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Australian Government
Australian Research Council

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

Quarterly newsletter



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MONASH
University



bio21
institute

MOLECULAR
HORIZONS



WEHI
brighter together

Image credit - Dr Sarah Piper



Welcome

Professor Patrick Sexton
Centre Director

Welcome to the inaugural quarterly newsletter of the ARC CCEMMP where we will provide regular updates on the activities and achievements of the Centre, its staff and students. As this is our first newsletter there is a lot of news as we have worked through the complexities of formally establishing the Centre as an entity under the auspices of the ARC. We have also begun the process of staff and, importantly, student recruitment, developed coursework, and, of course, prosecuted study of the cryo-EM of membrane proteins.



ARC funding for the Centre was announced in July 2020, however, the official establishment of the Centre required a formal agreement to be put in place with all of the Academic, Industry and Educational Partners, which was finalised and approved by the ARC on March 23rd, 2021. Key to this outcome was the appointment of Dr. Jackie How as our Centre Manager in September 2020 who has worked tirelessly with the Administrative Organisation (Monash) legal team and research office, and the respective counterparts in each of our Partner Organisations to finalise the Agreement (herding cats is child's play in comparison). In parallel, we have moved forward with development of the bespoke coursework that is a feature of our Doctoral Training Program that needed to be built from scratch to be ready for our first cohort of ICHDRs who commenced in April.

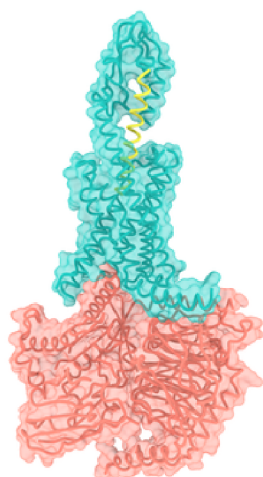
We are excited to be operational and we will provide more information on our key people and committees in future Newsletters.

A handwritten signature in black ink that reads "Patrick Sexton". The signature is fluid and cursive, with the first letters of the first and last names being capitalized and prominent.

Professor Patrick Sexton
Centre Director

A handwritten signature in black ink that reads "Jackie How". The signature is cursive and somewhat stylized, with the first letters of the first and last names being capitalized and prominent.

Dr Jackie How
Centre Manager



Did you know?

Cryo-EM is a specialised form of EM where samples are frozen in a non-crystalline form of ice, termed vitreous ice, which allows images of samples captured in their native state. Cryo-EM can be applied to purified proteins that are frozen in very thin vitreous ice so that only a single protein is present in any section of the ice generating an atomic level 2D picture of that protein. However, during freezing the protein will have multiple different orientations with each of these leading to 2D images of the protein at different angles. By capturing images of 100s, 1,000s or even 1,000,000s of particles, sophisticated software allows us to reconstruct these 2D images into a high-resolution 3D map of our protein of interest that we use to determine the structure of the protein

Wollongong Node

Professor Antoine van Oijen

Node Leader, University of Wollongong Node

Introducing ICHDR student Maddison Steele!

Maddie is a current PhD candidate based at the Wollongong node of the CCEMMP. Maddie has grown up in Wollongong, and while she enjoys travelling both around Australia and abroad, there is absolutely no place like home.

Maddie began her tertiary education with an undergraduate degree in Science, majoring in Medical Biotechnology at the University of Wollongong. Maddie fell in love with structural biology late in her degree. She chose to undertake a structural study of glutathione transferase Omega 1 using X-ray crystallography for her Honours project in 2020. Maddie decided to focus on the structural technique of cryo-EM after experiencing the FEI Titan-Krios at the new cryo-EM facility at the University of Wollongong, which she describes as “love at first sight”.



Supervisors: Prof David Adams (main), Prof Antoine van Oijen and Dr Simon Brown.

Opening of Molecular Horizons Institute and the Introduction to the CCEMMP to the University of Wollongong



From left: Prof Adams, Prof Sexton, Maddie Steele, Prof van Oijen, Dr Tulan, Dr Bouwer and Dr Jackie How.

On Friday 30th of April 2021, we were able to visit the UoW Node and attend the official opening of the Molecular Horizons Institute. The Institute was officially opened on Friday 30 April 2021 by the Governor-General, His Excellency General the Honourable David Hurley AC DSC (Retd). The ceremony dedicated the Molecular Horizons building to retiring UOW Vice-Chancellor Professor Paul Wellings CBE for his achievements and contributions to the University.

The CCEMMP Director presented an introduction to the Centre to the researchers and students at Molecular Horizons and Illawarra Health and Medical Research Institute (IHMRI).

