Australian Cancer Research Foundation Detector

THE GRANT

In 2015 ACRF awarded $2 million to The Australian Synchotron. The ACRF Detector funded would enable the shape and function of proteins to be analysed on the Australian Synchrotron's Micro Crystallography (MX2) beamline in a fraction of the time taken, providing a ten-fold increase in capacity crucial to accelerating cancer drug development.

The capital investment was used to significantly expand the capability of the Australian Synchrotron Micro Crystallography (MX2) beamline. This technology accurately analyses the 3D shape and functional interaction of proteins.

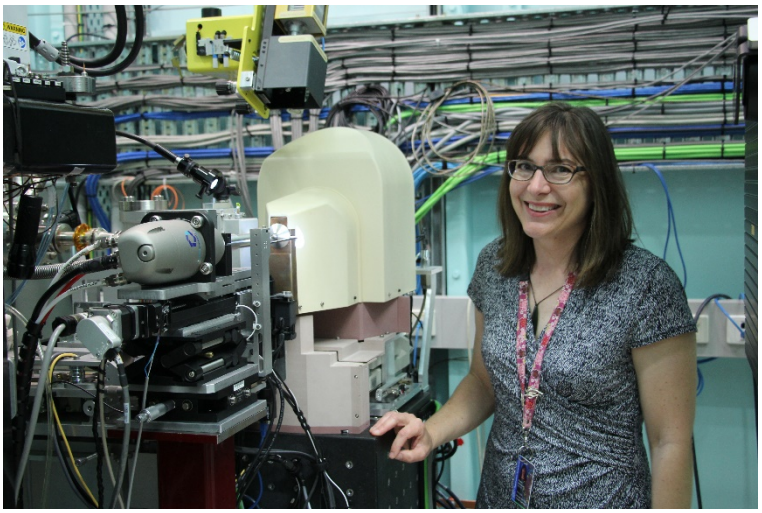
The brilliant light of the MX2 beamline allows researchers to investigate the arrangement and activity of molecules in cancer cells (and cancer treatments) at a level of detail that is not possible at any other Australian research facility.

By introducing the capacity to process large numbers of micron sized protein crystals, the ACRF Detector increases capacity of this crucial beamline, enabling many more research studies to take place, while producing data of greater accuracy and quality.

This means researchers will gain answers much faster, shortening the time from laboratory research to the clinical trial, which tests the performance of new cancer drugs.

The grant was awarded to an esteemed research consortium comprising of micro-crystallography experts from six research institutions across Australia. The ACRF Synchrotron became operational in 2016.

At the time the ACRF investment in this key cancer research technology, was available at only a handful of other synchrotron facilities around the world.



Dr Rachel Williamson with the ACRF detector on the MX2 beamline.

PROGRESS in 2019

The ARCF Eiger 16M Detector has been revolutionary for the MX2 Beamline at the Australian Synchrotron. From an operational perspective, 2019 saw the detector collect **51,064,846** diffraction images, associated with **71,460** datasets across **240** distinct group experiments. Approximately **5 petabytes** of raw (uncompressed) data were generated by the ACRF detector in 2019 prior to reduction, processing and analysis.

Use of the ACRF Detector on the Micro-Crystallography (MX2) beamline has continued to grow, and the Macromolecular Crystallography beamlines at the Australian Synchrotron (MX1 and MX2) produced a record number of publications in 2019. More than 210 peer-reviewed scientific journal articles were published in the highest quality journals such as *Science, Nature Immunology, Nature Cell Biology, Trends in Biochemical Sciences and Science Translational Medicine*.

Large numbers of protein structures from the Australian Synchrotron (~290) were also deposited in the worldwide Protein Data Bank (PDB). Scientific outputs from the MX2 beamline and the ACRF Detector have increased compared to 2018, with 89 PDB structures and 60 journal publications based on data acquired using the ACRF Detector.

In 2019 one of the applicants for the grant for the ACRF Detector (Peter Czabotar from the Walter and Eliza Hall Institute for Medical Research) received the Prime Minister's Prize for Innovation. Dr Czabotar has also been a productive user of the ACRF Detector during the year, with a publication resulting from use of the detector appearing in the journal *Nature Communications*.

