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ARC Industrial Transformation Training Centre for Cryo-electron Microscopy of Membrane Proteins

Quarterly newsletter

INSIDE THIS ISSUE:

Australian Government
Australian Research Council

MONASH University
THE UNIVERSITY OF MELBOURNE
bio21 institute
MOLECULAR HORIZONS
UNIVERSITY OF WOLLONGONG AUSTRALIA
WEHI brighter together

Image credit - Dr Sarah Piper

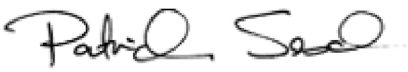
Welcome

Professor Patrick Sexton
Centre Director

Welcome to the 4th instalment of the ARC CCeMMP quarterly newsletter.

In this newsletter we welcome new staff and students joining the Centre, provide an update on activities over the last quarter and present recent highlights from our member nodes.

We commemorate the official launch of the Centre and our first research symposium. We review our first joint workshop with other ARC ITTCs that discussed the important topic "What does it mean to be an ethical researcher?" and we celebrate the success of our Node members who have received awards for their research and highly competitive research funding.

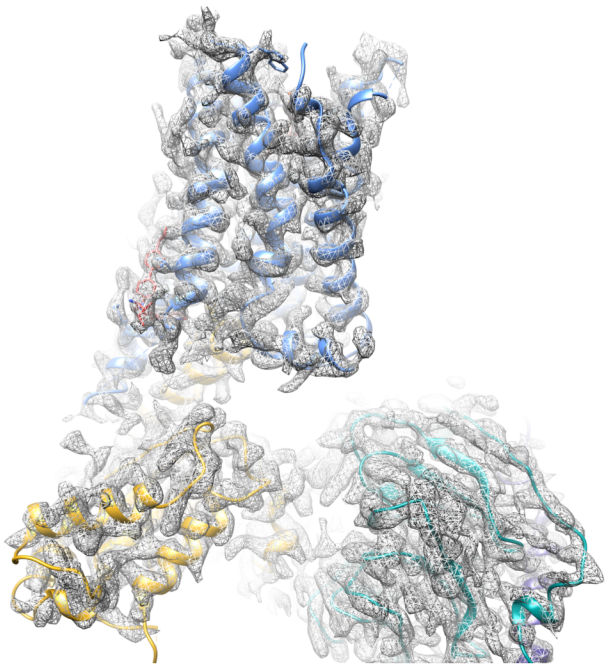


Professor Patrick Sexton
Centre Director

Dr. Jackie How
Centre Manager

Did you know?

G protein-coupled receptors (GPCRs) are the largest family of cell surface receptor proteins with >800 genes in humans that encode these proteins. However, even greater diversity occurs through multiple mechanisms including, post-translational modification, RNA editing, single nucleotide polymorphisms and formation of higher order protein complexes; hetero oligomers with other GPCRs or non-GPCR transmembrane proteins, and assembly into large signalosome complexes. GPCRs are the targets for >30% of current therapeutics but these cover only a fraction of the potential therapeutic GPCR targets. Advances in cryo-EM are providing novel opportunity for exploitation of this target class through structure-assisted drug discovery and development.



Centre updates

Official Centre launch

The Centre was officially launched on February 4th at the Bio21 Auditorium in hybrid fashion, welcoming all members, partners and senior staff from the academic institutions to participate and celebrate this tremendous milestone. The launch proceedings was overseen by Prof. Chris Porter (Director, MIPS, Monash) with speeches from Dr. Robert Munn (Australian Research Council), Prof. Mike Ryan (Pro Vice-Chancellor Research, Monash), Prof. Patrick Sexton, Prof. Isabelle Rouiller, Prof. Michael Parker and CCeMMP student Jack Tovey. The Centre offered tours of the cryo-EM facilities and an opportunity for networking.

