

C-Platform



Comprehensive X-ray crystallography (XRD) services for improving drug candidate compounds and understand protein functions.

Services based on experience with space experiments

X-ray crystallography (XRD: X-Ray Diffraction Structure Analysis) of protein-compound complexes can confirm the electron density map derived from the bound compounds. It has been reported that high-quality crystals provide a more detailed electron density map, which enables us to accurately determine the coordinates of not only the atoms that make up the bound compounds and protein molecules, but also the coordinates of the binding water, thereby enabling us to understand the important interaction sites.

Based on the experience obtained through technical support for JAXA space experiments, Confocal Science has developed crystallization devices for growing high-quality crystals, and has accumulated know-how in this area.

Technical Information:

The technologies provided by C-Platform are introduced in the following review. Y. Hashizume, K. Inaka, N. Furubayashi, M. Kamo, S. Takahashi and H. Tanaka, Methods for Obtaining Better Diffractive Protein Crystals: From Sample Evaluation to Space Crystallization, Crystals 2020, 10(2), 78; doi:10.3390/cryst10020078

◆ Services are customized for the user's demand

C-Platform can undertake the entire process as a single contract, or can optimize the processes. As a whole, more rational services with shorter periods and lower costs that match the budget can be provided.

Some of the processes provided by C-Platform have a strong R&D element, and it is sometimes difficult to guarantee the final result from the beginning. For this reason, a contract with milestones for each process is provided. Rough estimate without detailed information on the target protein can be provided.

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<Service and Process Overview>

1. Expression

E. coli, insect cells, or mammalian cells are available as hosts. DNA sequence is optimized for each system. The culture conditions are examined. Then, mass expression and purification of protein samples are performed.

2. Purification

A purification is applied to the expressed protein to obtain a stable and highly homogeneous protein sample. The quality of it is evaluated by the own methods and experience based on the consistency between the results of SDS/Native-PAGE electrophoresis, HPLC, DLS, etc. and the physical properties predicted from the amino acid composition of the protein sample. A tag cleavage is applied, if necessary. In the purification process, it is not necessary to disclose the details of the protein. If it is difficult to disclose the amino acid sequence, only the physicochemical properties of the protein calculated by C-Profile (sold separately) is required.

3. Search & optimize crystallization condition

For protein samples for which crystallization conditions have already been reported, those crystallization conditions and methods are used as a starting point. For unknown samples, searching and optimization of the crystallization condition and method will be based on our own experience. Confocal Science has experienced more than 500 protein samples using various crystallization methods (batch method, VD method, CD method, dialysis method, etc.). In addition, the crystallization condition will finally be optimized for the good cryoprotection.

4. Co-crystallization

Based on the optimized crystallization conditions, co-crystallization of the prepared protein with the given ligand compound will be applied. Sometimes crystallization conditions may vary greatly when adding the ligand, but based on the extensive experience, some countermeasure will be applied including the seeding and the post soaking. In the co-crystallization, it is not necessary to disclose any details other than information on the molecular weight and solubility of the compound.

Once an experimental protocol has been established, it is possible to order at any time to prepare complex crystals of the new compound with the prepared samples. Optionally, it is possible to deliver samples, optimized crystallization reagents, and crystallization device, and to allow customer to attempt co-crystallization at the customer's site without sending the compound.

5. Cryo-cooling the crystal and X-ray diffraction data collection

To obtain X-ray diffraction data, crystals must be frozen with cryoprotection. After optimizing the cryoprotection conditions, we perform the cryo-cooling, preparing about 5 frozen crystals per compound. Diffraction data will be obtained at Diamond Light Source in the UK. Beamtime is set aside according to crystal preparation, and data set is acquired with the simultaneous evaluation. Confocal Science also handles all the procedures related to the transportation of the crystals beyond the boarder.

6. Scaling

The obtained diffraction data sets are used for indexing, integration, and scaling. Diffraction data sets from multiple crystals are evaluated for subsequent analysis.

7. Phasing and structure determination

Based on the coordinate data in PDB, the phasing is applied to determine the structure by molecular replacement method. In this process, the customer is expected to disclose information related to the structure of the protein sample. If the molecular replacement method is not applicable, it is necessary to consult with the customer to solve the situation. Once the phase and structure have been determined, in most cases the electron density derived from the compound can be confirmed.

8. Refinement

Based on the determined coordinates, the refinement of the structural coordinate will be performed. If the structure of the compound is disclosed, the refinement including the atomic coordinates of the compound will be performed. Confocal Science has abundant experience in structural analysis, including ultra-high resolution structural analysis that exceeds 1.0 Å.

9. In space crystallization (optional)

Confocal Science offers high-quality protein crystallization in microgravity using "Kirara" space experiment of the Japan Manned Space Systems Corporation (JAMSS). (<https://www.jamss.co.jp/kirara/index.html>). Kirara is highly confidential, and it has been commissioned to create high-quality crystals for pharmaceutical companies in Japan and overseas.

Confocal Science has contributed to JAXA and NASA space experiments with more than 25 years and has world-leading know-how in space experiments.

※ Some of these processes will be outsourced as necessary.