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An ignored variable: solution preparation temperature in protein crystallization

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Protein crystallization is affected by many parameters, among which certain parameters have not been well controlled. The temperature at which the protein and precipitant solutions are mixed (i.e., the ambient temperature during mixing) is such a parameter that is typically not well controlled and is often ignored. In this paper, we show that this temperature can influence protein crystallization. The experimental results showed that both higher and lower mixing temperatures can enhance the success of crystallization, which follows a parabolic curve with an increasing ambient temperature. This work illustrates that the crystallization solution preparation temperature is also an important parameter for protein crystallization. Uncontrolled or poorly controlled room temperature may yield poor reproducibility in protein crystallization.

etermining protein structure is important because structural information is essential for understanding the biological function of proteins and for rational drug design. The prevailing method for solving protein structures is X-ray crystallography. Most structures have been solved using this method, which is becoming increasingly important as the percentage of structures obtained using this method increases. However, this X-ray crystallography is not trivial, due to difficulties in crystallization.

Producing high-quality protein crystals remains the rate-limiting step for structural determination¹⁻⁵. One problem often encountered in protein crystallization is notoriously poor reproducibility^{6,7}. For example, only a few people are known as having "green fingers" and have successfully crystallized difficult proteins, and often, others are unable to reproduce such results⁸⁻¹⁰. It is a common complaint that crystallization results differ even under the "same conditions"¹¹. This phenomenon is partially due to the influence of many uncontrolled or poorly controlled factors in protein crystallization. It is well known that many parameters, such as the protein and precipitant concentrations, temperature, and pH, among other factors, can affect protein crystallization^{12,13}. In addition to these parameters, certain other factors are often ignored and, therefore, not intentionally controlled. The temperature used to prepare the crystallization solution is such a factor. Temperature is a well-known parameter that can affect protein solubility¹⁴ and further influences supersaturation, which is the driving force of the crystallization process^{15,16}; temperature has also been demonstrated to affect protein crystal nucleation and growth¹⁷. As an important parameter, temperature has been extensively studied for its effects on protein crystallization¹⁸⁻²². However, in most investigations, "temperature" refers to the temperature after the solution is prepared (i.e., the temperature during crystallization and incubation in a controlled environment). The temperature prior to incubation (i.e., the temperature during crystallization solution preparation) is primarily ignored, and its effect has not been investigated. Typically, the ambient temperature for preparing the crystallization solution is room temperature, which is not accurately controlled and subject to change depending on environmental variations. Crystallization is undoubtedly influenced by temperature, and the temperature during crystallization solution preparation may also influence the final crystallization results. However, it is unclear whether and to what extent this temperature affects crystallization. To clarify this issue, we designed an experimental investigation to study the effect of the mixing temperature on crystallization of three model proteins, lysozyme, proteinase K and thaumatin. The results show that the ambient temperature of solution preparation



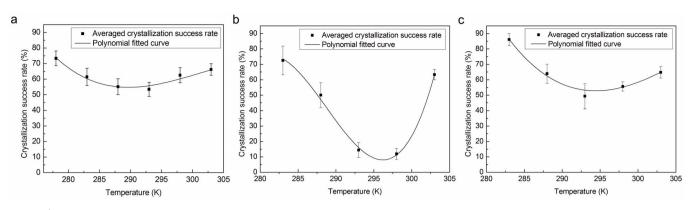


Figure 1 | The average crystallization success at different solution preparation temperatures. (a) Lysozyme; (b) Proteinase K; (c) Thaumatin. Based on the overall data, the crystallization success exhibited a parabolic curve trend as a function of the solution preparation temperature (Error bar for lysozyme: standard error of mean, n = 30; error bar for proteinase K and thaumatin: standard error of mean, n = 5).

did affect protein crystallization, which implies that the ambient temperature during solution preparation may partially contribute to the poor reproducibility of protein crystallization.

Results

The effect of the solution preparation temperature on crystallization success. Fig. 1 shows the crystallization success rate of three model proteins; clearly, the average crystallization success followed a parabolic curve as a function of the solution preparation temperature. For all test proteins, at a lower solution preparation temperature (278 K and 283 K), crystallization was most successful. At approximately 290 K ~ 297 K, crystallization was least successful; next, the success rate increased with increasing temperature. As determined using a one-way ANOVA analysis, the solution preparation temperature significantly affects protein crystallization success (P = 0.043, 0, 0.007 < 0.05 for lysozyme, proteinase K and thaumatin, respectively).