The Australian Synchrotron has continued to deliver excellent science outputs and outcomes in 2019. This is evidenced by a record number of **608** peer-reviewed journal publications resulting from research undertaken at the facility. Over $^{1}/_{3}$ of these publications (**214**) resulted from structures determined using the MX1 and MX2 beamlines, demonstrating the importance of these beamlines to the scientific program at the Australian Synchrotron. The number of journal articles published in 2019 that contain data taken using the ACRF Detector continues to increase in line with expectations based on the lead-time between data collection and publication. Starting with 4 publications in 2017, there were 31[1] in 2018 and more than **60** in 2019. Over the next few years we will see continued growth to the point where almost all MX2 publications (which account for the majority of publications on MX1 and MX2) will result from data collected using the ACRF Detector.

2019 saw at-least 88 research theses published containing results from Australian Synchrotron beamlines; the vast majority being for Doctoral Theses. This is a substantial underestimate of the actual total number of research theses, which usually numbers more than 150 per annum. More than $^{1}/_{3}$ of these research theses contain data from the MX1 and/or the MX2 beamlines. The *Australian Synchrotron Stephen Wilkins Thesis Medal* for the most outstanding doctoral thesis in 2019 was awarded to Dr Angus Cowan (WEHI) for his thesis: *Structural Investigations of Pro-apoptotic Bcl-2 Family Proteins.* This

research was centrally focused on understanding key biomolecular mechanisms associated with cancer and cell death, with his work also contributing to the development of the drug Venetoclax.

RESEARCHERS AND RESEARCH STUDENTS WORKING ON THE PROJECT

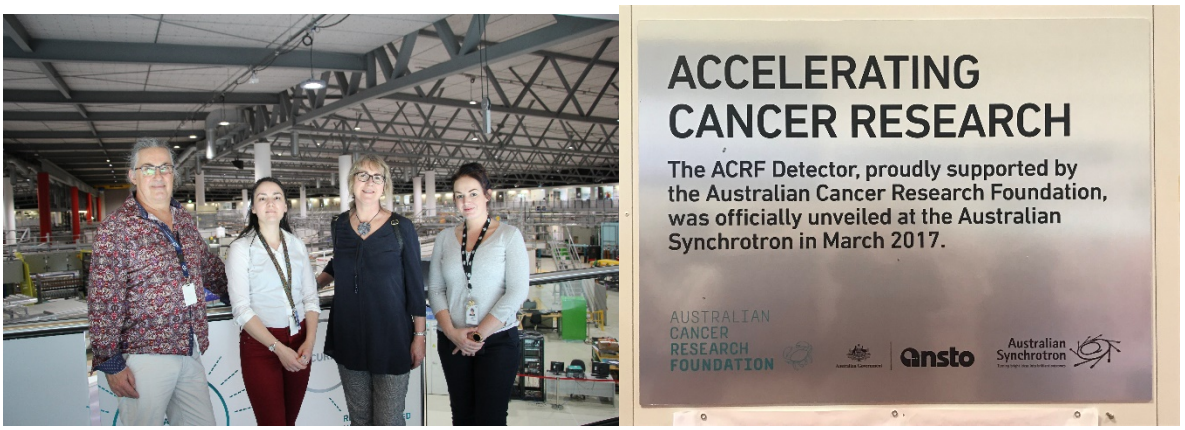
The ACRF Detector is available for use through the Australian Synchrotron peer-reviewed merit access program. Since the first experiment with the ACRF Detector on 12 February 2017, every researcher accessing the MX2 beamline has used the ACRF Detector. In 2019, MX2 hosted **1986** merit user accesses (for both on-site user visits and off-site remote access use). This amounts to approximately one third of all Australian Synchrotron user visits.

The researchers accessing the MX2 beamline and the ACRF Detector are part of larger research teams. Typically, a few researchers from a laboratory group will take part in a Synchrotron experiment. The larger group will be working on other aspects related to the structural determination undertaken at the Synchrotron, including protein identification, purification and crystallisation, as well as in the related biochemistry, molecular biology and pharmaceutical drug discovery aspects of these studies.

In addition, access to MX2 and the ACRF Detector typically takes the form of research groups bringing multiple samples on multiple occasions throughout the year. Accordingly, the 1986 user visits throughout the year corresponded to **378** individual researchers who accessed the MX2 beamline and the ACRF Detector in 2019. Of these typically about 50% are students and early career researchers.

