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Beamline 08ID-1, the prime beamline of the Canadian Macromolecular Crystallography Facility

Pawel Grochulski,[a,b]* Michel N. Fodje,[a,c] James Gorin,[a] Shaunivan L. Labiuk[a] and Russ Berg[a]

[a]Canadian Light Source Inc., 101 Perimeter Road, Saskatoon, Saskatchewan, Canada S7N 0X4, [b]College of Pharmacy and Nutrition, University of Saskatchewan, 110 Science Place, Saskatoon, Saskatchewan, Canada S7N 5C9, and [c]Biochemistry, University of Saskatchewan, 107 Wiggins Road, Saskatoon, Saskatchewan, Canada S7N 5E5. E-mail: pawel.grochulski@lightsource.ca

Beamline 08ID-1 is the prime macromolecular crystallography beamline at the Canadian Light Source. Based on a small-gap in-vacuum undulator, it is designed for challenging projects like small crystals and crystals with large cell dimensions. Beamline 08ID-1, together with a second bending-magnet beamline, constitute the Canadian Macromolecular Crystallography Facility (CMCF). This paper presents an overall description of the 08ID-1 beamline, including its specifications, beamline software and recent scientific highlights. The end-station of the beamline is equipped with a CCD X-ray detector, on-axis crystal visualization system, a single-axis goniometer and a sample automounter allowing remote access to the beamline. The general user program is guaranteed up to 55% of the useful beam time and is run under a peer-review proposal system. The CMCF staff provide 'Mail-in' crystallography service to the users with the highest-scored proposals.

Keywords: macromolecular crystallography; multiwavelength anomalous diffraction; mail-in crystallography; remote control; structural biology.

1. Introduction

The Canadian Light Source (CLS) is a 2.9 GeV national synchrotron radiation facility located on the University of Saskatchewan campus in Saskatoon. The CLS is funded by the Canadian government, provincial, industrial and academic sources. The initial $137.5 million project consisted of construction of the storage ring along with seven beamlines. This phase included the first macromolecular crystallography (MX) beamline: an insertion-device beamline, 08ID-1, the first Canadian Macromolecular Crystallography Facility (CMCF) beamline. The contribution was further matched by other governments and institutions to a total of $173.5 million.

The first beam at the sample position was observed in 2005 and the first data were collected in May 2006 (Grochulski *et al.*, 2006). In 2001, the CMCF beamline team submitted another Letter of Intent to the Canada Foundation for Innovation to build a second complementary beamline, named 08B1-1. This fully automated bending-magnet beamline is capable of screening large numbers of crystals and is equipped for remote control of data acquisition. At present, the CMCF consists of two beamlines: an insertion-device beamline, 08ID-1, and a bending-magnet beamline, 08B1-1. Currently the facility serves more than 60 macromolecular crystallographers located across Canada and USA.

2. Beamline overview

Beamline 08ID-1 is a highly efficient and flexible beamline, capable of satisfying the requirements of the most challenging and diverse of crystallographic experiments, *i.e.* small crystals and crystals with large unit-cell dimensions. The beamline is illuminated by a 20 mm-period asymmetric hybrid small-gap in-vacuum undulator (SGU). The undulator operates at a minimum gap of 5.5 mm ($B_{\rm eff}$ = 0.923 T). It is located upstream of the straight section and chicaned inboard by 0.75 mrad to accommodate a future ID beamline in the same straight section. The beam is defined by a front-end beam-defining mask (FE) with opening of 0.1 mrad (vertical) × 0.26 mrad (horizontal) (Fig. 1). The optics are comprised of a chemical-vapor-deposition diamond-based water-cooled fluorescence screen (FS) followed by water-cooled adjustable white-beam slits (WBS). Downstream from the slits there is a double-crystal Si(111) monochromator (DCM) which contains a cryogenically side-cooled first crystal, and a second sagittally bent crystal to focus the beam in the horizontal plane. Vertical focusing of the beam is achieved by a 1.1 m-long dynamically bendable ultra-low-expansion titanium silicate flat mirror (VFM). The active surface of the mirror has three stripes: platinum, palladium and un-coated. These can be translated into the beam, depending on the X-ray energy, to remove higher harmonics.

