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A simple technique to convert sitting-drop vapor diffusion into hanging-drop vapor diffusion by solidifying the reservoir solution with agarose

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A simple protocol to convert sitting-drop vapor-diffusion plating into a hanging-drop vapor-diffusion experiment in protein crystallization is reported. After making a sitting-drop plate, agarose solution was added to solidify the reservoir solution, and the plates were incubated upside down. Crystallization experiments with hen egg white lysozyme, thaumatin and glucose isomerase showed that the 'upside-down sitting-drop' method could produce single crystals with all the benefits of the hanging-drop crystallization method.

1. Introduction

The vapor-diffusion method is widely used for setting up protein crystallization because it produces a higher throughput than other methods and forms well ordered crystals (Chayen, 1998). Sitting- and hanging-drop vapor-diffusion methods are easy to perform and allow flexible screening with minimal sample volume. The sitting-drop technique has benefits over hanging-drop plating, such as cost and time efficiencies, but crystals often adhere to the hardware surface. The hanging-drop technique reduces the occurrence of hardware crystal adherence and improves the crystal shape and size because of the inverted position of the drop, but this method has the inefficiency of requiring silicone greasing and a cover slip. The newly developed Nextal crystallization device could avoid this problem, although this device is expensive (Nneji & Chayen, 2004).

Here a simple protocol to convert a conventional sitting-drop plate for hanging-drop protein crystallization processes is reported. We used agarose gel to solidify the reservoir solution of sitting-drop plates, enabling us to convert sitting-drop into hanging-drop plates by incubating the sitting-drop plates upside down. Agarose gel allows a diffusive environment for the mass transport of vapor from protein solution to reservoir solution since solidified agarose has a porous texture (González-Ramírez *et al.*, 2008).

2. Experiment and results

Crystallization trials were conducted on hen egg white lysozyme (Sigma L6876) prepared at 20 mg ml^{-1} in 50 m*M* Tris–HCl (pH 8.0) with the precipitant solution described by Chayen *et al.* (1993), thaumatin at 35 mg ml^{-1} in distilled water (Sigma T7638) as described by Malkin *et al.* (1996) and glucose isomerase (Hampton Research HR7-102) at 33 mg ml^{-1} in distilled water as described by Sleutel *et al.* (2009).

To obtain 'upside-down sitting-drop' plates, sitting-drop plates were set up using 24-well Cryschem plates (Hampton Research, HR3-160) with 4 µl drops over 500 µl of the initial reservoir solution. First, 2 µl of the protein solution was combined with 2 µl of the well solution. Then, 500 µl of a 2%(*w*/*v*) agarose (American Bioanalytical, Natick, MA, USA) solution was added. The agarose solution was prepared by dissolving agarose in distilled water and sterilizing the solution at 394 K for 15 min. The solution was kept melted in a 318 K drying oven until use. Once the reservoir solution had solidified as a result of the added agarose, the plate was sealed with transparent sealing tape (Hampton Research, HR3-511) and turned upside down. Finally, each drop was left to equilibrate against the solidified reservoir solution in the reservoir well at 288 K for lysozyme, or at 295 K for thaumatin and glucose isomerase.

The inverted Cryschem plate with solidified mother liquor and the outer shape of an individual well are shown in Fig. 1. Typical crystals of lysozyme, thaumatin and glucose isomerase formed *via* the 'upside-

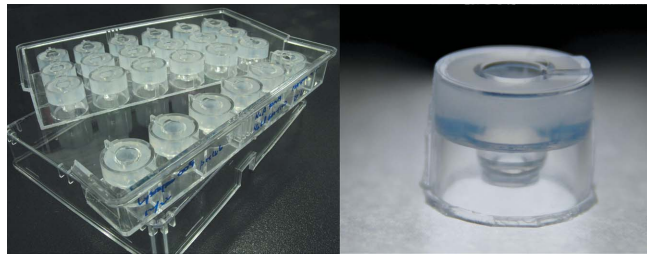

Figure 1
Inverted Cryschem plate with solidified mother liquor (left) and the outer shape of an individual well (right).

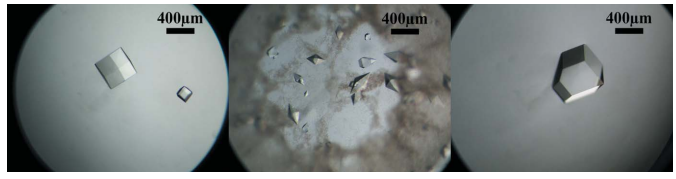

Figure 2
Crystals of hen egg white lysozyme (left), thaumatin (middle) and glucose isomerase (right) grown using the 'upside-down sitting-drop' vapor diffusion method.

down sitting-drop' method grew up to 425 × 425 × 223 µm, 351 × 213 × 213 µm and 744 × 531 × 584 µm after 72 h (Fig. 2).

3. Discussion

By placing the protein droplet in an inverted position, 'upside-down sitting-drop' plate crystallization can be used to avoid crystal adhesion to the hardware surface. One could observe the crystals through the transparent backside of the crystal plates, even in the presence of water droplets that often formed on the sealing tape surface inside the reservoir. Sealing with plastic tape made it easy to reseal each well after some crystals had been removed, in contrast to sealing with a cover slip and silicone greasing in conventional hanging-drop plates.

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References

Chayen, N. E. (1998). *Acta Cryst.* D**54**, 8–15.
Chayen, N. E., Radcliffe, J. W. & Blow, D. M. (1993). *Protein Sci.* **2**, 113–118.
González-Ramírez, L. A., Caballero, A. G. & García-Ruiz, J. M. (2008). *Cryst. Growth Des.* **8**, 4291–4296.
Malkin, A. J., Kuznetsov, Y. G., Glantz, W. & McPherson, A. (1996). *J. Phys. Chem.* **100**, 11736–11743.
Nneji, G. A. & Chayen, N. E. (2004). *J. Appl. Cryst.* **37**, 502–503.
Sleutel, M., Willaert, R., Gillespie, C., Evrard, C., Wyns, L. & Maes, D. (2009). *Cryst. Growth Des.* **9**, 497–504.