poster #7

### ProteinDJ: a high-performance and modular protein design pipeline

Dylan Silke<sup>1,2</sup>, Julie Iskander<sup>1,2</sup>, Junqi Pan<sup>1,2</sup>, Andrew P. Thompson<sup>1,2,3</sup>, Anthony T Papenfuss<sup>1,2</sup>, Isabelle S. Lucet<sup>1,2,3</sup>, Joshua M. Hardy<sup>1,2,3</sup>

<sup>1</sup>The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia.

<sup>2</sup>Department of Medical Biology, University of Melbourne, Parkville, VIC, Australia.

<sup>3</sup>ARC Centre for Cryo-electron Microscopy of Membrane Proteins, Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, 3052, Victoria, Australia

Leveraging artificial intelligence and deep learning to generate proteins *de novo* has unlocked new frontiers of protein design. By training deep learning models on protein sequences and experimental structures, we can sample new structural landscapes unexplored by evolution. This approach can be used to design bespoke binders that target specific proteins and domains. However, generating successful binders has a low in silico success rate, often requiring thousands of designs and hundreds of GPU hours to obtain enough hits for experimental testing. There is a lack of efficient open-source pipelines designed for high-performance computing (HPC) systems that can maximise hardware resources and parallelise the workflow efficiently.

Here, we present 'ProteinDJ' - an implementation of a synthetic protein binder design workflow that is deployable on HPC systems using the Nextflow orchestration domain-specific language and Singularity containerisation. It automatically batches and parallelises the workload across both GPUs and CPUs. Importantly, it allows central deployment of the workflow on shared HPC resources, rather than workstations that are restricted to individual research groups.

ProteinDJ is modular by design (see Figure 1) and currently includes RFdiffusion for fold generation, ProteinMPNN or Full-Atom MPNN for sequence design, and AlphaFold2 or Boltz-2 for prediction and validation of binder-target interfaces, with supporting packages for structural evaluation of designs. We have included structure-based filters to reject problematic designs mid-stream to minimise manual inspection of designs by the user. Our approach democratises protein binder design in an easy-to-use and robust implementation.

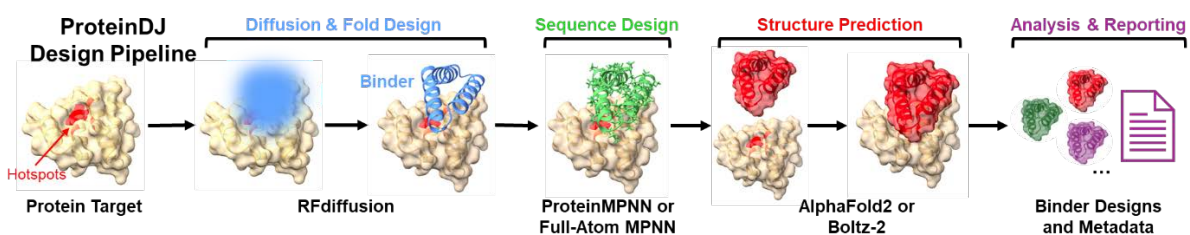


Figure 1. Overview of the ProteinDJ pipeline for *de novo* binder design. RFdiffusion is used to diffuse a fold/backbone near hotspots on a protein target. The user can choose from ProteinMPNN or Full-Atom MPNN for sequence design, and AlphaFold2 Initial Guess or Boltz-2 for structure prediction and validation

## Poster #8

### Advancing Materials Research with Cryogenic Focused Ion Beam (FIB)-SEM

K. Raghu<sup>1</sup>, F. L. Ng<sup>2</sup>, A. Tan<sup>3</sup>, M. Heller<sup>3</sup>, M. Etsuo<sup>4</sup>

<sup>1</sup>Carl Zeiss Pty Ltd, Research Microscopy Solutions, Sydney, Australia

<sup>2</sup>Carl Zeiss Pte Ltd, Research Microscopy Solutions, Singapore,

<sup>3</sup>Carl Zeiss Microscopy GmbH, Oberkochen, Germany,

<sup>4</sup>Carl Zeiss Co. Ltd, Tokyo, Japan.

Cryogenic Focused Ion Beam Scanning Electron Microscopy (Cryo-FIB-SEM) is a powerful technique for studying novel and heat-sensitive materials. This advanced method combines the high-resolution imaging capabilities of Scanning Electron Microscopy (SEM) with the precision of Focused Ion Beam (FIB) milling, all under cryogenic conditions. Analysing materials at low temperatures allows for the preservation of delicate structures and the examination of their behaviour in a near-native state, which is particularly crucial for materials that can undergo significant physical or phase changes during traditional preparation or imaging.

Cryo-FIB-SEM offers several distinct advantages for advanced research including processing at sub-room temperatures, where cryogenic temperatures enhance milling outcomes by reducing ion beam-induced local heating and electron beam-induced deposition. Additionally, it enables 3D volume imaging of heterogeneous structures at the nanoscale, providing valuable insights into fine structural details and phase transitions of these materials.

This presentation introduces the ZEISS Cryogenic-FIB-SEM. It highlights the principles and applications of cryogenic microscopy, with a particular focus on polymer, energy, and electronic materials. A key feature of the ZEISS FIB-SEM lies in its ability to combine in-situ imaging with milling, enabling precise end-pointing to the desired region of interest (ROI). The system's low-kV imaging and FIB processing minimize sample damage to heat-sensitive materials, as demonstrated in Figure 1(a). Additionally, with stage drift-reduction, the ZEISS Cryo-FIB-SEM ensures stable long-term cryo-milling for isotropic 3D tomography, as shown in Figure 1(b). Overall, the ZEISS Cryo-FIB workflow facilitates imaging and FIB processing of beam-sensitive materials for a range of novel & emerging applications.

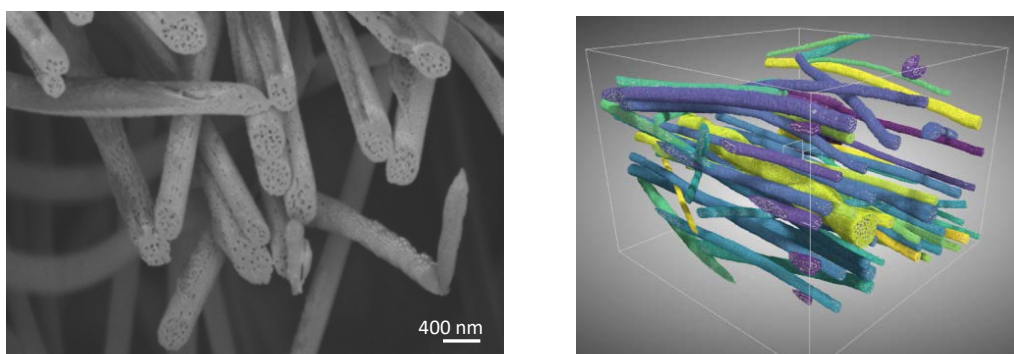


Figure 1: Polymer fiber imaged and processed at cryogenic temperature. (a) FIB cross-sectioning at  $-150^{\circ}\text{C}$  with 30kV and 15 nA. (b) 3D FIB tomography of polymer fibers immersed in water

## Poster #9

### Structural remodeling of flagellar filament promotes biofilm formation in oral pathogen *Treponema denticola*

Luca Troman<sup>1,2</sup>, Bindusmita Paul<sup>1,2</sup>, Ananya Padmakumar<sup>3</sup>, Jack Kim<sup>4</sup>, J Christopher Fenno<sup>5</sup>, M. Paula Goetting-Minesky<sup>5</sup>, Jillian F Banfield<sup>4</sup>, Eric C. Reynolds<sup>3</sup>, Paul D. Veith<sup>3</sup>, Debnath Ghosal<sup>1,2</sup>

<sup>1</sup>Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Melbourne, VIC, Australia.

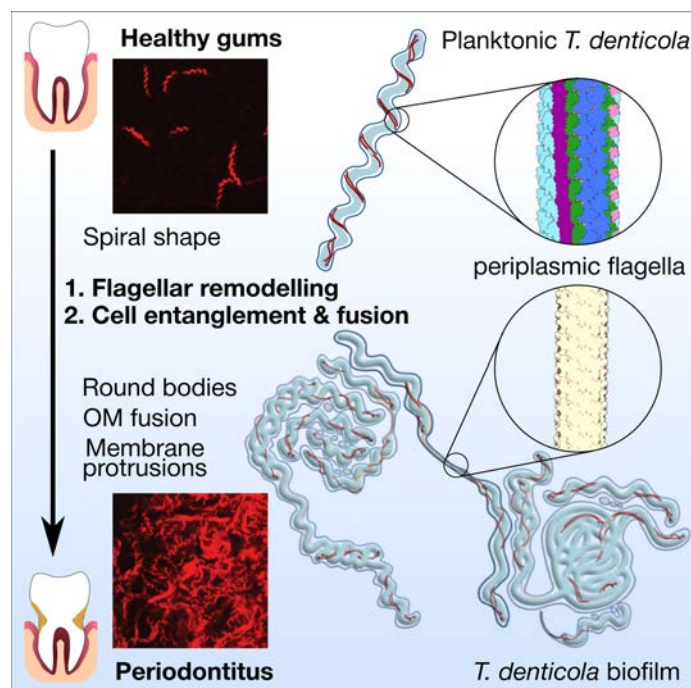
<sup>2</sup>ARC Centre for Cryo-electron Microscopy of Membrane Proteins, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Melbourne, VIC, Australia.

