

Introduction to single crystal X-ray analysis

II. Mounting crystals

Akihito Yamano*

1. Introduction

During data collection in single crystal structure analysis, a crystal is kept within the X-ray beam and rotated and oriented in various directions for hours to days. Therefore, the crystal mount should satisfy two contradictory conditions: it should hold the crystal steady for a relatively long period of time without blocking the incident X-ray beam. Some additional points should be considered when choosing the crystal mount. An ideal crystal mount, if such a thing exists, should meet all of the following conditions: (1) it should be completely transparent to the incident beam, (2) it shouldn't block any diffraction from the crystal, (3) it should produce no extra diffraction, (4) it should be stable throughout data collection, (5) it should allow the crystal to be completely bathed in the X-ray beam, and (6) it should not damage the crystal. It is almost impossible to satisfy all of these conditions simultaneously, but efforts should be made to satisfy as many as possible.

Conventionally, the crystal is glued to the tip of a glass fiber. This is a versatile method applicable to a wide range of crystals. However, there are some other useful options. By employing an appropriate mounting method, one can collect a good data set from a difficult crystal. In this article, the latest crystal mounting methods and tools will be introduced.

2. Shaping crystals

Shaping a crystal is sometimes necessary to reduce anisotropy or to reduce the size of the crystal so it can be completely bathed in the X-ray beam. The word "shaping" may be a little exaggerated. This is usually performed by cutting or trimming the crystal on a glass slide using a needle or a razor blade. Bond's method⁽¹⁾, grinding the crystal by continuously blowing it against a piece of sand paper, is not applicable to an organic crystal because it often results in cleavage of the crystal.

To avoid losing the crystal when cutting, a small amount of grease can be applied to the surface of the glass slide. N-hexane can be used to remove excess grease if desired. The trimming can also be done in a drop of liquid paraffin. In both methods, it is important to make sure that crystallinity is maintained when it is placed in grease or liquid paraffin.

Extremely fragile and delicate crystals do not allow any manipulation. If this is the case, the best results

are probably obtained when a crystal just large enough to provide the necessary intensity is used, along with a high brilliance X-ray source. The empirical absorption correction is getting robust and usually results in a publication quality structure despite the sample's anisotropy.

3. Mounting crystals

Conventionally, the crystal is glued to the tip of a glass fiber. This method is still applicable for a wide range of crystals. However, it is not appropriate for unstable, fragile or very small crystals. These days, sticking crystals to a polyimide film or a fiber loop and measuring under low temperature is becoming the common practice for diffraction experiments. With this method, the crystal can be attached and detached readily. Therefore, it is useful not only for handling difficult crystals but also to check multiple crystals for one of high quality.

Prof. Hope advanced and spread the usefulness of so called cryocrystallography, using oil instead of glue to mount a crystal at low temperature⁽²⁾. Cryocrystallography was first applied to unstable small molecule crystals containing volatile solvent, but soon it was extrapolated to protein crystallography⁽³⁾ because it dramatically extends the lifetime of protein crystals. Data collection in protein crystallography is now almost always done at low temperature. The development of cryocrystallography is one of the major factors in the rapid increase of the number of protein structures reported during the last 20 years. At first, the crystal was mounted directly to the tip of a glass fiber with oil such as Paraton-N, but later the loop method was introduced. The loop made it much easier to harvest fragile crystals without exposing them directly to ambient air⁽⁴⁾.

3.1. Sample pins

MiTeGen (<http://www.mitegen.com/>) provides sample pins of various sizes and shapes. They are composed of a solid stainless steel post and a piece of polyimide film precisely fabricated by lithography. The films come in various patterns, such as the original MicroMount (Fig. 1, middle)⁽⁵⁾, the MicroMesh (left) and MicroMount LD (right) etc. The MicroMesh works as a sieve allowing crystals to be scooped from the mother liquor. The MicroMount LD is developed especially for small molecule crystals. It has a long neck to maintain the distance between the sample loop and the tip of the stainless steel post to avoid unwanted diffraction from

*Application Laboratories, Rigaku Corporation.

