

X-ray Crystallography Facility – Practices and Procedures

How to submit a sample

Most of the samples submitted to this lab are grown by slow evaporation of solvent (see appendix A for advice on growing crystals). It's best to submit the sample in a small (e.g., 1 dram) sample vial. The crystallographer can usually extricate a single crystal from one of these – test tubes and, especially, nmr tubes are less ideal. If you are unsure whether your sample is “good enough” for submission, don't take a chance - any time you have a solid, have it examined with the microscope in the X-ray lab. It is usually not possible to judge the appropriateness of a sample by observation with the naked eye (many times, even observation via microscope is not conclusive). The only real requirement is that the sample is “big enough” to mount (the maximum size of a crystal sample is 0.5 mm because the diameter of the X-ray beam on the data collection instrument is 0.5 mm and crystals as small as 0.03 – 0.05 mm may work). It is very important that either you affix a label to your sample when it is submitted or make sure that the crystallographer does so. You will be asked to fill out a sample card:

Name:		Advisor:	
Structure:		Phone:	
		Formula:	
Optically Active?	Solvent:	Date:	

The entries are mainly self explanatory: in the large area under “Structure” you should draw a representation of what you think the structure is; “Optically Active?” is a yes-no question (is the compound optically active? Or not?); “Solvent” is the solvent from which the crystals were grown (sometimes the solvent is included in the unit cell contents and it's helpful to know what you're looking for). Actually, the only information that the structure solution software gets is the overall chemical formula (which should be entered in the “Formula” section). It is also helpful if you include something on the sample card, e.g., a lab notebook page reference, that also appears on your sample vial.

Scheduling

When your sample is accepted, your name will be added to the schedule on the white board in Rm B35-B. This schedule is only approximate; it can change for a variety of reasons: someone submits an air-sensitive sample, an earlier sample requires a repeat data collection, or someone later in the schedule than you offers a better bribe. But, generally, there is about a two week turn-around time from when you submit your sample to when your structure determination is completed. Any extenuating circumstances that might get your sample “bumped up” to an earlier data collection time should be made clear to the crystallographer. Such legitimate circumstances include things like your sample may decompose upon sitting or you have a group meeting in two days and need something to keep your boss off your back.

Reporting of Results

Once your sample has resulted in a successful structure determination, you will usually receive a phone call giving you the good news and inviting you to stop by the lab to see an ORTEP picture. Within about a week, you will receive a hard copy report detailing the X-ray experiment and listing an appropriate set of structural data: crystal co-ordinate, bond distances, bond angles. These reports are written as Mac Microsoft WORD documents and are saved forever. At any time, e.g. when writing your thesis or a publication manuscript or even as soon as the report is completed, you can obtain the report documents plus supporting graphics as electronic files. Beginning in October, 2007, these WORD report documents are stored on the webserver and can be downloaded.

The X-ray Facility Website

When a structure has been completed, an entry is made on the X-ray website at macxray.chem.upenn.edu. The website has a series of public links that give some background about the X-ray facility, that have examples of the use of the Jmol applet and that illustrate the archiving of X-ray data for structure determinations that have already been published. Under the “Chemistry Department Access” menu, there is a link to a set of password-protected pages devoted to each research group (Be sure to take a look at the “Instructions on accessing and using these pages”). In order to access a particular research group, you must obtain the group password from P.J. Carroll. These pages first display a pictorial index of completed structures that is linked to a page for each structure that has a java applet window in which a user can manipulate a graphical representation of his molecule in real time. A second page enables a user to investigate the unit cell using a Jmol-scripted display. There are also links to download the pdb file, the CIF, or the final report (in Microsoft WORD format) for the structure. PDB files are co-ordinate files that can be read by various molecule viewers (like RasMol, MacroModel, WebLabViewer, etc.). The CIF (Crystallographic Information File) is required for submission by many journals that publish X-ray structures. It can also be used as input to the program CrystMol, a molecule viewer that runs on both Mac’s and PC’s. (CrystMol can be downloaded from www.crystmol.com; we have a department-wide license for CrystMol – you can obtain the license code from P.J. Carroll).

