The Macromolecular Crystallography Beamline of SSRF Postprint

Authors: WANG Qi-Sheng, YU Feng, Sheng Huang, Sun Bo, Kun-Hao Zhang, LIU Ke, WANG Zhi-Jun, XU Chun-yan, WANG Si-Sheng, YANG Li-Feng, PAN Qiang-Yan, LI Liang, ZHOU Huan, Cui Yin, XU Qin, Earnest Thomas, HE Jian-Hua

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Abstract

The macromolecular crystallography beamline BL17U1 at the Shanghai Synchrotron Radiation Facility (SSRF) is the first dedicated macromolecular crystallography (MX) beamline at a third-generation synchrotron in China. It utilizes an in-vacuum undulator as a source and is energy-tunable from 5 to 18 keV. The beamline was commissioned and opened for users in April 2009. The experimental station was upgraded in 2011 with an advanced detector, a high precision goniometer and an automatic sample exchanger for high efficient and high-throughput data collection of protein crystals. The current set-up allows for remote operation of sample mounting, centering and data collection of pre-frozen crystals. In recent two years, the number of PDB depositions from this beamline exceeds 330 each year. In this paper, we describe the complete BL17U1 beamline with upgraded end station and how it is managed for user community.

Full Text

Preamble

The Macromolecular Crystallography Beamline of SSRF

WANG Qi-Sheng (汪启胜),[1,2] YU Feng (郁峰),[1] HUANG Sheng (黄胜),[1] SUN Bo (孙波),[1] ZHANG Kun-Hao (张坤浩),[1] LIU Ke (刘科),[1] WANG Zhi-Jun (王志军),[1] XU Chun-yan (徐春艳),[1] WANG Si-Sheng (王思胜),[1] YANG Li-Feng (杨利峰),[1] PAN Qiang-Yan (潘强岩),[1] LI Liang (李良),[1] ZHOU Huan (周欢),[1] CUI Yin (崔莹),[1] XU Qin (徐琴),[1] Thomas Earnest,[1] and HE Jian-Hua (何建华)[1],†

[1]Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, China
[2]University of Chinese Academy of Sciences, Beijing 100049, China

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The macromolecular crystallography beamline BL17U1 at the Shanghai Synchrotron Radiation Facility (SSRF) is the first dedicated macromolecular crystallography (MX) beamline at a third-generation synchrotron in China. It utilizes an in-vacuum undulator as a source and is energy-tunable from 5 to 18 keV. The beamline was commissioned and opened for users in April 2009. The experimental station was upgraded in 2011 with an advanced detector, a high-precision goniometer, and an automatic sample exchanger for efficient, high-throughput data collection from protein crystals. The current setup enables remote operation for sample mounting, centering, and data collection of pre-frozen crystals. In the past two years, the number of PDB depositions from this beamline has exceeded 330 each year. This paper describes the complete BL17U1 beamline with its upgraded end station and how it is managed for the user community.

Keywords: Macromolecular crystallography, Beamline automation
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Introduction

The Shanghai Synchrotron Radiation Facility (SSRF) is a national facility dedicated to scientific research requiring synchrotron light [**?**]. The phase-I beamlines serve the user community with a wide range of applications in physics, chemistry, and life sciences. Among them, BL17U1 is a dedicated macromolecular crystallography (MX) beamline. It is the first third-generation MX beamline in China and makes synchrotron beam time for MX more easily accessible to structural biologists in China. It was designed to meet various demands for crystal structure determination of both large unit cells and small crystals. The high flux, small beam divergence, and reasonably small beam size facilitate structural determination of large complexes with unit cells up to 1000 Å and small crystals down to a few tens of microns in size.

