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Original Article

Implementation of level-2 biosafety for a macromolecular crystallography beamline at SSRF

Huating Kong[1*], Minjun Li[1*], Qin Xu[1,2], Huan Zhou[1], Feng Yu[1], Qisheng Wang[1,3]

[1]*Shanghai Advanced Research Institute, Chinese Academy of Sciences, Shanghai 201204, China;* [2]*University of Chinese Academy of Sciences, Beijing 100049, China;* [3]*Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, China.* [*]*Equal contributors.*

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Abstract: Macromolecular crystallography is commonly used to determine the structure of biological macromolecules. Currently the beamlines at synchrotron radiation facilities play an important role in macromolecular crystallography, and have produced an enormous number of molecular structures to help solve scientific questions and support applications. Structure information makes significant contributions to the virus-related research as well. However, it is mandatory to be protected the operators under a compatible biosafety infrastructure when a pathological agent is set up in a beamline. Here a level-2 biosafety protection for a macromolecular crystallography beamline at Shanghai Synchrotron Radiation Facility (SSRF) is introduced. To fulfill the biosafety in a radioactive environment, a dedicated design is implemented. Since the beamline will be opened to the external users from nationwide research units, the management process and experimental method are also drawn up.

Keywords: Macromolecular crystallography, beamline, biosafety, in-situ, crystal structure

Introduction

Macromolecular crystallography (MX) is widely used to study the biological molecular structure to a resolution higher than 5 angstrom [1]. High-resolution structures are the key basis to elucidate bio-function. A beamline at a synchrotron radiation (SR) facility which adopted the MX technique is called MX beamline. Worldwide, there are more than 100 MX beamlines [2]. Nowadays, there are four MX beamlines with different parameters at Shanghai Synchrotron Radiation Facility (SSRF) [3] in user operation [4]. Up to date (March, 2021), more than 5400 structures have been deposited into the protein data bank (PDB) by using these four beamlines, especially the first MX beamline BL17U1 producing more than 3,500 entries. These structures contribute to a lot of scientific highlights, with more than 80 research papers published in *Nature*, *Science* and *Cell* [5-7].

Productive scientific achievements from these beamlines have attracted wide attention. The user community have increased gradually during the last ten years [8]. MX is also the main technique to study the precise three-dimensional structure of pathogen viruses, as shown in the structural biology field. High-resolution structural information can be used to understand the mechanism of virus replication, assembly, pathogenicity and immunity, which provide strong support for the development of antiviral drugs. But the researchers who focus on the virus-related projects find there are no MX beamline with biosafety available in China. Due to the potential bio-hazard, this kind of specimens cannot be delivered to other synchrotron facilities abroad. To help the community in investigating the precise three-dimensional structure of pathogens, an MX beamline with biosafety level-2 was proposed by the user expert community and was approved by the central government. Now this beamline (BL10U2) is one part of the SSRF Phase-II project initiated in December 2016, and it will be officially opened to users in 2022. This beamline had been certified and reported for the record by Shanghai Pudong Municipal Health Commission.

Table 1. Specifications of the beamline

Parameter	Value
Energy Range	7~18 keV
Energy Resolution	2×10^{-4} (@12.7 keV)
Beam Size	20×10 μm^2 (FWHM)
Beam Divergence	1.5×0.2 $mrad^2$
Photon Flux	2×10^{12} phs/s (@12.7 keV, 300 mA)
Biosafety Level	BSL-2
Screening Speed	>15 samples/hour
Highest Diffraction Resolution	≤0.8 Å

According to national standard "General Requirements for Laboratory Biosafety" (GB 19489-2008), four levels of biosafety corresponding to the risk groups are formulated. Biosafety Level 2 (BSL-2) is suitable for experiments involving moderate potential bio-hazard to individuals and the environment. BSL-2 is selected for this beamline, based on the scientific requirements, regulations as well as biosafety law. X-ray is delivered into the beamline endstation for the diffraction data collection with pathogen samples, thus the experimental platform is surrounded by a radioactive environment with the biosafety. (The experimental site is protected under both the radiation safety and the biosafety environment). This combination will offer a unique setup for virus structure determination. In this paper, the implementation of BSL-2 for the beamline BL10U2 at SSRF will be introduced in detail.

