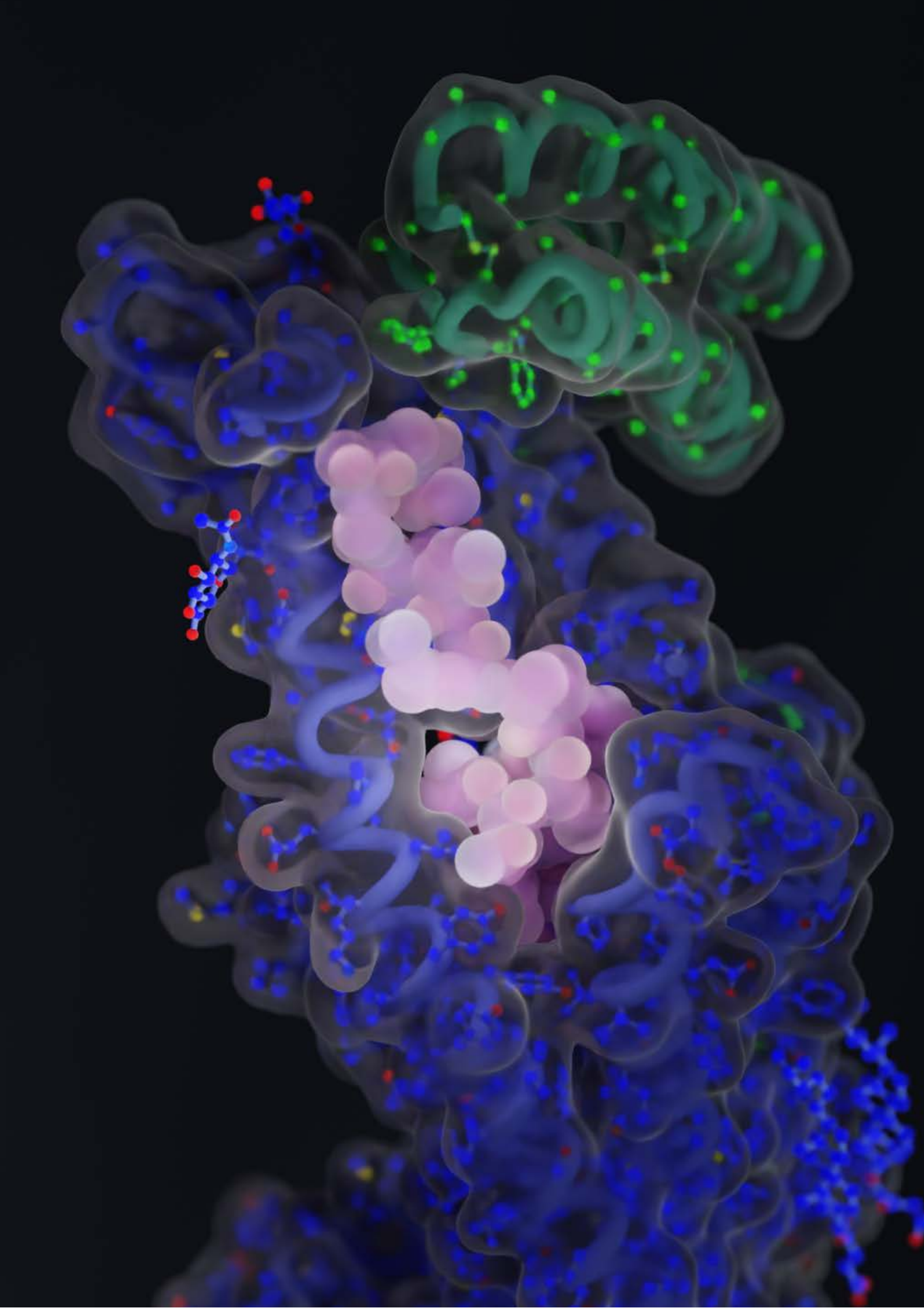
Centre for Cryo-electron Microscopy of Membrane Proteins

May
2022

ANNUAL REPORT

Year 1 (2021/2022)



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Centre Overview

The Centre for Cryo-electron Microscopy of Membrane Proteins (CCeMMP) is funded by the Australian Government through the Australian Research Council (ARC) Industrial Translation Research Program (ITRP). The Centre is an academic-industry partnership supported by the Industrial Transformation Training Centre arm of the scheme. Our core academic partners are Monash University (Administering Institution), The University of Melbourne, the Walter and Eliza Hall Institute of Medical Research (WEHI), and the University of Wollongong. Key goals of the Centre include training industry-ready, world-class graduates in cryo-electron microscopy of membrane proteins, and providing leadership and innovation in the application of cryo-EM to advance industrial expansion in structure-enhanced drug design. Expected outcomes include world-first structural biology knowledge and techniques, and the generation of next-generation cryo-EM researchers with entrepreneurial and technical skills desired by industry.

Vision

The CCeMMP core vision is to provide a world-leading workforce that can advance Australia's biotechnological capability and to build strong linkages with the drug discovery and development industries. Our Centre will train industry-ready, world class graduates in cryo-electron microscopy of membrane proteins. The Centre's graduates and research results will enable tomorrow's industrial expansion in structure-enhanced drug design.

Director's Report

Patrick Sexton, ARC CCeMMP Director

The Centre was approved for funding by the ARC in July 2020 with execution of the Participant's Agreement by all Academic, Industry and Educational partners on March 23rd, 2021 marking the formal establishment of the Centre. In this report I am excited to provide a summary of the first full year of operation through to the end of April 2022, as well as the key pre-establishment work that was vital to our ability to hit the ground running despite the many challenges posed by Covid-19, particularly in 2020 and 2021.

Key to the many highlights discussed below and in other sections of the report was the appointment of Dr. Jackie How as our Centre Manager in September 2020, who has worked tirelessly with all partners and members to operationalise the Centre.

From our original partner organisations, Medicago Inc. withdrew from the Centre due to a change in research direction. However, we have welcomed one new Industry Partner, Boehringer Ingelheim, and one new Educational Partner, LabArchives, to the Centre.

As is expected for a new Centre, much of our earlier activity was focused on finalising our Governance structure and the development and approval of Key Performance Indicators and our Strategic Plan. In parallel with this, a lot of effort, from many individuals, has supported the development and implementation of the bespoke coursework and training that is a feature of our 4-year Doctoral Training Program.

Covid-19, of course, has presented many challenges for the establishment and operation of the Centre, particularly with respect to student and staff recruitment and the implementation of the training program. Nonetheless, we have made great progress towards meeting the key objectives for Year 1, despite deferment of many of the originally planned professional development workshops to 2022.

We have expanded our membership within the Nodes of the Centre, including many new established investigators, early and mid-career researchers and student affiliates, bringing additional breadth to the research of the Centre and to our ability to engage with key stakeholders in industry, academia and the lay community.

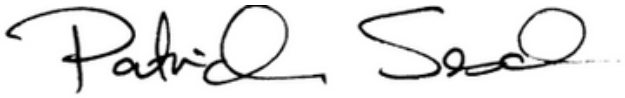
Some key engagement highlights include our monthly seminar series that has attracted an average audience of over 90, with nearly double this number viewing the recording of these presentations on our YouTube channel. We also partnered with SB Grid (Harvard) to deliver 2 webinar series to audiences spanning the globe.

Director's Report (cont.)

We have also implemented quarterly newsletters that provide regular updates on the activities and achievements of the Centre and its members, as well as delivering the latest updates through social media.

On the research front, our Centre members continue to provide leadership in the application of cryo-EM to solution of membrane protein structure with publications in leading journals including Science, Nature, Nat Cell Biol, Nat Commun, Plos Biol and Cell Reports, with 25 new structures available from the PDB and EMDB data bases. Our members have also been active in disseminating their research at conferences, and through presentations to academic and industry institutions. Congratulations also to our members who have been successful in securing national and international competitive grant funding, fellowships and industry funding, and also for the multiple awards that have been bestowed.

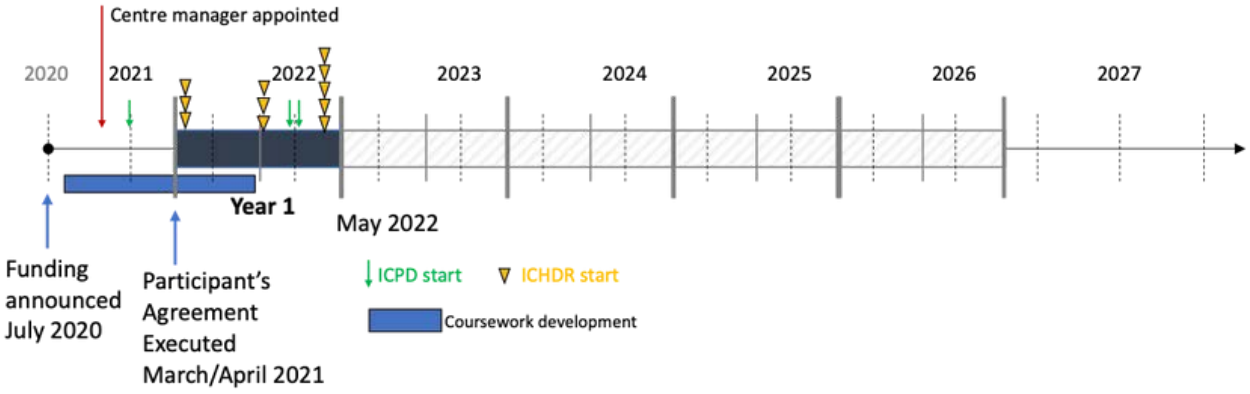
We are excited to be moving into our second year of formal operation and particularly looking forward to the increased opportunities for face-to-face interactions as many of the restrictions imposed by the Covid-19 pandemic are being relaxed.



Patrick Sexton
ARC CCeMMP Director

CCeMMP Snapshot

Centre Operational Timeline



Centre Partners

4 Academic Institutions

11 Industry partners

4 Education partners

Key Data

22 Publications

25 Structures published

$14M New funding leveraged

12 Academic presentations

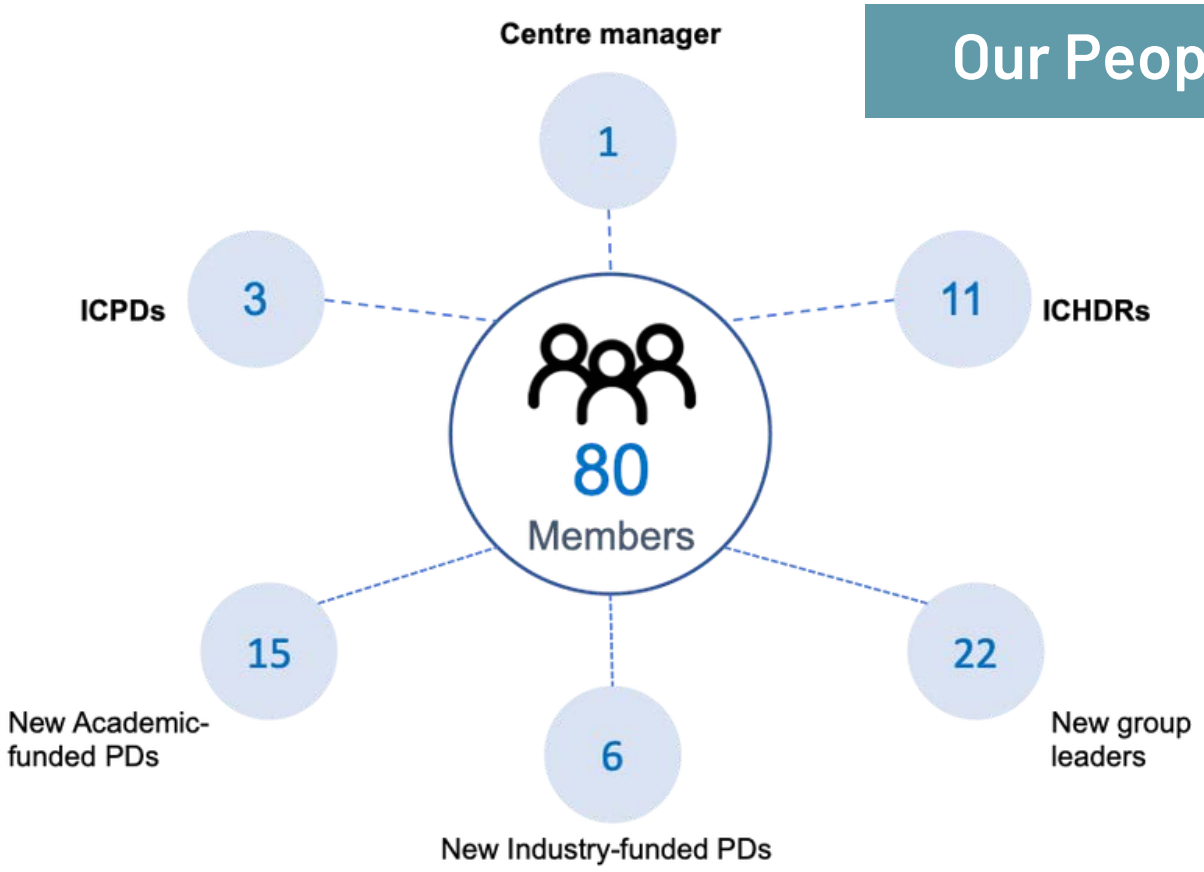
4 Industry presentations

28 Conference presentations

4 Career Fellowships

10 Awards to members

Our People



Education & Training

Education Overview

The Centre recognises the importance of providing opportunities for our members and students to grow both in their research capabilities and their professional development outside of research in entrepreneurship, innovation, commercialisation and business development. We will continue to identify and source professional opportunities for our members and students to allow steady growth and enrichment to their development and training. As part of this, the Centre will be running a professional development week called "CCeMMP EduWeek" once a year where all members are encouraged to participate in the educational activities. Some activities will be open to the wider community. These activities will change every year and feedback will be gathered from members and attendees to ensure that we are providing the best training and workshops as possible.