The beamline is tunable in the range of 5–18 keV, with a flux of approximately 4×10^{12} photons/s at 1 Å. The performance of the beamline was examined and verified by a committee of international experts. It became available for users in April 2009. In its initial stage, the end station was equipped with a MarDTB and Mar225 CCD (Charge-Coupled Device) detector due to budget constraints. In the end station upgrade project approved by the Chinese Academy of Sciences (CAS), the station was equipped with an ADSC 315 CCD detector and a high-precision goniometer from Crystal Logic. To improve operational efficiency and achieve full automation of experiments, a robotic sample exchanger from Rigaku was installed and integrated into the control system with a user-friendly interface. This paper describes the upgraded end station, user activity, productivity, and their fruitful results.

II. Beamline Overview

SSRF is a third-generation light source with a 3.5 GeV storage ring of 432 m circumference and 3.9 nm rad emittance, which has been in operation since 2009. Many performance improvements have been made to meet the experimental demands of users [**?**]. Currently, top-up mode with 240 mA ring current is the routine operational mode for users.

A home-made in-vacuum undulator with 80 periods and a period length of 2.5 cm [**?**] serves as the source. Parameters are optimized to cover the required energy range (5–18 keV) with easy tuning, allowing typical SAD (Single-wavelength Anomalous Dispersion) or MAD (Multi-wavelength Anomalous Dispersion) experiments and producing a brilliant beam of 10^{19} photons/[(mm^{2}mrad^{2}s(0.1 %BW)]. The source size is 380 µm × 25 µm (FWHM, H×V) and the flux in the central cone (80 × 20 µrad^2@12 keV) is 3.0 × 10^{14} ph/[s(0.1 %BW)]. A water-cooled aperture defines the size of the emitted photon beam. The beamline has an optical setup well suited for the finely collimated undulator beam. The layout is shown in Fig. 1 [FIGURE:1].

To build an easy-to-use and reliable crystallography beamline, the optical design pursues high stability and high flux. The beamline optical components include a double plane crystal monochromator (DCM) and a toroidal mirror that focuses the beam both vertically and horizontally. The DCM, a central unit of the optical system manufactured by ACCEL Instruments GmbH, is located 27.5 m from the source. Due to the extremely high heat-load power density on the first crystal of the DCM, it is cooled by liquid nitrogen (LN_2). The Si(111) flat first crystal, sized 150 mm(l) × 30 mm(w) × 30 mm(h), is mounted on an LN_2-cooled copper heat exchanger. The second flat crystal is sized 170 mm(l) × 30 mm(w) × 30 mm(h). The DCM deflects the beam upwards with a fixed 25 mm offset across the energy range. A separate water chiller is installed to help maintain internal thermal stability, which is vital for beamline stability. The temperature of the thermal stabilization system is monitored by PT100 sensors placed at different points in the DCM chamber.

The focusing mirror, manufactured by Thales SESO, has a toroidal surface obtained by bending a cylinder and deflects the beam horizontally at a 3.5 mrad grazing angle. It focuses the beam horizontally by dynamically tunable meridional curvature and vertically by a fixed sagittal curvature. In this optical geometry, the beam exiting from the toroidal mirror is parallel to the ground, which facilitates beamline alignment and experimental setup. The mirror dimensions are 1000 mm(l) × 70 mm(w) × 50 mm(h), with 50 nm rhodium coating on the silicon substrate. The mirror is mounted on a bender manufactured by Toyama Co., Ltd.

The beam position monitoring system for monitoring and diagnosing the beam includes a fluorescence screen, a wire-scan beam probe, and a quadrant beam position monitor (QBPM) [**?**]. The fluorescence screen can be inserted into the beam to inspect both white and monochromatic beams, and the wire can be

scanned to probe the beam profile; these are mainly used during commissioning, while the foil QBPM can remain in the beam for recording beam stability data. Two beryllium windows are located after the DCM and mirror, respectively, to protect the components in ultra-high vacuum. The beamline is tunable over the range of 0.69–2.48 Å, though it is usually operated in the 0.8–1.8 Å range. The flux of the focused beam at 1 Å is 3.8×10^{12} phs/s (see Table 1).