<sup>3</sup>Oral Health Cooperative Research Centre, Melbourne Dental School, Bio21 Institute, The University of Melbourne, Parkville, VIC, Australia.

<sup>5</sup>Department of Biologic and Materials Sciences and Prosthodontics, School of Dentistry, University of Michigan, Michigan, US.

<sup>4</sup>Department of Environmental Science, Policy and Management, University of California, Berkeley, CA, US.

*Treponema denticola* is a Gram-negative, motile oral spirochete implicated in periodontal diseases through the formation of biofilms. These structured microbial communities promote chronic infections by conferring resilience to antibiotics and host defenses, yet the structural adaptations underpinning their assembly remain poorly defined. Using an integrated, cross-scale approach combining confocal laser scanning microscopy with fluorescence in situ hybridization, cryo-electron tomography, single particle cryo-electron microscopy and phylogenomics, we reveal that *T. denticola* undergoes striking morphological transitions during biofilm formation. We found that the structural remodelling of periplasmic flagella underpins this process and resolve two distinct flagellar conformations at sub-3 Å resolution. Our analysis revealed unexpected complexity within planktonic flagella, which comprise seven different proteins, including four previously uncharacterized components. The complex asymmetric assembly is the first complete model of a periplasmic flagellum and uncovers mechanisms for curvature generation within the characteristic spiral filaments. In contrast, within biofilms we observe remodelling to only core flagellins and demonstrate their crucial role in mediating cell-cell contacts within biofilm. These findings reveal multi-scale structural and functional adaptations linking flagellar remodelling to stable biofilm architecture with broad implications across diverse bacterial pathogens.



## Poster #10

### Structural divergence in Hepatitis B core antigen virus like particle variants

Jaanaky Vigneswaran<sup>1</sup>, Anne Nguyen<sup>1</sup>, Jake baum<sup>1</sup>, Daniel Luque<sup>1,2</sup>

<sup>1</sup>*School of Biomedical Sciences, University of New South Wales, Sydney, Australia*

<sup>2</sup>*Electron Microscopy unit, University of New South Wales, Sydney, Australia*

Hepatitis B core antigen virus-like particles (HBcAg VLPs) are among the most widely used carriers for antigen presentation and other nanobiotechnology applications. Over the years, several engineered versions—including wild-type (Cohen and Richmond 1982), tandem-fusion (Peyret et al., 2015), and split-core (Walker et al., 2011) constructs—have been developed and applied in diverse contexts. While individual variants have been characterized in separate studies, a systematic head-to-head comparison of their biochemical, biophysical, and structural properties has been lacking. Here, we produced these HBcAg VLP variants in parallel and assessed their assembly and stability using a combination of biochemical and biophysical assays. To complement these findings, we are analysing by cryo-electron microscopy (cryo-EM) the structure of all variants, providing high-resolution three-dimensional insights into their morphology and assembly states. Having cryo-EM maps for all HBcAg VLP variants provides a unified structural framework that links biochemical and biophysical observations with molecular-level features, thereby elucidating the underlying structural reasons for why certain variants exhibit greater stability, solubility, and suitability for plug-and-display applications.

#### References

1. BJ Cohen, Richmond JE. [Electron microscopy of hepatitis B core antigen synthesized in E. coli. *Nature*. 1982;296.
2. Peyret H, Gehin A, Thuenemann EC, Blond D, El Turabi A, Beales L, et al. Tandem Fusion of Hepatitis B Core Antigen... *PLoS One*. 2015;10(4):e0120751.
3. Walker A, Skamel C, Nassal M. SplitCore: An exceptionally versatile viral nanoparticle for native whole protein display... *Sci Rep*. 2011;1:5.

## Poster #11

### Towards the Molecular Mechanisms of Ligand Activation in Kv7.5 Potassium Channel

Thomas Ficker<sup>1,2</sup>, Aidan Grosas<sup>1,2</sup>, Lezanne Ooi<sup>1,2</sup>, Gökhan Tolun<sup>1,2</sup>,

<sup>1</sup>*Molecular Horizons and School of Science University of Wollongong, Wollongong, Australia*

<sup>2</sup>*ARC Industrial Transformation Training Centre for Cryo-electron Microscopy of Membrane Proteins, Wollongong, Australia*

The regulation of neuronal excitability is essential for processes such as learning, memory, and cognition, as well as for the control of muscle contraction (1, 2). A key protein family involved in this regulation is the Kv7 voltage-gated potassium channel family, which consists of the five  $\alpha$ -subunits Kv7.1-5. Mutations in Kv7.5 are linked to epilepsy, neuropathic pain, and other neurological disorders (3, 4). While pharmacological modulators of Kv7 channels hold therapeutic promise, the structural basis of ligand activation of Kv7.5 remains poorly understood. This question is of particular importance because of the high conservation within the Kv7 family, making selective targeting challenging and raising the risk of off-target effects.

To address this, we expressed Kv7.5 homotetramers in Expi293F cells using the BacMam expression system, purified them via a GFP-nanobody resin, and started structural characterization in both apo and ligand-bound conditions using cryo-electron microscopy (cryo-EM). Four known modulatory ligands were selected for structural analysis, with the aim of comparing their binding interactions and conformational effects on channel gating. In parallel, patch-clamp electrophysiology and molecular docking will be used to complement the structural data and link observed conformational changes to functional modulation.

Through these studies, we hope to gain structural insights into the mechanisms of ligand-dependent activation of Kv7.5. Establishing this framework will be an important step towards understanding subtype-specific modulation within the Kv7 family and could help with the design of selective therapeutics for neurological disorders.

1. Rutecki PA. Neuronal excitability: voltage-dependent currents and synaptic transmission. *J Clin Neurophysiol.* 1992;9(2):195-211.
2. Brodal P, Brodal P. 32Neuronal Excitability. *The Central Nervous System: Oxford University Press;* 2016.
3. Huang Y, Ma D, Yang Z, Zhao Y, Guo J. Voltage-gated potassium channels KCNQs: Structures, mechanisms, and modulations. *Biochemical and Biophysical Research Communications.* 2023;689:149218.
4. Jones F, Gamper N, Gao H. Kv7 Channels and Excitability Disorders. *Handb Exp Pharmacol.* 2021;267:185-230.

## Poster #12

### Low voltage electron microscopy as a screening tool for Cryo-EM

Emad Shahnam<sup>1</sup>, Daniela Vieira<sup>1</sup>, [Jared Lapkovsky](#)<sup>1</sup>

<sup>1</sup>*Delong America, Montreal, Canada*

Cryo-EM enables structural biology researchers to determine macromolecular structures at near-atomic resolution. Yet, the technique faces practical challenges: sample preparation is labor-intensive, microscope time is scarce, and data collection is costly. To maximize efficiency, researchers rely on pre-screening methods that allow for rapid assessment of sample quality before committing resources to vitrification and high-resolution imaging. Low Voltage Electron Microscopy (LVEM) is emerging as a highly effective solution to this bottleneck.

Operating between 5-25 kV, LVEM combines the principles of Transmission Electron Microscopy (TEM) with the benefits of low-voltage operation, including exceptional image contrast of negatively stained samples such as protein complexes, viruses, liposomes, and organic nanoparticles (Figure 1). By enabling investigators to quickly evaluate particle integrity, distribution, and homogeneity, filter out unsuitable preparations and guide optimization of grid preparation, LVEM significantly increases the success rate of downstream Cryo-EM studies.

Recent work highlighted LVEM's capabilities in adeno-associated virus (AAV) research, demonstrating that LVEM could characterize unstained samples with data comparable to cryo-TEM (Figure 2) [1]. This unique capability enabled researchers to visualize particle morphology, integrity and payload status without staining artifacts, benefiting from a more accessible workflow.

Delong Instruments' LVEM instruments are designed for accessibility: fast, compact, easy to operate, and requiring no specialized laboratory infrastructure. Beyond Cryo-EM pre-screening, LVEM is also contributing to nanomaterial characterization, and cellular ultrastructural studies. As demand for Cryo-EM accelerates, LVEM offers a cost-effective and reliable screening tool, ensuring that only the highest-quality samples progress to vitrification and advanced cryogenic imaging. This integration streamlines workflows, conserves resources, and accelerates structural discovery.

