Australian Synchrotron Development Plan
Project Submission Form

Section A: Summary and Proponent Details

Project Title

High-performance macromolecular crystallography beamline (HPMX)

Spokesperson

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Executive Summary (approx. 100 words)

The high-performance macromolecular crystallography beamline (HPMX) will meet the needs of the MX community studying membrane proteins, large protein and nucleic acid complexes, naturally-occurring crystals and other challenging samples that are too small or weakly diffracting to be tested on the existing MX beamlines. The most important targets for the design of novel drugs include difficult large assemblies such as membrane-bound complexes, proteasomes, RNA/DNA polymerases, ribosomes and ever larger and biologically more relevant protein-protein, protein-RNA and protein-DNA complexes. Such structures rarely produce crystals of sufficient size for analysis using traditional MX beamlines. A micro-focus beamline well beyond the capability of the existing MX beamlines is essential for studying increasingly common sub-5μm crystals. Thus, the 5μm × 5μm focus of this beamline delivering greater than 10^{12} ph/s coupled with an ultra-high performance detector will provide a state-of-the-art facility for Australasian researchers, permitting high-impact science on these crystals to be carried out at the Australian Synchrotron.

Other proponents (add more rows if necessary)

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Section B: Detailed Description

B1: Description of Proposed Beamline/Development Project

1. Source

A 3m in-vacuum undulator is required to provide a source of sufficient brilliance and size for the HPMX beamline. A water-cooled copper mask will reduce the heatload of the transmitted beam to the downstream optics.

2. Monochromator

The double crystal monochromator will consist of two Si111 crystals with the first crystal indirectly cooled with liquid nitrogen and the second crystal indirect cooled via copper braids to the first crystal cage. This design will reduce vibration of the second crystal assembly and improve beam stability at the sample.

