

OCT. 2021, ISSUE 2



Australian Government  
Australian Research Council



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

*Quarterly newsletter*

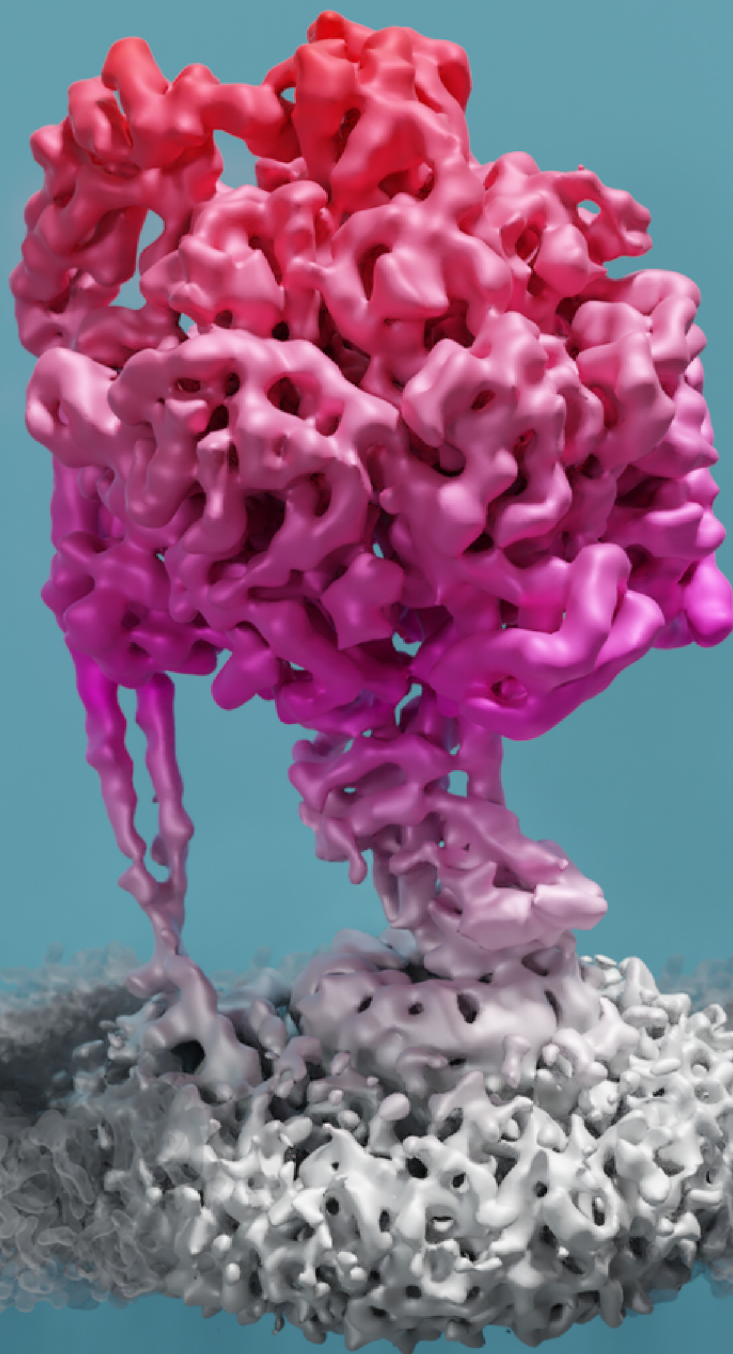
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MONASH  
University



bio21  
institute

MOLECULAR  
HORIZONS



UNIVERSITY  
OF WOLLONGONG  
AUSTRALIA



WEHI  
brighter together

Image credit - Dr Sarah Piper



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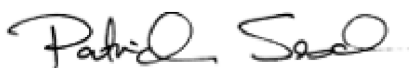
# Welcome

Professor Patrick Sexton  
*Centre Director*

Welcome to the second instalment of the quarterly newsletter of the ARC CCeMMP.

In this newsletter we welcome new staff and students joining the Centre, provide an overview of our bespoke Doctoral Training Program and present recent highlights from our member Nodes.