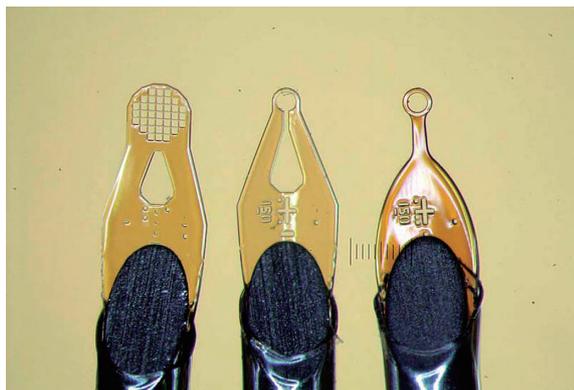


Fig. 1. MiTeGen pins. MicroMesh (left) is used for micro or thin crystals. The one in the center is the original MicroMount. MicroMount LD (right) was developed for small molecule crystals.

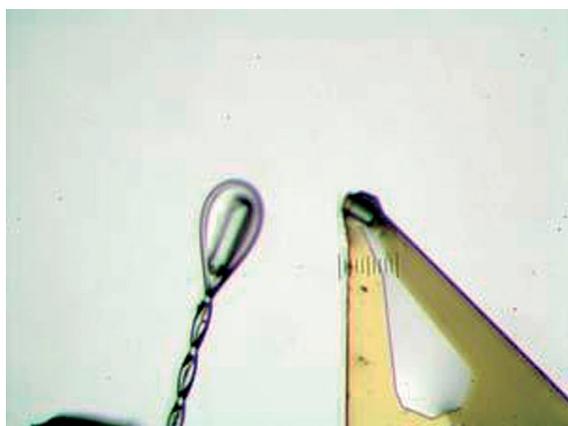


Fig. 2. CryoLoop from Hampton Research. CryoLoops are set on bases.

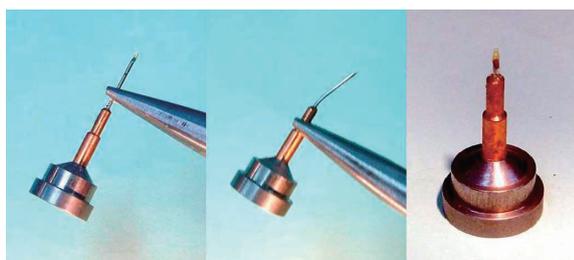


Fig. 3. Connecting a sample pin to a base. It is usually glued but it can be bent in the middle to eliminate adhesive and ease the removal the pin.

the metal, because in small molecule data collection the crystal is oriented in various directions for data completeness to high 2θ angles (Fig. 1. right).

Hampton Research (<http://www.hamptonresearch.com/>) supplies CryoLoops that are made of $10\mu\text{m}$ or $20\mu\text{m}$ synthetic fiber and a metal post. The $10\mu\text{m}$ fiber loop has a lower background but it is sometimes too soft. The $20\mu\text{m}$ loop is suited for most cases. It produces higher background than the $10\mu\text{m}$ loop, but this is negligible for most diffraction experiments. Figure 2 shows the MicroMount and the CryoLoop with a crystal mounted.

Usually sample pins are glued to the bases. However, if you bend it in the middle when you insert the pins to the base, you can eliminate adhesives (Fig. 3). This technique makes it easy to remove the pins for reuse of the bases. Recently MiTeGen released a new base that grips the sample pin as you insert it.

