

X-Ray Crystallography Laboratory Department of Chemistry Crystallographic Resources Inc

Getting Crystals Your Crystallographer Will Treasure

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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 makes the routine structures quick and easy for even non-specialized scientist to be able to perform this 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 that exist.

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 usually associated with directions in the crystal where there are only weak intermolecular interactions.

Where to start?

Concept of crystal growing

Properties of the compound

Solubility is the single largest and most used property needed to grow a single crystal. Generally one knows a fair amount of this from the synthesis and other aspects of working with the compound. Stability and reactivity need to be considered. One does not want to cause a reaction with the compound of interest in the solvent system that you are trying to grow the crystals.

Simple recrystallization is usually the first step in growing a good crystal. It is very important that the sample be pure and can be a solid! If you get oils, this could mean that the sample is not pure since contaminants often lower the melting points of solids and can cause them to be oils. So first thing to do is look at the recrystallization you performed to make the solid the first time. Did you get a solid and are these crystals well defined and good enough for x-ray study?

If these are not good enough crystals then the method you chose depends greatly on the physical and chemical properties of the sample. Solution methods require solubility of the solute in various solvent systems. Thermal, chemical and melting properties can also play a major role in choosing a method for crystal growing.

Patience is the major thing you need to remember.

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 x 0.3 mm, volume = 0.027 mm³

Typical unit cell is 12 x 12 x 12 Å; volume = 1728 Å³

Å = 10⁻¹⁰ meters = 10⁻⁸ cm = 100 pm (picometers)

Therefore in a typical crystals 1.6 x 10¹⁶ unit cells

1.3 x 10¹⁷ molecules for 8 molecules per cell.

MW= 206.2 then only 2.49 x 10⁻⁷ moles in the cell. 5.1 x 10⁻⁵ g, 0.051 mg

Unfortunately more than one crystal grows in the vessel so more material is needed.

Typically use a concentration that you would use in an NMR experiment.

What do I grow the crystals in and where?

Clean glassware is very important. The use of new glassware sometimes results in problems due to the lack of nucleation sites (see *crystal growth*), but this can also be helpful. Some crystallographers suggest that the new glassware contains a “variety of dusty contaminants” from the manufacturing process. This has not been observed here at Harvard. Most solution methods growing the crystals in vials that can fit inside one another are usually a good idea.

Consider the location of the set-up. You want the setup to be located out of the way, avoid vibrations and disturbances. Set it up so you can see if there are crystals growing without having to move the apparatus. Note if you grow them near a heater or cooler, in the sun or not. All these can change the way crystals are formed.

Keep the container covered so that no dust or dirt can enter and cause crystallization.

The use of vials that fit inside each other, this allows for the three most common experiments to be tried. The center vial, where the solute of interest is dissolved consists of either a glass tube or small flat bottom vial. Round tube have the advantage that they keep the material concentrated longer. These work well for complexes that tend to be round or ball shaped. These are usually tried first here at Harvard. Flat vials work better for more flat materials, and sometimes for compounds that form needles.

The outer vial is such that you can tighten the cap or have it loose (slow evaporation) but dirt and dust does not get in the system. The cap should be resistant to solvents.

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.



Recently Limited success using Teflon coated vials or silicon coated vials to help create larger crystals. J.R. Cox, L.A. Ferris, V.R. Thalladi, *Angew. Chem. Int. Ed.* **2007**, *46*, 4333-4336. *Caution, should be noted polymorphs have sometimes been observed.*

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 are 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 when only milligram quantities are available. 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 gives 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.

SLOW COOLING

This is the standard recrystallization method. This can work very well; follow the rule soluble hot, insoluble cold. Remember here we want to have the crystals form very slowly. We do not mind if material is still left in solution, we want the nice formed solid, not good yield. Slow reduction of temperature works the best.

To generate reduced temperature slowly, isolation of the material from environmental conditions can help. Generally though you most often put these crystals in the fridge or freezer. To reduce the time for the vial and solvent system to cool, one can place the crystallization vial into another container. Some people use a Styrofoam box, others a dewar with foam lid. We find that a jar with cotton in the bottom works well since you can still see if crystals are growing without disturbing the crystals. Sometimes this is hard with the cotton and some students use a plastic petri dish as isolation. This works better than the glass which conducts the cold quicker.

USE OF NMR TUBE

Often crystals have been received by allowing the solvent to evaporate slowly from the NMR tube. The cap fit tight enough to keep dirt out, but allows evaporation of the solvent and crystals form.

SUBLIMATION

This is probably the best method for getting x-ray quality crystals. Must be performed very slowly and with a small amount of material to get good results. Need to be careful not to have new crystals forming on already formed single crystals.

CHIRAL COMPOUNDS

Chiral compound tend to be more difficult to crystallize than racemic compounds. Nature prefers to have a center of inversion. Try to make derivatives which possess phenyl rings. If absolute configuration is needed try and have heavy atoms.

S-Alpha-Methylbenzlamine is good to use with carboxylic acids, can be generated from alcohol or aldehydes. Cheap and usually easily crystallized. Provides one known center, and then can determine other centers.