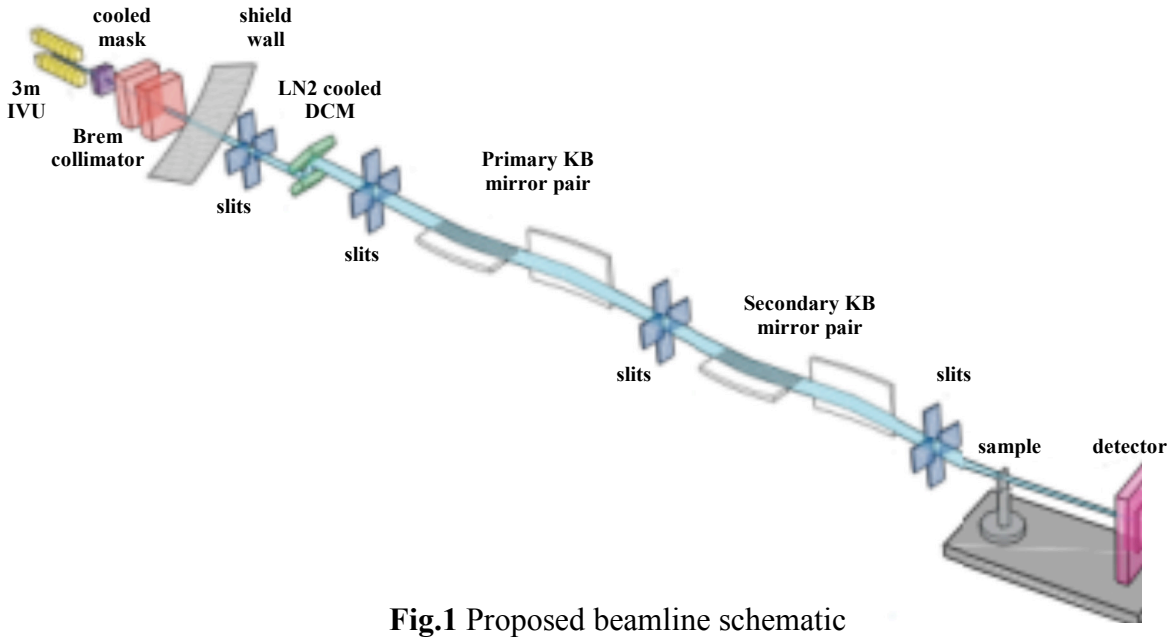

Fig.1 Proposed beamline schematic

3. Focusing elements

The beam will be initially focused *via* a pair of Pt- and Rh-coated silicon mirrors in Kirkpatrick-Baez (KB) geometry. These mirrors will be pre-ground to shape and equipped with hydraulic U-benders for precise focusing. This mirror set will produce a virtual source for a second KB mirror pair by focusing on set of X/Y slits immediately upstream of the KB set. The second set of mirrors will be adaptive mirrors to deal with imperfections in structure of the incoming beam and allow for the best possible focus at the sample. These mirrors will be sited in the endstation on a granite slab to minimize vibration. Temperature of the slab and surrounding environment will be tightly controlled via a process cooler to within 0.2 °C of ambient.

4. Endstation

The beam conditioning elements will need to be carefully constructed to reduce their length in the down-beam direction due to the requirement to place the final mirror tank as close to the sample as possible. At a minimum these elements must include slits, attenuators, beam position monitoring equipment and a fast shutter. The real-time video based beam alignment system that has been developed for the MX2 beamline will be ideal for this purpose due to its high precision, speed and compact size. A further alternative to be considered is the Microdiffractometer MD2 system, such as is in used at the APS and ESRF.

To reduce vibration the goniometer will be mounted on the same granite slab as the final focusing mirror tank. The sphere-of-confusion (SOC) of the goniometer must be less than 0.3μm and an alignment stage such as a membrane-based flexure driven by piezo-screw actuators may be required to allow collection of single crystal data from micro crystals.

Thermal stability of the endstation will be provided via the same process cooling system that feeds the optics hutch. Increased thermal stability of key endstation components will be provided by a secondary system consisting of water-cooled copper blocks clamped to components and fed from a chiller external to the hutch.

A pixel-array detector (such as a Pilatus 6M) will allow "shutter-less" data collection and allow for both low-noise and high-speed data collection. The large dynamic range and low point-spread function of such a detector is essential with a beam of such intensity and small size. The detector-Z stage will allow for a closest approach of less than 90 mm and a maximum crystal-to-detector distance of 1 m. Samples will be cooled to 100 K using a conventional cryocooler.

A sample mounting robot such as the Stanford Auto-Mounter (SAM) robot will be fitted to the endstation to allow for high-throughput and remote access use.

B2: Applications and Potential Outcomes to Australian Scientific Community
How does the project advance synchrotron-based research in Australia/NZ? What are the likely outcomes? Include specific examples where possible.

Frontier projects in protein crystallography

The proposed High Performance Macromolecular Crystallography facility will provide world best facilities for difficult yet high profile projects on the frontier of protein crystallography. Australasian scientists are prominent in fields of protein crystallography targeting a range of proteins important to human health such as membrane proteins and hormone:receptor complexes.

Australia's outstanding record in the field of structure-based drug design is founded on the ability to solve the structures of these molecules, yet it is well recognized that these projects rarely produce crystals of sufficient size and diffraction quality for use on conventional synchrotron beamlines. Currently several members of the Australasian community are forced to travel to facilities in Europe and the USA in order to collect data for these projects; although the AS cannot provide details, we understand that six groups have sought funding to travel abroad to collect microfocus data in the last year alone.

Specific Projects:

The following example projects were selected from a larger number of contributions due to space limitation. Usage will certainly not be restricted to these themes. Each of these high-profile projects displays a clear recent, or current, need for a microfocus beamline.

Prof Peter Metcalf (University of Auckland): Naturally occurring protein microcrystals

Micro-focus protein crystallography beam-lines are typically used to diffract small locally ordered regions of crystalline aggregates, and have been used recently to determine structures of pharmacologically important GPCR membrane proteins (1), previously impossible because of poor crystal quality. We use micro-beams for an even more challenging task - collecting data from micron sized crystals, work which requires both a stable micron-sized beam, and also tools to rapidly locate and centre the tiny crystals, which survive only briefly in the beam.

