Interim Report: MXe/MX3D proposal

Section A: Summary and Proponent Details

Project Title

MXe: A Flagship Crystallography Environment Serving Australasian Biotechnology and Smart Materials Research for the Next Decade

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Executive Summary (approx. 100 words)

Crystallographers are leading the use of the Australian Synchrotron in internationally competitive fundamental and translational research. To create a cutting-edge, high-speed Macromolecular Crystallography Environment (**MXe**) that supports this valuable research through 2020 requires investment in four areas: (1) Enhancement of small-molecule crystallography capabilities of beamline MX1 to rival world standards; (2) Upgrades to beamline MX2 to enhance its outstanding microfocus functionality; (3) Construction of a unique, leading-edge new undulator beamline, **MX3D**, aimed at providing an unprecedented high-throughput platform for automated **D**iffraction screening and **D**rug **D**esign; and (4) Provision of a high-performance, secure, integrated data-handling infrastructure for molecular structure research as performed on the X-ray diffraction and scattering beamlines.

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Section A2: Summary of current status.

A2-1: Current MX beamlines

MX1 and MX2

The two current MX beamlines at the AS are MX1 (Macromolecular crystallography) and MX2 (Micro-crystallography). A summary of their capabilities is shown below:

	MX1	**MX2**
Source	Bending Magnet	In Vaccum Undulator
Working Energy Range	6 – 17.6 keV	6 – 20 keV (Si111) 8 – 27 keV (Si311)
Beam @ Sample (H x V)	150 x 150 micrometers	30 – 30 micrometers
Monochromator	DCM (Sagittally bent 2nd crystal)	DCM. Two crystal sets (Si111 and Si311)
Flux @ 12.6 keV	1.5 x 10^{11} photons/sec	4 x 10^{12} photons/sec
Detectors	ADSC Quantum 210r	ADSC Quantum 315r
Fluorescence Detector	Vortex-ES Si drift detector	Vortex-ES Si drift detector
Micro Collimator		Beamsize 5, 7.5, 10, 20μm
Robotic sample Mount	Epson	Epson
Sample Storage	288 Samples	288 Samples
Horizontal focus	Sagitally bent DCM 2nd crystal	Horizontal focusing mirror (Mechanical bender) and second hirozontal focusing mirror (bimorph)
Vertical focus	VFM (bender)	VFM (bimorph)

A2-2: What are the existing beamlines used for?

MX1 :

- **Small molecule crystallography (SMX)**
- ~20% of allocated time is used by Small Molecule Crystallographers (SMX)
- This consists of ~40 individual users
- SMX use has increased 2 fold since 2009
- The increased user base is due to both speed and ease of use
- Now essential to some SMX users as datasets take 15 minutes on site, in contrast to 1 day or more in-house
- SMX go from data to structure on-site (average 20+ 'new' structures solved per 24 hour period) a further 20+ require further work off-site
- **Macromolecular Crystallography MX**
- ~75% of allocated time is used by Macromolecular Crystallographers (MX)
- MX1 is the 'work horse' beam line
- Most allocated time data is collected (~10% of time screening crystals)
- SAD/MAD experiments are possible and structure determination routine

- Many MX users utilize the in-house Auto-processing, and structure solution. This reduces the number of 'poorly' collected datasets.
- **Non-Standard Use**
- ~5% Allocated time used for 'non-standard' hard X-ray experiments (tomography, powder like diffraction, elemental detection etc)

MX2 :

- **SMX**
- ~10% of allocated time used by Small Molecule Crystallographers (SMX)
- Projects not possible on MX1, Small or weakly diffracting crystals
- Crystals with intrinsic defects requiring only a small (5μm) area of the crystal to be illuminated
- **MX**
- 85% of allocated time used by Macromolecular Crystallographers (MX)
- Projects that are not possible to undertake on MX1 I.e. small (>30μm) crystals
- Projects that require higher flux and smaller beam size
- Important to 'match' the crystal size with beam size
- Weakly diffracting crystals, intrinsic defects in the crystal etc..
- SAD/MAD routine (5μm crystal structure solved)
- Projects that 'fail' on MX1 can often produce data of sufficient quality to solve the structure on MX2
- 5% in-situ diffraction experiments (limited to a few tray types)
- This allows screening of crystals in trays without the need to identify a cryoprotectant. Increasingly popular but very time consuming as 30 minutes setup/reset time needed and around 2 hours per tray.

A2-3: Use of complementary Beam lines by the structural biology community:

Many of our users are using other beam lines at the Australian Synchrotron that are complementary to X-ray crystallography. Small Angle X-ray Scattering is one of the most commonly used techniques. With the advances in gene technology and protein production and purification, the ability to produce a protein sample to analyze has become relatively straightforward. Whilst producing the protein is commonplace, getting crystals of these proteins is not. This results in many users having samples suitable for small angle X-ray scattering (SAXS) experiments. The SAXS beamline at the AS is world leading in the field of biological SAXS (Bio-SAXS).

The synergy of SAXS and X-ray crystallography produces a far wider dynamic range of problems that can and will be studied. Whilst crystallography produces a snapshot of how a protein looks, in SAXS the protein may often adopt many shapes/forms with mobile regions in the protein shifting position. This fluid nature of the protein allows the users to visualize changes to their protein(s) in real time I.e. the addition of metal ions, binding partner protein, drug molecules. With SAXS large shifts of the overall shape of the protein (oligomerization, gross structural changes etc) can be observed where observing such shifts would not be possible in a crystal structure without damaging/destroying the crystal lattice. These

structural changes also provide information on conditions that produce a more stable form of the protein, and this information can be fed back into crystallization experiments.

A2-4: Oversubscription

- The number of shifts/experiments applied for on the current 2 MX beam lines by established users outstrips the available shifts (over 160% oversubscription).
- This results in fewer shifts being awarded than requested per experiment.
- The number of new users from both SMX and MX communities are increasing each run. Each year new users apply from established labs AND new institutes, both national and international (recent examples: China, Japan, Korea, Singapore).
- This increased user base adds further pressure onto the 2 existing MX beam lines, reducing further the time allocated per successful experiment, with more users being allocated zero shifts.

A2-5: Publications

In 2010 (the last full year statistics are available for) the Australian Synchrotron produced:

- 166 papers in total
- 50% of which result from work on MX and SAXS/WAXS
- With 37 of these publications in A^* or A journals
- To date, of the facilities 28 papers with an impact factor greater than 9.0 25 are from structural biology (89%).
- This includes 2 papers in Nature, 2 in Nature Immunity, one in Cell and 4 in Immunity.

A3-1: Proposed MX3Dbeamline

Summary of New capabilities on MX3D:
MX3D Differences to existing beam lines: MX3D will be a highly automated beam line allowing experiments not currently possible on MX1 and MX2 to be undertaken.

1. **Automated Tray Storing, Handling, & Screening**
2. **Automated Sample Tracking (trays, cassettes, pins)**
3. **New Software to perform the above tasks (to be written in-house) & Hardware (developed in-house)**
4. **High speed sample transfer, alignment and data collection**
5. **Automated Data Handling (collection, processing, structure solution)**
6. **Dedicated optics for the above applications**

Automated Tray Storing, Handling, & Screening

- As at crystallisation facilities barcodes will be used to track many thousands of trays and experiments.

- Users will be able to submit trays to undergo screening. Trays will arrive from crystallisation facilities around Australia and be stored in temperature-controlled incubators.
- For screening 25 plates can be loaded into a mounting system (similar to the racks found in-side crystal hotels).
- The robot gripper will select a plate (based on barcode information) and move the selected tray to the X-ray beam.
- Trays will then be screening in one of 2 ways:
    - Multiple hits (30+) each well will be screened.
    - If only one or two hits are found in a tray the location of the crystals will be pre assigned
- The diffraction quality of each and every crystal hit can be evaluated.
- This removes human 'error', which can damage a crystal during mounting.
- Any diffraction observed from in-situ diffraction room-temperature experiments is a true representation of the crystals nature.
- By pre-screening trays for diffraction quality the user saves both time and expensive material thus increasing user output..
- The new software needed will be written & implemented by beam line staff.

High speed sample transfer, alignment and data collection

- Number of modifications and new features must be implemented.
- Barcode readers for the samples and cassettes (we are currently working with Crystal Positioning Systems to implement 2D barcodes on their cassettes).
- Automated identification is essential for this system to work, as it would be impossible to track samples manually.
- All code written to track and manipulate samples available throughout the Australian Synchrotron.
- With developing expertise in robotics and automation beam line staff will be involved in generating robotics specifications/standards.
- We need a total redesign of the current MX end station and robot(s) positioning.
- We expect to collect data on 'every' crystal mounted, with 30 – 60 seconds per dataset

Challenging projects:

Current projects are being undertaken by the MX user community that require the screening and data collection on thousands of crystals to achieve the desired outcome (3D crystal structure). This requires the Synchrotron (MX) to modify existing technology and develop new methodologies, which will assist in tracking and analyzing the data from these projects.
For example, two extremes where thousands of crystals must be screened:

1) Rational Drug Design (Fragment Screening): In this example a library of compounds (from many hundreds to thousands) are soaked into crystals (or co-crystallised) in to the protein of interest. The proteins used in fragment screening have already been shown to crystallize readily with suitable diffraction qualities (resolution/survivability in the beam). With a library containing 500 fragments to would be necessary to collect 1000+ datasets (to ensure each fragment is visualized). Using the current beam lines this would

require 10 days, on the highly automated MX3D beam line this could be achieved in 16 hours.

2) Membrane proteins/Protein Complexes: As these type of projects often result in small weakly diffracting crystals, it is often necessary to screen hundreds/thousands of crystals in order to find a crystal suitable for data collection (or to merge data from many crystals). With a time frame similar to above.

Automated Data Handling (collection, processing, structure solution)

Currently diffraction data from the beam lines is automatically processed and reduced to a standard reflection file (.mtz). Users know they have 'complete' data before removing the sample and moving onto the next.

- This approach will no longer be viable on MX3D
- The number of samples and speed of data collection will far outstrip even the most experienced crystallographer.
- Existing automatic data processing will be taken a stage further with MX3D:
- The reduced data file will be 'fed' into a series of programs. Depending on how the structure is to be solved (molecular replacement, SAD/MAD etc).
- Resulting in 'automatic' structure determination, allowing users to quickly analyze their structures.
- Important for cases such as fragment screening, (is the fragment bound), and SAD/MAD experiments (has the structure been solved).

Why can these experiments not be done on MX1 or MX2:

Current beamlines:
MX1 high throughput
MX2 micro crystallography

New beamline:
MX3D tray screening and ultra-high throughput

The rate-limiting step in macromolecular crystallography is the production of crystals suitable for diffraction experiments. In-tray screening is a new technique for rapidly assessing crystallisation trays to find conditions and micro-crystals using X-ray diffraction. This can greatly reduce the time required to produce crystals that arte suitable for use on MX1 or MX2. Building MX3D will greatly increase the output of MX1 and MX2 via helping users to produce better crystals.
In addition, fragment screening requiring ultra-high throughput will now be possible and this will reduce the load on MX1, which is facing increasing demand from the SMX community.

While manual tray screening can be done on MX2 it is far too slow for high-throughput use. It takes ~30 minutes to switch the beamline from normal mode to tray screening mode, about 2 hours to shoot a plate (assuming 20 drops are tested per plate) and ~30 minutes to reset the beamline. In contrast a plate should take 240 seconds to shoot 20 drops on MX3D with minimal

changeover time. Given the heavy oversubscription of MX2 it is not possible to use this beamline for the large volume of tray screening needed. MX1 is unusable for tray screening as the flux is too low and beam size too large. To use tray screening effectively large numbers of trays need to travel from crystallisation facilities to the AS, be screened in a highly automated manner and the data uploaded for the users to analyze. This is not technically feasible on MX2.
While both MX1 and MX2 have sample changing robots these are not fast enough for ultra-high throughput screening and collection needed for fragment screening studies. As with tray screening the large amount of beamtime needed to carry out extensive fragment screening is just not available on MX1 or MX2.

Original Duration	Start	Finish	Before Funding	Funding Announced Day 1	Year 1 (by month)	Year 2 (by month)
Conceptual Design 1	01/02/2011	01/04/2011				
Preliminary Design 1	01/05/2011	01/06/2011				
Final Design						
Open Tender To build	Day 1					
Close Tender To Build						
Contract Awarded						
IVU Contract						
Robotics Contract						
Optics Contract						
Hutch Contract						
Acceptance of Final Design						
All PDRs Complete						
All FDRs Complete						
Payment (mirror substrates) 10%						
Payment (DCM goniometer & vessel) 10%						
Payment (motion controls) 5%						
Payment (Slits, Diagnostics, Shutter) 5%						
Payment (mirror vacuum vessel) 5%						
Payment (mirror optics & bender) 5%						
Hutch's Installed						
Robotics FAT						
Hutches Validated						
Completion FAT						
Delivery of Components to Site						
IVU Installed						
Optics Installed						
Completion of Installation						
Cold Commissioning						
PSS Validation						
Hot Commissioning						
Expert Users						
Reduced User Program						
Full User Program						

Gantt chart of proposed MX3D build schedule.

A3-2: Capability to build the new beamline

Build Speed:

Summary of beam line design: (See section B-3 for full details)

- Extensive consultation has been undertaken with the crystallographic community (both macro-molecular and small molecule crystallographers). We have clear requirements from both communities as to what is needed/expected of a new beam line. We now have a detailed understanding of the experimental needs (in-terms of equipment) and believe that our setup fulfills the scientific requirements of our community.
- We would be able to go to tender as soon as funding is announced.
  The tender would require vendors to utilize hardware and software that are included on the Australian Synchrotrons Standards list:
  If non-standard hardware/software must be included in a design, we require a detailed reason why. For non-standard hardware we require the tender document to include costs for spares and maintenance of these parts in the contract document.

- The skills for optics design are available in-house: detailed optics plans (regarding positioning and tolerances) and ray tracing have been already been undertaken in-house (TCD & NK).
- We expect MX3D to utilize 'Turn Key Optics' as this will significantly reduce the time requirements: design, fabrication and installation allowing for an extremely fast overall build schedule.
- We believe the use of 'off the shelf' components where needed and the advanced state of the beam line plans mean we can build a new beam line within a tight time schedule. The performance of such components has already been proven on MX2 and its upgrades.
- Due to these reasons, if funding is approved for a third MX beam line we are confident that it will either be the first beam line completed or first equal with BioSAXS.

A3-2: Worldwide trends in MX beamlines:

MX3D will be a world leading beam line with the possibility to undertake experiments on samples that it would not previously have been possible to solve.
Much of what is planned for MX3D has also been suggested at other synchrotrons around the world. One such common element is for MX to work with other synchrotron techniques (XAS, SAXS/WAXS, IR etc) to form the basis of a 'life science centre' with adequate support facilities on site.

<u>Summary of worldwide MX beamline development trends:</u>

NSLS-II is building 2 new MX beam lines: AMX (Highly Automated Beam line), FMX (tunable 1μm beam) in the first stage of the build, with a number of other beam lines planed for later stages.

Diamond Light Source (DLS) has plans to build 2 or 3 new MX beam lines to complement their existing suite of beam lines, they will include high throughput small molecule crystallography, and *in-situ* screening. This new science would dramatically improve/increase a users ability to discover the best crystallisation conditions in the shortest possible time, cutting the time required to cycle between initial hit and usable crystals.
At **Soleil** the planned MX beam line will allow the redox state of an enzyme/protein to be analyzed by combining MX with XAS.
The **ESRF** has the most ambitious plans for expansion to its MX beam lines. With automation, screening of crystals prior to data collection and the possibility to 'choose' which beam line best suits the crystal.
The 'new' storage ring **PETRA** as well as concentrating on MX and SAXS/WAXS will build support laboratories making the synchrotron a place where protein can be produced, purified, and experimentally characterized (by crystallographic means or in solution by SAXS/WAXS).

All of the upgrade/new beam lines have the following common features:

- A center for structural biology will embrace other synchrotron-based disciplines than MX to stimulate multidisciplinary approaches to large biomedical problems.
- Able to deal with extremely small crystals in the 1-5um range
- A UV-Vis micro spectrophotometer BioXAS
- An energy-dispersive X-ray detector
- High throughput at multiple wavelengths
- Highly automated - increase efficiency, expand on remote access
- Robotic Screening
- Rapid data acquisition crucial for very short-lived samples
- Small beam (where needed): Signal to Background (noise) greatly improved allowing data to be collected on weakly diffracting crystals
- Small beam (where needed): Scan areas of the same crystal (I.e. multi-protein complexes) to locate the 'best' area to collect data
- In-Situ screening of crystals in crystallisation plates
- Rapid information on which crystal condition to 'screen around' I.e. what is salt, poorly diffracting protein, suitable for data collection crystal
- Un-necessary to screen for cryo-conditions on each crystal hit
- A robotic system has been realised that allows 'automatic' crystal mounting from the crystallisation tray, allowing a 'test-shot' to be taken on the crystal before being mounted and a complete data set collected.
- Evaluation of many crystals prior to data collection will become the norm (currently users will collect many datasets of the same protein, only 1 or 2 of which will be used).
- Crystals of biological macromolecules, show considerable variation in the quality of their diffraction, are mechanically fragile, and therefore susceptible to damage during transfer (from the crystallisation trays to the sample holders)
- Automation of synchrotron beam lines thus not only increases scientific output but it also maintains the high-level impact of the science performed.
- Tuneable (5 – 20 keV) end-station: adjustable beam size (10 um – 200 um) and specialisation for very low-resolution data collection and detection of very weak anomalous signals.

Section B: Detailed Description

B1: Description of Proposed Beamline/Development Project

Introduction

In order to support the Australasian Crystallography community's need to improve the rate of high-profile research (both fundamental and translational), we provide a vision for enhancement of the capabilities of the existing MX beamlines, integration with other related beamlines, and **MX3D** - a new undulator beamline for automated diffraction, screening and drug development. The community's work on high-value challenging structures, and on medically-urgent drug design projects require large amounts of crystal screening. MX3D will provide access to automated screening in crystal trays, automated collection after pre-alignment of crystals and ultra-high throughput data collection due to the double-tong robot and shutterless data collection. This flagship Macromolecular Crystallography Environment, **MXe**, will place the Australian Synchrotron at the forefront of world diffraction capabilities. A technical case, including detailed design considerations and projected budget is provided for each of four aspects to this project:

1. Enhancement of SMX capabilities on MX1

A key strategic objective of MXe is to enhance the capabilities of the existing high-throughput beamline (MX1). The drivers for this are three-fold: to better service our steadily-increasing existing small-molecule crystallography (SMX) user community for whom the current MX1 setup is inadequate for many sophisticated experiments; to expand our user base and access new SMX science (charge-density, high- or low-temperature phase changes); and to improve useability for macromolecular crystallography (MX) users.

1.1. Optics modifications

The focused beam-at-sample size on MX1 is approximately 150 μm x 150 μm, which is suitable for well-diffracting SMX crystals of 50-100 μm in size. To allow the beamline to accommodate the smallest SMX crystals (1-10 μm) which are currently studied on MX2, a micro-collimator with apertures of 100, 50, 20, 10 μm will be installed on MX1. However, this will necessitate increasing flux. Modifying the optics on MX1 to include a double multilayer monochromator (DMM) will produce around 50 times the existing flux of MX1 and allow for faster exposures and shutter-less data collection (in combination with a pixel-array detector) for MX experiments and SMX experiments that can be done at 13 keV. However, the DMM will be fixed at 13keV and adding a second DMM for 17.4 keV is not feasible because of the need to use sagittal focusing. The low Bragg angle of a DMM requires small a radius of curvature for sagittal focusing, which must be permanently carved into the substrate. The DMM will operate in fixed energy mode, as incorporating a multiple energy option would require a separate focusing element with a tailored curvature and/or multilayer period for each - this is technically too ambitious and is also operationally ineffective to have multiple pairs of multilayer substrates incorporated into the beamline. The first substrate (flat) will be mounted in the existing double crystal monochromator (DCM), however the second (focusing) element will have a separate vessel and motion system as it will be 716 mm further downstream (i.e. larger than the radius of the DCM vessel). The existing white beam and bremsstrahlung stops will remain. Switching modes between the DMM and DCM will allow the existing DCM optics to continue to provide

user-changeable energies and anomalous dispersion experiments such as MAD and SAD to be carried out.

The properties of the MX1 beamline with a sagittally focusing DMM using a 29.8 Å period multilayer coating have been modeled (using SHADOW, including slope error effects), allowing for a 1% bandpass:

- The horizontal acceptance should be limited to 0.8 mrad to maintain a cleanly focused beam in the horizontal plane. The horizontal divergence at the sample position will be below 1.55 mrad, or even less if the beam is further slitted down.
- The focal size of the full beam would be 140 x 150 µm. The predicted vertical size of the beam is due to anticipated tangential slope errors of the second DMM element, which should be specified at 2 to 3 microradian RMS in order to achieve this focal size. Whilst this would push the technology for large toroidal mirrors, this specification would be quite achievable for the small substrate in this application. Slope errors have little effect on horizontal focussing.
- The upgrade can supply up to 1 x 10^{13} ph/s in the full focussed beam at 13 keV, which is at least a 50x increase over the current DCM configuration.
- The final beamsize can also be controlled and reduced where needed by user-interchangeable apertures at the sample position, which are already in service at the beamline. The flux deliverable into a 150 x 150 µm beam would be approximately 6 x 10^{12} ph/s, a 25x increase over the current beamline. Notwithstanding with focal size, divergence and bandpass, these levels of flux are on par with undulator beamlines and the upgrade will lead to a dramatic improvement in capability for many applications.

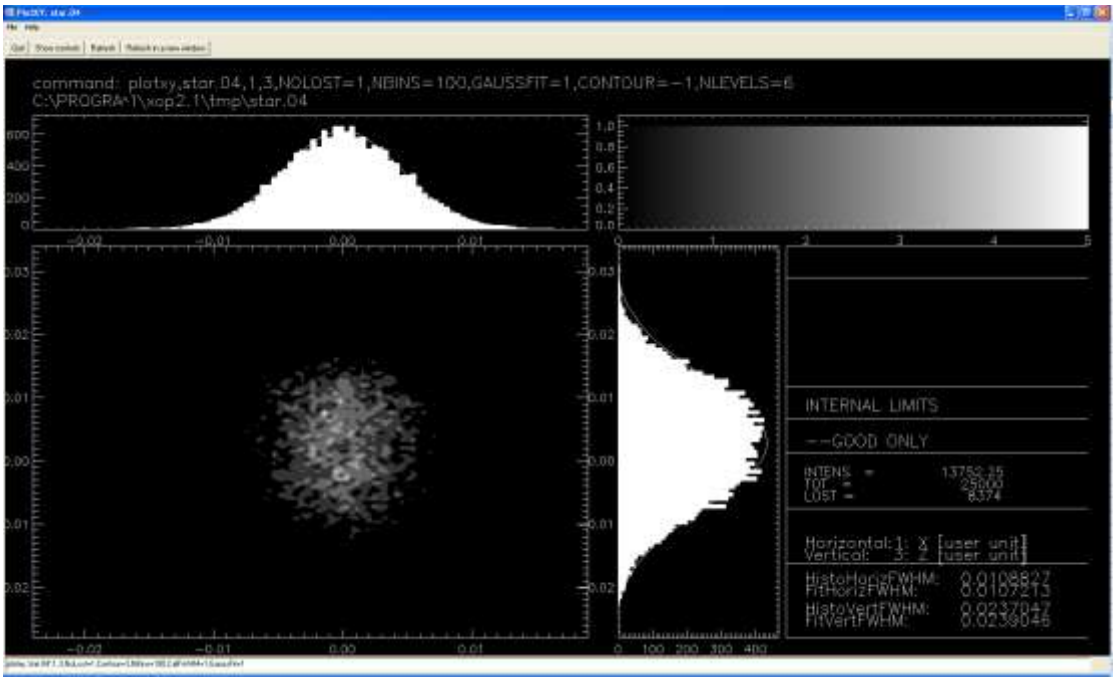


Figure 1.1: *Cross-section of beam at focal spot for a 0.8 mrad horizontal beamline acceptance.*

This 50-fold increase in flux, when combined with a micro-collimator, pixel-array detector, and high-range attenuation wheel, will allow MX1 to service the needs of both MX and SMX users.

1.2 Installation of a pixel-array detector (Pilatus 2M)

The installation of a pixel-array detector offers two significant advantages over the current CCD detector on MX1: speed of data collection and increased dynamic range.

A pixel array detector (e.g. Pilatus 2M) allows shutterless data collection: the sample is rotated at constant velocity with continuous detector read out. Removing the requirement for synchronizing shutter control with crystal rotation allows one to collect data using zero beam attenuation. Combining this with removal of 'dead-time' of 1-2s per exposure results in a significant acceleration of data collection (reducing the time for a 360° dataset collected in standard mode with 1s 1° oscillations of 400 seconds to 60 seconds in shutterless mode).

Figure 1.2. *Pilatus 2M detector (Dectris)*

Strongly-diffracting SMX samples, such as inorganic complexes, can result in overloaded high-angle reflections, requiring the use of extensive attenuation and the loss of weak reflections at low-angle. The increased dynamic range of a pixel-array detector (20 bits) compared with that of a CCD (16 bits) enables the collection of very intense reflections without overloads and also the collection of both extremely intense and weak reflections in a single dataset.

1.3. Mini-Kappa goniometer head and 2-theta capabilities

The existing goniometer heads on both MX1 and MX2 allow sample rotation around only a single axis. Installation of the Bruker MK3 Mini-Kappa goniometer head, alongside the implementation of a 2-theta detector approach on MX1 will deliver on one of the SMX community's keenest requests; a 4-circle stage to allow data-completeness to high resolution.

Figure 1.3. *Mini-kappa goniometer head.*

The Bruker MK3 has a low circle of confusion (< 3 μm) and will be installed with the STAC alignment and control software package, which provides users with an integrated GUI for sample alignment, and concurrently prevents collisions of the kappa head with existing beamline equipment.

Data collection with the Mini-Kappa goniometer, in conjunction with STAC software, will allow SMX crystallographers to re-orient crystals while keeping the crystal in the X-ray beam, to perform collections on multiple crystals with inherited alignment, to misalign crystals to avoid the blind zone, and to align crystals along an axis for highest completeness.

1.4. Robot upgrades

The following upgrades will be carried out on the MX1 robot systems. These will require some engineering and programming by the beam-line scientists, but will ultimately lead to a dramatic increase the throughput of MX1 (and also to MX2). These changes will reduce the time required to mount and dismount samples to approx. 20 seconds, down from the current 300 seconds. This will allow faster crystal screening (automated or with user intervention) allowing considerably more efficient use of the beam-lines and collection of data on only the best of the crystals available to the users. To implement this, we must:

- *Move and Replace Dewar*

The current positions of the Dewar vessels mimic the SSRL MX beamlines, with the Dewar away from the goniometer. The upgrades will move the Dewar to underneath the goniometer head that will greatly reduce sample mounting time.

- *Install Double Tongs*

Double tongs have been implemented on SAM robots at the Photon Factory (KEK). MX beamline staff are currently working with scientists at KEK on a redesign of the MX SAM robots to incorporate these tongs on the MX beamlines. Mounting using the KEK double tongue robot requires about 10 seconds compared to 120 seconds with single tongs

- *Change SAM robot control code*

Currently it takes 5 minutes to change samples (from mounting one crystal to mounting the next). New code will be deployed to cool the tongs for the first crystal and mount it, return to the dewar and place the next crystal on the dumbbell ready to mount. The tongs will remain cold and reduce time needed to take the sample from the goniometer. The "outgoing" sample will be placed on the dumbbell, the "incoming" sample (already on the dumbbell) will be mounted on the goniometer. While the new sample is being centered and diffraction images are recorded the robot will return to the dewar and replace the "outgoing" sample in the cassette and take the next sample ready to mount and place it on the dumbbell. If data collection is undertaken (and not only screening) the robot will return to its home position to warm and dry the tongs. Changes to the hutch air-conditioning will reduce hutch humidity and allow the tongs to remain in the dewar for a long period of time.

- *Install Barcode Reader*

In order to track crystal samples accurately during both screening and data collection, barcode readers will be implemented on all beamlines. This will allow the 2D barcode (present on all new sample bases) to be read as the sample is moving in to the goniometer position. Cassettes, dewars and shippers will also be tagged using RFID tags for tracking.

1.5. Endstation improvements and ancillary equipment

The following upgrades to the endstation hardware are required in order to more closely meet the needs of the SMX community to perform world-class experiments:

- Deployment of two-theta movement for detector (integrated with the Mini-Kappa goniometer head to give a 4-circle environment)
- Modifications to detector mount to allow for smaller crystal-detector distance for high resolution data collection (essential for many SMX experiments)
- Upgrades to A-frame allow both CCD and pixel-array detector to be mounted on the detector cage
- Enhanced attenuation wheel to allow greater range and control over flux at sample (vital

after optics modifications)

- Improved hutch AC system to reduce humidity.
- Installation of a helium cryojet (allowing low temperature studies)
- Programmable temperature control of cryojet to allow for SMX phase transition studies
- Purchase and installation of a high-pressure mini-diamond anvil cell for the SMX community
- Improved crystal visualization for the smallest SMX samples (cameras, lenses, lighting)

MX1 Upgrade Draft Budget

Top-level budget:

Item	***Cost***
Optics (inc installation)	$330,000
Endstation	$1,036,650
Ancillaries	$150,000
Subtotal:	$1,516,650
Contingency (10%)	$151,665
Total:	**$1,668,315**

Breakdown budget:

Optics:	
Upgrade of DCM to add focussing 13keV DMM	$250,000
Added control systems for DCM	$35,000
Freight	$8,000
On-site installation and Commissioning	$37,000
Total:	**$330,000**

Endstation:	
Pilatus 2M detector	$834,750
Mini-kappa	$46,000
Moving robot dewar	$12,000
Single channel current amplifiers	$12,000
Double tongs for robot	$15,000
Upgrade hutch AC	$22,000
Barcode reader and database IOC	$8,500
A-frame modifications for Pilatus detector	$5,400
Improved sample cameras	$22,000
Mini diamond anvil cells	$50,000
Enhanced attenuator wheel	$9,000
Total:	**$1,036,650**

Ancillaries:	
Helium cryojet	$150,000
Total:	**$150,000**

2. MX2 upgrades to enable microfocus work

With MX1 upgraded to improve its capability for SMX studies and MX3D (see below) installed for ultra-high throughput and tray screening, the MX2 beamline will be upgraded to accommodate even the most difficult MX experiments. These experiments require extremely high brilliance and small beam size.

The MX2 beamline upgrade will substantially improve the beamline's performance and stability and requires the following six modifications:

2.1. Installation of a large pixel-array detector

A large pixel-array detector (Pilatus 6M or equivalent) will significantly improve the data quality from the MX2 beamline. The large dynamic range and high sensitivity of this detector will allow for faster data collection (single fine-phi sliced pass rather than traditional high- and low-resolution passes). The lower detector readout noise will reduce systematic error from finely phi-sliced data. For data collected from very large assemblies where reflections are close together on the surface of the detector, the 1 pixel point-spread function will allow for collection of higher resolution data than a conventional CCD detector. A pixel-array detector will also provide the ability to conduct shutterless data collection. The larger detector area allows for the measurement of higher angle reflections at the same detector distance. This will be of particular help in low energy data collection where air-absorbance is an issue. A computer cluster for real-time data integration is required and solutions will be supplied with the detector by the vendor

2.2. Installation of a replacement vertical focusing mirror substrate

A replacement vertical focusing mirror (VFM) mirror substrate will improve the focal size of the beam, increase the flux density of the collimated beam at the sample and allow for faster user-controlled changes in the beam size. This will allow for higher quality data to be collected on smaller samples and more datasets to be collected from larger crystals.

2.3. Addition of a fine pitch piezoelectric motor to the microfocussing horizontal focusing mirror

Horizontal beam position in MX2 is controlled using the fine pitch of the first horizontal mirror (HFM1). Changing the HFM1 fine pitch also changes the section of the beam that illuminates the microfocussing horizontal focusing mirror (HFM2) and this currently causes two problems. Firstly, the ends of a mirror have far higher figure errors and illuminating the ends of HFM2 produces considerable streaking at the sample position. Secondly, the change of angle from HFM1 has the effect of changing the effective source position at HFM2, which affects the accuracy of the beam steering system: the beam can be in the same place on the beam steering YAG but produce a different beam position at the sample. The simple fix for this is to add a fine pitch piezoelectric motor to HFM2. This piezo will then be used for beam steering and will both allow accurate control of the area of HFM2 illuminated and also remove the effect of changing source position.

2.4. Implementing thermal control of cabins and hutches

The effect of thermal instability in the optics and endstation hutches is to produce drift in both the real and apparent beam position. In order for MX2 to be useable for true microcrystal studies,

the current level of beam stability must be significantly improved. Changes in air temperature between the endstation hutch and user cabin currently lead to large changes in the temperature of endstation components. The resulting thermal expansion changes the height of the rotation axis and moves the endstation relative to the X-ray beam. New air conditioning equipment will thus be installed to keep a constant air temperature in both the hutches and user cabin. The airflow in the endstation hutch will be increased using diffuser "socks", as these will allow a large increase in the volume of air introduced to the hutch without creating drafts at the sample point. This approach has been used on the microfocus MX beamlines at DIAMOND, SOLEIL and SLS. The required air temperature stability is in the order of 1 degree Celsius. By dehumidifying the hutches, icing of the robot will be reduced.

2.5. Reducing beam vibration

The effect of vibration on MX2 is to introduce beam movement, move the sample in the beam and reduce the clarity of the optical sample alignment camera. The liquid nitrogen cryo-cooler acts as an "antenna" that introduces environmental vibration into the double crystal monochromator (DCM) via the rigid liquid nitrogen cooling lines. This transmitted vibration drives the DCM to vibrate at characteristic harmonic frequencies that can be detected in the monochromatic X-ray beam. In order to reduce the magnitude of harmonic vibration, the DCM will be modified to replace the double flexure "crystal2" perpendicular stage and the "roll2" and "pitch2" single flexures. The cryo-cooler will have a new mount designed and installed to isolate it vibrationally from the technical floor. The endstation slit-base assembly will be modified to reduce its susceptibility to vibration. The optical camera mount will also be modified in a similar manner.

2.6. Upgrading the endstation and introducing ancillary equipment

The following upgrades to the endstation hardware are required in order to better meet the needs of the community:

- Improved crystal visualization (cameras, lenses, lighting)
- Exhaust system for the cryojet dewar and robot dewar as they significantly cool the hutch when the dewars are filled with liquid nitrogen.
- Beamline modifications to accommodate an in-line Raman spectrophotometer.
- Installation of a 266nm laser for laser phasing of protein crystals.
- Modifications to detector mount to allow for smaller crystal-detector distance for high resolution data collection (e.g. essential for publication-standard resolution for SMX studies).
- Deployment of two-theta movement for detector
- Upgrades to A-frame allow both CCD and pixel-array detector to be mounted on the detector cage

MX2 Upgrade Draft Budget

Top-level budget:

Item	***Cost***
Optics (inc installation)	$235,222
Endstation	$1,870,530
Subtotal:	$2,105,752
Contingency (10%)	$210,575
Total:	**$2,316,327**

Breakdown budget:

Optics:	
Addition of fine pitch to MHFM	$41,500
New VFM substrate	$101,222
DCM vibration upgrade	$77,500
Cryo-cooler vibration isolation	$15,000
Total:	**$235,222**

Endstation:	
Pilatus 6M detector	$1,727,250
Endstation thermal control	$55,000
Endstation vibration reduction	$22,000
Improved sample visualisation	$22,000
Inline spectrophotometer	$15,000
Installation of laser for Se phasing	$16,780
A-frame modifications for Pilatus detector	$9,500
Enhanced attenuator wheel	$3,000
Total:	**$1,870,530**

3. MX3D – a high-throughput beamline for automated Diffraction, screening and Drug Design

MX3D is flagship undulator beamline for expediting translational outcomes from macromolecular crystallography. It will have three modes of operation: (1) as a facility for in-tray screening, (2) as a high-throughput screening beamline and (3) as a standard crystallography beamline. All features of the beamline have been designed to facilitate these functions.

- The beamline will be powered by an in-vacuum undulator, at least 3m in length, that will provide a beam that can be varied in size between 25x25µm and 100 µm x 100µm to allow for both standard crystallography experiments and high-throughput screening.
- The endstation will be connected to an adjacent cabin that will contain an crystal tray storage system (96-well SBS-footprint) with the ability to remotely transfer trays into the hutch.
- Inside the hutch a robotic arm will hold crystal trays for in-tray screening, transfer trays to the cabin and also transfer cassettes from a cassette storage dewar to the SAM dewars.
- The SAM robot will be modified to use the double-tong design (as outlined for MX2 above).
- A pixel-array detector (e.g. Pilatus 6M) will allow for shutterless data collection.
- The endstation will also include an "alignment" platform consisting of goniometer plus stages, cryojet, robot and camera so that users can pre-align their crystals before their beamtime starts. This will allow automated data collection where users queue cassettes for collection.

Fragment screening involves measuring 100s or even 1000s of data sets. The combination of the brilliant source, the high-speed robot and the shutterless data collection will reduce the time required for each dataset by more than tenfold.

Due to the high degree of automation for tray and crystal screening, access to the beamline will be rapid. Samples will be sent to the beamline and queued for collection. Users will use a web-based system to request access and to enter the queue. This will provide rapid access to beamtime and reduce the demand for rapid access on the MX1 and MX2 beamlines.

The combination of a brilliant source with variable beam size, a high level of robotic automation and shutterless data collection will create a world-class crystallography beamline that will also address the rate-limiting step of protein crystallization and service the growing need of the Australasian crystallographic community for high-throughput screening, turning crystal structures into pharmaceuticals.

The beamline will be capable of full remote operation, like its forerunners MX1 and MX2.

3.1. Source

An in-vacuum undulator in a long straight section is required to provide a source of sufficient brilliance and size for MX3D. A water-cooled copper mask will reduce the heat load of the transmitted beam to the downstream optics.

The key requirements for the source are:

- Small spot size at the sample position e.g. 25µm x 25µm (full-width half-maximum)
- High flux of >4e12 ph/s at the sample in this spot size.

A small spot size at the sample position is required in two major applications: (1) In-tray screening, where the aim is to produce measurable diffraction from micro-crystals of weakly-scattering crystals (e.g. membrane proteins, glycosylated receptors, flexible proteins) and the small beam improves the signal-to-noise ratio. (2) Standard MX mode, where the small beam also improves signal-to-noise ratio as most protein crystals have a shortest dimension comparable to this spots size (i.e. 25 µm). Here the goal is to illuminate the crystal with a beam that matches the crystal size, thus avoiding scattering from the large amounts of material surrounding the crystal (mounting loop, cryo-protectant etc) which otherwise swamps the weaker high angle reflections.

High flux is required for weakly scattering micro-crystals and to minimize exposure time in ultra-high throughput screening. For example, on MX2, crystals of the most weakly diffracting membrane crystals currently require about 30 seconds of non-attenuated beam per image. While this is the worst-case scenario, it is clear that the achieving the flux needed for rapid, automated rastering of crystallization trays requires a long in-vacuum undulator (IVU).

Given the performance of the IVUs supplied by Neomax it is likely that a period shorter than the 22mm used in 3ID1 will be viable for MX3D. A period of 19.5mm will allow the bottom of the 5^{th} harmonic to be used to collect data at 13keV at a gap of 7.2mm, a calculated K of 1.1711 and B_0 of 0.643 T. The device would have a K_{max} of 1.25 at 6.8mm gap and 1.3 at 6.6mm. Such a device would be expected to provide an increase in flux of more than 50% over a u22 device. Also, with 4m straight sections it may be possible to extend the length of the device to greater than 3m (as with 3ID1) and a 3.6m 19.5 mm period IVU (providing a gap of 7.2mm is acceptable) would be expected to produce almost twice the flux of the current MX2 source. Further modeling of the IVU properties is required to ensure no gaps in the tuning curves with a period shorter than the existing 22mm device is a viable proposition. The combination of in-tray screening and ultra-high thoughput requires at least the levels of flux produced by MX2.

A bending magnet or a short straight section source will be incapable of satisfying the requirements of MX3D.

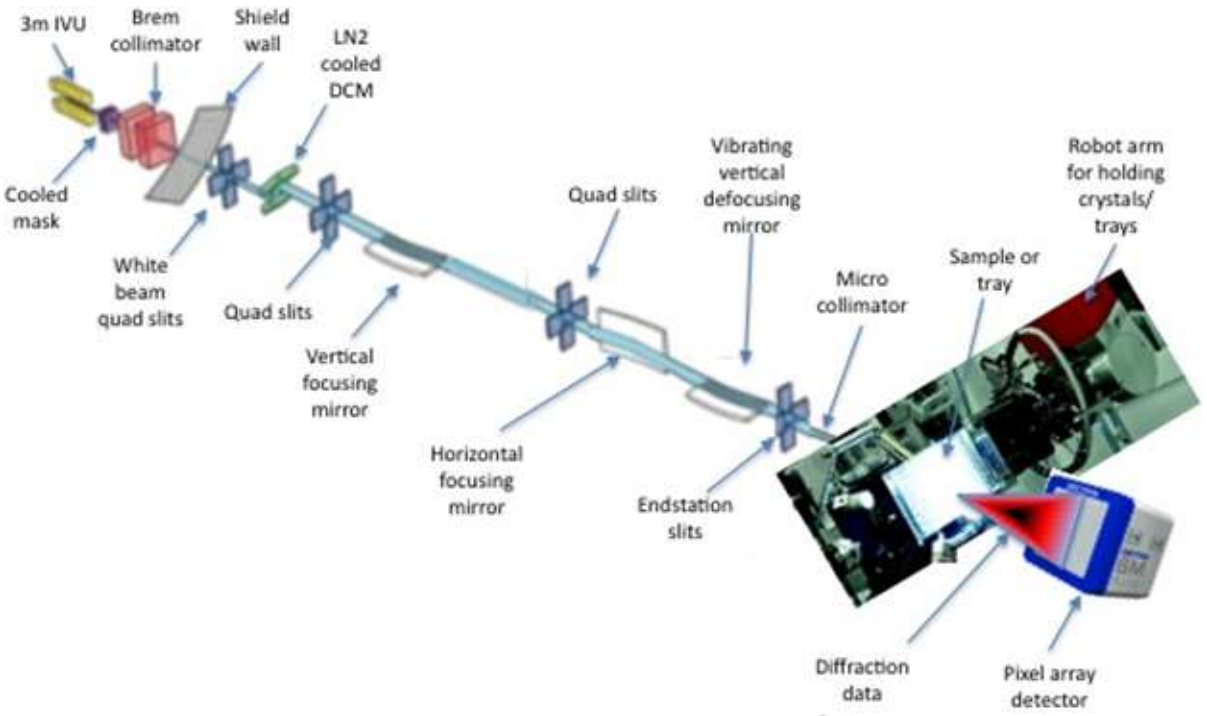


Figure 3.1. *Proposed MX3D beamline Schematic*

- *Monochromator*

The double crystal monochromator at 28m will consist of two Si111 crystals, with the first crystal directly cooled with liquid nitrogen and the second crystal indirectly 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. The translation stages of the second crystal will avoid the use of single pivot flexures and double-flexures in order to reduce both the intrinsic vibration of the system and its sensitivity to external vibration. Given the required energy range (6 to 18 keV), only one set of crystals is needed in the DCM.

- *Focusing elements*

The beam will be initially focused *via* a pair of Pt- and Rh-coated vertical and horizontal (at 32 and 33.87m, respectively) focusing silicon mirrors in Kirkpatrick-Baez (KB) geometry. These mirrors will be pre-ground to shape and equipped with bimorph electronics for precise focusing. The VFM, HFM and vertical defocusing mirrors will be 300, 800 and 150mm long, respectively. These mirrors will be of extremely high quality and will be ion-beam polished to better than 0.5 µrad RMS with voltage and 2 µrad peak-to-valley. These mirrors will produce the 25x25µm focus at the sample. This optical design is extremely simple and will mean that the mirrors are not moved, bent or adjusted during normal use. In order to allow change of beam size from 25x25µm to up to 100x100µm, it will be possible to defocus the beam using a vertical defocusing mirror (at 34.5m) that utilizes a novel high-frequency vibration system to increase the vertical beam size. Horizontal defocusing will be achieved by translation of the HFM lateral to the beam and compensation to bring the beam back onto the same angular

path as before using DCM roll2. The advantage of this design is that there will be no change in source position or angular change when the beam size is changed. On return to the 25x25µm size, the beam will be in the same position as there will have been minimal adjustment of the mirrors. This system should provide the stable, reliable beam needed for ultra-high throughput.

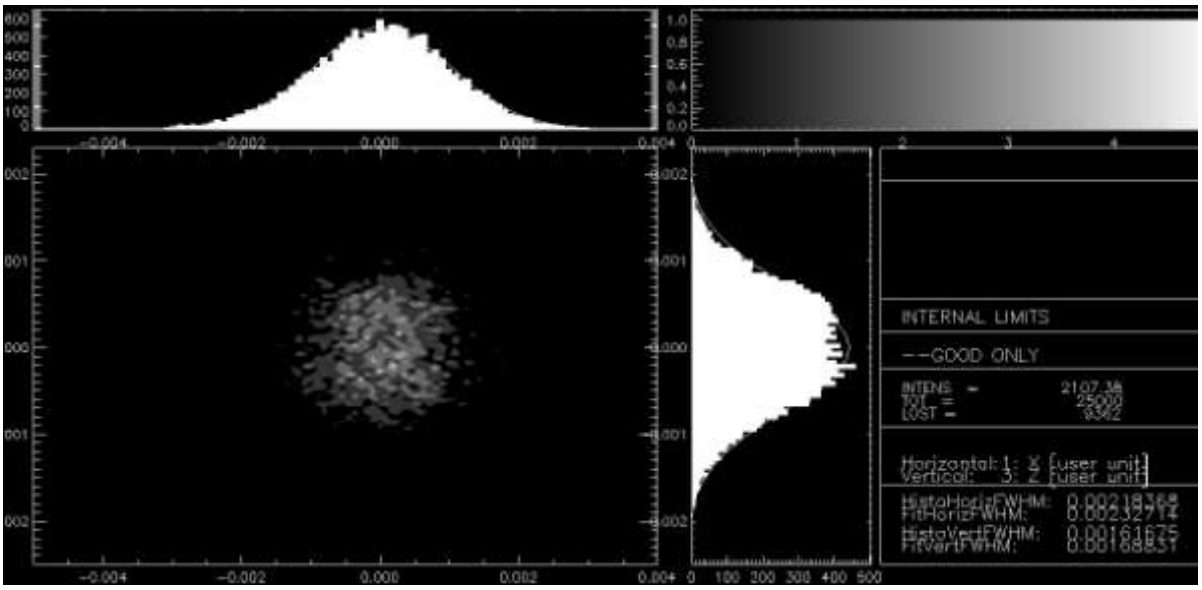


Figure 3.2. *Ray-tracing of beam at sample position with calculated flux assuming 0.5 µrad RMS figure error on mirrors.*

3.2. Endstation

The endstation (with the sample at 35m from the source) has been designed to facilitate both automated in-tray screening and rapid, high-throughput screening.

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.

- *In-tray screening*

The endstation will be connected to a tray storage enclosure outside the hutch with an automated slot that will allow for trays to be remotely moved from the tray storage enclosure into the hutch (similar to the system at X06DA at the SLS).

Trays from crystallization facilities will be sent to the beamline and stored in the tray storage system for screening. Tray-cassettes that hold 12 trays (supplied by Rigaku) will be shipped by users in transfer containers to the beamline. These containers will be specially designed to keep the temperature of the trays constant and to protect them from shock and inversion. The tray-cassettes will then be placed in the tray storage system and the crystallization drops imaged. Users will have access to the pre-experiment optical images to be able to check that crystals have not been damaged in transit

The endstation will contain a robot arm capable of holding trays for in-tray screening (such as a Stäubli 6-axis robot). This robot will both transfer trays from the hutch access slot and position the trays in the x-ray beam for data collection.

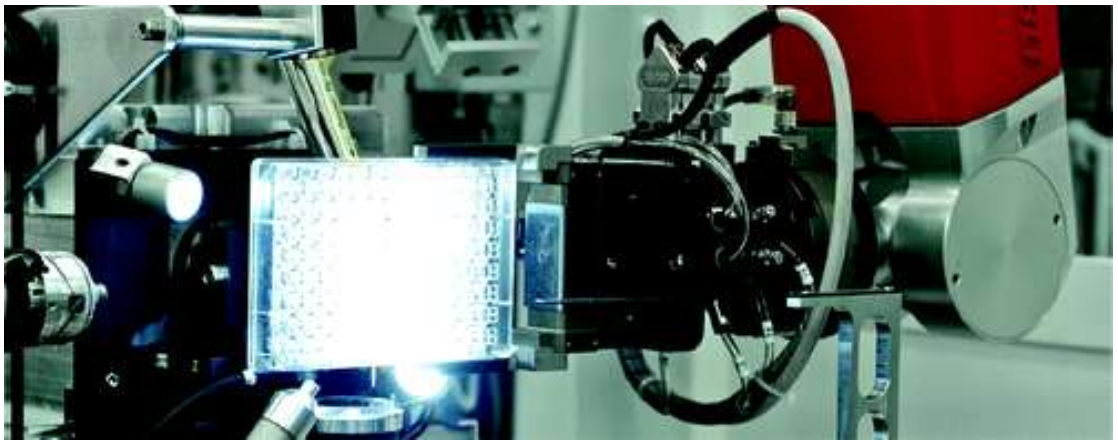


Figure. 3.3 *Tray screening in X06DA at the SLS. Proposed configuration for the MX3D endstation. (from Bingel-Erlenmeyer et al. (2011) LS Crystallization Platform at Beamline X06DA—A Fully Automated Pipeline Enabling in Situ X-ray Diffraction Screening Crystal Growth & Design 11, 916-923)*

Optical and diffraction images will be automatically sent back to the crystallization facility as the trays are screened. These can then be viewed (e.g. with CrystalTrak) as a separate inspection and users will have access to images of the region of interest in the drop, the optical images from the beamline and the diffraction images.

- *Rapid, high-throughput screening*

The SAM robot will be modified to use the double-tong design (described for MX2) to provide extremely rapid sample changes. This will reduce shutter opening times for pre-aligned samples from 90 seconds using a single tong robot to be less than 10 seconds from initiation of sample exchange.

The endstation will contain a cassette storage dewar holding up to 12 cassettes in addition to the SAM dewar holding three 'active' cassettes. The tray screening robot (above) will be used to transfer cassettes between the robot dewar and the storage dewar. This will allow samples to be queued for automated collection and user changeover. The robot dewar will be situated below the goniometer to reduce sample exchange times. The robot dewar will be lid-less and a gentle flow of N_2 gas will reduce icing of the robot arm.

- *Detector*

A large pixel-array detector (e.g. Pilatus 6M) will allow for shutterless data collection. This will allow collection of a complete dataset in under 60 seconds for many crystals, compared to around 6-7 minutes when using a shutter. The increased dynamic range of the pixel-array detector should also remove the need for second pass low-resolution datasets as the number of overloaded reflections should be greatly reduced.

- *Pre-alignment platform*

The endstation will also include an “alignment” platform consisting of goniometer plus stages, robot, cryojet and camera so that users can pre-align their crystals before their beamtime starts. Users will be able to access this platform separately from the beamline and mount and centre their samples. The centering information will be stored and when the samples are mounted on the beamline SAM robot the sample stages will move to those pre-aligned settings. This allows for rapid data collection from crystals where automated centering fails. This system also allows automated data collection where users queue cassettes for collection.

MX3D Build Budget

Top-level budget:

Item	***Cost***
In Vacuum Undulator	$1,200,000
Optics (inc installation)	$3,080,820
Endstation	$3,681,250
Hutches	$1,000,000
Ancillaries	$498,400
Subtotal:	$9,460,470
Contingency (10%)	$946,047
Total:	**$10,406,517**

Breakdown budget:

Optics:	
Design, Engineering, Controls, Software	$650,490
Mirror systems	$841,910
Monochromator	$466,650
Bremsstrahlung Stop	$23,360
Photon Shutter	$46,710
Transport Tubes	$23,360
Cooling water and Compressed air systems	$23,360
Cryocooling system	$163,300
Beamline Diagnostics	$154,630
Support Stands	$11,630
Ion pumps and controllers	$69,980
Vacuum gauges and controllers	$23,360
Vacuum Valves	$34,980
Turbo pump and controller for DCM	$26,450
Be Window on gate valve	$14,280
Vacuum Bellows	$23,360
Cable Management	$7,530
Beam conditioning	$51,000
Control system hardware	$100,000
Mirror power supply	$25,350
Freight	$15,210
On-site installation and Commissioning	$283,920
Total:	**$3,080,820**

Endstation:	
Motor controllers (32 channels)	$96,000
User and aux. computers (3 user cabin, 2 in-hutch)	$16,000
Quad Current Amps (3)	$34,500
Single channel current amplifiers	$12,000
HV and electronics for Ion Chamber	$6,000
Ion Chamber	$4,000

Tray entry labyrinth	$175,000
Motion stages for tray transfer	$28,000
Beam focussing system	$15,000
Slits	$15,000
Electronics racks	$15,000
Experimental gas equipment	$10,000
Pixel-Array detector	$1,727,250
Goniometer	$200,000
Detector support	$250,000
VME rack, bridge, VFC, scalar	$50,000
Sample changing robot	$180,000
Tray screening robot	$250,000
Tray storage and imaging unit	$400,000
Control hardware	$100,000
Sample imaging	$42,000
Thermal stability systems	$18,000
Storage and furniture	$25,000
Software	$12,500
Total:	**$3,681,250**

Hutches:	
Optics hutch	$450,000
Endstation hutch	$450,000
Utilities	$35,000
Cables, racks, cable trays etc	$35,000
Personal safety systems	$30,000
Total:	**$1,000,000**

Ancillaries:	
Microscopes	$35,000
User cabin	$250,000
Portable pumping equipment	$25,000
Tools	$6,500
Crystal handling tools	$1,200
Surveillance cameras	$20,000
User data storage array	$123,400
Remote access servers and video	$22,300
Liquid nitrogen dewars	$15,000
Total:	**$498,400**

4. Integrated data handling

The existing MX1, MX2, SAXS/WAXS and the proposed MX3D and BIOSAXS beamlines will form a complementary suite for the Structural Biology community allowing the opportunity for a highly-streamlined process for comprehensive structural analysis and investigation of solid- and solution-state samples.