The pandemic continues to provide challenges for the Centre. Those of us in Melbourne have the unwanted World record of having spent the most time in lockdown, while our colleagues in Wollongong have also been impacted by the outbreaks in NSW. Nonetheless, as vaccination rates steadily move towards key threshold levels, there is clear light at the end of the tunnel, and we look forward to moving towards more normal modes of operation in 2022. Thanks to all staff and students for their incredible efforts in implementing our training program (more below), and in driving cutting edge membrane protein cryo-EM research.

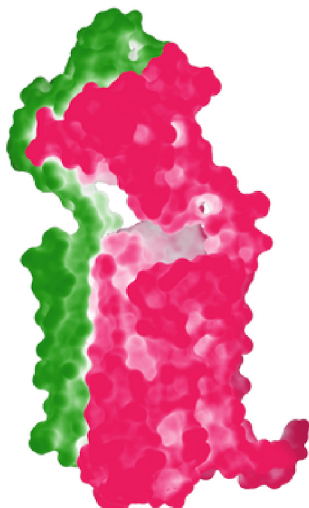


Professor Patrick Sexton  
Centre Director



Dr Jackie How  
Centre Manager

## Did you know?



Cells are the basic units of life. Cells may have individual roles, such as those of the immune system that circulate in our bodies and respond to foreign invasion, but more commonly are part of larger aggregates that make up our vital organs. Cells are separated from each other and the environment by membranes, a barrier that is comprised of a lipid bilayer with embedded or associated proteins.

These membrane proteins allow the cell to sense their external environment and can control the internal cell environment through regulation of the movement of substances in and out of the cell, or by changing the activity within the cell. A major class of membrane proteins are termed receptors, and these receptors are the conduits of intracellular communication and allow the coordinated control of cells that underpins complex physiology. Disruption of normal cellular function leads to disease and altered behaviour of membrane proteins can contribute to disease, but targeted regulation of these proteins can also lead to disease resolution. As such membrane proteins are the largest class of therapeutic drug targets.



# Centre updates

## New Partners



We are excited to announce that Boehringer Ingelheim (BI) has formally joined the Centre as a Partner Organisation. BI is a major pharmaceutical company headquartered in Ingelheim, Germany. BI will be working with the Monash Node on two project areas, supporting postdocs and students within the Centre.

## CCeMMP seminar series

We are pleased to announce the commencement of the Centre's virtual and in person seminar series. Seminars will be held on the second Tuesday of each month between February and December each year from 10am to 11am. The seminars commenced on October 12th with Professor Patrick Sexton speaking about 'Using cryo-EM to interrogate the structure and dynamics of GPCRs' with an audience of 146 people.

A seminar committee with representation from all Nodes, plus a member of our ICHDR cohort, will oversee this program. Seminars will be chaired by ICHDRs as part of their training in scientific engagement. The committee welcomes ideas for speakers from all node members and affiliates. Please email any suggestions to Jackie How ([ccemmp@monash.edu](mailto:ccemmp@monash.edu)), including suggested speaker topic and why you believe they will be a good speaker for the Centre.

All sessions are recorded and available at <https://ccemmp.org/news/ccemmp-seminar-series/>



## 2021 ICHDR cohort update

Our ICHDRs have completed their Biochemistry rotation and are now one week into their Data and Processing rotation. All ICHDRs completed self-directed learning on key tasks relevant to the rotations prior to attendance

*From left: Our ICHDRs Dongju Lee, Hai-Tian Chen, Qinghao Ou, Isabella Russell and Jack Tovey.*



