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J. Appl. Cryst. (1998). **31**, 945–953

Macromolecular Cryocrystallography: Cooling, Mounting, Storage and Transportation of Crystals

Sean Parkin[a]† and Håkon Hope[b]*

[a]*Biology and Biotechnology Research Program, L-452, Lawrence Livermore National Laboratory, 7000 East Avenue, Livermore, CA 94550, USA, and* [b]*Department of Chemistry, University of California, Davis, CA 95616, USA. E-mail: hhope@ucdavis.edu*

(*Received 16 December 1997; accepted 6 April 1998*)

Abstract

Simple methods are presented for handling, mounting, storage and transportation of crystals at cryogenic temperatures. They are easy to learn and have a number of technical and operational advantages over currently popular methods. In particular, the temperature of the crystal throughout all manipulations is known; it is shown never to rise above that of the warmest component of the cryogenic system, typically the cold gas stream of the low-temperature apparatus. Crystals can be mounted and inspected in the home laboratory prior to transportation to a synchrotron, giving dramatic savings in experimental time and effort. Provided appropriate care is taken, crystals remain frost free throughout any number of mount–dismount cycles.

1. Introduction

Low-temperature methods have long been used in small-molecule crystallography to simplify handling, prolong crystal life and improve data quality. Until recently, it was thought that the high water content of biocrystals would preclude their cooling to cryogenic temperatures unless the internal water structure could be modified in some way. Some time ago one of us demonstrated that biocrystals could be cooled to cryogenic temperatures by a simple procedure, without ill effects (Hope, 1988; Hope *et al.*, 1989). In recent years, there has been a growing interest in cryomethods in biocrystallography, mainly because cooling leads to greatly diminished radiation damage and hence to better data. That far fewer crystals are needed is also an important consideration.

Most biocrystals cannot be picked up from their growth medium and cooled directly; they usually require some treatment prior to cooling to help preserve the crystallographic integrity of the specimen. On occasion, a crystal can be successfully cooled simply by placing it in a cold gas stream. Many crystals, however, require a more elaborate treatment.

Although we are not aware of any systematic study of the effect of cooling rate, it is generally thought that the most successful procedure requires the most rapid cooling. A belief held by some is that dunking the crystal in liquid propane results in faster cooling than that provided by liquid N_2. Teng & Moffat (1996) described measurements that purported to support this notion. However, the sample used in those experiments was quite large compared to a typical crystal in a diffraction experiment. It is well known that in liquid N_2 a gaseous envelope tends to form around larger objects, effectively providing insulation. Because of the size and composition of their test object, the observations cannot be expected to represent the behaviour of normal-size crystals. In contrast, measurements with small thermocouples, coated with glue to simulate crystals, have shown that the transition from room temperature to 140 K is twice as fast in liquid N_2 at 77 K (0.6 s) as in liquid propane at 100 K (1.2 s) and over three times faster than gas-stream cooling (2.0 s with a stream of 100 K) (Hope *et al.*, 1994; Walker *et al.*, 1998). These results, coupled with the greater complexity and safety considerations of liquid-propane techniques, provided the impetus for the development of simple methods for pre-cooling treatment, cooling with liquid N_2 and handling of cold crystals. They have been used in special circumstances for many years in the crystallography laboratory at UC Davis and routinely at Lawrence

† Present address: Biochemistry Department, Duke University, Durham, NC 27710, USA.

Files: h:\iuoc/jacj/wb0058/wb0058.3d, h:\iuoc/jacj/wb0058/wb0058.sgm Paper number: J980530–WB0058 Paper type: CN N TE-9813/10(3)11

Livermore National Laboratory since 1994. In this paper, we describe relatively simple procedures that we have developed for handling, cooling, storage and transport of cold crystals. We also describe a set of useful implements that are an integral part of the methods. Although we have not previously published a description of these methods, they have been presented at a number of international conferences (Parkin & Hope, 1993; Parkin *et al.*, 1996; Hope, 1996; Parkin, 1997). This paper was written at the request of a number of people who have used and adapted these techniques to suit their own particular experimental set-ups. These methods now constitute the preferred *modus operandi* at a number of institutions (*e.g.* SSRL, 1996).

Note our use of the term 'cooling' rather than 'freezing'. This is because 'freezing' in physics denotes a liquid-to-solid phase transition, which is precisely the process we wish to suppress. We also deliberately avoid the colloquial use of 'freezing' to mean 'very cold'. Further, we tend to use the term 'antifreeze' rather than the more common 'cryoprotectant', although at least in the context of this paper they are synonymous.

2. Procedures

2.1. *Overview*

Most biocrystals contain a large fraction of water, typically 50% or more. If this water is converted into ice the crystals will be destroyed. Early cooling experiments were aimed at modification of the internal water (Petsko, 1975), presumably because it was thought that destructive ice formation begins with internal nucleation. In our experience, the water structure within most crystals does not appear to favour nucleation, but rather, ice growth is initiated in the aqueous layer at the crystal surface. Unless this layer is removed or modified, ice growth may propagate into the crystal, thereby destroying it. Even if ice formation is confined to an external layer, diffraction rings due to polycrystalline ice will render parts of the diffraction pattern worthless. It is therefore always necessary to prevent ice formation.