Since 2003 we developed methods to collect data from micron-sized protein crystals, using the 5x15 micron focus PX1 beamline at SLS, visiting once or twice each year. These visits typically involve a week away from Auckland including about 50 hours of traveling, 48 hours at the beam and leave us exhausted!

We used the data to determine the atomic structures of insect virus polyhedra, stable virus containing protein crystals that form inside infected cells. We have determined structures from the two main polyhedra types that produce micron sized crystals (2,3) and recently in addition of 400nm 'nano-crystal' polyhedra using both powder and micro-crystal diffraction methods. In future we intend to use these structures as stable platforms to create protein based devices, using protein engineering methods and exploiting the extreme stability of polyhedra. We are enthusiastic about the possibility of collecting micro-crystal data in Melbourne on a regular basis and strongly support the plan to build a 5 micron focus beam line.

(1) Rasmussen et al et Kobilka. "Crystal structure of the human beta2-adrenergic G-protein-coupled receptor". Nature 450 (2007): 383–7.
(2) Coulibaly et al et Metcalf. "The molecular organization of cypovirus polyhedra." Nature 446, no. 7131 (2007): 97-101.
(3) Coulibaly et al et Metcalf. "The structure of baculovirus polyhedra reveals the independent emergence of infectious crystals in DNA and RNA viruses". Proc Natl Acad Sci (USA) (2009) under review

Dr Fasseli Coulibaly (Monash University): Structural virology.

I study viral occlusion bodies that constitute the main infectious forms of many insect viruses. These natural crystals function as crystalline armors analogous to bacterial spores that allow the virus to remain viable for years in the environment. Despite decades of research, their structure remained elusive until recently because the crystals are produced in vivo, in infected cells, and are thus intrinsically limited in size (<15 μm in diameter).
I recently determined the structure of two types of occlusion bodies, called viral polyhedra, by X-ray microcrystallography with colleagues from the University of Auckland (see Metcalf above 2,3). These studies revealed the molecular architectures of viral polyhedra and suggested the molecular bases for their outstanding robustness. Importantly, we found that polyhedra could have converged to the same function and overall structure from different precursors. I have now engaged in the structural study of a third class of occlusion bodies, the spheroids of poxviruses that are remarkable by their ovoid shape and distinctive molecular organization. These structural studies of natural crystals may allow the development of improved bioinsecticides and I investigate the development of engineered microcrystals for biotechnological applications for instance as novel vaccines.
Because the diffracting power of spheroids is even weaker than that of polyhedra, this project is critically dependent on a high-flux, focused X-ray beam of the highest quality and will benefit enormously from the proposed beamline.

Richard Kingston (University of Auckland): Viral architecture

My laboratory uses biophysical techniques to investigate virus architecture, assembly and replication. X-ray diffraction studies on viral proteins form a large part of this work. Because many of the proteins under study self-associate into large assemblies, the crystals we generate are often of small size; have large unit cell dimensions; and diffract X-rays only weakly. For example, we are currently preparing crystals of a retroviral capsid protein to enable us to visualize the basic building blocks of the authentic viral capsid. However despite optimization, the crystals remain extremely small. Convenient access to a microfocus beamline would greatly increase the tractability of this and similar projects.

Dr Jacqui Gulbis (WEHI): Potassium channel structures

Potassium currents provide electrical activity vital to organ function, and are responsible for K^+ flux across cell membranes. Crystals of solubilised integral membrane channel assemblies are

typically columns of protein molecules separated by micelle-like lattice contacts. Diffraction from lipid / detergent-mediated lattices is characteristically weak, and may suffer from anisotropy, marked diffuse scattering and high local variability in spot profile. Consequently, in contrast to lattices dominated by protein-protein contacts, data testing and collection from crystals of lipophilic proteins is rarely feasible with laboratory standard X-ray detectors. To comparatively assess crystals and collect full native or derivative datasets we often require access to synchrotron radiation with a high intensity microfocused beam with an option for fine phi slicing to enhance spot profiling. As protein-detergent crystals can be extremely radiation sensitive, a means to attenuate the beam to a suitable intensity for each crystal is desirable.