3.2. Oil

The crystal can be directly frozen from its mother liquor but usually it is immersed in a drop of oil. There are various kinds of oil used for this purpose: paraffin oil, silicon oil/grease, Paraton-N, etc. Paraton-N was originally from Exxon but is now available from Hampton Research. Other kinds of oil can be purchased from reagent vendors. Silicon grease has a high viscosity and can be used as a glue to pick up a relatively stable crystal. Since silicon has relatively strong diffraction, it is important to apply the minimal amount. Paraffin oil has a number of good features: it is unlikely to dissolve crystals, has low viscosity, is transparent and is inexpensive. Paraton-N is also a mixture of hydrocarbons but it is as viscous as silicon grease and sometimes causes difficulty during crystal manipulation. If this is the case, the viscosity can be adjusted by adding some paraffin oil. In all cases, it is important to check the stability of the crystals in oil.

Epoxy glue composed of two separate liquids can be used to harvest an air sensitive crystal. Apply a small amount of the base resin to the tip of a needle and touch the crystal with the resin. The crystal is captured and wrapped with the resin to prevent evaporation of solvent when you take out the crystal. If the resin does not work, try the hardener. This method can be used even in the presence of organic solvent and is therefore very useful when the crystal contains volatile solvent such as ethyl acetate.

3.3. Freezing crystals

There are two major methods for freezing crystals. One is to use the gaseous cold nitrogen stream of the low temperature system and the other is to use liquid nitrogen. The former method is demonstrated in Fig. 4.

1. Roughly center the sample pin prior to mounting a crystal.
2. Suck up crystals and mother liquor using a pipette and inject them into a drop of paraffin oil. This way, crystals are not exposed directly to ambient air.
3. Pick up a crystal with a CryoLoop or MicroMount.
4. Block the cold gaseous nitrogen stream using a piece of thick paper. Room temperature is therefore maintained at the sample.
5. Place the sample pin with the crystal onto the goniometer head with a magnetic base and quickly remove the piece of paper blocking the cold stream. The crystal is flash frozen.
6. Center the crystal.

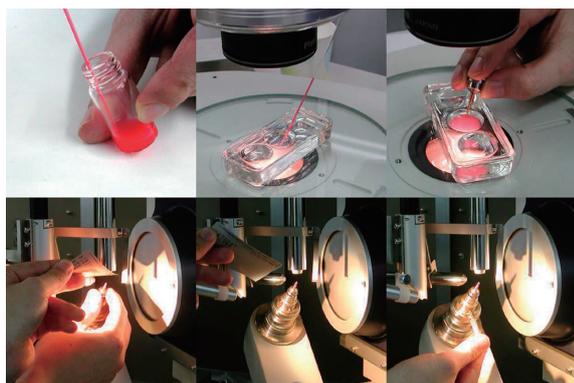


Fig. 4. Freezing procedure using gaseous cold nitrogen. (1) Roughly center the sample pin. (2) suck up crystals with a pipette (upper left), (3) inject them into a drop of paraffin oil (upper middle), (4) scoop a crystal (upper right), (5) block the cold stream with a piece of paper and attach the sample pin to the goniometer head (lower left), (6) remove the paper quickly (lower middle) and (7) center the crystal (lower right).



Fig. 5. Freezing crystals using liquid nitrogen. (1) Roughly center the sample pin, (2) suck up crystals with a pipette, (3) inject them into a drop of paraffin oil (upper left), (4) pick up a crystal with a sample pin attached to a CrystalWand (Hampton Research) (upper middle), (5) rapidly plunge into liquid nitrogen (upper right), (6) chill the CryoTong (Hampton Research) and (7) grab the sample pin (lower left), (8) attach the sample pin to the goniometer head with a magnet (lower middle), (9) center the crystal (lower right).

For relatively stable crystals, blocking step 4 can be skipped. Small molecule crystals usually do not require blocking.

When the crystals must be frozen more rapidly, liquid nitrogen can be used^{(6),(7)}. The freezing sequence is shown in Fig. 5.