PEER REVIEWED JOURNAL PUBLICATIONS

In 2019, there were 214 peer reviewed journal publications containing data from the MX1 and MX2 beamlines, with a substantial proportion of these published in high-impact journals. Of note were macromolecular crystallography studies published in *Science*, and numerous others published in high-impact journals such as *Nature Immunology, Nature Cell Biology, Trends in Biochemical Sciences and Science Translational Medicine Journal of the American Chemical Society*, *Angewandte Chemie International Edition*, *Proceedings of the National Academy of Sciences of the United States of America*, and *Nature Communications*. Of these 214 publications, we have identified at-least 60 publications containing data sourced from the ACRF Detector in 2019.



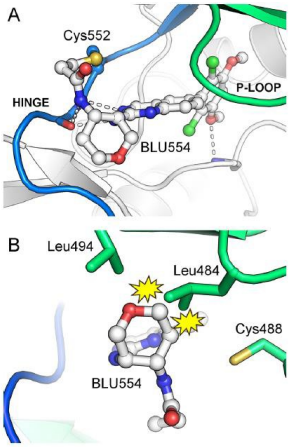
It was a privilege to be shown around the Synchotron and see the ACRF Detector.

Picture here – ACRF's Victoria Bonsey with Dr Daniel Hausermann, Dr Danielle Martin and Jaye Muir.

SIGNIFICANT CANCER-RELATED RESEARCH FINDINGS 2019

The following summaries are a selection of significant cancer-related findings published in 2019 from the MX beamlines. The majority of these made use of the ACRF Detector, again indicating the rapid impact the installation of the detector has had.

Xiaojing Lin, et al., Rotational freedom, steric hindrance and protein dynamics explain BLU554 selectivity for the hinge cysteine of FGFR4,
***ACS Medicinal Chemistry Letters*, 10, 1180 (2019).**

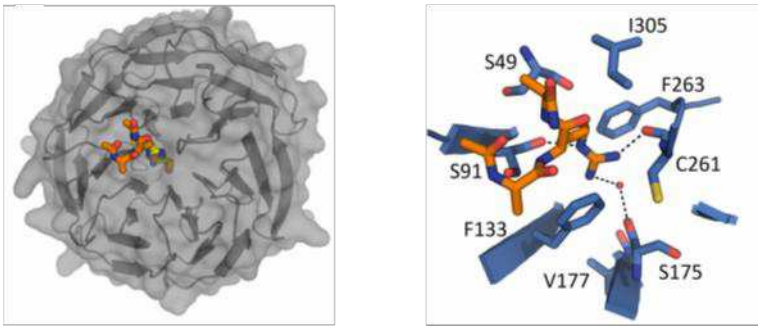


X-ray crystal structure of FGFR4-BLU554. (A) Covalent and hydrogen bonding. (B) Modelled FGFR1 binding pose high-lighting P-loop clashes.

Aberration in FGFR4 signalling drives carcinogenesis and progression in a subset of hepatocellular carcinoma (HCC) patients, thereby making FGFR4 an attractive molecular target for this disease. Selective FGFR4inhibition can be achieved through covalently targeting a poorly conserved cysteine residue in the FGFR4 kinase domain. This study **using the MX2 beamline and the ACRF detector** and led by researchers from the *University of Auckland*, investigated the structure of FGFR4 in covalent complex with the clinical candidate BLU554 and with a series of four structurally related inhibitors that define the inherent reactivity and selectivity profile of these molecules. Collectively, their results suggest that rotational freedom, steric hindrance, and protein dynamics explain the exceptional selectivity profile of BLU554 for targeting FGFR4.

(**PDB Structures:** *6NVG, 6NVH, 6NVI, 6NVJ, 6NVK, 6NVL*)

Matthew L. Dennis, et al., Fragment screening for a protein-protein interaction inhibitor to WDR5,
***Structural Dynamics*, 6, 064701 (2019).**