Performance of the beamline

The beamline BL10U2 is located at the 10th port of the SSRF storage ring [9], the BL in the name is short for beamline, and the capital letter U is standing for the Undulator which is a kind of the source device [10]. This beamline is located downstream and canted with the other beamline in a same straight section, thus the last number of this beamline identify is 2.

The undulator device can produce high brilliant synchrotron radiation beam in a wide energy range. The beamline will be collimated by aperture and slits, then the monochromator is used to select the energy of the photon for the experiment. Mirrors are used to deflect and focus the beam at the sample position. The parameters of the beamline are listed in **Table 1**.

In the experimental endstation, there are the diffractometer for mounting and rotating the sample, the robot to mount the sample automatically, and the large area detector. Two operation modes are offered in this beamline: normal mode and BSL-2 mode. In normal mode, since the in-situ date collection is not required, the robots will be used to transport samples. The diffraction intensity is recorded by the detector Eiger X 16M [11]. To resolve the structure of the sample, the phase must be obtained. There are several methods that can be used, such as the MR, MAD/SAD. A lot of academic software implementing these methods are accessed easily at the beamline [12-15].

The beamline works under a radioactive environment, thus the design and the operation must comply with the specified operation process [16]. For the user's experiment, they bring their sample and mount the sample on the diffractometer, therefore the endstation is required to manage the radioactive safety and biosafety at the same time. It is the first time BSL-2 is offered at an MX beamline in China.

Architecture design

Because the beamline is located at synchrotron radiation facility, the space for the endstation is relatively small, but the equipment for the diffraction experiment is complicated. Compared with the radiological department in most hospitals, there are several differences in this beamline. (1) More complex radiation protection facilities, due to the higher potential radiation dose in SSRF. (2) Single experimental process: macromolecular crystallography. (3) The samples are well sealed in the crystallization plate, which is relatively safe. (4) The users should submit their proposals online. In the proposal, the users are required to report the details on their experimental plan, pathogeny agents, risk, emergency plan, and personnel qualification, et al. (5) The lab was divided into five rooms to complete different functions, including transferring sample, wearing PPE, mounting sample and collecting data. For this beamline endstation, the area is about 35 m^2. The endstation is made of lead containing metallic materials to block the X-ray. This combination of the radioactive and biosafety make the nested hutch as shown in **Figures 1** and **2**.

The area is divided into different rooms based on the experimental procedures, such as deliv-

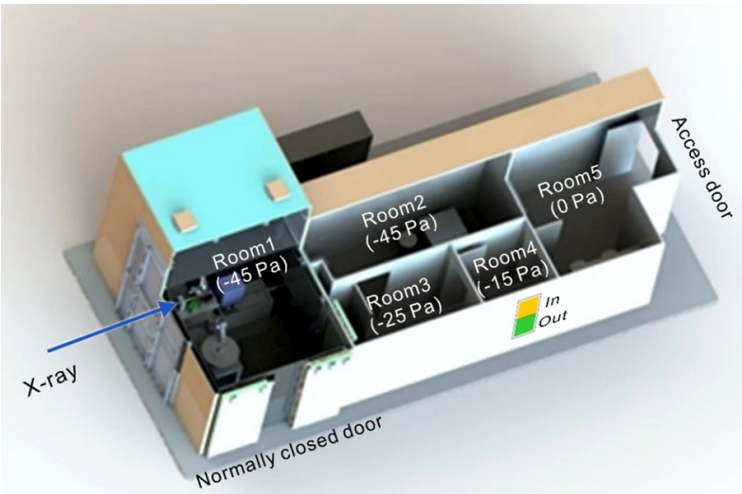

Figure 1. Layout of the Biosafety area. Five rooms and corresponding air pressure are shown in the figure.

