Crystallization Laboratory

Objectives

- Experience setting up crystallization trials.
- To optimize protein and precipitate concentrations for lysozyme crystallization.
- To learn to differentiate precipitates, microcrystals, crystals, etc.
- To obtain a crystal or two for diffraction demonstrations.

Introduction

We will pretend that we don't know the conditions for crystallization of lysozyme. Each student will set up hanging drops in one half of a 24-well tissue culture plate. Each row will be set up with a different protein concentration. Each column will be set up with a different concentration of precipitant (NaCl). Every few days the tray will be examined under the microscope. Changes, such as the appearance of precipitate, or hopefully crystals will be recorded. The size of crystals will be measured.

Apparatus

(If you have them at hand, please bring to the lab the following pipettemen: P20, P1000 (and some tips for each). We will provide as many as we have, but if you have your own, you will be delayed less by sharing.)

Microfuge tubes (3).
Pipettemen: 20 µL, 1 mL, please bring to lab. 20 µL pipette tips (20); 1000 µL pipette tips (4).
Non-siliconized cover slips for practice (5).
Tissue culture plate (24 well).
Siliconized cover slips (13).
(Marker pens).
(Kim wipes).

Reagents

Buffer: 0.1 M sodium acetate, pH 4.6 (10 ml).
Stock precipitant: 10% w/v sodium chloride, 0.1M sodium acetate, pH 4.2 (20 ml).
Stock protein: 100 mg/ml lysozyme in acetate buffer (100 µL).

Method

A) Serial dilutions of protein

Each student is provided with a microfuge tube containing 100 µL of 100 mg/ml lysozyme solution.

- Label 3 microfuge tubes "33"; "10"; "3".
- Transfer 35 µL protein stock to tube "33".
- Add 70 µL buffer to tube "33" --> 33 mg/ml solution.
- Transfer 35 µL from tube "33" to tube "10".
- Add 70 µL buffer to tube "10" --> 10 mg/ml solution.
- Transfer 35 µL from tube "10" to tube "3".
- Add 70 µL buffer to tube "10" --> 3 mg/ml solution.

B) Preparation of the tissue culture plate.

Each drop will be hung on a microscope cover slip that is sealed to a well. The seal is made by a bead of grease drawn around the rim of each well; in this case, the plates are provided pre-greased. As the hanging drops are easily disturbed, the tray is fully prepared before adding the first drop. Read the next two sections before starting.

- Label the side of the lid with your name, date, "lysozyme", etc.
- Practice applying a non-siliconized cover slip to a well with gentle pressure; since we will be using the left 3 columns of the plate, apply this cover slip to the right side. Check the seal by looking at the surface from an acute angle. Remember that you will not be able to tip a filled tissue culture plate.

C) Addition of mother liquor

Note that molded into the plastic are row labels, A-D, and column numbers, 1-6.

- To all wells in columns 1, add 1.0 mL precipitant.
- To all wells in columns 2, add 0.65 mL precipitant.
- To all wells in columns 3, add 0.5 mL precipitant.
- Change pipette tip.

Holding the plate in one hand, and pipetting with the other, the following aliquots can be added without changing tip, if the aliquots are discharged gently down the side of each well, and contact is avoided with the mother liquor in the well and the grease on the rim.

- To all wells in columns 2, add 0.35 mL buffer.
- To all wells in columns 3, add 0.5 mL buffer.

The wells of columns 1 & 4 now contain a mother liquor of 10% salt.
The wells of columns 2 & 5 now contain a mother liquor of 6.5% salt.
The wells of columns 3 & 6 now contain a mother liquor of 5% salt.

D) Hanging drops

In the following protocol, use 100 mg/ml lysozyme for row 1 (top), 33 mg/ml for row 2, 10 mg/ml for row 3, and 3 mg/ml for row 4.

Some skill is required to invert a filled cover slip. Practice using a siliconized cover slip, adding 20 µL of water. The same cover slip can be used for practice repeatedly after drying with a kim wipe.

- Microfuge all of your protein solutions at maximum speed for a minute, and thereafter avoid pipetting from the bottom of the tube.
- Hold a siliconized cover plate between your forefinger and thumb.
- Place 10 µL of the of protein solution in the middle of the cover slip.
- Take 10 µL of mother liquor from a well, and add it to the drop on the cover slip. Steady your hand, and try not to touch the cover slip with the pipette tip or spread the drop.
- With a quick, but smooth motion, invert the coverslip, trying not to move the drop. If the drop moves slightly, it can sometimes be coaxed back with gentle shaking.
- Place the cover slip, drop-down, over a well.
- Press down gently to seal the cover slip to the top of the well.
- Repeat for all 12 wells in columns A, B, and C.
- Inspect all grease seals.
- With light pressure, cover with the lid and store.

E) Inspection

(Once crystals start growing, we will leave 10 minutes early from a couple of classes in the coming weeks to come back to the laboratory to inspect the crystals.)
After several days, the plate is inspected under the microscope. Crystals are distinguished by sharp edges, and clarity. Precipitates are cloudy or white. Note any changes that you see. Crystals should start to form within a few days, but may take a month, depending on the conditions.
Move the plate gently, the drops are easy to dislodge. To calibrate the cross-hairs, inspect a transparent ruler using a known magnification.