A comprehensive strategy for efficient generation of well-diffracting crystals

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A special advantage of protein crystallography is the quick determination capability of target protein structures. The high-throughput structure determination of protein-compound complexes for pharmaceutical science is one of the most valuable examples. Furthermore, inhibitors for protein functions are important in cell biology because these compounds can be utilized as chemical probes for modulating cellular functions. However, the high-throughput structure analysis requires well-diffracting crystals. Thus, the preparation of well-diffracting crystals is a critical issue to be addressed in the field of macromolecular crystallography, particularly in this cryo-EM era. In addition, protocols of well-diffracting crystal preparation should be integrated into an automated crystal structure determination system with the (MR-)native SAD method, which is being developed in Photon Factory using BL-1A and BL-17A. We have so far developed a standard protocol to obtain well-diffracting crystals based on our successful experiences: histone chaperon TAF-Iβ, CagA, which is an effector protein of Helicobacter pylori, and phosphatidylinositol 6-phosphate 4-kinase β (PI5P4Kβ) (1, 2, 3, 4, 5). Here, we would like to present the standard protocol used in our group. Our method has supported many PF users.

First, we have used an integrated crystallization robot for the initial crystallization screening (6). If crystals appeared at several conditions, we evaluate their crystal quality based on the snapshot images of X-ray diffractions and choose good crystals. It is critically important to select crystals with high reproducibility because many crystals are needed for screening experiments. The micro-seeding method is often helpful to obtain crystals with high reproducibility. When crystal quality is insufficient, cryoprotectant screening, one of the post-crystallization treatments, is applied to improve the crystal quality. The crystal quality improvement of the tandem SH2 domain-CagA peptide, full-length LTTR (CbnR)-promoter DNA complex, shark PI5P4Kβ, a novel FAD-dependent C-glycoside-metabolizing enzyme, C-deglycosylation enzymes are part of our successful examples (7, 8). Notably, the crystal quality of a full-length CbnR (LTTR)-promoter DNA complex was dramatically improved from 6.9 Å to 3.6 Å, resulting in obtaining the first crystal structure of the full-length LTTR-promoter DNA (55bp) complex (8).

Anaerobic crystallization technique is sometimes useful when no crystals were obtained under aerobic conditions, particularly in the case of redox-related proteins. We have several successful examples of anaerobic crystallization with our anaerobic chamber and developed a standard protocol for anaerobic crystallization (7, 9). Since it is rather difficult to set up the equipment for the anaerobic crystallization in each laboratory, we can support the anaerobic crystallization as a collaboration.

In this presentation, we'll show our strategy for obtaining well-diffracting crystals for high-throughput structure determination. We believe that our strategy would apply to many other proteins.

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