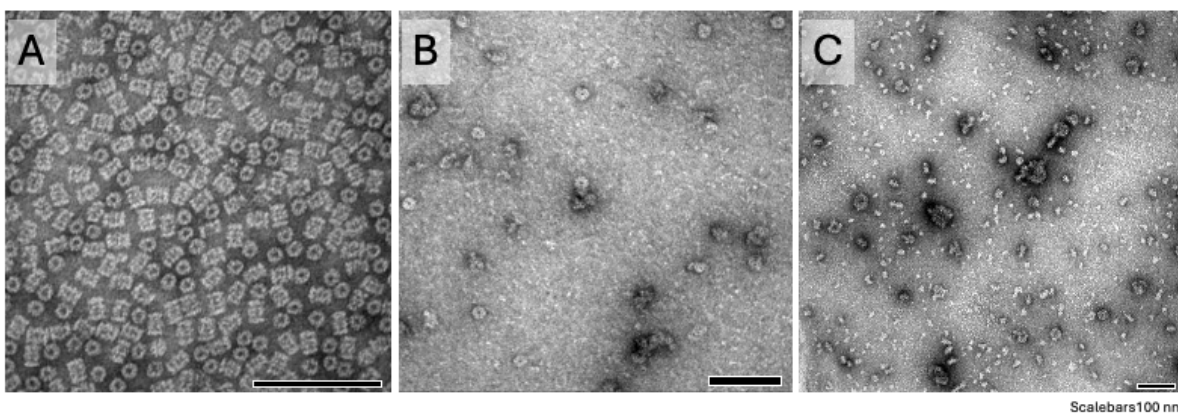
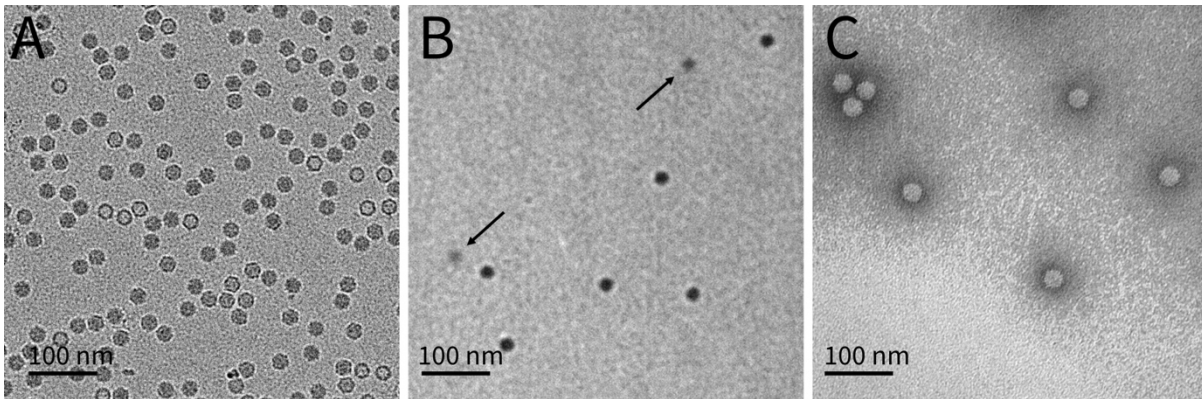


Figure 1. Images captured on a LVEM 25E (Delong Instruments, Czech Republic) operated in TEM mode at 25kV of negatively stained samples of a) Proteosome 20S b) 700kDa protein c) Sarcoendoplasmic reticulum calcium ATPase



Figure

2. AAV Imaged under a) Cryo-TEM b) LVEM with unstained sample c) LVEM with negatively stained sample. (Ausman, Kevin D., et al. "Low Voltage Electron Microscopy: An Emerging Tool for AAV Characterization." *Journal of Pharmaceutical Sciences* (2025))

1. Ausman, K. D., Marini, J. C., Zhou, Y., Matusheski, N. V., Fisher, M. T., & Schilke, K. F. (2025). Low voltage electron microscopy: An emerging tool for AAV characterization. *Journal of Pharmaceutical Sciences*, *114*(2), 567–576. <https://doi.org/10.1016/j.xphs.2025.01.013>

## Poster #13

### Cryo-EM structures of *Salmonella* phage P22 annealase ERF reveals mechanistic details of SSA DNA recombination

Jhonnatan Reales-Gonzalez<sup>1,2</sup>, Mariakatarina Lambourne<sup>1,2</sup>, Jordan Nicholls<sup>1,2</sup>, Jodi Brewster<sup>1,3</sup>, Gökhan Tolun<sup>1,2</sup>.

<sup>1</sup>*School of Chemistry and Molecular Bioscience, and Molecular Horizons, University of Wollongong, Australia*

<sup>2</sup>*ARC Industrial Training Centre for Cryo-electron Microscopy of Membrane Proteins*

<sup>3</sup>*Teva Pharmaceuticals, Sydney, Australia*

Single strand DNA annealing proteins (a.k.a., annealases) catalyse the annealing reaction during Single Strand Annealing (SSA), which is a homologous DNA recombination pathway conserved virtually in all life from bacteriophages to humans. Despite their ubiquity, our understanding of how these proteins work is limited [1].

The Essential Recombination Function (ERF) protein, the annealase of the *Salmonella* phage P22, defines a family of its own [2], making it a model system to study SSA. We have successfully purified the full-length ERF protein, and for the first time after more than half a century of its discovery, we obtained its high resolution cryo-electron microscopy (cryo-EM) structures. Our structures reveal that this protein forms oligomers with a ring-like structure, each monomer presenting a conserved fold among annealases. Importantly, we have obtained the cryo-EM map of the complex of ERF bound to its ssDNA substrate at a 2.5 Å resolution, revealing its DNA binding mechanism. Our results revealed that DNA binds into a positively charged groove in ERF, that runs along the oligomer. Remarkably, we have captured an alternative oligomeric state, that was predicted to be a DNA annealing intermediate. Unlike the structure previously revealed by our group on Redβ annealase from phage λ [1], ERF may form transient complexes that release the DNA once the complementarity of the DNA has been found.

Unravelling ERF's mechanism of action will provide pivotal insights into our understanding of how recombination is catalysed, and is expected to have profound implications in its potential biotechnological applications [3].

1- Newing, T. P., Brewster, J. L., Fitschen, L. J., Bouwer, J. C., Johnston, N. P., Yu, H., & Tolun, G. (2022). Redβ177 annealase structure reveals details of oligomerization and λ Red-mediated homologous DNA recombination. *Nature communications*, 13(1), 5649.

2- Iyer, L. M., Koonin, E. V., & Aravind, L. (2002). Classification and evolutionary history of the single-strand annealing proteins, RecT, Redβ, ERF and RAD52. *BMC genomics*, 3, 1-11.

3- Usman, S. S., Uba, A. I., & Christina, E. (2023). Bacteriophage genome engineering for phage therapy to combat bacterial antimicrobial resistance as an alternative to antibiotics. *Molecular Biology Reports*, 50(8), 7055-7067.

## Poster #14

### The structure of PhoH2 reveals the architecture of an ATP-dependent RNA helicase/RNase machine

Jordan Nicholls<sup>1,2</sup>, Emma S.V. Andrews<sup>3</sup>, Tim Newing<sup>1,2</sup>, Jodi Brewster<sup>1,2</sup>, Vickery L. Arcus<sup>3</sup>, Gokhan Tolun<sup>1,2</sup>

<sup>1</sup> *Molecular Horizons, University of Wollongong, Wollongong, Australia*

<sup>2</sup> *Australian Research Council Centre for Cryo-electron Microscopy of Membrane Proteins, University of Wollongong, Wollongong, Australia*

<sup>3</sup> *Te Aka Mātuatua, School of Science, University of Waikato, New Zealand*

PhoH2 proteins are ubiquitous bacterial proteins involved in the stress-response pathway of several notable pathogens, namely *Mycobacterium tuberculosis*, the causative agent of tuberculosis. They act to induce a state of symptomless latent infection, which is non-infectious but displays high antibiotic resistance. It is estimated ~25% of the global population is infected with latent tuberculosis [1]. PhoH2 carries out this role through its two domains: an N-terminal PIN nuclease domain, fused via a long flexible linker to a C-terminal PhoH helicase/ATPase domain [2]. It is hypothesised that PhoH2 targets growth-related RNA which is unwound by the helicase domain before the nuclease domain acts to cleave it, but how they are coordinated within a single assembly has remained unclear due to the lack of high-resolution structural information.

To address this, we expressed and purified recombinant PhoH2 from *Mycobacterium smegmatis* and determined its structure using single-particle cryogenic electron microscopy at 3.2 Å resolution (Figure 1). The resulting structure reveals a heptameric double-tiered ring in which the PIN domains form the lower tier and the PhoH helicase and linker domains form the upper tier. Notably, the linker is structured and adopts an OB-fold-like architecture. The PIN-domain active site is located on the inside of the ring, and the ATP-binding site is located at the interface between helicase subunits.

Overall, this architecture suggests a structural basis for the coupling of ATP hydrolysis to RNA degradation. This is significant as a novel characterisation and structural determination of a PhoH2 protein, and identifies several mechanistically important sites which warrant further investigation. These findings additionally represent an important intermediate step in structure-based drug design.

