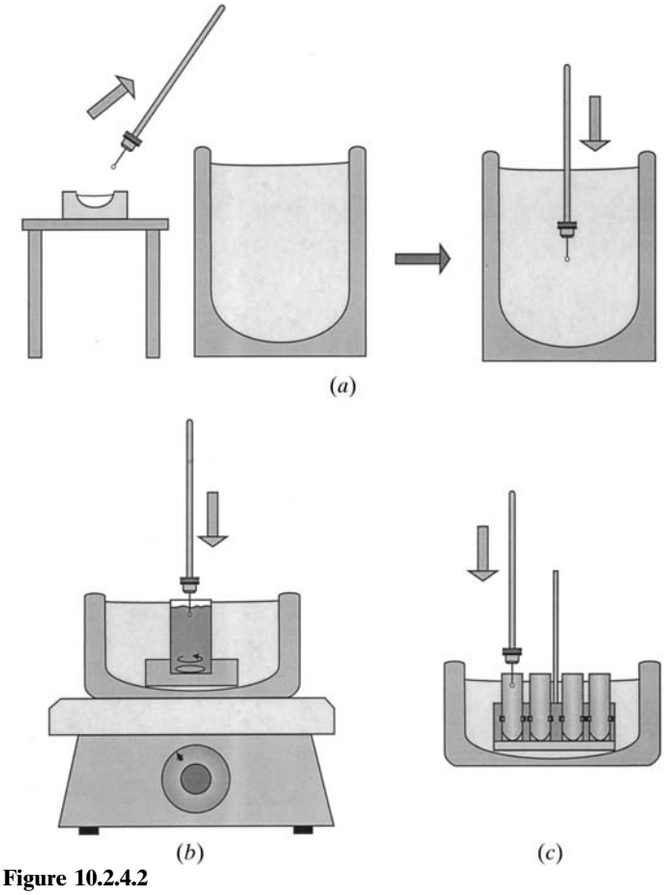

Figure 10.2.4.2
Flash cooling in a liquid cryogen. (*a*) Cooling in liquid nitrogen. The loop assembly is attached, *via* a magnet mount, to a short rod and the crystal is captured. It is then quickly plunged into a nearby Dewar of liquid nitrogen. (*b*) One method of flash cooling in a liquid cryogen such as propane. The cryogen is placed in a weighted container, which itself stands in a Dewar of liquid nitrogen. The Dewar rests on a stir plate, which mixes the liquid cryogen to ensure a uniform temperature. When the temperature of the cryogen is just above its melting point, the loop assembly is plunged into the liquid. (*c*) A variation on cooling in propane or a similar cryogen. The cryogen is placed into small plastic vials designed for cryogenic storage. Just before the cryogen freezes, the loop assembly is plunged directly into a vial. A holder for the vials allows multiple samples to be prepared sequentially.

gas around the crystal. To minimize drying of the sample during transfer, the crystal container and the Dewar are located as close as possible. If necessary, drying can be further reduced by using a portable humidifier to add moisture in the area.

Other cryogens, such as propane, can be tested if results with liquid nitrogen are not satisfactory. Two methods for flash cooling in these other cryogens are illustrated in Figs. 10.2.4.2(*b*) and (*c*). In the first (Fig. 10.2.4.2*b*), the liquid cryogen is held in a small container with a weighted base, which is placed in a Dewar of liquid nitrogen to cool the cryogen. The cryogenic liquid is mixed using a magnetic stir bar to ensure a uniform temperature throughout the sample. Since the boiling points of these cryogens are well above their melting points, it is possible in the absence of stirring to have relatively warm, and therefore less effective, cryogen near the top of the container. When a temperature probe indicates that the cryogen is just above its melting point, the crystal is mounted and plunged quickly into the liquid. A variant of this technique (Fig. 10.2.4.2*c*) calls for plunging the loop assembly directly into cryogen-filled plastic vials, which are used for low-temperature transfer and storage of the crystals (see Section 10.2.5). The cryogen is then allowed to solidify around the crystal before it is placed on the X-ray camera or stored for later use. With this technique, it is more difficult to ensure that the temperature of the cryogen is uniform throughout the container. Other mechanisms for flash cooling in liquid cryogens have been described (Hope *et al.*, 1989; Abdel-Meguid *et al.*, 1996), and devices for combining xenon derivatization with flash cooling (Soltis *et al.*, 1997) are available commercially.