ering the sample into the experimental hutch, opening and checking the crystals, mounting the sample on the diffractometer, collecting the diffraction images and disposing the sample. The room 1 is connected to the beamline endstation. For data collection, the users need to follow the beamline radioactive operation which requires searching the hutch and closing the endstation door before opening the photon shutter to allow the x-ray to irradiate the sample. It means that the users do not need to touch the sample after they mount the sample. Except the sample loading, all the equipment is controlled by the dedicated software system. In the room 2, the users can check the crystallization plate in a Class II Type A2 biological safety cabinet. The function of room 3 is mainly for the sample transferring. The Room 4 is for changing the lab cloth and wearing personal protective equipment, including disposable laboratory gowns, N95 respirator, head cap, disposable gloves, protective eyewear and disposable shoe covers. Users operate the software to carry out their data collection in the room 5. All rooms are maintained at temperatures of 19-25°C and humidity of 45-65%.

According to the National standard "Architectural and Technical Code for Biosafety Laboratories" (GB 50346-2011), National standard "General Requirements for Laboratory Biosafety" (GB 19489-2008) and General biosafety standards for microbiological and biomedical laboratories of Ministry of Health of the People's Republic of China (WS 233-2002), we design the negative pressure gradient, according to the standard of enhanced BSL-2 laboratory. There is a negative pressure gradient from room five to one. In the room five the pressure is normal, and the air pressure in the room two is -15 Pa. Then the pressure is setting at -25 Pa in the room three. For the room four and five, where the sample is observed or irradiated, the pressure is reduced to -45 Pa. The pressure can be adjusted according to experimental requirement by control system. Heating Ventilation Air Condition (HVAC) is equipped with fresh air system. High Efficiency Particulate (HEPA) filters are used to clean the air before exhausted.

There were two entrances and exit doors for the whole experimental Room, one is for transferring the equipment in and out and will be closed during experiments, the other is equipped with the access control for the operator's entering. The sample is transferred into the lab by the upper transfer window, as shown in the **Figure 1** in yellow. Currently the bio-hazard sample must be disinfected and disposed after the data collection, then it will be transferred out through the window below, as shown in the **Figure 1** in green.

Operation management and experimental method

The beamline will be opened to the user community, and the users need to apply for the beam time to conduct their research. To make sure the beamline carries out the data collection and structure determination effectively and safely, the operation procedures of the experiment must be carefully considered and checked. The operation procedures consist of the proposals management, sample list, sample transportation, sample processing and environment disinfection, laboratory people qualification and equipment training (**Figure 3**). According to the laws and regulations on biosafety from government, including National standard "General Requirements for Laboratory Biosafety" (GB 19489-2008), Code for man-