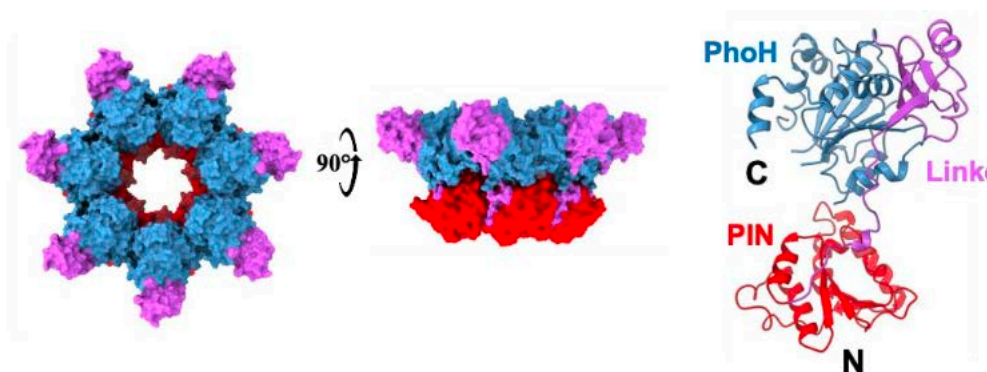


Figure 1. Heptameric PhoH2 protein is a double-tiered ring composed of an N-terminal PIN domain (nuclease, red) fused via a linker (purple) to a C-terminal PhoH domain (helicase, blue).

1. Ding, C., et al., Prevalence trends of latent tuberculosis infection at the global, regional, and country levels from 1990-2019. *International Journal of Infectious Diseases*, 2022. **122**: p. 46-62.

2. Andrews, E.S.V. and V.L. Arcus, PhoH2 proteins couple RNA helicase and RNase activities. *Protein Sci*, 2020. **29**(4): p. 883-892.

## Poster #15

### Fate of the Human Replisome at DNA Damage Lesions

Declan Fisher<sup>1</sup>, Jordan Nicholls<sup>1</sup>, Lisanne Spenkelink<sup>1</sup> and Jacob Lewis<sup>1</sup>

<sup>1</sup>*Molecular Horizons, School of Chemistry and Molecular Bioscience, University of Wollongong, Wollongong, NSW, 2522, Australia*

Complete DNA replication is constitutively threatened by intrinsic and extrinsic species that can inflict a broad spectrum of DNA damage lesions. Imprecise DNA replication can compromise genome stability, and if left unrepaired, exacerbates the risk of uncontrolled cell division which may ultimately drive malignant transformation. The human replisome possesses unique enzymatic capabilities that allow structural and composition reconfiguration to tolerate physical impediments to fork progression. While these frontline mechanisms are crucial for preserving genome fidelity, the modes of recognition and actions of the replisome after lesion encounter remains unclear. This project aims to explore the mechanisms of DNA damage tolerance by examining the response of the human replisome after confronting various DNA lesions, each of which represent a distinct topological challenge. The core human replication factors will be individually purified from recombinant insect cell cultures and assembled onto a forked, linearised DNA template harbouring a chemically defined lesion. Stalled fork products will be enriched by glycerol gradient sedimentation and captured by cryo-electron microscopy (cryo-EM). Cryo-EM studies have successfully defined the precise architecture of the human replisome during elongation, providing mechanistic rationale into how several accessory proteins sustain rapid and sustained replisome progression. Acquiring high-resolution snapshots of stalled replisome complexes may uncover how structural plasticity enables DNA damage tolerance to safeguard genome stability.

## Poster #16

### Employing Xe Plasma FIB for Fast and Precise Sample Preparation

Samuel Záchaj<sup>1</sup>, Martina Zánová<sup>1</sup>, Evgenia Zagoriy<sup>2</sup>, Linhua Tai<sup>2</sup>, Hyunmin Kim<sup>2</sup>

<sup>1</sup>TESCAN GROUP, Brno, Czech Republic,

<sup>2</sup>European Molecular Biology Laboratory Heidelberg, Heidelberg, Germany

Cryo-electron tomography is becoming increasingly accessible for a wide range of samples, from single cells to complex tissues, paving the way for new discoveries in structural biology. While focused ion beam (FIB) instruments utilizing Gallium ion sources are widely employed for high-resolution and precise milling, their slower processing speed can hinder throughput in large volume applications. Modern Xe-based plasma FIB offers a fast and comparably precise alternative for the demanding workflows.

This study explores optimized workflows for cryo-TEM sample preparation using the Xenon-based plasma FIB-SEM. Fast and precise ion beam milling with Xenon plasma not only accelerates sample preparation, but also enables the analysis of larger specimen volumes, enhancing our understanding of biological structures in a broader context. Additionally, we present novel approaches to the existing cryo-FIB workflows, inspired by related materials science techniques, such as the use of different materials as protective layers for FIB milling and 3D volume imaging, or the use of low-energy ion beams for sample processing.

## Poster #17

### Investigating the structural rearrangements of the respiratory chain following heme depletion

Natalie Hooper<sup>1</sup>, Benedicta Sherrie<sup>1</sup>, Alice Sharpe<sup>1</sup>, Charles Bayly-Jones<sup>1</sup>, Luke Formosa<sup>1</sup>

<sup>1</sup>*Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton, VIC 3800, Australia*

The electron transport chain (ETC) couples the transfer of electrons through the respiratory chain in a series of redox reactions, to generate adenosine triphosphate (ATP) by ATP synthase, in a process known as oxidative phosphorylation (OXPHOS). The ETC is composed of four membrane-bound protein complexes, located in the inner membrane of the mitochondria. While traditional depictions of the ETC show each complex in isolation, in reality, they form higher-order molecular weight complexes, known as super complexes (SC).

As part of their structure and/or catalytic function, respiratory CII, CIII and CIV all require heme, a small molecule, composed of a ferrous iron ( $\text{Fe}^{2+}$ ) chelated in an organic porphyrin ring. Following heme deprivation in cells, we have discovered a novel higher molecular weight respiratory supercomplex that we have denoted as  $\Delta^{\text{Heme}}\text{SC}$ .

To investigate this novel complex, we have generated a HEK293T *ALAS1* knockout cells to induce formation of the  $\Delta^{\text{Heme}}\text{SC}$ . These cells have been observed to form the  $\Delta^{\text{Heme}}\text{SC}$  that contains respiratory CI and CIII, but not CIV, suggesting novel interacting proteins may be associated that lead to the higher molecular weight observed. Immunoprecipitation-mass spectrometry has been used to isolate the complex to determine the composition of this complex. In addition, isolated  $\Delta^{\text{Heme}}\text{SC}$  will be subjected to cryogenic electron microscopy and single-particle analysis to reconstruct a high-resolution structural snapshot. This work provides insights into better understanding the regulation of bioenergetics and the consequences of heme loss in the mitochondria.

## Poster #18

### Thinking Outside the Box in Making Cryo-EM More Accessible for Everyone

Heather Berensmann<sup>1</sup>, Theo Humphreys<sup>2</sup>

<sup>1</sup> Hitachi High-Tech America Inc, Environmental Business Division, Hillsboro, Oregon, United States of America

<sup>2</sup> Fred Hutchinson Cancer Center, Electron Microscopy Shared Resource, Seattle, WA

The iterative nature of grid screening for single particle analysis (SPA) requires a specialized microscope that is easy to learn, provides minimal operational downtime, and supports maximum efficiency. However, the financial ramifications associated with such a system can pose significant accessibility barriers for many laboratories and can be limited to selective applications. By improving the versatility of a thermionic (tungsten, LaB6 source) TEM, while preserving the full functionality of all room temperature capabilities, cryo-EM entry becomes more attainable for a wider demographic. However, the process of screening vitrified proteins using a low-kV thermionic TEM can present inherent challenges; one of which can be maintaining a stable, low temperature environment within the microscope column. To comprehensively evaluate the efficacy of Hitachi's HT7800 thermionic TEM for cryo-screening, two separate samples were investigated, each targeting different aspects of the system's performance. The first experiment aimed to assess the TEM's ability to maintain appropriate conditions over an extended amount of time by screening apoferritin for over 9 hours. The second experiment tested the instrument's ability to sustain optimal conditions in untraditional, non-laboratory environments by screening vitrified virus-like particles (VLPs) at The Microscopy & Microanalysis Conference (M&M 2024) in Cleveland, Ohio, USA. Through the experimental evaluations of Hitachi's innovative retractable cold finger design, coupled with Simple Origin's transfer holder and SerialEM [1], Hitachi's HT7800 TEM demonstrated to be an effective screening tool in multiple environments. Thereby expanding the HT7800's versatility for a wider array of applications and providing greater accessibility to cryo-EM technology for everyone.