Table 1 . Specifications of beamline BL17U1

Parameter	Specification
Source Type	Undulator, 25 mm × 80 periods, 6–12 mm gap
Monochromator	Cryo-cooled double flat crystal Si(111)
X-ray energy range (keV)	5–18
Wavelength range (Å)	0.69–2.48
Flux at sample (at 12.4 keV, 240 mA) (phs/s)	3.8×10^{12}
Focused beam size (FWHM) (µm)	67 × 23 (H×V)
Typical beam divergence (mrad)	0.3 × 0.1
Goniometer	Single-axis
Cryo capability (K)	100
Sample mounting	Manually/Rigaku ACTOR robot
Detector model	CCD, Q315r
2Theta capability	Yes

Measured using a calibrated ion chamber close to the sample position with the 7th harmonic of U25.

III. Experimental Environment

In the experimental hutch, the monochromatic beam passes through a rotary attenuator consisting of aluminum foils of various thicknesses to provide an attenuated X-ray beam based on user requirements. The first ionization chamber probes the intensity, which is directly linked to the piezoelectric-based optimization program on the second crystal of the DCM to maintain maximum intensity and a constant beam position. The overview of the end station, the environment around the sample, and one tab of the graphical user interface are shown in Fig. 3

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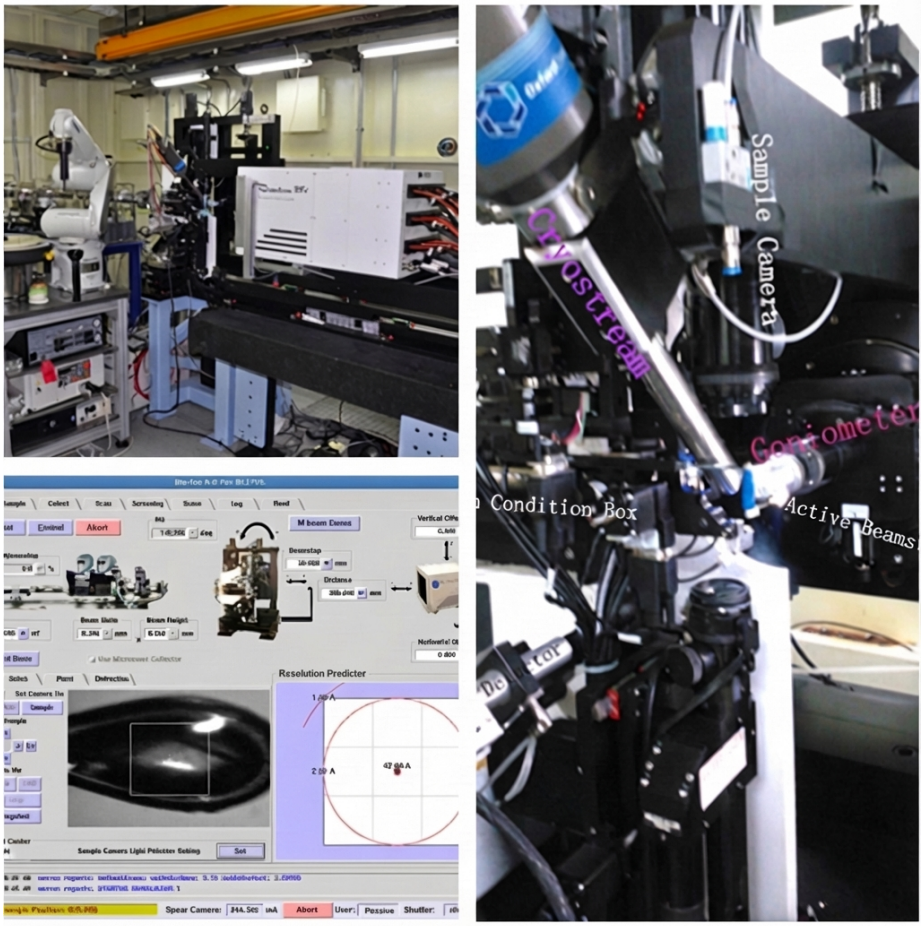