10.2.5. Transfer and storage

Crystals flash cooled in a liquid cryogen must be placed for data collection in the cold gas stream of a cryostat without any substantial warming. One common transfer method (Rodgers, 1994, 1997) is shown in Fig. 10.2.5.1. Once the loop assembly has been plunged into the Dewar of liquid nitrogen, it is inserted into a small plastic vial of the type normally used for cryogenic storage, ensuring that the sample remains below the liquid surface during the operation. There is then sufficient liquid nitrogen in the vial to keep the sample cold as it is transferred to the cryostat gas stream. Again, the magnetic mounting system is used to reduce the time required for transfer. For X-ray cameras with vertical spindles, as shown in Fig. 10.2.5.1, some means of pointing the magnetic mount downward is required to prevent the nitrogen from spilling out of the vial. The goniometer illustrated has a detachable arc extension (Engel *et al.*, 1996; Litt *et al.*, 1998) that provides this capability. When the loop assembly is attached to the magnet, the vial is quickly withdrawn, exposing the crystal to the gas stream. The arc slide can then be returned to the normal position and the arc extension removed.

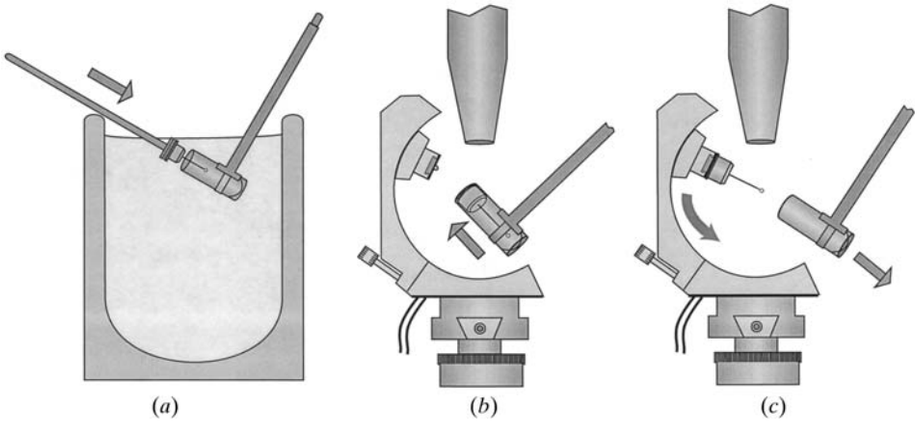

Figure 10.2.5.1
Transfer of the flash-cooled crystal and loop assembly to a goniometer using a cryovial. (*a*) The loop assembly with a flash-frozen crystal is placed in the vial, which is held by a rod-shaped tool. The operation is carried out beneath the surface of the liquid nitrogen in a Dewar. (*b*) The loop assembly is transferred to the goniometer using the magnetic mounting system. (*c*) The vial is withdrawn, exposing the crystal to the cold gas stream. With this arrangement of goniometer and cryosystem nozzle, it is necessary to use a device that allows the magnetic mount to point downward. Here, a detachable arc extension provides this ability. After crystal transfer, the arc slide can be returned to the normal position and the extension removed.