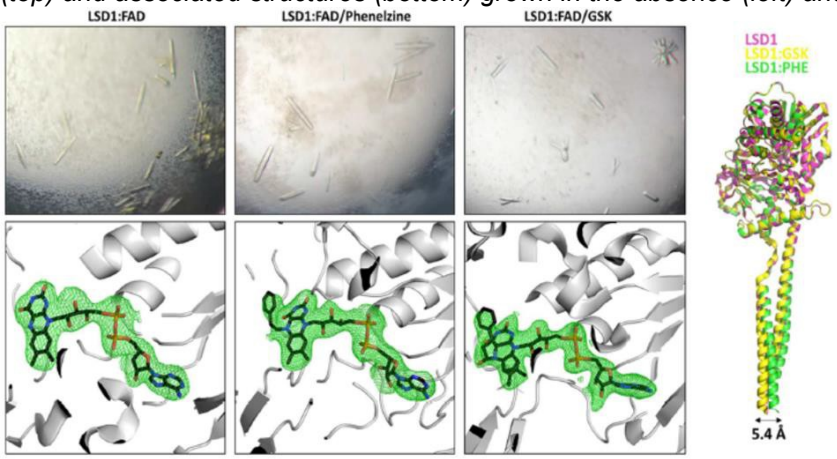
Overlay of WDR5 co-crystal structures bound to the MLL peptide (left). Binding mode of the MLL1 peptide (right).

The WD40-repeat protein WDR5 scaffolds various epigenetic writers and is a critical component of the mammalian SET/MLL histone methyltransferase complex. Dysregulation of the MLL1 catalytic function is associated with mixed-lineage leukemia. Antagonism of the WDR5-MLL1 interaction by small molecules has been proposed as a therapeutic strategy for MLL-rearranged cancers. Small molecule binders of the "WIN" site of WDR5 that cause displacement from chromatin have been additionally implicated to be of broader use in cancer treatment. In this study by researchers from *CSIRO, Monash Institute of Pharmaceutical Sciences, the Cancer Therapeutics CRC, Bio21 Institute, the Walter and Eliza Hall Institute of Medical Research,* and *the University of Melbourne*, a fragment screen with Surface Plasmon Resonance was used to identify a highly ligand-efficient imidazole containing compound that is bound in

the WIN site. The subsequent medicinal chemistry campaign — guided by a suite of high-resolution co-crystal structures with WDR5 (**determined using the MX2 beamline and the ACRF detector**) — progressed the initial hit to a low micromolar binding molecule. This system represents a means to overcome potential permeability issues of WDR5 ligands that possess highly basic groups like guanidine. The series reported here furthers the understanding of the WDR5 WIN site, and functions as a starting point for the development of more potent WDR5 inhibitors that may serve as cancer therapeutics.
(**PDB Structures:** *6PG3 – 6PG9, 6PGA – 6PGF*)

Abel H. Y. Tan, et al., Lysine-Specific Histone Demethylase 1A Regulates Macrophage Polarization and Checkpoint Molecules in the Tumour Microenvironment of Triple-Negative Breast Cancer, *Frontiers in Immunology*, 10, 1351 (2019).

LSD1 protein crystals (top) and associated structures (bottom) grown in the absence (left) and presence of



phenelzine (middle) and GSK2879552 (right). Overlay of the 3 structures is shown on the right.

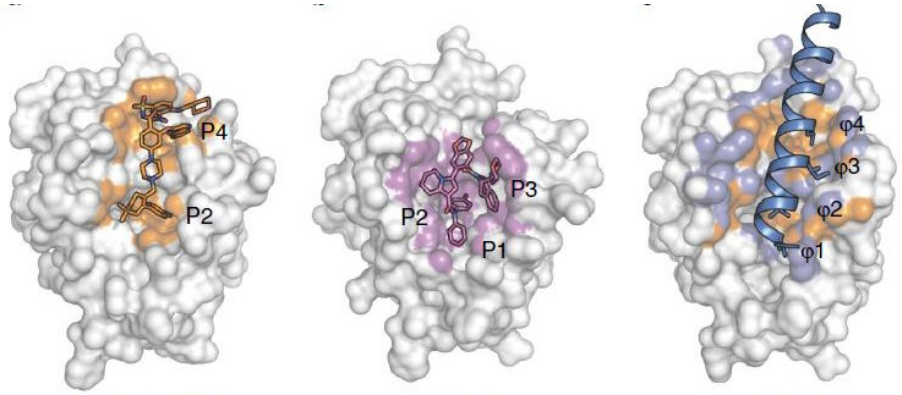
The triple-negative subtype of breast cancer (TNBC) accounts for 15–20% of cases. TNBCs have a particularly high immune cell infiltrate compared to other breast cancer subtypes, but these immune cells are often functionally impaired. The tumour microenvironment (TME) of any cancer contains a complex mixture of immune cells with both pro- and anti-tumour properties. Tumour-associated macrophages (TAMs) are a major immune cell subset in the TME. Lipopolysaccharide (LPS) and IFN-g- induced M1 macrophages secrete pro-inflammatory cytokines that contribute to tumour cell cytotoxicity. Conversely, IL-4- and IL-13-induced M2 macrophages produce anti-inflammatory cytokines that can suppress other immune cells in the TME and promote tumour progression.

Lysine-specific demethylase 1 (LSD1) is a protein that is essential for myeloid cell differentiation, reactivating key immune checkpoint regulators, and producing cytotoxic T cell chemokines. This study by researchers from the *University of Canberra*, and *Charles Sturt University* showed that M1 macrophage polarization reduced the expression of LSD1, and the nuclear REST corepressor 1 (CoREST). Crystal structures determined using the Australian Synchrotron showed that he LSD1 inhibitor phenelzine targeted both the flavin adenine dinucleotide (FAD) and CoREST binding domains of LSD1, while the LSD1 inhibitor GSK2879552 only targeted the FAD domain. Phenelzine treatment reduced nuclear demethylase activity and increased transcription and expression of M1-like signatures both *in vitro* and in a murine triple-negative breast cancer model. These findings suggest that inhibitors must have dual FAD and CoREST targeting abilities to successfully initiate or prime macrophages toward an anti-tumour M1-like phenotype in triple-negative breast cancer.
(**PDB Structures:** 6NR5, 6NQM, 6NQU)

Richard W. Birkinshaw, et al., Structures of BCL-2 in complex with venetoclax reveal the molecular basis of resistance mutations, *Nature Communications*, 10, 2385 (2019).

BCL-2 surface contacts with either venetoclax, S55746 or BaxBH3 peptide.

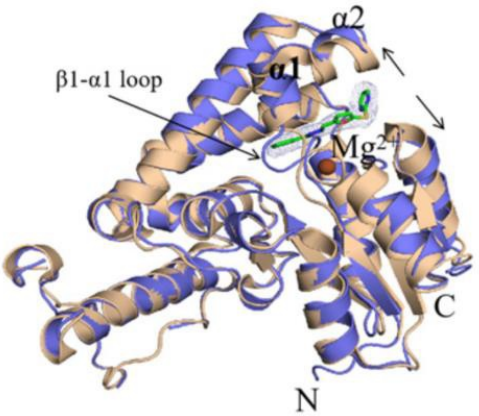
Venetoclax is a first-in-class cancer therapy that interacts with the cellular apoptotic machinery promoting apoptosis. Treatment of patients suffering chronic lymphocytic leukaemia with this BCL-2 antagonist has revealed emergence of a drug-selected BCL-2 mutation (G101V) in some patients failing therapy. To understand the molecular basis of this acquired resistance researchers from *the Walter and Eliza Hall Institute of Medical Research, the University of Melbourne, the Peter MacCallum Cancer Centre,* and *the Victorian Comprehensive Cancer Centre* **used the MX2 beamline and the ACRF detector** to describe the crystal structures of venetoclax bound to both BCL-2 and the G101V mutant. The pose of venetoclax in its binding site on BCL-2 reveals small but unexpected differences as compared to published structures of complexes with venetoclax analogues. The G101V mutant complex structure and mutant binding



BCL-2 G101V Ven | BCL-2 G101V S55746 | BCL-2 WT Bax-BH3

assays reveal that resistance is acquired by a knock-on effect of V101 on an adjacent residue, E152, with venetoclax binding restored by a E152A mutation. This provides a framework for considering analogues of venetoclax that might be effective in combating this mutation.
(**PDB Structures:** 6O0K, 6O0L, 6O0M, 6O0O, 6O0P)

Jothi Anantharajan, et al., Structural and functional analyses of an allosteric Eya2 phosphatase inhibitor that has on target effects in human lung cancer cells,
***Molecular Cancer Therapeutics*, 18, 1484 (2019).**