New Educational Partner

The Centre also expanded their educational partners to include LabArchives. The inclusion of LabArchives to the Centre is to provide a platform for members and students to access for training materials and research data management. As part of our commitment to ensure the Centre is managing the research data to meet the Australian Code for Responsible Conduct of Research, we work closely with LabArchives to provide training and support to the Centre members and students to encourage and consolidate data management skills.

labarchives
Research Notebook

Doctoral Training Program

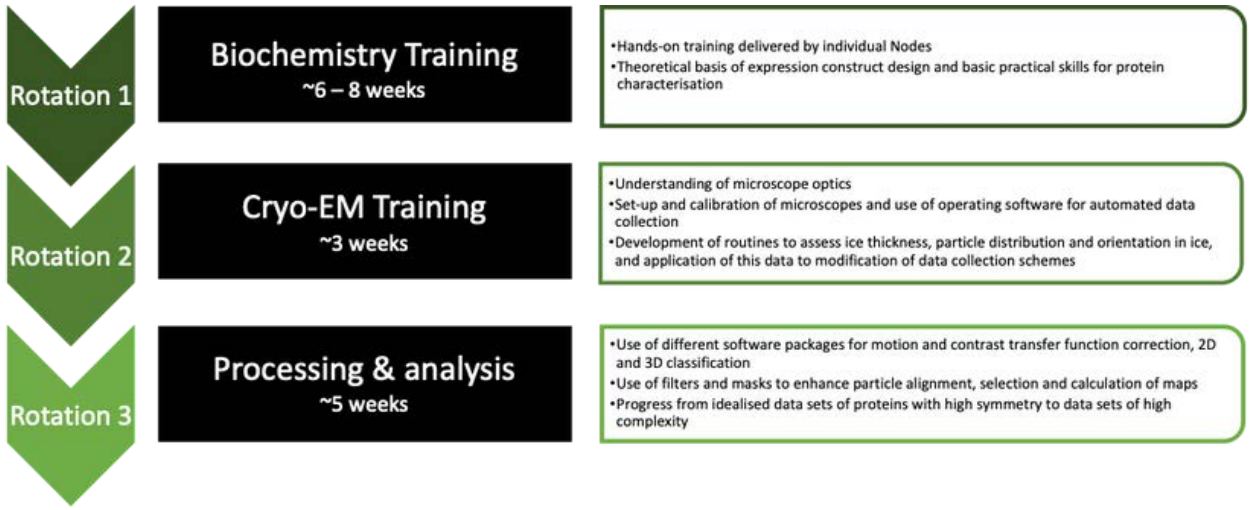
The Centre has developed a unique 4-year Doctoral Training Program including practical rotations through the key technical areas of competency required for application of cryo-EM to determination of membrane protein structure. This program is aligned with the core aims of CCeMMP and the Australian Research Council ITTC scheme: 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.

Key Features of the CCeMMP Doctoral Training Program

- 4-year PhD Program
- 3 technical rotations delivered by Industry, ICPDs and Node members
- Approximately 5 months delivered in Year 1
- Coursework Unit “The Process of Drug Discovery” delivered in Year 2
- 3-12 months embedded placement with Industry Partner in Year 3
- Up to 9 months of additional Industry-linked experiential training
- Participation in leadership opportunities
- Seminar committee organisation and chairing
- Organisation of scientific symposiums
- Outreach and public engagement

Students also undertake professional development training and 'masterclasses' to develop skills outside of research activities. These skills will provide them with foundational understanding to engage with industry partners on drug discovery, including understanding of commercialisation and entrepreneurship. These activities will primarily be conducted for a week every year under the banner of “CCeMMP EduWeek”, but will be supplemented by ad hoc workshops.

Technical Training Rotations



Biochemistry Rotation

This training focuses on the protein biochemistry of membrane proteins, including expression, solubilisation and purification of stabile protein complexes and their reconstitution in detergent micelles or nanodiscs.

Cryo-EM & Imaging Rotation

This training focuses on cryo-EM imaging of exemplar membrane proteins using 120 kV, 200 kV and 300 kV instruments. ICHDRs are 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 & Analysis

This rotation focuses on the principles and practice of cryo-EM data processing, modelling and analysis. ICHDRs are introduced to 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.

Our ICHDRs

Maddison Steele

Maddie is a current PhD candidate based at the Wollongong node of the CCeMMP. 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".

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 they are excellent drug targets. Dongju's interests include travelling and learning new languages while travelling, tennis, swimming, baking and making coffee.

Riya Joseph

Riya is a Biology BS-MS graduate from the Indian Institute of Science Education and Research, Mohali, India. Her Master's thesis involved purifying a cholesterol-dependent cytolysin (CDC) and studying the role of cholesterol in its pore formation. Riya's PhD project is focused on a novel family of pore-forming toxins consisting of nearly 300 uncharacterised proteins that are related to the CDCs. The project aims to investigate the structure and function of these proteins by cryo-EM, crystallography and other biophysical/biochemical approaches. Riya is also interested in music and loves visiting mountains.

Qinghau 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.

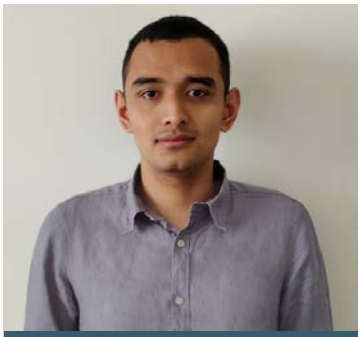
Education & Training

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.

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 longstanding fascinations with the Humanities: history, literature, linguistics, and art.

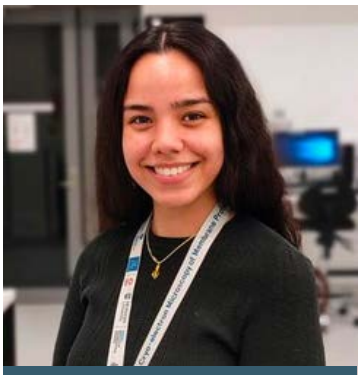


Alok Pradhan

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 completed 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.

Minakshi Baruah

Minakshi is working on developing cryo-EM approaches that will aid in the structure determination of inactive and antagonist bound GPCRs at Monash University Node. Her research project could open up new applications of cryo-EM for GPCR drug discovery and development. Apart from playing around with GPCRs, Minakshi loves to travel and try different cuisines. She also enjoys outdoor gaming and cooking is one of her passions.



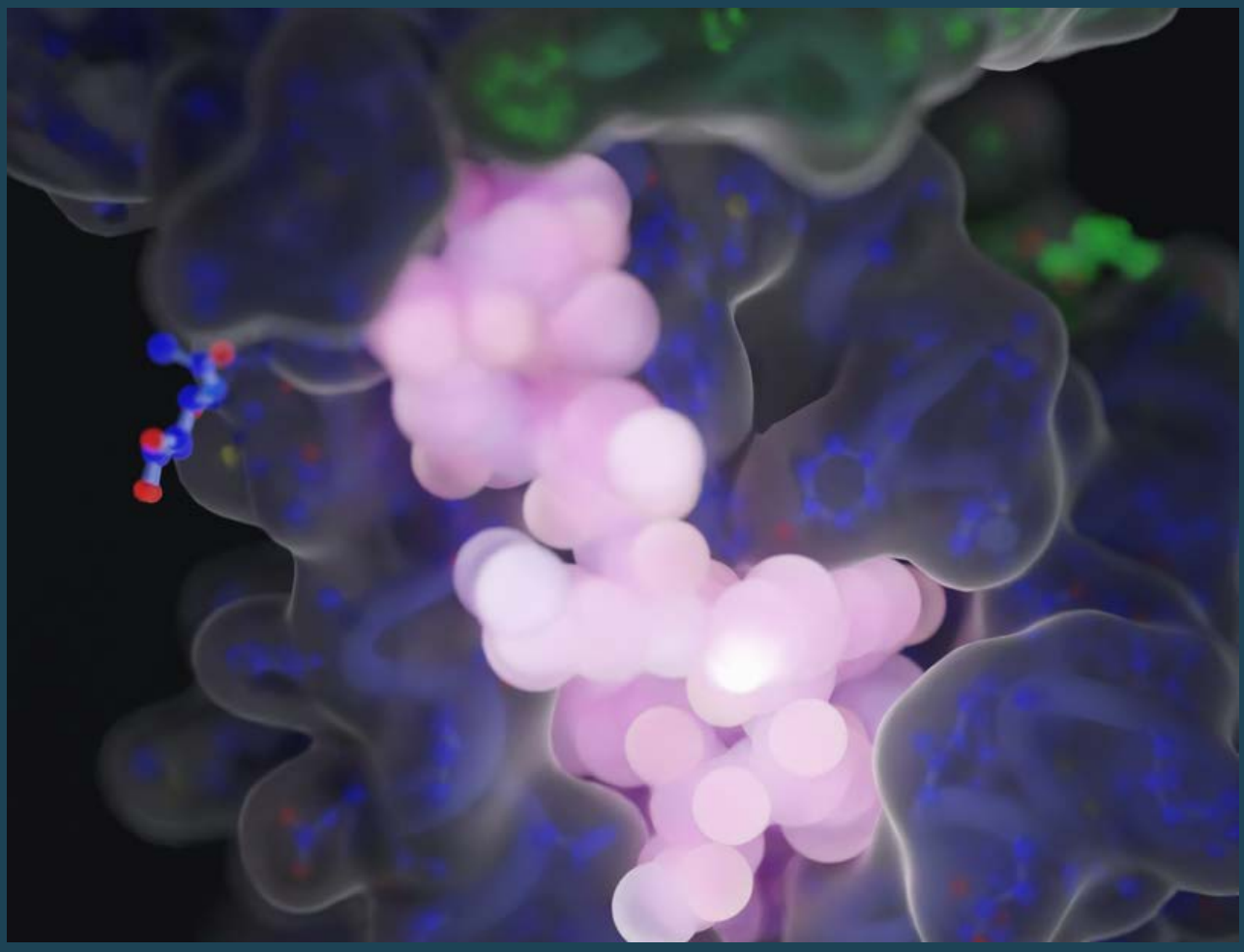
MariaKatarina Lambourne

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 during her Honours year 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.

Maria Elena 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. 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.

Research



Research Overview

We undertake academic and academic-industry partnered research to advance cryo-EM and its application to study of membrane proteins, implemented across key membrane protein targets, to deliver on key objectives of the Centre aligned to both the broader ARC Linkage Program objectives, and the core objective of the Industrial Transformation Training Centre scheme.

Research Programs

Innovative research for the successful implementation of cryo-EM in membrane protein structure-based drug design

Theme 1: Technological Advancements (all nodes)

- Application of 3D variability analysis (3DVA) to understanding of membrane protein conformational dynamics and mechanism of drug action. ARC CCeMMP researchers were the first to apply this analysis to understanding of GPCR function and this has become a routine part of the analysis of membrane protein function (1,3,5,7,8,19).
- Integrated cryo-EM and HDX-MS for understanding of conformational transitions1.
- Systematic optimisation of vitrification and imaging of detergent solubilised GPCRs (4).
- Establishment and evaluation of GPCR complex stabilisation technologies that do not require patented technologies (2).
- Evaluation of 200kV imaging with the latest direct electron detectors (e.g. Glacios-Falcon 4) and demonstration that resolutions supporting drug discovery, including key structural waters, can be achieved on optimised samples for GPCRs (2,14). This decreases the cost and increases the breadth of access of cryo-EM for drug discovery programs. Partnered with Thermo Fisher Scientific.
- General application of cryo-EM to dynamic protein structures (22).
- Partnership between WEHI's New Medicines and Advanced Technologies theme and the Monash Industry Team Initiative (MITI) to build new cryo-EM sample preparation devices.

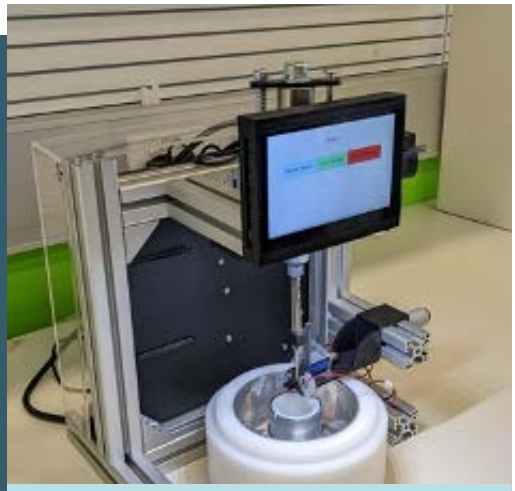
Theme 1: Research Highlight

Construction and Testing of a Cost-Effective Plunge Freezer for Cryo-EM Sample Preparation

Project supervisors: Dr Andrew Leis, cryo-EM facility manager, WEHI and Dr Shabih Shakeel, Lab Head, Division of Structural Biology, WEHI.

WEHI's New Medicines and Advanced Technologies theme funded a project to build a cost-effective plunge freezing device coupled with a temperature-controlled cryostat for cryo-EM sample preparation. Through the Monash Industry Team Initiative (MITI) program, two engineering students from Monash University, Lucile Naegele and Wes Flavell were recruited, who built a plunge freezer device and a cryostat based on published plans (Rubinstein et al., Acta Cryst. D75, 1063–1070, 2019 and Russo et al., Rev Sci Instrum 87:114302, 2016). The plunge freezer device was built at the cost of about $1700 (excluding salaries), and the cryostat at about $1500 (excluding salaries). A commercial plunger freezer can cost anywhere between ~$90k (FEI Vitrobot) to ~$900k (SPT Labtech Chameleon). We built two of these devices, one for WEHI and the other for cryoEM facilities around Australia, including Bio21.

In the plunge freezer design, an inexpensive, piezo-driven ultrasonic device creates an aerosol from a droplet of protein solution. Unlike conventional blotting techniques, the near-simultaneous 'spray and plunge' principle operates on the millisecond timescale and therefore has the potential to solve longstanding issues with the preferred orientation of proteins at the air-water interface. Several new improvements were made to enhance the robustness and portability of the design, as well as the user-friendly nature of the touchscreen interface.



WEHI's cryo-plunger

We used the device to solve the structure of the protein kinase PINK1, and we now plan to test the device on other proteins and protein crystals intended for analysis by micro-electron diffraction. We have also made adjustments to the cryostat design, including a simpler and cheaper circuit design that makes the whole device more compact, thus, improving its portability.

Overall, we have built a robust, modular, cost-effective solution for our cryoEM sample preparation needs. There is scope to improve the design further to incorporate several novel features such as time-resolved modality.