Prof Jamie Rossjohn (Monash University): Processes central to infection and immunity.

Our work in infection has focussed on the interactions between an AB5 toxin from pathogenic *E. coli* that specifically interacts with sugars that are acquired via dietary intake by humans. Whilst the interaction is very specific between the toxin and the non-human sugar is very specific, the affinity is very low, and thus it is very difficult to grow large crystals of such complexes.

The human adaptive immune system is critically dependent on the interactions of T-cell Receptors (TCRs) with Antigen (Ag) presenting molecules such as the Major Histocompatibility Complex (MHC) molecules or MHC-like molecules that are associated with foreign antigens (eg from viruses). This MHC restricted response, the discovery of which was recognised by the 1996 Nobel Prize to Zinkernagel and Doherty, shows remarkably specificity yet is dominated via very weak interactions. We still do not understand the structural basis of MHC-restriction, and as the affinities for the TCR-MHC or TCR-MHC-like interactions are very low (much lower than Antibody-Ag) interactions, it is extremely difficult to grow crystals of these complexes, and the crystals that do form are often fragile and very small. Thus, the ability to collect data on these microcrystals will be greatly enhanced by ready access to a dedicated micro-focus beamline.

Recent relevant publications: Byres *et al* Nature 2008, Borg *et al* Nature 2007, Paton *et al* Nature 2006

Prof Geoffrey B Jameson (Massey University): Protein clusters of regulation and substrate trafficking

To better understand processes at the cellular level, we are looking to move away from analysis of individual components to analysis of multi-component clusters, such as found in fungal gene clusters of secondary metabolism, where the product of one enzyme becomes the substrate of the next. Indole-diterpene pathways are of particular interest. In collaboration with Emily Parker at the University of Canterbury, we have also begun investigations into regulation of protein activity by a non-covalently associated regulatory protein whose gene is co-located in the operon expressing the protein of interest. Metabolic pathways found in microorganisms but not in humans are a particular focus here.

These new ventures require a very intense source coupled with a large pixel-array detector with very rapid read out, such as the proposed Pilatus 6M, allowing shutterless data-collection with fine phi-slicing to optimise signal:noise from small crystals with large unit cells.

Dr Daniela Stock (Victor Chang Cardiac Research Institute): Membrane protein complexes

Our work focuses on structures of biological rotary motors and other large and dynamic macromolecular assemblies. Both the trans-membrane nature and the size and dynamics of these protein complexes represent extreme challenges to crystallisation and structure determination. In the past we used high-end undulator beamlines with microfocus optics at APS (14-ID and 23-ID) and at ESRF (ID 14-4) to be able to collect useful data sets that ultimately allowed structure determination. A high-brilliance microfocus beam allows to expose only small parts of a crystal that might be better ordered than others and also allows to collect more isomorphous data from the same crystal by shifting the crystal in the beam after a few images. One essential requirement for SAD data collection is the inverse beam mode, i.e. the spindle needs to be rotated by 180º after every image, so the crystal decay has minimal effect on the anomalous signal. This puts extreme requirements on the mechanics of the spindle axis.
One of our current foci is on the structure determination of A-ATPases. H^+-ATPases are central to biological energy conversion. They use a rotary catalytic mechanism to either convert energy derived from respiration or photosynthesis into the universal biological energy carrier ATP, or in reverse by building up transmembrane ion gradients used to power membrane transport processes. While eukaryotes have both F-type ATP synthases to synthesise ATP and V-ATPases to build up ion gradients, eubacteria and archaea have typically only one enzyme for both tasks. The bacterial A-ATPase is related to eukaryotic V-ATPases, but has additional regulatory mechanisms to allow functioning as an ATP synthase. Thus, by studying A-ATPases we can gain insight into the structure and function of eukaryotic V-ATPases, into the mechanism of both ATP synthesis and hydrolysis and can create a basis for the development of new antibacterials.
Another project involves structural studies of the bacterial flagellar motor, a mega-Dalton membrane protein complex that is responsible for the motility of most bacteria. Similar to ATP synthase it is powered by a transmembrane ion gradient, but unlike the A-ATPase it can switch its sense of rotation while the ion gradient runs in the same direction. Switching from counter-clockwise to clockwise rotation allows the bacterium to change its trajectory towards chemical attractants and away from repellents. Studying these mechanisms will not only provide insights into fundamental biological processes but will also provide another basis for the development of antibacterials.