Appendix A

Growing Crystals That Will Make Your Crystallographer Happy

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Practically every chemist knows the necessity of recrystallization as a method of purification. What is less well known, however, is methods by which "X-ray quality" crystals may be obtained. The purpose of this monograph is to briefly and informally outline some of the methods which can be used to obtain single crystals suitable for X-ray diffraction studies. This monograph is meant neither to be rigorous nor exhaustive, but rather, a

sort of practical "how to" cookbook. A good general reference which covers similar material can be found in "Crystal Growing", Chemistry in Britain, 1981, 17, 222-225 by Peter G. Jones.

The Goal

The goal in growing crystals for a single crystal X-ray diffraction experiment is to grow single crystals (obviously) of suitable size. The optimum size for a crystal is one which has dimensions of 0.2 – 0.4 mm in at least two of the three dimensions. Most potential structure determinations are thwarted by a lack of suitable crystals.

General Considerations

The factors during crystal growth which affect the size of the crystals are, solubility of compound in the solvent chosen for recrystallization, the number of nucleation sites, mechanical agitation to the system, and time.

Solvent.

Choose a solvent in which your compound is moderately soluble. If the solute is too soluble, this will result in small crystal size. Avoid solvents in which your compound forms supersaturated solutions. supersaturated solutions tend to give crystals which are too small in size.

Nucleation.

The fewer sites at which crystals begin to grow will result in fewer crystals each of larger size. This is desirable. Conversely, many nucleation sites results in a smaller average crystal size, and is not desirable. In many recrystallizations ambient dust in the laboratory provide sites of nucleation. It is important to minimize dust or other extraneous particulate matter in the crystal growing vessel.

Mechanics.

Mechanical disturbance of the crystal growing vessel results in smaller crystals. Let the crystals grow with a minimum of disturbance. This means: Don't try to grow crystals next to your vacuum pump, and don't pick up the vessel everyday to check on how your crystals are growing. Set up the crystal growing attempt, in a quiet, out of the way place and forget about it (if possible!) for a week.

Time.

This is related to mechanics. Crystals fully recognize that patience is a virtue and will reward those who practice it.

Techniques

Crystal growing is an art, and there are as many variations to the basic crystal growing recipes as there are crystallographers. The recipes given below are ones which I have either tried or I have read about and sound reasonable. 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? etc. etc.

Slow Evaporation.

This is the simplest way to grow crystals and works best for compounds which are not sensitive to ambient conditions in the laboratory. Prepare a solution of the compound in a suitable solvent. The solution should be saturated or nearly saturated. Transfer the solution to a CLEAN crystal growing dish and cover. The covering for the container should not be air tight. Aluminium foil with some holes poked in it works well, or a flat piece of glass with microscope slides used as a spacer also will do the trick. Place the container in a quiet out of the way place and let it evaporate. This method works best where there is enough material to saturate at least a few milliliters of solvent.

Slow Cooling.

This is good for solute-solvent systems which are less than moderately soluble and the solvent's boiling point is less than 100 deg C. Prepare a saturated solution of the compound where the solvent is heated to just its boiling point or a just below it. Transfer the solution to a CLEAN large test tube and stopper. Transfer the test tube to a Dewar flask in which hot water (heated to a temperature of a couple of degrees below the solvent boiling point). The water level should exceed the solvent level in the test tube, but should not exceed the height of the test tube. Stopper the Dewar flask with a cork stopper and let the vessel sit for a week. A more elaborate version of this involves a thermostated oven rather than a Dewar flask.

Variations on Slow Evaporation and Slow Cooling.

If the above two techniques do yield suitable crystals from single solvent systems, one may expand these techniques to binary or tertiary solvent systems. The basic rationale for this is by varying the solvent composition one may inhibit growth of certain crystal faces and promote the growth of

other faces, yielding crystals of suitable morphology and size. If you choose this route for growing crystals, it is absolutely necessary to record the solvent composition you use! If crystal growing is an art, growing crystals from binary or tertiary solvent mixtures is that much more imprecise. Remember reproducibility is paramount in science.

Vapor Diffusion.

