X-Ray Crystallography Laboratory

What is a crystal structure?

The definition of a crystal Structure.

The determination of the connectivity of the atoms in a compound and the way the molecule (or molecules) pack to form a solid crystalline material.

What information do we get?

A crystal structure provides positive identification of a single crystal taken from a pure batch of material. This provides absolute proof (*provided it was done properly*) that the compound or complex is the stated material. It provides the exact connectivity of the atoms and the bond distances and angles between these atoms in the solid state which result in the complete identification of the compound. It also provides Inter and Intra molecular interactions which may provide insight into the chemistry and properties of the compound.

Why have crystal structures become so popular?

Rarely incorrect and now faster to achieve results! With the advance in technology for x-ray crystal structure determination and the increased speed of computers, single crystal studies are rapidly becoming more routine. The ease of new programs make the routine structures quick and easy for even non-specialized scientist to be able to perform these analysis. The positive identification of the compound leaves no interpretation of the data leading to incorrect assignments of the structure. Answers basic questions regarding bonding within the molecule which can explain the chemistry and properties.

What do we need to bring to the Laboratory?

A single crystal is required in the determination of an x-ray Structure. A single crystal consists of atoms which possess long-range three dimensional order. Typically appear as regular polyhedral shapes with well defined boundaries. Examples include: Table salt, sugar, gems, quartz and metals.

We can not perform analysis on non-crystalline materials. These amorphous material contain only short range order, or random ordered atoms. Example: Glass.

Twined crystals are usually thought of as single crystals that are grown such that they contain a

boundary between them. Twinned crystals are for the experienced crystallographer and should be avoided if possible.

Crystal size

Ideal size of a crystal is one which occupies the entire x-ray beam, here at Harvard the beam is 0.5 mm generally. This means that the ideal crystal would be a sphere 0.45 mm in diameter. Although this is the ideal size, one can perform x-ray determination on smaller or larger (by cutting) crystals. The capabilities of this depend on the x-ray source, the arrangement of the atoms in the lattice and what atoms are there as well as the diffraction power of the crystals. Unfortunately the diffraction power of crystals is still relatively unknown until you try the crystals in the diffractometer.

Unfortunately, the shapes of crystals depend on both the internal symmetry of the material and on the relative growth rate of each of the faces. In general, the faces of the crystal that grow most rapidly are those to which the crystallizing particles are bound most securely. These rapidly growing faces are usually the smaller, less well developed faces. Thus, the larger faces are

How much material is needed?

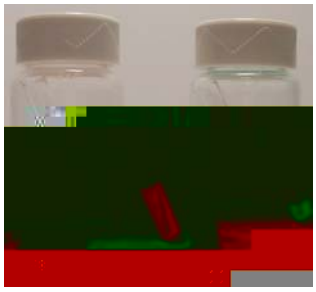
Simple rule is that you only need one single crystal. The concentration of the solutions tends to be near what you would expect in order to run an NMR experiment. The most important issue is that the compound is insoluble in the final resultant mixture of solvents that is attained in the vessel of choice.

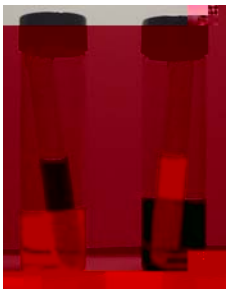
If the crystal for x-ray diffraction is to be 0.3 x 0.3 x0.3 mm, volume = 0.027 mm^3

Typical unit cell is 12 x 12 x12 Å; volume = 1728 $Å^3$

Å = 10^{-10} meters = 10

Unfortunately the choice of vial does not follow the above general guidelines. So if you have trouble with one system, try the other, exceptions have been noted here at Harvard.





Solvent Choice

Consider your solvents carefully. Like dissolves like.

Remember if the compound is polar, then polar solvent with the compound is layered with non-polar solvents.

Avoid solvents in which your compound forms supersaturated solutions since these solutions tend to give crystals which are too small in size (micro crystals).

For compounds soluble in non-polar solvents, evaporation may be the best or layering with polar solvent, this is harder to accomplish.