Figure 1: Figure 3

A. End Station Hardware

A micro-goniometer (Crystal Logic, LA, USA) is installed on a separate stand from the detector support table so as not to affect the sample-beam alignment when changing the detector distance. The air-bearing spindle has vertical motion for accurate beam-sample alignment. The beam-conditioning components located in the goniometer arm include a fast shutter synchronized with the rotary axis, two slits, and one micro ionization chamber. The slits combined with the micron ion chamber are used for auto-optimization when the energy is changed or after long-term operation. An active beam stop is installed after the sample to block the beam and monitor the intensity [**?**].

The sample is visualized from a top view and an on-axis view. Optical positioning relies on the on-axis camera, which is a dual-lens system with two fixed magnifications: low magnification for easily finding the loop and high magnification for precise crystal positioning. The digital image is displayed in a browser window and integrated into the BluIce control software. Crystal centering is performed via a click-to-center procedure from the software interface.

The Mar 225 CCD was replaced by an ADSC Q315r detector (Area Detector System Corporation, Poway, USA) in October 2010. The sample-detector distance is adjustable from 85 to 1000 mm, corresponding to a maximum detectable resolution of 0.98 Å at 1 Å wavelength for the circle inscribed in the ADSC detector square face. A resolution predictor is automatically updated when the detector position changes.

The Oxford Cryosystem 700 (Oxford, UK) keeps the sample at 100 K in standard operation. A Hitachi vortex Si-drift detector can be moved pneumatically close to the sample for MAD scans and X-ray excitation experiments to determine the optimal energy for data collection. To meet the need for data collection on extremely small crystals, a micro-collimator offering 20, 10, and 5 µm apertures is under development.

Samples can be mounted either manually or by the ACTOR sample changer (Rigaku, USA), which has a capacity of 80 pre-cooled samples in its storage Dewar. Uni-pucks with 18 mm standard pins are recommended. Routine calibration and maintenance ensure reliability, and an increasing number of crystals have been screened easily since the installation of ACTOR, as shown in Fig. 4 [FIGURE:4].

B. Software

The EPICS (Experimental Physics and Industrial Control System) Input Output Controllers (IOCs) run on VME crates for motor control and signal readout. Linux-based IOCs also run for other module devices, such as the Galil motor controller and axis network devices. Our strategy is to put as much as possible under EPICS to minimize code while maintaining good flexibility. The beamline components controlled by EPICS were implemented by the control

group [**?**] of the Phase I beamlines. The operator interface of EPICS is suitable only for engineering-level operation, but BL17U1 users requested an integrated control and data acquisition system including all components and functions of the beamline and end station with an intuitive tabbed graphical user interface (GUI). The Blu-Ice/DCS (Distributed Control System), developed at SSRL [**?**] and implemented on many beamlines, is extensively utilized by the international user community. Based on the DCSS (distributed control system server) protocol, the EPICS gateway program translates messages between the EPICS CA (Channel Access) protocol and the DCS protocol. A few new DHS (distributed hardware servers) have been developed for specific devices, such as the sample exchanger and fluorescence detector. Sequencing and scientific functions are implemented by the script engine, including automatic beamline optimization, energy synchronization and undulator gap adjustment during energy scans, click-to-center functionality, and more. More details on the control and data acquisition system can be found elsewhere [**?**]. Automatic crystal centering, especially for positioning tiny crystals, is required by users, and this function is being integrated. The functional system with intuitive GUI ensures that the beamline is easy to use and reliable.