(excerpted and paraphrased from Stout and Jensen p. 65). This method is good for milligram amounts of material. A solution of the substance is prepared using solvent S1 and placed in test tube T. A second solvent, S2, is placed in a closed beaker, B. S2 is chosen such that when mixed with S1 the solute will become less soluble. The test tube containing S1 is then placed in the beaker and the beaker is sealed. Slow diffusion of S2 into T and S1 out of T will cause crystals to form. If S2 is more volatile than S1 the solvent level will increase and prevent microcrystalline crusts from forming on the sides of T.

Solvent Diffusion (Layering Technique).

This method also is good for milligram amounts of materials which are sensitive to ambient laboratory conditions (air, moisture). Dissolve the solute in S1 and place in a test tube. Slowly dribble S2 into the tube so that S1 and S2 form discreet layers. This will only be successful if 1) The density of S2 < S1 and 2) Care is exercised in creating the solvent layer. I have found that a syringe is the best way to add the second solvent. The narrower the tube, the easier it is to build up the layer. Five millimeter NMR tubes are excellent vessels to use for this crystal growing technique. CH₂Cl₂/Et₂O is a good solvent combination to try this method (if your compound is insoluble in ether).

Reactant diffusion.

This is similar to the other diffusion methods except that solutions of the reactants are allowed to diffuse into one another. If the product of the reaction is insoluble, crystals of the product will form where the reactants mix. There is mention in the literature of this technique being used with diffusion in silica gels (see *Acta. Cryst.* 19, 483, (1965)).

Sublimation.

Andrea Sella (a.sella@ucl.ac.uk) suggests a couple of ways to grow crystals of somewhat volatile air sensitive crystals. The first way is to simply seal a sample under vacuum into a glass tube and placing the tube into an oven for a few days or weeks. Larger crystals tend to grow at the expense of

smaller ones. If it doesn't work raise the temperature of the oven or move to another hotter one. In some cases a tube furnace can be used. Andrea uses an all glass furnace which was originally designed by Prof. Geoff Cloke of Sussex University (email him for the details if you want to try this method). The tube can be placed close to one end of the furnace so that there is a mild temperature gradient.

The second method is to seal off a long piece of glass tubing at one end and put a joint with a vacuum adapter (or a Young's/Ace/Kontes valve) at the other. The sample is placed at the sealed end of the tube and is followed by a glass wool plug which cleans the tube as it is pushed along and serves to prevent bulk/crude material blowing along the tube itself. The tube is then evacuated and inserted into a length of copper pipe which will serve to produce a temperature gradient. The sealed end of the tube (where the sample is) and the copper jacket are then heated in an oil bath (vertical set-up) or in a tube furnace (either horizontal or vertical). The tube can be left under either dynamic or static vacuum (it's worth trying both). Andrea learned this method from his Ph.D. supervisor M. L. H. Green of Oxford.

Convection.

One may attempt to grow crystals by convection by creating a thermal gradient in the crystal growing vessel. The idea behind this method is that the solution becomes more saturated in the warm part of the vessel and is transferred to the cooler region where nucleation and crystal growth occur. To create the convection one may use either local heating or local cooling. The velocity of the convection current is proportional to the thermal gradient across the vessel. Care must be taken to not make the gradient too large, or the convection will be too rapid and inhibit crystal growth.

To use local cooling, simply take a flat bottomed crystal growing dish (similar to what one would use for slow evaporation) and place one side of it against the heat sink. Normally, simply placing the vessel against a cold outside window will suffice. Notice that the heat sink needs to be against the side of the vessel. Laying the vessel on a cool surface will not induce convection.

A Thiele tube with a resistive heater attached to it makes a good apparatus to grow crystals by local heating convection. This method was contributed by Professor Clarence Pfluger (cpfluger@keene.edu) He writes, First, use a solvent in which the solute has limited solubility at room or perhaps somewhat below room temperature, but of course increased solubility at elevated temperature. The Thiele tube is filled with this solvent and a small container with the compound in question is hung in the large part of the tube, slightly below the top "entrance" of the smaller 'side' tube. Wrap

a section of the bottom of the small tube with nichrome wire to make a heating element (adjustable, using a Variac or such). When the bottom part of the smaller tube is heated, the solvent will begin to circulate, delivering heated solvent to the container with the compound which slowly builds up the solute concentration in the solvent. Since the tube has a temperature gradient ranging from the temperature of the solvent at the heater element to almost room temperature near the bottom of the 'large tube' crystals will grow, if the gods are with you, in this part of the tube. Obviously one can also wrap part of the Thiele tube with small plastic tubing with tap water flowing through it to enhance the temperature gradient, if needed.