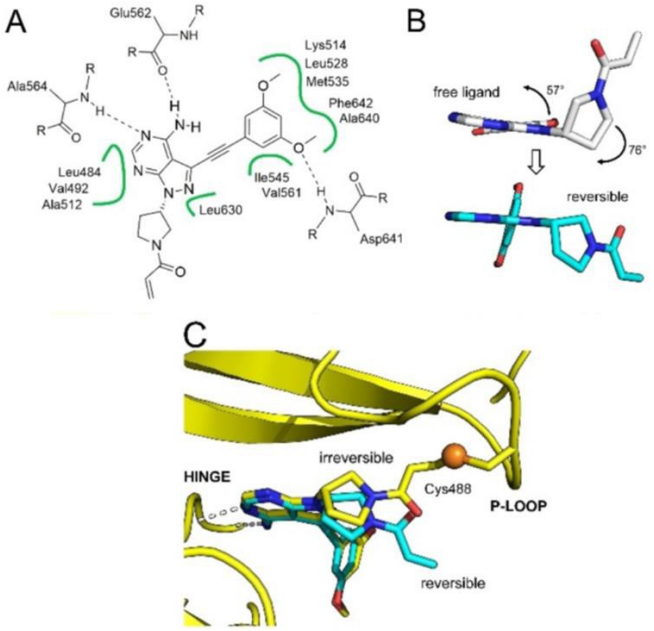


Crystal structure of the EYA2 ED–9987 complex.
(Superposition of the inhibitor bound (blue) and apo (orange) crystal structures)

EYA proteins (EYA1-4) are critical developmental transcriptional cofactors that contain an EYA domain (ED) harbouring Tyr phosphatase activity. EYA proteins are largely downregulated after embryogenesis but are re-expressed in cancers, and their Tyr phosphatase activity plays an important role in the DNA damage response and tumour progression. The authors of this study from *the A*STAR Institute (Singapore), the University of Colorado,* and *the National Institute of Health (Maryland, USA)*, used the Australian Synchrotron to determine the crystal structure of the EYA2 ED in complex with NCGC00249987 - a small-molecule allosteric inhibitor that specifically inhibits the Tyr phosphatase activity of EYA2. They demonstrated that NCGC00249987 binds to an induced pocket distant from the active site. NCGC00249987 binding leads to a conformational change of the active site that is unfavourable for Mg binding, thereby inhibiting EYA2's Tyr phosphatase activity. Further, the authors demonstrated that NCGC00249987 specifically targets migration, invadopodia formation, and invasion of lung cancer cells, but that it does not inhibit cell growth or survival. The compound had no effect on lung cancer cells carrying an EYA2 F290Y mutant that abolishes compound binding, indicating that NCGC00249987 is on target in lung cancer cells. These data suggest that the NCGC00249987 allosteric inhibitor can be used as a chemical probe to study the function of the EYA2 Tyr phosphatase activity in cells and may have the potential to be developed into an antimetastatic agent for cancers reliant on EYA2's Tyr phosphatase activity. (**PDB Structures:** 5ZMA)

Maria Kalyukina, et al., TAS−120 Cancer Target Binding: Defining Reactivity and Revealing the First Fibroblast Growth Factor Receptor 1 (FGFR1) Irreversible Structure,
***ChemMedChem*, 14, 494 (2019).**

TAS-120 in complex with FGFR1: A) Binding interactions between FGFR1 and TAS-120 in a reversible



binding mode. B) TAS-120 conformational change on reversible binding with torsional angle changes indicated. C) TAS-120 irreversible (covalent) binding to FGFR1.

Lung cancer is a leading cause of death worldwide, accounting for ~19% of all cancer-related mortalities. Non-small cell lung cancer (NSCLC) accounts for ~80% of cases, with smoking-related squamous cell carcinoma (SCC), a subtype of NSCLC, linked to amplification of fibroblast growth factor receptor 1 (FGFR1) in approximately 20 % of patients. FGFR1 amplifications can also occur in a wide range of other cancers, including" prostate, oesophageal, SCC head and neck, bladder, colorectal, and osteosarcoma. FGFR1 is a receptor tyrosine kinase (RTK); somatic mutations in this group of receptors and aberrant activation of their signalling pathways is seen in tumours, contributing to proliferation, angiogenesis, migration, and survival. Understanding the details of RTK signalling in the genesis and maintenance of disease is critical in delivering effective treatments to the clinic.