New UoW members joining CCEMMP

- Dr James Bouwer
- Dr Simon Brown
- Dr Lezanne Ooi
- Prof Nicholas Dixon
- Dr Gokhan Tolan
- Dr Jodi Brewster
- Dr Rocio Finol Urdaneta

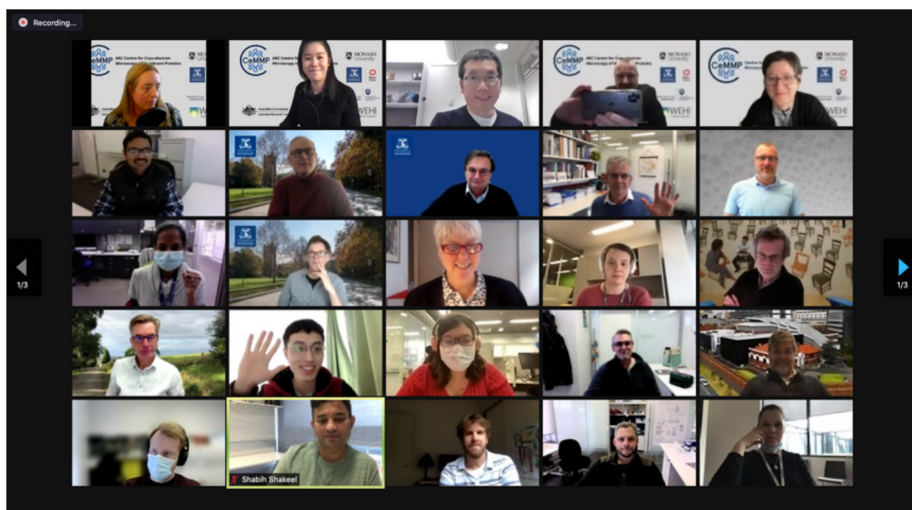
UoM/Bio21 Node

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

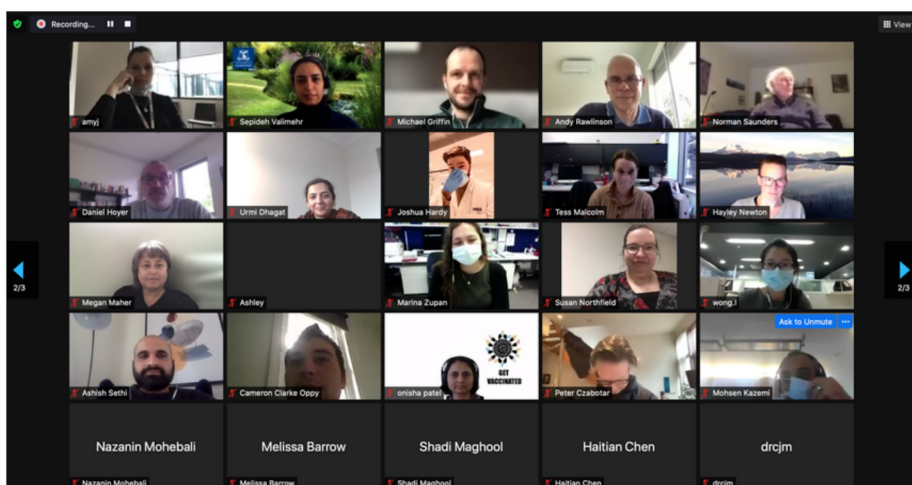
WEHI Node

A/Professor Isabelle Lucet
Node Leader, WEHI Node

Introduction to the CCeMMP to the UoM/Bio21 and WEHI Nodes



On Wednesday 18th of August 2021, the CCeMMP Director presented a virtual introduction to the Centre to the researchers, students and industry partners at The University of Melbourne, The Bio21 Institute and the WEHI. A/Prof Isabelle Rouiller and A/Prof Isabelle Lucet presented on their Nodes and Prof Michael Parker (Director, Bio21) presented on the facilities at Bio21. There were over 60 attendees at the presentations.



New UoM and WEHI members joining CCeMMP

UoM/Bio21 members

- A/Prof Eric Hanssen
- Dr Debnath Ghosal
- Dr Alisa Glukhova
- Dr Josh Hardy

WEHI members

- Dr Alisa Glukhova
- Dr Josh Hardy

WEHI affiliates

- Dr Shabih Shakeel
- Dr Winne Tan

Monash Node

A/Professor Denise Wootten

Node Leader, Monash University Node

Introducing ICPD research fellow Dr Matt Belousoff!