Another device (Mancia *et al.*, 1995) for achieving the correct transfer geometry is shown in Fig. 10.2.5.2. This 'flipper' mechanism can be extended to permit transfer of the crystal. The device is then rotated about the hinge to reorient the loop assembly for data collection. The

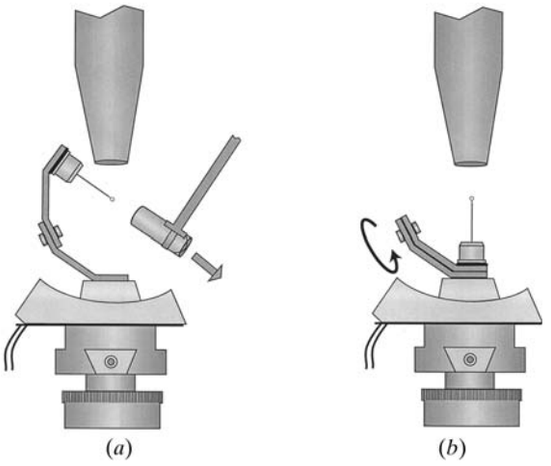

Figure 10.2.5.2
Transfer using an alternative device for achieving the correct magnetic mount orientation. (*a*) A hinged mechanism is extended to orient the magnetic mount downward, and the loop assembly is attached. (*b*) The mechanism is rotated about the hinge to place the mount in the normal orientation for data collection.

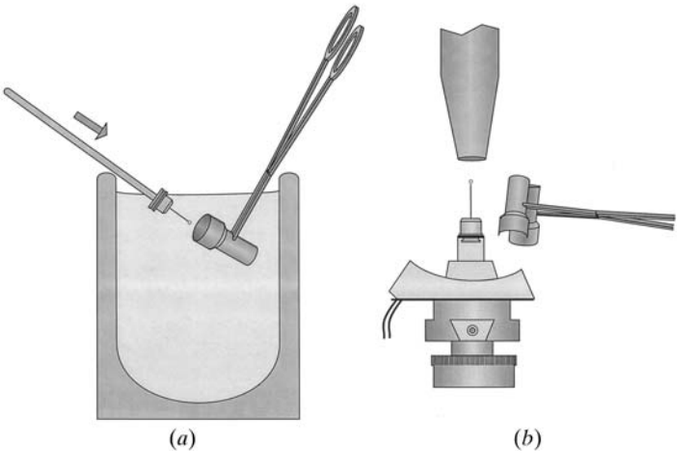

Figure 10.2.5.3
Transfer using tongs. (*a*) The crystal is inserted into a split metal cup that allows the base to be held securely. (*b*) The tongs are inverted and used to place the loop assembly on the magnetic mount. The jaws of the tongs are then opened to separate the halves of the cup, and the tongs are withdrawn.

hinge is positioned so that rotation does not translate the crystal, keeping it in the cold stream during reorientation.

When cooling in other liquid cryogens such as propane, the same cryovial transfer system is used. Flash cooling in cryovials (Fig. 10.2.4.2*c*) permits direct transfer using the magnetic mounting system. Usually, the liquid cryogen has been solidified in the vial, and it is allowed to melt at least partially before placing the crystal on the goniometer. Any remaining solid then melts and drips away (although it is often necessary to remove the last drop on the crystal with filter paper). When cooling in a larger volume of cryogen (Fig. 10.2.4.2*b*), the crystal can be 'hopped' rapidly from the cooling cryogen to the surrounding vat of liquid nitrogen. A drop of cryogen transfers with the crystal, keeping it from warming. The loop assembly can then be placed in a cryovial and transferred to the goniometer.

Another device that does not use cryovials has been introduced (Parkin & Hope, 1998) to facilitate transfer from liquid nitrogen. The device consists of a split metal cup attached to handles that allow the cup to be opened and closed. When closed, the two halves of the metal cup form a cavity that can accommodate and grasp the loop assembly. As shown in Fig. 10.2.5.3, the loop assembly is inserted after first cooling the tongs in liquid nitrogen. The thermal mass of the tongs prevents warming as the crystal is then placed on the goniometer. The tongs are opened and removed to expose the crystal to the gas stream.

Any of these transfer procedures can be reversed in order to return the loop assembly to liquid nitrogen without thawing the crystal. The assembly and cryovial can then be placed in a Dewar designed for long-term storage. Some opening should be present in the loop-assembly bases, or the cryovials should be notched, to allow free movement of liquid nitrogen. The vials are conveniently held and organized using aluminium canes, which take up to five samples and have tabs that hold the loop assemblies in place. For even more secure long-term storage, loop assemblies with threaded bases that screw into the cryovials are available.

The ability to store samples for long periods of time permits a number of crystals to be flash cooled under consistent conditions, which can be important for maintaining isomorphism, and crystals can also be stockpiled for later data collection at a synchrotron X-ray facility. In fact, crystals should be prescreened for quality in the laboratory before synchrotron data collection to make efficient use of time on the beam line. Finally, crystals that degrade in growth or harvest solutions, or that contain macromolecules in unstable or transient states, can be conveniently preserved by flash cooling and storage in liquid nitrogen.

References

Abdel-Meguid, S. S., Jeruzalmi, D. & Sanderson, M. R. (1996). *Crystallographic Methods and Protocols*, edited by C. Jones, B. Mulloy & M. R. Sanderson, pp. 55–87. New Jersey: Humana Press.

Bishop, W. H. & Richards, F. M. (1968). *Properties of liquids in small pores. Rates of diffusion of some solutes in cross-linked crystals of β-lactoglobin. J. Mol. Biol.* **38**, 315–328.

Dewan, J. C. & Tilton, R. F. (1987). *Greatly reduced radiation damage in ribonuclease crystals mounted on glass fibres. J. Appl. Cryst.* **20**, 130–132.

Engel, C., Wierenga, R. & Tucker, P. A. (1996). *A removable arc for mounting and recovering flash-cooled crystals. J. Appl. Cryst.* **29**, 208–210.

Fink, A. L. & Petsko, G. A. (1981). *X-ray cryoenzymology. Adv. Enzymol. Relat. Areas Mol. Biol.* **52**, 177–246.

Garman, E. F. & Mitchell, E. P. (1996). *Glycerol concentrations required for cryoprotection of 50 typical protein crystallization solutions. J. Appl. Cryst.* **29**, 584–587.

Garman, E. F. & Schneider, T. R. (1997). *Macromolecular cryocrystallography. J. Appl. Cryst.* **30**, 211–237.

Gonzalez, A. & Nave, C. (1994). *Radiation damage in protein crystals at low temperature. Acta Cryst*. D**50**, 874–877.

Haas, D. J. (1968). *X-ray studies on lysozyme crystals at −50 °C. Acta Cryst.* B**24**, 604.

Haas, D. J. & Rossmann, M. G. (1970). *Crystallographic studies on lactate dehydrogenase at −75 °C. Acta Cryst.* B**26**, 998–1004.

Henderson, R. (1990). *Cryoprotection of protein crystals against radiation damage in electron and X-ray diffraction. Proc. R. Soc. London Ser. B*, **241**, 6–8.

Hope, H. (1988). *Cryocrystallography of biological macromolecules: a generally applicable method. Acta Cryst.* B**44**, 22–26.

Hope, H. (1990). *Crystallography of biological macromolecules at ultra-low temperature. Annu. Rev. Biophys. Biophys. Chem.* **19**, 107–126.

Hope, H., Frolow, F., von Böhlen, K., Makowski, I., Kratky, C., Halfon, Y., Danz, H., Webster, P., Bartels, K. S., Wittmann, H. G. & Yonath, A. (1989). *Cryocrystallography of ribosomal particles. Acta Cryst.* B**45**, 190–199.

Litt, A., Arnez, J. G., Klaholz, B. P., Mitschler, A. & Moras, D. (1998). *A eucentric goniometer head sliding on an extended removable arc modified for use in cryocrystallography. J. Appl. Cryst.* **31**, 638–640.