Crystallization Methods and Protein Crystal Properties

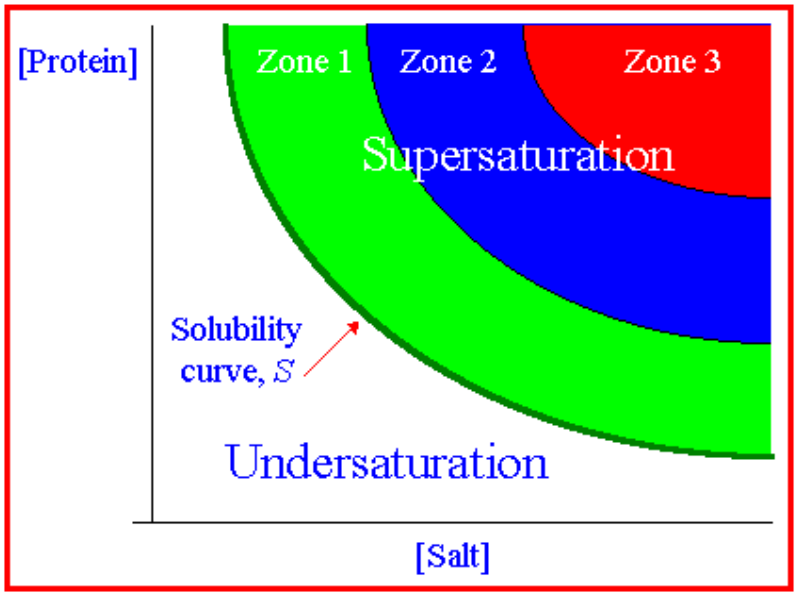
(adapted from http://www.bioc.rice.edu/~bios482/Xtal_PPT/)

Four major steps in crystallization

- Obtain large amounts of pure protein samples
- Choose a protein buffer in which the protein is both soluble and stable
- Bring protein solution to supersaturation where spontaneous nucleation can take place
- Crystal growth now begins

Supersaturation

Supersaturation can be achieved by adding more of a substance (to a solution) than can normally be dissolved. This is a thermodynamically unstable state, achieved most often in protein crystallography by vapor diffusion or other slow evaporation techniques.



Zone 1 - Metastable zone.
The solution may not nucleate for a long time but this zone will sustain growth.
It is frequently necessary to add a seed crystal.

Zone 2 - Nucleation zone.
Protein crystals nucleate and grow.

Zone 3 - Precipitation zone.
Proteins do not nucleate but precipitate out of solution.

Diagram from the website for The University of Reading, Course FS460
Investigating Protein Structure and Function

Nucleation

A phenomenon whereby a “nucleus”, such as a dust particle, a tiny seed crystal, or commonly in protein crystallography, a small protein aggregate, starts a crystallization process.

Nucleation poses a large energy barrier, which is easier to overcome at a higher level of supersaturation.

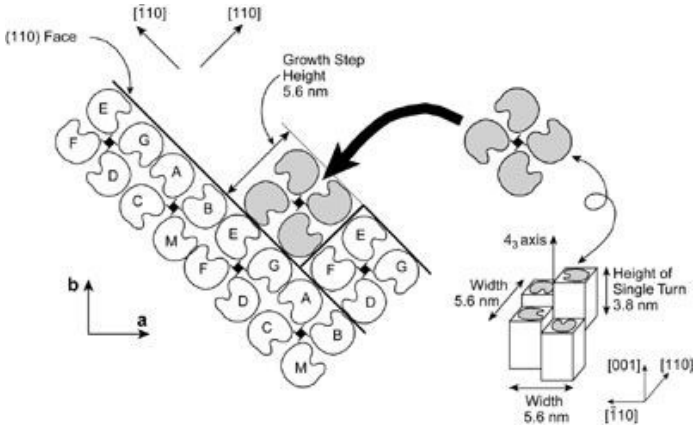
Common difficulties:

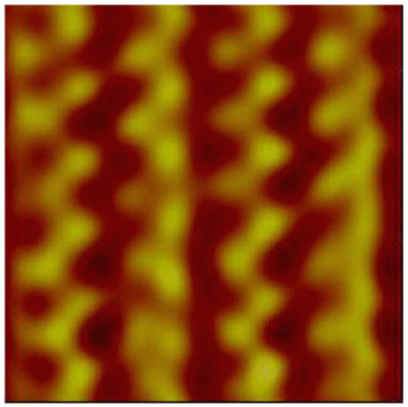
1. If supersaturation is too high, too many nuclei form, hence an overabundance of tiny crystals.
2. In supersaturated solutions that don’t experience spontaneous nucleation, crystal growth often only occurs in the presence of added nuclei or “seeds”.

Crystal Growth

Adding single molecules to the surfaces of the nucleating lattice.

Illustrated here through the work of Li and Nadarajah of The Macromolecular Crystallization Laboratory at the University of Toledo.





AFM image of individual lysozyme molecules on the (110) face of a tetragonal crystal. (Li and Nadarajah)

H. Li, M.A. Perozzo, J.H. Konnert, A, Nadarajah & M.L. Pusey, Acta Crystallographica, D55, 1023-1035 (1999).

***The** growth steps and growth units of Lysozyme. The growth steps are at least bimolecular in height. The minimum growth unit for this step must be a tetramer corresponding to a single turn of the 4_3 helix as shown here.(Nadarajah)*

Major factors that affect crystallization

1) Purity of proteins

2) Protein concentration

3) Starting conditions (make-up of the protein solution)

4) Precipitating agent (precipitant)

5) Temperature

6) pH

7) Additives: Detergents, reducing agents, substrates, co-factors, etc.

1) Purity of proteins

Sources of heterogeneity (other than unrelated proteins and nucleic acids as contaminants):

- Partial proteolysis products
- Oxidation of cysteines
- Deamidation of Asn and Gln to Asp and Glu
- Post-translational modifications
- Oligomerization
- Isoforms
- Misfolded population
- Structural flexibility

3) Starting conditions (make-up of the protein solution)

The main point is to KNOW what your starting conditions are for purposes of reproducibility.

4) Precipitating agent (precipitant)

Salts

- **Ammonium sulfate**
- **Sodium chloride**
- **Potassium phosphate**

Organic reagents

- **MPD**
- **Isopropanol**

Polyethylene glycol

- **PEG 4000**
- **PEG 6000**
- **PEG 8000**

5) Temperature

Temperature affects protein stability and also the dynamics of how protein solution reaching supersaturated states.

Ideally:

- **An individual crystal screen should be kept at constant temperature**
- **Each set of conditions should be screened at several temperatures**
- **The easiest are 4° C and room temperature, also try 12 or 15° C**

6) pH

Surface charges affect “crystal packing”.
(Crystal packing refers to the spatial arrangement of molecules within the crystal, particularly in reference to their relationships to one another.)
Hydrophobic interactions are less important than electrostatic interactions in crystal packing.

7) Additives:

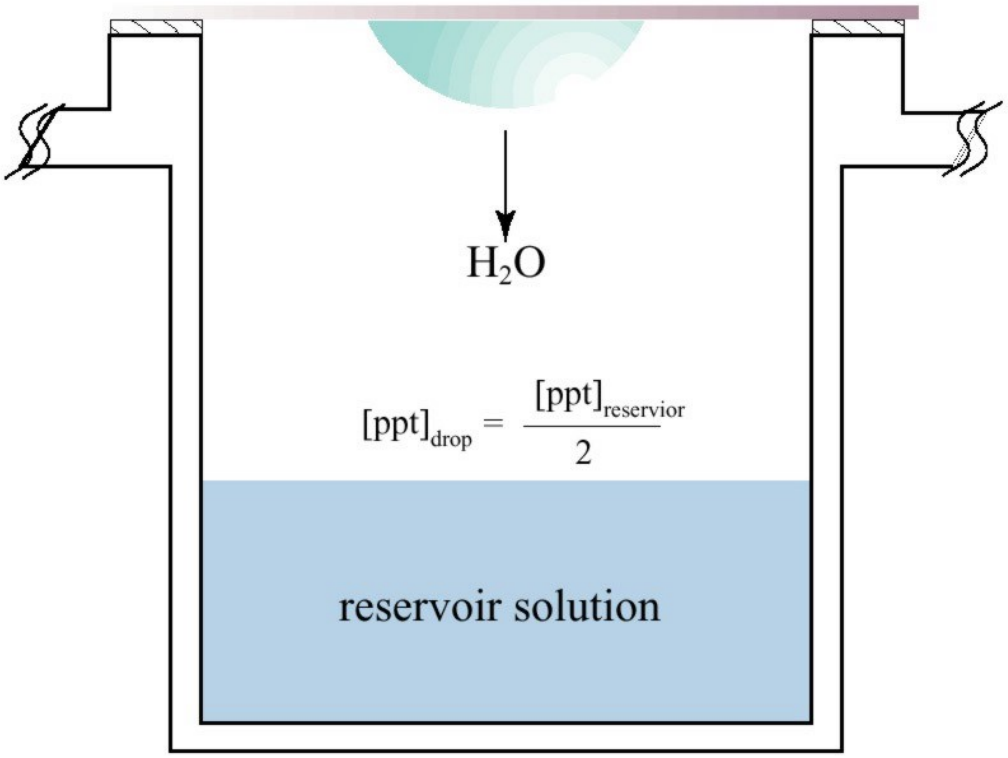
Sometimes you can increase the stability of your protein, and/or the homogeneity of its conformation by having relevant additives present in the crystal screen:

- **Detergents**
- **Reducing agents**
- **Substrates**
- **Co-factors**
- **etc.**

Common Methods for Crystallization:

Vapor Diffusion
Slow Evaporation
Dialysis

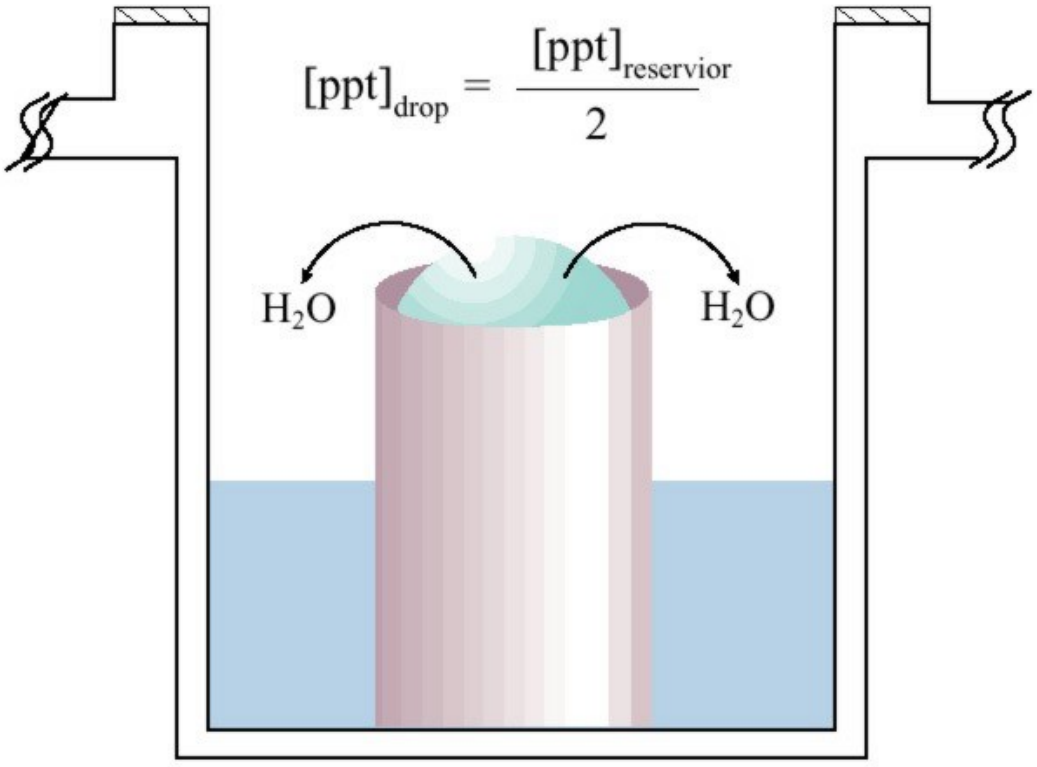
Hanging Drop Vapor Diffusion



Most popular method among protein crystallographers.