The monochromatic beam passes through an evacuated filter box, a beam position monitor (BPM) used for steering the beam, a set of vertical and horizontal slits, another BPM and a fast shutter which are encapsulated in the exposure box (EB). The end-station is equipped with an on-axis visualization system from Maatel integrated with the end-station by Accel (now Bruker) and a mini-beam apparatus (Fischetti *et al.*, 2009) (Fig. 2). The mini-beam apparatus allows selection of a beam size suitable to a specific crystal size by choosing an appropriate pinhole, although the most popular beam size is

Table 1
Beamline details.

Beamline name	08ID-1
Source type	Small-gap in-vacuum undulator
Mirrors	1 m ULE glass with Pt, none, Rh stripes
Monochromator	Cryo-cooled Si(111)
Energy range (keV)	6.5–18
Wavelength range (Å)	0.69–1.9
Beam size (uncollimated) (µm)	150 (H) × 50 (V)
Beam size (collimated, typical) (µm)	100
Flux (uncollimated) (photons s^{-1})	5×10^{12}
Flux (collimated, typical) (photons s^{-1})	2×10^{12}
Goniometer	Huber 410 single-axis
Cryo capability	LN_2 available
Sample mounting	SAM
Detector type	CCD
Detector model	Rayonix MX300
2Θ capabilities	0–20°

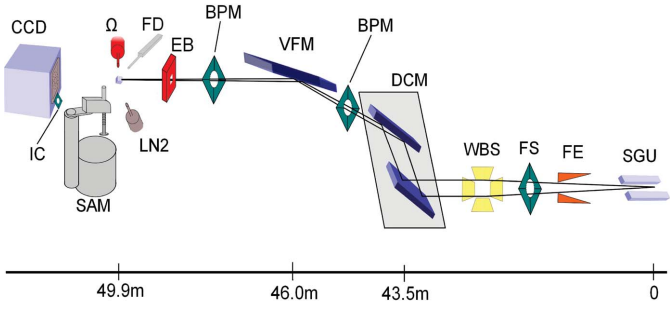
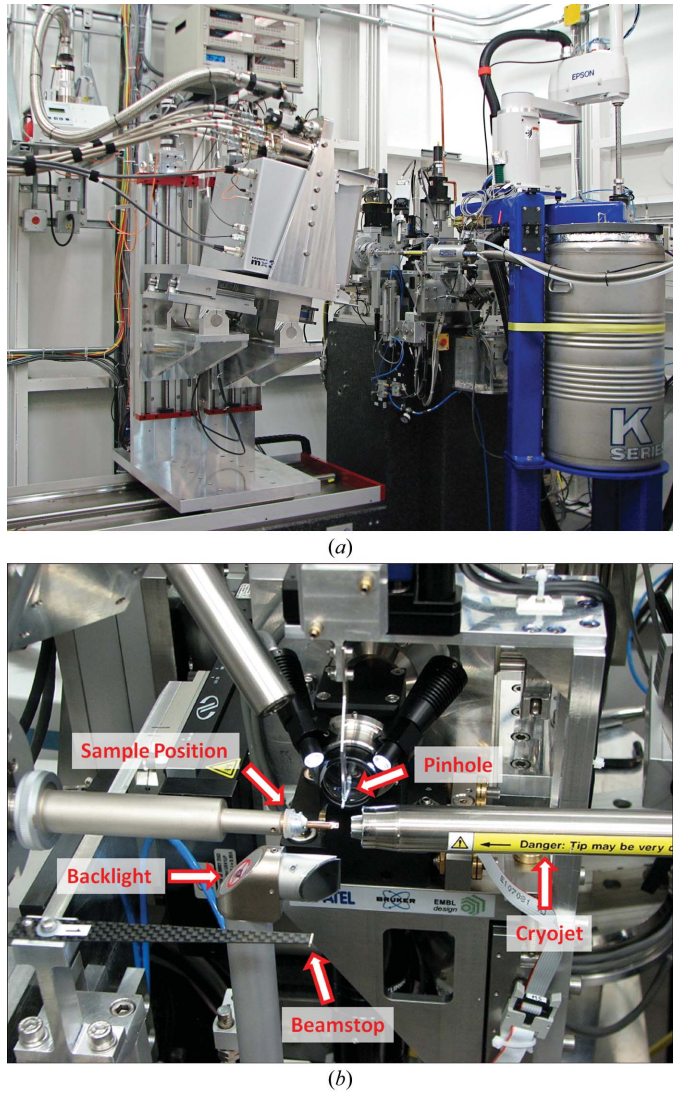