Conceptually, the simplest procedure would be to remove all water from the surface of the crystal. In many cases this can be readily accomplished. A crystal can be placed under an inert oil (possibly saturated with water) and the aqueous layer removed with a wick or gently teased away with a needle (Hope, 1988, 1990). Requirements for the oil are: low optical distortion during microscopy, absence of crystal formation on cooling and low scattering power. Exxon Paratone-8277 (previously known as Paratone-N) is a mixture of saturated hydrocarbons that satisfies the criteria well. It is best to avoid preparations containing Si or F because of the higher scattering power of these elements. The main function of the oil is to serve as a shield against loss of water from the crystal before it is cooled.

In our hands, roughly half the crystals we have tried survive oil treatment. The remainder either lack sufficient mechanical strength, have a tendency to lose water or crack, or tenanciously cling to their aqueous coating so that it cannot be removed. Such crystals require modification of the aqueous layer with some form of antifreeze. A brief wash in mother liquor spiked with antifreeze (*e.g.* glycerol, MPD, PEG *etc.*) may modify the aqueous layer at the crystal surface so that the liquid droplet around the crystal forms a glass on cooling. If the concentration of this antifreeze is high enough (typically 15–30%, depending on the species), the crystal may be cooled successfully. We have always been able to find suitable recipes by a combination of trial and error and expeditious work.

When a crystal at equilibrium with its mother liquor is transferred to a different environment (cryoprotective antifreeze in this case), the chemical potentials of species within the crystal are no longer the same as in the surrounding solution. There is thus a thermodynamic driving force for the crystal to re-establish equilibrium; this is simply a manifestation of Le Chatelier's principle. Any change in osmotic pressure, for example, will alter the balance between rates of water diffusion into and out of the crystal. Either way, the crystal may be destroyed. The main point to observe is that exposure to any added antifreeze should be short enough to keep crystal damage to a minimum, but long enough to modify the liquid layer on the crystal surface so that ice formation is prevented.

Some laboratories still advocate extended soaking in antifreeze in an attempt to modify the internal water structure (Rodgers, 1997). Although such incorporation has been observed (Garman *et al.*, 1996; Kuzin *et al.*, 1997; Parkin, 1997), we have never needed to soak crystals for extended periods. At the time of this writing, we do not believe that it is a prerequisite for success, though we have on occasion resorted to sequential brief washes in increasing concentrations of antifreeze. Perversely, advocates of long soaks have used brief washes when a satisfactory long soaking regimen could not be found (Rodgers, 1997). More detailed guides to the search for suitable conditions have been presented by Garman & Mitchell (1996) and Mitchell & Garman (1994).

2.2. *Equipment*

We have developed a set of tools that are used in conjunction with the techniques described in this paper. Since they are a prerequisite for the use of these techniques, we describe them first.

2.2.1. *Mounting pin*. The mounting pin depicted in Fig. 1 is a hollow copper rod whose outer diameter matches the bore in a standard goniometer head, typically 3 mm (0.125″). The tip bore is about 0.5 mm (0.02″) and the main body bore is 1.5 mm (0.06″). We have

found that it is important to use copper, rather than brass, because proper functioning of the tip relies on the high heat conductivity of copper. Mounting pins for use with the transfer tongs described below must be fitted with a collar to prevent the pin from sliding too far into the cavity of the transfer tongs. Loop mounts, originally described by Teng (1990), are generally the most useful for crystals in aqueous antifreeze, while straight glass fibres are better for oil mounting. Our loop mounts are made of hair or some other fibre threaded through a fine glass capillary. They can be easily adjusted in size to fit any given crystal. The capillary (or fibre) is then held within the mounting pin with regular electronic solder, which requires gentle heating of the pin from the wide end. It is well to keep in mind that the original purpose of the loop was to prevent deformation of the crystal, not to serve as an aid in picking it up. For blocky crystals the danger of deformation is much lower than for thin plates, so it is often not necessary to suspend the crystal in a film. Instead, the loop just serves as a platform to hold the crystal in place. In such cases it is not necessary to make the loop so large that the crystal will fit inside; it is not even desirable. Because of the detrimental effects of foreign scattering material around the crystal, it is generally better to make the loop just wide enough to keep the crystal from falling off until it has been cooled. Specialized mounts, such as microspatulas (Hope *et al.*, 1989), are made from very thinly blown glass bubbles glued to a glass fibre with RTV silicone sealant (obtainable from a hardware store).

2.2.2. *Soldering aid*. We have used two different tools to heat the mounting pin above the melting point of solder: a modified soldering iron and a small butane burner (*e.g.* Blazer, Blazer Corp. NY, USA). Special care should be taken not to overheat the mounting pin, as scaling of the bore hole will eventually render it unusable. On the other hand, a carefully handled pin can be reused for years.