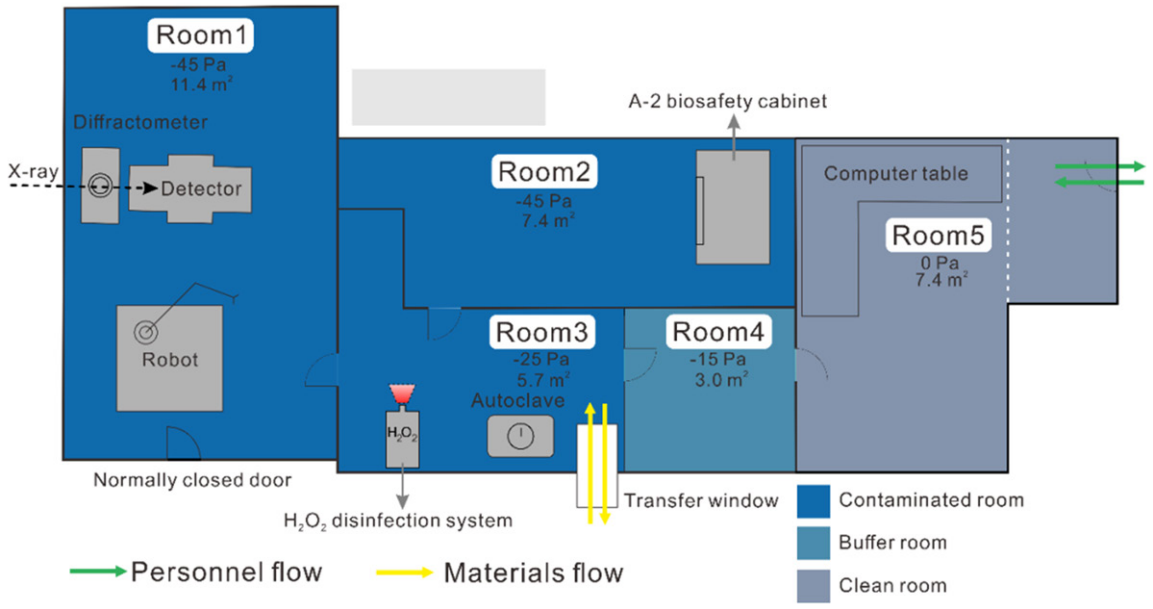

Figure 2. Floor plan of the Biosafety area. Major instruments were represented in the figure. The lab is connected to outside through a transfer window. The lab was divided into five rooms, including three contaminated rooms, a buffer rooms and a clean rooms, which are indicated in the corresponding colours. Green lines and yellow lines show the routes for personnel flow and materials flow through the lab, respectively.

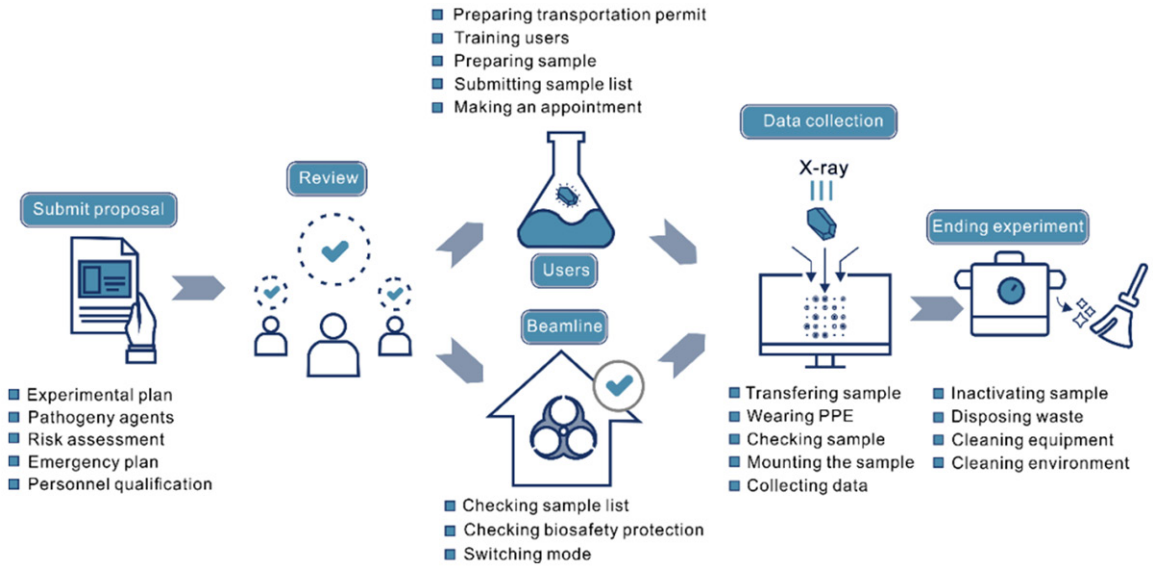

Figure 3. Experimental flow chart procedures of proposal submittion, review, experimental prepration, in-situ data collection and experiment ending performed in BL10U2.

