Crystallizing Lysozyme

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References

Understanding Crystallography - Part 1: From Proteins to Crystals
https://www.youtube.com/watch?v=gLsC4wlrR2A

Protein Crystallization for X-ray Crystallography
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3182643

Introduction to protein crystallization
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3943105/

Protein crystallography for non-crystallographers, or how to get the best (but not more) from published macromolecular structures
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4465431/

Crystallizing lysozyme from Hampton Research
https://hamptonresearch.com/uploads/support_materials/HR7-110_UG.pdf

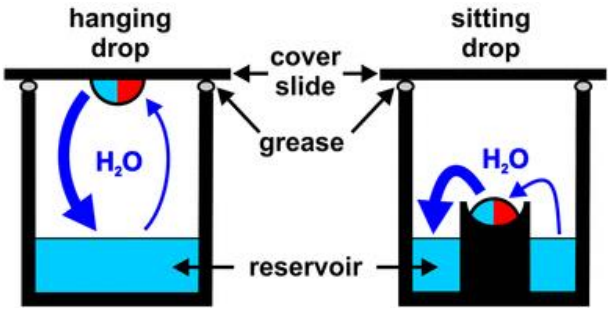
Crystallization kit for Lysozyme
https://hamptonresearch.com/product-Lysozyme-524.html

Purpose

The purpose of this demo is to become a protein crystallographer by growing crystals of hen egg white lysozyme! Crystals will be grown under various conditions where you will probe the effects of protein concentration and buffer condition on lysozyme crystallization in both hanging and sitting drop format. Crystal growth will be monitored with a standard microscope and, if desired, an automated microscope.

Usually, a crystallization drop is prepared by mixing equal parts (1:1 ratio) of purified protein and precipitant solution (buffer containing a precipitant), and the drop is surrounded by a reservoir solution of the precipitant solution. The precipitant that successfully crystalizes a protein varies widely; the most commonly successful precipitant is polyethylene glycol (PEG), followed by ammonium sulfate. When diluting the precipitant, its concentration drops in half in the reservoir, which initiates water vapor diffusion.

For the hanging drop method, the protein drop is suspended above the well solution from a seal and makes no contact with the crystallization plate. For the sitting drop method, the protein drop sits directly on a surface of the crystallization plate above the crystallization condition.



Materials Needed

- Gloves (PPE) {optional}
- Crystallization trays and accessories

Hanging drop: VDX Plate 24 well hanging drop plate (Hampton Research #HR3-170)
https://hamptonresearch.com/uploads/cg_pdf/CG101_Hanging_Drop_Vapor_Diffusion_2020.pdf

Sitting drop: Cryschem 24 well sitting drop plate (Hampton Research #HR3-159)
https://hamptonresearch.com/uploads/cg_pdf/CG101_Sitting_Drop_Vapor_Diffusion.pdf

Grease
https://hamptonresearch.com/product-Dow-Corning-Vacuum-Grease-Dupont-Molykote-268.html

Crystal Clear Sealing Film (or Tape)
https://hamptonresearch.com/product-Crystal-Clear-Sealing-Film-566.html
or
https://hamptonresearch.com/product-Crystal-Clear-Sealing-Tape-271.html

Film Sealing Paddle
https://hamptonresearch.com/product-Film-Sealing-Paddle-632.html

"Unbreakable" Cover Slip
https://www.fishersci.com/shop/products/fisherbrand-unbreakable-cover-slips/12547

- Zeiss Stemi 2000-C Stereo Microscope
- Purified Protein

In this case, Lysozyme from chicken egg white (Sigma Aldrich #L6876-5G)

Stock solution 1: 20 mg/mL Lysozyme

20 mg Lysozyme + 1 mL 0.1 M sodium acetate buffer pH 4.5

Stock solution 2: 50 mg/mL Lysozyme

50 mg Lysozyme + 1 mL 0.1 M sodium acetate buffer pH 4.5

Stock solution 3: 100 mg/mL Lysozyme

100 mg Lysozyme + 1 mL 0.1 M sodium acetate buffer pH 4.5

- Pipettes (1,000 µL and 2 µL) & Pipette tips (1,000 µL and 2 µL)
- Waste container (for tips and such)
- Crystallization solutions A – C

  Solution A. 1 M sodium chloride, 0.1 M sodium acetate pH 4.5

  Solution B. 0.6 M sodium chloride, 0.1 M sodium acetate pH 4.5, 25% ethylene glycol

  Solution C. 0.6 M sodium chloride, 0.1 M sodium acetate pH 4.5, 25% glycerol

- Buffer Recipes

0.1 M sodium acetate pH 4.5 (100 mL):
50 mL of distilled water
0.82 g sodium acetate anhydrous
Adjust to pH 4.5 using glacial acetic acid
Add distilled water until volume is 100 mL
Filter with 0.2 µm PES filter