Official Centre launch

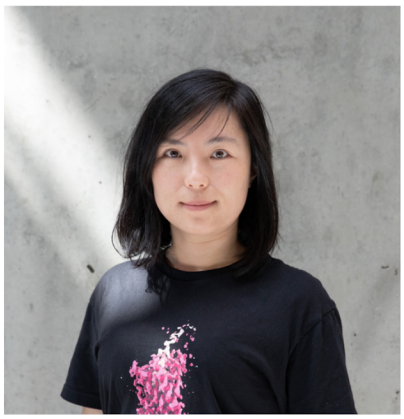
CCeMMP Annual Research Symposium

The Centre held its first annual research on the 3rd of February. The event was open to the public and was run virtually due to ongoing Covid restriction, with over 220 attendees. The symposium was split into three sessions with 12 speakers from across the four Nodes of the Centre, and was chaired by Centre postdocs Dr. Sepideh Valimehr, Dr. Aidan Grosas and Dr. Nazanin Mohebali. The symposium showcased some of the leading research, key findings and collaborations of the four Nodes. Dr. Maria Flocco from our industry partner, AstraZeneca, was the keynote speaker and shared insights into the work from an industry perspective.

Meet the speakers

Dr. Matt Belousoff
Monash Node
GPCR s at 200 k V

Dr. Katrina Black
WEHI Node
Ion currents through Kir potassium channels are gated by anionic lipids



Dr. Xin (Cindy) Zhang
Monash Node
Structural insights into glucagon-like peptide-1 receptor activation and allostery by small molecules

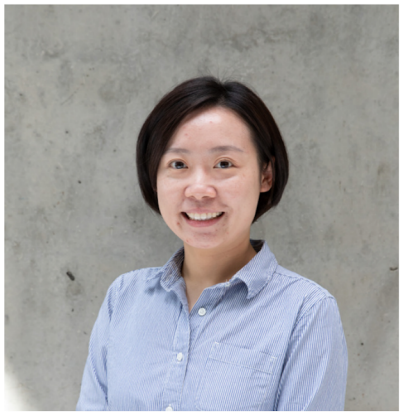
Jason Cao
Monash Node
Toward a structural understanding of amylin receptor phenotype: implications for therapeutic development for obesity

Dr. Sepideh Valimehr
UoM Node
Understanding the molecular mechanisms of controlling AAA+ ATPase p97 activity

Dr. Nazanin Mohebali
Monash Node
TACAN interaction with membrane lipids provides insights into its potential function in mechanosensation

Meet the speakers

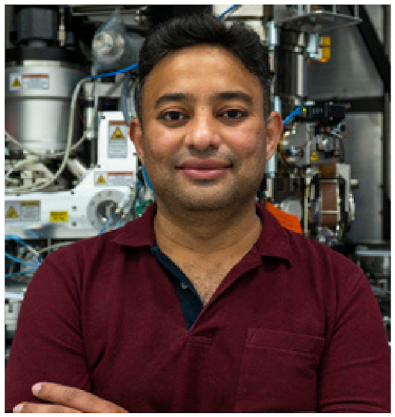
Bronte Johnstone
UoM Node
Optimising the sample & grid preparation of a novel pore-forming toxin for cryo-EM



Jackie Lu
Monash Node
Agonist-bound GPR52 G protein complex structures solved by cryo-EM

Sarah Piper
Monash Node
Joining forces: Cryo-EM and MD to decipher selectivity in the VPAC receptor family

Luidi (Aileen) Zhang
Monash Node
Structural insights into the inactive and active states of the A3 adenosine receptor



Dr. Shabih Shakeel
WEHI Node
Structures of Protein Complexes Involved in Fanconi Anemia DNA Repair

Dr. Anuja Bony
UoW Node
Analgesic a-conotoxin binding site on the human GABAB receptor

Keynote Speaker
Dr. Maria Flocca
AstraZeneca
Cryo-EM in drug discovery: expanding the target space accessible to structural studies

ITTC Panel and Workshop
What does it mean to be an ethical researcher?

In early April, five ARC training Centres hosted part two of the "What does it mean to be an ethical researcher?" workshops. This was the second event of the two planned events that comprised discussion from an interactive panel of ethics experts followed by a guided workshop where students and researchers were split into small groups and were guided through key scenarios facilitated by a mentor. The event was a success with more than 80 virtual and in person attendees at the panel event, and over 50 attendees at the in-person facilitated workshop.

This event explored the ethics and responsibilities that Centre researchers (especially students and postdoctoral researchers) have as they engage in research across academia and industry, and the importance of addressing what their personal responsibilities are versus that of the company or institution they work for.