IV. Ancillary Facility

An auxiliary laboratory near the beamline has been built for macromolecular crystallography users. The lab provides facilities for users to prepare protein crystal samples on-site. It is equipped with numerous instruments, including incubator shakers, centrifuges, an AKTA (GE Healthcare Life Sciences) purifier, an ultraviolet spectrophotometer, a nanoparticle size analyzer, a nanoliter high-throughput liquid handling system, stereomicroscopes, and more. Users can carry out protein expression, protein purification, and crystal growth experiments in the lab. Two thermostatic chambers at 277 K and 291 K are available for protein purification, crystal growth, and crystal sample storage. Tools for flash-freezing crystals and soaking heavy atoms are also provided for sample preparation. Users can harvest diffraction data immediately after sample preparation, reducing effects from transportation and other environmental changes. Uni-pucks are also provided to novice users.

Beamline control and data processing are performed from the control area adjacent to the experimental hutch. All computers are managed by OpenLDAP (Open Lightweight Directory Access Protocol) for account authorization. Data storage is 16 TB over a 1 GB/s connection to all beamline computers. Data processing and analysis software commonly used by protein crystallographers are available, including HKL2000 [**?**], iMosflm [**?**], XDS [**?**], CCP4i [**?**], PHENIX [**?**], SHELX [**?**], and other related programs. Since beam time is extremely oversubscribed, users are encouraged to process datasets as soon as possible and back up their data to their own disks after their beamtime slot. Due to limited storage, diffraction images are saved on the server for one month.

V. Facility Access

Users must complete typical safety training and pass the related examination to conduct experiments on the BL17U1 beamline. Beam time for general users can be obtained through four parallel mechanisms, varying mostly in lead time before scheduling, ranging from half a year to one week. The first is the regular user proposal access program. SSRF receives proposals of this type twice yearly, with deadlines on March 30 and September 30, respectively. Proposals are reviewed and scored by an external panel based on scientific merit and technical feasibility of the research plan. Proposals must be submitted online at http://ssrf.sinap.ac.cn/proposals.

The second mechanism is fast access mode, which can be submitted at any time by contacting beamline staff during the year, providing access on a very short time basis for a particular experimental visit. This mode accounts for less than 10% of total user beam time. Since 2010, the third mode, called awarded beamtime, has been allocated to users who performed outstanding work on the beamline, based on the SCI impact factor of their papers. Beamtime for this access mode is now about 20% of total user beamtime due to explosive growth in publications. Since 2012, the fourth mechanism, long-term proposals for two-year periods with a deadline of June 30, has been accepted by the beamline and allotted through peer review. This type accounts for about 15% of total user beamtime.

Typically, an allocated beamtime slot is three-quarters of an eight-hour shift to utilize the beamline efficiently. Over the past five years, an average of 22% of requested beamtime has been satisfied, as shown in Fig. 5 [FIGURE:5].

Operation of the beamtime has also stimulated research enthusiasm from pharmaceutical companies. Industrial user needs are valued by the facility, so 10% of user beamtime is reserved for these users. Fourteen pharmaceutical companies have already carried out projects on the beamline.

Since the end of 2012, remote access has been opened to users after a series of tests [**?**]. Users with previous experience on the beamline and robot are welcome to use remote access for data collection. Authorized users may access the beamline control software GUI remotely to conduct their experiments via a secure NoMachine NX session. Users can fully operate the beamline from their home laboratory as if they were on-site. Remote access will become routine in the very near future.

VI. Productivity

Commissioned in April 2009, BL17U1 is now in its 6th year of operation. The beamline now accommodates over 180 groups annually, including users from academic institutions and pharmaceutical companies. It has been productive both in terms of publications and Protein Data Bank (PDB) depositions over the past five years. The number of structures keeps increasing year by year.

According to statistics from Biosync (http://biosync.sbkb.org), BL17U1 ranked No. 1 in 2012 in the number of structures determined from data collected at a single beamline. Considering the delay in depositing structures to the PDB, the number for 2013 is still increasing and will likely exceed that of 2012.

The number of published articles is growing explosively. Experiments on BL17U1 have yielded several important results in structural biology \cite{16-19}. Up to September 2014, 33 papers were published in *Cell*, *Science*, and *Nature*. Fig. 6 [FIGURE:6] shows statistical data up to October 10, 2014.

