“Crystal growth is achieved by the slow dehydration of the water of solvation from the sample in a controlled manner that prevents precipitation and takes the sample out of solution and into a crystalline state.”
1840 F.L. Hunfield (German)  
First documented protein crystallization Earthworm hemoglobin. Obtained plate-like crystals when he pressed the blood of an earthworm between two slides of glass and allowed the blood to dry very slowly.

1851 Otto Funke (German) published a series of papers describing the growing of hemoglobin crystals, by diluting blood with water, alcohol or ether and allowing slow evaporation.

First crystals screen experiments
The exact forces governing crystal nucleation are difficult to understand and pinpoint because of the many factors that affect the solubility of the sample in the solvent in which it is dissolved.

These factors include the buffer used in the experiment; the pH of the solution; the concentration of the sample and counter ions; the type and concentration of the precipitant used to bring the sample out of solution; the temperature, the surface area of droplet, and the gravity of the system used.

In general, when no homologous proteins have been crystallized, all these factors have to be explored before suitable conditions are identified. If a homologous protein has been crystallized, the conditions for the new protein should explore those published and be expanded from this starting point.
Lecture 3

Graph showing the relationship between protein concentration and logarithm of solubility. The graph illustrates the concept of supersaturation and undersaturation. The x-axis represents the crystallizing agent concentration, which is the square root of ionic strength. The y-axis represents the protein concentration. The graph includes a saturation curve.
Concept of crystal growth

Solution

Nucleation

Solid State

Crystal or Precipitation

Concept of crystal growth

Solution

Nucleation

Solid State
Concept of crystallization methods

Crystals are grown by slow, controlled precipitation from an aqueous solution under conditions that **DO NOT** denature the protein.

Precipitant solution (Also referred to as mother solution or mother liquor or reservoir solution)
Variables that effect crystallization

**Precipitants:** Ionic compounds (salts)
Organic compounds (Polyethylene Glycol)

**Sample:** Concentration
Buffer

**Environment:** Temperature
pH
Magnetic fields
Gravity

**Methods:** Surface area
volume
Fundamental tips (essential for successful crystal growth)

Keep EXCELLENT NOTES (from expression onwards)

Don’t screen too many variables at once

It is important to minimize dust or other extraneous particulate matter in the crystal-growing vessel.

Let the crystals grow with a minimum of disturbance. Should be left undisturbed for at least a week.

Do not pick up the vessel everyday to check. The sample will precipitate or produce micro crystals.
Methods in Crystallization:

1- Hanging drop
2- Sitting drop
3- Sandwich drop
4- Free interface diffusion
5- Batch (robots)
6- Microbatch - under oil
7- Microdialysis (buttons)

linbro plates

Hanging drop

Micro-bridges (Sitting drop)

Crystal screen kits

Dialysis buttons
Sitting and Hanging Drop

Most popular techniques. Easy to perform and requires a small amount of sample. Allows a wide range of conditions to be screened.

Uses linbro plates (referred to as trays)

Allows 24 or 96 individual crystallization conditions to be screened.

2 to 40 µl droplet of the sample is mixed with an equal amount of the precipitant solution. (Mixed 1:1)
Sitting Drop

Drop is placed on a bridge/post sitting inside a well, with the precipitant at the bottom of the well.
Hanging Drop

Drop suspended from a cover slip over the precipitant at the bottom of the well.

Both methods allow vapor equilibration of the drops with the precipitant (500-1000 µl).
The sealing of the each crystallization well is essential to prevent air evaporation of the drop.

The wells are sealed by creating an interface between the cover slip and the rim of each well on the linbro plate using vacuum grease, oil or sealing tape in the case of the sitting drops.

The initial precipitant concentration in the droplet is less than that in the reservoir, thus over time the reservoir solution will draw water from the droplet in a vapor phase such that equilibrium will exist between the droplet and the reservoir. During this equilibration process the sample is also concentrated, increasing its relative supersaturation, thus slowly bring the sample out of solution and into a crystalline state. drop methods are excellent for crystallization condition screening and optimization.
The major advantages of the sitting and hanging drop techniques are speed and simplicity.

The disadvantage of the sitting drop technique is that crystals can sometimes adhere to the sitting drop surface, making removal difficult.

The hanging drop method avoids the problems of surface crystal adherence, but droplet volume is limited compared to sitting drops.
Crystal screen kits

Can be commercially purchased.

The kits are designed to use linbro plates with Sitting or Hanging Drop methods.

Screens various conditions that have produce xtals before. Membrane, PEG, salt screens.
Sandwich Drops

The same protocol as the sitting and hanging drop method.

But here the drops of sample mixed with precipitant solution are sandwiched between two cover slips.

This method is rarely used, but does have the advantage of reducing the exposed surface area of the drop.

This reduces the rate at which the precipitant reservoir solution draws water from the droplet and slows down the equilibration process.
**Batch**

The method used by NASA.

The sample is mixed with the precipitant and appropriate additives to create a homogeneous crystallization medium requiring no equilibration with a reservoir. Therefore supersaturation is achieved directly rather than by diffusion.

Advantages to the technique are speed and simplicity.

A batch experiment can be readily performed in a capillary using 10 µl, test tube 300 µl, or a plate <500µl solutions.
Microbatch (ROBOTS)

The same as the batch method, but uses extremely small drop volumes (<2 µl).

Crystallization set up droplet is covered by oil.

This prevents the evaporation of the very small drop.
Free Interface Diffusion

The sample is placed in direct contact with the precipitant.

Creating a boundary interface between sample and precipitant.

The sample and precipitant diffuse into one another and crystallization occurs either at the interface or on the side of droplet.

The technique allows the screening of crystallization conditions in relation to the gradient of sample/precipitant concentration.

The technique can readily be performed in capillaries and thus only small amounts of sample are used.
Dialysis

The earliest methods used by crystallographers. (other than evaporation)

Sample placed in a dialysis cell.

As simple as a dialysis tubing.

Or a button
(defined volume typically between 10 –1000 µl )

Sealed with a dialysis membrane at one or both sides.
The dialysis cell is placed in suitable container (typically a glass beaker ~250ml of volume) holding the precipitant or crystallization media.

The cell is placed in the container the beaker is sealed with parafilm to prevent evaporation.

The dialysis cell permits water and some precipitants to exchange while retaining the sample in the cell.

The technique allows for salting in and salting out of the sample, as well as using a pH change to induce crystallization.

For example, a sample requiring a high ionic strength for solubility can be dialyzed against a solution of low ionic strength to salt it out.
When is a crystal good enough for X-ray crystallography

As a rule of thumb, the optimum size for a crystal is that at least two of the three crystal lengths measure 0.1 - 0.4 mm.

However, crystals as small as .05mm in all dimensions have been successfully used to determine structures when data is collected at microfocussed, highly intense synchrotron beamlines.

Only truly answered when you see the diffraction pattern.

Assignment: “Why is crystal growth linked to NASA?”