Theme 2: Cell Surface Receptors (G protein-coupled receptors) (Monash, WEHI and UoW nodes)

Over 800 genes encoding GPCRs are present in the human genome, with splice variants, differential post-translational processing and formation of heteromeric complexes between distinct GPCRs, as well as between GPCRs and other modulatory proteins (e.g. receptor activity-modifying proteins; RAMPs), greatly amplifying the repertoire of GPCR phenotypes. Thus, GPCRs are the largest and most important class of cell surface membrane proteins, and they are involved in virtually all physiological processes. GPCRs are naturally dynamic proteins that function to allosterically communicate signals from the extracellular surface to changes in intracellular function. Not surprisingly, these membrane proteins are one of the largest target classes for drug discovery and development. Understanding the molecular interactions that govern ligand affinity and regulation of protein function is a key requisite of structure-assisted drug discovery and development.

GPCRs transduce signals via different families of heterotrimeric G proteins, and other regulatory/scaffold proteins, exemplified by arrestins. The diversity in transducer engagement has led to a class of ligands termed biased agonists that activate different spectrums of signaling from the same receptor. Understanding the molecular basis for drug efficacy and biased agonism is also key for optimal development of therapeutics.

The ability to capture high resolution structure of GPCRs in different states, in complex with different transducer and other regulatory proteins, and to ligands of different pharmacology is an important challenge for structural biology and is a key focus of much of the research within the Centre.

Subproject 1: Understanding Receptor Activation and Transducer Coupling

We have extended the spectrum of GPCR states amenable to structural resolution in study of the CGRPR to provide understanding of the transition between apo receptor, ligand-bound receptor and ligand- and transducer-bound receptor, as well as integrating cryo-EM and HDX-MS to probe dynamics of different states (1).

We solved peptide liganded complexes of therapeutically important amylin and calcitonin receptors and revealed that distinct template peptides utilise distinct activation mechanisms, gaining insight from high-resolution cryo-EM structure and conformational dynamics of individual complexes (19). This work, along with pharmacological characterisation of different peptides acting at this receptor family (17), will form the basis for a collaborative project with Novo Nordisk.

We provided the first structures of a receptor (CCK1R) bound to Gs and Gq proteins providing insight into G protein selectivity across different receptor classes and modulation of ligand affinity and G protein selectivity (3), as well as the identification of a novel allosteric modulator of the CCK1R (16). This work is also contributing to the collaborative project with Astex Pharmaceuticals described below.

Subproject 2: Understanding the Molecular Basis for Biased Agonism

We are extending investigation into the molecular basis for biased agonism at the incretin peptide receptor family that has provided new insight into key atomic interactions and also the distinct dynamics of receptors with ligands of distinct pharmacology (5,6,7,8,11). We have projects in this space that are being developed collaboratively with Boehringer Ingelheim, and we are currently progressing discussions for a project with Servier.

Subproject 3: Allosteric and Bitopic Ligand Regulation of GPCRs

We have been pursuing pharmacological and structural studies with a focus on muscarinic and adenosine receptors (9,12,13,15,18). The work on the A1 adenosine receptor published in Nature revealed an unexpected site for allosteric ligand binding with the structural work linked to in vitro and in vivo pharmacological validation of the potential for targeting this site. A separate component of this research area is investigation of allosteric regulation of GPCRs through dimerization. A project in this latter space is being developed with Dimerix.

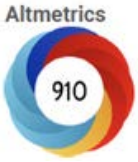
Subproject 4: Development of Biochemical Approaches for Determination of Inhibitor-bound GPCR Structures

The small size of apo and inactive GPCRs has made these particularly challenging for cryo-EM. We are currently exploring different methods, both biochemical and in cryo-EM data processing, to advance this area of research. We have collaborative projects in this space with Pfizer, Astex and Boehringer Ingelheim.

Subproject 5: Development of Approaches for Determination of Orphan GPCR Structures

Orphan GPCRs lack known natural ligands but in many cases have been implicated in disease. Generating structures of orphan receptors can potentially open up opportunities for structure-based design. We currently have collaborative projects with AstraZeneca and Boehringer Ingelheim in this research area.

Theme 2: Research Highlights



Structure and Dynamics of CGRP Receptor in Apo and Peptide-bound Forms

Josephs TM, et al. Science 372:eabf7258, 2021. doi:10.1126/ science.abf7258


Tracy Josephs

Researchers from the Monash University Node have harnessed cutting-edge technology to discover the progression of molecular events that lead to migraine – something that, until now, has remained a mystery. The discovery has filled one of the most important gaps in our understanding of how migraines are activated. Published in the prestigious journal Science, the breakthrough study was led by a team of researchers from the Monash Institute of Pharmaceutical Sciences (MIPS) and the recently established ARC Centre for Cryo-EM of Membrane Proteins (CCeMMP). One of the most common causes for migraine is abnormal levels of activation of the target for an extremely potent vascular regulator, calcitonin gene-related peptide (CGRP).

The newest and most exciting treatments for migraine act by blocking this activity, but how CGRP activates its receptor at the molecular level has been poorly understood. In this study, the researchers applied cryo-electron microscopy (cryo-EM) to, for the first time, show how the binding of CGRP to the receptor leads to receptor activation that, in turn, leads to the onset of migraine. Until now this simply wasn't possible as proteins such as the CGRP receptor were too small and too mobile to be captured and studied by any method.

Lead author of the study, ARC CCeMMP member Dr Tracy Josephs from MIPS said: "To really understand what triggers migraines, we need to be able to study structure and dynamics using unmodified forms of the receptor - this has been a major technical hurdle in understanding the progression of molecular events that link CGRP binding to activation of the cellular signalling pathways that govern migraine pain, and one the team have now overcome." "Based on the structures and data from complementary biophysical techniques, we showed that initial binding of CGRP to the receptor caused unexpectedly small conformational changes in the most prevalent form of the receptor. It was the coordinated change in dynamics of the external (CGRP binding) face of the receptor and the intracellular face that was the key, and visualising this would not have been possible by other methods."

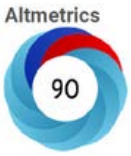
Migraines affect approximately five million Australians. It is a neurological condition that can cause multiple, debilitating symptoms including intense headaches, nausea, vomiting, difficulty speaking, numbness or tingling and sensitivity to light and sound.

Professor Patrick Sexton, co-lead on the study and Director of the ARC CCeMMP said: "This is an example of the enormous benefits of fundamental basic research in addressing major unmet medical needs".

The team from MIPS and ARC CCeMMP worked with collaborators from the University of Tokyo, the University of Otago and the Hudson Institute of Medical Research.

The data underpinning this work was also used in the "CCeMMP EMPIAR data processing challenge" (https://ccemmp.org/news/empiar-challenge/) that was run as an international outreach activity.

Theme 2: Research Highlight



A Structural Basis for Amylin Receptor Phenotype

Cao J, et al. Science 375:eabm9609, 2022 doi:10.1126/science.abm9609.

Jason Cao

Australian researchers have announced a discovery which ultimately could play a major role in reducing obesity. In a report published in the leading international research journal, Science, scientists from the Monash Institute of Pharmaceutical Sciences (MIPS) and the ARC Center for Cryo-EM of Membrane Proteins (CCeMMP), affiliated with Monash University, have pinpointed how a promising group of anti-obesity drugs known as DACRAs (dual amylin and calcitonin receptor agonists) activate various receptors in the body. Previously the activation process had not been fully understood, which limited the clinical advancement of this class of weight-loss drug. "Our work opens up opportunities for the design and development of more effective DACRA therapies that could be used to better treat the spectrum of overweight and obese patients, and those with related metabolic disease," said Monash University Professor of Drug Discovery Biology and ARC CCeMMP director Patrick Sexton

MIPS structural biologist doctoral candidate Jason Cao said the breakthrough was made by using cutting-edge technology called cryo-electron microscopy (cryo-EM). "To really understand how these drugs may work, we needed to visualize molecular level details of how the different types of template peptides were bound to each of the four different target receptors," he said. Cao said cryo-EM enabled the scientists to "capture molecular details of the interactions that drive activity" along with "information on the dynamics of the protein that are critical to selectivity and function."

The researchers said they were surprised by their findings because it had been assumed the "molecular architecture of the amylin receptors would dictate how the peptides worked." Instead, they saw "dramatic differences in the conformations and dynamics of the individual receptors when bound to different peptides."

The quest for such discoveries has become ever more urgent, with World Health Organization estimating that by 2025, approximately 167 million people -- adults and children -- will become less healthy because they are overweight or obese. “The team has revealed structural details on how calcitonin and amylin receptors are activated” said Professor Wootten, co-lead on the study and leader of the Monash node of the ARC CCeMMP. “This work will support the development of next generation DACRA drugs as weapons to address the rising obesity epidemic”.
The team from MIPS and ARC CCeMMP worked with collaborators from the University of Tokyo, and the University of Otago.

Theme 3: Other Membrane Proteins (WEHI, UoM and UoW nodes)

Subproject 1: Applying Cryo-EM to Understand Receptor Tyrosine Kinase Structure and Functions and Developing Drug Discovery Opportunities

Ephrin Receptor Tyrosine kinases collectively governs cell-cell recognition in the nervous, vascular, immune, and skeletal systems. Their dysregulation is strongly linked to the onset and progression of cancer. However, the development of therapeutics against Eph receptors remains at a relatively early stage due to a poor understanding of their structure and regulation. We are exploring the capability of cryo-EM to generate structures of this emerging class of drug targets. We have developed a cryo-EM research program on two members of Ephrin Receptor family, EphA10 and EphB6, leveraging on their unique features that can be exploited for drug discovery. Catalyst Therapeutics is an Industry partner for this project subtheme.

Subproject 2: Applying Cryo-EM to Receptor-ligand Interactions and Mechanisms of Signal Transmission in Wnt Signalling Pathways.

We have expanded our initial program on signalling receptors to include the study of Frizzled receptors (FZD), a class F GPCRs (overlapping with Theme 2), and their co-receptors ligands LRP5/6. The activation of FZD by Wnt proteins triggers a complex network of downstream signalling cascades. Wnt signalling is important in embryonic development and the maintenance of healthy tissue homeostasis in adults. Because of this, different members of Wnt signalling cascades are recognised as promising targets for the treatment of different human cancers.

Subproject 3: Cryo-EM on Membrane Proteins Involved in Chronic Pain

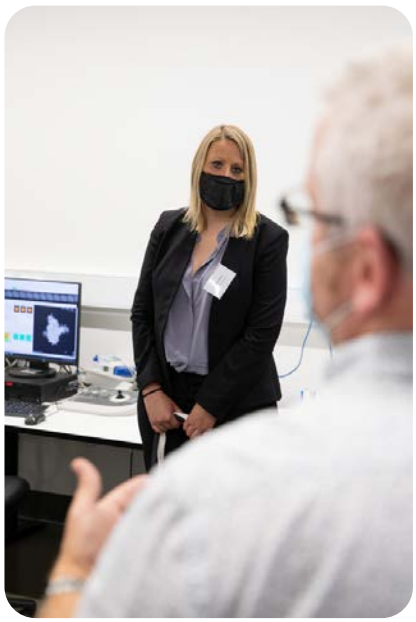
Although several GPCR structures have been determined, the heterodimeric nature of the GABABR has hindered efforts for its structural elucidation. Cryo-EM is ideally suited to circumvent hurdles associated with recombinant protein production and heteromeric arrangement. Research around the investigation and development of cryo-EM techniques to enable structural resolution of the interactions. Knowledge obtained by the structural work will be used iteratively to computationally model and design novel compounds. We are currently in the process of producing GABABR protein constructs, characterising them using biophysical approaches, and acquiring initial cryo-EM data for structure determination.

Additional work is ongoing in characterisation of other membranes, including viral spike proteins, host cell receptors that mediate viral entry, amyloid proteins in Alzheimer's disease, pore-forming toxins and ion channels, however, these are early phase projects within the Centre at this stage.

Joint Research Projects with Industry Partners

Many of our projects include collaborative Industry-partnered research, one of the key objectives of the Centre, the ARC ITTC scheme and the broader ARC Linkage program umbrella. Examples of established collaborative projects are noted below.
Thermo Fisher Scientific is a broad partner within the Centre for technical innovation for membrane protein cryo-EM (2,14).
Other current (or in development) industry-partnered research projects:

- Astex Pharmaceuticals: Systematic investigation of factors driving resolution of receptors and low affinity ligand interactions.
- AstraZeneca: Methods for orphan receptor structure determination.
- Boehringer Ingelheim: Investigation of molecular mechanisms for biased agonism at incretin receptors. Structure determination of orphan GPCRs.
- Catalyst Therapeutics: Developing small molecules/ligands to interrogate Ephrin Receptor Pseudokinase family conformational dynamics.
- Dimerix: Structure determination of GPCR oligomers.



Engagement

Industry Engagement

The Centre is strongly linked to drug discovery and technology industries and continues to work closely with industry to advance the science of membrane protein cryo-EM. The Centre has strong collaborations with local and international collaborators and companies and continues to build on relationships with current, and connect with new, partners.

Boehringer Ingelheim

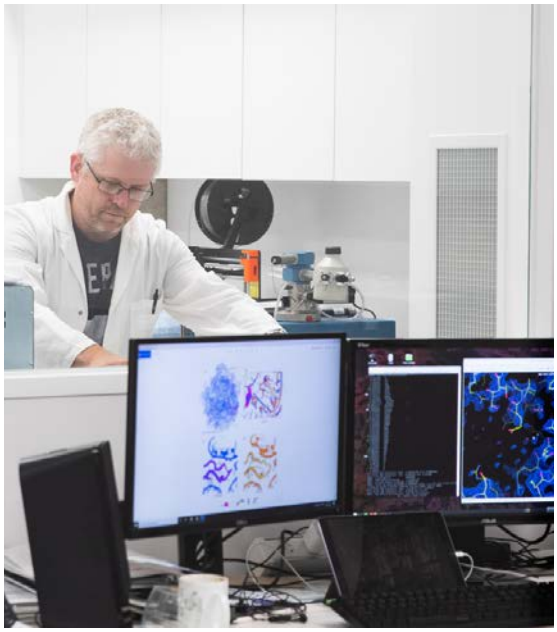
New Partner

In 2021, the Centre expanded its industry partners to include Boehringer Ingelheim. Boehringer Ingelheim is a major pharmaceutical company headquartered in Ingelheim, Germany. BI will be working closely with the Monash Node on two project areas, supporting postdocs and two students within the Centre. The inclusion of BI will expand our strategic and research activities and impact.