Instructions for constructing a similar device may be found in the literature. See *J. Appl. Cryst.*, 1971, 4, 333.

An inexpensive thermal gradient device can be constructed which can be used to obtain good crystals of "difficult to crystallize" compounds. This was suggested by Chuck Barnes (chemclb@mizzou1.missouri.edu) on the sci.techniques.xtallography newsgroup. According to Chuck, his advisor came up with this design after he had seen a rather elegant device described in the literature (Watkin, D.J., *J. Appl. Cryst.*, 1972, 5, 250). To make the device do the following: Cut off the tip of a Pasteur pipet about one cm above the beginning of the narrowing part, and then heat seal the small end, you get a nice cheap thermal gradient vial. Place a slurry of your material in a relatively poor solvent in the vial and centrifuge to pack the undissolved material in the tip. After centrifuging, you have a pellet of solid (~5mm) in the tip, covered by clean "poor" solvent. Seal the vial with teflon tape and parafilm. I make the heater from the small cylinder shaped ceramic resistors which are usually available from the electronics shop. I found some green ones of 100 ohms which are a good size. Place just the tip of the vial with the solid pellet in the heater, with the vial at ~45 degrees from vertical. Apply voltage to get maybe 50 C at the resistor, and you have a nice thermal gradient up the vial. With luck, you get crystals growing up the vial. It seems important to make sure all the amorphous material is packed down...no powder where you want the crystals to grow. Chuck adds about this method, " This has given me excellent crystals at times, and has on occasion given superior crystals (smaller mosaic spread) even when crystals were available from evaporation."

Co-crystallants.

If you are not getting suitable crystals from other crystallization methods, you can try to cocrystallize your compound with a "crystallization aid". In the literature (see *J. Amer. Chem. Soc.* 1988, 110, 639- 640) triphenylphosphine oxide (TPPO) has been used as a cocrystallant for organic molecules which are proton donors. Obviously, this method of crystallization

is not for all compounds, but for those it is, the results may be rewarding. Essentially this method of crystal growing is the same as slow evaporation except that an equimolar amount of TPPO is added to the solution. I suppose that the other crystallization methods outlined above would also work with this method.

Counterions.

If your compound is ionic, and is not giving suitable crystals with a given counterion, perform a metathesis reaction to change the counterion. Very often the crystal quality will improve when a different counterion is used. Ions of similar size tend to pack together better and subsequently give better crystals. In addition, you might also get more desirable physical properties for your crystal. For example, the sodium salt of your compound might be hygroscopic, but the tetramethyl ammonium salt might not be or at least, less so. When selecting possible counterions, avoid ions which contain large non-rigid groups (like tetrabutyl ammonium). Use ions which have very limited conformational flexibility. A word of warning: Make sure that whatever ion you use for your metathesis does not react with your ion of interest.

Ionization of Neutral Compounds.

Anthony Linden (alinden@oci.unizh.ch) suggests that if your compound is neutral and contains proton donor or acceptor groups, better crystals may be grown by first protonating or deprotonating (as applicable) your compound. The ionic form of your compound could then take advantage of hydrogen bonding to yield better crystals. This will alter the electronic properties of your compound, but if a general confirmation of the success of a synthesis is what is needed from the structure determination, then this is not a problem.

I hope this compilation of crystal growing techniques proves to be useful. It's generally not possible to predict which method will work best for a given compound; choose the method which sounds appealing to you and try it. You will save yourself a lot of time if you do purification and recrystallizations before attempting to grow single crystals of the material. If you are sure your compound is pure and a crystal growing technique is not giving suitable crystals, change the solvent or the method. If one system does not yield suitable crystals after a couple of tries, it is not going to yield good crystals after the tenth or hundredth time. Be intuitive, imaginative, but above all else stick to the basics – no dust, disturbance and a lot of patience.

References

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