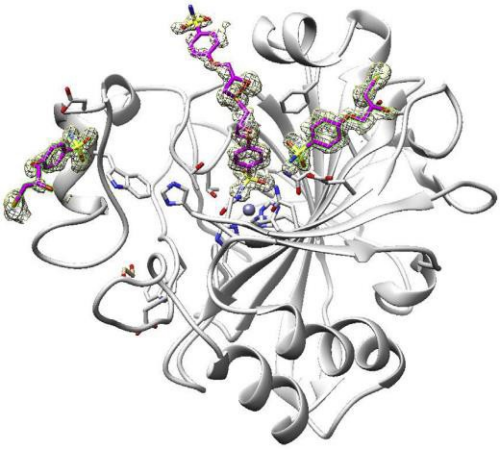
TAS-120 is an irreversible inhibitor of the fibroblast growth factor receptor (FGFR) family, and is currently under phase I/II clinical trials in patients with confirmed advanced metastatic solid tumours harbouring FGFR aberrations. This inhibitor specifically targets the P-loop of the FGFR tyrosine kinase domain, forming a covalent adduct with a cysteine side chain of the protein. Researchers from the University of Auckland used mass spectrometry experiments to characterise an exceptionally fast chemical reaction in forming the covalent complex. The structural basis of this reactivity was revealed by a sequence of three X-ray crystal structures, generated in part **using the MX2 beamline and the ACRF Detector**: a free ligand structure, a reversible FGFR1 structure, and the first reported irreversible FGFR1 adduct structure. We hypothesise that the most significant reactivity feature of TAS-120 is its inherent ability to undertake conformational sampling of the FGFR P-loop. In designing novel covalent FGFR inhibitors, such a phenomenon presents an attractive strategy requiring appropriate positioning of an acrylamide group similarly to that of TAS-120.
(**PDB Structures:** 6MZQ, 6MZW)

Damiano Tanini, et al., Synthesis of novel tellurides bearing benzensulfonamide moiety as carbonic anhydrase inhibitors with antitumor activity,
***European Journal of Medicinal Chemistry*, 181, 111586 (2019)**

X-ray crystal structures of hCA II with compound 8 in the active site and two monomers (halves of the symmetric compound 8) bounded out of the active site.

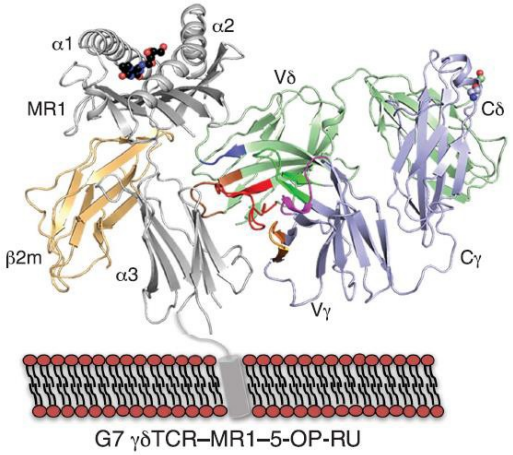
The human isoform carbonic anhydrase (hCA) IX enzyme is found overexpressed in a wide selection of hypoxic tumours acting as a key player in cancer cells survival, proliferation and metastasis, and for this reason it has been validated as a pharmaceutical target. Medicinal Chemists have sought to identify molecular moieties able to inhibit the CA isozymes and to discriminate among the various isoform expressed in humans.

Researchers from *the University of Florence, the Universita degli Studi di Firenze, CSIRO,* and *the "Petru Poni" Institute of Macromolecular Chemistry (Romania)* synthetized a novel series of -hydroxy tellurides bearing the benzenesulfonamide group as potent inhibitors of carbonic anhydrase enzymes. They made



use of the Australian Synchrotron in their study to determine the first X-ray structure of a -hydroxy telluride derivative co-crystallised with hCA II. The potent effects of these compounds against the tumour-associated hCA IX with low nanomolar constant inhibition values led to an evaluation of their activity *in vitro* using a breast cancer cell line (MDA-MB-231). Compounds 7e and 7g induced significant toxic effects against tumour cells after 48 h incubation in normoxic conditions killing over 50% of tumour cells at 3 mM, but their efficacy decreased in hypoxic conditions reaching the 50% of the tumour cell viability only at 30 mM. These unusual features make them interesting lead compounds to act as antitumor agents, not only as carbonic anhydrase IX inhibitors, but also in different pathways, where hCA IX is not overexpressed.
(**PDB Structures:** 6PGX)

Jérôme Le Nour, et al., A class of γδ T cell receptors recognize the underside of the antigen-presenting molecule MR1, *Science*, 366, 1522 (2019).