A. MAD/SAD: ECF Transporter

The energy-coupling factor (ECF) transporters constitute a novel family of conserved membrane transporters in prokaryotes with domain organization similar to ATP-binding cassette transporters. Yigong Shi's group solved the 3.5 Å structure of an ECF transporter [**?**] believed to be specific for hydroxymethyl pyrimidine. These structures enabled them to propose a plausible working model for the transport cycle of ECF transporters. There are no mammalian homologues for the S-protein components of ECF transporters, and the molecules have notably high substrate-binding affinity, suggesting potential targets for much-needed new antibiotics. This project performed on BL17U1 took advantage of MAD and SAD methods for structure determination.

B. High Resolution: LGN/NuMA and LGN/mInsc Complexes

Asymmetric cell division requires the establishment of cortical cell polarity and orientation of the mitotic spindle along the axis of cell polarity. Par3/Par6/aPKC protein complexes are involved in establishing and maintaining cell polarity. NuMA/LGN/Gαi protein complexes regulate spindle orientation. The adaptor protein mInscuteable (mInsc) connects Par3/Par6/aPKC and NuMA/LGN/Gαi protein complexes. Therefore, these protein complexes play important roles in asymmetric cell division. Previous studies confirmed that in invertebrates, the adaptor protein mInscuteable (mInsc) connects the Par3/Par6/aPKC complex and NuMA/LGN/Gαi complexes, playing a key role in the asymmetric cell division process. However, the molecular mechanisms of LGN-NuMA and LGN-mInsc complex interactions were not yet clear. Minjie Zhang' s group solved the structures of the LGN/mInsc and LGN/NuMA complexes. Their results suggest that the Par3/mInsc/LGN and NuMA/LGN/Gαi complexes may play sequential and partially overlapping roles in asymmetric cell division. The molecular basis for LGN binding to NuMA and mInsc is now well understood based on the 1.10 Å high-resolution structures of the LGN/NuMA and LGN/mInsc complexes [**?**].

C. Large Cell Constants: EV71

Human enterovirus 71 (EV71) is the leading causative agent of severe hand-foot-and-mouth disease (HFMD) in infants and young children. Uncoating is essential in the EV71 life cycle, characterized by conformational changes in the capsid to facilitate RNA release into host cells. Rong Chen' s group solved

the EV71 virus structure in uncoating status, which crystallized in space group I23 with large cell dimensions of a=b=c=591.38 Å [**?**]. Comparison of the full virion and uncoating intermediate structures enabled them to map the conformational changes associated with uncoating and provide a more detailed picture to understand the early steps of EV71 uncoating.

VII. Discussion and Perspective

As the first dedicated MX beamline at a third-generation synchrotron radiation facility in China, BL17U1 is well designed and operated. To gain experience, we conducted a verification process at the beginning to check performance. The beamline performance is routinely checked by collecting datasets from lysozyme crystals and solving the structure by S-SAD (Sulfur-SAD) at 1.5 Å. This routine self-inspection can assure that all beamline components are functioning well, particularly avoiding misalignment between the rotation axis and the beam. Beamline operation has attracted nationwide users and several groups from neighboring countries. Although we have tried our best to offer as much beam time as possible to users, the beamline remains oversubscribed. Our flexible access modes, streamlined data collection software with intuitive interface, and hardware allow the beamline to be competitive and as productive as beamlines at more modern synchrotrons worldwide.

Efficient beamtime scheduling, sometimes within a few hours of a request, accelerates the progress of users' research projects. 24-hour on-site support is available during user experiments. Our group, consisting of members from many disciplines, contributes to high-quality support for users.

In addition, hardware and software are continuously improved to meet requirements from more challenging projects. For example, a smaller beam size will be achieved by implementing compound refractive lenses to facilitate data collection from micro-crystals. New functions such as rastering for centering crystals from opaque backgrounds and a data processing pipeline are being developed and evaluated to achieve greater scientific productivity.

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