1. Roughly center the sample pin prior to mounting a crystal.
2. Suck up crystals and mother liquor using a pipette and inject them into a drop of paraffin oil. This way, crystals are not exposed directly to ambient air.
3. Affix the sample pin to the CrystalWand

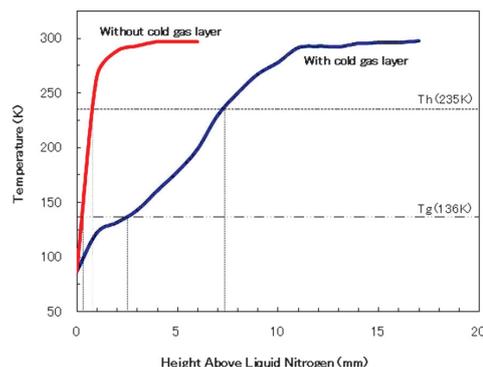


Fig. 6. Temperature distribution with and without the cold gaseous layer on the surface of liquid nitrogen. A steep temperature change can be achieved by removing the gaseous layer.



Fig. 7. Typical tools for low temperature measurement. From front left to right in the left picture: a goniometer head with a magnetic base, goniometer bases (MiTeGen and Hampton Research), cap for the bases. Upper left to right, MicroMount (MiTeGen) and CryoLoops (Hampton Research). The right picture includes tools used for freezing by liquid nitrogen and storing crystals at liquid nitrogen temperature. From left to right, Vial Clamp, CryoTong and CrystalWand (Hampton Research).

(Hampton Research) and pick up a crystal with a CryoLoop or MicroMount.

4. The crystal is then rapidly plunged into liquid nitrogen. The plunging speed determines the freezing rate.
5. CryoTong (Hampton Research) is chilled completely in liquid nitrogen.
6. Grab the sample pin with the tong in liquid nitrogen and place it on the goniometer head.
7. Center the crystal.

When this method is employed, it is very important to minimize the cold gaseous nitrogen layer on top of the liquid nitrogen⁽⁸⁾. Figure 6 shows the temperature distribution with and without the gaseous layer. When the layer is removed, the slope of the temperature near the surface of the liquid nitrogen becomes steep. Therefore, assuming that the plunging speed is constant, the freezing speed dramatically increases. Especially when the major ingredient of the crystallization mother liquor is water, it is necessary to maximize the freezing speed to avoid the formation of ice; otherwise, you get a lot of unwanted reflections. The cold layer can be

eliminated by blowing gaseous nitrogen or sucking. An easier method is to fill the container to its brim.

Figure 7 summarizes tools used for low temperature experiments. When only the cold stream of a low temperature system is used, the sample pin, the goniometer base and the goniometer head with a magnet are sufficient.

4. Summary

Low temperature data collection using a CryoLoop or MicroMount has many advantages: (1) crystal mounting becomes easier, (2) thermal vibration is reduced and (3) X-ray damage can be minimized. Crystals difficult to handle with conventional mounting methods can often become measurable with low temperature measurement. Recently we managed to measure a good data set from a crystal that readily sublimates and disappears in less than a minute. The crystal was scooped with its mother liquor

and frozen with liquid nitrogen. This crystal structure could not have determined if only the conventional mounting method was available.

References

- (1) W. L. Bond: *J. Sci. Instr.*, **22** (1951), 344.
- (2) H. Hope: Annu. Meet. Am. Crystallogr. Assoc. Abstract (1985), PA3: 24.
- (3) H. Hope: *Acta Cryst.*, **B44** (1988), 22–26.
- (4) E. F. Garman and T. R. Schneider: *J. Appl. Cryst.*, **30** (1997), 211–237.
- (5) R. E. Thorne, Z. Stum, J. Kmetko, K. O'Neill and R. Gillilan: *J. Appl. Cryst.*, **36** (2003), 1455–1460.
- (6) L. J. Walker, P. O. Moreno and H. Hope: *J. Appl. Cryst.*, **31** (1998), 954–956.
- (7) T. Teng and K. Moffat: *J. Appl. Cryst.*, **31** (1998), 252–257.
- (8) M. Warkentin, V. Berejnov, N. S. Hussein and R. E. Thorne: *J. Appl. Cryst.*, **39** (2006), 805–811.