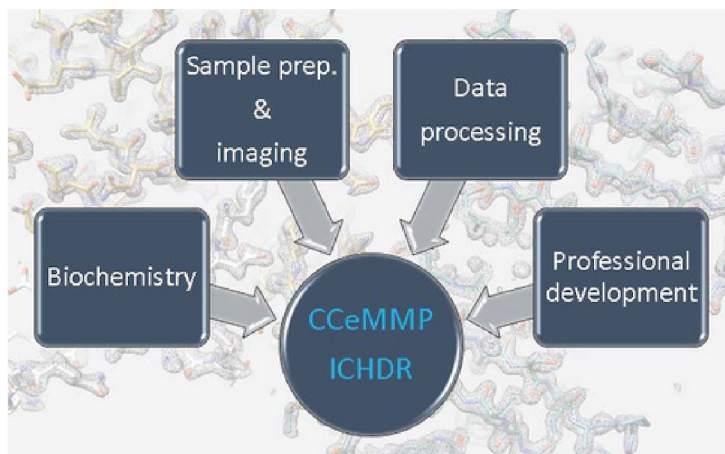
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# Doctoral Training Program

Associate Professor Denise Wootten  
*Node Leader, Monash University*

## About the program

We have designed a Doctoral Training Program that is in line with the Australian Research Council and CCEMMP core aims: to train industry-ready, world class graduates in cryo-electron microscopy of membrane proteins. In doing so, we expect that our graduates will go on to drive cutting edge structural biology knowledge and techniques, address knowledge gaps and develop entrepreneurial and technical skills that are desired by industry.



The program comprises 3 main training rotations:

### Biochemistry rotation

This training will focus on the protein biochemistry of membrane proteins, including expression, solubilisation and purification of stable protein complexes and their reconstitution in detergent micelles or nanodiscs. This training is delivered by the Nodes.

### Cryo-EM and imaging rotation

This training will focus on cryo-EM imaging of exemplar membrane proteins using the 200 kV and 300 kV instruments. ICHDRs will be trained to evaluate particle behaviour in vitreous ice, identify optimal areas of ice for data collection and in the collection of tilt data for 3D reconstruction of particles.

### Data processing and analysis

This rotation will focus on the principles and practice of cryo-EM data processing, modelling and analysis. ICHDRs will cover different software packages for motion and contrast transfer function correction, 2D and 3D classification and progress from idealised data sets of proteins with high symmetry to data sets of high complexity.

ICHDRs will also undertake professional development training and 'masterclasses' to develop skills in entrepreneurship, innovation, commercialisation and business development. These skills will provide them with foundational understanding to engage with industry partners on drug discovery.

We are very excited to see our first ICHDR cohort going through these rotations and to see their development as they progress through their training. Many thanks and recognition should be given to the Coursework Committee for their work in designing and organising the rotations.



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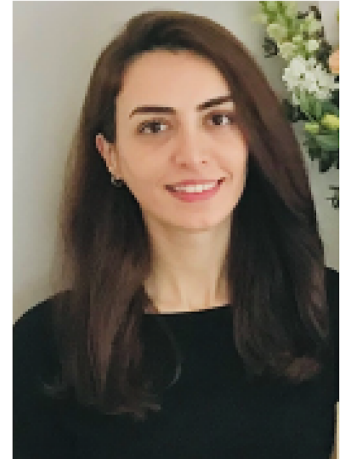
# UoM/Bio21 Node

A/Professor Isabelle Rouiller

*Node Leader and Deputy Director, UoM and Bio21 Node*

## Introducing research fellow Dr Sepideh Valimehr

CCeMMP welcomed Dr. Sepideh, one of our Centre-funded ICPD fellows who will be commencing at the Bio21 Institute in December. Sepideh received her PhD from The University of Melbourne under A/Prof. Isabelle Rouiller's supervision. Her research project was about understanding the molecular mechanism of the AAA+ ATPase p97 using Cryo-EM and biophysical techniques. From this she has discovered her strong interest in using microscopes to look at the proteins. Outside of the lab, Sepideh loves cycling and hiking to enjoy the beauty of nature.