agement of secondary biosafety protection laboratory in Shanghai and general biosafety standards for microbiological and biomedical laboratories of Ministry of Health of the People's Republic of China (WS 233-2002), the operation rules of this beamline have been set up, mainly including the standard operation procedure (SOP), bio-hazard risk assessment, research project management, personal training and qualification, inspection and so on. A biosafety committee certified by Shanghai Pudong Municipal Health Commission is established and will supervise the experimental activity. The biosafety committee is comprised of one

director, one executive deputy director and several committee members. The legal person of SSRF serves as the director, and the vice director in charge of safety concurrently serves as the executive deputy director. Heads of departments related to biosafety serve as committee members.

Proposal management

This beamline is managed by the large research infrastructures administration of SSRF, which is one among many facilities in the Chinese Academy of Sciences. The user proposals are submitted online and reviewed by the external expert committee with members come from different research fields. In the proposal, the users are required to report the details on their experimental plan, pathogeny agents, risk, emergency plan, and personnel qualification, *et al*. For this committee, the scientific backgrounds of the experts include the structure biology, crystallography, virology and biosafety. In the reviewing stage, the research risk will be evaluated in general, and case by case. To some special cases, such as novel pathogeny agents, the biosafety committee will make the final decision based on the law and situation.

After the proposals are approved, the users are required to apply for the transportation permission from the authority. The personnel involved in the experiment should be trained online and onsite, finally qualified by beamline official. The users need to make an appointment in advance to allow the beamline staff to have enough time to confirm the integrity of biosafety protection.

Sample list

Based on a questionnaire survey of the research interesting from the relevant research groups in China, combined with the "List of Human Pathogenic Microorganisms" released by the Ministry of Health of China in 2006, 39 species of viruses under specific experimental activity: operation of inactivated materials, are acceptable for this beamline, such as Sabia Virus, Flavivirus, et al. In this beamline, only inactivated virus crystals are allowed to be operated. Therefore, BSL-2 practice, containment equipment, and facilities are enough for handling the specific 39 species of inactivated virus crystals. For structure determination, the virus samples need to be prepared in the crystal form. Those samples are required to be prepared in the qualified wet lab from users' side.

In-situ data collection

Two operation modes will be offered in this beamline. One is the normal mode that the sample is held in a loop and cryo-protected, the other one is in-situ data collection, which record diffraction in situ from crystals well sealed in their crystallization plates at room temperature. To reduce the biohazard risk, the in-situ data collection is recommended [17]. In this mode, the virus crystals are sealed in the tiny cells of a crystallization plate, and the samples are irradiated by the X-ray to produce the diffraction pattern on the detector. The in-situ data collection scheme is shown in the **Figure 4**. The sample will be observed by the on-axis microscope. Since the sample is surrounded by liquid and the sealing membrane, it might not be possible align the crystal precisely. In order to overcome the inaccuracy of optical observation, a grid scan [18] with low dose X-ray is optional.

During the experiment, the users are required to follow the Standard Operation Procedure (SOP). After the experiment, the samples and related materials are required to be sterilized by high pressure sterilization using autoclave, and then the waste will be disposed by the qualified agency. The equipment and the experimental area will be cleaned by ultraviolet lamp sterilization for 1 h or hydrogen peroxide vapor room decontamination for 1 h if necessary.

Prospective and discussion

MX is a powerful technique to resolve the high-resolution structure of bio-macromolecules which is curial for function interpretation. It has been already proven that the MX beamlines can play an important role in the research related to viruses [19-21]. Recently, SARS-CoV-2 caused a worldwide outbreak, and it is still a serious threat to public health security. The crystal structures determination of several vital proteins from the SARS-CoV-2 virus give insight into the process of SARS-CoV-2 virus invasion and replication [22-26]. The subsequent structural studies are also helpful on drug design, antibody optimization and vaccine design [27-30].