Figure 1
Layout of the CMCF 08ID-1 beamline including distances between major components. From right to left: SGU, small-gap in-vacuum undulator; FE, front-end beam-defining mask; FS, fluorescence screen; WBS, white-beam slits; DCM, double-crystal Si(111) monochromator; BPM, beam-position monitor; VFM, vertically focusing mirror; BPM; EB, exposure box which contains vertical and horizontal slits, BPM, fast shutter, mini-beam apparatus and OAV system; FD, fluorescence detector; LN2, cryojet; Ω, single-axis goniometer; SAM, Stanford automounter; IC, ion chamber; CCD, X-ray detector.

100 µm. Crystals are mounted on a single-axis Huber 410 goniometer (Ω) equipped with an *x*/*y* stage, allowing automatic centering of the loop. Diffraction data are collected on a Rayonix MX300 CCD detector mounted on a holder allowing a 2Θ offset of up to 20°. The beamline is equipped with a Röntek Spectrometer System XFLASH 101A to perform X-ray spectroscopy for multiwavelength anomalous dispersion (MAD) experiments, and crystals are kept under a nitrogen vapor stream by an Oxford Cryojet (LN2) allowing temperatures of 100 K. The shutter–Ω synchronization is accomplished by using a Parker GV6K programmable controller allowing exposure times to be greater or equal to 0.1 s. A typical exposure time at the beamline is 1 s, allowing fast screening of the crystals, efficient data collection at a single wavelength, as well as MAD experiments. The focused and uncollimated beam size of 150 µm (H) × 50 µm (V) delivers a flux of 5×10^{12} photons s^{-1} measured by an ion chamber (Table 1) at 12.66 keV using the sixth harmonic of the SGU at a ring current of 250 mA.

3. Ancillary facilities

CMCF users have access to a wet laboratory, a shipping Dewar storage room and a sample preparation room. The CMCF beamlines are equipped with a 128-core computing cluster with 8 TB of short-term and 30 TB of long-term storage. The beamline is equipped with a Stanford automounter (SAM) that accepts the SSRL cassettes and/or universal pucks (Cohen *et al.*, 2002). The automounter can contain up to 192 samples at a time.



(*a*)

(*b*)

Figure 2
Photograph of the end-station including CCD detector at 2Θ = 10° and SAM automounter (*a*), and close-up view of the sample position (*b*).

3.1. Beamline software

As with all facilities at the CLS, the low-level software of the beamline has been developed in EPICS. An interactive graphical user interface (GUI) for data collection (MxDC) has been developed locally (Fodje *et al.*, 2010). The GUI contains the following tabs: Beamline Setup, Samples, Screening, Data Collection, Fluorescence Scans and Results. The software allows for automatic centering, crystal screening, excitation and fluorescence scans. Strategy calculations, data collection and data processing are automated *via* a data processing module (DPM) known as *AutoProcess*, which is based on the *XDS* (Kabsch, 1993), *Pointless* (Evans, 2006), *CCP4* (Collaborative Computational Project, 1994), *BEST* (Bourenkov & Popov, 2006) and *XDSSTAT* (Diederichs, 2006) packages.

4. Facility access

Beam time is distributed such that at least 55% is devoted to general user programs. Up to 25% is reserved for commercial usage of the beamline, and the rest is devoted to upgrades and for use by the beamline staff. There are two calls for proposals per year and a peer-

review committee reviews proposals based on scientific merit and beamline suitability. Approved proposals are valid for two years, but principle investigators can re-submit proposals should they wish to seek a better score from the peer-review committee. Beam time is scheduled by beamline staff upon submission of a Beam-time Request or Mail-in Request against an active proposal (http://cmcf.lightsource.ca/).