CCeMMP welcomed Dr. Matthew Belousoff, the first of our Centre funded ICPD Fellows, who commenced January 2021. Matthew, working with CCeMMP colleagues across the Nodes, has been integral to the major task of developing bespoke training in the theoretical and practical use of our high-end cryo-EM instrumentation and in the process of 3D reconstruction of the cryo-EM maps that define membrane protein structure. Matthew is also heavily involved in supervision of our ICHDRs and in the research program of the Node.



Introducing ICHDR Isabella Russell!



Isabella is investigating the potential use of engineered constitutive activity in GPCRs as an approach to solve structures of orphan (no known natural ligand) GPCRs. When not in the lab, Isabella can usually be found at the ice rink. Whether it's playing, refereeing or goaltending, she just loves ice hockey! The ice rink is also an excellent place for her to knit in-between games, and she is currently trying to knit socks. The project is a collaboration with our Industry Partner Organisation, AstraZeneca.

Supervisors: Prof Patrick Sexton, A/Prof Denise Wootten, Dr Xin Zhang and Dr Matt Belousoff.

Introducing ICHDR Jack Tovey!

Jack is investigating the utility of alternative approaches to solubilisation and reconstitution of GPCRs on complex formation, robustness and resolution of cryo-EM structures to support structure determination with low affinity/low efficacy ligands. The project is a collaboration with our industry partner, Astex. Outside of the lab Jack can often be found exploring their other long-standing fascinations with the Humanities: history, literature, linguistics, and art.

Supervisors: A/Prof Denise Wootten, Prof Patrick Sexton, Dr Jesse Mobbs, and Dr Matt Belousoff.



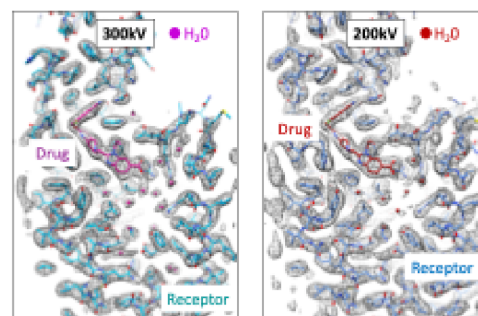
New Monash members and affiliates joining CCeMMP

- Dr Emma Del Maso
- Dr Anandhi Anandan
- Dr Chris Langmead
- Wessel Burger
- Jianjun (Jason) Cao
- Liudi (Aileen) Zhang
- Jessica Lu
- Dr David Thal
- Dr Paul Stuppel
- Dr Rachel Johnson
- Dr Peter Keov
- Prof Arthur Christopoulos
- Dr Darren Riddy
- Dr Cindy Zhang
- Dr Sebastian Furness
- Dr Elva Zhao
- Dr Jesse Mobbs
- Dr Sarah Piper
- Dr Thomas Coudrat
- Dr Matthew Belousoff

Working with industry

Evolving cryo-EM structural approaches for GPCR drug discovery in partnership with Thermo Fisher Scientific.

High resolution structure for small, dynamic, membrane proteins such as G protein-coupled receptors (GPCRs) have required the use of the most advanced cryo-EM instrumentation that rely on high energy electron beams generated with 300kV microscopes. Such microscopes are very expensive, costing up to AUD 10 million, in addition to the specialised laboratories that need to be built to house the instruments. This limits the routine application of cryo-EM within many drug discovery programs.



CCeMMP scientists at the Monash Node, led by CCeMMP ICPD Fellow, Dr. Matthew Belousoff, have been collaborating with our Industry Partner Organisation, Thermo Fisher Scientific, to explore the extent to which the latest developments in detector technology and image acquisition can enable the use of 200kV instruments (that are <50% of the cost of 300kV instruments) to support structure determination for small membrane proteins such as GPCRs. This work demonstrated that 200kV imaging can deliver the structural resolution required for structure-based drug discovery, including visualisation of structural waters within the drug binding pocket. This work, recently published in the journal Structure [2], opens the way for broader use of cryo-EM for membrane protein drug discovery with routine structure determination that integrates 200kV and 300kV imaging.

CCeMMP has state-of-the-art Thermo Fisher Scientific 200kV and 300kV cryo-EM instruments, equipped with the latest generation direct electron detectors (K3 or Falcon 4), within each of the Univ. Wollongong, Bio21/WEHI and Monash Nodes.

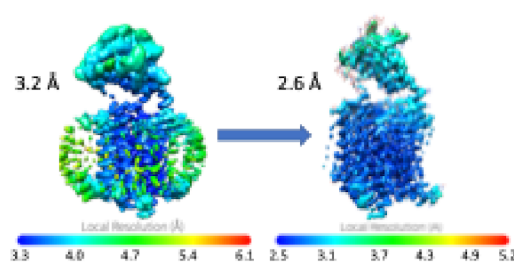
Outreach activities

CCeMMP EMPIAR data processing competition

Structural cryo-EM is a highly dynamic field with rapid advancement in instrumentation for image acquisition and the software used to analyse the data to generate high-resolution information on the 3D structure and dynamics of proteins.

