Comprehensive strategy for efficient generation of well-diffracted crystals

Miki Senda, Toshiya Senda
Structural Biology Research Center, Photon Factory (PF), Institute of Materials Structure Science (IMSS), High Energy Accelerator Research Organization (KEK)
miki.senda@kek.jp, toshiya.senda@kek.jp

Crystallography is still one of the most powerful tools for obtaining detailed 3D structural information of proteins. Its special advantage is high-throughput structure determination of a large number of protein-compound complexes, particularly for pharmaceutical science. In order to achieve the high-throughput crystal structure determination, protein crystallization is the most important process. When obtained crystals have insufficient quality, we need to optimize the crystallization conditions in a try-and-error manner. Therefore, a robust strategy for obtaining well-diffracted crystals has been awaited. We have so far accumulated enough experiences of crystal quality improvement using cryoprotectants; crystal structures of histone chaperone TAF-Iβ, CagA oncoprotein from *Helicobacter pylori*, and GTP-sensor PI5P4Kβ were determined at atomic resolution via optimizing cryoprotectant (Table 1). On the basis of these experiences, we propose a standard protocol for obtaining well-diffracted crystals. The protocol is composed of two parts; the first one is a crystallization optimization to obtain crystals with high reproducibility and the second one is a soaking experiment using cryoprotectant (REF 1, 2, 3, 4, 5). Qualities of several crystals have been improved using our strategy when original crystals were of poor quality. Screening of suitable cryoprotectant is the most important part of our strategy because it's impossible to expect a suitable cryoprotectant from crystallization conditions. We believe that our strategy would be applicable to many other proteins.

References

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Table 1 Our successful examples of crystal quality improvement

	Protein name	Before [Å]	After [Å]	Cryoprotectant
1	TAF-Iβ	5.5	2.3	Trehalose
2	CagA	7.5	3.1	trehalose+PEG1000 (Multi-step soaking)
3	PI5P4Kβ (human)	3.5	2.1	polyvinylpyrrolidone + ethyleneglycol
4	BphA4	1.6	1.1	Sucrose + PEG200
5	tandemSH2–CagA peptide	2.3	2.1	Diethyleneglycol
6	CbnR_DBD–DNA complex	2.9	2.5	Glycerol
7	Protein X–DNA complex	7.5	3.6	Erythritol