Working with Industry

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

ThermoFisher SCIENTIFIC
The world leader in serving science



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. The work, recently published in the journal Structure2, opens the way for broader use of cryo-EM for membrane protein drug discovery with routine structure determination that integrates 200kV and 300kV imaging.

Presentations to Industry

Part of the Centre's commitment is to engage with the community in industry to bridge industry and academia to understand where the knowledge gaps are and how to address them.

Technical Workshop: Dr. Matthew Belousoff

Model fitting to cryo-EM maps using molecular dynamics (MD). Sanofi Aventis (Germany), January 2021.

Research Seminar: Prof. Patrick Sexton

Using cryo-electron microscopy to probe G protein-coupled receptor function. Pfizer (USA), May 2021.

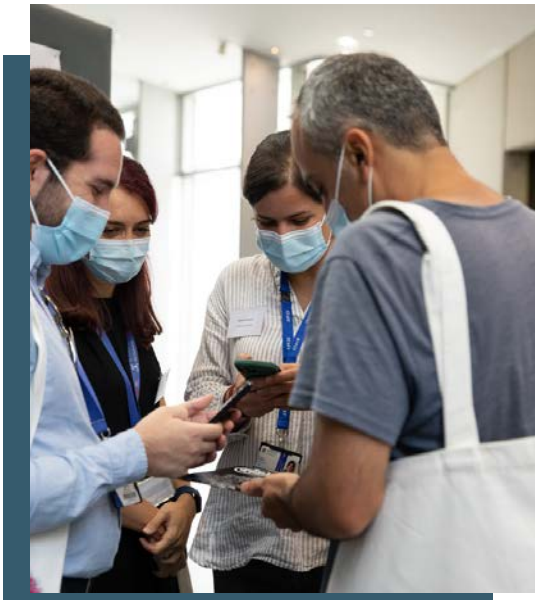
Invited Speaker: Dr. Natalie Diepenhorst

ACvA Industry Collaborations with Academia panel

Workshop: Prof. Michael Parker

Structural biology for COVID-19 drug discovery
CSIRO Infection Disease Resilience Mission - therapeutics for COVID-19 workshop, March 2021.

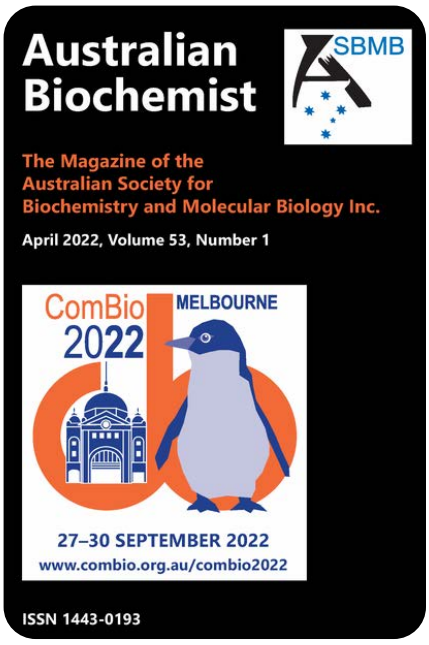
Media Engagement



The Centre will continue to prioritise and build on media engagement through both traditional and social media. The Public Engagement and Outreach Subcommittee is newly formed and will take charge in ensuring that we have clear communication channels with our members and partners for updates on our research and other activities to the wider community, to ensure awareness and allow increased engagement with Centre research.

CCeMMP Members in the News

- 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
- Dr. Simon Brown - https://www.panasas.com/press/panasas-university-of-wollongong-establish-partnership-to-support-cryo-em-research/ (July 2021)
- Dr. David Thal - https://www.monash.edu/news/articles/monash-university-researchers-unlock-the-key-that-could-lead-to-the-development-of-non-opioid-painkillers-to-treat-chronic-pain (Sept 2021)
- Dr. Isabelle Rouiller - https://research.unimelb.edu.au/research-updates/new-microscopy-technique-to-transform-field-of-structural-biology



Engagement

Digital & Social Media

Twitter

331,783
tweet impressions

424
followers

112
tweets

21,699
profile visits

ccemmp.org

7,398
website visitors

27,013
website hits

Newsletter

501
reach

5
newsletters

LinkedIn

418
post impressions

155
connections

Eventbrite

80
subscribers

Centre Newsletters

The Centre releases monthly newsletters to engage and update the public and scientific community on the activities and achievements of the Centre. These activities include promotions, new recruits and members, funding and awards, publications and engagements with current and new partners.

Community & Outreach

External facing events

1 Symposiums
6 CCeMMP seminars*^
12 SBGrid seminars [2 webinar series]
3 Centre info sessions
2 Professional workshops

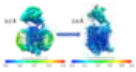
Internal events

1 Centre launch
1 Joint ITTC Ethics workshop [2 separate sessions]

90* Average seminar attendance
180^ Average YouTube views/seminar

4 Newsletters

1 External competition
EMPIAR data processing challenge



CCeMMP Seminar Series

In 2021, the Centre established a seminar series centred around the advancement of research in the cryo-EM and membranes proteins field. Speakers are selected by the CCeMMP Seminar Subcommittee with feedback from members. Seminars are scheduled monthly and are open to all of the scientific and public community to increase awareness of what the researchers in the field are doing. To date we have had 6 speakers:

Professor Patrick Sexton, Monash University

Associate Professor Michelle Dunstone, Monash University

Professor Radostin Danev, The University of Toyko

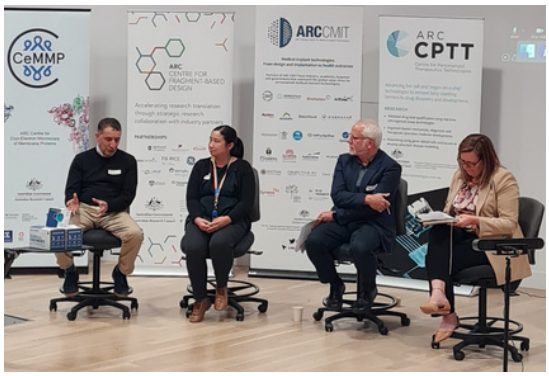
Assistant Professor Oliver Clarke, Columbia University

Dr Doreen Matthies, Eunice Kennedy Shriver National Institute

Assistant Professor Christopher Barnes, Stanford University

Joint Centre Ethics Workshops

Five ARC Industrial Transformation Training Centres jointly held two ethics sessions around the theme "What does it mean to be an ethical researcher?". The sessions aimed to explore the different aspects of what it means to be an ethical researcher and the inherent responsibilities that researchers have to themselves and the diverse stakeholders across academia, industry, funders and the general public.



In December 2021, the first session was held as a webinar comprised a panel of the five Centre Directors (a "Directors Forum"), moderated by Prof. Megan Munsie, who is a researcher who has specialised in ethics across her career. The panel was an opportunity for all Centre members to hear from their Directors, specifically their thoughts, expectations, and experiences with research ethics. The panel's purpose was to also generate topics of interest and contention that would shape the conversation of the following workshop with the students and postdocs from the Centres. In April 2022, the second session was held as an in-person workshop where member and students across the five Centres listened to an expert panel regarding case studies and scenarios that presented complex ethical issues. After the panel, the Centre students and members were split into small groups moderated by mentors to discuss pre-prepared case studies and to encourage them to identify ethical issues and to provide potential solutions.

A joint session between five Centres encouraged cross-discipline networking and to engage in topics outside of their research areas.

Supporting Equity, Diversity & Inclusion

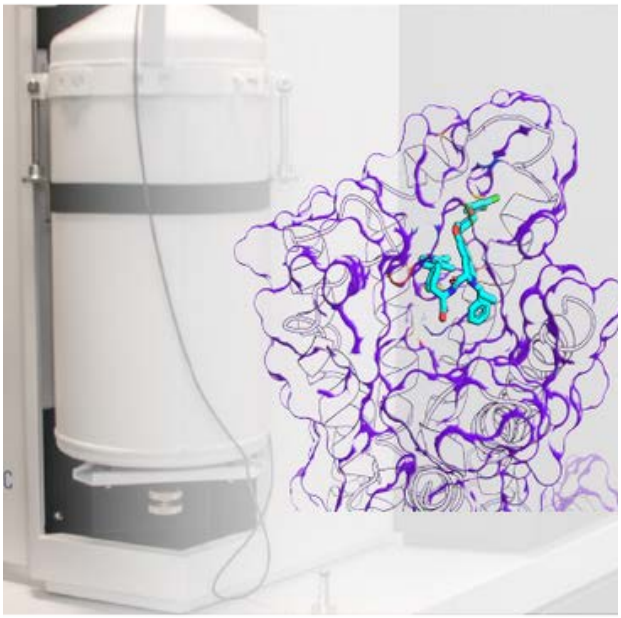
The ARC Centre for Cryo-electron Microscopy of Membrane Proteins (CCeMMP) is committed to supporting and advocating the advancement of gender, diversity and inclusion. We value a diverse environment and aim to foster a culture in which staff and students of all genders, cultural backgrounds, faiths and heritage participate equally. As part of our ongoing commitment, we have established an Equity, Diversity and Inclusion policy and will actively expand our activities and initiatives. We actively seek opportunities in which we can encourage, engage and educate our members and senior management to be conscious of strategies to recognise inequities and how to build on a culture of inclusivity.

Some of these opportunities include:

- Supporting the full cost of members to attend the Australian Society for Medical Research (ASMR) Professional Development Webinar for Gender Equity; attended by 6 members.
- Upcoming workshop "Leading in an Age of Inclusion" has been organised for members in June 2022 that is compulsory for all members to attend.

Introduction of CCeMMP to UoW, UoM and WEHI

The CCeMMP Director, Prof. Patrick Sexton presented an introduction to the Centre to the researchers and students at Molecular Horizons and Illawarra Health and Medical Research Institute (IHMRI). 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.



SBGrid Seminar Series

The Centre was involved in organising 2 of the SBGrid seminar series in partnership with The University of Wollongong (Molecular Horizons), University of Otago and Harvard University.

CCeMMP PhD Information Night

The Centre held an information night for interested domestic and international students on the 28th of September 2021. There were over 65 students who attended to learn about the Centre Doctoral Training Program and the research at each of the Nodes.

Centre Events

Centre Launch

After multiple deferments due to the Covid-19 pandemic, the Centre was formally launched in February 2022, almost a year after execution of the Participant's Agreement and commencement of operations. The members, partners and senior staff from the academic institutions, attending in-person and virtually, celebrated this tremendous milestone. The launch proceedings were 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 launch was held at the Bio21 Institute of the University of Melbourne Node and was followed a tour of cryo-EM facilities housed at the Ian Holmes Imaging Centre.

Centre Student Research Presentations

Our Centre students delivered presentations of their research to date in February 2022 to Centre members and industry partners, chaired by the Coursework Committee Chair and Monash Node Leader, Prof. Denise Wootten. In this session, students were able to demonstrate their understanding of, and progress in, their research projects and invite feedback from both partners and Centre members.

CCeMMP Virtual Symposium

The Centre held its first public research symposium in February 2022. The event was held free of charge and was run virtually due to ongoing Covid restrictions, with over 220 attendees. The symposium showcased some of the leading research, key findings and collaborations of members from the four Nodes. Dr. Maria Flocco from our industry partner, AstraZeneca, was the keynote speaker and shared insights into the field from an industry perspective. 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. Maria Flocco

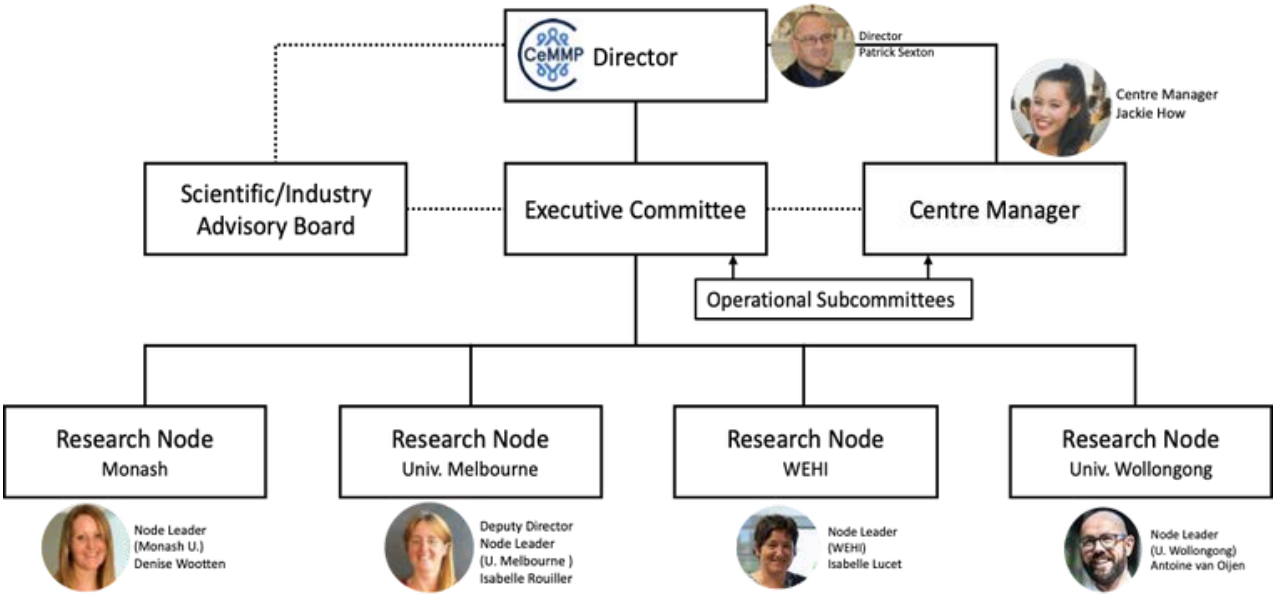
Governance

Committees & Boards

The senior governance scheme for the centre is outlined below. Strategic direction and planning are overseen by the Executive Committee that is supported by the Centre Manager and Operational Subcommittees. The Subcommittees are responsible for implementation of strategy and report back through the Executive. A Scientific and Industry Advisory Board has been established to review and provide advice on the strategic direction and performance of the Centre and to support the Centre to achieve its purpose.