1. Crystal screen buffer is the well solution (0.5 - 1 mL)
2. Drop (on siliconized glass cover slip) is 1/2 protein solution, 1/2 crystal screen buffer (6-10 μL). So, the concentration of precipitant in the drop is 1/2 the concentration in the well.
3. Cover slip is inverted over the top of the well and sealed with vacuum grease (airtight).
4. The precipitant concentration in the drop will equilibrate with the precipitant concentration in the well via vapor diffusion.

Sitting Drop Vapor Diffusion



Same basic principles as in hanging drop method, except the drop containing your sample sits on a bridge within the well. This allows for a larger sample size (20 - 40 μL), however protein is frequently precious to the crystallographer, so there isn't that much demand for a larger sample size.

Interpreting the Results of the Crystallization Experiment

Clear Drop

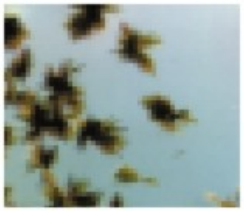
Skin/Precipitate

Precipitate

Precipitate/Phase

Quasi Crystals

Microcrystals

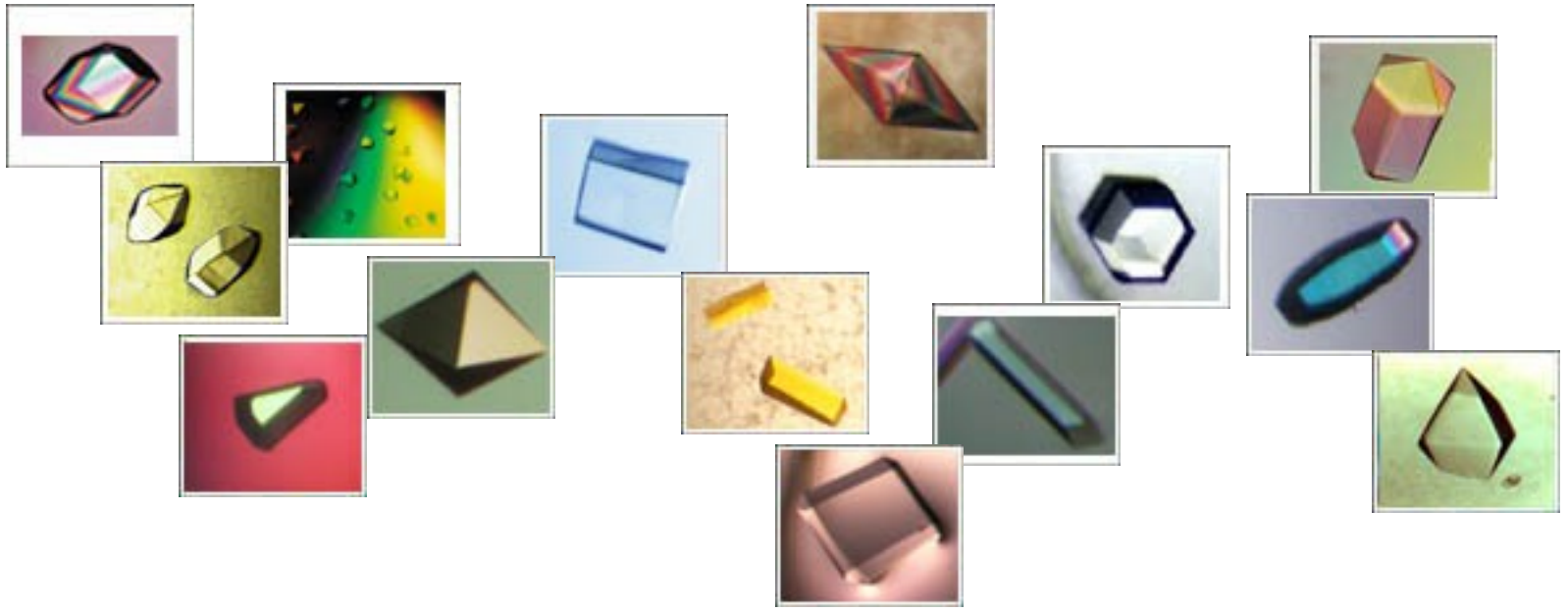


Needle Cluster

Plates

Rod Cluster

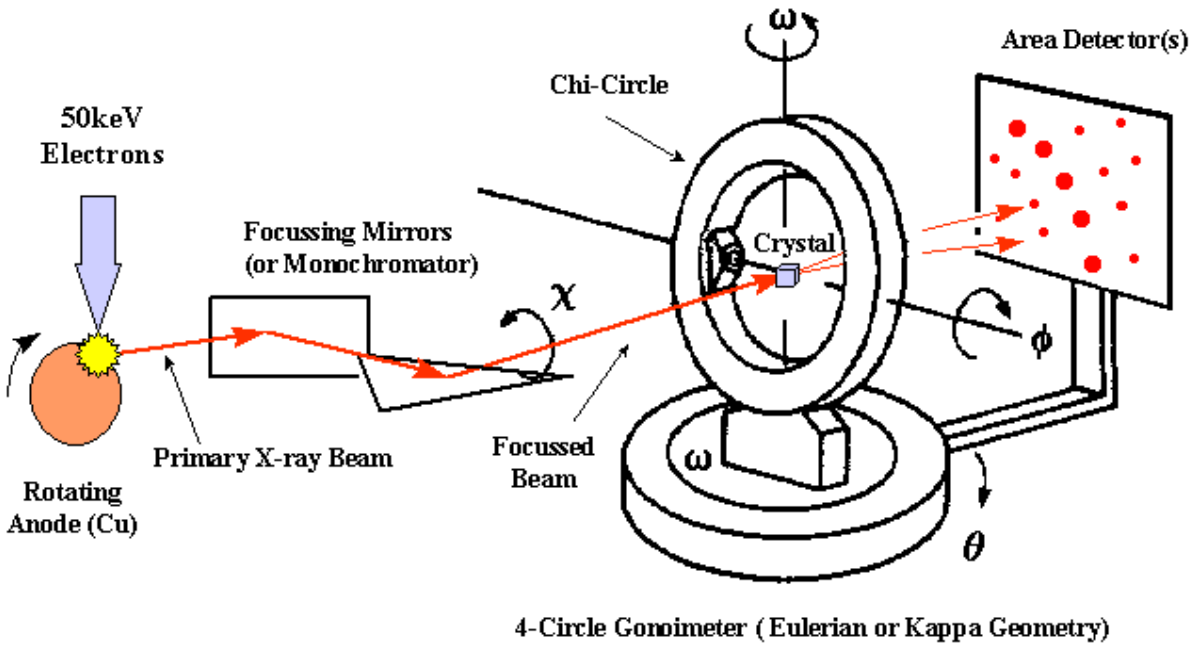
Single Crystal



View the Hampton Crystal Gallery to see in which labs each of these crystals originated and which biological molecule(s) they represent.
http://www.hamptonresearch.com/stuff/gallery.html

The oscillation equipment

Rotates the crystal about an axis (ϕ) perpendicular to the x-ray beam (and normal to the goniometer). The diffraction pattern from a crystal is a 3-D pattern, and the crystal must be rotated in order to observe all the diffraction spots.



4-Circle Goniometer (Eulerian or Kappa Geometry)

Crystal Mounting

Capillary tubes
(Glass or Quartz)

Cryo-loops
(thin nylon)

Properties of protein crystals

- Soft, easy to crush
- Contain large solvent channels
  - Relatively large organic and inorganic molecules can diffuse inside
- Anisotropic physical properties
  - Birefrigence due to anisotropic refraction indices
- Ability to diffract X-ray due to regular spaced lattices