

Canadian Macromolecular Crystallography Facility

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Principal Contacts

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Beamline Overview

Status	Approved and funded as of December 31, 2001
Source	Small-gap in-vacuum undulator
Monochromator	Double crystal
Spectral range	6.5–18 keV
Flux at sample at 12 keV (7 th harmonic)	$\sim 7 \times 10^{12}$ photons/s with Si(111) monochromator
Brilliance at 12 keV (7 th harmonic)	$\sim 10^{18}$ photons/mm ² /mrad ² /0.1% bandwidth
Energy Bandwidth ($\Delta E/E$): with Si(111) monochromator with Si(220) monochromator	1.4×10^{-4} 5.5×10^{-5}
Focused spot size (horizontal \times vertical) at sample	0.15 mm \times 0.05 mm (FWHM)
Beam divergence at sample	<1 mrad

Science

Crystallography is the use of X-ray diffraction to determine the structure of crystals of molecules. A single crystal is made up of molecules packed in a repeating three-dimensional arrangement. All repeating units, called unit cells, are identical and contain one or more molecules. When such a crystal is placed in a focused

beam of X-rays, diffraction of the primary beam occurs. The measurement of the position and intensity of the diffracted X-ray beams, called reflections, produces an X-ray diffraction data set.

The position and spacing of the reflections are determined by the three-dimensional arrangement of the unit cells. The larger the unit cell is, the more closely spaced are the reflections. The intensities are determined by the electron density distribution within the unit cell. Unfortunately, the electron density distributions cannot be derived directly from the diffraction intensities. To obtain these distributions, a second property of diffracted X-rays—their phases—must be determined. Since there is no practical way to measure the phases of reflections, they have to be determined indirectly. Three general methods are used to establish the phases of reflections: molecular replacement (MR), multiple isomorphous replacement (MIR), and multiple wavelength anomalous dispersion (MAD). In recent years, MAD has become the method of choice, but it requires a synchrotron source.

Single crystal X-ray diffraction of biological macromolecules has arguably become the most important experimental technique to elucidate the atomic-level structure of biological macromolecules. It has produced the detailed structures of thousands of proteins and other macromolecules, structures that have contributed to the understanding of fundamental processes in virtually all fields of biological and medical sciences.

Since there are a large number of macromolecular crystallographers in Canada, each with large numbers of samples, a high level of productivity will be needed.

Studying small crystals of large biological molecules requires hard X-ray sources with extraordinarily high brilliance. A precisely and readily tunable X-ray energy source is needed in order to use MAD phasing.

The X-ray optics must be superior—with both horizontal and vertical focusing—to get a very small beam size at the sample. Moreover, the beam must have a small divergence at the sample (small crossfire). At the CLS, the optimum beamline length (for the desired divergence and horizontal focused spot size) is greater than the longest site in the current building. Consequently, the protein crystallography (PX) beamline team has requested the longest possible beamline site.

Taken together, these various requirements have led to the selection of an in-vacuum small gap undulator (SGU)

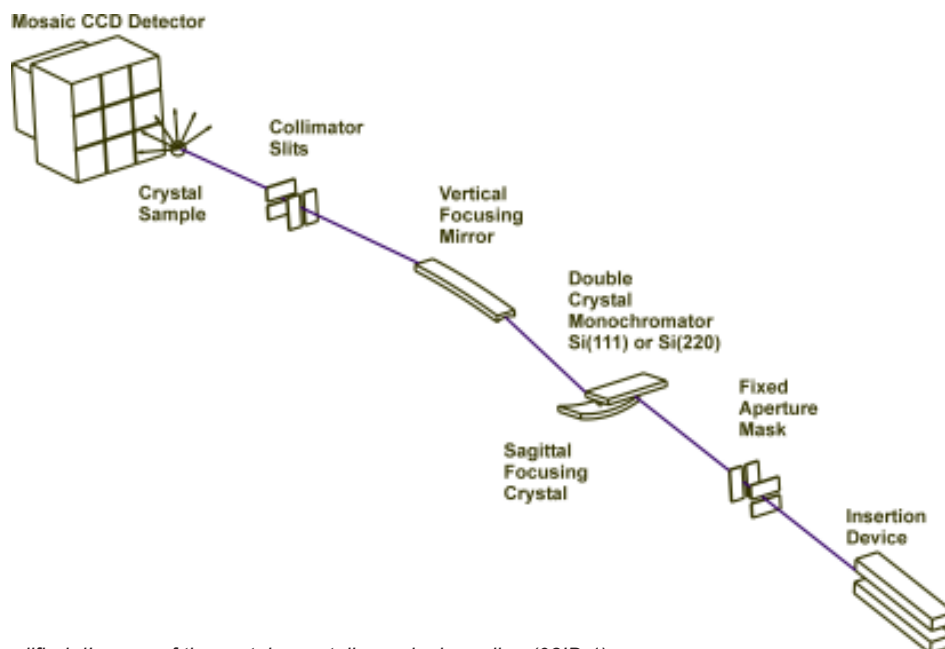


Figure 11-1 Simplified diagram of the protein crystallography beamline (08ID.1).

coupled to a beamline and endstation design that is optimized for high efficiency and productivity.