2.2.3. *Mounting-pin holders*. For cooling by immersion in liquid N_2, some form of mounting-pin holder is also required. We have used three such devices, curved-nose haemostat tongs, a draughtsman's mechanical pencil and a large pin vice fitted with a modified goniometer-head extension (Fig. 2).

2.2.4. *Transfer tongs*. Crystals to be immersed in liquid N_2 require additional steps to exclude the possibility of warm-up or frost formation during later handling. For this purpose we have designed special transfer tongs. These are haemostat tongs that have been fitted with grooved blocks of stainless steel (Fig. 3). When the tongs

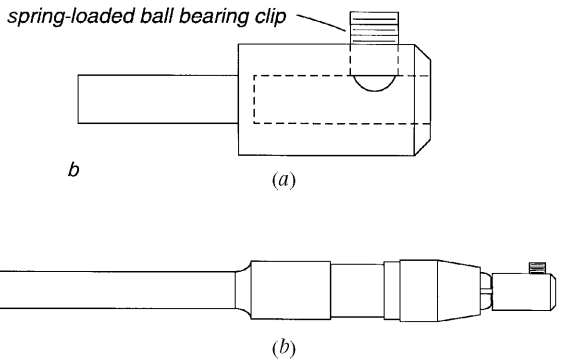


Fig. 2. A goniometer-head extension with its set screw replaced by a spring-loaded ball-bearing clip (*a*) is held in a large pin vice and provides a sturdy handle (*b*) for the mounting pin.

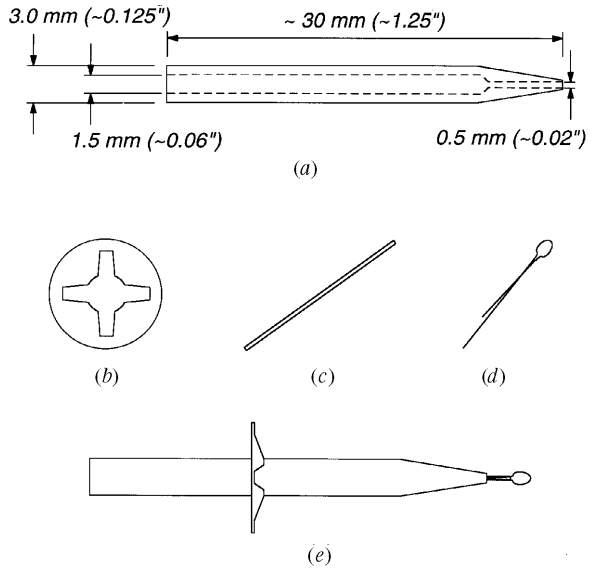


Fig. 1. Components of the mounting-pin assembly: (*a*) copper pin, (*b*) steel collar, (*c*) glass capillary, (*d*) fibre loop, (*e*) assembled mounting pin.

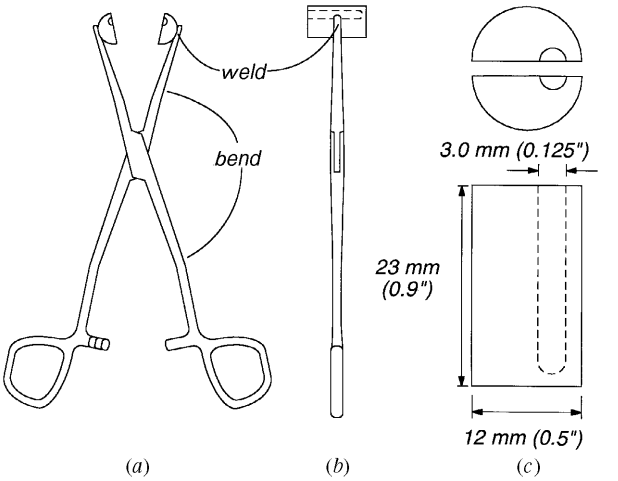


Fig. 3. Standard haemostat tongs which have been modified by the addition of grooved stainless-steel blocks. Some precision is required in the manufacture of the blocks to prevent gaps which could allow warmer air into the crystal cavity. The tongs must be bent slightly, as indicated, to enable them to be clipped shut. (*a*) Front, (*b*) side, (*c*) blocks.

are closed, the grooves form a cylindrical cavity. With the pin in place there is a small enclosed chamber sufficient to house a mounted crystal. During transfer, the crystal is well protected; the heat capacity of the blocks prevents the temperature from rising more than a few degrees during the time it takes to mount the crystal (§ 2.5). Although the chamber will initially be filled with liquid N_2, it is not necessary to conserve this liquid for the transfer to be successful; the heat capacity of the blocks yields sufficient protection. These tongs require some precision in fabrication. They must hold the mounting pin snugly, without any gaps that would allow warmer air to leak into the crystal chamber. The tongs have the supreme advantage that the geometry of the experimental set-up is irrelevant. This negates the need for extended-arc goniometer heads (Rodgers, 1997; Garman & Schneider, 1997), bulky rotation-axis inverters (Goodwill *et al.*, 1997) or other elaborate solutions to problems associated with alternative methodologies.