At present there are up to four 8 h shifts per week set aside for Mail-in crystallography and it is provided to users with the highest peer-review scores. The data are collected by experienced CMCF staff members according to instructions provided by investigators.

Table 2
Occupancies of sulfur atom solutions as output by *PHENIX* or *SHELX*.

Processing† Phasing	*Autoprocess AutoSol*	*Autoprocess SHELX*	*HKL2000 AutoSol*	*HKL2000 SHELX*	*Mosflm AutoSol*	*Mosflm SHELX*
Normal beam	1.22	1.00	1.26	1.00	1.33	1.00
	1.15	0.97	1.20	0.85	1.23	0.94
	1.09	0.91	1.18	0.78	1.20	0.87
	1.07	0.90	1.16	0.72	1.20	0.64
	1.05	0.88	1.12	0.72	1.14	0.63
	1.03	0.76	1.10	0.59	1.12	0.62
		–	–	–	–	–
		0.30	0.19	0.29	0.32	0.21
		0.29	0.16	0.28	0.16	0.09
	(44)‡	(43)	(43)	(41)	(42)	(29)
Inverse beam	1.15	1.00	1.33	1.00	1.33	1.00
	1.09	0.90	1.32	0.94	1.26	1.00
	1.05	0.89	1.30	0.92	1.20	0.97
	1.04	0.76	1.16	0.84	1.18	0.96
	0.96	0.76	1.10	0.79	1.18	0.94
	0.95	0.67	1.06	0.69	1.09	0.75
	–	–	–	–	–	–
	0.15	0.34	0.18	0.33	0.26	0.30
	0.12	0.31	0.17	0.31		0.21
	(42)	(44)	(44)	(37)	(42)	(33)

† All data had a resolution range of 50–1.91 Å, a completeness of 95.7–98.6% (outer shell 63.5–89.7%), anomalous multiplicity of 8.8–9.3 (outer shell 2.0–2.7) and $R_{\rm merge}$ of 0.035–0.080 (outer shell 0.141–0.536). ‡ Values in parentheses represent correlation coefficient (CC) from *SHELX* or figure of merit (FOM) from *AutoSol*.

5. Highlights

The first data were collected at the 08ID-1 beamline in 2006 and the beamline was open to the general user by the peer-review process in August 2009. During the commission period between 2007 and 2009 the beamline was used rather extensively, resulting in 13 Protein Data Bank (PDB) deposits in 2008, 55 deposits in 2009 and, so far, 90 deposits in 2010.

Although beamline 08ID-1 is relatively new, it has already successfully taken on an array of challenging projects, including structures from crystals containing relatively large unit cells to membrane-associated proteins, MAD and S-SAD (sulfur single-wavelength anomalous diffraction) phasing. To date, more than 140 coordinates of crystal structures collected at the 08ID-1 beamline are deposited in the Protein Data Bank and more than 70 scientific articles have reported data collected here.

5.1. Ryanodine receptor

Because of their complexity and large size (~2.2 MDa), constructing accurate structural models of these tetrameric Ca^{2+} channels that control muscle contraction has been difficult. Data from beamline 08ID-1 have been used to determine the crystal structures of small fragments of up to 217 residues (Lobo & Van Petegem, 2009). More recently, the crystal structure of a larger 559-residue fragment of the N-terminal disease hotspot region, having relatively large unit-cell dimensions ($a = b = 170.8$ Å, $c = 301.2$ Å), was solved and combined with a lower-resolution cryo-electron microscopy structure (Tung *et al.*, 2010). This is a critical step in understanding why this region in particular is so important and provides insights into how the receptor is affected by disease mutations.

5.2. MATE family transporter

Multidrug and toxic compound extrusion (MATE) family transporters are the last known multiple-drug resistance transporters to have their crystal structures solved (He *et al.*, 2010). This large study involved crystals from multiple mutants of this membrane protein collected at a number of beamlines. Statistics from the datasets obtained at beamline 08ID-1 were comparable with those obtained from other well established facilities.