As shown in Fig. 1, the crystallization results were affected by the crystallization solution preparation processes. Crystallization solution preparation includes two procedures: (1) preparing the protein and precipitant solutions and (2) mixing the two solutions. Because both the protein and precipitant solutions were stable at the tested temperatures and, hence, crystallization could not occur in any of these solutions, mixing was the only procedure wherein nucleation might occur and induce different final crystallization results. The total time from mixing the solutions to placing the dispensed plate into the temperature controller was low (approximately 80 s); the induction time for protein crystal nucleation should also be low for the solution preparation process to affect the final crystallization results. The induction time for crystallization nucleation strongly depends on supersaturation²³, thus, it is helpful to determine the supersaturation conditions for the crystallization solution after mixing. Because the solubility data of lysozyme under certain conditions had been reported²⁴, we quantitatively studied the effect of solution preparation temperature on concentration, solubility and supersaturation of model protein, lysozyme.

The initial crystallization solution concentration at different solution preparation temperatures. The designed concentration of the crystallization solution (after mixing) was 20 mg ml⁻¹ HEWL and 40 mg ml⁻¹ NaCl. Because the crystallization solution was exposed to air before the crystallization plate was sealed, the actual initial concentration of the solution soon after sealing the plate should change due to solvent evaporation.

To discern the initial concentration of the crystallization solution, we measured the level of crystallization solution evaporated in the same ambient environment. The crystallization solution evaporation level versus the different preparation temperatures is shown in Fig. 2. The figure shows that the level of evaporation increases with higher solution preparation temperatures; thus, the initial concentration of the crystallization solution changes.

The initial concentration of each droplet can be estimated based on the evaporation levels in the different samples. Fig. 3 shows the estimated initial concentrations of both lysozyme and NaCl when the crystallization plate was sealed. The figure shows that the initial concentration at different solution preparation temperatures differed. The vapor pressure was clearly higher at higher solution preparation temperatures; thus, evaporation was more rapid, and hence, the initial solution concentration was greater.

The effect of solution preparation temperature on solubility and supersaturation. Supersaturation (σ) is a critical parameter for protein crystallization because it is the driving force for the crystallization. Supersaturation is determined based on the solution concentration (*C*) and solubility (*C_s*) (we use a simple expression: $\sigma = \frac{C}{C_s}$), and the latter is correlated with temperature. During crystallization solution preparation, the solution preparation temperature (i.e., mixing temperature) can affect the crystallization

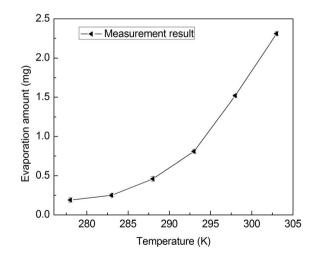


Figure 2 | The evaporation levels at different solution preparation temperatures. The designed crystallization solution concentration was 20 mg ml⁻¹ HEWL and 40 mg ml⁻¹ NaCl, which is the same or the crystallization experiments. The initial volume of the crystallization solution was 240 μ l. The experiment was repeated 3 times. The results show that the evaporation levels increased with an increasing solution preparation temperature.



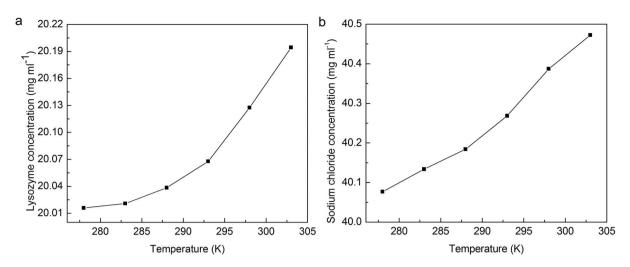


Figure 3 | The initial concentration of lysozyme and sodium chloride at different solution preparation temperatures upon sealing. The designated crystallization solution concentrations before they were dispensed into the wells were 20 mg ml⁻¹ HEWL and 40 mg ml⁻¹ NaCl. Due to solvent evaporation after dispensing, the evaporation levels different solution preparation temperatures; hence, the initial concentration of the crystallization solution upon sealing differed.

solution supersaturation level upon sealing, because the solution preparation temperature will influence solubility (C_s) in most cases.