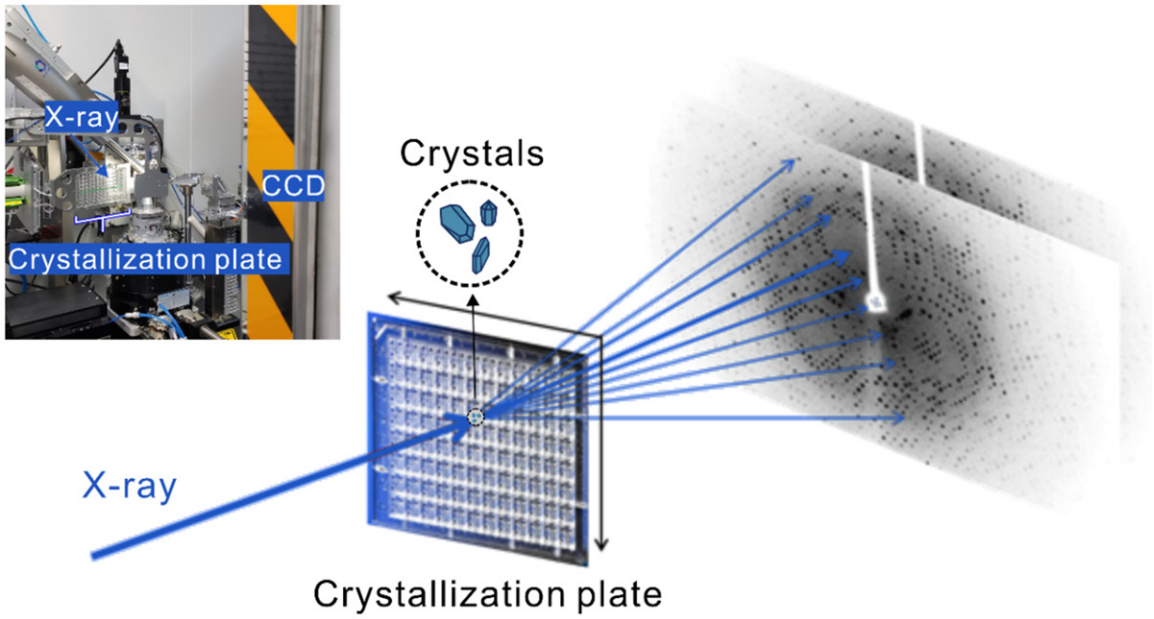

Figure 4. In-Situ data collection scheme. The Crystals are sealed in the crystallization plate and moved to the X-ray, and the detector records the diffraction images.

In the "List of Human Pathogenic Microorganisms" released by the Ministry of Health of China in 2006, SARS-CoV under specific experimental activity of operation of inactivated materials, belongs to the second category of pathogenic microorganisms. In this beamline, only inactivated SARS-CoV-2 virus crystal is allowed to be operated. Therefore, BSL-2 practice, containment equipment, and facilities are enough for handling inactivated SARS-CoV-2 virus crystal. Because of the lack of higher biosafety levels on the current MX beamlines, there is no crystal structure of the whole virus determined by MX in this pandemic. Moreover, due to China's large population, high density, relatively limited health conditions and increasingly extensive communication with the outside world, the rapid spread of new infectious diseases around the world has posed great challenges to the prevention and control of major infectious diseases in China. Therefore, the research and control of major infectious diseases is a major strategic demand in China. It can be expected that this beamline BL10U2 will server in the structure determination related to pathogenic agent better. Considering the proposal, design, construction and registration of this beamline were carried out in strict accordance with the requirements of BSL-2 MX beamline, it is difficult to find more potential biomedical applications in the short term. But we will strive to look for more biomedical applications to expand the functions of this beamline, by adding more experimental equipment.

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Disclosure of conflict of interest

None.

Address correspondence to: Qisheng Wang, Shanghai Advanced Research Institute, Chinese Academy

of Sciences, No.99 Haike Road, Pudong District, Shanghai 201204, China. Tel: +86-20304309; E-mail: wangqs@sari.ac.cn

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