This beamline is the initial phase of the Canadian Macromolecular Crystallography Facility (CMCF). The beamline team is planning to build a PX beamline with a performance comparable to the best facilities anywhere in the world. To this end, CLS PX Beamline Scientist Pawel Grochulski spent five months (in 2001) at the APS working on the construction of the 22-ID SER-CAT beamline with Gerd Rosenbaum and his colleagues. This beamline is itself an improved version of one of the most productive and advanced PX beamlines in the world, the SBC-CAT beamline at the APS.

Due to the large demand for PX beamlines in Canada, the PX beamline team, with support from the Alberta Synchrotron Institute and scientists in British Columbia, has submitted a letter of intent for a second PX beamline.

Layout and Instrumentation

A schematic layout of the PX beamline is presented in Figure 11-1. An in-vacuum small-gap undulator (SGU) located in the “08” straight section will illuminate the PX beamline. The SGU will be a hybrid undulator 1.6 m in length, with a 20 mm period and a minimum gap of 5 mm. The predicted performance of the undulator is

shown in Figure 11-2. At 12 keV generated from the seventh harmonic using a Si (111) crystal monochromator, the expected flux in the 0.15 mm × 0.05 mm (horizontal × vertical) spot on the sample will be approximately 7×10^{12} photons/s.

The main optical elements of the PX beamline include a primary aperture (water-cooled mask), a double crystal monochromator with a cryogenically cooled first crystal and a sagittally bent second crystal, a mirror, Bremsstrahlung collimators and stop, a synchrotron radiation photon beam stop, adjustable guard slits, and a photon shutter. The beam will be horizontally focused by the sagittally bent crystal and vertically focused by the 1.0 m long mirror, which will also remove harmonics. A number of beam position monitors and reference detectors will be included in the optics configuration. The beamline will be separated from the front end by a beryllium window during commissioning.

The endstation (Figure 11-3) will be equipped with a goniostat, a CCD detector, a sample cryocooling system, a fluorescence detector, a timing shutter, video cameras, and an automated robotic system for crystal mounting and alignment. Advanced user interfaces, advanced control software, and fully automated robotic sample-handling systems will allow remote observation and data collection in “FedEx” mode.

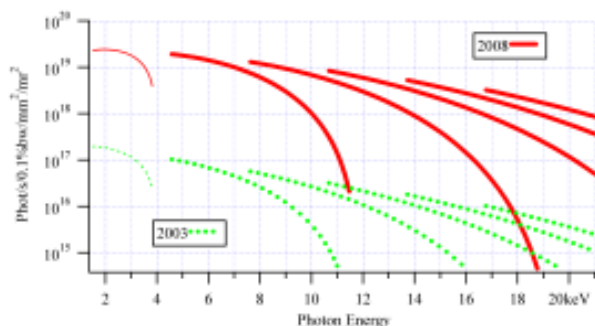


Figure 11-2 Brightness of the SGU in 2003 (dotted line) and in 2008 (solid line). The thicker lines show the harmonics that will be used to cover the 6.5–18.0 keV range.

Performance

General specifications for the PX beamline are in the Beamline Overview at the beginning of this chapter.

Most of the Canadian PX research community is anticipated to need X-ray energies between 6.5 and 18 keV. This range encompasses the absorption edges of the heavy atoms most commonly used for crystal structure analysis: the K edges of Mn, Fe, Co, Ni, Cu, Zn, Ga, Ge, As, Se, Br, Kr, Rb, Sr, Y, Zr, Nb, and Mo, as well as the L_{III} edges of all elements with an atomic number greater than 62 (samarium to uranium). The most valuable absorption edges for resonant crystallography are between 11.5 and 12.66 keV: the K edge of Se and the L_{III} edges of Pt, Au, and Hg.