Executive Committee

The Executive Committee (ExC) provides support to the Centre Director in the establishment of the Centre, and for the day-to-day operations of the Centre through policy development, risk management, the review and monitoring of progress and performance of the CCeMMP. The Terms of Reference for this Committee are included in the CCeMMP Governance Plan.



Science & Industry Advisory Committee

The Science and Industry Advisory Committee (SIAC) supports the Centre through regular review of its planning and operation. SIAC provides advice on the strategic direction and performance of the CCeMMP and provides support to the Centre to achieve its purpose. The Terms of Reference for this Committee are included in the CCeMMP Governance Plan.

Prof. Alastair Stewart (Univ. Melbourne) [Chair] – ARC ITTC Director, Biotech, Academia; Dr. Lisa Dube (MTPConnect) – Industry and Government; Dr. Cathy Drinkwater (Biocurate Ltd) – Biotechnology Investment; A. Prof. Rado Danev (Univ. Tokyo) – Academic Thought Leader, cryo-EM; Dr. Raymond Schrijver (Thermo Fisher Scientific, Pharma/cryo-EM) – cryo-EM & Industry; Dr. Leigh Farrell (Industry Consultant, former Cetara) – Drug Development; Prof. Rebekah Brown (Monash Univ.) – DVC Research; Dr. Anne-Laure Puaux (WEHI) – Academic & Industry Engagement; Dr. Jackie How (CM; secretariat)

Operational Subcommittees

Coursework & Training

The Coursework and Training subcommittee supports the Centre Director and Executive Committee through the development of the advanced training provided to the ICHDRs, ensures that training meets AQF Level 10 standards and aligns with best practice for education. The committee also oversees the implementation of professional development, entrepreneurship and innovation activities for the students and adopts a continual review and improvement model for training and coursework. The Terms of Reference for this committee are included in the CCeMMP Governance Plan.

HDR Recruitment

The HDR Recruitment subcommittee is responsible for ICHDR recruitment and oversight of candidature and progress of enrolled students. The committee undertakes ranking of expressions of interest, coordinates interviews of competitive candidates and liaises with project leaders within Nodes to provide final ranking and recommendations of placement and scholarship offers to the Executive Committee. The Terms of Reference for this committee are included in the CCeMMP Governance Plan.

Outreach & Public Engagement

The Outreach and Public Engagement subcommittee (OPEC) assists the Centre Director and Executive Committee in promoting Centre activities and achievements.

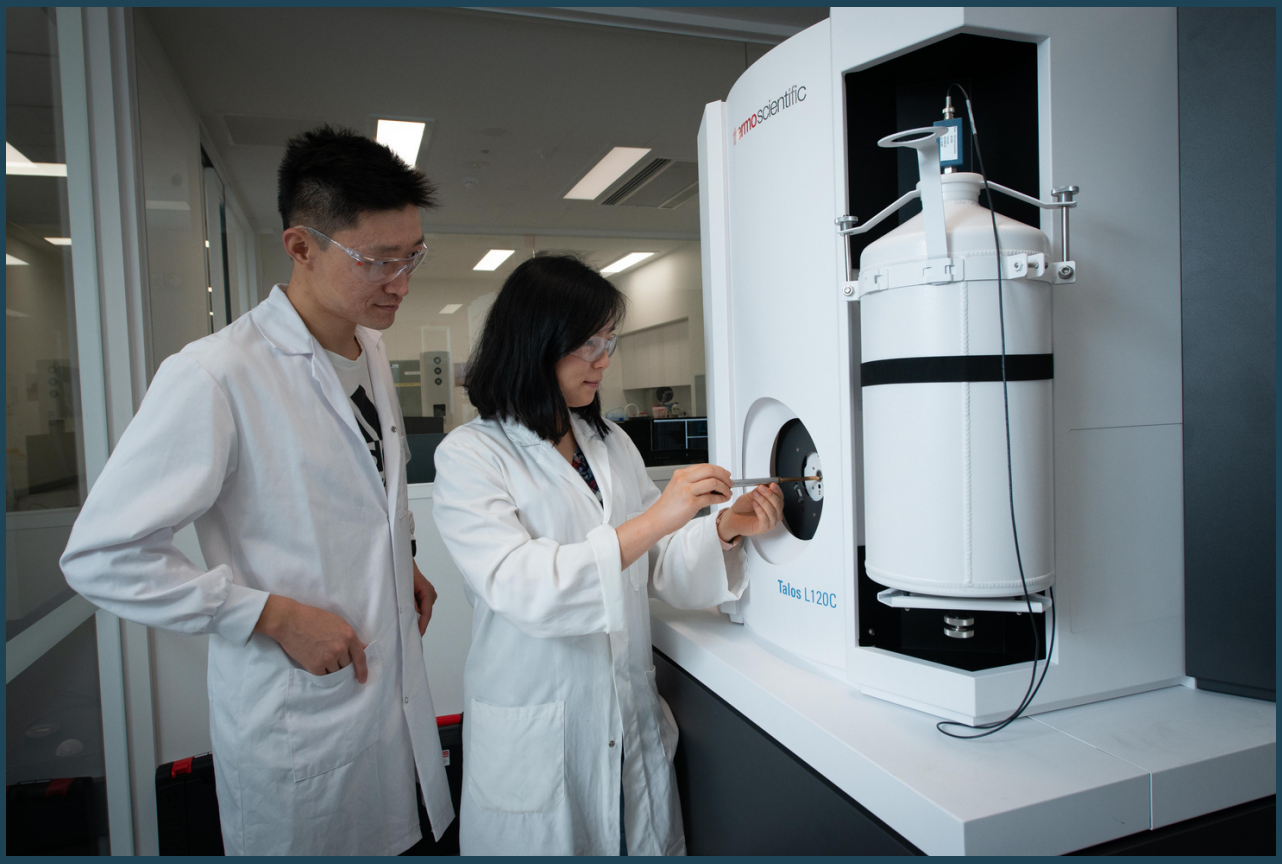
Partnership Engagement

The Partner Engagement subcommittee (PEC) provides support to the Centre Director and Executive Committee through the identification and management of partnerships with stakeholders, in particular with Industry and Biotechnology companies. The PEC includes the Centre Director, Centre Manager and nominated representatives from the business development offices of each of the research nodes. The Terms of Reference for this committee are included in the CCeMMP Governance Plan.

Seminar

The Seminar subcommittee assists the Centre Director and Executive Committee in organising and inviting national and international speakers in the cryo-EM field to a monthly CCeMMP seminar series. The Seminar Committee is comprised of a representative from each of the Nodes; Monash University, The University of Melbourne, University of Wollongong and WEHI, and an ICHDR student. The Committee is supported by the Centre Manager.

