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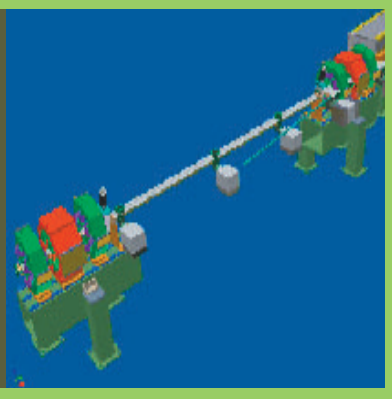


Beamline summaries

IMAGE: Computer Aided Design (CAD) render of a beamline emerging from a section of the Australian Synchrotron storage ring.

The Australian Synchrotron has the capacity for more than 30 beamlines to service a vast range of scientific disciplines.

Chapter 10

Beamlines 1 and 2: crystallography of macro- and small molecules

01/02

Potential Research Fields

Beamline 1

Life sciences

- Biological research and drug design
- Biotechnology and bio-sensors
- Plants and crops

Physical sciences

- Agricultural technology
- Food technology

Beamline 2

Life sciences

- Biological research and drug design
- Biotechnology and bio-sensors

Physical sciences

- Sustainable environment
- Advanced materials
  - Functional polymers
  - Nanomaterials and composites
  - Biomaterials
- Earth sciences
- Oil and gas production and distribution
- Food technology
- Chemical reactions and catalysts

Introduction

In the first phase it is proposed to construct two beamlines to characterise biological macromolecules. Beamline 1 will be designed for high-throughput protein crystallography. Beamline 2 (Protein Micro-crystal & Small Molecule X-ray Diffraction), will also be capable of characterising small molecules via a separate end station.

This is deemed the most cost-effective start-up approach, but it is anticipated that the user base for both requirements will expand rapidly and in the next suite a dedicated beamline for small molecule studies will be justified, leaving beamline 2 solely for protein crystallography.

Protein Crystallography

The major activity on beamlines 1 and 2 will be protein crystallography, where single crystals are analysed by x-ray diffraction using the multiple wavelength anomalous dispersion (MAD) technique. As described in chapter 3, protein crystallography provides the primary structural information on the complex macromolecules that drive biological processes. The scale of this activity worldwide is very large, because of the enormous number of different proteins expressed by the genome, as well as many viruses and nucleic acids.

It is envisaged that beamline 1 will be able to be used by researchers who are not specialists in crystallography. It will be best suited to larger protein crystals and for initial assessment of more complex crystals which might then be further investigated on the fine-focus beamline 2.

Beamline 2 will provide high flux and brilliance and improved beam focus. The characteristics are important for obtaining diffraction data from extremely small and weakly diffracting protein micro-crystals (10–30 micron size, which are, in general, difficult to grow and diffract weakly) – an area of research that is predicted to grow rapidly in the next few years. It will be a fine focus beamline (80 micron beam size) with slits capable of reducing the spot size to as little as 5 microns. It is intended to be used by specialist protein crystallographers.

A key feature is to make user access as friendly as possible, and in certain cases to provide full service at beamline 1, including crystallisation, data analysis and the ability to operate the system remotely.

User Community

Currently, there are about 45 protein crystallography groups in Australia and New Zealand and this number is growing steadily. The larger laboratories are centred around the State capitals and Auckland, and at a number of smaller laboratories elsewhere in both countries. Australian laboratories currently access overseas synchrotrons, principally the BioCARS facility at the Advanced Photon Source, near Chicago, through the Australian Synchrotron Research Program (ASRP).

The specialist protein crystallography community from its beginning in the late 1970s has formed a cohesive and cooperative group with good communications between the chief investigators.

By 2007 it is expected that there will be a much larger community that will include non-specialist protein crystallography users, who would use the beamlines for routine structure determination and view the beamlines as a routine analytic tool for biology.

Research Applications

Macromolecular research applications

The new field of proteomics has emerged that involves the systematic characterisation of the gene products of entire organisms. A key component of proteomics is the three-dimensional structural elucidation of proteins, termed structural genomics, which is being undertaken by worldwide public and commercial consortia. Once the structures of complexes of multiple proteins are solved and large proteins are broken down into their functional domains, it is possible to study their mechanisms or ligand binding properties at atomic resolution.

