

Guidelines for the Use of the Protein X-ray Crystallography Facility (IBC-PXF)

A. Precautions before use

1. First-time users should apply for an account on the Academia Sinica online reservation system (link displayed below) and request the use of the facility through the reservation system.

(<https://reservation.iis.sinica.edu.tw/servlet/SignInHandler>)

2. Please refer to page 4 of this guideline for all service items, fees, and relevant notes.
3. Users who require preliminary consultation or detailed discussions may contact designated personnel for each service item on the reservation system. The contact information for three facility personnel is as follows:

Dr. Kai-Fa Huang: (Tel) 02-27855696 ext. 3090; (Email) huangkf@gate.sinica.edu.tw

Ms. Hui-Ling Shih: (Tel) 02-27855696 ext. 3140; (Email) mbce00326@gate.sinica.edu.tw

Dr. Tzu-Ping Ko: (Tel) 02-27855696 ext. 3090; (Email) kotping@gate.sinica.edu.tw

B. Submission guidelines for protein crystallization screening

1. Before submission, please check the crystallization conditions for proteins that are similar or identical to your sample to facilitate the selection of crystallization screening conditions.
2. Select the desired crystallization screening conditions on the online reservation system and fill in the relevant sample information. All crystallization screening suites for our facility can be found in the link below.
([https://www.ibr.sinica.edu.tw/facilities/protein/List of screening suite-20230410.pdf](https://www.ibr.sinica.edu.tw/facilities/protein/List%20of%20screening%20suite-20230410.pdf))
3. After submitting the application, the facility staff will confirm the date for pre-crystallization testing (PCT) and on-site operation within one working day. The priority of reservation dates will be determined by the order in which user applications are received.
4. During PCT testing, users should bring ~10 μ l of protein samples (concentration of ~10 mg/ml) to the facility at the appointed time, where they will be guided by the facility staff for testing.
5. To avoid clogging of the liquid dispenser device needle, users' samples should be centrifuged beforehand, and the sample buffer should avoid high salt or detergent content. If impurities or precipitates in the user's sample cause the needle to clog, the user will be

responsible for the cost of purchasing a new needle.

6. If a user has reserved a crystallization screening time but fails to deliver the sample on the scheduled day, unless there are special reasons, they will still have to bear the basic maintenance fee of NT\$1000 for that day.
7. During crystallization screening, if there is an unexpected situation with the sample, the user's opinion may be urgently required. Therefore, users should try to be present during the screening process. Otherwise, if any unforeseen circumstances occur with the sample during the screening process, the user will be solely responsible for any consequences.

C. Submission guidelines for X-ray data collection, crystal structure determination, molecular simulation, and integrated service

1. In the priority of case handling, when both IBC users (case number CA○○○○) and non-IBC users (case number DI○○○○) submit their cases at the same time, the cases commissioned by IBC users will be processed first. When both are IBC users or non-IBC users, the priority will be based on the time of case submission, i.e., the case with a smaller case number will be given priority.
2. For crystal structure determination, molecular simulation, and integrated service, a collaborative model is generally adopted. When publishing papers, the facility staffs who have contributed to the intellectual property (IP) will share authorship.

D. Instructions for using Room 314 on the third floor of IBC

1. Users are not allowed to take any items from Room 314 without permission. If borrowing is necessary, please inform the manager (Dr. Kai-Fa Huang or Ms. Hui-Ling Shih) and fill out the borrowing register.
2. It is prohibited to bring toxic, radioactive, infectious, biological specimens, or corrosive substances into the room. Liquid nitrogen and organic solvents should be used in designated areas.
3. Eating and drinking are prohibited in this facility. Wearing gloves to open doors, drawers, operate microscopes, and other instruments is also prohibited. Using the facility's computers for internet access, watching videos, installing software, or any other non-experimental activities are also prohibited.
4. If a user violates the above rules, they will be informed of their first violation and the service of this facility will be suspended for two weeks. In the case of a second violation,

生化所蛋白質X-光結晶學設施 (IBC PXF)
IBC Protein X-ray Crystallography Facility

the service will be suspended for two months, and in the case of a third violation, the user will lose the right to use this facility's services.