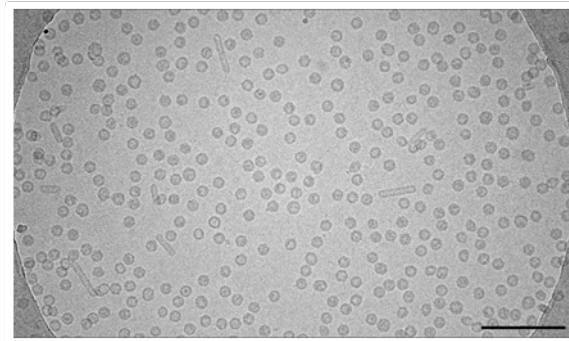
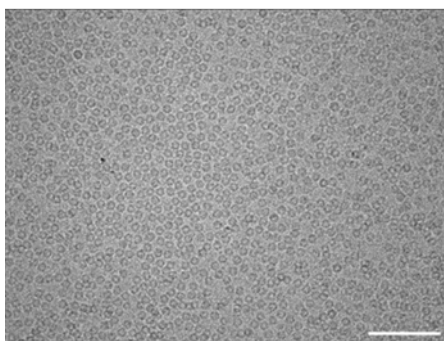


Figure 1. Vitrified Apoferritin particles imaged approximately nine hours after the Simple Origin transfer holder was loaded into the microscope column. Images were taken with AMT's Nanosprint15-MarkII sCMOS camera. Image is digitally enlarged to show detail. Scale bar equivalent to 100 nm.

Figure 2. Vitrified Virus-like particles (VLPs) imaged during the M&M 2024 conference in Cleveland, Ohio, USA. Images were taken with AMT's Nanosprint15-MarkII sCMOS camera at x70,000 magnification. Scale bar equivalent to 200 nm.

1. Mastrorade D, 2005, Journal of Structural Biology, 152(1): 36-51.

## Poster #19

### **Sparse imaging applied to low-magnification cellular tomography and high-resolution sub-tomogram averaging**

Sylvain Trépout and Georg Ramm

*Ramacioti Centre for CryoEM, Monash University, Clayton, VIC, Australia*

In cryo-electron microscopy, the electron beam generates damage to the biological specimens, hampering the recovery of the fragile sample's structure. Sparse imaging is a recent and emerging method to prevent such damage. It consists in i) the collection of downsampled images in which only few pixels are acquired, and ii) the computational reconstruction of the missing pixels that were not collected to create the entire image. Because only few pixels are collected, mechanically the number of electrons used is low, hence the reduction of electron beam damage. This acquisition method fits particularly well with the collection pattern of scanning transmission electron microscopy/tomography (STEM/STET) since the beam can be driven at different sample locations during scanning. This work presents for the first time the application of sparse imaging on bacteria observed in cryo-STET [1]. The benefits, consequences and limitations of sparse imaging are presented for better use of the method in future cellular tomography works. Building upon this experience, the sparse strategy was applied to sub-tomogram averaging using *in silico* sparse high-resolution cryo-transmission electron tomography data. Until sparse imaging is performed at high-magnification for high-resolution structural biology, this work presents preliminary data where different sparse samplings are compared using different metrics (e.g. 3D alignment, CTF estimation, polishing) to understand the effects of sparse sampling on high-resolution data.

1. Trépout S., 2025, bioRxiv, <https://doi.org/10.1101/2025.07.28.667105>.

## Poster #20

### Insights into *Drosophila* EAAT2: Unveiling unique Cl<sup>-</sup> dependent taurine transport and K<sup>+</sup> coupling in the EAAT glutamate transporter family

Yan Jiang<sup>1</sup>, Qianyi Wu<sup>1,2</sup>, Chelse Briot<sup>1</sup>, Hariprasad Venugopal<sup>3</sup>, Renae M Ryan<sup>1</sup>

<sup>1</sup>*School of Medical Sciences, Faculty of Medicine and Health, University of Sydney, New South Wales 2006, Australia.*

<sup>2</sup>*Present address: Weill Cornell Medicine, 1300 York Avenue, New York, NY, USA 10044.* <sup>3</sup>*Ramaciotti Centre for Cryo-Electron Microscopy, Monash University, Clayton, VIC, Australia*

The precise regulation of synaptic glutamate concentrations is vital for maintaining neuronal function and preventing excitotoxicity. Mammalian glutamate transporters, known as Excitatory Amino Acid Transporters (EAATs), play a crucial role by transporting glutamate into glial cells and nerve terminals, thus terminating excitatory neurotransmission<sup>1</sup>. EAATs couple one glutamate to 3 Na<sup>+</sup> and an H<sup>+</sup>, followed by counter-transport of one K<sup>+</sup>. Despite their importance, mammalian SLC1 family members have not been known to transport taurine, an organic osmolyte essential for cell volume regulation. Interestingly, the isoform EAAT2 from *Drosophila melanogaster* (dEAAT2) transports taurine alongside aspartate<sup>3</sup>. This study aimed to characterise the substrate transport mechanism of dEAAT2, shedding light on its function and relationship with other SLC1 members.

We show that dEAAT2 operates as an electroneutral exchanger, activating an uncoupled Cl<sup>-</sup> conductance like its mammalian counterparts but lacking Na<sup>+</sup>/H<sup>+</sup>/K<sup>+</sup> dependence. Our investigation reveals a unique Cl<sup>-</sup> dependent taurine uptake mechanism in dEAAT2, not observed in other SLC1 transporters. High-resolution structures of dEAAT2 bound with taurine and aspartate reveal that a Cl<sup>-</sup> ion substitutes for the second carboxyl group on aspartate, facilitating taurine accommodation within the substrate-binding pocket. Strikingly, through two single point mutations, we fully reconstitute human EAAT-like coupling into dEAAT2, where aspartate transport becomes electrogenic and coupled to Na<sup>+</sup>/H<sup>+</sup>/K<sup>+</sup>. Our findings explain the divergence of substrate recognition and ion coupling among SLC1 members and provide mechanistic insight into the complete transport cycle of human EAATs.

#### References

1. Vandenberg RJ, & Ryan RM (2013). Mechanisms of glutamate transport. *Physiological Reviews* 93: 1621–1657.
2. Zerangue N, & Kavanaugh MP (1996). Flux coupling in a neuronal glutamate transporter. *Nature* 383: 634–637.
3. Besson MT, Ré DB, Moulin M, & Birman S (2005). High Affinity Transport of Taurine by the *Drosophila* Aspartate Transporter dEAAT2. *Journal of Biological Chemistry* 280: 6621–6626.

## Poster #21

### ***In situ* cryo-EM with FIB milling to resolve the ultrastructure of cryptophyte antennae and the identity of the photosystem-linking protein**

Gesa Gruening<sup>1</sup>, Paul Curmi<sup>1</sup>

<sup>1</sup>*University of New South Wales, Sydney, Australia*

Cryptophytes are single-celled algae that thrive in photon-limited environments through highly efficient light harvesting. Their antennae, composed of phycobiliproteins (PBPs), broaden the spectral absorption range and channel excitons to the photosystem<sup>1,2</sup>. Evidence of quantum beats suggests a mixture of coherent and incoherent transfer steps, with coherence likely playing a key role in the linking protein that connects the antenna to the photosystem. Yet, the molecular identity of this linking protein and the ultrastructure of the cryptophyte antenna complex remain unresolved.

The main goal of this project is the application of **state-of-the-art in situ cryo-electron microscopy (cryo-EM) with focused ion beam (FIB) milling** to study cryptophyte cells in their native environment. This approach will allow us, for the first time, to directly visualize both the ultrastructure of the antenna complex and the single-particle structure of the linking protein. These structural insights are essential to reveal how cryptophyte light-harvesting systems are organized at the molecular level and how coherence is preserved during exciton transfer.

Building on the cryo-EM results, we will integrate molecular dynamics simulations and quantum mechanics/molecular mechanics (QM/MM) calculations to map possible exciton transfer pathways and evaluate the quantum role of the linking protein.

By combining advanced cryo-EM imaging with computational modeling, this project will provide a structural and mechanistic understanding of cryptophyte light harvesting. Beyond cryptophytes, it may uncover a general photosynthetic design principle in which coherent linking proteins enable efficient energy transfer—offering inspiration for quantum technologies and bio-inspired photovoltaics.

1. Rathbone, H.W., Davis, J.A., Curmi, P.M.G., 2020, Coherent Processes in Photosynthetic Energy Transport and Transduction. In: Larkum, A., Grossman, A., Raven, J. (eds) *Photosynthesis in Algae: Biochemical and Physiological Mechanisms*. *Advances in Photosynthesis and Respiration*, vol 45. Springer, Cham.

2. Rathbone, H.W. et al., 2023, Molecular dissection of the soluble photosynthetic antenna from the cryptophyte alga *Hemiselmis andersenii*, *Commun Biol* **6**, 1158.

## Poster #22

### Dragonfly software as an integrated platform for AI-based electron microscopy data processing

Denis Korneev<sup>1</sup>, Georg Ramm<sup>1</sup>

<sup>1</sup>*Ramaciotti Centre for Cryo-EM, Monash University, Clayton, Australia*

Virtually all kinds of modern electron microscopy require some image processing to transform the raw data to a form, which is suitable for visual perception.

AI-based image processing is intensively developing now, transforming from a high-end instrument for professionals only to a general tool, which is routinely used by typical users of an electron microscopy facility. Nowadays, it is mostly used for images denoising and segmentation, but the potential applications are even broader.

Despite the recent explosive growth of the AI field, including the new large language models and other breakthroughs, classic convolutional neural network with supervised training (typically, U-Net) still are a workhorse in segmentation of large 3D datasets. A typical iterative workflow includes some data preparation (alignment, filtration, etc), labelling, model training, model application, and then manual correction of the results with their subsequent use to train an improved model. The last step can be repeated for a few times, depending on the required accuracy.