Panel members (from left): George Kiossoglou, Catherine Nie, Andrew Gooley, Megan Munsie (Chair) and Andrea O'Connor (Zoom).

CCeMMP students and members (from left): Riya Josephs, Jason Cao, Qinghao Ou, Hai-Tian Chen, Jack Tovey and Dongju Lee.

Wollongong Node

Professor Antoine van Oijen
Node Leader, University of Wollongong Node

Introducing ICHDR student MariaKatarina Lambourne!

MariaKatarina (MK) is investigating the role of potassium ion channels that control neuronal excitability in health and disease at the Wollongong node of the CCeMMP. The "structure-function" relationship has always piqued MK's interest throughout her undergraduate years as she completed a Bachelor of Bionanotechnology at the University of Wollongong. However, she truly fell in love with cryo-EM after she generated her first-ever 3D model, which occurred in her Honours year while investigating annealases (DNA recombination proteins promoting annealing) with the Tolun Lab. Outside of the science bubble, she is passionate about issues affecting her home country of Kiribati, whether that be Kiribati politics or climate change. **She has also recently been awarded the Sydney Protein Group Greg Ralston Travel Award ($600).**

Supervisors: Prof. Lezanne Ooi, Dr. Gökhan Tolun, Dr. Aidan Grosas

Dr. Lisanne Spenkelink awarded The Robin Anders Young Investigator Award at Lorne Proteins

Dr. Lisanne Spenkelink was awarded one of the The Robin Anders Young Investigator Award at the Lorne Proteins 2022 Conference. This prestigious award is given to early-career researchers who have outstanding track records for this stage of their careers and have interesting research stories to share.

New UoW members joining CCeMMP

- MariaKatarina Lambourne

UoM/Bio21 Node

A/Professor Isabelle Rouiller
Node Leader and Deputy Director, UoM and Bio21 Node

Introducing ICHDR student Maria Eleni Georgopoulou!

Marialena obtained her BSc and MSc degrees from the Department of Biochemistry and Biotechnology, University of Thessaly, Greece. Her MSc thesis work introduced her to the fascinating world of structural biology. Her project included characterising human angiogenin interactions with DDX39B and DDX39A, key enzymes in tumorigenesis. Marialena's PhD project, as a member of the Parker lab, is focused on studying a transmembrane protein involved in Traumatic Brain Injury (TBI). Marialena will investigate the structure of that protein in the presence of inhibitors by Cryo-EM, crystallography and other biophysical/biochemical approaches with the aim of discovering drugs for treating TBI, for which there are currently only poor treatment options. When not in the lab, Marialena likes to explore her artistic nature by singing, drawing and poetry writing.
Supervisors: Prof. Michael Parker, Dr. Claire Weekley, Dr. Tracy Nero, Prof. Eric Hanssen

A/Prof. Michael Griffin awarded Cancer Council funding

A/Prof. Michael Griffin was awarded Cancer Council funding for his team's research in *Extending treatment success in pancreatic and bowel cancers*. His team have developed antibodies that inhibit the activity of IL11 and will test if these antibodies can extend the effectiveness of treatment with EGFR inhibitors for pancreatic and bowel cancers. If successful, this could establish the basis for much needed clinical trials of this new combination treatment for patients with advanced pancreatic and bowel cancers.

New UoM members joining CCeMMP

- Abolfazl Mirzadeh
- A/Prof. Megan Maher
- Marialena Georgopoulou

WEHI Node

A/Professor Isabelle Lucet
Node Leader, WEHI Node

Dr. Josh Hardy and Dr. Katrina Black awarded *The Robin Anders Young Investigator Award* at Lorne Proteins

Dr. Josh Hardy and Dr. Katrina Black were recipients of the The Robin Anders Young Investigator Award at the Lorne Proteins 2022 Conference. This prestigious award is given to early-career researchers who have outstanding track records for this stage of their careers and have interesting research stories to share.

Dr. Alisa Glukhova awarded the Australian Academy of Science's 2022 Gottschalk Medal

Dr. Alisa Glukhova has been awarded the Australian Academy of Science's 2022 Gottschalk Medal for her group's research in how cells function and respond to threats to find better treatments for debilitating conditions including heart and nerve diseases. Her collaborative research provided the first structural insights into the activation mechanism of the A1 adenosine receptor, a target for pain management and heart disease, opening possibilities for structure-based drug design. This distinguished award recognises outstanding early-career researchers who are leading significant research in the medical sciences.