1 M sodium chloride, 0.1 M sodium acetate pH 4.5 (100 mL):
50 mL of distilled water
5.84 g NaCl
0.82 g sodium acetate anhydrous
Adjust to pH 4.5 using glacial acetic acid
Add distilled water until volume is 100 mL
Filter with 0.2 µm PES filter

0.6 M sodium chloride, 0.1 M sodium acetate pH 4.5, 25% ethylene glycol (100 mL):
50 mL of distilled water
3.50 g NaCl
0.82 g sodium acetate anhydrous
25 mL ethylene glycol
Adjust to pH 4.5 using glacial acetic acid
Add distilled water until volume is 100 mL
Filter with 0.2 µm PES filter

0.6 M sodium chloride, 0.1 M sodium acetate pH 4.5, 25% glycerol (100 mL):
50 mL of distilled water
3.50 g NaCl
0.82 g sodium acetate anhydrous
25 mL glycerol

Adjust to pH 4.5 using glacial acetic acid
Add distilled water until volume is 100 mL
Filter with 0.2 µm PES filter

Procedure

1. Place 500 µL of each crystallization solution in their respective reservoir on the sitting or hanging drop plates (see Crystal Tray Layout below)

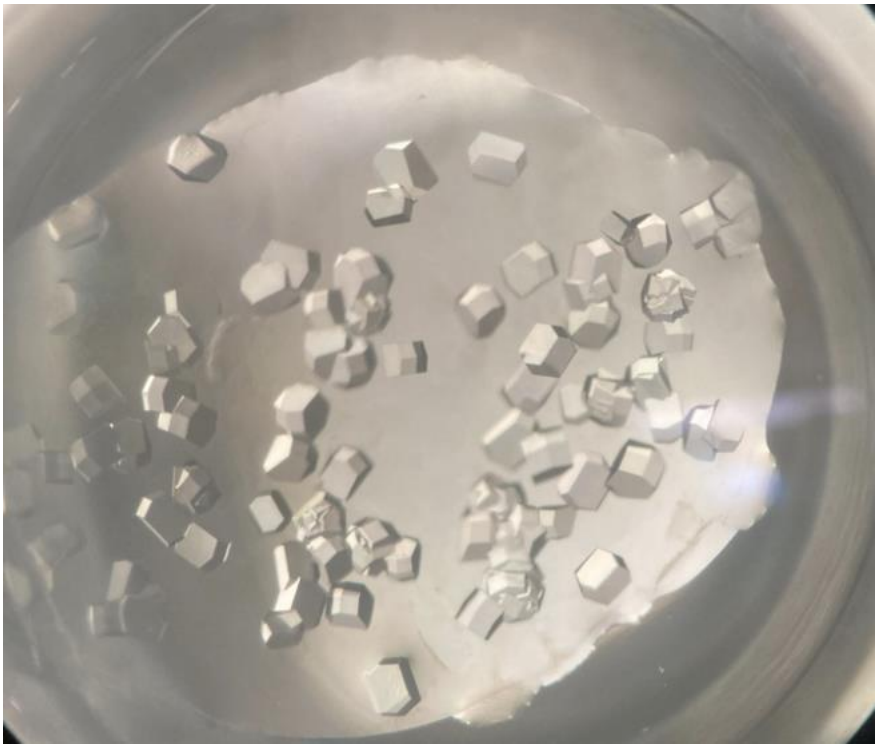
2. Add 2 µL of crystallization solution to the cover slip (hanging drop) or to the center well (sitting drop)

3. Add 2 µL of protein stock solution directly to the 2 µL of crystallization stock solution (no need to mix)

4. Seal the wells with either the sealant+cover slips (hanging drop) or cover tape (sitting drop). Make sure the seal is tight and firm.

5. Start timer and watch for crystal growth. Crystals begin to form within ~30 min and are clearly visible and well developed within an hour or so.

6. Check growth under a visible light microscope (Zeiss Stemi 2000-C) for crystal growth. It should look something like this:



Questions

1. Which buffer produced the most and "best" looking crystals?

2. Which protein concentration was best, and why?

3. Did sitting vs hanging drop methods produce any noticeable difference (if tested)?

Crystal Tray Layout

Column		Row	
1	20 mg/mL Lysozyme	A	Solution A
2	50 mg/mL Lysozyme	B	Solution B
3	100 mg/mL Lysozyme	C	Solution C
4	N/A	D	N/A
5	N/A		
6	N/A		

Example:

Sitting Drop Plate Organization

Example layout of 24 well sitting drop plate: