

Crystal Growing Tips and Methods

X-Ray Crystallography Facility

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General Tips

- The purer your sample, the better results you will get
- Make sure your glassware is clean and free of dust and other impurities during crystallization
- Once you set up the crystallization, don't disturb it but keep an eye on what's happening
- Give your sample a few days to grow crystals. High quality crystals take time to form
- NEVER dry out your crystals

How do crystals form and grow?

Crystals grow from saturated solutions that are close to the critical point of nucleation. At such a concentration, molecules will slowly clump together and fall apart until they reach a critical size and form the initial nucleation site for a crystal. If the concentration becomes too high, nucleation occurs rapidly and can result in powdery material or microcrystals. If the concentration of the solution is too low, then no crystals will form.

If your crystals are too small:

- The sample was not given enough time to grow crystals
- The solution became supersaturated too quickly, resulting in many nucleation points
 - Dilute your initial solution more
 - Slow the diffusion of your anti-solvent by using colder temperatures
 - Use a different solvent/solvent combination

Choosing a solvent(s)

- "Solvent" will refer to a solvent that dissolves your compound, "Anti-solvent" will refer to solvents that do not dissolve your compound
- An ideal solvent is one that partially dissolves your material and results in well-defined crystals
- Determine what solvents your material is highly soluble, marginally soluble, and insoluble in
- Solvents that partially dissolve your compound are preferred over solvents that readily dissolve your compound as nucleation can readily occur with small changes in the temperature or concentration
- When using two or more solvents, be sure that they are miscible
- For some methods (vapor diffusion, layer diffusion) be sure that the vapor pressures and density of the solvents are compatible with the method
- Use of volatile solvents can result in crystals containing the solvent and are therefore prone to drying out and cracking very readily

General Considerations

A good sized crystal ranges from 0.1 – 0.3 mm in all dimensions. Larger crystals can be cut down.

Saturated solutions should be created by slow addition of solvent until all the material barely dissolves in solution. Can also be created by filtering a solution that still contains undissolved material or heating the solution until all the sample is dissolved.

A concentrated solution should be about the same concentration as used in NMR samples.

Choose your glassware with care. Ensure that it is appropriate for the scale of your crystallization and allows for easy removal of crystals once they form.

Crystal growing is more of an art than a science. Some compounds crystallize readily and other compounds require a lot of effort. Just because crystals didn't readily form, don't give up. Try a new method, new solvents or both.

Methods

Slow Evaporation

What's occurring: Gradual loss of solvent results in the concentration of the solution slowly increases until it becomes saturated and nucleation occurs which grow into crystals.

Pros: Very easy to perform

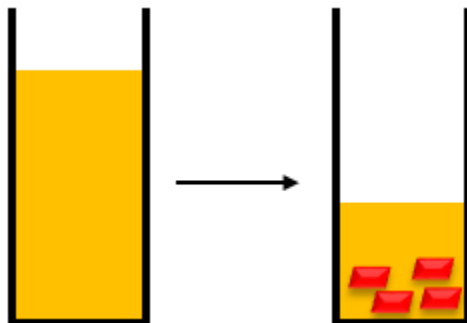
Cons: Not good for highly volatile solvents, requires a large amount of material, not ideal for air-sensitive compounds, not ideal for performing in a dry box

How to Perform:

Single solvent: prepare a saturated solution of your compound using a moderately good solvent. Place a cap on top of the vial and loosely screw the cap on but don't tighten. Alternatively stick a needle through the cap of the vial or septum to allow for slow evaporation.

Two solvents: The anti-solvent should have a higher vapor pressure than the good solvent. Prepare a saturated solution of your compound using both solvents. Loosely screw a cap onto the vial or stick a needle through the cap of the vial or septum. As the good solvent evaporates, the ratio of anti-solvent to good solvent will increase resulting in the sample becoming less soluble in the solution and forming crystals.

Tips: Control the rate of evaporation by varying the size of a needle in the vial cap or septum, or changing the temperature of the solution. Large surface areas result in fast evaporation and should be avoided. This method can be performed under air-free conditions on a Schlenk line using a Schlenk tube and slowly flowing inert gas over the solution. If all the solvent is allowed to evaporate, any resulting crystals may dry out and not be useable for diffraction.



Slow Cooling

What's occurring: As the solution cools and becomes more saturated, nucleation sites form which grow overtime to form crystals.

Pros: Very easy to perform

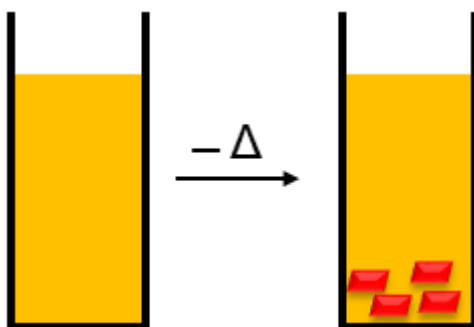
Cons: Not ideal for small amounts of material, requires use of solvent or solvent mixture with a moderate solubility

How to Perform:

Cooling from room temperature: Generate a saturated solution of the compound using a solvent of moderate solubility. Once the sample is fully dissolved, seal the container and slowly cool the solution in a refrigerator or freezer. Can be done with a mixture of good solvent and anti-solvent to generate ideal level of saturation.

Cooling from hot solvent: Generate a saturated solution of the compound using a solvent or solvent mixture of moderate solubility by heating the solution near the boiling point. Allow the solution to slowly cool back to room temperature by placing the container in a Dewar of warm water or in a hot/warm sand bath.

Tips: Slowing the rate of cooling in a freezer can be added by placing the container in sand



Vapor Diffusion

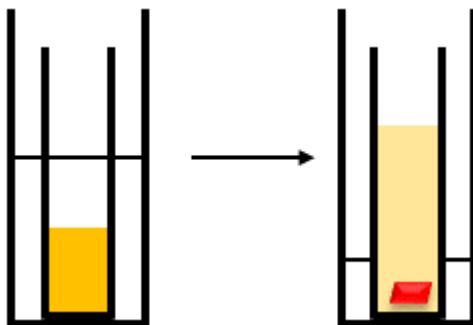
What's occurring: An anti-solvent slowly condenses into a solution of compound, lowering the solubility of the compound in solution which results in the formation of nucleation sites

Pros: Excellent for small quantities of material

Cons: Limited number of solvent/anti-solvent combinations available, can take longer to grow crystals than other methods.

How to Perform: Prepare a concentrated solution of compound in a small vial. Place this vial into a larger container and add the anti-solvent to the larger container. Seal the larger container and allow several days for crystal growth.

Tips: Be sure that the solvents are miscible and anti-solvent has a lower vapor pressure than the compound solution or else it won't diffuse. Multiple crystallizations can be set up in the same large container to maximize the possibility of crystals. Using anti-solvents with higher vapor pressures (eg. hexanes vs pentanes) may allow for slower diffusion. The set up can also be placed in a cold environment to allow for slower diffusion of the anti-solvent but may result in the solution becoming supersaturated too quickly.



Layer Diffusion

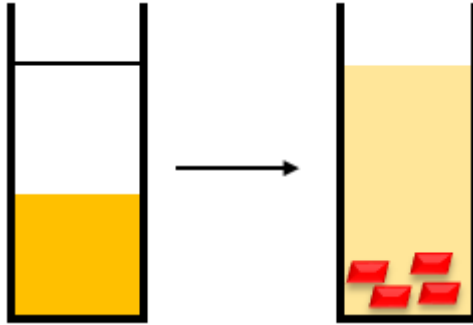
What's occurring: Slow mixing of solvents gradually generates a saturated solution, causing nucleation sites to form.

Pros: Excellent for small quantities of material

Cons: Can be complicated to set up, limited number of solvent/anti-solvent combinations available

How to Perform: Prepare a concentrated sample of compound and add to the bottom of a container. Carefully add anti-solvent down the side of the container such that the two solvents form distinct layers and do not mix. Seal the container and leave for several days.

Tips: Be sure that the solvents are miscible, better results if the anti-solvent is the least dense. Layering of the anti-solvent is best performed using a syringe and needle to add the solvent down the side of the container. Methods to help form a clean layer between the solvents include adding a buffer layer of pure solvent before addition of anti-solvent or freezing the layer of solvent before addition of the anti-solvent. Start with a 1:4 or 1:5 ratio of solvent to anti-solvent and adjust as necessary. Using containers with smaller surface areas between the layers allows for slower diffusion and possibly better crystals.



Seeding

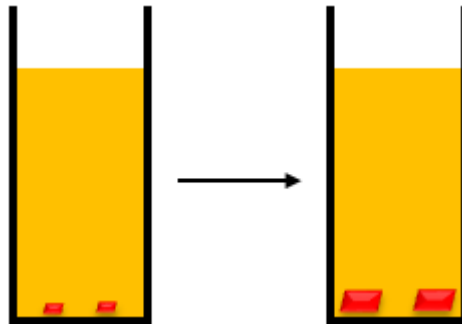
What's occurring: The seed crystals give already well-developed nucleation sites that allow for continued depositing of material.

Pros: Can work well for growing very large crystals

Cons: Requires already well-defined crystals that are too small for diffraction

How to Perform: Place a few (but not too many) small crystals into a saturated solution of your material. Leave the sample alone for several days.

Tips: Transfer the seed crystals to the saturated solution using a pipette. The use of many seed crystals will limit the size of the final crystals



Convection

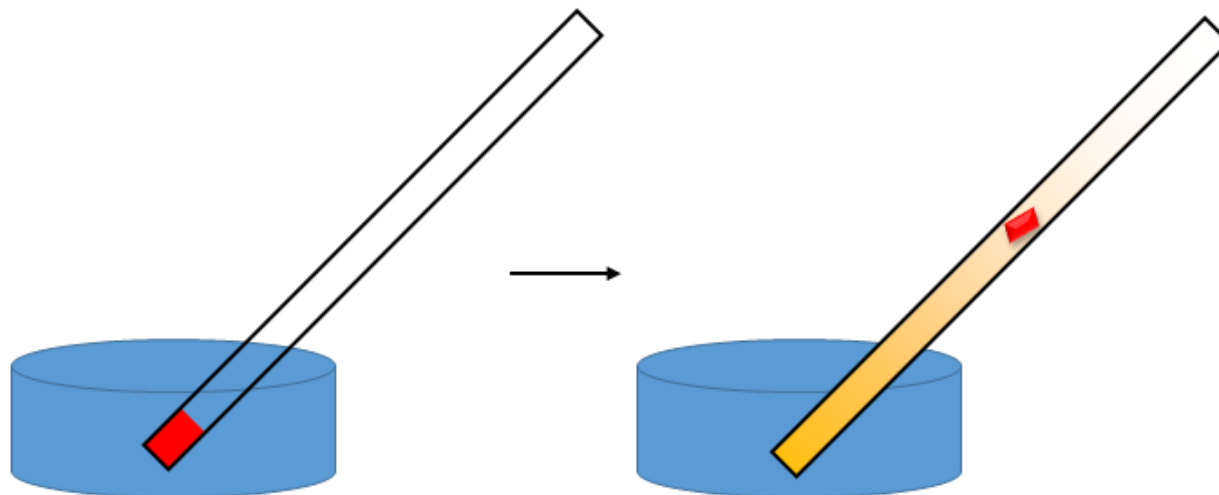
What's occurring: A saturated solution is formed by gentle heating. The presence of a thermal gradient results in the saturated solution slowly diffusing to a cooler portion of the apparatus through convection at which point the solution cools and nucleation occurs and crystals can grow.

Pros: Excellent for small quantities of sample depending on apparatus, can result in high quality crystals

Cons: Can require custom made apparatus, getting the right thermal gradient can require a lot of effort.

How to perform: Place a slurry or heavily saturated solution of material in the bottom of the apparatus and carefully layer additional solvent on top. Gently warm the area of the apparatus with the material such that a thermal gradient forms between two areas of the apparatus and slow convection occurs.

Tips: Apparatus can be made from pipettes or by using a Thiele tube if present. Can also use a crystallization dish at room temperature if one side is kept cold. Undissolved material should remain at the bottom of the apparatus and not flow with the convection current. Material may be packed by centrifuge. See references and examples: <http://web.mit.edu/x-ray/crystallize.html>; D.J. Watkin *J. Appl. Cryst.* **1972**, 5, 250; H. Hope *J. Appl. Cryst.* **1971**, 4, 333.



Special Methods

Scratching

To use if nucleation is not occurring or you are getting a lot of small crystals. Using a needle, glass scorer, or other hard edge to scratch the bottom of the container. The rough edges of the scratch results in an initial nucleation site that can result in crystallization.

Sublimation

This method only works if your material readily sublimates. Controlling the temperature and pressure of the sublimation can allow for precise conditions for growing high quality crystals. Crystals that grow too quickly are prone to twinning.

Diffusion of Reagents

A specialized form of Layer Diffusion. This method works when the products of a reaction are highly insoluble and can't redissolve to form crystals. The two layers will contain the reagents and the reaction will occur at the interface, ideally forming crystals. Extra care is needed when preparing, use of a buffer layer of pure solvent is recommended

Co-Crystallization

Addition of compounds that can undergo hydrogen bonding can help form highly ordered structures that allows for improved crystals. If your compound has hydrogen bond donors, use of hydrogen-bond acceptors such as $\text{Ph}_3\text{P}=\text{O}$ can work (see Etter M.C.; Baures P.W. *J. Am. Chem. Soc.* **1988**, 110, 639).

If your compound contains hydrogen bond acceptors, consider using protic solvents or even addition of an acid to protonate your neutral compound to form an ionic complex.

If your compound is an ionic complex, try changing your anion or cation that have more rigid groups (eg. Me_4N^+ , $(\text{PPh}_3)_2\text{N}^+$, PPh_4^+ , BPh_4^-).

Gels

Gels provide a medium for the transport of molecules during crystallization and can eliminate convection and sedimentation. Gels are often used for protein crystallization and typically used with aqueous solutions. There are a handful of reports of using gels as a crystallization medium for growing crystals of small molecules in organic solvents. Extensive examination of crystallization techniques with poly(ethylene) oxide in a range of organic solvents reported in Choquesillo-Lazarte D.; Garcia-Ruiz J.M. *J. App. Cryst.* **2010** *44*, 172-176. Gels were used with evaporation, layer diffusion, and vapor diffusion techniques.

Clathrates and Guest/Host Molecules

The use of a host lattice to help crystallize a guest molecule has been well established with porphyrins (C.E. Strouse; et. al. *J. Am. Chem. Soc.* **1993**, *115*, 9480-9497) and more recently with metal-organic frameworks (M. Fujita; et. al. *IUCrJ*, **2016**, *3*, 139-151). This method takes advantage of large voids present in the crystal structure of the host molecules such that the guest molecule can fit inside and can be used for molecules that don't crystallize or can only be synthesized in very small quantities.

References and Other Resources:

P. G. Jones *Chemistry in Britain*, **1981**, *17*, 222–225

P. van der Sluis; A. M. F. Hezemans; J. Kroon *J. Appl. Cryst.*, **1989**, *22*, 340–344

B. Spingler; S. Schnidrig; T. Todorova; F. Wild *CrystEngComm*, **2012**, *14*, 751-757

See guide by Richard J. Staples (Michigan State University),
<https://www2.chemistry.msu.edu/facilities/crystallography/xtalgrow.pdf>

See site by Peter Muller (MIT), <http://web.mit.edu/x-ray/crystallize.html>

See guide by Olga Chetina,
<https://community.dur.ac.uk/crystallography.group/imagesgroup/GrowCrystals.pdf>

Several versions of Paul Boyle's "Growing Crystals That Will Make Your Crystallographer Happy" can be found online and is a good resource