2.2.5. *Modified goniometer head*. Transfer of the mounting pin is simplified considerably by a modification of the goniometer head to allow side entry, rather than the common end entry. The modification incorporates a slot, as shown in Fig. 4. Further simplification is possible by replacing the set screw used to lock the mounting pin in place with a small ball-bearing plunger (Fig. 4*b*). Magnetic mounts that are popular in many laboratories allow the same freedom and provide an equivalent solution to the same problem. Side entry is of crucial importance since repositioning of the cold stream or large movements of the goniometer head must be avoided if control of crystal temperature is to be maintained. (*cf.* Rodgers, 1997).

2.2.6. *Liquid-nitrogen container*. For liquid-N_2 cooling, a volume of about 1 l is appropriate. We have used two types of container: a low-profile glass Dewar with a wide opening and a styrofoam box with 5–10 cm walls. Both are satisfactory. It is important that the container has a wide opening for easy access and that it can be placed within easy reach of the microscope. The main problem with open containers is the relatively rapid formation of snow in the cryogen. As far as possible the container should be kept free of such snow because it tends to percolate around and may stick to the crystal. To this end, the liquid N_2 should be completely replaced every few minutes.

2.2.7. *Storage vials*. For storage and shipping we have adapted a commonly available style of biological-sample cryovessel (Fig. 5). The screw cap has been modified with a 5 mm (3/16″) hole to accommodate the shaft of the mounting pin. This hole also serves as a vent when the vial is full of liquid N_2. A lip on the inside of the cap is also removed. The vial has a small ring of magnetic rubber (the sort used for refrigerator magnets) attached to its rim which provides a steadying force while the cap is attached. The vials can be stored in cryocanes, common in biological laboratories.

2.3. *Cold-stream–mounting-pin ineraction*

The crystal must be protected from deposits of snow or ice for the duration of the measurements. The flow characteristics of the cold stream and the interaction between the stream and the crystal mount are of the utmost importance for precise control of crystal temperature. Bear in mind that commercial low-temperature machines monitor temperature some distance back inside the delivery tube; the actual crystal temperature may be higher and much less stable than indicated. In our set-up, the cold stream is angled relative to the mounting pin; it only envelops the crystal and the tip of the mounting pin, and does not touch the

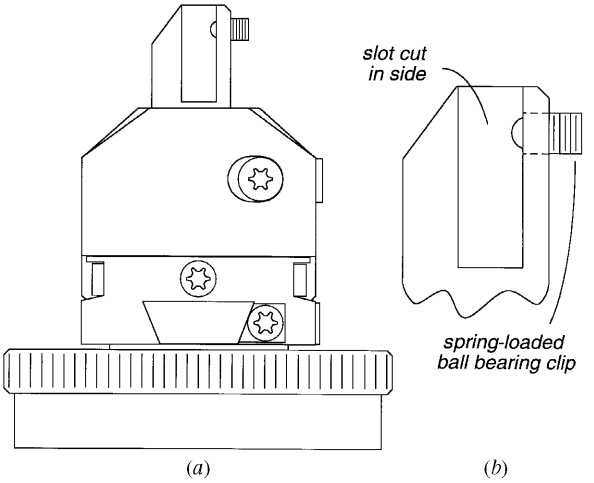


Fig. 4. (*a*) Modified goniometer head. The side of the mounting-pin hole has been removed to leave a slot to allow side entry of the mounting pin. In addition, the standard set screw has been replaced by a spring-loaded ball-bearing clip (*b*).

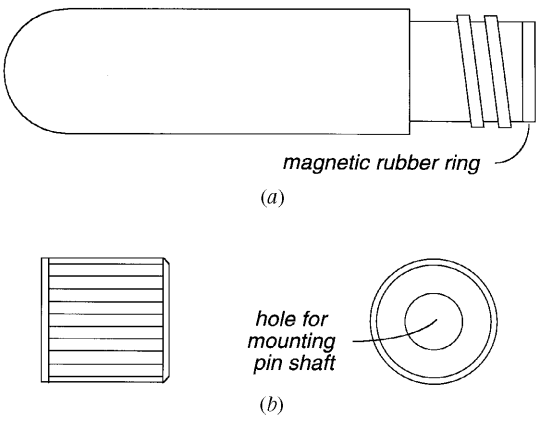


Fig. 5. Cryogenic storage vial modified for crystal storage. A magnetic rubber ring is glued to the lip of the vial and a hole is drilled in the cap to accommodate the mounting pin. (*a*) Storage vial, (*b*) vial screw cap (side and top views).

goniometer head (Fig. 6). The stream should be as close to laminar as possible so that turbulence around the crystal mount does not result in room air reaching the crystal or its immediate vicinity. The more laminar the flow, the sharper the temperature gradient from room air to cold stream, and hence the larger the region of lowest temperature within the cold-stream cross section. To this end, we have found that coaxial nozzle heaters are more effective than warm, dry outer streams. While delicate flow balancing of dual streams can prevent moisture from approaching the crystal, there is a definite added cost in experimental effort and machine complexity. Resistive nozzle heaters have been used in our laboratories for about 25 years. The nozzle supplied with the Siemens LT-2 low-temperature machine requires simple modification for satisfactory operation. This entails insertion of a plastic tube (*e.g.* Teflon, 7 mm inner diameter, ~70 mm long) inside the nozzle until it is flush with the end. The gap between the tube and the original nozzle should be filled with epoxy putty. More complex designs (*e.g.* Kottke *et al.*, 1996) are not necessary. A properly designed low-temperature machine should be capable of producing temperatures below 100 K with a liquid-N_2 consumption well below $1\,l\,h^{-1}$. When the cold stream is angled and copper mounting pins are used, there is no need for baffle heaters to protect the goniometer head, or for dry-air-purged tents around the equipment, even in a humid environment. It is, however, necessary to shield the cold stream from air currents.