Dragonfly software [1] includes all tools for such a workflow, providing a large selection of filters and sophisticated instruments for data labelling, as well as an artificial intelligence unit itself. A possibility to 3D-render the results with subsequent application of morphological operations to correct them, dramatically improve the workflow efficiency.

Using the Dragonfly software for the last five years, we optimized the workflow and found plenty of non-obvious tricks and bypasses to improve the segmentation pipeline. We believe that our findings can be helpful for many facilities and research groups in electron microscopy.



Figure 1. AI-segmented FIB-SEM dataset. Mouse muscle. The sample was provided by Dr Meagan McGrath.

1. <https://dragonfly.comet.tech>

## Poster #23

### Characterisation of a novel intermediate conformation of the archaeal glutamate transporter Glt<sub>Ph</sub>

Amy Cheng<sup>1</sup>, Yan Jiang<sup>1</sup>, Renae Ryan<sup>1,2</sup>

<sup>1</sup>School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Australia

<sup>2</sup>School of Biomedical Engineering, Faculty of Engineering, The University of Sydney, Australia

The excitatory amino acid transporters (EAATs) are widely expressed in the central nervous system where they facilitate the rapid clearance of glutamate from the synaptic cleft<sup>1</sup>. The EAATs facilitate the concentrative transport of glutamate into surrounding astrocytes and neurons by coupling to the co-transport of 3 Na<sup>+</sup>, 1 H<sup>+</sup>, and the counter-transport of 1 K<sup>+</sup><sup>2</sup> and operates via a twisting elevator mechanism<sup>3,4</sup>. The Na<sup>+</sup>-coupled archaeal transporter, Glt<sub>Ph</sub>, is a homologue of the human EAATs, which belong to the solute carrier 1 (SLC1A) transporter family. SLC1A transporters assemble into homotrimers, where each protomer consist of a transport domain that binds the substrate and a scaffold domain that forms the trimerisation interface. After substrate and Na<sup>+</sup> binding, the transport domain compacts and undergoes rotation and vertical translocation from the extracellular to the intracellular side of the membrane, where the substrate is released<sup>1</sup>. During the transport cycle, a Cl<sup>-</sup> permeation pathway opens between the transport and scaffold domain, additionally allowing the thermodynamically uncoupled Cl<sup>-</sup> conductance<sup>5,6</sup>.

Here we have examined two single cysteine residues in human EAAT1 and Glt<sub>Ph</sub> whose function is influenced by oxidation and reduction. We expressed the single-cysteine mutants of EAAT1 in *Xenopus laevis* oocytes for functional characterisation using two-electrode voltage clamp electrophysiology. The EAAT1 mutants exhibit increased Cl<sup>-</sup> conductance, the activity of which can be modulated by oxidising/reducing agents. We have employed single particle cryo-EM to solve the structure of the corresponding single-cysteine mutant of Glt<sub>Ph</sub>, revealing a novel intermediate conformation that precedes Cl<sup>-</sup> channel opening.

1. Vandenberg RJ, Ryan RM. Mechanisms of Glutamate Transport. *Physiol Rev.* 2013;93(4):1621-57.
2. Zerangue N, Kavanaugh MP. Flux coupling in a neuronal glutamate transporter. *Nature.* 1996;383(6601):634-7.
3. Ryan RM, Vandenberg RJ. Elevating the alternating-access model. *Nat Struct Mol Biol.* 2016;23(3):187-9.
4. Reyes N, et al. Transport mechanism of a bacterial homologue of glutamate transporters. *Nature.* 2009;462(7275):880
5. Chen I, et al. Glutamate transporters have a chloride channel with two hydrophobic gates. *Nature.* 2021;591(7849):327
6. Wadiche JI, et al. Ion fluxes associated with excitatory amino acid transport. *Neuron.* 1995;15(3):721-8.

## Poster #24

### Structural Insights into Hepatitis B Core Antigen Nanoparticles for Multi-Stage Malaria Vaccinology

Anne Nguyen<sup>1</sup>, Daniel Luque<sup>1,2</sup>, Michael Johnson<sup>1</sup> and Jake Baum<sup>1</sup>

<sup>1</sup>*School of Biomedical Sciences, University of New South Wales, Sydney, Australia*

<sup>2</sup>*Electron Microscopy unit, University of New South Wales, Sydney, Australia*

Malaria caused an estimated 263 million cases and 597,000 deaths worldwide in 2023. Although the first two malaria vaccines, RTS,S and R21, have been approved—both based on a virus-like particle (VLP) platform targeting the circumsporozoite protein (CSP)—neither provides fully efficacious or durable protection. Their limited, stage-specific effectiveness underscores the need for multi-stage vaccine strategies capable of targeting multiple points in the parasite's complex lifecycle.

Hepatitis B core antigen virus-like particles (HBcAg VLPs) have long been used in vaccinology and nanobiotechnology as a versatile scaffold for antigen display. Their capacity to present multiple antigens simultaneously makes them particularly attractive for next-generation malaria vaccines. Several engineered HBcAg variants, including split-core and tandem-fusion designs, have been developed, but their relative potential for multi-antigen presentation in the malaria context has not been systematically assessed.

In this study, we produced these HBcAg VLP variants in parallel and examined their assembly, stability, and antigen presentation capacity using biochemical and biophysical assays. To complement these findings, we are employing cryo-electron microscopy (cryo-EM) to determine the high-resolution structures of each variant, revealing detailed insights into their morphology and assembly states. By linking structural features with functional performance, this integrated framework clarifies why certain variants offer superior engineering flexibility, solubility or stability. These observations directly inform the rational development of HBcAg-based multi-antigen nanoparticles as candidate platforms for next-generation multi-stage malaria vaccines.

## Poster #25

### Structural and functional studies on TIR domain-containing proteins from Gram-negative bacteria

Jobichen Chacko<sup>1</sup>, Jieyu Song<sup>1</sup>, Chen-Yang Lai<sup>1</sup>, Weixi Gu<sup>1</sup>, Mohammad K Manik<sup>1</sup>, Mitchell Sorbello<sup>1</sup>, Sulin Li<sup>1</sup>, Jeffrey D Nanson<sup>1</sup>, Bostjan Kobe<sup>1</sup>

<sup>1</sup>*School of Chemistry and Molecular Biosciences, Institute for Molecular Bioscience, and Australian Infectious Diseases Research Centre, The University of Queensland, Brisbane, QLD, Australia*

Toll/interleukin-1 receptor (TIR) domains play critical roles in immune responses in humans, plants and bacteria. Bacterial TIR-containing proteins have NADase activity and are involved in bacterial anti-phage defence and interference with the host innate immunity<sup>1,2</sup>. The hydrolysis of NAD<sup>+</sup> leads to the production of small molecules that activate downstream signalling pathways, or to cell death through NAD<sup>+</sup> exhaustion. These proteins have to self-associate into a filamentous assembly to form the NADase active sites at the interface of two domains. To understand the molecular basis of the activity of bacterial TIR domain-containing proteins, we studied the proteins AbTir from *Acinetobacter baumannii* and VpTir filaments from *Vibrio parahaemolyticus*. We determined the crystal structure of the inactive form of AbTir from *Acinetobacter baumannii* (2.65 Å resolution) and the cryo-EM structure of active filamentous form bound to an NAD<sup>+</sup> mimic<sup>1</sup> (2.74 Å resolution). We also determined the cryo-EM structure of the filamentous form of VpTIR from *Vibrio parahaemolyticus* (3.98 Å resolution). Biochemical assays reveal that AbTir produces the cyclic variant of ADPR (2'cADPR), whereas VpTir only the linear form of ADPR. We also show that VpTir form assemblies with dsDNA, whereas AbTir does not. We are optimising cryo-EM workflows to uncover how VpTir binds to DNA. The DNA function may be relevant to the activation of anti-phage defence by bacterial TIR-domain proteins. Integrating structural, enzymatic, and ligand-binding analyses, our work broadens our understanding of bacterial TIR domains and sheds light on the evolutionary links to TIR domain-mediated immune signalling in humans and plants.