Prof Michael Parker (SVI): Membrane receptors

The Parker lab has been working on a variety of membrane-associating proteins for over 20 years with a recent focus on membrane-bound receptors. Many of these receptors consist of multiple subunits and often crystallise as microcrystals with large unit cells. A couple of examples are described below.

Alzheimer's disease (AD) is a neurodegenerative disorder characterised by the presence of extracellular amyloid plaques in the brain. The major component of these plaques are aggregates of a 4kDa peptide, Abeta, a peptide that is proteolytically derived from the membrane-bound

amyloid precursor protein (APP) by a family of proteases referred to as secretases. Abeta aggregates have been shown to be toxic to neurones. We have recently produced microcrystals of an APP construct including the sites of action of each of the secretases. We have crystallised a subunit of the gamma-secretase complex called nicastrin. This is an important target as this subunit binds the APP C-terminal product of beta-secretase cleavage and recruits this to the gamma-secretase complex where the neurotoxic Abeta peptide is generated. We have generated microcrystals (5-30 microns) of nicastrin.

Granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3) and interleukin-5 (IL-5) are members of a family of cytokines that regulate the survival and proliferation of haematopoietic cells. The actions of GM-CSF and IL-3 are particularly important in various forms of myeloid leukaemia. We have recently determined the structure of the GM-CSF receptor using synchrotrons which reveals a hexamer comprising 2 molecules each of GM-CSF, receptor alpha chain and receptor beta chain (Hanson et al., 2008). An unexpected and novel dodecameric complex was also observed involving the interaction of 2 hexamer complexes and offers exciting insights into a novel receptor activation mechanism. We now plan to extend our structural studies to the IL-3 and IL-5 receptors. Microcrystals of binary and ternary forms of these receptors are already at hand.
All these crystals have been to APS GM-CA where we saw some limited diffraction.
(1) Hansen et al et Parker (2008) The structure of the GM-CSF receptor complex reveals a distinct mode of cytokine receptor activation. Cell, 134, 496-507.

Dr Peter Turner (University of Sydney, on behalf of the Australasian Small Molecule Crystallography community): Challenging microscopic samples

The determination of the relatively small atomic structures comprising microporous and mesoporous materials, hydrogen storage materials, novel metal oxides and ceramics, superconductors, minerals, 'smart' materials, piezoelectric materials, novel magnetic materials, photonic devices, information storage materials, molecular switches and sensors, biomimetic materials, and pharmaceutical materials is crucial to their rationalisation, development and utilisation. Such materials all too often crystallise as no more than 'powder material' of micron size or smaller particles. A world class micro-focus beamline at the Australian Synchrotron Facility would then provide Australian chemical, biochemical, pharmaceutical, geochemical and materials researchers with a leading capability to obtain structures from highly challenging samples of national and international scientific significance.

B3: Match to Selection Criteria

Projects should meet as many as possible of the following criteria:

Meet the demands of an identified group of researchers for new techniques

Frontier Protein Crystallography

Provision of a high flux micro-focus beamline at the Australian synchrotron will provide facilities to Australian and New Zealand scientists equal to world best standards. Similar facilities currently exist at the Swiss Light Source (SLS), the Advanced Photon Source (APS) and the European Synchrotron Radiation Facility (ESRF). Construction of the proposed High Performance Macromolecular Crystallography beamline at the Australian synchrotron will provide Australasian scientists with cutting-edge facilities to perform high-profile research.