4.1 Data handling architecture

Fast data collection places a huge pressure on the experimentalist, however experienced they are. It is essential, in terms of efficiency, to ensure that users are aware as soon as possible if either there are technical issues with their data, or if they have already collected the data necessary to solve their structural question. To ensure best-practice, an X-ray experiment evaluation system must provide output while the crystal still is at or near the beamline. At the end of each data collection (on any beamline), users will receive a report, describing the protocols used, the data collected and the methods used in automated processing and structure determination by using the AUTOPROCESS/AUTORICKSHAW system. AUTORICKSHAW makes use of publicly-available MX software and is based on several distinct computer-coded decision-makers for a number of standard phasing protocols.

Systems for automatic data processing and databases to track and store information are likely to evolve and develop throughout the lifetime of the beamlines. However, at the heart of the system will be a relational database holding metadata relating to the sample such as sequences and identifiers, optical images collected during the experiment such as sample views, details of beamline set up such as X-ray wavelength or detector distance, references to the location of the raw data in storage arrays and the results from down stream data reduction and analysis. At the front end will be dynamic web applications allowing the user to choose different ways of representing their data. Behind this system series of EPICS triggers and scripts will push data from the beamlines to a variety of data analysis software and direct output from these programs to the database.

A nascent version of this system is currently running on the MX beamlines. The user initiates collection of a diffraction dataset through the Blu-Ice control system. This event triggers data reduction using the open source XDS software. Metrics from XDS describing data quality are harvested and uploaded to a mySQL database. These results are displayed in tabular form at the beamline giving the user an overview of what they have collected and to compare the quality of different datasets. Information from the database is uploaded along with the diffraction images to TARDIS for long-term storage.

The sensitive nature of the intellectual property that is typically associated with translational products requires careful control of IT security. The MX beamlines have already managed this situation by using highly-restricted disk access and providing one-time logins to users. During the development of this more sophisticated setup, a keen eye will be kept to ensure data integrity.

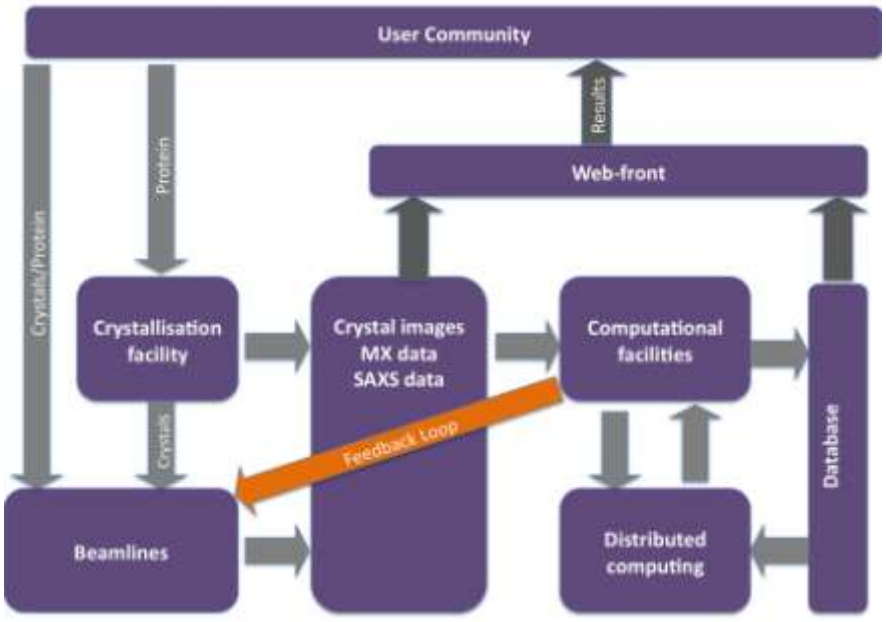


Figure. 4.1. *Flow diagram of the Integrated User Facility*

4.2 Modes of User Access

Here are a number of typical experimental scenarios where Australiasian scientists can benefit from an integrated facility. A particularly powerful approach would allow combination of these approaches to obtain data from both diffraction and solution scattering experiments of the same sample, with user uploaded metadata being used to phase the diffraction data and to model the scattering data. Final results from both beamlines will be stored in a database with common sample identifiers to allow the different experimental and their metadata to be considered together.

Scenario 1: User has been unable to find conditions for stable purification and concentration of their protein.

The user submits their barcoded protein samples (as pure as possible) to the BioSAXS high-throughput automated buffer screening protocol and submits metadata to a database. Experimental data are passed to the database, providing useful data that can analysed in a systematic way to provide conditions for stabilising their protein during purification and crystallisation.

Scenario 2: User has successfully purified components of a molecular complex.

The user can submit samples of the components for analysis of the complex at various concentrations and ratios of components using BioSAXS to determine the most likely ratio and concentrations to yield a complex suitable for crystallisation trials. For example, even in its optimal buffer conditions, the complex may not be soluble at high concentration – thus dictating the concentration for trials.

Scenario 3: User has soluble and stable sample ready for crystallisation trials.

A user contracts a crystallisation facility (e.g. C3, Monash or other participating facility) to screen their protein sample against many hundreds of crystallisation conditions. During these

experiments several promising-looking microcrystals are noted and flagged for X-ray analysis. These trays are sent to the synchrotron and the objects of interest are automatically tested for X-ray diffraction on the MX3D beamline. The images from these experiments will be automatically analysed for diffraction quality and the resulting data uploaded to the crystallisation facility's database for the consideration of the end user. Any crystallisation hits can thus be immediately assessed for their suitability for data collection. If the crystals are promising, but not yet ready for data collection, crystallisation hits would then be further improved.

Scenario 4: User has purified soluble protein, and has produced crystals of diffraction quality and wishes to look for small molecule binding partners.
The user can submit their protein to a crystallisation facility for fragment screening soaks or co-crystallisation. The protein will be crystallised in the presence of defined cocktails of small molecules. Barcoded trays will be submitted to the synchrotron along with metadata, diffraction data measured, and electron density maps automatically produced using the AUTOPROCESS/AUTORICKSHAW software. The maps will then be analysed to see which, if any, compounds are bound to target protein. These compounds can then be used as leads for drug development. All data will be stored in the database for systematic analysis

Scenario 5: User has crystal structures of two components of a complex but is unable to crystallise the complex.
The user will submit the complex to BioSAXS and the known crystal structures of the components can be modelled into the SAXS envelope to determine possible binding modes, which can then be tested by mutagenesis or other techniques.

Scenario 6: User has crystals that vary in diffraction quality, both within the crystal and between crystals.
The user will submit their crystals to the high-throughput cassette screening facility on MX3D, where each crystal will be subjected to diffraction imaging (snapshots at two orthogonal angles). The resolution and quality of the diffraction will be assessed for each crystal, to determine which crystals should be used for further data collection. Once suitable crystals are found, diffraction data from these can be collected on MX2 (if areas of the crystal showed heterogeneity or the crystals were small), MX1 or MX3D.

Scenario 7: User has small crystals that are difficult to locate in a loop by optical means.
The user will use the exiting rastering protocol in the new J-BluIce control software on MX2 whereby the whole loop is scanned in a grid-like fashion to determine the location of the best crystal diffraction. Data collection will then continue as normal.

Budget for Data Integration
The cost of integration (principally staff time) will be absorbed in the MX3D beamline design budget.

Summary Budget for the MXe Project

The total cost of the project is shown below. Each item includes 10% contingency.

Projected Cost for MXe Project:

Item	***Cost***
MX3D Beamline total build cost	$10,406,517
MX1 Upgrade	$1,668,315
MX2 Upgrade	$2,316,327
- Existing budgeted upgrades*	$266,222
- EOU funding*	-$266,222
Total:	**$14,124,937**

*Some of the projects proposed in the MXe project have already been funded *via* the Australian Synchrotron EOU (Essential Operating Upgrades) fund. These include the MX2 VFM substrate replacement, MX2 DCM upgrade and the MX1 Mini-kappa. The total cost of the already-funded projects is $266,222.00 leaving a total budget of $14,124,937 required for the project.

B2: Applications and Potential Outcomes to Australasian Scientific Community

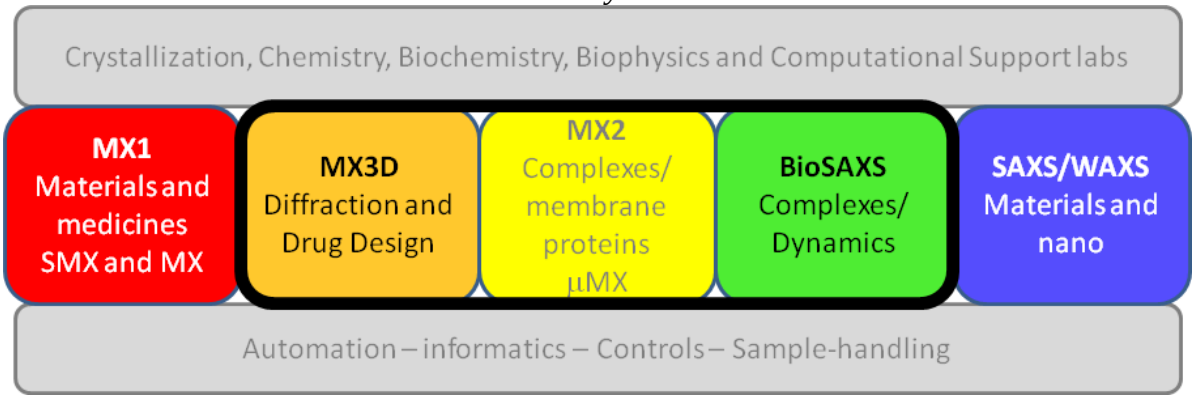
How does the project advance synchrotron-based research in Australia/NZ? What are the likely outcomes? Include specific examples where possible.

The rapidly expanding, world-class research carried out by the Australasian Structural Biology research community is severely testing the current capabilities of the Australian Synchrotron. The challenge for the future is to maintain this flow of research that is leading to exposure in the highest profile journals and media sources. For three specific examples of such research please see The Science Case for the Development of the Australian Synchrotron, pp7-8, and http://www.synchrotron.org.au/index.php/aussyncbeamlines/macromolecular-crystallography/highlights-mx. In summary, there have been 57 papers published in 2010 from MX beamlines of which 37 are ERA rated A or A*. Two MX papers have appeared in Nature, one in Cell, each attracting subsequent press, radio and TV exposure. There have been 113 structures deposited to the PDB to date.

