

## Interpretation of the Crystallization Drop Results

Using a stereo microscope carefully examine the droplets, scan the focal plane for small crystals and record observations. If crystals are obtained during an initial screen the conditions may be optimized by varying the pH and concentrations of precipitant or salt. In the absence of crystals inspect any droplets with precipitate for microcrystallinity. Use a high power microscope to examine amorphous material between crossed polarizing lenses. True amorphous precipitates do not glow. Birefringent microcrystalline precipitates can glow as a result of the plane of polarization. It may be possible to use streak seeding to produce larger crystals from microcrystalline precipitates. If the amorphous material is precipitate repeat the screen but, reduce the sample concentration or dilute the precipitant with water. If the droplets remain clear leave the screen for a few weeks but, continue to observe the samples. Increasing the sample concentration may optimize the conditions. If small crystals, not suitable for x-ray diffraction, are grown it may be possible to use seeding techniques to grow larger crystals.

### Interpretation of the Crystallization Drop Results

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### Result Interpretation and Type of Screen

The major difficulty in the crystallization of proteins is the identification of conditions that, after optimization, will produce crystals suitable for X-ray diffraction analysis. Since good quality crystals are not usually obtained in the first screen, it is necessary to get leads from whatever results that are produced. The approach to "reading" the crystallization drop will depend somewhat on the type of screen used.

The **sparse matrix** screen is used for the identification of those areas in variable space that have some chance of producing crystals. The crystallization experiments are examined with a stereomicroscope 1) immediately after setup, 2) each day for the first week, and 3) once a week for several weeks. When storage space is available the experiment may be continued for as long as 2 years. The conditions that cause precipitation or crystal growth are identified, as is the rate at which they occur, because the rate of formation is important for optimization of the precipitant concentration.

The advantage of the sparse matrix screen is that it tests many parameters with a limited amount of protein. This might result in the identification of different crystal forms, but often only information about the solubility of the protein is obtained. Therefore, careful examination of the results is required in order to identify promising leads.

Unlike the sparse matrix screen, with a systematic screen the trends in precipitation behavior are easy to identify or infer. In a systematic screen the results can be plotted in a phase diagram. The phase diagram shows the different zones for soluble protein (Score=0), denaturation (Score=3), precipitation (Score=3-4), and nucleation (Score=6-9).

In the final stages of optimization, when we are aiming for highly ordered, big crystals, it is better not to disturb the crystal growth process, so wait one or two weeks before checking the experiment.

### The Stereomicroscope

For the examination of the crystallization conditions use a Stereomicroscope. The Stereomicroscope should have an observation platform that is smooth and big enough to support the tray when looking at all the drops. For transmitted light use a separate light source and a fibre-optic light guide with bright and dark field illumination. If the only microscope available is one with the light source in the observation platform, place a Perspex (plexiglass) plate between the platform and the tray, and work quickly to prevent heating of the drop during examination. When examining the drops always use the same light brightness, and do not use a polarizer because precipitate color is an important indicator (dark = bad sign, light/transparent = good sign). Examine and store the experiments at the same temperature to prevent irreproducible results.

### Examination of the Crystallization Experiments

In order to save space in the lab book, and for easy identification, use a score of one or more numbers to classify the contents of the drop, such as that described below. Examples of these scores are shown with coloured photographs in the above book.

**Example:** A score of 49 indicates gelatinous precipitate (score 4) with crystals (score 9). The scoring is not based on quality; a higher number does not necessarily mean a better result.

Check the entire depth of focus in the drop, first at 40 to 50x magnification and then more closely at 100x. Use polarized light to check the optical properties of crystalline material in the drop. Crystalline material, as opposed to amorphous precipitate, is birefringent, i.e., it splits (polarizes) transmitted light into two orthogonal beams. Birefringent precipitates, like crystals, sparkle or glisten.

Be sure to remove the tray from the observation platform before applying and removing the polarizer or analyzer. Without the tray, adjust the two polarizers so that the field of illumination is uniformly dark. If a crystal is birefringent, the analyzer will transmit some of the light passing through the crystal. The intensity of the transmitted light passes through maxima and minima when the crystal is rotated. This effect is not always clearly visible because of interference from the plastic tray with the polarized beam. A birefringent crystal in a plastic tray will have a different color than the background. Birefringence is a property of both protein and salt crystals and is even pronounced for salts.