The solubility of many proteins is affected by the temperature, and 86% of the examined proteins provide interpretable results for temperature-dependent solubility¹⁴. In general, the temperature-dependence of solubility (i.e., the sensitivity of the dissolution enthalpy) for many proteins may range on the order of approximately 10% per K^{25} .

The temperature-dependence of lysozyme solubility can be determined from the literature. The following relationship between solubility C_s and temperature T was derived from published data²⁴ via polynomial fitting.

$$C_{s} = 0.0004(T - 273)^{3} - 0.0089(T - 273)^{2} + 0.1302(T - 273) + 0.3437$$
(1)

The solubility data include lysozyme in a NaCl solution and dissolved in a sodium acetate buffer at pH 4.6 in the temperature range between 274.7 K \sim 296.6 K. Based on our analysis using Equation (1), the solubility decreases by 10.4% when the temperature rises from 293 K to 294 K. Thus, the solubility changes approximately 10.4% per degree, and the difference in supersaturation is 0.729. Because the smallest difference in supersaturation that can change lysozyme crystallization was reported to be 0.018²⁶, thus, the considerable solubility change due to a temperature difference (1 K) will certainly produces differences in crystallization success.

For more detailed information on the supersaturation status upon mixing the solution, we estimated the supersaturation level at different mixing temperatures when the mixed solution concentration is 20 mg ml⁻¹ HEWL and 40 mg ml⁻¹ NaCl. Fig. 4 shows the level of supersaturation at different temperatures. As shown, supersaturation decreases as a function of temperature, which indicates that supersaturation is greater at lower temperatures, and supersaturation is sensitive to temperature variations.

Greater supersaturation typically indicates that crystallization is more likely. However, in our experiments, the above supersaturation was maintained over a short time period because the crystallization solution was immediately transferred to the temperature controller (the temperature was 293 K) after the crystallization trials were set up in the plates. The mixing time to the time that the temperature reached the crystallization temperature (293 K) was limited. During this period, can crystallization occur? We have attempted to decrease the time period between mixing to placing the crystallization plate in the temperature controller; the time period was approximately 80 s. Further, another period of time should also be considered, the period of time from placing the plate into the temperature controller to the time when the crystallization droplet temperature reaches the intended temperature. We measured the time necessary for the crystallization plate to increase from three lower temperatures (278 K, 283 K and 288 K) to 293 K, which was approximately 10.7 min to 16 min. Therefore, during the time period of approximately $12 \sim 17.3$ min, the crystallization solution was at the intended crystallization temperature. This time should be sufficient for lysozyme crystal nucleation when supersaturation is appropriate.

The effect of evaporation on supersaturation. During crystallization solution preparation, solvent evaporation can affect the crystallization solution supersaturation level upon sealing, because evaporation will increase the solution concentration. In the above result, we did not consider evaporation during the solution preparation process. Evaporation would definitely occur because, when the

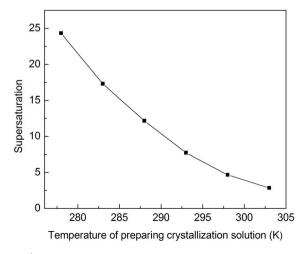


Figure 4 | The supersaturation level at different solution preparation temperatures. The solution concentrations were 20 mg ml⁻¹ HEWL and 40 mg ml⁻¹ NaCl. The temperature strongly influenced supersaturation. At a lower temperature, the supersaturation was greater.

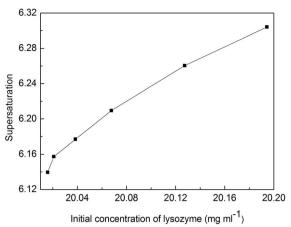


Figure 5 | Supersaturation at different lysozyme concentrations in our experiment. The initial lysozyme concentration upon sealing the crystallization plate differed from the intended concentration due to evaporation before sealing the plate. At higher solution preparation temperatures, the initial lysozyme concentration was greater (refer to Fig. 2); hence, supersaturation increased.

solutions were dispensed to the crystallization plate, the solutions were exposed to open air. The evaporation level depended on the solution preparation temperature; therefore, the solution preparation temperature can affect the solution concentration (as shown in Fig. 3) and further change the solution supersaturation level. Would this concentration difference produce a difference in crystallization? To answer this question, we estimated the supersaturation level at the intended crystallization temperature (293 K) based on the concentration after evaporation and solubility at the intended temperature.