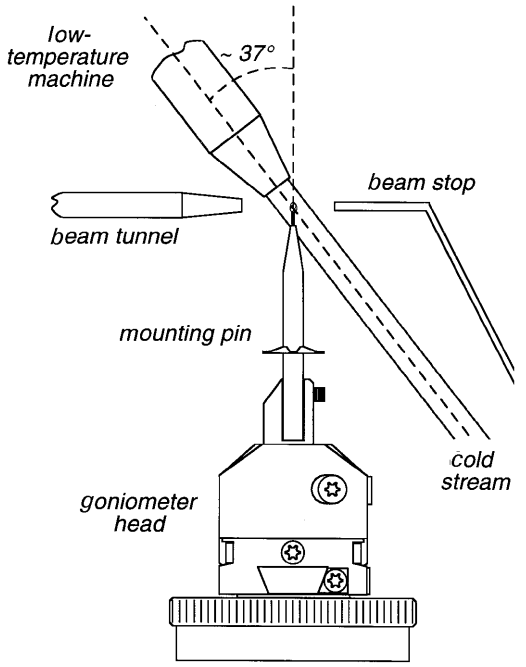


Fig. 6. The interaction between the cold stream, crystal and mounting pin. The tip of the copper mounting pin juts approximately 1 mm into the cold stream. When the cold stream is angled appropriately, ice formation is suppressed without the need for further frost-prevention measures.

2.4. *Crystal handling*

After selection of a crystal and possible mechanical removal of unwanted fragments, the next step in preparation for cooling involves removing the crystal from its growth (or storage) medium. This has commonly been done with a small pipette, sucking up the crystal and some of the surrounding liquid. Since the introduction of the crystal-mounting loop (Teng, 1990), handling the crystal with a loop has become popular, offering significant advantages over a pipette. Overall operation is less cumbersome; it is possible to lift just one crystal from a drop, with only a very small amount of liquid being removed. This makes it easier to preserve other crystals that might be present in the same drop. A loop for crystal handling can be made sturdier than a mounting loop, as there is no concern about interference with the X-ray beam. A relatively heavy handle is also helpful in steadying hand movements.

The crystal should be deposited either in a drop of oil, or in a drop of mother liquor placed on a microscope slide. In the latter case there should also be a drop of modified mother liquor (*i.e.* cryoprotective antifreeze) near the first drop. Removal or modification of the liquid layer on the crystal, either by the oil-drop technique or by antifreeze modification, is the next step.

2.4.1. *Oil treatment*. The crystal is transferred to a small drop of oil on a microscope slide. With a piece of pre-moistened filter paper, any visible drops can be wicked away. It is also often possible to remove the liquid by rapidly moving a needle away from the crystal. Since most biocrystals are fragile it is generally not a good idea to push the crystal itself, or otherwise touch it with a hard implement. When the aqueous layer has been adequately removed, it is best to pick up the crystal with a straight glass fibre rather than a loop, because a loop tends to take along an excessive amount of oil. The crystal should be cooled as soon as possible after pick-up. Not all crystals tolerate long exposure to oil, presumably because water can diffuse into the oil. This effect can be minimized by keeping the oil in contact with water. Note that oil with water should be treated gently, as it is easy to distribute small drops of water in the oil, making it unusable. An advantage of oil comes from the fact that the crystal is encapsulated in a more-or-less spherical drop, resulting in nearly isotropic absorption. A disadvantage is the introduction of extraneous material into the X-ray beam. Oil is not well suited to plates or needles as the hardening of the oil on cooling can deform thin crystals.