These developments regularly reset what is achievable with cryo-EM. CCeMMP is engaged in research that often pushes the boundaries of what is currently feasible for membraneprotein cryo-EM and this creates an excellent opportunity to engage with the broader research community to test the how the most recent developments in software and data processing approaches can be applied to the targets studied within the Centre. As such, CCeMMP will regularly run competitions for the cryo-EM community that challenges them to improve on select published research from CCeMMP, using our “raw” data that is deposited on the public EMPIAR database.

The first of these competitions was a challenge to improve the resolution of a structure published early this year in Science (see publications [1]). From submissions from across the globe, the winning entry was from Dr. Simon Fromm (EMBL in Germany) who improved the EM map from the published resolution of 3.2 Å to 2.6 Å. Through these competitions we both expand awareness of CCeMMP, and gain insight into potential alternative approaches to data processing and the value of the most recent software advances for work within the Centre.



Recent Centre Activities and Achievements

Presentations to Academia

- Research Seminar: Prof. Patrick Sexton "Using cryo-electron microscopy to probe G protein-coupled receptor function" Biozentrum Institute, Univ. Basel (Switzerland), April 13th, 2021
- Research Seminar: Dr. Sarah Piper "Dynamic drug targets: Cryo-EM structures of G-protein coupled receptors" Center for Soft Nanoscience/Institute of Medical Physics & Biophysics (Univ. Muenster, Germany). June 21st, 2021.

Presentations to Industry

- Technical workshop: Dr. Matthew Belousoff; Model fitting to cryo-EM maps using molecular dynamics (MD). Sanofi Aventis (Germany), January 19th 2021
- Research Seminar: Prof. Patrick Sexton "Using cryo-electron microscopy to probe G protein-coupled receptor function" Pfizer (USA), May 7th 2021

Conference presentations

- Prof. Patrick Sexton: Keystone Symposium: Frontiers in cryo-electron microscopy. Feb. 4th-5th, 2021. From apo to active to small molecule drug discovery: GPCR structure using cryo-EM
- Prof. Patrick Sexton: 2nd NovAliX Virtual Conference. Biophysics in drug discovery 2021. March 9th-12th, 2021. Using cryo-EM for GPCR drug discovery and development
- Prof. Patrick Sexton: Cryo-EM in Drug Discovery Symposium. SciLife Labs, Stockholm. June 8th-9th, 2021. Using cryo-EM for GPCR drug discovery and development

Publications

[1] Josephs TM, Belousoff MJ, Liang Y-L, Piper SJ, Cao J, Garama DJ, Leach K, Gregory KJ, Christopoulos A, Hay DL, Danev R, Wootten D, Sexton PM. Structure and dynamics of the CGRP receptor in apo and peptide-bound forms. *Science* 372: eabf7258, 2021. [doi:10.1126/science.abf7258].

[2] Zhang X, Johnson RM, Drulyte I*, Yu L, Kotecha A*, Danev R, Wootten D, Sexton PM, Belousoff MJ. Evolving cryo-EM structural approaches for GPCR drug discovery. *Structure* S0969-2126(21)00124-6, 2021. doi: 10.1016/j.str.2021.04.008. on-line ahead of print.

[3] Mobbs J, Belousoff MJ, Harikumar K, Piper SJ, Xu X, Furness SGB, Venugopal H, Christopoulos A, Danev R, Wootten D, Thal DM, Miller LJ, Sexton PM. Structures of the human cholecystinin 1 (CCK1) receptor bound to Gs and Gq mimetic proteins provide insight into G protein selectivity. *PLoS Biol* 19: e3001295, 2021. doi: 10.1371/journal.pbio.3001295.

New cryo-EM structures

- Apo CGRPR (PDB:7KNT; EMDB:22962) [1]
- CGRP:CGRPR (PDB:7KNU; EMDB:22963) [1]
- PF06882961:GLP-1R:Gs [Krios-K3] (PDB:7LCI; EMDB:23274) [2]
- PF06882961:GLP-1R:Gs [Krios-F4] (PDB:7LCJ; EMDB:23275) [2]
- PF06882961:GLP-1R:Gs [Glacios-F4] (PDB:7LCK; EMDB:23276) [2]
- CCK8:CCK1R:mGqsi:scFv16 (PDB:7MBY; EMDB:23750) [3]
- CCK8:CCK1R:Gs:Nb35 (PDB:7MBX; EMDB:23749) [3]