By curve fitting the published data^{24,26,27}, we can determine the relationship between the lysozyme solubility C_s and the NaCl concentration C_{NaCl} with lysozyme and NaCl dissolved in sodium acetate buffer pH 4.6 at 293 K (NaCl concentration between 20 and 50 mg ml⁻¹). The relationship can be expressed as shown in Equation (2).

$$C_s = -0.0016C_{NaCl}^3 + 0.2146C_{NaCl}^2 - 9.6437C_{NaCl} + 148.06$$
(2)

Using the lysozyme concentration and Equation (2), the supersaturation (σ) can be calculated when the crystallization solution temperature is 293 K. Fig. 5 shows the supersaturation results at different initial lysozyme concentrations. An increasing supersaturation trend can be observed as a function of the lysozyme concentration, which indicates that the crystallization solution exhibits greater supersaturation due to evaporation at a higher solution preparation temperature.

The protein concentration may affect the crystallization solution supersaturation, and the induction time for crystallization nucleation decreased with higher concentrations²³. Based on Fig. 3, the initial concentration at a higher crystallization solution preparation temperature (293 K, 298 K and 303 K) was higher than at a lower temperature (278 K, 283 K and 288 K). When the crystallization plates were placed into the temperature controller, the supersaturation was higher with a higher concentration of crystallization solution. Lysozyme nucleation was easy at the higher temperature during solution preparation due to a higher initial concentration and super-saturation; thus, crystallization was more successful.

Discussion

The above results indicate that the solution preparation temperature can affect the level of supersaturation at two stages: (1) the time

lization temperature (293 K); and (2) the time period after reaching the crystallization temperature through the end of the experiment. During the first stage, lower solution preparation temperatures yield greater supersaturation (Fig. 4). In the second stage, the inverse trend dominates (Fig. 5). Although the first stage is shorter than the second stage, the variation in supersaturation magnitude is much greater such that the difference in supersaturation during the first stage may affect the final crystallization results. During the second stage, the supersaturation variation is low (but sufficiently large to generate differences) due to the difference in solution preparation temperature, but the time is sufficiently long for the difference to have an effect. Hence, both stages can affect the final crystallization results. Because the two stages followed different trends, it is understandable that the final crystallization success rate may follow a combined trend (i.e., a parabolic trend as a function of the solution preparation temperature), as demonstrated by the experiments herein (Fig. 1a). Hence, we anticipate that, at a lower solution preparation temper-

period from mixing the lysozyme and NaCl solutions through the

crystallization solution temperature change to the intended crystal-

ature, the solution more easily forms nuclei before the crystallization plate is placed into the temperature controller. At a higher solution preparation temperature, crystallization is easier after the crystallization plate is placed into the temperature controller. Consequently, the data exhibit a parabolic curve for crystallization success as a function of the solution preparation temperature.

Herein, we show that the crystallization solution preparation temperature affects the final crystallization results. During protein crystallization, the solution preparation temperature effects are typically ignored, and the preparation temperature is either not controlled or poorly controlled. A lab's temperature can vary throughout the day and year. Throughout a day, the room temperature variation range may be greater than 5 K. Throughout a year, the variation range may be much greater depending on the room conditions, season, and geological region, among other factors. Even in a thermostatic room, the temperature is not strictly stable but changes all the time, and the variation range may be greater than 1 K. Hence, the ambient temperature may differ during crystallization solution preparation in different crystallization experiments. Such differences in the solution preparation temperature can, as shown in this study, produce different final crystallization results. Therefore, the phenomenon observed herein may be one of many reasons for poor reproducibility in protein crystallization.

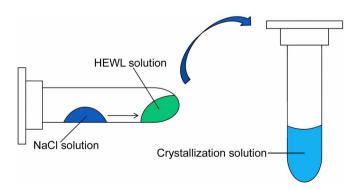


Figure 6 | A schematic of crystallization solution preparation. Before the experiment, the HEWL and NaCl solutions were pipetted into an Eppendorf tube at different positions without mixing (left). Next, the Eppendorf tube with the solutions was maintained horizontally in the temperature controller. After a one-hour treatment, the crystallization solution can be generated by turning the Eppendorf tube upright such that NaCl solution moves downwards and mixes with the HEWL solution (right).