Performance



Key Performance Measures

22 Publications

25 Structures published

$14M New funding leveraged

12 Academic presentations

28 Conference presentations

4 Career Fellowships

10 Awards to members

Industry Related Performance Measures

4 Research Co-publications

5 ICHDR partnered Research projects

6 Industry funded postdocs

2 White papers

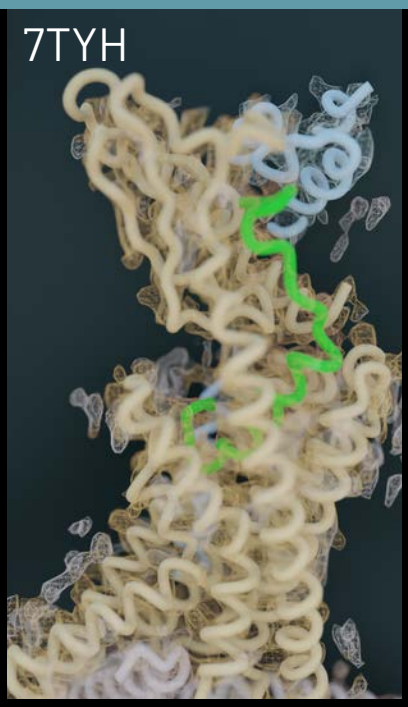
5 Project Agreements

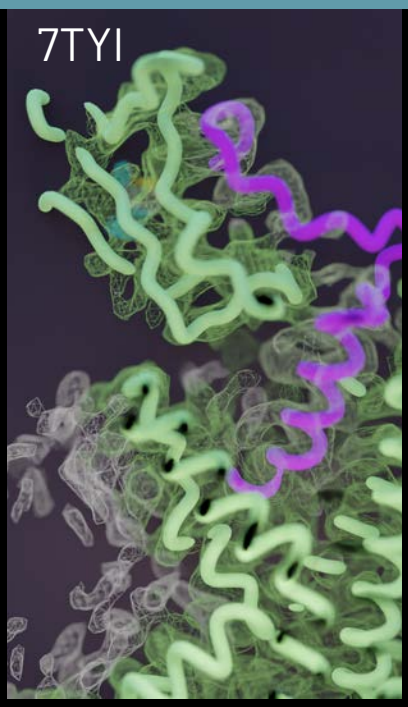
1 New industry Partner Organisation

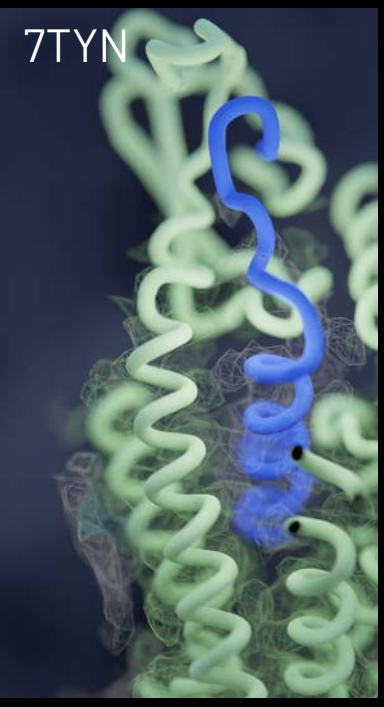
4 Industry presentations

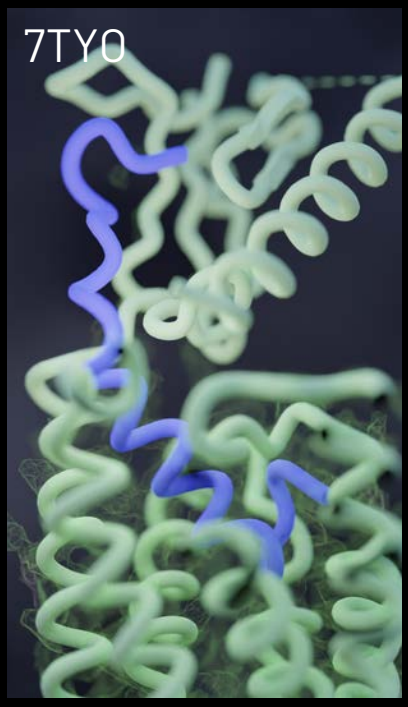
1 Spin-out company

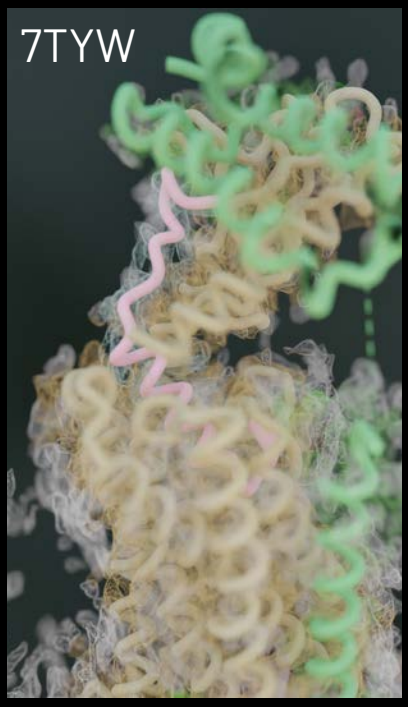
Structures Solved in Year 1

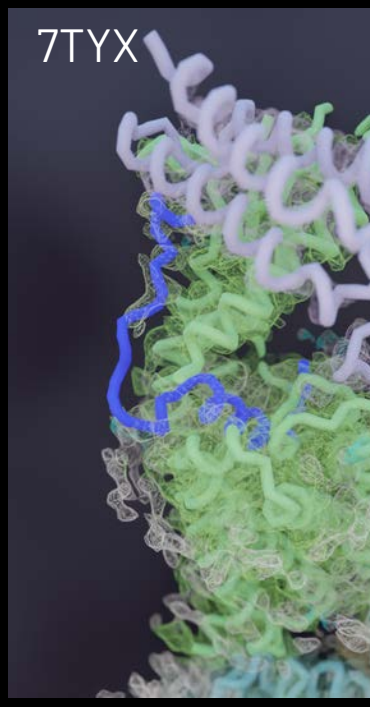


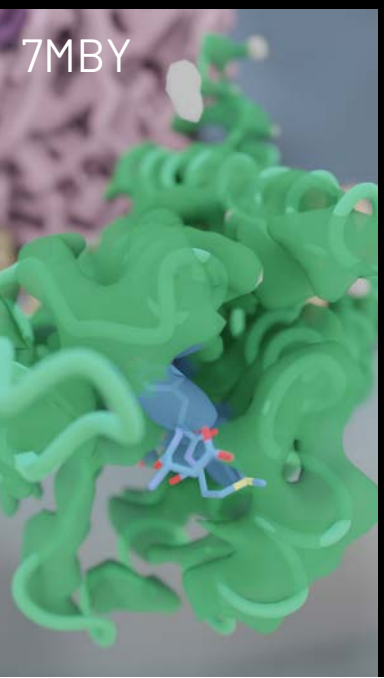


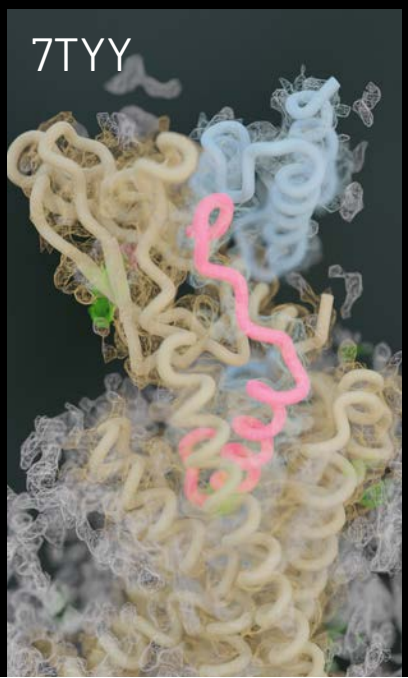


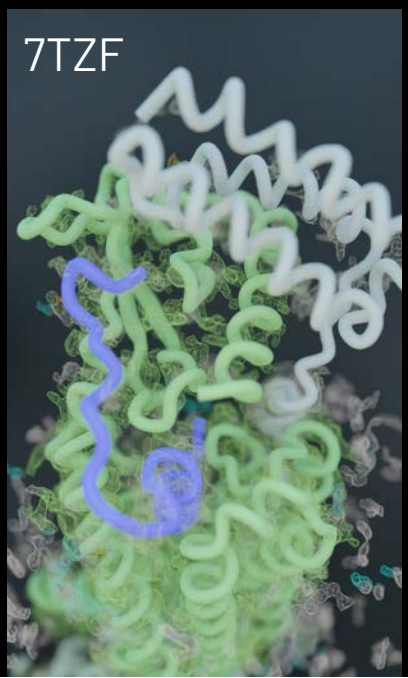


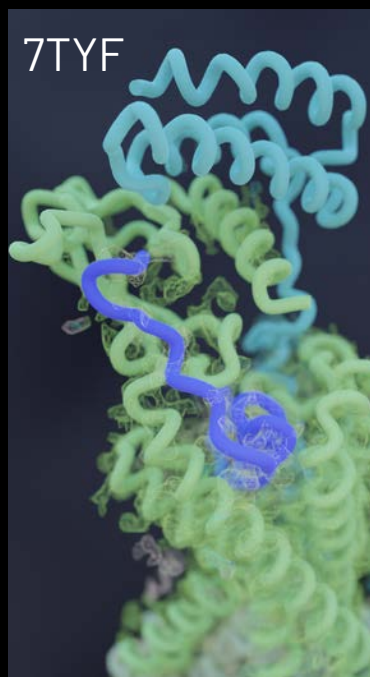










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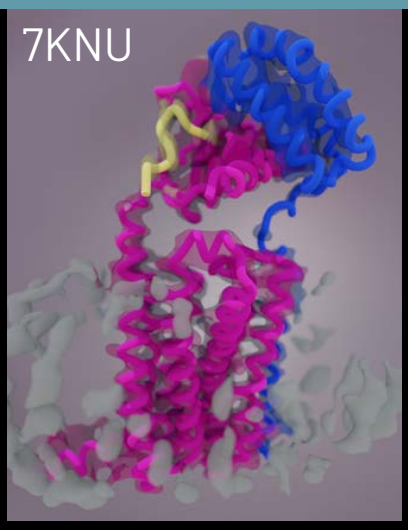
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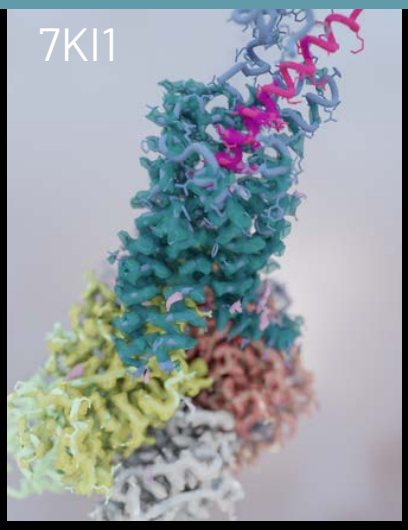
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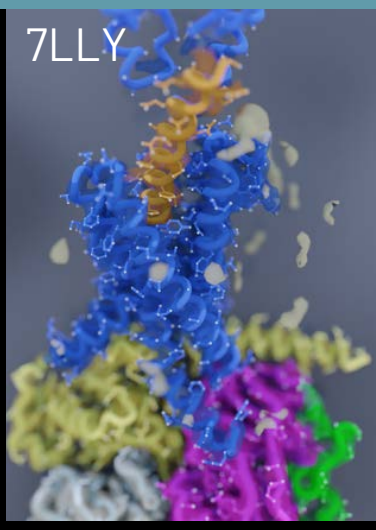
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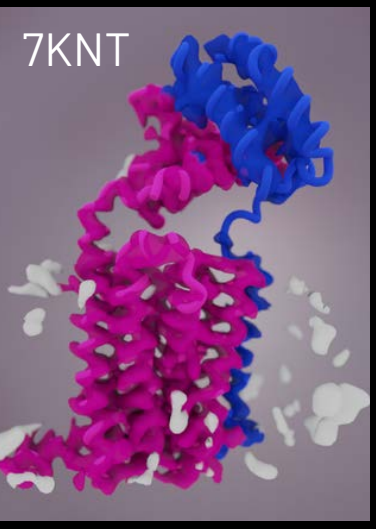
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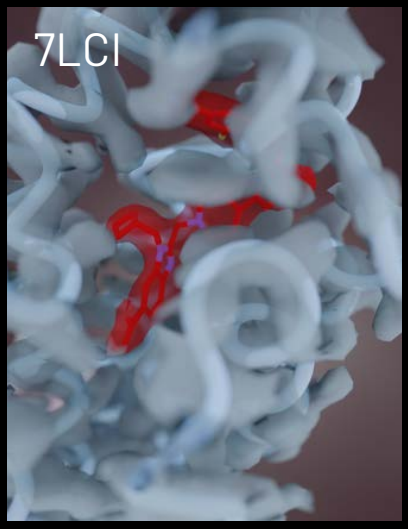
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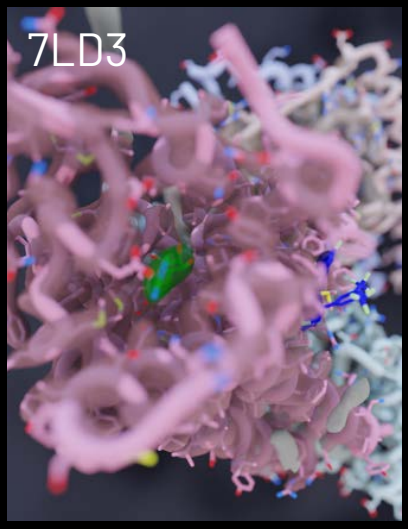
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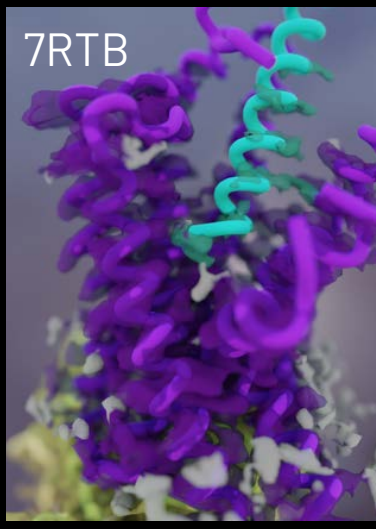
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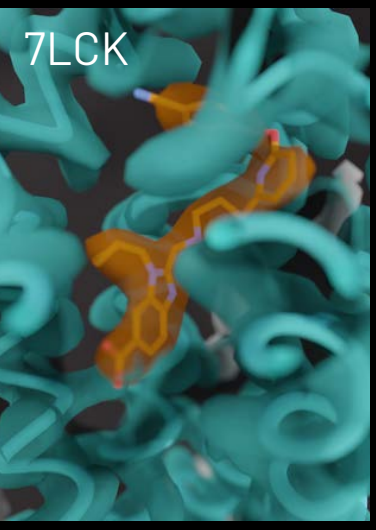
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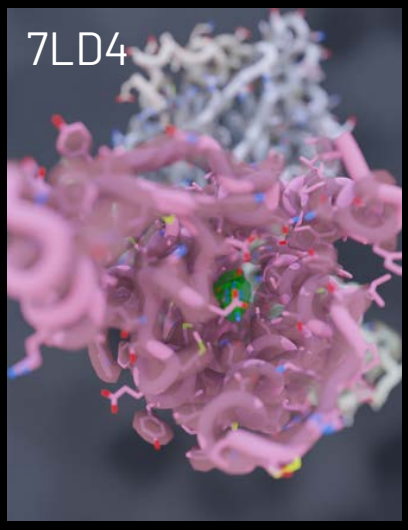
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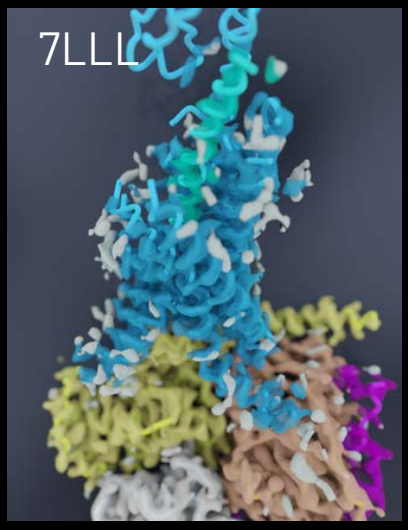
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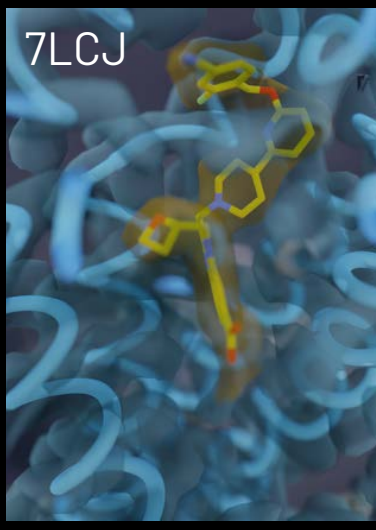
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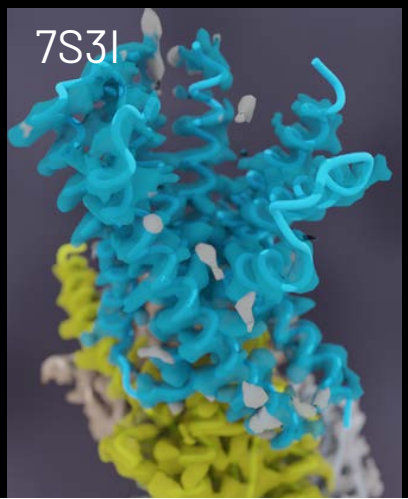
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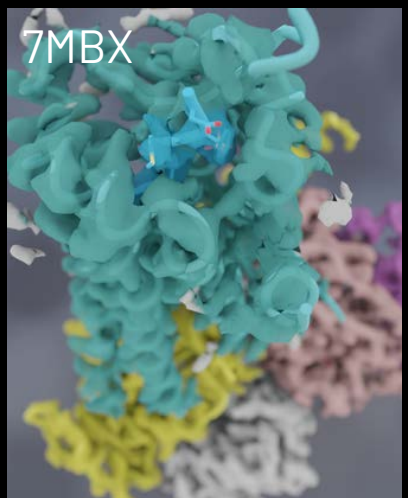
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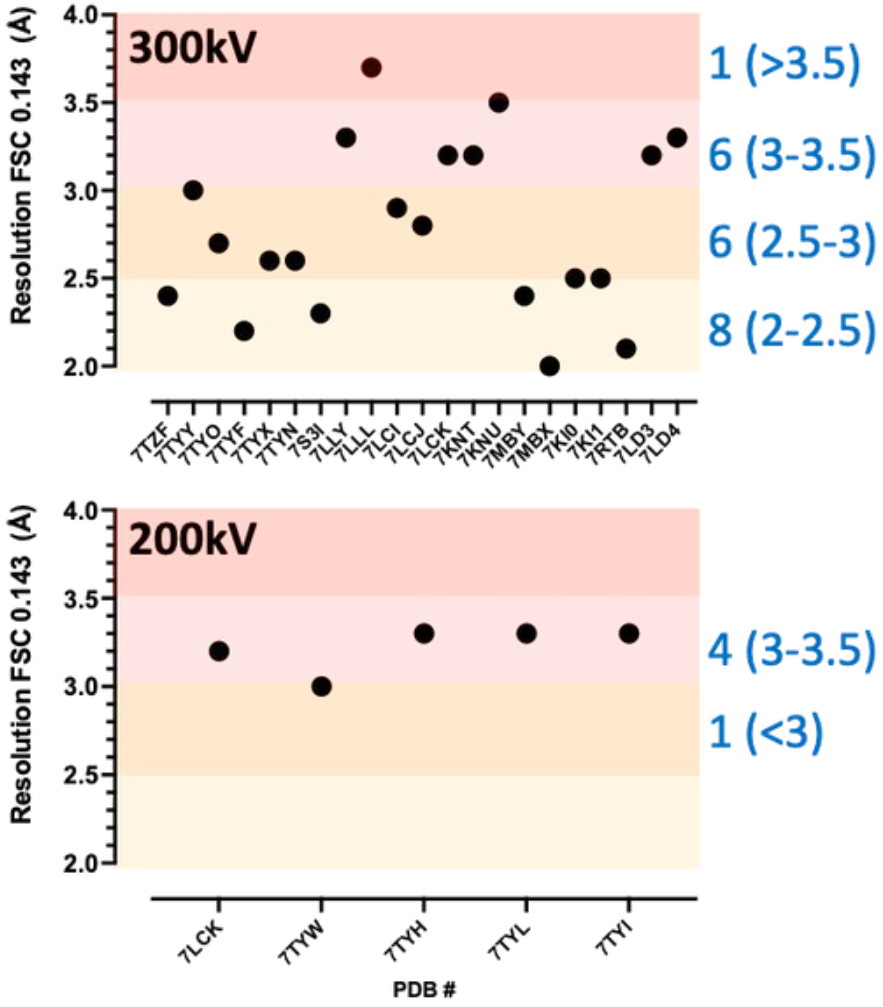
Structure Resolution (Å)

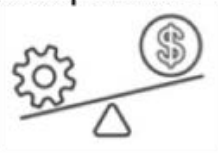


The upper graph depicts the global resolution of structures solved following imaging on a 300 kV Krios microscope. The lower graph depicts the global resolution of structures solved following imaging on a 200 kV Glacios microscope.