5.3. S-SAD phasing

Application of the S-SAD phasing method using a standard test protein has demonstrated the efficacy of using the method at beamline 08ID-1 (Labiuk *et al.*, 2009). Data were collected at 7.00 keV from bovine insulin crystals, either with normal or inverse beam, and data processed using *AutoProcess*, *HKL2000* (Otwinowski & Minor, 1997) and *Mosflm*/*Scala* (Leslie, 1992; Evans, 1997). All six S atoms were identified by *AutoSol* in *PHENIX* (Gursky *et al.*, 1992; Adams *et al.*, 2002) in the known cubic $I2_13$ space group using normal-beam data processed with *AutoProcess*. *AutoSol* was able to build 45 of 51 side-chains with an overall model-map correlation of 0.75. Subsequently, in all cases, the sulfur positions could be reliably solved using either *AutoSol* in *PHENIX* or *SHELX* (Sheldrick, 2008) (Table 2). Occupancies are presented for the top solutions output from each procedure, the first six being correct and the next-best positions shown (in output) to demonstrate a sharp cut-off in all cases. Successful structure solution combining the main processing and phasing programs available at the CMCF demonstrates the efficacy of using this powerful method for well diffracting crystals.

5.4. Teichoic acid polymerase

Teichoic acid polymerase is a critical enzyme that is localized to Gram-positive bacteria cell walls and is necessary to the full integrity and virulence of some bacteria. Thus, a key enzyme responsible for teichoic acid biosynthesis makes an intriguing target for novel anti-infectives. Data collected at beamline 08ID-1 were used to determine the 2.7 Å native structure of a teichoic acid polymerase construct from *Staphylococcus* containing the catalytic domain as well as the membrane-targeting region, with or without substrate (Lovering *et al.*, 2010).

5.5. Flagellar secretion apparatus

Bacterial flagella are complex structures and one of the components thought to comprise the export pore for flagellar assembly is a membrane-spanning protein called FlhA. A selenium *K*-edge MAD experiment allowed the solution of the cytoplasmic domain of FlhA from *Helicobacter pylori*, a bacterium that plays a role in peptic ulcer

diseases, gastritis and even adenocarcinomas (Moore & Jia, 2010). The structure provided clues about the likely orientation of this fragment in relation to the export pore and comparison with other related structures such as that found in *Salmonella*. This is one of several studies successfully conducted at the CMCF making use of MAD or SAD phasing with various heavy atoms.

6. Discussion and conclusions

In summary we have developed and maintain the 08ID-1 beamline which is one of the CMCF MX beamlines located at the Canadian Light Source. The beamline is capable of measuring data from crystals with cell dimensions approaching 1000 Å owing to small divergence at the sample, large 2Θ offset and up to 1 m sample-to-crystal distance. Owing to large flux density and small divergence on the sample, crystals of size less than 50 µm are routinely being measured at the beamline, with the smallest so far being $\sim$5 µm $\times$ 10 µm $\times$ 50 µm. The software at the beamlines has been developed locally. It is extremely robust, reliable and versatile allowing performance of all operations from one console. Besides usual operation of the beamline, it includes a data-processing module known as *AutoProcess*.

The recently installed Maatel's on-axis visualization system allows centering of crystals of very small size (<10 µm). To be able to measure data from such crystals, the goniometer needs to have a sphere of confusion of less than 1 µm. Therefore, an upgrade of the Huber 410 goniometer to an air-bearing goniometer is planned. It will not only improve the sphere of confusion but will speed the centering of crystals since the speed of the air-bearing can reach 360° s^{-1}. The *SHADOW* (Sánchez del Rio & Dejus, 2004) calculated focused beam size in the vertical direction is about 20 µm. Therefore, in order to produce an appropriate flux density for work with smaller crystals (less than 20 µm), we are planning on using monocapillary optics to generate an intense microbeam of about 5 µm (Gillilan *et al.*, 2010). The capillary will become an integrated part of the mini-beam apparatus.

The number of scientific papers for which data were collected at the 08ID-1 beamline exceeds 80 at present, and the number of PDB entries exceeds 180 structures. During 2010 several high-impact papers, including studies of membrane proteins, transporters and important enzymes, were reported that are described in the *Highlights* section of this paper.

Detailed instructions on how to access and use the beamline are available on the beamline website (http://cmcf.lightsource.ca/).

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