Table 1 | The initial experimental conditions used for proteinase K and thaumatin

Protein	Initial concentration	Buffer	Precipitant
Proteinase K	20 mg ml ⁻¹	0.1 M Tris-HCl pH 8.5	2.0 M Ammonium sulfate
Thaumatin	20 mg ml ⁻¹	0.1 M Potassium phosphate pH 7.0	2.8 M Potassium sodium tartrate

Methods

Crystallization experiments at different solution preparation temperatures. Hen egg-white lysozyme (HEWL, six-times-recrystallized, Seikagaku Kogyo Co., Japan) and NaCl were dissolved in 0.1 M sodium acetate buffer (pH 4.6) to obtain solutions at the initial concentrations 40 mg ml⁻¹ and 80 mg ml⁻¹, respectively. The solutions (120 μ l HEWL and 120 μ l NaCl solutions) were pipetted to different positions in an Eppendorf tube (Fig. 6) and sealed; the tube was moved into a temperature controller and maintained at a particular temperature for 1 hour. To ensure that the solution would not mix during the procedure, the tube was maintained horizontally. After treatment, the temperature of both solutions reached the controller temperature; next, the Eppendorf tube was turned upright for the NaCl solution to move and mix with the HEWL solution at the bottom of the tube due to a gravitational force.

The temperature controller was previously described²⁶, and the temperature can be accurately controlled within 0.1 K. Six mixing temperatures were used: 278, 283, 288, 293, 298 and 303 K. The crystallization experiment was repeated 30 times at each solution preparation temperature. For each experiment, we determined the crystallization success rate by dividing the number of droplets that yielded crystals by the total number of droplets (i.e., 30×96). Next, the success of each of the 30 experiments was averaged to yield the average success rate for the solution preparation temperature.

After mixing, the mixed solution was immediately dispensed into the microbatch crystallization plate (HR3-267, Hampton Research Co., USA) using a crystallization robot (Screenmaker 96 + 8^{TM} , Innovadyne Technologies, Inc., USA). Two microliters of the mixed solution was dispensed into each of the 96 wells to produce 96 identical crystallization droplets. The droplets were sealed using the Crystal Clear Sealing Tape (HR4-506, Hampton Research Co., USA) 80 s after dispensing and placed into the temperature controller for incubation at 293 K for 2 days to generate crystals. The crystal images were collected by an automated crystal image reader (XtalFinder, XtalQuest Inc., China).

The crystallization experiments of proteinase K and thaumatin (Sigma-Aldrich company, USA) was set up following the procedures described above. And the protein and precipitant solutions were prepared according to the conditions shown in Table 1. For these two proteins, the crystallization experiments were all repeated 5 times at each solution preparation temperature (283, 288, 293, 298 and 303 K). For each experiment, we also determined the crystallization success rate following the procedures described above.

Evaporation test at different solution preparation temperatures. In this mechanism study, lysozyme was utilized, and we must determine the level of solution evaporation at different preparation temperatures. Therefore, we attempted to obtain the level of crystallization solution evaporated at different solution preparation temperatures.

The solution preparation procedure was similar to the crystallization experiment. The HEWL (120 μ l) and NaCl solutions (120 μ l) were pipetted into an Eppendorf tube without mixing; the tube (with the solutions) was then sealed and weighed using a microbalance (BT125D, Sartorius Scientific Instruments Co., Beijing, China), which was used as the initial weight. After one hour in the temperature controller at a temperature designed for mixing, the Eppendorf tube was turned upright to mix the solution. After mixing, the Tube lid was opened, and the tube weight (with the mixed solution) was measured using the microbalance; the weight was then recorded every 10 seconds. The total weighing time for each tube was 5 minutes. Each solution experiments was used for this measurement experiment, and we repeated the measurement 3 times at each solution preparation temperature.

- D'Arcy, A., Mac Sweeney, A., Stihle, M. & Haber, A. The advantages of using a modified microbatch method for rapid screening of protein crystallization conditions. *Acta Cryst.* D59, 396–399 (2003).
- 2. Helliwell, J. Macromolecular crystal twinning, lattice disorders and multiple crystals. *Crystallogr. Rev.* 14, 189–250 (2008).
- 3. McPherson, A. Macromolecular crystallization in the structural genomics era. *J. Struct. Biol.* **142**, 1–2 (2003).
- 4. Moreno, A., Saridakis, E. & Chayen, N. E. Combination of oils and gels for
- enhancing the growth of protein crystalls. *J. Appl. Crystallogr.* 35, 140–142 (2002).
 Newman, J. One plate, two plates, a thousand plates. How crystallisation changes
- with large numbers of samples. *Methods* **55**, 73–80 (2011). 6. Chernov, A. A. Protein crystals and their growth. *J. Struct. Biol.* **142**, 3–21 (2003).