Leveraged Research Funding

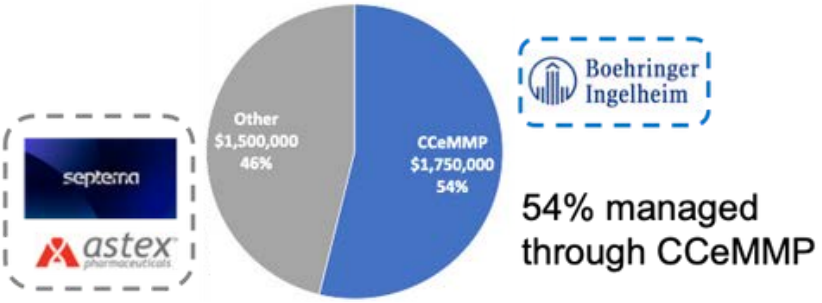
$11M

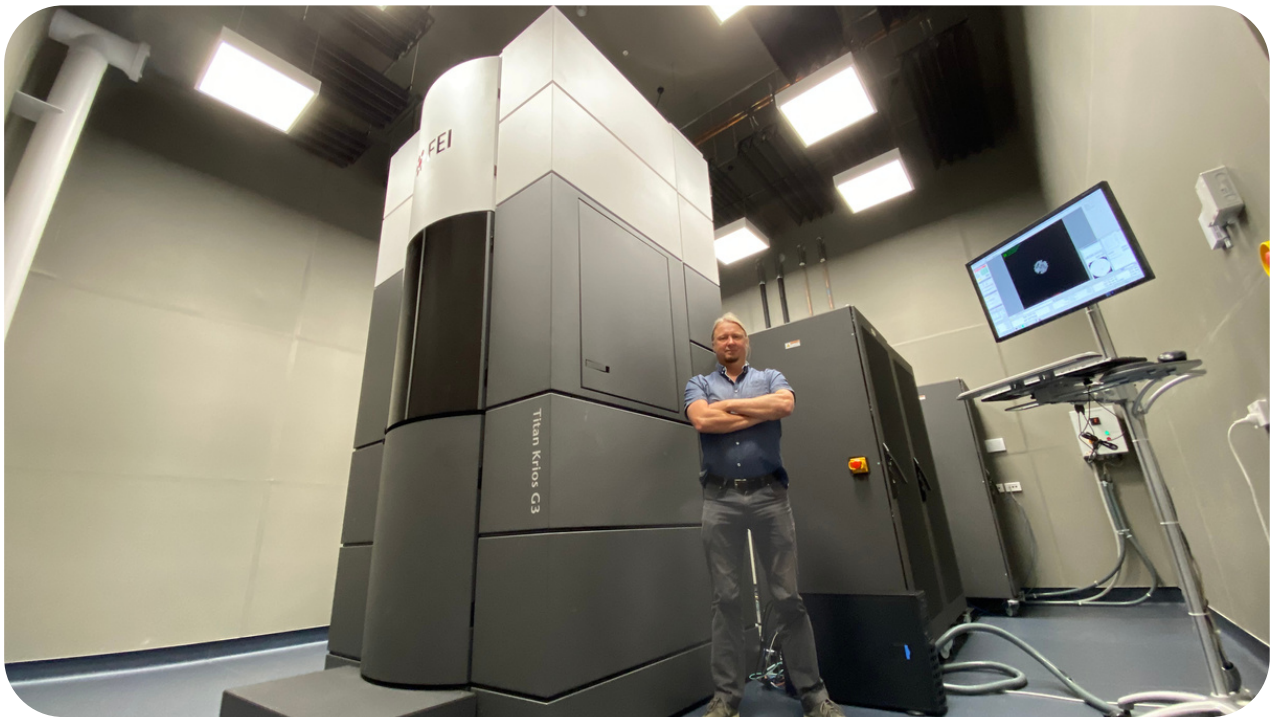
Leveraged funding:
Competitive



$3.2M

Leveraged funding:
Industry





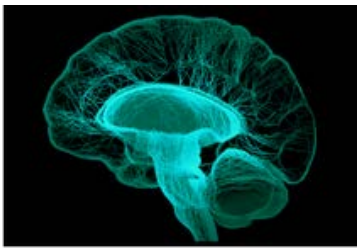
Activities & Achievements

Septerna – New GPCR Biotechnology Company

Prof. Patrick Sexton and Prof. Arthur Christopoulos together with Prof. Robert Lefkowitz (Duke University, 2012 Nobel Laureate) are the scientific co-founders of the exciting new biotechnology company, Septerna Inc. Prof. Denise Wootten joined the team of Scientific Advisors for the company.

The venture has attracted US$100 million (AUD$140 million) Series A financing and is dedicated to discovering and advancing novel small molecule medicines targeting GPCRs using a proprietary platform for protein reconstitution in near-native environments and cryo-EM structure assisted drug discovery and development. The Series A financing was led by Third Rock Ventures with significant support by Samsara BioCapital, BVF Partners, Invus, Catalio Capital Management, Casdin Capital and Logos Capital.

Neuromedicines Discovery Centre (Monash University Node)



Monash launched the new Neuromedicines Discovery Centre (NDC) to drive novel psychiatric drug discovery for the treatment of mental health disorders. The Centre is led by CCeMMP members NDC Director Prof. Arthur Christopoulos and NDC Deputy Director Prof. Chris Langmead, in collaboration with world leading researchers from Monash University, collaborators from The University of Melbourne and The Florey Institute of Neuroscience and Mental Health.

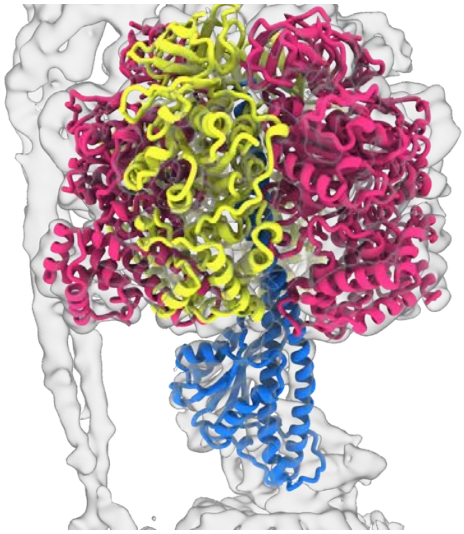
The Centre will involve researchers from Monash's Institute of Pharmaceutical Sciences (MIPS), the Turner Institute for Brain and Mental Health, The Monash School of Clinical Sciences Department of Psychiatry and BehaviourWorks Australia, along with collaborations with the University of Melbourne's Department of Psychiatry, Phoenix Australia, the Melbourne Neuropsychiatry Centre and the Florey Institute of Neuroscience and Mental Health.

Opening of Molecular Horizons Institute. (Univ of Wollongong Node)

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

National Competitive Funding

- Prof. Michael Parker, A. Prof. Isabelle Rouiller, Prof. Patrick Sexton, Prof. Peter Czabotar - ARC LIEF grant LE210100122 - Hydrogen-deuterium exchange system – a missing link in protein analysis.
- Prof. Antoine van Oijen, Dr. James Bouwer, A. Prof. Isabelle Rouiller, Prof. Eric Hanssen - ARC LIEF grant LE210100166 - High-throughput camera system for biological cryo-electron microscopy.
- Prof. Antoine van Oijen, Dr. James Bouwer - ARC LIEF grant LE210100042 - Cryo-focused ion beam facility for soft and hard materials.
- A. Prof. Michael Griffin - ARC LIEF grant LE210100130 - New biomedical capabilities for the Melbourne magnetic resonance facility.
- Prof. Antoine van Oijen. ARC Discovery Project grant DP210100167 - Roadblocks in DNA replication.
- Prof. Patrick Sexton, Dr. Tracy Josephs - ARC Discovery Project grant DP210101504 - Probing the role of dynamics in protein modulation of GPCR phenotype.
- A. Prof. Michael Griffin - 2021 National Drug Discovery Centre - MRFF subsidised small molecule screen.
- Prof. Peter Czabotar - NHMRC Investigator grant APP2009062 -Mining the molecular machinery of cell death for novel drug targets.
- Prof. Antoine van Oijen – 2021 ARC Discovery Project (CIA) Understanding chaperone function, one molecule at a time
- Prof. Michael Parker – 2021 ARC Discovery Project (CIA) How do signals cross the cell membrane: the betacommon receptor family
- A/Prof. Isabelle Rouiller – 2021 ARC Discovery Project (CIC) Metabolite regulation of mitochondrial fission.
- Prof. Patrick Sexton - National Institutes of Health (NIH) R01 grant (2022-2025) - Impact of membrane composition on cholecystokinin receptor structure and function.
- Dr. Greg Stewart - MedTech Actuator Menzie Fellowship.
- Dr. Shabih Shakeel - WEHI's New Medicine and Advance Technology (NMAT).
- Dr. Chris Langmead – Therapeutics Innovation Australia voucher.
- Dr. Simon Brown - Marsden Grant 21-UOO-076 (NZD).



Career Fellowships

- Assoc. Prof. Peter Czabotar – 2021 NHMRC Investigator Award. Mining the molecular machinery of cell death for novel drug targets.
- Dr. Josh Hardy – 2021 NHMRC Investigator Award. Harnessing cryo-electron microscopy to study the engineering of microtubule networks in cancer and neurogenesis.
- Dr. Tracy Josephs – 2021 NHMRC Investigator Award. A personalised pharmacogenomic approach to inform autosomal dominant hypocalcaemia treatment.
- Dr. Lisanne Spenkelink – 2021 NHMRC Investigator Award. The rapid evolution of a genome-editing tool to develop new biomolecule to improve medical treatments.

A/Prof Peter Czabotar

Dr. Josh Hardy

Dr. Tracy Josephs

Dr. Lisanne Spenkelink

Awards

- Dr. Tracy Josephs - Certara New Investigator Award at the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists 2021 meeting
- Dr. Lisanne Spenkelink - The Robin Anders Young Investigator Award at Lorne Proteins 2022
- Dr. Katrina Black - The Robin Anders Young Investigator Award at Lorne Proteins 2022
- Dr. Josh Hardy - The Robin Anders Young Investigator Award at Lorne Proteins 2022
- Dr. Alisa Glukhova - Australian Academy of Science 2022 Gottschalk Medal
- Dr. Jodi Brewster - JBC Herbert Tabor Early Career Investigator Award
- Yao Lu - GW-NSW Georgina Sweet Fellowship 2021

Dr. Katrina Black

Dr. Alisa Glukhova

Dr. Jodi Brewster

Yao (Jackie) Lu

Other Achievements

- Prof. Arthur Christopoulos – Clarivate Analytics Highly Cited researchers – categories: Pharmacology and Toxicology; Biology and Biochemistry
- Prof. Patrick Sexton – Clarivate Analytics Highly Cited researchers – categories: Pharmacology and Toxicology; Biology and Biochemistry
- Prof. Denise Wootten – Clarivate Analytics Highly Cited researchers – categories: Cross-disciplinary
- Prof. Patrick Sexton - Doctor of Science "The structure and function of G protein-coupled receptors".
- Prof. Eric Hanssen promoted to Level E.
- Prof. Lezanne Ooi promoted to Level E.
- A/Prof. Michael Griffin promoted to Level D.
- Dr. Katie Leach appointed Director of of Functional Biology and Pharmacology at AdAlta Ltd

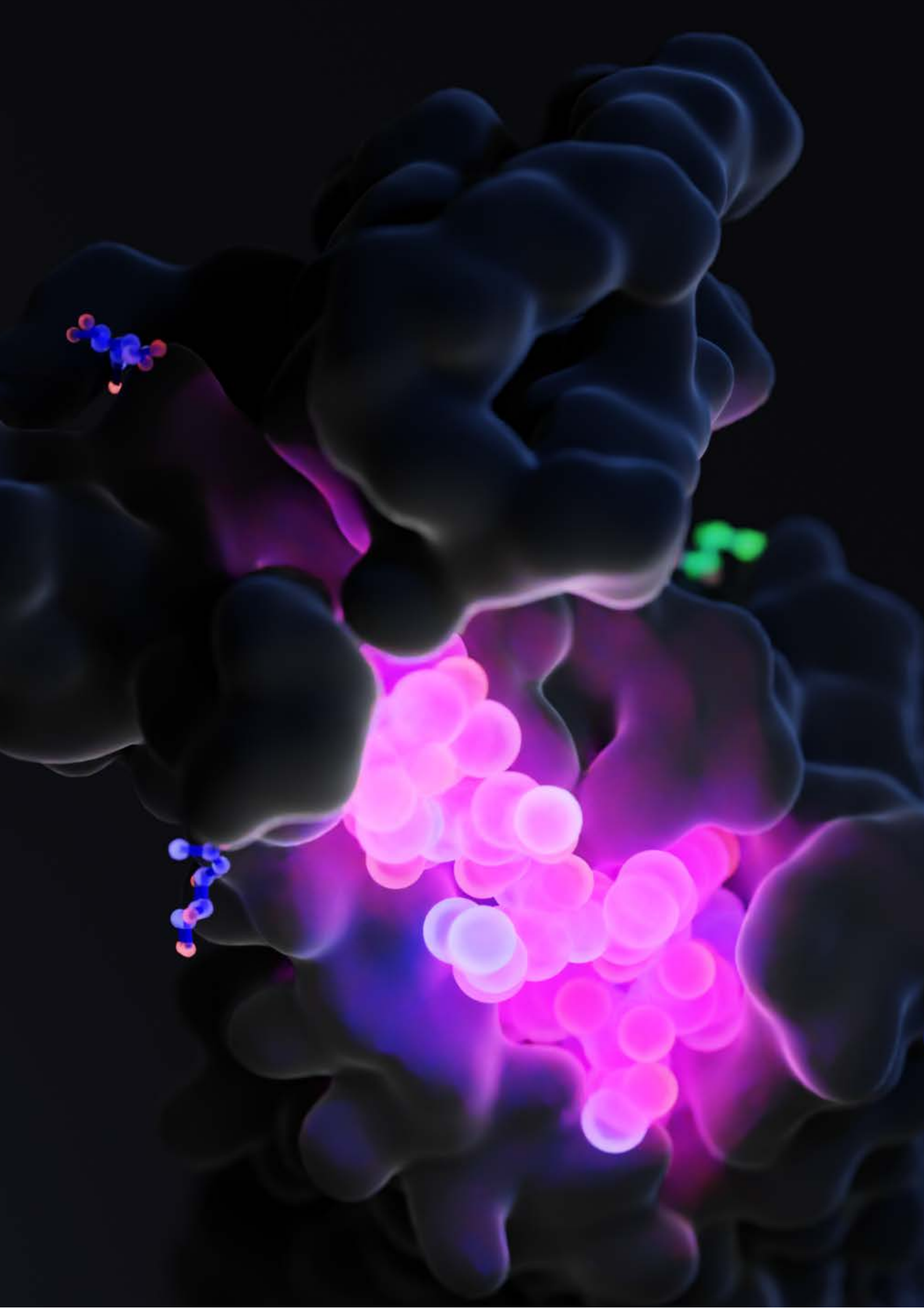
Clarivate Analytics Highly Cited researchers.
Left to right; Prof Arthur Christopoulos, Prof, Patrick Sexton, Prof Denise Wootten

