A Comprehensive Strategy for Efficient Generation of Well- Diffracting Crystals

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Protein crystallography offers the advantage of rapid determination of target protein structures. Its high-throughput structure determination of protein-compound complexes for pharmaceutical science is a valuable example. However, for high-throughput structure analysis, well-diffracting crystals are required. In the field of macromolecular crystallography, especially in this cryo-EM era, the preparation of well-diffracting crystals is a critical issue. Our group has developed a standard protocol for obtaining well-diffracting crystals based on our successful experience with the histone chaperone TAF-Iβ, CagA, which is an effector protein of Helicobacter pylori, and phosphatidylinositol 5-phosphate 4-kinase β (PI5P4Kβ) (1, 2, 3, 4, 5). We are confident in presenting our standard protocol, which has helped many PF users.

To initiate our protocol, we use an integrated crystallization robot for the initial crystallization screening (6). When crystals appear under several conditions, we evaluate their crystal quality based on the snapshot images of X-ray diffraction and select a good one. Obtaining crystals with high reproducibility is crucial to facilitate soaking experiments with compounds. The micro-seeding method is often helpful in ensuring high reproducibility. In cases where crystal quality is insufficient, cryoprotectant screening, one of the post- crystallization treatments, is used to improve crystal quality. We have successfully improved the crystal quality of full-length LTTR (CbnR)-promoter DNA complex, a novel FAD-dependent C-glycoside-metabolizing enzyme, C-deglycosylation enzymes, and other proteins using this approach (7, 8, 9). In particular, we were able to improve the crystal quality of a full-length CbnR (LTTR)-promoter DNA complex from 6.9 Å to 3.6 Å, resulting in the first crystal structure of a full-length LTTR-promoter DNA (55bp) complex (7).

Anaerobic crystallization is sometimes useful when crystals have not been obtained under aerobic conditions. We have several successful examples of anaerobic crystallization using our anaerobic chamber and have developed a standard protocol for anaerobic crystallization (10, 11). As it is rather difficult to set up the equipment for anaerobic crystallization in each laboratory, we can support anaerobic crystallization as a collaboration.

In this presentation, we will show our strategy for obtaining well-diffracting crystals for high- throughput structure determination. We are confident that our strategy is applicable to many other proteins.

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