Improve heavy atom and crystallization

Have heavy atom present such as Bromide or Iodide (Si, Cl S also work). For alcohols and amines you can make a derivative using *p*-Bromobenzoate. This usually increases the ability to form good crystals as well as determination of chirality. Include aromatic components in derivative when possible.

THERMAL GRADIENT

Thermal gradient methods can produce very high quality crystals. Such methods include slow cooling of sealed, saturated solutions, refluxing of saturated solutions, and gradient (zonal) heating. Gradient heating is used primarily for crystallizing solid solutions or mixtures. Small 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 your 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^+ , $(\text{Ph}_4\text{P})_2\text{N}^+$, 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 proven to be the only way to get single crystals of the material.

Melting the compound and letting it recrystallize! This can be tried in a melting point tube.

Seeding a solution with similar crystallized material. Sometimes you have a similar compound that gives good crystals and you can use one of these crystals as a seed.

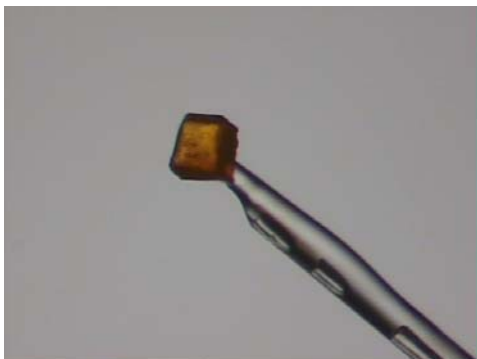
Protein crystallographers use different techniques to grow their crystals. Some people have used these techniques to grow single crystals of small molecules. Current limits on this technique are the molecule should have a high solubility in water or alcohols or mixture. Advances are being made by companies to help crystal growing of small molecules using these techniques, even with small amounts of organic solvents in the solution. See Hampton Research, <http://www.hamptonresearch.com/>

See for example: *Principles of Protein X-ray Crystallography*, by Jan Drenth, 2nd ed., Springer-Verlag, New York, 1994. ISBN 0-387-98587-5.

Key Factors to Good Crystals

- ☞ Solvent- Choosing the right solvent or solvent system is very important.
- ☞ Nucleation- generating only enough nucleation sites that you get a few large crystals and not lots of small ones.
- ☞ Mechanics-the physical method that takes place to get the crystal, diffusion, evaporation, gas-solid change. The location of the apparatus that is growing the crystal.
- ☞ Time-the longer it takes to grow the crystals generally the better. Unfortunately this does not always apply.

☞ Patience, Patience



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 ($\text{CH}_3\text{CH}_2\text{OH}$) – Soluble hot, insoluble cold
Methanol (CH_3OH) – Soluble hot, insoluble cold
Iso-propanol

Dichloromethane (CH_2Cl_2)
1,2 - Dichloroethane ($\text{ClCH}_2\text{CH}_2\text{Cl}$)
Ethyl Acetate ($\text{CH}_3\text{CO}_2\text{C}_2\text{H}_5$)
Acetone, ($\text{CH}_3(\text{CO})\text{CH}_3$)
Toluene ($\text{C}_6\text{H}_5\text{CH}_3$)
Benzene (C_6H_6)
Tetrahydrofuran, THF ($\text{C}_4\text{H}_8\text{O}$)
Acetonitrile (CH_3CN)

Hexane(s)
Heptane
Pentane
Diethyl Ether

Rare solvents to try

Methoxybenzene
Pyridine
Dilute HCl. Or phosphate buffered PH, acetic acid, trifluoroacetic acid, formic acid
DMF Often to soluble, no material crystallizes out; used when compound insoluble in all
Other choices (inorganic clusters)

DMSO Often to soluble, no material crystallizes out; used when compound insoluble in all other
choices (inorganic Clusters)

To help get organics and peptides to dissolve in water, use of solubilizing agents, alcohols,
acetonitrile, Dichloromethane, DMSO can be used.

Sample holding, vials: ½ Dram VWR # SC66011-020

Tube, Culture, Rimless Pk 72 6 x 50 mm VWR # 6820-068

Second solvent/ environment/ dirt reducing vial.

Vial S, Scint, VWR Tinfoil VWR SC 66022-106A

Be sure you get the caps.

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>

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Chemistry and Crystal Growth, J. Hulliger, *Angew. Chem. Int. Ed. Engl.* (1994) 33, 143-162 ; *Angew. Chem.* (1994) 106, 151-171.

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. <http://www.hamptonresearch.com/>

Richard J. Staples, Crystallographic Resources Inc and Michigan State University, Department of Chemistry: Originated at Harvard University, Department of Chemistry and Chemical Biology, 1998-2006, taken in part from the lecture given at MIT, 1998 *Getting Crystals Your Crystallographer Will Treasure*. Distributed as part of Chem 154. Updated to include more material for *Crystallography for Organic Chemists*, ACS PRF summer school, 2004, 2005 and 2007. Last updated 1-2011.