Prof Eric Hanssen

A/Prof Michael Griffin

Prof. Lezanne Ooi

Dr Katie Leach



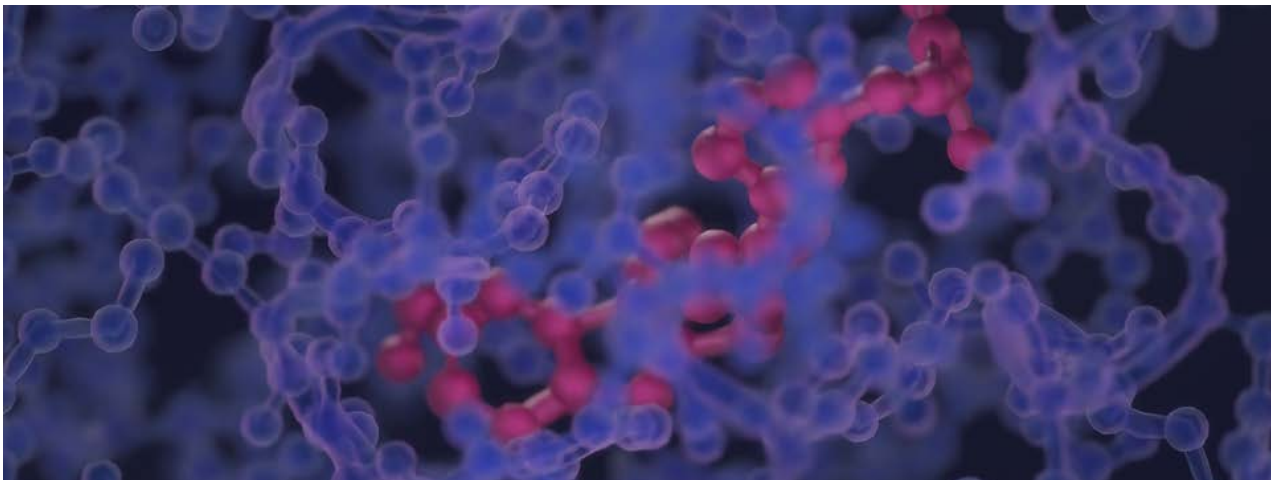
Conference Presentations

- Prof. Patrick Sexton, Keystone Symposium: Frontiers in cryo-electron microscopy. 4-5 February 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. 9-12 March 2021. Using cryo-EM for GPCR drug discovery and development
- Prof. Patrick Sexton, Cryo-EM in Drug Discovery Symposium. SciLife Labs, Stockholm. 8-9 June 2021. Using cryo-EM for GPCR drug discovery and development
- Prof. Patrick Sexton, RegPep23. 23rd International Symposium on Regulatory Peptides. Hybrid meeting. Acapulco, Mexico. 15-19 August 2021. Mechanisms of signaling by peptide-activated G protein-coupled receptors
- Prof. Patrick Sexton, Dr. GPCR Summit 2021. Virtual meeting. 13-19 September 2021. Structure and dynamics of GPCRs: lessons from cryo-EM
- Dr. Elva Zhao, ASCEPT 2021 Virtual Annual Scientific Meeting, December 2021. Understanding the physiological consequences of biased agonism at the GLP1R
- Dr. Xin (Cindy) Zhang, ASCEPT 2021 Virtual Annual Scientific Meeting, December 2021. Structural insights into allosteric modulation of the human glucagon-like peptide-1 receptor
- Wessel Burger, ASCEPT 2021 Virtual Annual Scientific Meeting, December 2021. Orthosteric or Allosteric: Implications for determining mechanisms of probe dependence
- Dr. Greg Stewart, ASCEPT 2021 Virtual Annual Scientific Meeting, December 2021. GPR52 and its role in cognition,
- Dr. Tracy Joseph, ASCEPT 2021 Virtual Annual Scientific Meeting, December 2021. RAMPing up dynamic at G protein coupled receptors
- Prof. Denise Wootten, CGRP 2022 Conference, 11 April 2022. The structure of CLR and CTR; new insights from cryo-EM
- Prof. Denise Wootten, 6th ERNEST Meeting, 30 March 2022. Structural and mechanistic insights into class B1 GPCR activation, signalling and allostery
- Dr. Onisha Patel, Lorne Protein 2022 Conference. Structural basis for small molecule targeting of Doublecortin Like Kinase 1 with DCLK1-IN-1
- Dr. Josh Hardy, Lorne Protein 2022 Conference. Viral metamorphoses: using cryo-EM to understand the maturation of flaviviruses
- Dr. Lisanne Spenkelink, Lorne Protein 2022 Conference. The E. coli helicase does not use ATP during replication
- Dr. Katrina Black, Lorne Protein 2022 Conference. Ion currents through Kir potassium channels are gated by anionic lipids
- Dr. David Thal, Lorne Protein 2022 Conference. Structural and dynamic mechanisms of allostery at the M4 muscarinic acetylcholine receptor
- Dr. Sarah Piper, Lorne Protein 2022 Conference. Dynamic drug targets: Using Cryo-EM data and MD simulations to create realistic 3D animations of GPCR complexes
- Dr. Alisa Glukhova, Lorne Protein 2022 Conference. Understanding ligand binding to adenosine receptors using cryo-EM
- Prof. Michael Parker, Manchester-Melbourne Symposium, virtual meeting on Neurodegeneration and inflammation, February 2021. Microglia receptors and neuroinflammation

- A/Prof. Isabelle Rouiller, Asia-Pacific Cryo-EM Symposium, December 2021. Analyzing the dynamic properties of the AAA ATPase p97 using single particle cryo-EM
- Dr. Shabih Shakeel, Fragile Nucleosome Series, 9 March 2021. Mechanistic Insights into the Fanconi Anemia DNA Repair Pathway
- Dr. Matthew Belousoff, - CryOZ 30 November 2021. How to deal with a mountain of electron micrographs
- Prof. Lezanne Ooi, International Society for Neurodegenerative Diseases, Canada 6th Annual Meeting, October 2021. Analysis of neurodegenerative phenotypes using induced pluripotent stem cells
- Dr. Tracy Josephs, 16th FAOBMB Congress, Christchurch, New Zealand, 22-25 November 2021. G protein-coupled receptor structural dynamics
- Dr. Lisanne Spenkelink, Sydney Protein Group Postdoc Symposium, 20 May 2021. Helicases and Nucleic Acid-based Machines
- Dr. Simon Brown, ACCS Knowledge Share, 9 September 2021. Using cryoSPARC demonstration
- Dr. Onisha Patel, Crystal 33 SCANZ virtual conference, 25-27 May 2021.

Academic Seminar Presentations

- Dr. Sarah Piper - Research Seminar, University of Muenster, Germany
- Prof. Patrick Sexton, Research Seminar, Biozentrum Institute, University of Basel, Switzerland
- Dr. Elva Zhao, Invited talk at Australia Biochemistry Seminar Series
- Dr. James Bouwer, SBGrid consortium Australasian seminar series
- Prof. Eric Hanssen, SBGrid consortium Australasian seminar series
- Prof. Eric Hanssen, Life Science Seminar Series Burnet Institute
- Prof. Eric Hanssen, CNRS Lyon, France
- Prof. Eric Hanssen, Peter Doherty Institute
- Prof. Eric Hanssen, Thermo Fisher workshop, University of Adelaide
- Dr. Debnath Ghosal, WEHI Seminar Series
- Dr. Alisa Glukhova, WEHI Seminar Series
- A/Prof. Isabelle Rouiller, Faculty of Medicine, University of Melbourne



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 29: 963-974.e6, 2021. doi: 10.1016/j.str.2021.04.008. *Industry collaboration (Thermo Fisher Scientific)

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 cholecystokinin 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.

4. 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.

5. 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 18: 256-263, 2022. doi: 10.1038/s41589-021-00945-w.

6. 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 13: 92, 2022. doi: 10.1038/s41467-021-27760-0.

7. 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.

8. 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.

9. Draper-Joyce CJ, Bhola 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 597: 571-576, 2021. doi: 10.1038/s41586-021-03897-2.

10. 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.

11. Yuliantie E, van der Velden WJC, Labroska V, Dai A, Zhao F, Darbalaei S, Deganutti G, Xu T, Zhou Q, Yang D, Rosenkilde MM, Sexton PM, Wang M-W, Wootten D. Insights into agonist-elicited activation of the human glucose-dependent insulinotropic polypeptide receptor. Biochem Pharmacol 192: 114715, 2021. doi: 10.1016/j.bcp.2021.114715.

12. Burger WAC, Gentry PR, Berizzi AE, Vuckovic Z, van der Westhuizen ET, Thompson G, Yeasmin M, Lindsley CW, Sexton PM, Langmead CJ, Tobin AB, Christopoulos A, Valant C, Thal DM. Identification of a novel allosteric site at the M5 muscarinic acetylcholine receptor. ACS Chem Neurosci 12: 3112-3123, 2021. doi: 10.1021/acschemneuro.1c00383.

13. Choy KHC, Luo JK, Wannan CMJ, Laskaris L, Merritt A, Syeda WT, Sexton PM, Christopoulos A, Pantelis C, Nithianantharajah J. Cognitive behavioral markers of neurodevelopmental trajectories in rodents. Transl Psychiatry 11: 556, 2021. doi: 10.1038/s41398-021-01662-7.

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

15. Brown AJH, Bradley SJ, Marshall FH, Brown GA, Bennett KA, Brown J, Cansfield JE, Cross DM, de Graaf C, Hudson BD, Dwomoh L, Dias JM, Errey JC, Hurrell E, Liptrot J, Mattedi G, Molloy C, Nathan PJ, Okrasa K, Osborne G, Patel JC, Pickworth M, Robertson N, Shahabi S, Bundgaard C, Phillips K, Broad LM, Goonawardena AV, Morairty SR, Browning M, Perini F, Dawson GR, Deakin JFW, Smith RT, Sexton PM, Warneck J, Vinson M, Tasker T, Tehan BG, Teobald B, Christopoulos A, Langmead CJ, Jazayeri A, Cooke RM, Rucktooa P, Congreve MS, Weir M, Tobin AB. From structure to clinic: Design of a muscarinic M1 receptor agonist with potential to treatment of Alzheimer's disease. Cell 184: 5886-5901.e22, 2021. doi: 10.1016/j.cell.2021.11.001. *Industry collaboration (Sosei Heptares)

16. Harikumar KG, Coudrat T, Desai AJ, Dong M, Dengler DG, Furness SGB, Christopoulos A, Wootten D, Sergienko EA, Sexton PM, Miller LJ. Discovery of a positive allosteric modulator of cholecystokinin action at CCK1R in normal and elevated cholesterol. Front Endocrinol (Lausanne) 12: 789957, 2021. doi: 10.3389/fendo.2021.789957.

17. Fletcher MM, Keov P, Truong TT, Mennen G, Hick CA, Zhao P, Furness SGB, Kruse T*, Clausen TR*, Wootten D, Sexton PM. AM833 is a novel agonist of calcitonin family G protein-coupled receptors: pharmacological comparison to six selective and non-selective agonists. J Pharmacol Exp Ther 377: 417-440, 2021. doi: 10.1124/jpet.121.000567. *Industry collaboration (Novo Nordisk)

18. 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.

19. 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.

20. 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.

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Structures Published in Publicly Accessible Databases (PDB & EMD)

- Apo CGRPR (PDB:7KNT; EMD:22962)[1]
- CGRP:CGRPR (PDB:7KNU; EMDB:22963)[1]
- PF06882961:GLP-1R:Gs [Krios-K3](PDB:7LCI; EMD:23274)[2]
- PF06882961:GLP-1R:Gs [Krios-F4](PDB:7LCJ; EMD:23275)[2]
- PF06882961:GLP-1R:Gs [Glacios-F4](PDB:7LCK; EMD:23276)[2]
- CCK8:CCK1R:mGqsi:scFv16 (PDB:7MBY; EMD:23750)[3]
- CCK8:CCK1R:Gs:Nb35 (PDB:7MBX; EMD:23749)[3]
- Ex4-D-Ala:GLP-1R:Gs:Nb35 (PDB:7S3I; EMD-24825)[5]
- Oyxn:GLP-1R:Gs:Nb35 (PDB:7LLY; EMD-23436)[6]
- Ex4:GLP-1R:Gs:Nb35 (PDB:7LLL; EMD-23425)[6]
- Sem:GLP-1R:Gs:Nb35 (PDB:7KIO; EMD-22882)[7]
- Tasp:GLP-1R:Gs:Nb35 (PDB:7KI1; EMD-22883)[7]
- Peptide-19:GLP-1R:Gs:Nb35 (PDB:7RTB; EMD-24680)[8]
- MIPS521:ADO:A1R:Gi2 (PDB:7LD3; EMD-23280)[9]
- ADO:A1R:Gi2 (PDB:7LD4; EMD-23281)[9]
- AMY3R/rAmy/Gs complex (PDB:7TZF; EMD:26208)[19]
- AMY2R/sCT/Gs complex (PDB:7TYY; EMD:26199)[19]
- AMY1R/sCT/Gs complex (PDB:7TYW; EMD:26196)[19]
- CTR/hCT/Gs complex (PDB:7TYO; EMD:26190)[19]
- AMY2R/hCT/Gs complex (PDB:7TYH; EMD:26179)[19]
- AMY1R/rAmy/Gs complex (PDB:7TYF; EMD:26178)[19]
- AMY2R/rAmy/Gs complex (PDB:7TYX; EMD:26197)[19]
- CTR/sCT/Gs complex (PDB:7TYN; EMD:26188)[19]
- CTR/rAmy/Gs complex (bypass motif)(PDB:7TYL; EMD:26184)[19]
- CTR/rAmy/Gs complex (CT-like state)(PDB:7TYI; EMD:26180)[19]