### The protein is not precipitated.

#### Drop is clear.

(Score=0)

- Check the entire depth of focus; try dark-field illumination to check for gelatinous precipitate.
- If the drop remains clear for more than two weeks, repeat the condition with a higher protein or precipitant concentration.
- If limited amount of protein is available, place the clear drops over a reservoir with a higher precipitant concentration.
- In an optimization step, clear drops can be seeded with crystals from neighboring drops.

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### **Drop contains non-protein particles.**

(Score=1)

- Glass has an irregular shape without birefringence.
- Dust on the cover slips, in and around the drop: Clean the cover slips carefully with a lint free tissue before setting up the drops.
- Dust in the drop, but also in the well: Filter the stock solutions.
- Fibres from clothes. Fibres are colorful under polarized light.
- Bacterial growth: Add sodium azide to the protein and well solution.

### **Precipitate shows no birefringence and has no edges.**

#### **Drop is mostly clear but contains precipitated protein.** (Score=2)

- This could indicate that the protein or precipitant concentrations (or both) do not favour nucleation and crystal growth.

#### **The protein is fully precipitated with a dark color.**

(Score=3)

- Identify unfavourable conditions (pH, precipitant, or additive). These conditions should be avoided in future experiments as should any pH where the protein denatures. (Box 2 conditions for The Solubility Tool Kit can be pH adjusted. Alternatively Molecular Dimensions' Clear Strategy Screens can also be pH adjusted.)
- If the protein precipitated within 1 day, repeat the condition with half the precipitant concentration. With viscous solutions like PEG the protein can precipitate when the protein and well solution are mixed. The drop will contain regions of heavy precipitate and regions that are clear.
- If the heavy precipitate is formed after a few days, the condition does not favor crystallization.
- In older drops a skin of denatured protein can appear on the surface of the drop. If the surface has a film that is not wrinkled it could be phase separation.

### **Gelatinous protein precipitate.**

(Score=4)

- Gelatinous precipitate is sometimes hard to see because it is white or transparent. Check the drop with dark-field illumination: the structural elements appear bright on a dark background.
- Gelatinous precipitate is a good starting point, when no crystals are found. Optimize the condition by changing one of the parameters (pH, precipitant concentration, or additive).

### **Phase Separation.**

(Score=5)

- The protein is visible as droplets within the drop or as an "oil" film on the surface of the drop. This can be seen best at the interface of the drop and cover slip. Temperature has an important influence on the equilibrium, so be careful not to heat the drop during examination. Sometimes crystals grow in the highly concentrated protein phase of the phase separation. Mounting crystals from this protein phase can be a problem, because of the unknown composition of the protein phase surrounding the crystal. Try cryofreezing the crystal directly from the drop.

### **Precipitate shows birefringence or has edges.**

(Score=6-9)

#### **Spherulites: transparent, birefringent clusters.**

(Score=6)

- Spherulites can look like droplets, or like transparent clusters. When using polarized light, spherulites show dark and light parts. This is a good starting point for optimization.
- When crystals grow too fast, the edges disappear. Lower the protein or precipitant concentrations (or both) or increase the drop volume.
- Try streak seeding to optimize the conditions. Add an additive or inhibitor when the optimized condition doesn't yield crystals.

#### **Small structures, where you might see edges.**

(Score=6)

- Microcrystals are small particles where edges and birefringence are visible if the magnification is high enough.

#### **Crystal grown in one dimension.**

(Score=7)

- Needles, too small for data collection.
- Optimize pH and precipitant concentration.
- Try a lower protein concentration.
- Shower of needles grown from one nucleation point; try seeding.
- If the crystal is damaged during macroseeding, a lot of small crystals are formed.

#### **Crystal grown in two dimensions.**

(Score=8)

- Try optimizing; use additives or seeding.

#### **Crystals grown in three dimensions.**

(Score=9)

- Once the crystals are bigger than 0.05 mm, write down the size (e.g., 0.4 x 0.2 x 0.1 mm)
- Check for salt crystals in the well. If the drop also contains dark precipitate (scored) then check if the conditions being used could possibly form salt crystals. Avoid phosphate.
- Mount the crystal and check diffraction quality.
- If the crystals are not suitable for X-ray diffraction analysis, try adding additives or inhibitors. Also go back to the initial screen and check for conditions that may lead to another crystal form.