Small Molecule Diffraction

A second end station on beamline 2 will determine three-dimensional small-molecule (and macromolecular) structures to resolutions from normal to high (3 to 0.4 Angstroms) of weakly diffracting crystals and of crystals for charge density studies.

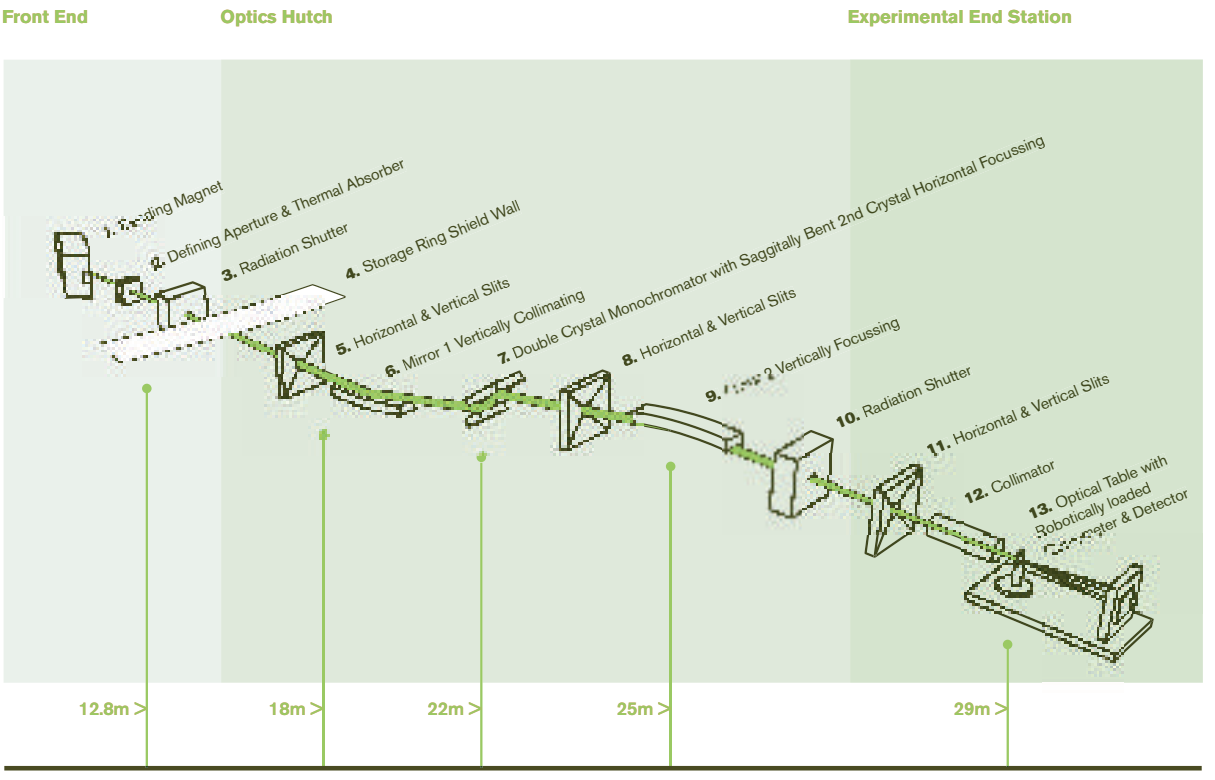
The use of the brilliant, highly collimated and tunable x-ray radiation from the synchrotron will either enable structural studies that are impossible with conventional laboratory sources, or provide improved precision and accuracy. Unlike any other x-radiation source, synchrotron light is tunable and this will provide contrast between isoelectronic species and also sensitively discriminate between oxidation states.

Applications of the small molecule facility will include the structure determination of micro-crystals with dimensions of a few microns or less. No other technique has the ability to assign unambiguously atom-to-atom connectivity, stereochemistry, absolute configuration, and charge density distributions.

Moreover, the facility will allow the resolution of disorder, and thus an understanding of its relation to physical properties, the identification of super-structure and structures under change (for example, pressure), which is not possible with laboratory x-ray sources. This will allow the structure elucidation of the ever increasing complexity of molecules/materials, for which it is difficult to grow large crystals for conventional x-ray studies.