#### References.

Manik MK. et al., 2022, Cyclic ADP ribose isomers: Production, chemical structures, and immune signaling. *Science*, 377: eadc8969  
Nimma S. et al., 2021, Structural Evolution of TIR-Domain Signalosomes. *Front Immunol*, 12: 784484

## Poster #26

### **STRUCTURAL BASIS OF IL-1R8– MAL TIR-DOMAIN CO-ASSEMBLY AND ITS ROLE IN TLR4 SIGNAL ATTENUATION**

Nada Alenzi<sup>1,2</sup>, Weixi Gue<sup>1,2</sup> Bostjan Kobe<sup>1,2</sup>

<sup>1</sup>*School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Queensland, Australia*

<sup>2</sup>*Institute for Molecular Bioscience (Division of Chemistry and Structural Biology) and Centre for Infectious Disease Research University of Queensland Brisbane, Queensland 4072*

Interleukin-1 receptor 8 (IL-1R8, also known as SIGIRR) is a key negative regulator of innate immune responses, particularly those initiated by Toll-like receptor 4 (TLR4). TLR4 signaling requires the adaptor protein MAL, which nucleates higher-order filamentous complexes via its Toll/interleukin-1 receptor (TIR) domain to recruit downstream effectors such as MyD88. The precise mechanism by which IL-1R8 dampens TLR4 signaling remains unclear. Here, we employed cryo-electron microscopy (cryo-EM) to determine the structure of co-assembled filaments formed by the TIR domains of IL-1R8 and MAL at 4.3 Å resolution. Our structural analysis reveals that IL-1R8 integrates into MAL filaments through a lateral interface. Notably, this lateral interface is also utilized by TLR4 for MAL binding. These findings suggest a competitive binding model where IL-1R8 attenuates TLR4 signaling by occupying the lateral interface on MAL, thereby modulating the composition and signaling capacity of the MAL filament scaffold without disrupting filament formation. This mechanism enables selective tuning of immune signaling, preserving complex integrity while limiting downstream activation. Our work provides new structural insights into the diverse modes of TIR domain interactions and highlights IL-1R8 as a modulator of innate immunity through a novel lateral binding mechanism. Understanding this regulatory interaction opens avenues for targeted therapeutic strategies to control inflammation driven by TLR4 signaling.

## Poster #27

### LLM-based and retrieval augmented SerialEM control code generation for fast prototyping cryo-EM imaging techniques

Chung-Han Tsai<sup>1</sup>, Melanie Rug<sup>1</sup>

<sup>1</sup>Centre for Advanced Microscopy, The Australian National University, Canberra, Australia

State-of-the-art generative AI large language models (LLMs) have the capability of computer software source code generation. Provided with plain English prompts, LLMs can generate SerialEM scripts that control cryo-electron microscopes (cryo-EM). Yet, they often miss facility-specific conventions and local human expert knowledge. We leverage a computing technique, called RAG-LLM<sup>1</sup> (retrieval-augmented generation-large language model) with our own local Python-based SerialEM code base. This allows us to achieve complex microscopy imaging tasks easier by using human natural language, and to eventually execute our cryo-EM operational tasks automatically.

We build a local testbed by using AI software framework, LangChain<sup>2</sup>, and Ollama<sup>3</sup> to construct a RAG-LLM system with our local Python-based SerialEM scripts indexed in. We use the latest open-source LLMs, called 'gpt-oss-120b'<sup>4</sup> (OpenAI), and 'embeddinggemma:300m'<sup>5</sup> (Google) for the RAG-LLM system. We then compare our RAG-LLM software test system with the off-the-shelf OpenAI ChatGPT 5 Plus platform for the essential coverage of small-scale imaging tasks. The evaluation also includes syntax checking and the correctness of accessing SerialEM script commands. We find that the SerialEM control code generation capability from the local, private RAG-LLM software test system is close to ChatGPT 5 Plus.

A local RAG-LLM allows facilities to establish a private knowledge base in more rigorous and accessible way as well as to fast prototype new cryo-EM imaging techniques through automatically generated and customised SerialEM scripts for specific instrument tasks. The initiative of leveraging local RAG-LLM prepares us for higher automation in microscopy imaging and operating microscopy facilities.

1. Lewis P, Perez E, Piktus A, Petroni F, Karpukhin V, Goyal N, Küttler H, Lewis M, Wen-tau Y, Rocktäschel T, Riedel S, Kiela D. Retrieval-Augmented Generation for Knowledge-Intensive NLP Tasks [Internet]. 2020 May 22 [cited 2025 Sep 15]. Available from: <https://ai.meta.com/research/publications/retrieval-augmented-generation-for-knowledge-intensive-nlp-tasks/> arXiv
2. LangChain. [Internet]. [cited 2025 Sep 15]. Available from: <https://www.langchain.com/>
3. Ollama. [Internet]. [cited 2025 Sep 15]. Available from: <https://ollama.com/>
4. OpenAI. Introducing GPT-OSS [Internet]. [cited 2025 Sep 15]. Available from: <https://openai.com/index/introducing-gpt-oss/>
5. Lisak A, Gonzalez L. Introducing EmbeddingGemma: The Best-in-Class Open Model for On-Device Embeddings [Internet]. Google Developers Blog; [cited 2025 Sep 15]. Available from: <https://developers.googleblog.com/en/introducing-embeddinggemma/> Hugging Face

## Poster #28

### Structural Analysis of Filamentous Prefoldin Chaperons in Thermophilic Bacteria and Archaea

Juanfang Ruan<sup>1</sup>, Dominic Golver<sup>2</sup>, Daniel Luque<sup>1,3</sup>

<sup>1</sup>*Electron Microscope Unit, Mark Wainwright Analytical Centre, UNSW Sydney, Sydney, Australia*

<sup>2</sup>*School of Biotechnology and Biomolecular Sciences, UNSW Sydney, Sydney Australia*

<sup>3</sup>*Department of Molecular Medicine, School of Biomedical Sciences, UNSW Sydney, Sydney, Australia*

Prefoldins are molecular chaperones that capture unfolded or misfolded polypeptides and deliver them to downstream folding systems. While typically functioning as heterohexameric complexes, certain hyperthermophilic bacteria and archaea have evolved filamentous prefoldin assemblies whose structures and biological roles remain largely unexplored. These organisms thrive under extreme conditions where protein stability is continuously challenged, making prefoldin filaments attractive models for understanding protein quality control in thermophiles.

In this study, we used cryo-electron microscopy (cryoEM) to investigate the structural organization of filamentous prefoldins. High-resolution reconstructions revealed repetitive subunit arrangements forming extended, helical filaments stabilized by inter-subunit coiled-coil interactions. Comparative analysis between bacterial and archaeal prefoldin filaments suggested both conserved and lineage-specific features, highlighting structural adaptations that may underlie their stability at high temperatures.

Our findings indicate that filament formation may provide an expanded binding surface for client proteins, potentially enhancing capture efficiency under stress conditions. The structural insights also suggest a modular architecture that could enable dynamic assembly and disassembly in response to cellular demands.

## Poster #29

### Optimized preparation of graphene-supported grids for high-resolution cryo-EM

Sepideh Valimehr<sup>1</sup>, Eric Hanssen<sup>1,2</sup>

<sup>1</sup> *Ian Holmes Imaging Centre and ARC Industrial Training Centre for Cryo Electron Microscopy of Membrane Proteins, The Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Victoria, Australia*

<sup>2</sup> *Department of Biochemistry and Pharmacology, The University of Melbourne, Parkville, Victoria, Australia*

Cryogenic electron microscopy (cryo-EM) has emerged as a leading technique for structural biology, yet specimen preparation remains a major bottleneck. Conventional holey carbon grids often suffer from sample loss, preferred particle orientation, and beam-induced motion, limiting data quality. Graphene, with its single-atom thickness, high electrical conductivity, and mechanical stability, offers a promising alternative support film for cryo-EM. Here, we present an optimized workflow for the preparation of graphene-coated grids suitable for high-resolution cryo-EM imaging. Our method emphasizes improved graphene transfer strategies to achieve uniform coverage and enhanced protein adsorption without compromising particle integrity. Using this approach, we were able to determine the high-resolution structure of apoferritin, validating the robustness and reproducibility of the method. This strategy provides a reliable route to generate high-quality graphene supports, paving the way for more accurate structural studies of challenging biomolecular specimens.

## Poster #30

### Structural Insights into A Novel Chi-like Phage Targeting Multiple *Klebsiella* spp

Yan Li<sup>1</sup>, Afif P. Jati<sup>1</sup>, Matthew Belousoff<sup>5</sup>, Hariprasad Venugopal<sup>4</sup>, Sylvain Trepout<sup>4</sup>, Denis Korneev<sup>4</sup>, Rhys A. Dunstan<sup>1</sup>, Fasséli Coulibaly<sup>2</sup>, Trevor Lithgow<sup>1\*</sup>

*1. Biomedicine Discovery Institute and Department of Microbiology, Monash University, Melbourne, Australia*

*2. Monash Proteomics & Metabolomics Facility, Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Melbourne, Australia*

*3. Ramaciotti Centre for Cryo-EM, Monash University, Melbourne, Australia*

*4. School of BioSciences, The University of Melbourne, Melbourne, Australia*

*5. Drug Discovery Biology, Faculty of Pharmacy and Pharmaceutical Sciences, Monash Institute of Pharmaceutical Sciences (MIPS), Monash University, Melbourne, Australia*

The Caudovirales order of bacteriophages (phages) is known for its structural complexity. These phages are sophisticated molecular machines, with a protein capsid that houses a double-stranded DNA genome, linked to a tail structure that interacts with bacterial receptors and facilitates DNA delivery into the host bacterium. Until recently, Caudovirales were categorized into three prominent families: Siphoviridae (long, non-contractile tails), Myoviridae (long, contractile tails), and Podoviridae (short, non-contractile tails).