## New UoM members joining CCeMMP

- Dr Sepideh Valimehr

# Wollongong Node

Professor Antoine van Oijen

*Node Leader, University of Wollongong Node*



## Dr Lisanne Spenkelink awarded NHMRC Investigator Grant

Lisanne Spenkelink has recently been awarded \$650,740 as part of her NHMRC Investigator Grant for the rapid evolution of a genome-editing tool to develop new biomolecule to improve medical treatments. Lisanne's research will aim to specifically improve CRISPR-Cas9 technology and developing a new evolutionary tool that helps deliver more accurate results using Biotechnology

## New UoW members joining CCeMMP

- Dr Lisanne Spenkelink

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# Monash Node

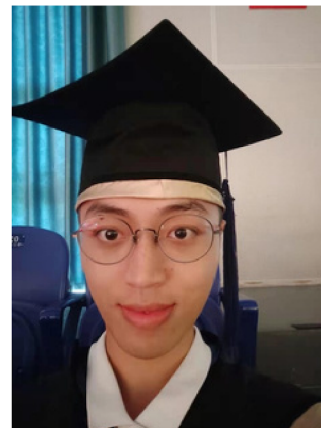
A/Professor Denise Wootten

Node Leader, Monash University Node

## Introducing ICHDR Qinghao Ou!

Qinghao is investigating the structural basis for activation of incretin receptors. The project is a collaboration with our Industry Partner Organisation, Boehringer Ingelheim. Qinghao has always been interested in GPCR signalling and the structural basis behind molecular interactions during GPCR signalling. Outside of research, he likes to cook, go fishing, and play computer games.

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



## Introducing ICHDR Dongju Lee!



Dongju is investigating approaches to determine the structure of an orphan GPCR that has substantial potential to be targeted to treat select CNS disorders. The project is a collaboration with our industry partner, Boehringer Ingelheim. Dongju was attracted to GPCRs, as developing the three-dimensional structures of them is very challenging and are excellent drug targets. Dongju's interests include travelling and learning new languages while travelling, tennis, swimming, baking and making coffee.

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

## Introducing research fellow Dr Nazanin Mohebali!

CCeMMP welcomed Dr. Nazanin Mohebali, a new postdoctoral fellow funded through the Sustaining Innovation Postdoctoral Fellowship scheme of our Partner Organisation, Astex Pharmaceuticals. Nazanin completed her PhD on "structural and functional characterization of TACAN, a novel protein involved in sensing pain" in A/Prof. Isabelle Rouiller's lab at Bio21. In her PhD, Nazanin utilized different membrane mimetic systems such as saposin-lipid nanoparticles, nanodisc, and peptidisc to optimize sample preparation conditions of TACAN for structure determination using cryo-EM.





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## Dr Tracy Josephs awarded NHMRC Investigator Grant

Tracy Josephs has recently been awarded \$650,740 as part of her NHMRC Investigator Grant on A personalised pharmacogenomic approach to inform autosomal dominant hypocalcaemia treatment. Tracey's research is characterising how mutations in autosomal dominant hypocalcemia can change drug behaviour and is employing hydrogen deuterium mass spectrometry to screen novel pharmacophores for favorable characteristics as apart of her drug design platform.

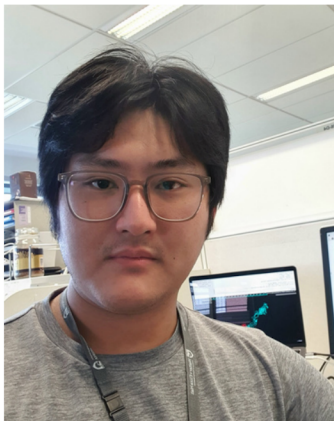


## New Monash members and affiliates joining CCeMMP

- Dr Katie Leach
- Dr Brian Cary
- Dr Yan Jiang
- Dr Tracy Josephs
- Yao Lu
- Dr Gregory Stewart
- Dr Natalie Diepenhorst
- Cameron Fairweather
- Dr Nazanin Mohebal

## WEHI Node

A/Professor Isabelle Lucet  
*Node Leader, WEHI Node*



## Introducing ICHDR Hai-Tian Chen!

Hai-Tian's research project is focused on applying Cryo-EM for understanding the molecular structure and signal transduction of two Ephrin Receptor Pseudokinases, EphA10 and EphB6. Understanding their 3D structures will allow us to understand the conformational dynamics of both pseudokinases at the plasma membrane. The project is a collaboration with our industry partner, Catalyst Therapeutics. Hai-Tian enjoyed the feeling of making little discoveries and inventions which led him to his PhD in cutting edge research.