- Newman, J., Xu, J. & Willis, M. C. Initial evaluations of the reproducibility of vapor-diffusion crystallization. *Acta Cryst.* D63, 826–832 (2007).
- Chayen, N. E. Crystallization with oils: a new dimension in macromolecular crystal growth. J. Cryst. Growth 196, 434–441 (1999).
- Chayen, N. E. Turning protein crystallisation from an art into a science. *Curr.* Opin. Struct. Biol. 14, 577–583 (2004).
- 10. Brzozowski, A. M. & Walton, J. Clear strategy screens for macromolecular crystallization. *J. Appl. Crystallogr.* **34**, 97–101 (2001).
- 11. Yin, D. C. *et al.* Uncertainties in crystallization of hen-egg white lysozyme: reproducibility issue. *Cryst. Res. Technol.* **43**, 447–454 (2008).
- 12. McPherson, A. Introduction to protein crystallization. *Methods* **34**, 254–265 (2004).
- 13. Neer, A. Protein crystallization and phase diagrams. Methods 34, 266-272 (2004).
- Christopher, G. K., Phipps, A. G. & Gray, R. J. Temperature-dependent solubility of selected proteins. J. Cryst. Growth 191, 820–826 (1998).
- Baird, J. K., Hill, S. C. & Clunie, J. C. Kinetics of protein crystal nucleation and growth in the batch method. J. Cryst. Growth 196, 220–225 (1999).
- Narayanan, J. & Liu, X. Y. Protein interactions in undersaturated and supersaturated solutions: a study using light and X-ray scattering. *Biophys. J.* 84, 523–532 (2003).
- 17. Ward, K. B. *et al.* Dynamic telerobotic control of crystallization experiments. *J. Cryst. Growth* **122**, 235–241 (1992).
- Lin, Y. B. *et al.* An extensive study of protein phase diagram modification: increasing macromolecular crystallizability by temperature screening. *Cryst. Growth Des.* 8, 4277–4283 (2008).
- Zhang, C. Y. *et al.* Cycling temperature strategy: a method to improve the efficiency of crystallization condition screening of proteins. *Cryst. Growth Des.* 8, 4227–4232 (2008).
- Astier, J. P. & Veesler, S. Using temperature to crystallize proteins: a mini-review. Cryst. Growth Des. 8, 4215–4219 (2008).
- Kitano, K., Motohashi, K., Yoshida, M. & Miki, K. A novel approach to crystallizing proteins with temperature-induction method: GrpE protein from thermus thermophilus. J. Cryst. Growth 186, 456–460 (1998).
- Bartling, K., Sambanis, A. & Rousseau, R. W. Multiwell microbatch crystallization on a thermal gradient. Cryst. Growth Des. 5, 1559–1564 (2005).
- Drenth, J. & Haas, C. Nucleation in protein crystallization. Acta Cryst. D54, 867–872 (1998).
- Forsythe, E. L., Judge, R. A. & Pusey, M. L. Tetragonal chicken egg white lysozyme solubility in sodium chloride solutions. J. Chem. Eng. Data 44, 637–640 (1999).
- Berg, M., Urban, M., Dillner, U., Muhlig, P. & Mayer, G. Development and characterization of temperature-controlled microreactors for protein crystallization. *Acta Cryst.* D58, 1643–1648 (2002).
- Chen, R. Q., Yin, D. C., Lu, Q. Q., Shi, J. Y. & Ma, X. L. Sensitivity of lysozyme crystallization to minute variations in concentration. *Acta Cryst.* D68, 584–591 (2012).
- 27. Chen, R. Q. et al. Promoting protein crystallization using a plate with simple geometry. Acta Cryst. D70, 647–657 (2014).

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Author contributions

D.-C.Y. and R.-Q.C. designed research; R.-Q.C., Q.-Q.L., Q.-D.C., C.-Y.Z., H.H., Y.-M.L. and D.-W.L. performed research; R.-Q.C. and D.-C.Y. wrote the main manuscript text and L.-B.A. prepared Figure 1 and Figure 6. All authors reviewed the manuscript.

Additional information

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