Hydrogen bonding is very important in the crystallization process. Hydrogen bonding provides energy to the lattice and generally better packing, but not always. Consider whether a hydrogen bonding solvent might help or hinder the crystallization. Amides generally do better with hydrogen bonding solvents.

It is amazing that some solvents tend to direct crystal growth better than other solvents. Benzene is such a solvent. We have had lots of luck using some benzene in the solvent mixture to generate x-ray quality crystals. The aromatic rings fill holes that may form in the lattices, but most of the time, we do not see the benzene co-crystallized with the compound. For organic complexes ethyl acetate works well.

Avoid highly volatile solvents, CH_2Cl_2 and diethyl ether. Unfortunately these often work very well. They also tend to lead to creation of crystals by slow evaporation.

Avoid long alkyl chains in the solvent, these cause disorder in the lattice if solvent is trapped in the lattice, since there are many conformations allowed and therefore all atoms are not in the same place throughout the lattice.

Table 1. shows some typical solvents that are used and considered when growing crystals in the organic world.

Crystal Growth

Producing good quality crystals of a suitable size is the first and most important step in determining any crystal structure. Crystallization is the process of arranging atoms or molecules that are in a fluid or solution state into an *ordered* solid state. This process occurs in two steps, nucleation and growth. Nucleation may occur at a seed crystal, but in the absence of seed crystals usually occurs at some particle of dust or at some imperfection in the surrounding vessel. Crystals grow by the *ordered* deposition of material from the fluid or solution state to a surface of the crystal. More information on crystal growth: *Crystal Growth of Organic Materials*, edited by Myerson, Green, and Meenan, ACS Proceedings Series, 1996.

The main focus for growing crystals is to create an environment that changes slowly over time. This change should produce an environment in which the compound becomes supersaturated and eventually grows a solid, crystal material. This change in environment is most generally accomplished (with small molecule) by addition of a second solvent in which the compound of interest does not dissolve.

Changing the nucleation process is the largest thing one can do, one avoid dust or glass fragments(from pipette) to be the nucleation site. If using new glass and getting lots of small crystals, scratch the glass to create only a few sites so the crystal might grow larger.

If a sample only yields small crystals, the method should generally be altered so as to slow down the growth step. Slowing the crystal growth sometimes requires changing the method used to grow the crystals. Or lowering the temperature at which the crystals are grown.

Physical disturbance of the crystal growing vessel can result in smaller crystals being formed. Choose a location to grow the crystals where there is no vibrations from elevators, doors, rotovaps, vacuum pumps etc… You should set the crystals where you can view them without having to move them, or if you do, wait one week before checking on the crystals.

Patience! Some methods work in a few hours, and other methods require weeks or even months for success.

CRYSTALLIZATION METHODS

The techniques chosen will largely depend on the chemical properties of the compound of interest: Is the compound air sensitive, moisture sensitive? Is it hygroscopic? Can it form hydrogen bonds, does it react with certain solvents etc...

VAPOR DIFFUSION

This is by far the best crystallization method to use. Very good for milligram amounts. Requires volatile solvents, but done properly one generates a less desirable solvent system which then allows for slow crystal growth.

Vapor diffusion is carried out by dissolving a small amount of the sample in a small vial, then

placing this inner vial inside a larger vial that contains a small volume of a solvent system in which the sample is insoluble. The outer vial is then sealed. **DO NOT DISTURB THE VESSEL.** Vapor from the solvent of the outer vial then diffuses into the solution in the inner vial, causing the compound to grow crystals. The vertical surfaces of the inner vial should not touch the outer vial to keep the outer solution from rising by capillary action and filling the inner vial.

Sometimes this is combined with slow cooling, or placed in a fridge to slow the diffusion of the solvents, giving more time for the crystals to grow.

SOLVENT LAYERING

This is a simple concept. You layer one solvent over top of a second solvent. The two solvents should be miscible in one another. One solvent your compound is insoluble, the other it is soluble. Dissolve some of your compound in the soluble solvent and then layer the two *very carefully*. Must have solvents that can be layered, enough of a difference in properties that an interface develops between the two solvents as you set it up. **DO NOT DISTURB THE VESSEL**. Can use a third solvent to create a buffer to slow the diffusion rate, which controls the rate of crystallization. Use benzene at the interface! Rate of crystal growth depend on concentration level and solubility of the compound in the resulting mixed solvent system.

Sometimes this is combined with slow cooling, or placed in a fridge to slow the mixing of the solvents, giving more time for the crystals to grow.

SLOW EVAPORATION

Evaporation is by far one of the easiest methods for crystallizing organic and organometallic small molecule compounds. The choice of solvent is important because it can greatly influence the mechanism of crystal growth, when the crystal begins to form and because the solvent may be incorporated into the crystalline lattice. The rate of crystal growth can be slowed either by reducing the rate of evaporation of the solvent, less open area or by cooling the solution. Keep the solution clean by covering it, simple thing to use is a Kimwipe, but some slow the process by putting a rubber septum in then inserting a needle.

If this method provides an oil, this could be not because the compound is impure, but the compound is too soluble in the solvent chosen for evaporation.

This method does not generally provide the best crystal, since the crystallization proceeds only when there is only a small amount of solvent left, causing the crystals to grow upon each other. Also the crystals tend to adhere to the glass walls, which can make it more difficult to retrieve the crystals without damaging the crystals.

crystals may sometimes be grown larger by zonally refluxing a supersaturated solution. Larger crystals may be grown either by decreasing the thermal gradient or by cyclic heating and cooling of the sample.

Thermal gradient heating sometimes works indirectly, if you set you crystallization apparatus by the cooling vent, one side of the apparatus is cooler than the other and this changes the crystallization properties and can cause crystal formation.

COUNTERIONS OR IONIZATION

Probably the best thing one can do to promote crystallization of an anion or cation is to change the counter Ion. Counter ions which are generally the same size usually pack well.

The counter ions most likely to cause difficulties are Et_4N^+, Bu_4N^+, BF_4^-, and PF_6^-. Some alternative counter ions that are usually ordered are triflate, BPh_4^-, Me_4N^+, $(Ph_4P)_2N^+$, and Ph_4As^+.

If the compound is neutral and does not crystallize or is liquid, consider creating an ion. Deprotonation or protonation can be performed to generate a salt which then may crystallize. Good to confirm the identity of the material.

CO-CRYSTALS AND CLATHRATE

Some have had success with growing compounds in the presence of other compounds, or co-crystallization. This incorporation of another molecule typically occurs with the solvent of crystallization.

The use of triphenylphosphine oxide (TPPO) has been seen to be a useful co-crystallant for some years in inorganic chemistry and has been reported to be useful for organic molecules which are proton donors. (see *J. Amer. Chem. Soc.* **1988**, *110*, 639- 640) .

A final group of co-crystals can be thought of as being formed by incorporating the compound of interest or guest molecule into the small vacant regions in the lattice around large, rigid host molecules. This lattice of host/guest molecules is called a clathrate. Structures of porphyrin-based clathrates are very common.

REACTANT DIFFUSION

This is performed when the compound is very insoluble and difficult to work with after it is formed. Perform the final reaction on a small scale compared to the surface area of the two reactants. Layer one reactant on the top of the other reactant and allow diffusion to control the reaction rate and crystal formation.

ODD METHODS

There are many odd methods that have been known to work. Some of these methods have

All of the crystals should appear to be the same, if there are a few very nice ones and most are poor (or shape differs) then one of three things exists.

1) If the compound is chiral, then the very nice crystals will probably be those of the trace amount of racemic compound present, or is an impurity.
2) Could be that there is more than one compound in the bulk material. This could be caused by decomposition during crystal growth or synthesis
3) Could be that the compound has two or more different packing arrangements that are similar in energy for the solvent system/crystallization used. Polymorphs

Do they look crystalline and single under cross polarized light? As you rotate the polarize, the crystals should turn light to dark (all the crystal) at some point. One can often see cracks, dislocations, and even twinned crystals clearly under the polarized light. If there appears to be a rainbow effect of colors, then they may not be single. Do not give up yet. If the crystals appear to be large and no pieces on them, or cracks, then typically we try the crystals. Once in a while these types of crystals work, although not very often.

Mount and evaluate the crystal on the diffractometer is the only way to know for sure whether the crystal will diffract or not. This requires about 20 - 30 minutes. Once you know what to look for in evaluation, experience, this can even take less time.

Crystal Mounting General

Crystal mountings must be rigid to hold the sample in the same orientation and must minimize the amount of extraneous material that is in the incident and diffracted beam paths. The sample support is usually made from an amorphous material such as glass that is held in a metal pin and clamped on a goniometer head. Solid glass fibers may be used; however, fibers pulled from glass tubing are actually small capillary tubes and are more rigid than solid glass fibers. These narrow

also holds the crystal in place by freezing the crystal in place on the fiber or loop. See mounting at Harvard.

Very reactive compounds must be mounted in a glove bag or glove box and sealed in capillary tubes. Crystals of these compounds are usually wedged in capillary tubes or are held in place by a small amount of grease. Capillary tubes containing unstable compounds must be sealed by melting the ends of the glass tube.

Capillaries introduce two kinds of problems. The curvature of the capillary distorts the image of the crystal when centering the sample on the diffractometer. Also, the glass itself significantly increases both the background scattering and the absorption of the incident beam of X rays. It is crucial that the capillaries be made out of thin glass similar to that found in commercially available capillaries. Thick glass capillaries absorb X rays so much that very little scattered radiation will leave the capillary.

Nylon Loops have begun to be used by small molecule crystallographers, although macromolecular crystallographers have used them for years, because of the ease of mount very small crystals in the loops and the low background they provide. With the advance in detectors, the smaller crystals are now more routinely studied. The loops provide stability in the low temperature stream. One must be careful that the loop size is not such that the crystal bends in the low temperature stream and causing the crystals to move within the x-ray beam. Experience has shown that loops made from 0.2 micron nylon and are 0.1 – 0.3 mm in diameter are suitable for many small crystals.

Limitations to Crystallography

- Requires single crystals. This by far is the greatest limitation to x-ray diffraction analysis. No crystal, no information.
- Crystal quality governs quality of results obtained.
- Only one crystal of the bulk material. Remember that we are looking at one small crystal in the entire bulk of the material.
- Chirality can not be generally determined with only C,N O atom present. Must know one chiral center or have a heavy atom present to tell, and only then if the data is good.

Table of typical solvents used for growing single crystals of organic compounds.

Water – alanine, organic acids
Ethanol (CH_3CH_2

Table of vapor pressures.

The lower number (solvent) will diffuse into the higher number (solvent).

Solvent	Vapor pressure
Water	21.0
Diethyl ether	34.6
Pentane	36.1
Dichloromethane	40.7
Acetone	56.5
Chloroform	61.3
Methanol	64.1
Hexane	68.7
Ethyl acetate	77.1
Ethanol	78.4
Benzene	80.1
Acetonitrile	81.8
Heptane	98.4
Toluene	110
Octane	125

Literature to crystal growing

"*Crystal Growing*", Peter G. Jones, *Chemistry in Britain, 17* (**1981**) 222-225.
See WWW site by Dr Paul D. Boyle, http://www.xray.ncsu.edu/GrowXtal.html

Crystals and Crystal Growing, Alan Holden and Phylis Singer, Anchor Books-Doubleday, New York, **1960**

The Growth of Single Crystals, R. A. Laudise, Solid State Physical Electronics Series, Nick Holonyak, Jr. *Editor*, Prentice-Hall, Inc., **1970**

Protein Crystallization Techniques, Strategies, and Tips , Terese M. Bergfors, International University Line, **1999-2000**, ISBN 0-9636817-5-3

Hampton Research Catalog, many good discussions regarding crystal growth and crystal growing.