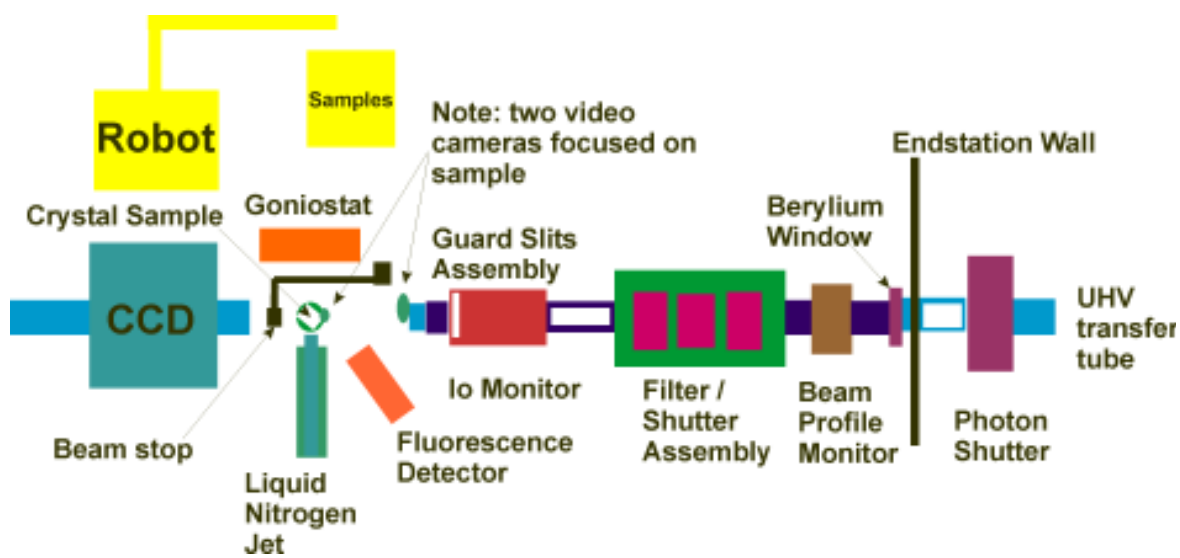


Figure 11-3 The PX experimental station.

Monochromator specifications

The monochromator will have a standard spectral range of 6.5–18.0 keV, a 31 mm beam offset, and a Bragg-angle range of 6.5–38 degrees with 2 μ rad Bragg-angle reproducibility. It will use either a Si (111) or a Si (220) double crystal; the first will be cryocooled and the second one sagittally bent. The temperature stability must be to less than 1 °C. The monochromator will have motorized tune, twist and roll control (4 mm range with 50 nm resolution), and will be supported by a vibration-isolation base isolated from the vacuum envelope.

Mirror specifications

The ultralow-expansion plane-mirror substrate will have a 1000 mm \times 90 mm \times 42 mm (L \times W \times H) clear aperture, with 2 Å r.m.s. roughness and 1 μ rad surface figure error. It will have “Pt, none, Pd” coating stripes (each 30 mm in width). The supports will be motorized and encoded, with independent and dynamic bending mechanisms at both ends. Aberration correction will be via elliptical bending.

Beamline Team Research Activities

The Beamline Team (BT) members listed in Table 11-1 are very active, with seventy refereed publications to date in 2001. Short summaries of the research activities of some members of the BT are presented in this section.

Canadian consortium in the X8-C PRT at NSLS

The Canadian consortium participating in the X8-C PRT at NSLS (Brookhaven) consists of nine principal investigators. They are (alphabetically): A. Berghuis, M. Cygler, S. Evans, L. Howell, Z. Jia, S.-X. Lin, E. Pai, J. Sygusch, and A. Vrielink (replaced by B. Shilton). This group had access to 30% of the beamtime: 69 days between September 1 of 2000 and August 31 of 2001. N. Strynadka used three additional days, for a total of 72 days.

During the three years of Canadian participation in this PRT, significant beamline upgrades were implemented. The MAR detector was replaced by the Quantum-4 CCD detector (from Area Detection Systems Corp.); a new goniostat with a two-theta arm was installed on a heavy stage; an Oxford Cryosystems cryocooler was added; and the computers were continually upgraded. A full range of crystallographic software was installed for rapid data processing, MAD phasing, and electron density map viewing. Small improvements to the optics were made and new beamline control hardware was also installed.

FedEx-type service for data collection is being implemented. The beamline staff consists of a Ph.D. scientist and a technician. A night technician is shared between all crystallography beamlines.

In 2000–2001, the Canadian consortium published 41 papers that directly resulted from data collected at the NSLS X8-C beamline.

Alberta Synchrotron Institute

The Alberta Synchrotron Institute (ASI) supports four projects (designated PX1–PX4) in protein crystallography and structural biology.

PX1: Beamline development at the CLS

The ASI will contribute directly to the design, construction and implementation of the protein crystallography beamline at the Canadian Light Source.

The ASI will also contribute towards CLS expertise in robotic handling of crystals through the provision of trainees to be located at major synchrotrons in the USA. These trainees will bring their expertise back to the CLS and will establish international collaborations in this technology. A firm of engineering consultants (FSDG/UMA) has been contracted to assess the status of robotics at several American synchrotrons and to advise on the development of the technology for the CLS.