Converting such high-profile biomedical projects into tangible health outcomes for Australasian citizens requires expedition of the more translational aspects of this research. Simply put, we need not only to be able to screen crystals of more medically-relevant target proteins, but we need to be able to provide a platform to support drug design projects which convert the science into medicines, and to do it faster.

To meet this challenge we have a coherent vision of a suite of beamlines finely-tuned to each of the most highly impacting areas of synchrotron-based research (in terms of published output and worldwide recognition of the AS). At the core we have a set of diffraction and scattering capabilities, backed by well-equipped support labs and infrastructure that offer a one-stop-shop for cutting-edge biological research. These facilities are also integrated with partner beamlines providing a comprehensive suite of X-ray methods for characterization of molecular structure.

***MX3D** fits clearly into an integrated vision of world-class structural biology facilities at the Australian Synchrotron*



An important feature of this vision is that many of the pieces are in place, in train, or capable of being put in place at modest cost. The most significant missing piece is the cutting-edge beamline tuned to the intense demands of high-throughput crystallography. **MX3D** not only addresses this need, but provides an opportunity to optimize the use and output of the existing

MX1 and MX2 beamlines. The investment in high-throughput sample-handling infrastructure will also benefit the proposed BioSAXS beamline where analogous developments are planned.

The need for ultra-high-throughput

Many of the serious challenges of Structural Biology encountered during the 1990s have been addressed through the application of high levels of parallelization to a series of processes. High-throughput methods allow a successful, brute-force resolution of the resulting combinatorial explosion of possible experiments ("Structural Genomics").

For example, to achieve diffraction to suitable resolution to solve the Nobel-prize winning structure of the ribosome, many, many thousands of crystals were screened at synchrotrons across the world. Fifteen years later almost all Structural Biology labs recreate this same approach to solve structures of their new important targets: membrane proteins that control which substances can enter or leave a cell; complexes of proteins from the human cell nucleus that direct cell fate; receptor proteins that maintain our metabolism in balance or govern our immune system. Each of these projects, currently ongoing at the Australian Synchrotron, requires the screening of hundreds of crystals in frequent cycles, to allow progress to their goal.

Structure-guided drug design, a methodology which has become embedded in translational biomedical research over the last 20 years, has similar high-throughput demands. If one is to evaluate the binding of many hundreds of candidate inhibitor molecules, or fragments of molecules which may eventually be combined to build a new drug, one requires not only to screen for diffraction, but to collect complete datasets for each of those samples.

The days of waiting for months to gain the data to allow one to make the next incremental step towards a high-value goal should be behind us.

The current beamline characteristics and peripheral infrastructure have provided an excellent level of throughput compared to previous times, but times change quickly, and without significant investment in ambitious technology as outlined in this proposal, Australasian structural biologists will find it increasingly difficult to take on the challenges of global significance that allow them to compete on the world stage.

MX3D - Automated Diffraction screening and Drug Design

- *In-tray screening*

Researchers will be able to ship crystallization trays from national or institutional facilities directly to the synchrotron where crystals can be screened for diffraction *in situ* in the crystallization drop. This capability offers a mind-boggling increase in efficiency in both time and material as less work must be done to move from crystallization hit to evaluation of diffraction.

- *Automated mounting, centering and data collection*

MX3D will transform structure-guided drug design by drastically reducing dead time (both mechanical and due to human interaction) involved in collecting data on uniform sets of large numbers of samples. With these developments, a much higher proportion of photons will be interrogating molecular structure rather than hitting the beamline shutter.

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 (thirty-to-forty user groups are accessing the MX beamlines each round, and each of these consists of a number of subprojects). Each of these high-profile projects displays a clear recent, or current, need for MX3D.

- ***Professor Michael Parker, ARC Federation Fellow, St Vincent's Institute, Melbourne***

The proposed MX3D beamline would dramatically enhance the productivity of crystallography at SVI where the predominant focus is structure-based drug discovery, some of which involves Industry collaborations.

HIV integrase – a target for new drugs to treat HIV/AIDS
(In collaboration with Avexa Ltd, Monash Institute of Pharmaceutical Sciences and Syn|Thesis)
In this project novel binding sites for drugs were discovered using a fragment screening approach. In this approach hundreds of data sets were collected from crystals soaked in solutions of small molecular weight compounds. The work, recently published in *ChemMedChem*, showed the power of fragment screening in revealing new ligand binding sites for drug discovery. In all, the fragment screening campaign took over two years. With access to the proposed MX3D beamline it is expected that this time could have been reduced to months.

HCV NS5b polymerase – a target for developing drugs to treat hepatitis C
(In collaboration with Biota Holdings)
In this project novel ligand binding sites were revealed using fragment screening. Surprisingly, some of the sites were cryptic in the structure of the uncomplexed protein and required conformational changes caused by the fragments to reveal these new sites. Again hundreds of data sets were collected over a period of a couple of years and the proposed MX3D beamline would likely cut this screening time to months.

Focal Adhesion Kinase – a target for certain cancers
(In collaboration with the CRC for Cancer Therapeutics)
In this project many diffraction data sets have been collected to progress a medicinal chemistry program of converting hits to leads and to optimise lead compounds. Co-crystallisation of proteins with compounds can produce crystals with very different diffraction properties, some more favorable for structure determination than others. This necessitated the collection of many data sets and even more crystals in the search for the best data sets. The proposed In-tray screening capability together with the high throughput capabilities of MX3D would have markedly accelerated progress in this project.

GM-CSF receptor – a target for drugs to treat leukemias, asthma and rheumatoid arthritis
(In collaboration with Prof Angel Lopez, CCB, Adelaide)
We recently determined the crystal structure of a GM-CSF receptor complex and published the results in the prestigious journal *Cell* in 2008. This project required hundreds of crystals to be screened and could not have been feasible without crystallisation robotics at the CSIRO C3 Facility and the crystal mounting robots at the APS IMCA CAT beamline. With funding from a 5 year NHMRC program grant we have extended our studies to other cytokine complexes and are meeting the same difficulty of poorly diffracting crystals and the need for extensive

diffraction screening. MX3D would greatly facilitate this program by providing access to high throughput crystallography.

- ***Professor Jennifer Martin ARC Australian Laureate Fellow, Institute for Molecular Biology, University of Queensland***

Prof Martin's current research program focuses on developing inhibitors targeting DsbA and DsbB as potential antivirulence drugs to treat bacterial infection (ARC Laureate Fellowship) and understanding the molecular mechanisms of Type II diabetes mellitus (NHMRC Program grant with Professor David James, Garvan Institute). In the future, her research program will concentrate increasingly on intact membrane proteins, including DsbB and SNARE proteins associated with insulin activity. Membrane proteins are important targets for crystallography studies, as they represent the largest class of drug receptors, but they are under-represented in the protein structure database (~1%) because of the challenges involved in crystallizing them and solving their structures. The next generation features of the MX3D beamline, including in-tray-screening and high-throughput screening, will therefore be essential to underpin and accelerate this research.

DSB Inhibitors

The crystal structure of the soluble *E coli* DsbA protein was solved by Professor Martin (Martin *et al* 1993 *Nature*) using synchrotron data measured at Brookhaven in the USA. This was one of the first structures solved by selenium-MAD methods. The crystal structure of *E coli* DsbB in complex with DsbA was solved to 3.7 Å resolution by Kenji Inaba *et al* (*Cell* 2006) using data measured at SPRING-8 in Japan. Professor Martin is developing inhibitors of bacterial DsbB and DsbA using structure-based methods. To enable this research, she will need access to the proposed MX3D features to improve the resolution of the *E coli* DsbB membrane protein crystals, solve the structures of inhibitor complexes with *E coli* DsbB and *E coli* DsbA and solve the structures of DsbB proteins from other pathogenic organisms.

SNARE Proteins

SNARE proteins are essential for the insulin-regulated uptake of blood glucose into fat and muscle cells. In response to insulin signaling, vesicles containing the GLUT4 glucose transporter are trafficked to the cell surface, where the SNARE membrane proteins on the vesicle and plasma membranes form a complex that enables the vesicle to dock and fuse, delivering GLUT4 at the right place and right time. A related SNARE system operates in neurotransmission. Professor Martin has reported important findings on how SNARE proteins are regulated by Munc18 proteins (Hu *et al Proc Natl Acad Sci USA* 2007; Hu and Christie *et al Proc Natl Acad Sci USA* 2011). However, current studies are limited by using soluble versions of SNARE proteins, that have the transmembrane domain removed. Professor Martin's goal is to investigate the regulation of SNARE-mediated vesicle fusion using intact SNARE membrane proteins. To achieve this goal in a timely, internationally competitive manner will require access to a high brightness synchrotron beamline optimised for microcrystals and with the facility for in-tray screening, such as is currently available at Diamond in the UK or ESRF in France.

- ***Dr Tom Peat, Group Molecular & Health Technologies, CSIRO, Parkville***

High-throughput Crystallography for Drug Design

At CSIRO, fragment screening projects are performed in conjunction with numerous industrial partners (e.g. Avexa, Schering-Plough, CRC-Cancer Therapeutics) and these projects often require hundreds of data sets (over 350 data sets for the Avexa project alone). As these are industrial collaborations addressing well-defined markets, there are tight timelines for results and thus the need for a protein crystallography beamline that can screen crystals as well as be optimised for high throughput data collection.

In addition, CSIRO scientists are involved in other high throughput projects which have required even larger data sets. For example, the SAMPL project with Stanford University and OpenEye Scientific Software was designed to validate computational drug design output – a key step in improving the accuracy of widely used computational methods. SAMPL required the generation of thousands of crystals and over 1500 data sets collected at the Australian Synchrotron. The data collection occurred over the period of a year, whereas implementation of a beamline such as MX3D would reduce this to months, and free up beamtime for other valuable projects.

Challenging High-Profile Structures

Recently, several samples (e.g. VAP-1, antibody:target complexes) have behaved such that slight variations in the crystallization conditions make substantial differences to the diffraction quality, while having no discernable effect on the visible light optical properties of the crystals. Instead of manually going individually through hundreds of crystals (and associated cryo-protectants), with MX3D it will be possible to defocus the beam and scan the crystals *in situ* to determine quickly the best conditions and thereby revolutionise the whole process.

Similarly, the integral membrane proteins studied at CSIRO are notorious for giving small, weakly diffracting crystals. The ability to screen these crystals for diffraction quality and then focus the beam to small dimensions for data collection is crucial for our ability to solve these structures.

The building of **MX3D** will increase productivity, bring in entirely new capability (*in situ* screening) and keep Australian Synchrotron diffraction science at the forefront of crystallography. Most importantly it will allow Australasian scientists to perform the optimum experiments to achieve world-class goals.