In 1936, a Siphoviridae phage named Chi ( $\chi$ ) was discovered to infect *Salmonella enterica* Typhi. Cryo-EM and immuno-EM analyses of a Chi-like phage YSD1 revealed it to be a flagellotropic phage and provided insight into its capsid, tail tube, and flagellum-binding tail fiber. More recently, a cryo-EM study revealed  $\chi$ 's nearly complete structure. Our current work investigates a Chi-like phage named PIN2, isolated from south-eastern Australia. Structural analysis shows PIN2 shares Chi and YSD1's capsid and tail components but lacks a flagellum-binding tail fiber. Unlike traditional Chi-like phages, PIN2 infects *Klebsiella* — a non-flagellated host — using an alternative receptor and infection mechanism.

Based on near-complete cryo-EM reconstructions at sub-nanometer resolution, this study defines the overall architecture of the Chi-like phage PIN2, including its capsid, neck, tail, and tail tip modules. Our ongoing work focuses on map interpretation and model refinement to further clarify the infection mechanism. These findings deepen our understanding of Chi-like phage diversity and offer a structural foundation for future phage therapy strategies.

## Poster #31

### Solving peptide structures using electron crystallography

Amanda Bower<sup>1</sup>, Sulin Li<sup>1</sup>, Felipe Kremer<sup>2,3</sup>, Lara Malins<sup>1</sup>, Hongyi Xu<sup>1</sup>

<sup>1</sup> Research School of Chemistry, the Australian National University, Acton, Australia

<sup>2</sup> Centre for Advanced Microscopy, the Australian National University, Acton, Australia

<sup>3</sup> Department of Electronic Materials Engineering, the Australian National University, Acton, Australia

Microcrystal Electron Diffraction (MicroED) is an emerging method for structural elucidation for peptides and small molecules that remain inaccessible by traditional crystallography. Reproducible workflows are critical for broad application, particularly for compounds that form only micro- or nano-crystals. Here, we present a comprehensive MicroED workflow optimized for cyclic peptide natural products and their analogues<sup>1</sup>.

The workflow begins with controlled crystallization strategies, enabling the generation of abundant, high-quality nanocrystals directly from solvents commonly used in NMR experiments. Sample preparation followed a deposition-and-evaporation approach adapted from general TEM workflows. To mitigate vacuum-induced degradation, TEM grids were pre-cooled to  $-50$  °C prior to insertion and further stabilized at  $-175$  °C during data collection. This cooling strategy was critical for preserving diffraction quality and preventing sample dissolution under electron beam exposure.

Data collection was carried out in continuous rotation mode using *InsteaDMatic*<sup>2</sup> on a JEOL F200 TEM equipped with a Gatan Rio16 camera, yielding high-quality diffraction patterns from multiple submicron crystals. Data processing employed crystallographic pipelines adapted for electron scattering, including intensity integration, phasing by direct methods, and refinement protocols originally developed for X-ray crystallography. In cases of limited resolution, simulated annealing and molecular replacement approaches were applied to enhance structure determination.

Together, this integrated workflow demonstrates how the alignment of crystallization, cooling, and data acquisition strategies expands the applicability of MicroED to challenging peptide systems. These advances not only provide detailed insights into solid-state conformations and packing of bioactive peptides but also establish a foundation for extending MicroED to broader applications in drug discovery and structural chemistry.

1. Li, H. *et al.* Cyclopeptide Avellanins D–O with Antimalarial Activity from the Mariana Trench Anemone-Derived *Hamigera ingelheimensis* MSC5. *J. Nat. Prod.* **87**, 2695–2708 (2024).
2. Roslova, M. *et al.* *InsteaDMatic*: towards cross-platform automated continuous rotation electron diffraction. *J Appl Crystallogr* **53**, 1217–1224 (2020).

## Poster #32

### Structural elucidation of peptide hormone amyloid fibrils via cryo-EM

Evan R. Bissett<sup>1</sup>, Thomas H. Ficker<sup>1,2</sup>, Aidan B. Grosas<sup>1,2</sup>

<sup>1</sup>*Molecular Horizons, School of Science, Faculty of Science, Medicine and Health, University of Wollongong, NSW, 2522, Australia*

<sup>2</sup>*ARC Industrial Transformation Training Centre for Cryo-Electron Microscopy of Membrane Proteins, University of Wollongong, NSW, 2522, Australia*

Amyloid is a fibrillar protein conformation often associated with disease, yet it can also play essential roles in biology. Peptide hormones are key regulators of metabolism and utilise a functional amyloid state to concentrate and store peptides in a stable, inert conformation until external signals trigger release into receptor-active hormone monomers. Despite the fundamental biological importance of peptide hormones, their amyloid structure and high-resolution insights into their mechanism of assembly/disassembly remain poorly understood. To gain an understanding of the structure-formation relationship of peptide hormone amyloid, the human tachykinin peptides Substance P, Neurokinin A, and Neurokinin B were characterised using circular dichroism spectroscopy, as well as negative-stain and cryo-electron microscopy, as these peptides have previously been observed to form amyloid with non-canonical features when examined by spectroscopic techniques. Five high-resolution tachykinin peptide amyloid structures were solved, with NKA and NKB forming two polymorphs each, and SP presenting with a single fibril type. These structures reveal the first nanotubular amyloids from proteins of eukaryotic origin and indicate the existence of a new structural class of amyloid fibril. Furthermore, the structure of these nanotubes is unique compared to other nanotubular peptide aggregates of largely synthetic origin, presenting with amyloid defining features and small inner pores relative to the thickness of the fibril. The organisation of the peptides within the fibril suggest that the amyloid state prepares hormone monomers for rapid release in a conformation primed for tachykinin receptor activation. These findings mark a major step toward structurally defining the peptide hormone functional amyloidome.

## Poster #33

### High-resolution cryo-EM using a common 120-keV LaB<sub>6</sub> electron microscope equipped with a sub-200-keV direct electron detector.

Hariprasad Venugopal<sup>1\*</sup>, Jesse Mobbs<sup>2,3</sup>, Cyntia Taveneau<sup>6</sup>, Daniel R. Fox<sup>3,4,5</sup>, Ziva Vuckovic<sup>6</sup>, Sahil Gulati<sup>7</sup>, Gavin Knott<sup>8</sup>, Rhys Grinter<sup>3,4,5</sup>, David Thal<sup>2,3</sup>, **Stephen Mick<sup>7</sup>, Cory Czarnik<sup>7</sup> and Georg Ramm<sup>1,8\*</sup>**

<sup>1</sup> Ramaciotti Centre for Cryo-Electron Microscopy, Monash University, Clayton, VIC, Australia.

<sup>2</sup> Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC, Australia

<sup>3</sup> Australian Research Council Centre for Cryo-Electron Microscopy of Membrane Proteins, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC, Australia.

<sup>4</sup> Department of Microbiology, Biomedicine Discovery Institute, Monash University, Clayton, VIC 3800, Australia

<sup>5</sup> Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Victoria, Australia

<sup>6</sup> GlycoEra AG, Einsiedlerstrasse 34, 8820 Wädenswil.

<sup>7</sup> GATAN, Inc., Pleasanton, CA, USA.

<sup>8</sup> Department of Biochemistry and Molecular Biology, Monash Biomedicine Discovery Institute, Monash University, Melbourne, VIC, Australia.

\*Corresponding Authors

High-resolution cryo-electron microscopy (cryo-EM) requires 200-300-keV cryo-capable transmission electron microscopes (cryo-TEMs) with a field emission gun (FEG) source, stable column, autoloader and direct electron detector (DED). These specialised cryo-EM setups are expensive to establish and maintain. Advances in 100-keV imaging and the development of sub-200-keV optimised DEDs, promises a more affordable solution to a major cryo-EM application, i.e., single particle analysis (SPA) (1, 2).

Recently, we explored, whether a standard 120-keV TEM, with a LaB<sub>6</sub> thermionic source, available in many laboratories worldwide, can be upgraded with a sub-200-keV optimised DED (GATAN, Alpine) and its suitability for high-resolution imaging using a standard side entry cryo-holder (3). Using this imaging configuration, we successfully achieved a 2.65 Å resolution reconstruction for apoferritin. We were able to solve a challenging, asymmetric, 153 kDa membrane protein GPCR (M4 muscarinic acid receptor) to a resolution of 4.4 Å. We even managed to achieve 4.3 Å resolution for a small protein target, haemoglobin (64 kDa). Importantly, all these results were achieved using an automated data collection routine implemented through SerialEM, making it feasible to collect large cryo-EM data sets with a side entry cryo-holder (3).

Overall, these findings point to a promising, widely accessible path for obtaining high-quality cryo-EM structures. We believe this imaging configuration offers many laboratories the opportunity to establish effective cryo-EM SPA screening capabilities without investing in costly specialised cryo-TEMs. This could help to considerably lower the economic entry barrier for cryo-EM SPA and contribute to the “democratisation” of cryo-EM.

#### References:

1. K. Naydenova. et al., 2019, IUCrJ 6, 1086-1098.
2. G. McMullan, K. Naydenova. et al., 2023, Proceedings of the National Academy of Sciences 120, e2312905120.
3. H. Venugopal. et al., 2025, Science Advances 11, eadr0438.