Crystallization

- Standard crystallization
- Protein crystallization
Crystal Growth 1

Whatever their external appearance, crystals are solids containing atoms arranged in a pattern that repeat periodically in 3 dimensions. Arguably the most difficult aspect of crystallography is obtaining crystals!

- Crystal growth methods
  A.J. Blake: http://www.nott.ac.uk/~pczajb2/growcrys.htm#Introduction
  P. D. Boyle: http://www.xray.ncsu.edu/GrowXtal.html
  http://www.cryst.chem.uu.nl/growing.html
Crystallization is the process through which the atoms, molecules or ions arrange themselves in a repeating pattern. While sometimes it may seem that crystallization is more of an art than a science, there are several methods that generally produce crystals. For small molecules, these methods are typically based on reducing the solubility of the sample.
The solubility of most compounds decreases as the temperature is lowered thus the cooling of a saturated solution will often produce crystals. Since rapid cooling may cause the precipitation of amorphous solid or microscopic crystals, it is often wise to surround the flask with an insulating medium to slow the rate of cooling.
Crystal Growth. Evaporation

The slow concentration of dissolved samples is one of the most general ways of obtaining crystals – when the concentration exceeds the solubility, nucleation and crystallization/precipitation may follow. This can be done a variety of different ways such as evaporation of the solvent (in open or closed systems). For insoluble samples, Soxhlet extraction is often a particularly effective method of concentration.
Crystal Growth. Diffusion.

The solubility of a compound in one solvent can be reduced through the slow introduction of another solvent in which the solute is not soluble (an anti-solvent). This can either be done by direct contact between the saturated solution and the anti-solvent (“layering”) or by allowing the vapors of the anti-solvent to slowly diffuse into a saturated solution.
There are a large number of other methods for obtaining crystals of sufficient size and quality, including: sublimation, convection, the controlled cooling of melted solid, heating and cooling of micro-crystalline material, co-crystallization and many more.
I. Protein crystals
II. Protein crystals


Sperm whale myoglobin
Protein crystals

- Regular arrays of protein molecules
- Few crystal contacts
- Protein crystals contain active protein
- Enzyme turnover
- Ligand binding

Example of crystal packing
Protein crystals contain lots of solvent typically 30 to 70% solvent by volume "Wet crystals"
Packing of protein molecules into crystal lattice
Protein crystallization

‘Hanging drop’:

Example:

Protein: 10mg/ml
in 10 mM Tris buffer, pH7.5

Reservoir solution:
2M ammonium sulphate
in 100mM citrate buffer, pH5.5
Hanging drop
Zavěšená, visící kapka

Heavy atom doping (phase problem)
Phase diagram of protein crystallization

- Protein 2µl + reservoir 2µl
- Coverslip & grease
- H₂O
- Reservoir ~1 ml

Graph:
- Crystallizing agent concentration
- Protein concentration
- Precipitation
- Nucleation
- Clear (metastable)
- Supersaturation
- Undersaturation
Old:
• sealed capillary
-> crystal stays at 100% humidity

Modern:
• “flash cooling” to T=100°C in nitrogen stream

Problem: Radiation damage
Crystal Selection and Mounting

Size
- between around 0.1 – 0.5 mm

Shape
- must be a single crystal
- the closer to spherical, the better

Mosaicity

The goniometer head allows the crystal to remain in the X-ray beam in every orientation.

Some ways to mount crystals: a) on a glass fibre; b) on a “two-stage” fibre; c) on a fibre topped with several lengths of glass wool; d) within a capillary tube; e) in a solvent loop.