User Community

The use of synchrotrons for small molecule studies is in its infancy in Australia and New Zealand, and it is difficult to predict the user community for 2007 reliably. The number of research groups in the small molecule community who use single-crystal x-ray diffraction data



BEAMLINE 1 High throughput Protein Crystallography

Figure BL1.1 *Schematic of the high throughput protein crystallography beamline*

for molecular structure determinations is conservatively estimated to be at least 100, and so the number of potential users of the synchrotron is certain to be significantly greater than 100.

Currently the level of awareness in the small molecule community of the powerful capabilities of a synchrotron is relatively low. International experience suggests that once the synchrotron is fully operational, the community's appreciation and use of synchrotron light will increase dramatically. Demand for the dedicated single-crystal facility at SRS, UK, is over-subscribed by more than a factor of two. The new UK synchrotron under construction (Diamond) will have a dedicated small molecule single-crystal facility, reflecting a recognition of the importance of synchrotron investigations of small molecule structure for the benefit of UK science, industry and the economy. The same benefits would be provided by small molecule studies at the Australian Synchrotron. Small-molecule structure elucidation supports a wide range of chemical, geochemical, material science and medical R&D activities in Australia and New Zealand.

Beamline Design

Beamline 1 will be sourced from a bending magnet and will be operational on first light from the synchrotron. Beamline 1 is intended to be a highly automated work-horse with crystals robotically loaded and centred and to be capable of being operated remotely. Because of the very large scale on which proteomics research will be conducted, it is expected that throughput will need to be high It will use the MAD technique, in which the wavelength of the x-ray beam is scanned over a range of frequencies close to the x-ray absorption edge of certain of the metallic atoms in the protein crystals. In addition, emphasis will be placed on rapid automatic data collection and interpretation. The data will be automatically handled by sophisticated software to provide detailed crystal structure information in real time.

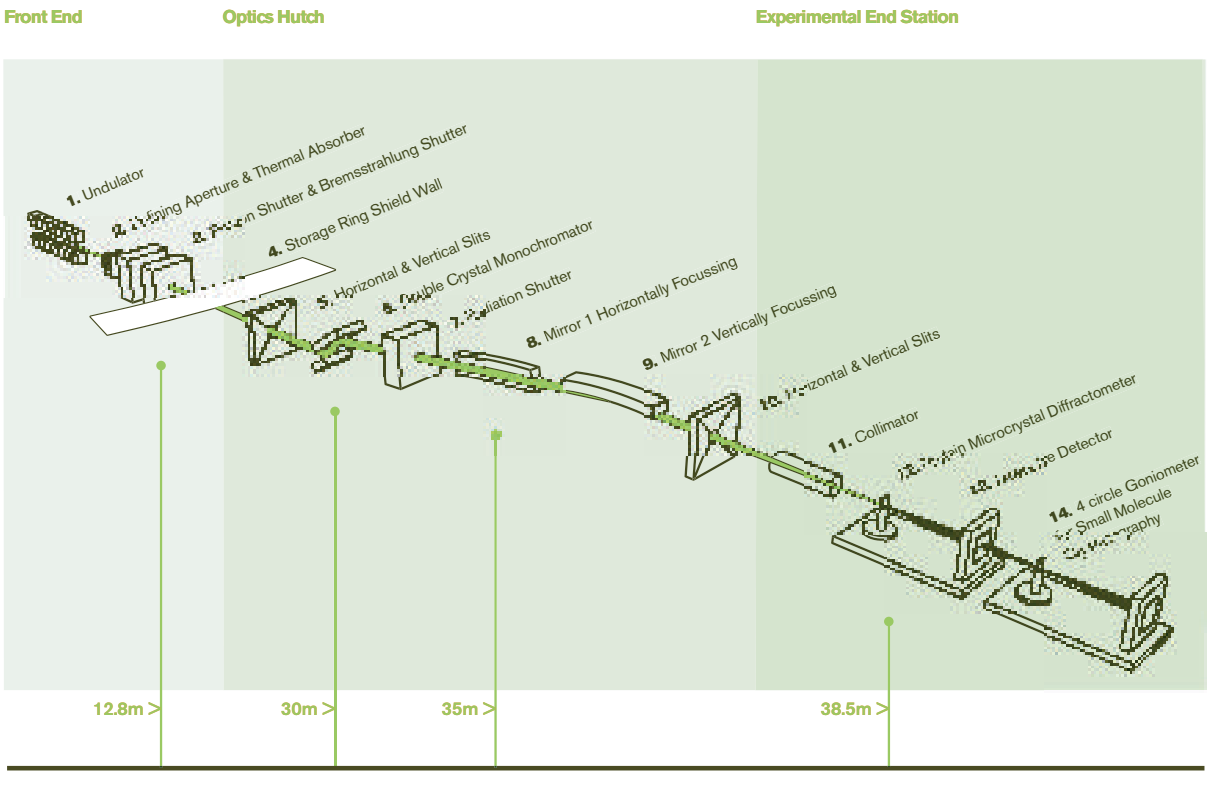
At a later stage, consideration will be given to sourcing the beamline from an undulator, to provide higher flux at the sample.