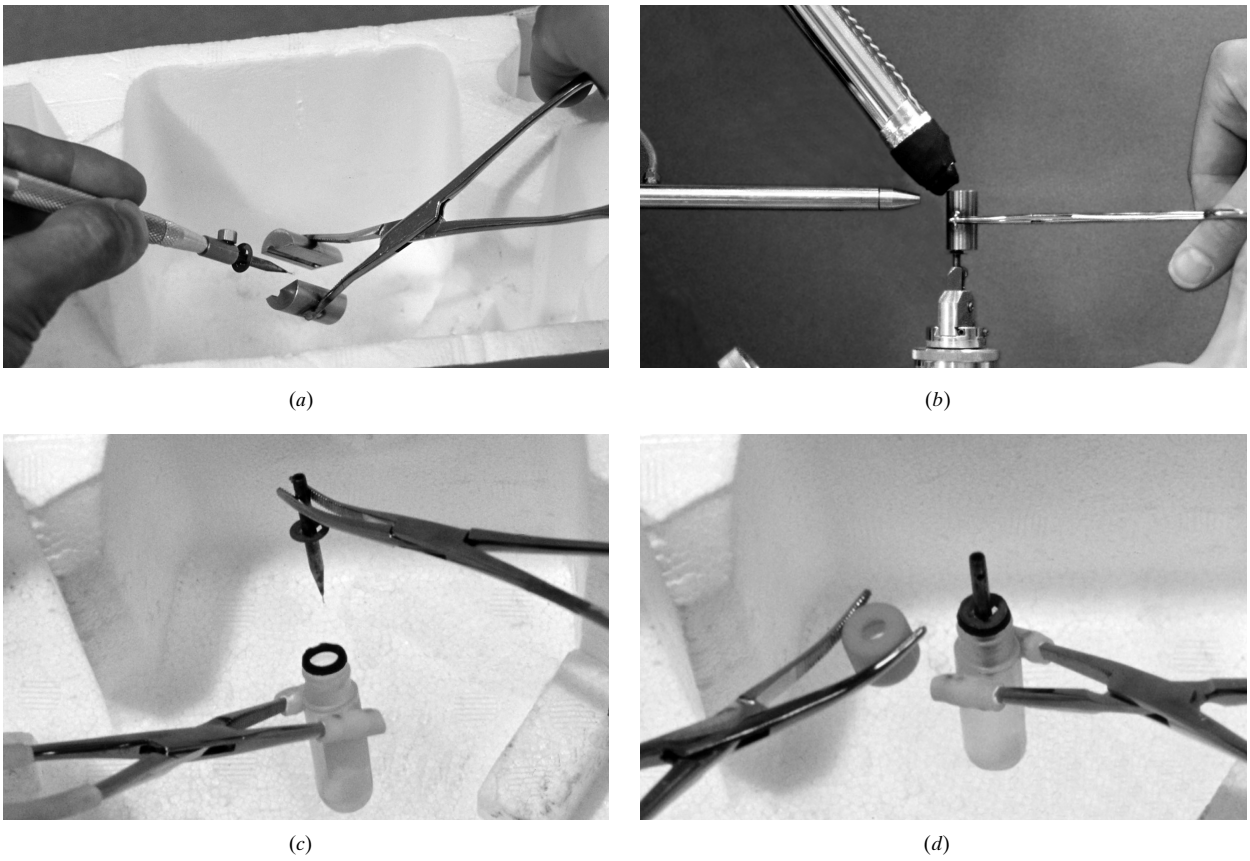
2.4.2. *Antifreeze*. If the oil method fails, or for some other reason is deemed undesirable, the liquid layer adhering to the surface of the crystal must normally be

modified to prevent the formation of ice. In some cases, the mother liquor already contains sufficient amounts of antifreeze so that no further modification is needed and the crystal can be mounted and cooled as is. However, the normal situation is that the mother liquor will freeze on cooling below 200–150 K. If no diffusion of antifreeze into the crystal is contemplated, the process of disturbing the surface liquid does not take much time when an agent of high solubility and low molecular weight is used; typically a few seconds will suffice. We have used glycerol, MPD, ethylene glycol and various PEGs with good results. Our current preference is to increase initially the amount of any suitable organic species present in the mother liquor. In the absence of such, we try glycerol first. The minimum required concentration of antifreeze should be determined before any crystal-cooling experiments are started. Usually, addition of 15–30% of antifreeze to a sample of the mother liquor will suffice. The test is to cool a drop of the modified solvent; if it remains clear there is enough antifreeze.

2.5. *Crystal mounting and cooling*

Removal of a crystal from oil with a straight glass fibre is typically quick and easy because of the relatively high viscosity. Conversely, aqueous antifreeze solutions can have very low viscosity and removal can be tricky even with a loop. Once the crystal is picked up, we recommend immediate cooling, especially for crystals in antifreeze, because drying of the drop can be surprisingly rapid.

The simplest cooling method is to seat the crystal on the goniometer with the cold stream deflected and then rapidly remove the flow obstruction. This operation is greatly speeded up by side entry to the goniometer head. We have found that with some practice it is not necessary to deflect the cold stream. On the other hand, not all crystals will survive the relatively slow rate of cooling involved. Much more rapid cooling is achieved by plunging the mounted crystal into a reservoir of liquid N_2 placed next to the microscope. Once the crystal is in the liquid N_2, there is ample time for the next steps,



(*a*) (*b*) (*c*) (*d*)

Fig. 7. (*a*) The crystal is cooled by rapidly plunging it beneath the surface of liquid N_2 (cryogen omitted here in the interest of clarity). Pre-cooled tongs are then clasped snugly around the shaft of the mounting pin. The steel collar prevents the crystal from being smashed against the top of the bore hole. (*b*) The mounting pin is inserted sideways into the slotted goniometer head. Once the pin is seated, the tongs are opened and drawn downwards in the direction of the cold stream. (*c*) After removal from the goniometer by reversing the mounting procedure, the crystal is transferred under liquid N_2 to a pre-cooled vial (cryogen omitted for clarity). (*d*) Once the crystal is in the vial, the lid is attached. The magnetic ring prevents unwanted movement of the crystal within the vial (cryogen omitted for clarity).

either transfer to a diffractometer or to a storage container.