*Supervisors: A/Prof Isabelle Lucet, Dr Josh Hardy and Dr Onisha Patel*

## Dr Josh Hardy awarded NHMRC Investigator Award

Joshua Hardy has recently been awarded \$650,740 as part of his NHMRC Investigator Grant on harnessing cryo-electron microscopy to study the engineering of microtubule networks in cancer and neurogenesis. Josh's research will look at understanding how cellular proteins control microtubule formation and dissociation so that we can switch them on or off when needed.



# Working with industry

## White paper with Thermo Fisher Scientific: AN0177-EN-09-2021

Deep-learning-based particle picking of detergent-solubilized GPCRs using crYOLO Software.

Authors: Matthew Belousoff, Denise Wootten, Patrick Sexton.


# Outreach activities

## SBGrid Australasian III Mini-Series

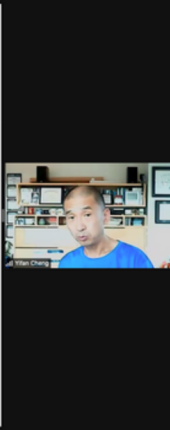
### *Cryo-electron microscopy: From Sample to Structure*

**“Resolution revolution”**

- The “resolution revolution” in single particle cryo-EM changed the landscape of structural biology;
- Structure determination is no longer a major obstacle in understanding biological question;



- Significantly improved resolution towards ~1Å;
- Significantly improved structure determination pace;
- Rapid structure determination pace advanced our understanding of diseases;



SBGrid in partnership with The University of Wollongong (Molecular Horizons), University of Otago and CCeMMP are excited to kick off their third Australasian regional mini-series, this time with focus on cryo-electron microscopy. The series comprises of five lectures and nine speakers this session should appeal to both

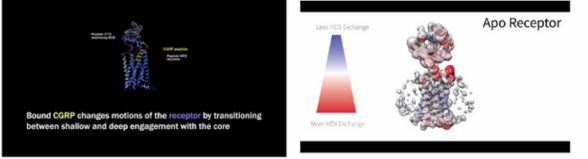
novice and expert structural biologists. Professor Yifan Cheng (University of California, San Francisco) was the keynote speaker for the first seminar on the 27th of October. Our Node members, James Bouwer and Eric Hanssen will be leading the panel discussion on the 24th of November on *Regional CryoEM infrastructure in Australia*.

## CCeMMP PhD Information Night

The Centre held an information night for interested domestic and international students on the 28th of September. There were over 65 students who attended to learn about the Centre Doctoral Training Program and the research at each of the Nodes.

**Examples of recent work from the Monash node**

Understanding GPCR activation and GPCR dynamics using cryo-EM and HDX-MS




Bound CGRP changes motions of the receptor by transitioning between shallow and deep engagement with the core

Less HDX Exchange      Apo Receptor      More HDX Exchange

Liang et al., *Nature* (2018) 561 (7724), 492-497  
Josephs et al., *Science* (2021) 372, 6538

Combining cryo-EM and HDX-MS to give complementary information on receptor dynamics





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# Recent Centre Activities and Achievements

## Conference presentations

[1] RegPep23. 23rd International Symposium on Regulatory Peptides. Hybrid meeting. Acapulco, Mexico. August 15th-19th, 2021.

\*Mechanisms of signaling by peptide-activated G protein-coupled receptors  
Prof. Patrick Sexton

[2] Dr. GPCR Summit 2021. Virtual meeting. September 13th – 19th, 2021.

\*Structure and dynamics of GPCRs: lessons from cryo-EM  
Prof. Patrick Sexton

## Publications

[1] Danev R, **Belousoff MJ**, Liang Y-L, **Zhang X**, **Wootten D**, **Sexton PM**. Routine sub-2.5 Å cryo-EM structure determination of B-family G protein-coupled receptors. *Nat Commun* 12: 4333, 2021. doi: 10.1038/s41467-021-24650-3.

