Seeding

• Why seed?

• What to use as a seed?

• How to handle it?
Why Seed?

• A seed provides a template on which further molecules can assemble. Energetically, it is more favorable to add to an already existing crystal plane than it is to create a new nucleus.

• Seeding allows to control the nucleation and some characteristics of the resulting crystal because it will inherit many of the characteristics of the seed from which it is originated.

• At given proper environment, time, and patience, the seed will enlarge into a crystal.
Seeding and the phase diagram


Seeding and the phase diagram

**Metastable Zone**
The critical supersaturation is not yet reached. Spontaneous nucleation does not occur unless it is induced by vibrations or introduction of a particle that will promote heterogeneous nucleation.

**Nucleation Zone**
Here crystals appear spontaneously. Important to control the nucleation. When increasing the supersaturation, the number of crystals increases and they appear earlier.

**Precipitation Zone**
Precipitation occurs at very high supersaturation. Insoluble protein rapidly separates from the solution into an amorphous state.
Seeding and the phase diagram
Heterogeneous nucleation

Disadvantages

Foreign solid substances promote a surface where protein molecules can adhesively bind at a lower degree of supersaturation than the homogeneous nucleation occur.

In protein crystallization, this is more often a problem than an advantage. Also siliconized glass surfaces can provide embedded heterogeneous seeds and the crystal can be found attached to them.

The interaction with the glass can be stronger than the internal lattice forces. So that the crystals break when an attempt is made to dislodge them.

Advantages

Adhesion to a regular surface may provide a suitable template to start an ordered protein layer, leading to the nucleation of a crystal.

Heterogeneous nuclei can also be used as seeds.
Surfaces promote adhesion because it is energetically easier to add to a surface than to create a new nucleus.
Microseeding

Only invisible small splitters were used as seeds.

Application to improve crystal quality

Control of crystalization by avoiding the nucleation zone.

Therefore the crystals were crushed and suspended in a slurry of mother liquor. The actual seeds are invisible with a microscope optic.
If spherulites are observed, then the conditions are located at the upper border of the nucleation zone. Most often happened at high concentrations of crystallizing agent.
How to microseed

1. Preparation for hanging drop
2. Centrifugation 1 min 10,000 g
3. Number of dilution steps are variable

- small seed crystal of insufficient quality for X-ray data collection
- microseed crystals in reservoir buffer
- glass capillary to smash the crystal
- transfer of seed crystals to find the optimal concentration
- siliconized coverslide

Preparation for hanging drop

Centrifugation 1 min 10,000 g
Optimization by reducing the number of seeds in microseeding

Examples
Optimization by reducing the number of seeds in microseeding
The fundamental difference between micro- and macroseeding is that, in macroseeding, you can see the seed.

To be successful with macroseeding, it is obviously necessary to choose a high quality crystal, this is another main difference to other types of seeding.

It is most efficient to build a macroseeding procedure in several discrete steps, each of which utilizes the results of the previous ones.
How to Macroseed

A. Pick up a crystal from drop

B. Wash crystal repeatedly in stabilizing solutions.

C. Transfer crystal to pre-equilibrated drop
How to prepare streak seeding

A cat whisker is used to gently touch a crystal and dislodge seeds.

The seeds remain attached to the whisker and are transferred to a new protein-precipitant drop.
Result of streak seeding

Self-nucleated crystals appear away from the streakline.
How to combine streak seeding and macroseeding

Cat whisker as an useful tool for streak seeding

Gently touch a crystal with the whisker to pick up seeds. Streak the whisker across a Pre-equilibrated drop.

Place a tubin on a syringe tip This will hold a standard X-ray Glass capillary

Transfer of seeding crystals use Four intervening wash steps Should be used.
Macroseeding

Examples
How to Macroseed

**Negative example**, to be successful with macroseeding, it is obviously that choosing a **good crystal is a critical factor** in the final crystal quality.
Combination of Macroseed and Microseed

Crystal obtained from macroseeding, crushed and then used for microseeding.