2.6. *Transfer from liquid nitrogen to diffractometer*

In preparation for mounting a crystal cooled in liquid N_2, the tongs are pre-cooled in the nitrogen bath, *i.e.* submerged until bubble formation has ceased. As soon as the crystal has been picked up it is quickly dunked beneath the surface of the liquid. The mounting pin is then inserted into the tong blocks (Fig. 7*a*) and the tongs are clipped shut. After removal of the mounting-pin holder, the crystal, held securely in the tongs under liquid N_2, is carried to the diffractometer. For crystals grown below room temperature, the mounting and cooling operations can be carried out in a cold room, so the crystals need never be exposed to the ambient temperature of the X-ray laboratory. At the diffractometer the mounting tongs are lifted out of the liquid-N_2 Dewar and the mounting pin is seated in the goniometer head (Fig. 7*b*). If the goniometer head has been fitted with a ball-bearing clip, the tongs can be unhooked, opened and gently drawn away in the direction of cold-stream flow. Essentially, the same operations are possible with magnetic mounts. The relative positioning of the mounting pin, crystal and cold stream is now set and the crystal is ready for optical alignment and X-ray exposure (Fig. 6).

2.7. *Crystal storage and transportation*

Storage of crystals at cryogenic temperatures requires transfer to a suitable container that will maintain the sample at low temperature. Transportation includes the added complication that the container must be portable and provide sufficient protection from mechanical shock. Use of readily available materials is also desirable. The cryovial shown in Fig. 5 forms the basis of our solution to this problem. The storage-system components also include ordinary cryogenic canes or racks, commonly used for storage of biological samples.

To get the crystal into the storage vial, it is first removed from the diffractometer using pre-cooled tongs and placed under liquid N_2 in an exact reversal of the procedures described for crystal mounting. In the liquid-N_2 bath the mounting pin is gripped with a pair of curved-nose haemostat tongs and the mounting tongs are removed. A pre-cooled vial is positioned under the crystal and the mounting pin is inserted (Fig. 7*c*). The magnetic ring holds the mounting pin centred while the screw cap is attached (Fig. 7*d*). It is then clipped into a storage cane and transferred to a Dewar. We have tested the sturdiness of these storage vials by dropping a cold-mounted crystal (concanavalin A) 3 m onto bare concrete. The crystal remained attached to its mount and was not damaged.

3. Performance

Reproducibility is of prime importance in scientific experiments. In work involving crystal cooling, this means maintaining control of crystal temperature throughout all operations. Inadequacies of other mounting techniques are that they involve steps in which it is difficult or impossible to know the crystal temperature with certainty at all times. Movements of the cold stream away from its data-collection position (Rodgers, 1997) should be avoided as the act of repositioning it can affect crystal temperature in an unpredictable way. In the case of liquid propane, large temperature gradients can develop within the cryogen. Moreover, once the crystal 'popsicle' (Rodgers, 1997; Garman & Schneider, 1997) has been mounted, the frozen hydrocarbon must be removed. Whether this is achieved by melting or sublimation, control of crystal temperature may be lost. The crux of our methods is the specially designed tongs. They allow a crystal to be mounted on a diffractometer of arbitrary geometry without the need for repositioning of any of the apparatus. To determine reproducibility and control, we have measured the temperature at the crystal position during crystal-mounting and dismounting operations.

3.1. *Warm-up rates for crystal-mounting tongs*

To measure the warm-up rates with the crystal-mounting tongs, a fine thermocouple [home made, 0.003″ (0.076 mm) Cu-constantan wires], referenced in liquid N_2, was threaded through a copper mounting pin so that the thermocouple junction was about 2.5 mm above the tip of the pin. The pin was then clasped in the tongs and submerged in liquid N_2 until the whole

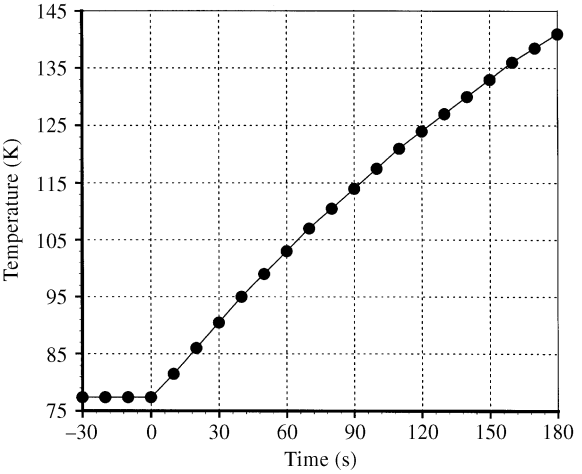


Fig. 8. A plot of temperature *versus* time for a fine thermocouple held at the position of a mounted crystal within the mounting tongs. The thermocouple was coated with grease to simulate the bulk of a typical crystal. The initial warming rate is approximately 0.5° per second for the tongs described here.

reached 77 K. Following this, the tongs were removed from the liquid N_2 and the temperature rise *versus* time was monitored. The results are shown in Fig. 8. Performance is dependent on both the size and composition of the blocks, but it is clear that the warming rate is sufficiently slow to allow a crystal to be mounted without undue crystal heating. It typically takes less than 5 s to transfer the crystal to the goniometer. In this time the crystal temperature rises just a few degrees, so in general it is still colder than a typical cold gas stream.