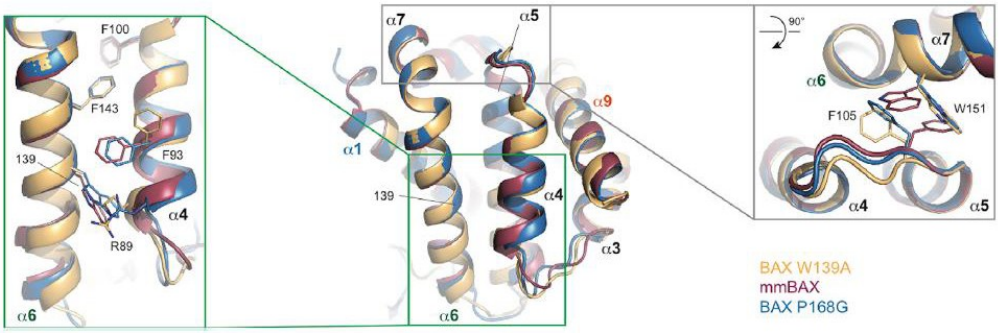


Cartoon representation of the G7 gdTCR–MR1–5-OPRU ternary complex:

The ligands bound by **γδ** T cell receptors (TCRs) are less well characterized than those of their αβ TCR cousins, which are antigens presented by major histocompatibility complex (MHC) and related proteins. Le Researchers from *Monash University, the University of Melbourne, Peter MacCallum Cancer Centre, the Walter and Eliza Hall Institute of Medical Research, the University of Queensland, the Hudson Institute of Medicine, the Alfred Hospital, Melbourne,* and *Cardiff University* identified a phenotypically diverse **γδ** T cell subset in human tissues that reacts to MHC-related protein 1 (MR1), which presents vitamin B derivatives. These MR1-reactive **γδ** T cells were found in both health and diseased tissues (for example they were found to be highly enriched in a tumour infiltrate from a Merkel cell carcinoma patient); suggesting roles in both physiology and pathology.

A crystal structure of a **γδ** TCR–MR1–antigen complex determined using the Australian Synchrotron revealed that some of these TCRs can bind underneath the MR1 antigen-binding cleft instead of recognizing the presented antigen. This work thus uncovers an additional ligand for **γδ** T cells and reconceptualizes the nature of T cell antigen recognition. (**PDB Structures:** 6MWR)

Michael A. Dengler, et al., BAX Activation: Mutations Near Its Proposed Non-canonical BH3 Binding Site Reveal Allosteric Changes Controlling Mitochondrial Association, *Cell Reports*, 27, 359 (2019).



BAX α1 and α6 Mutations Reduce Cross-Linkage to tBID and Alter BAX Conformation

Apoptosis is controlled primarily by three factions of the BCL-2 protein family. The ‘‘BH3-only proteins,’’ which possess a single domain related to BCL-2, convey stress signals, but the essential apoptosis

effectors are BAX and BAK, which, when activated, permeabilize the mitochondrial outer membrane (MOM).
To elicit apoptosis, BAX metamorphoses from an inert cytosolic monomer into homo-oligomers that permeabilize the mitochondrial outer membrane (MOM). A long-standing puzzle is that BH3 domains apparently activate BAX by not only its canonical groove but also a proposed site involving helices a1 and a6.

Mutagenesis studies by researchers from the *Walter and Eliza Hall Institute of Medical Research* and *Melbourne University* reveal that late steps like oligomerization require activation through the groove but probably not earlier steps like MOM association. Conversely, a1 or a6 obstruction and alanine mutagenesis scanning implicate these helices early in BAX activation. The a1 and a6 mutations lowered BH3 binding, altered the BAX conformation, and reduced its MOM translocation and integration; their exposure of the BAX a1-a2 loop allosterically sequestered its a9 membrane anchor in the groove. The crystal structure of an a6 mutant determined **using the MX2 beamline and the ACRF Detector**, revealed additional allosteric effects. The results suggest that the a1 and a6 region drives MOM association and integration, whereas groove binding favours subsequent steps toward oligomerization. (**PDB Structures:** 6EB6)