Beamline 2 will be driven from an undulator to produce the high brightness that is required for a highly focussed beam. Like beamline 1 it will have robotic loading and centring of the crystals and will also have MAD capability.

A second end station will be installed in a separate hutch on beamline 2 that will be able to collect high resolution, low signal-to-noise data from large protein complexes and virus crystals (the maximum unit cell size of biological materials to be studied is planned to be about 1,200 Å) as well as small molecules.

The design of the two beamlines has been developed according to the following principles:

- As far as possible the beamlines will use common elements and equipment to reduce cost and for efficiency of maintenance. The small molecule end-station, in particular, would use well-established and essentially 'off the shelf' technology.



BEAMLINE 2 Protein Microcrystal & Small Molecule X-ray Diffraction

Figure BL2.1 *Schematic of the protein microcrystal and small molecule x-ray diffraction beamline*

- Requirements for beam spot size and divergence will vary depending on the crystals to be analysed, so flexibility will be built into the beam optics.
- The designs will be based on the current international best practice, particularly the beamlines at the Swiss Light Source.
- Each line will commence operations with a flat panel detector to ensure rapid start-up. Once each beamline is fully operational the flat panel detector will be replaced with a single pixel type. This will be staged so that the flat panel from beamline 1 will be used to start up beamline 2.
- The beamlines will be automated and made as user friendly as possible, recognising that the users are unlikely to be specialists in these synchrotron based techniques.
- Provision will be made to enable remote operation of the facility, and for rapid interpretation of the data. Robotic operation will be added after initial commissioning.

It is envisaged that at commencement both functions on beamline 2 will be housed in the same end station, but later, as demand for small molecule studies increase, they will be housed in separate end stations for greater experimental flexibility.

The combination of small molecule crystallography with protein micro-crystallography will provide equipment and personnel benefits for both applications. Both fields have convergent detector needs – wide solid-angle, linearity, high dynamic range and low noise. Small molecule studies require a four-circle kappa goniometer, and although not the conventional choice it provides recognised benefits for protein data collection. A four-circle goniometer provides full diffraction sphere coverage, and this has been shown to facilitate and accelerate the determination of protein structures. Precision studies of small molecules require shorter wavelengths than would normally be used by protein crystallography. Shorter wavelengths minimise absorption and additionally charge density studies are now beginning to be extended to proteins; this is likely to have a significant impact on rational drug design programs.

Ancillary equipment will include cryostats providing a temperature range of at least 15 K to 1000 K and diamond anvil cells for high pressure studies.

BEAMLINES 1 & 2

Beamline 1 – High-throughput Protein Crystallography	
Source	Bending magnet
Energy range	2–23 keV
Resolution	$<1 \times 10^{-4}$
Nominal beam size at sample (horizontal × vertical)	300 × 200 microns
Features	Robotic loading, MAD capability

Beamline 2 – Protein Micro-crystal and Small Molecule X-ray Diffraction	
Source	In-vacuum undulator
Energy range	5.5–20 keV
Resolution	$<1 \times 10^{-4}$
Nominal beam size at sample (horizontal × vertical)	80 × 30 microns
Features	Robotic loading, MAD capability, 2 end stations