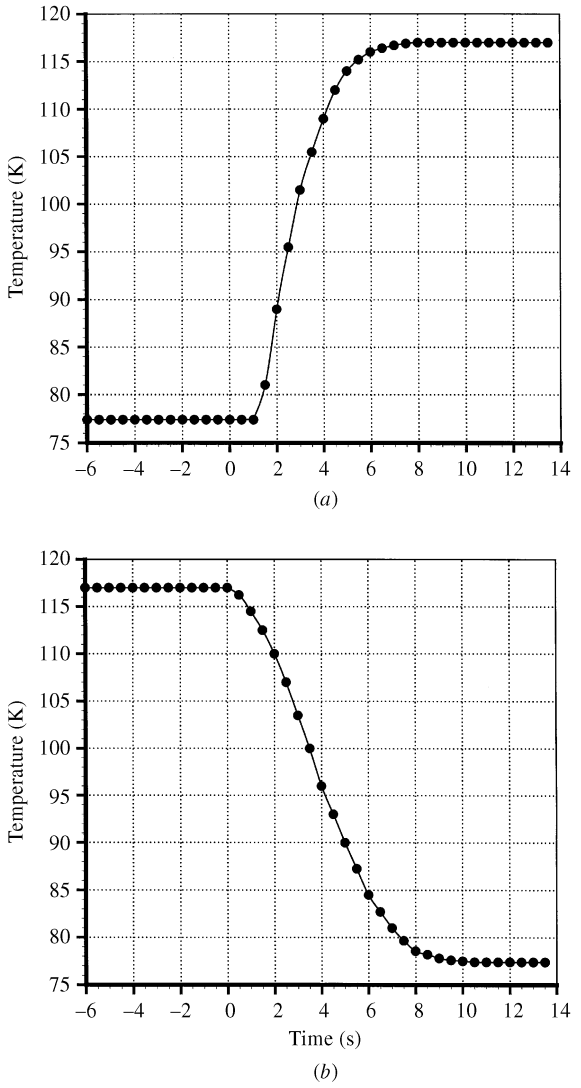


Fig. 9. A plot of temperature *versus* time for a fine thermocouple undergoing the crystal-mounting process. The thermocouple–mounting-pin assembly was lifted out of the liquid N_2 at time $t = 0$ s and mounted less than 2 s later. The temperature remained at 77.4 K for about 1 s after removal, presumably because of a small volume of liquid N_2 initially trapped in the cavity. A plot of temperature *versus* time for the dismounting process. The thermocouple–mounting-pin assembly was removed from the goniometer at time $t = 0$.

3.2. *Temperature history of a crystal during mounting and dismounting*

The temperature experienced by a crystal during the mounting and dismounting operations using stainless-steel tongs was measured. A representative run for mounting is shown in Fig. 9(*a*). At no time did the temperature rise above that of the cold gas stream. A controlled temperature response was also obtained for the dismount procedure (Fig. 9*b*). It is, however, important to have the opening of the tongs parallel to the cold stream rather than crosswise, so as to prevent turbulence. In transfer to the storage cryovials, the temperature never rises above 77 K, which is not surprising considering the system remains submerged under liquid N_2 throughout.

4. Concluding remarks

The techniques described in this paper represent the current status of methods that have been evolving in our laboratories for over 25 years. The major impetus for their development was the desire for simple, easy-to-learn procedures. We firmly believe that fewer and simpler experimental steps lead to fewer failures and less frustration, and hence to better results. Of paramount importance, however, is the need for reproducibility. These methods suffer from none of the drawbacks associated with other techniques currently in use and are easier to implement. This is evident in the speed at which the basic principles have been adopted by other groups.

We thank all those who have helped in the design, modification, construction and testing of the tools and techniques discussed in this paper, with special mention of Felix Frolow (Weizmann Institute), Wasyl Krimetz (UC Davis), Mark Knapp, Sergei Trakhanov and Bernhard Rupp (Lawrence Livermore National Laboratory) and of Mike Soltis and Henry Bellamy (Stanford Sychrotron Radiation Laboratory). Part of this work was performed under the auspices of the US Department of Energy, contract No. W-7405-ENG-48, at the Lawrence Livermore National Laboratory.

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Files: h:\iuoc/jacj/wb0058/wb0058.3d, h:\iuoc/jacj/wb0058/wb0058.sgm Paper number: J980530–WB0058 Paper type: CN N TE-9813/13(3)11