MX2 – High Performance Macromolecular Crystallography

- *Microfocus Beamline Capability*

Australasian scientists are prominent within the international protein crystallography community with a number of investigators targeting high profile complexes fundamental to our understanding of biology and human health. Australia's continuing record in these fields is founded on the ability to solve structures for challenging molecules, yet these projects often produce crystals of insufficient 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.

Studies of membrane proteins, large protein and nucleic acid complexes, naturally-occurring crystals and other challenging projects often involve crystals that are too small and weakly diffracting to be studied with the existing MX beamlines. The proposed upgrade of MX2 to a stable high-intensity 5μm×5μm microfocus beam, with the ability to scan sample loops for optimal diffraction, will provide to the Australasian MX community a world-standard capacity to address such projects.

Specific Projects:

Studies of naturally occurring protein crystals: Prof Peter Metcalf (University of Auckland), Dr Fasseli Coulibaly (Monash University)

Prof. Metcalf and Dr Coulibaly regularly visit the 5x15μm micro focus PX1 beamline at the Swiss Light Source in order to collect data on micron-sized protein crystals that form inside virally infected insect cells. This work has culminated in multiple high profile publications in prestigious journals including Nature and PNAS. Recent developments at MX2 of the Australian Synchrotron have indicated that it can compete with PX1 to some extent, but the requested upgrades are essential to meeting this high standard.

Studies of viral architecture: Dr Richard Kingston (University of Auckland)

Dr Kingston studies virus architecture, assembly and replication. Many of the proteins targeted in this work self-associate into large assemblies generating crystals that are small and weakly diffracting with large unit cell dimensions. Access to a highly reliable microfocus beam will greatly increase the tractability of this work.

Potassium channel structures: Dr Jacqui Gulbis (Walter and Eliza Hall Institute)

Potassium currents provide electrical activity vital to organ function, and are responsible for K^+ flux across cell membranes. Diffraction from crystals of integral membrane channel proteins are characteristically weak and often suffer from anisotropy and marked diffuse scattering. Access to a high intensity microfocus beam greatly enhances analysis of these crystals through the ability to translate crystals within the beam in order to sample different regions. The implementation on MX2 of automated rastering of the loop to identify optimum diffraction will expedite this process.

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

The human adaptive immune system is critically dependent on the interactions of T-cell Receptors with Antigen presenting molecules such as the Major Histocompatibility Complex (MHC). This MHC restricted response, the discovery of which was recognised by the 1996 Nobel Prize to Zinkernagel and Doherty, shows remarkable 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 interactions are very low, 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 optimum high-resolution data on these microcrystals will be greatly enhanced by ready access to an upgraded MX2 beamline.

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

The work of Prof Jameson includes the analysis of multi-component clusters involved in cellular

processes, such as those found in fungal gene clusters of secondary metabolism where the product of one enzyme becomes the substrate of the next. Crystals from these ventures are often small with large unit cells requiring a very intense microfocus source coupled with a large pixel-array detector with very rapid read slicing to optimise the signal-to-noise ratio.

Membrane protein complexes: Dr Daniela Stock (Victor Chang Cardiac Research Institute)
Dr Stock's work focuses on structures of biological rotary motors and other large and dynamic macromolecular assemblies such as ATPases and the bacterial flagella motor. Studying these mechanisms will not only provide insights into fundamental biological processes but will also provide another basis for the development of antibacterials. In the past Dr Stock has used high-end undulator beamlines with microfocus optics at APS (14-ID and 23-ID) and at ESRF (ID 14-4) to collect data. A high-brilliance microfocus beam is essential for this work as it allows exposure to small parts of a crystal that might be better ordered than others and also the collection of more isomorphous data from the same crystal by shifting the crystal in the beam after a few exposures.

Challenging microscopic samples: Dr Peter Turner (University of Sydney, on behalf of the Australasian Small Molecule Crystallography community):
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. World class microfocus facilities at the Australian Synchrotron would then provide Australasian chemical, biochemical, pharmaceutical, geochemical and materials researchers with a leading capability to obtain structures from highly challenging samples of national and international scientific significance.

MX1 – Cutting Edge Materials Characterisation

- *(Not-so-)Small Molecule Crystallography*

The structural characterisation of biomimetic materials, hydrogen and carbon dioxide storage materials, superconductors, micro-magnets, 'smart' materials, piezoelectric materials, negative thermal expansion materials, advanced catalysts, photon harvesting and photonic devices, information storage materials, molecular switches and sensors is a critical requirement in understanding and developing their properties. Research chemists are now preparing from simple building blocks discrete supramolecular assemblies with molecular weights comparable to small proteins and unit cells with edges in excess of 100 Å. When crystals of materials such as these are obtained, they are frequently very small, disordered, and/or twinned, and very weakly diffracting. A bright light source is essential.

In general, data to genuinely atomic resolution (better than 0.87 Å resolution) are invariably needed to provide the precision in metrical details necessary to understand subtle physical and chemical properties. In the case of charge-density studies, providing rigor to quantum-mechanical calculations, very much higher resolution (0.65 Å or better) is required. For materials

applications, and elsewhere, where sites may be occupied by atoms or ions of more than one element access to absorption edges and anomalous dispersion are needed for element identification and determination of site occupancy. All these emerging areas require equipment far beyond that typically found in-house for chemical crystallography and in many cases beyond that of newer 30-50W microfocus anode technologies that are now being taken up in-house by the chemical crystallography community.

Despite a configuration that is currently less than ideal for SMX, use of MX1 by the SMX community is increasing rapidly, especially as systems previously intractable to structure elucidation are now accessible with the brilliance of synchrotron radiation. Use of MX1 (and also MX2) by, in particular, the Monash University and University of Tasmania groups has generated many publications in high-impact A* journals.

The needs for SMX align remarkably closely with those for MX, with the exceptions that data to very high resolution are invariably required, and access to higher (>350 K) and lower (down to 14 K)) temperatures and to high pressures up to GPa levels are needed more often than in the MX community at present. With expanded temperature and pressure capabilities, MX1 will complement the microfocus beamline MX2 and the high-throughput MX3D beamline capable of examining and harvesting diffraction data from crystallization plates *in situ*.

Expansion of the capability of MX1 to address the needs of our highly active SMX community forms an extremely important strand to the **MXe** project. There is extremely high quality advanced materials science research underway in Australasia that will immediately make high-impact use of the new MX1 facilities which become available with the provision of a new **MX3D** beamline.

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

MXe – A Flagship Macromolecular Crystallography Environment

This proposal forms part of an integrated vision of a new high-throughput beamline, and developments to the existing MX1 and MX2 beamlines in concert with the SAXSWAXS and proposed BioSAXS beamlines. Upgrades to the MX2 beamline will allow it to focus on its high-quality micro crystallography capability – for characterization of challenging samples which fundamentally require high levels of user interaction to perform the best experiments. In parallel, upgrades to MX1 allow it to adequately serve the rapidly growing, high-impact Australasian small-molecule crystallography synchrotron user community.

Expansion of existing single-crystal analysis capacity

The Australasian crystallography community has a long-standing collaborative attitude to beamtime. As subscription rates at the MX1 and MX2 beamlines have risen, beamtime applicants have been restrained in their demands for beamtime to ensure that enough is available for every deserving project. Despite this altruistic approach, requests for beamtime now clearly outstrip the available capacity: in round 2011_2 at least four projects with good feasibility and significance were denied beamtime. For the first time, limits were put on the beamtime available to existing Program allocation holders. If the PAC were adamant on making a point to prove oversubscription, there would be considerably more projects denied time. Simply put, there is too much high-quality structural biology and materials characterization being performed for the current setup. This is due to:

- Expansion in the Australian and New Zealand crystallographic community, e.g. new laboratories established at La Trobe University, Adelaide
- The increasing use of robotics in macromolecular crystallization. This increases the success rate of crystallization and output of crystals thus increasing the demand for beamtime.
- The growing demand for beam time on MX1 and MX2 due to the establishment of Drug Discovery screening projects and other projects requiring high-throughput crystallography faciltites.
- The growing use of MX1 by the small-molecule crystallography community, with frequent use by users from Monash, Tasmania, Sydney, New South Wales and Adelaide.

MX3D – automated Diffraction, screening and Drug Design

MX3D will provide facilities to Australian and New Zealand scientists equal to or better than world-best standards. Somewhat similar facilities currently exist at the Swiss Light Source (SLS) and are proposed as a new beamline at the DIAMOND light source. However MX3D will be a significant advance on existing beamlines and will push back the frontiers of ultra-high throughput data collection. This novelty will open the door to highly valuable inhibitor screening and drug design projects.

Take advantage of the existing third generation light source

Upgrades to MX1 and MX2 will make optimum use of the existing light source to improve output quality and efficiency for all crystallographers.

For practical use of high-throughput methodology, MX3D will require an extremely intense X-ray source with a small source size to allow focusing to a 30μm x 30μm 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 focusing, and to support shutterless data collection, a three meter in-vacuum undulator is essential and hence a 3rd generation source.. Also, to allow ultra-high throughput the exposure times for data collection in "standard-mode"collection needs to be kept as short as possible, even with weakly diffracting samples.

Will position Australasian scientists at the leading edge of their field

Current groups working on fragment screening must spend many hours collecting data and using large amounts of beamtime on the existing beamlines. MX3D will fundamentally change the way these techniques are carried out in Australasia and provide a distinct competitive advantage for local researchers compared to counterparts in the USA and Europe.

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 Australasian structural biology community maintains its position at the leading edge of this important field.

This proposal forms part of a complementary suite of proposals aimed at placing 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 MX3D can feasibly be carried out within a three-year timeframe. The beamline components such as the IVU, hutches, optics and robotics will be based on existing technology and the facility has all of the necessary experience in beamline construction.

Upgrades to MX1 and MX2 are relatively straightforward to implement technically and are easily feasible within three years of funding becoming available.

B4: Potential Users

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

MXe/MX3D will address the following current and predicted needs of the crystallographic community in Australia and New Zealand.

- The totally unmet need for in-tray crystal screening that will allow researchers to overcome the current bottleneck to production of crystals suitable for data collection.
- The totally unmet need for an ultra-high throughput facility for drug design and fragment screening.
- Expansion of the capacity for single crystal analyses at the Australian Synchrotron, both protein crystallography and small molecule, to address an actual and increasing shortfall in beamtime availability.
- Provision of world-standard small molecule crystallography facilities to support our highly-productive smart materials research community.
- Fulfilling an integrated approach to world-class Structural Biology and Materials Characterization research.

An indicative list of the organisations that would use this facility:

The Walter and Eliza Hall Institute

St Vincent's Medical Research Institute

Burnet Institute

Monash Institute for Medical Research

Ludwig Institute for Cancer Research

University of Melbourne

Monash University

Latrobe University

Charles Sturt University

University of Auckland

Waikato University

University of Otago

Massey University

University of Canterbury

University of Tasmania

Australian National University

University of Western Australia

Curtin University
University of Wollongong
CSIRO
University of Sydney
University of New South Wales
Victor Chang Cardiac Research Institute
Centenary Institute
Griffith University
University of Queensland
University of the Sunshine Coast
University of Adelaide
Flinders University