This proposal links closely with the SAXS-CD proposal being developed. The capability proposed here will allow entire assemblies to be structurally characterised in three dimensions to complement the solution-state one-dimensional SAXS-CD data. It is important to note that SAXS-CD data of multi-component systems can not always be modelled using three-dimensional structural information of the individual components, as obtained by X-ray (and NMR) methods.

This proposal also dovetails with plans (*q.v.*) to upgrade the existing MX1 and MX2 beamlines in terms of providing a wide suite of structural biology capability at the Australian Synchrotron.

Expansion of existing single-crystal analysis capacity.

Currently requests for beamtime on MX1 and MX2 at the Australian Synchrotron saturate the available capacity. It is anticipated that supply will soon outstrip demand due to:

- Expansion in the Australian and New Zealand crystallographic community, e.g. new laboratories now being established at La Trobe University and in Adelaide.
- The increasing use of robotics in macromolecular crystallization. This will increase the success rate of crystallization thus increasing the demand for beamtime.
- The growing demand for beam time on MX1 and MX2 due to the establishment of Fragment Based Drug Discovery projects and other projects requiring high-throughput crystallography faciltites.
- The growing use of the MX beamlines by the small-molecule crystallography community.

We note that the original National Science case for the Australian Synchrotron (Chapter 4) indicated that MX would ultimately be served by two in-vacuum undulator beamlines and this bid is thus entirely consistent with the anticipated provision of support by the synchrotron for the highly successful and expanding Australasian structural biology community.

Take advantage of the existing third generation light source

This beamline will require an extremely intense X-ray source with a small source size to allow focusing to a 5μm x 5μm spot size at the sample. Comparable microfocus MX beamlines at other facilities such as X06A of the SLS, ID23-1 of the ESRF and 24-ID-C of the APS all use undulators as sources. Due to the requirement for high flux to allow for losses due to microfocussing a three meter in-vacuum undulator is required and hence a 3rd generation source.

Will position Australasian scientists at the leading edge of their field

Current groups working on medically-relevant projects for which only small, weakly diffracting crystals are achievable are forced to travel to overseas facilities to collect data. This places a distinct disadvantage on these researchers compared to counterparts in the USA and Europe. The High Performance Macromolecular Crystallography beamline will provide a local facility for these groups to perform experiments essential to their work.

It is imperative that the single-crystal beamline facilities are expanded to in order to keep up with the anticipated demand. This will ensure that the Australian Crystallographic community maintains its position at the leading edge of this important field.

This proposal has been conceived as part of a concerted approach to providing a world-class Structural Biology facility, along with the proposals for a new SAXS-CD beamline, and Major Facilities upgrades to existing beamlines (MX1 and MX2 and SAXS/WAXS). This will place the Australian Synchrotron's integrated Structural Biology capability at the forefront of world science.

Can be demonstrated to be feasibly constructed within a 3 year time-frame

The construction of the HPMX beamline can feasibly be carried out within a three-year timeframe. The beamline components such as the IVU, hutches and optics will be based on existing technology and the facility has experience in beamline construction.

B4: Potential Users

Does the project address a clearly identified need in the community? The need may be actual or potential.

The High Performance Macromolecular Crystallography Beamline will address the following current and predicted needs of the crystallographic community in Australia and New Zealand.

- The unmet need for a high-energy microfocus beamline to enable work on high impact projects with small, weakly diffracting crystals of large protein assemblies.
- Expansion of the capacity for single crystal analyses at the Australian Synchrotron, both Protein Crystallography and small molecule, to address an anticipated very large shortfall in beamtime availability.
- Fulfilling an integrated approach to world-class Structural Biology research.

An indicative list of the organisations that would use this facility are listed below.

Australian National University

CSIRO

Curtin University

Flinders University

Griffith University

Latrobe University

Massey University

Monash University

St Vincent's Medical Research Institute

The Walter and Eliza Hall Institute of Medical Research

University of Adelaide

University of Auckland

University of Canterbury

University of Melbourne

University of New South Wales

University of Otago

University of Queensland

University of Sydney

University of Tasmania

University of Western Australia

University of Wollongong

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