Table 11-1 CMCF beamline team

Université Laval	Shen-Xiang Lin (CHUL Research Centre)
McGill University	Albert Berghuis (Biochemistry) James Coulton (Microbiology and Immunology)
McMaster University	Daniel Yang (Biochemistry)
Queen's University	Zongchao Jia (Biochemistry)
NRC	Mirek Cygler (BRI, Montreal) Michelle Loewen (PBI, Saskatoon)
Simon Fraser University	Frederic Pio (Microbiology and Immunology) Mark Paetzel (Molecular Biology and Biochemistry)
University of Alberta	Michael James (Biochemistry) Mark Glover (Biochemistry) Bart Hazes (Medical Micro-biology and Immunology)
University of British Columbia	Gary Brayer (Biochemistry) Natalie Strynadka (Biochemistry) Michael Murphy (Microbiology and Immunology)
University of Calgary	Barry Phipps (Biological Sciences) Hans Vogel (Biological Sciences) Leslie Tari (Biological Sciences) Anthony Shryvers (Microbiology and Infectious Diseases)
University of Lethbridge	Steve Mosimann (Biochemistry)
University of Manitoba	Peter Loewen (Microbiology)
Université de Montréal	Jürgen Sygusch (Biochemistry) Patrick Hallenbeck (Microbiology and Immunology)
University of Ottawa	Stephen Evans (Biochemistry)
University of Saskatchewan	Louis Delbaere (Biochemistry) Pawel Grochulski (Biochemistry) Scott Napper (Biochemistry) Wilson Quail (Chemistry) David Sanders (Chemistry) Emil Hallin (CLS)
University of Toronto	Emil Pai (Biochemistry) Lynne Howell (Biology and Biochemistry) David Rose (Medical Biophysics) Gilbert Privé (Medical Biophysics) James Rini (Molecular and Medical Genetics and Biochemistry) Frank Sicheri (Molecular and Medical Genetics and Biochemistry) Aled Edwards (Banting and Best Dept. of Medical Research)
University of Western Ontario	Brian Shilton (Biochemistry) Marie Fraser (Biochemistry)

Software development supported by the ASI will be in the areas of advanced graphical user interfaces, integrated control software, high-capacity network data transfer and storage, and automated robotic sample handling for remote data collection. The graphical interface being adopted is the BLU-ICE system from SSRL.

PX2: Interim access to PX beamlines

The first CLS beamlines are scheduled to come on line in January, 2004. To assist in meeting the immediate research needs of protein crystallographers in Alberta, the ASI has established an interim access agreement and joined a consortium for a newly developed PX beamline at the ALS. This consortium includes protein crystallographers from UC Berkeley (Alber et al.) and UC San Francisco (Agard et al.).

PX3: Technology training

This ASI project will provide training in two major areas that are crucial for the operation of a modern competitive protein crystallography beamline: (i) software development (for integrated beamline control software, graphical user interfaces, and high-capacity data transfer and storage) and (ii) robotic sample handling.

PX4: Collaborative initiatives

This ASI project, “Development of Advanced Experimental Endstation Technology for High-Throughput Protein Crystallography for Structural Genomics Projects”, is designed to facilitate collaboration among several protein crystallography groups. An initial

application has been made to the International Access Fund of the Canada Foundation for Innovation. The principal universities (and institutions) involved are Stanford University (SSRL), the University of Alberta (ASI), and the University of Saskatchewan (CLS).

Beamline Team and Design Team

Table 11·1 lists the core group of Canadian researchers who support this beamline, while Table 11·2 gives the design team for the PX beamline. The milestones for the CMCF are presented in Table 11·3. ✨



Jim Rini (University of Toronto) describing his research on glycoprotein structure and mechanisms at the CLS Users' Meeting on November 17, 2001.

Table 11·2 CMCF beamline design team

Canadian Light Source	Ingvar Blomqvist Emil Hallin Pawel Grochulski
University of Saskatchewan	Louis Delbaere (Biochemistry)
University of Alberta	Ernst Bergmann (Biochemistry; Alberta Synchrotron Institute) Bart Hazes (Medical Microbiology and Immunology)
University of British Columbia	Natalie Strynadka (Biochemistry)
University of Toronto	Emil Pai (Biochemistry) James Rini (Biochemistry)
BRI (NRC) Macromolecular Structure Group	Mirek Cygler Joe Schrag
University of Georgia	Gerold Rosenbaum (SER-CAT)

Table 11·3 CMCF milestones for 2000–2001

February, 2000	FAC approval of the proposal
November, 2000	PX beamline development scientist hired
December, 2000	Orientation Meeting
January–May, 2001	CLS PX beamline development scientist at APS
April, 2001	Decision of the design team to build the first beamline on a small gap undulator
August, 2001	LOI for a second PX beamline submitted to the CLS
October, 2001	Submission of the Preliminary Design Report and Preliminary Safety Report for the first PX beamline