[2] **Cary BP**, **Zhao P**, Truong TT, **Piper SJ**, **Belousoff MJ**, Danev R, **Sexton PM**, **Wootten D**, Gellman SH. Structural and Functional Diversity among Agonist-Bound States of the GLP-1 Receptor. *Nat Chem Biol* (in press, October 1st 2021).

[3] Deganutti G, Liang Y-L, **Zhang X**, Khoshouei M, Clydesdale L, **Belousoff MJ**, Venugopal H, Truong TT, **Glukhova A**, Keller AN, Gregory KJ, **Leach K**, **Christopoulos A**, Danev R, Reynolds CA, **Zhao P**, **Sexton PM**, **Wootten D**. Dynamics of GLP-1R peptide agonist engagement are correlated with kinetics of G protein activation. *Nat Commun* (in press, September 24th 2021)

[4] **Zhang X**, **Belousoff MJ**, Liang Y-L, Danev R, **Sexton PM**, **Wootten D**. Structure and dynamics of semaglutide and taspoglutide bound GLP-1R-Gs complexes. *Cell Reports* 36: 109374, 2021. doi: 10.1016/j.celrep.2021.109374.

[5] **Johnson RM**, **Zhang X**, **Piper SJ**, Nettleton TJ, Vandekolk TH, **Langmead CJ**, Danev R, **Sexton PM**, **Wootten D**. Cryo-EM structure of the dual incretin receptor agonist, peptide-19, in complex with the glucagon-like peptide-1 receptor. *Biochem Biophys Res Commun* 578: 84-90, 2021. doi: 10.1016/j.bbrc.2021.09.016.

[6] Draper-Joyce CJ, Bholra R, Wang J, Bhattarai A, Nguyen ATN, Cowie-Kent I, O'Sullivan K, Chia LY, Venugopal H, Valant C, **Thal DM**, **Wootten D**, Panel N, Carlsson J, Christie MJ, White PJ, Scammells P, May LT, **Sexton PM**, Danev R, Miao Y, **Glukhova A**, Imlach WL, **Christopoulos A**. Structural basis of analgesic action of an adenosine A1 receptor allosteric modulator. *Nature* On-line, September 8th, 2021. doi: 10.1038/s41586-021-03897-2.

[7] Deganutti G, Atanasio S, Rujan R-M, **Sexton PM**, **Wootten D**, Reynolds CA. Exploring ligand binding to calcitonin gene-related peptide receptors. *Front Mol Biosci* 8: 720561, 2021. <https://doi.org/10.3389/fmolb.2021.720561>.

[8] **Drulyte I**, **Zhang X**, **Johnson R**, **Koh A**, **Masiulis S**, **Unger S**, **Pechnikova E**, **Wootten D**, **Sexton P**, **Belousoff M**. Thermo Scientific™ Glacios cryo-TEM: A versatile 200kV tool for structure-based drug discovery. *Microsc Microanal* 27 (Suppl. 1): 3256-3258, 2021. doi:10.1017/S1431927621011223.

## New cryo-EM structures

- Ex4-D-Ala:GLP-1R:Gs:Nb35 (PDB:7S3I; EMD-24825) [2]
- Oyxn:GLP-1R:Gs:Nb35 (PDB:7LLY; EMD-23436) [3]
- Ex4:GLP-1R:Gs:Nb35 (PDB:7LLL; EMD-23425) [3]
- Sem:GLP-1R:Gs:Nb35 (PDB:7KIO; EMD-22882) [4]
- Tasp:GLP-1R:Gs:Nb35 (PDB:7KI1; EMD-22883) [4]
- Peptide-19:GLP-1R:Gs:Nb35 (PDB:7RTB; EMD-24680) [5]
- MIPS521:ADO:A1R:Gi2 (PDB:7LD3; EMD-23280) [6]
- ADO:A1R:Gi2 (PDB:7LD4; EMD-23281) [6]