Monash Node

A/Professor Denise Wootten
Node Leader, Monash University Node

Introducing ICHDR student Alok Pradhan!

Alok graduated with a Masters of Research (Biological Science) from the University of Wollongong where he worked on the structural determination of proteins in the E. coli replisome in Prof. Nicholas Dixon's lab. In his PhD project at the Monash Node, he will be using cryo-EM to determine the structures of GPCR heteromers. This project is being done in collaboration with our industry partner Dimerix. When not in the lab, he likes to seek new adventures, goes on hikes, climb rocks and does nature and wildlife photography. He also loves Economics.
Supervisors: *A/Prof. Denise Wootten, Prof. Patrick Sexton and Dr. Matt Belousoff*

Prof. Patrick Sexton awarded National Institutes of Health Research Grant

Laurence Miller and Patrick Sexton (Co-I) were recently awarded a National Institutes of Health (NIH) R01 grant (2022-2025) for the project *Impact of membrane composition on cholecystokinin receptor structure and function*.
Cholecystokinin acts on type 1 receptors (CCK1Rs) are key regulators of satiety, an important action in prevention and treatment of obesity. While CCK1R agonists can acutely reduce feeding, such agents with high potency and long duration of action tend to be associated with side effects and potential toxicity not tolerated for chronic therapy of healthy people. It is also now clear that a subset of the population is refractory to effects of CCK agonists, due to impact of membrane cholesterol on receptor conformation and dysfunctional stimulus-activity coupling, likely negatively affecting previous clinical trials. This project will combine cryo-EM structure determination, biochemistry and pharmacology to understand how drug action is regulated at the CCK1R.

New Monash members and affiliates joining CCeMMP

- Dr. Fabian Bumbak
- Dr. Sheng Yu Ang
- Alok Pradhan

Outreach activities

SB Grid and ARC CCeMMP webinar series "Cryo-EM of membrane proteins"

Scientific Organisers: Patrick Sexton (ARC Centre for Cryo-electron Microscopy of Membrane Proteins, Monash University), Jamaine Davis (Meharry Medical College), and Piotr Sliz (Harvard Medical School).

As previewed in our last Newsletter, we partnered with SB Grid (Harvard) to deliver a public webinar series that spanned cutting edge research on cryo-EM of membrane proteins, highlighted by the opening and closing Keynote speakers, Nieng Yan (Princeton) and Chris Tate (MRC), workshops on sample preparation, data processing and protein modelling to infrastructure access. Live audiences ranged from 150 to >250 participants.

March 15th - Opening Keynote: Nieng Yan, Princeton University
The molecular choreography of Cav/Nav channels – A mission impossible by today's AI

March 22nd - *Sample Preparation: From single particle cryoEM to MicroED, tomography and FIB milling.*
Panel discussion with: Sara Weaver, Ph.D., University of California, Los Angeles; Lisa Eshun-Wilson, Ph.D., Scripps Research; William Nicolas, Ph.D., Caltech

March 29th - *Single Particle CryoEM: from data to structure*
Shaun Rawson, Ph.D., Harvard Medical School - Effective on-the-fly and downstream processing of CryoEM data
Tom Goddard, UCSF Resource for Biocomputing, Visualization, and Informatics - Using AlphaFold protein structures in ChimeraX for cryoEM modeling

April 12th - *Regional CryoEM infrastructure in the United States.*
Panel session moderated by Melissa Chambers (Vanderbilt):
Liz Wright (UW-Madison); Caleigh Azumaya (Fred Hutch); Craig Yoshioka (PNCC); Bob Grassucci (Columbia).