E. Acknowledgments

1. Users who use the services provided by this facility must acknowledge the facility's contribution, as exemplified below:

“We acknowledge the services provided by the Protein X-ray Crystallography Facility at the Institute of Biological Chemistry, Academia Sinica, Taiwan”

Summary of service items and fee about the IBC Protein X-ray Crystallography service

Service items and fees (NTD/per unit)

	Only for IBC users
A. Robotic crystallization screening with 96-well plate⁽¹⁾	
A.1 Sitting-drop plate with 1 drop/per well (TW brand)	A.1 (550/plate)
A.2 Sitting-drop plate with 1 drop/per well (Greiner)	A.2 (1050/plate)
A.3 Sitting-drop plate with 3 drops/per well (Intelli-plate)	A.3 (850/plate)
A.4 Additive screening with sitting-drop plate (TW brand)	A.4 (550/plate)
A.5 Additive screening with sitting-drop plate (Intelli-plate)	A.5 (850/plate)
A.6 Screening with user-prepared plate	A.6 (500/plate)
A.7 Screening with user-prepared conditions ⁽²⁾	A.7 (640/plate)
A.8 Screening with user-prepared plate and conditions	A.8 (340/plate)
B. Manual crystallization screening with 96-well plate^{(1),(3)}	
B.1 Sitting-drop plate with 2 drops/per well (Intelli-plate)	B.1 (570/plate)
B.2 Sitting-drop plate with 1 drop/per well (TW brand)	B.2 (340/plate)
C. Incubators for crystallization plates	
Incubators for crystallization plates ⁽⁴⁾	(20/plate/month)
D. Crystal inspection with UV-imaging system	
Crystal inspection with UV-imaging system ⁽⁵⁾	Free of charge
E. Crystal refinement, soaking and co-crystallization⁽⁶⁾	
E.1 24-well hanging-drop plate (TW brand)	E.1 (200/plate)
E.2 24-well hanging-drop plate without sealant (int'l brand)	E.2 (250/plate)
E.3 24-well hanging-drop plate with sealant (int'l brand)	E.3 (350/plate)
E.4 96-well sitting-drop plate (TW brand)	E.4 (150/plate)
E.5 24-well sitting-drop plate (int'l brand)	E.5 (250/plate)
E.6 48-well sitting-drop plate (int'l brand)	E.6 (350/plate)
E.7 96-well sitting-drop plate (int'l brand)	E.7 (350/plate)
F. Stock solutions for crystallization condition optimization	
Stock solutions of various polymers, organics, salts and buffers (2-22 ml/per unit)	(200/per unit)
G. Customized crystallization strategy	Consultation
H. Design of expression construct for increasing crystallization probability	Consultation
I. X-ray data collection	
I.1 Crystal shipping to synchrotron radiation center	I.1 (200/puck) ⁽⁷⁾
I.2 X-ray diffraction test with synchrotron light source ⁽⁸⁾	I.2 (400/condition)
I.3 X-ray data collection with synchrotron light source ⁽⁹⁾	I.3 (1500/condition)
J. Structure determination, model building, refinement and validation	
J.1 Crystal structure determination ⁽¹⁰⁾	Collaboration. User should pay the manpower cost at 400/per hour .
J.2 Model building, refinement and validation for X-ray crystallographic data ⁽¹⁰⁾	
J.3 Model building for cryo-EM data ⁽¹¹⁾	
K. Integrated service (from purified protein to validated crystal structure)	Collaboration ⁽¹²⁾
L. Homology modeling, molecular docking and simulation	Collaboration. User should pay the manpower cost at 400/per hour .

⁽¹⁾A pre-crystallization test (PCT) of protein concentration is included. Free for 3-month storage of the plates in our incubators.

⁽²⁾The facility provides the sitting-drop Intelli-plate.

⁽³⁾The facility provides all the consumables and reagents, but users should set up the crystallization plates themselves.

⁽⁴⁾User gets 3-month free storage if the plates are set up via the core service.

⁽⁵⁾Only the UV-compatible plates are allowed.

⁽⁶⁾The facility provides cover slides, tape and other relevant tools, **but not** crystallization solutions.

⁽⁷⁾Each puck can be loaded with up to 16 crystals.

⁽⁸⁾User should pay the fee of crystal shipping.

⁽⁹⁾User should upload the preliminary diffraction data for full data set collection and pay the fee of crystal shipping. All publications resulting from the use of TPP synchrotron beamtime should acknowledge TPP.

⁽¹⁰⁾User should upload the crystal picture, X-ray diffraction image and data-collection statistics and the facility has the right to decide whether a case will proceed.

⁽¹¹⁾The facility has the right to decide whether a case will proceed.

⁽¹²⁾User must provide manpower and pay the usage fee for the fee-based service items.