April 19th - Melanie Ohi, University of Michigan – *Cryo-EM analysis of membrane spanning complexes*

April 26th – Closing Keynote: Chris Tate (MRC) - *Insights into GPCR activation from cryo-EM structures*

Recent Centre Activities and Achievements

Presentations to Industry

- Dr. Natalie Diepenhorst - *Invited speaker ACvA Industry Collaborations with Academia panel*

Conference presentations

- A/Prof. Denise Wootten, CGRP 2022 Conference - *The structure of CLR and CTR; new insights from cryo-em*, 11th April 2022
- A/Prof. Denise Wootten, 6th ERNEST Meeting - *Structural and mechanistic insights into class B1 GPCR activation, signalling and allostery*, 30th March 2022

Lorne Protein 2022 Conference

- Dr. Onisha Patel - *Structural basis for small molecule targeting of Doublecortin Like Kinase 1 with DCLK1-IN-1*
- Dr. Josh Hardy - *Viral metamorphoses: using cryo-EM to understand the maturation of flaviviruses*
- Dr. Lisanne Spenkelink - *The E. coli helicase does not use ATP during replication*
- Dr. Katrina Black - *Ion currents through Kir potassium channels are gated by anionic lipids*
- Dr. David Thal - *Structural and dynamic mechanisms of allostery at the M4Â muscarinic acetylcholine receptor*
- Dr. Sarah Piper - *Dynamic drug targets: Using Cryo-EM data and MD simulations to create realistic 3D animations of GPCR complexes*
- Dr. Alisa Glukhova - *Understanding ligand binding to adenosine receptors using cryo-EM*

Presentations to Academia

- Fragile Nucleosome Series - Dr. Shabih Shakeel, *Mechanistic Insights into the Fanconi Anemia DNA Repair Pathway*, March 9th 2022
- MIPS Seminar - Dr. Tracy Josephs - *A pharmacogenomic and structural-dynamic approach to inform autosomal dominant hypocalcaemia treatment*, 23rd March 2022
- MIPS DDB Seminar - Dr. Katrina Black, March 2022

MIPS EMCR Symposium, 12 April 2022

- Dr. Brian Cary - *Prolonged signaling of backbone-modified glucagon-like peptide-1 analogues with diverse receptor trafficking*
- Dr. Nazanin Mohebali - *Utilizing membrane mimetic systems in structure determination of GPCRs using cryo-EM*
- Dr. Sarah Piper - *Dynamic drug targets: Using Cryo-EM data and MD simulations to create realistic 3D animations of GPCR complexes*

Media

- Australian Biochemist Magazine. Dr Josh Hardy and Dr. Stephanie Nguyen - *Taking Care of Your Mental Health During a PhD*, April 2022
- Asia & Pacific News. Jason Cao and Prof. Patrick Sexton - *Aussie researchers' finding to help in battle against obesity*, 25th March 2022

Publications

[1] Gregory KJ, Jörg M. Chemical biology-based approaches to study adenosine A2A - dopamine D2 receptor heteromers. ***Purinergic Signal***. 2022 Mar 29. doi: 10.1007/s11302-022-09860-8.

[2] Cao J, **Belousoff MJ**, Liang YL, **Johnson RM**, **Josephs TM**, Fletcher MM, **Christopoulos A**, Hay DL, Danev R, **Wootten D**, **Sexton PM**. A structural basis for amylin receptor phenotype. ***Science***. 375(6587):eabm9609, 2022. doi: 10.1126/science.abm9609.

[3] Zhao P, Truong TT, Merlin J, **Sexton PM**, **Wootten D**. Implications of ligand-receptor binding kinetics on GLP-1R signalling. ***Biochem Pharmacol***. 199:114985, 2022. doi: 10.1016/j.bcp.2022.114985.

[4] Milburn JE, Harikumar KG, **Piper SJ**, Raval S, **Christopoulos A**, **Wootten D**, **Sexton PM**, Miller LJ. Secretin amino-terminal structure-activity relationships and complementary mutagenesis at the site of docking to the secretin receptor. Mol Pharmacol. 101(6):400-407, 2022. doi: 10.1124/molpharm.122.000502.

[5] Newing, T, **Brewster JL**, Yu H, Johnston NP, Fitschen LJ, **Tolun G**. Structure of phage λ Redβ177 annealase shows how it anneals DNA strands during single-strand annealing homologous DNA recombination. Preprint. https://doi.org/10.1101/2022.04.09.487726

CCeMMP cryo-EM structure image gallery

Illustrated are images of the solved protein structures within the EM maps that are shown as transparent surfaces, coloured according to the protein chain, for each of the complexes. Images were created by Dr. Sarah Piper in Blender using the publicly available PDB and EMDB files.