[10] Treatment and Manipulation of Crystals

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General Considerations

Throughout a crystal structure analysis, it is necessary to physically and chemically manipulate the crystals being studied. These operations include crystal mounting prior to data collection, preparation of heavy-atom derivatives, and changing the solvent for low-temperature diffraction studies or studying the contrast between proteins, nucleic acid, and solvent in the crystal. The prime objective of all of these manipulations is to preserve the inherent order of the crystals.

Protein crystals are usually grown from an aqueous solution, and when they have finished growing they are in equilibrium with the solution that surrounds them. Any rapid change in the equilibrium may result in damage to the crystals. Thus, the first rule in crystal manipulation is not to make dramatic changes in the crystallizing solution (or mother liquor, as it is generally termed).

The preparation of derivatives often entails placing a crystal in a new solution which contains the heavy-atom compound of interest. It is important to establish a synthetic mother liquor to which the crystals may be transferred without reducing their order. Crystals which are grown in high salt or polyethylene glycol may often be stabilized by slowly increasing the concentration of the precipitant such that they will not dissolve when they are moved. Crystals which are grown from low salt present a greater problem since they are often in equilibrium with a significant protein concentration in the solution. Occasionally they may be stabilized by the addition of a low concentration of polyethylene glycol.

Crystal Mounting

The purpose of crystal mounting is to isolate a single crystal from its growth medium so that its diffraction properties may be studied. It is vital that the manipulation of the crystal introduce as little damage as possible to its three-dimensional order.

Protein crystals usually contain a large proportion of the aqueous medium in which they were grown. This varies from as little as 29% for edestin to as much as 95% for tropomyosin.[1] The solution forms an inte-

[1] B. W. Matthews, *J. Mol. Biol.* **33,** 491 (1968).

gral part of the crystal lattice. Its loss results in physical shrinkage of the crystal and reduction of the three-dimensional order as detected by X-ray diffraction. The most important aspect of crystal mounting is to preserve the crystal in its state of hydration. This may be accomplished by sealing the crystal in a thin-walled (0.001 mm thick) glass or quartz capillary tube.[2,3] The crystal manipulations are most easily viewed using a binocular dissecting microscope and should be performed quickly to minimize the dehydration of crystals. The exact procedures used will depend on the strength and morphology of the crystals and also the vessel in which they are grown. It is generally easier to mount a crystal from a shallow depression slide (routinely used for growing crystals by vapor diffusion) than from a straight-sided vial. The ease of mounting should be considered when growing crystals. The important steps in crystal mounting are illustrated in Fig. 1.

The first step in mounting the crystals is to dislodge them from the surface on which they grew. Robust crystals may be nudged gently with a glass rod or with the open end of the capillary. A fragile or soft crystal may be loosened by squirting a narrow jet of the crystallizing medium at its base. A controlled way of doing this is to use a pipettor set at ~25 μl. Once the crystal is free it may be drawn gently into the capillary using suction from a small volume (0.25 ml) glass syringe or mouth aspirator (Fig. 1a). The end of the capillary should be broken off cleanly; this facilitates drawing up the crystal and reduces the chance of a hairline crack running up the tube which could cause the crystal to dry out. Quartz tubes may be cleaved by scratching them with a diamond marking pencil and bending gently at that point. Glass tubes, which tend to be more fragile, may be broken cleanly by running a thin band of melted wax around the tube and fracturing just beyond the wax. It is good practice to acid-wash the glass capillaries prior to using them in order to remove any alkaline deposits remaining from their manufacture.

Next, the capillary should be inverted to allow the crystal to fall to the inner meniscus. Then any gross excess solution may be returned to the crystallizing droplet without losing the crystal. Conserving the crystallizing solution in this way may allow several crystals to be mounted from a small droplet.

The crystal should be drawn further into the capillary (2–3 cm) and rotated until it lies in roughly the required orientation. Then the surrounding solution may be removed using thin strips of filter paper or by the

[2] M. V. King, *Acta Crystallogr.* **7**, 601 (1954).

[3] K. C. Holmes and D. M. Blow, "The Use of Diffraction in the Study of Protein and Nucleic Acid Structure." Wiley (Interscience), New York, 1966.

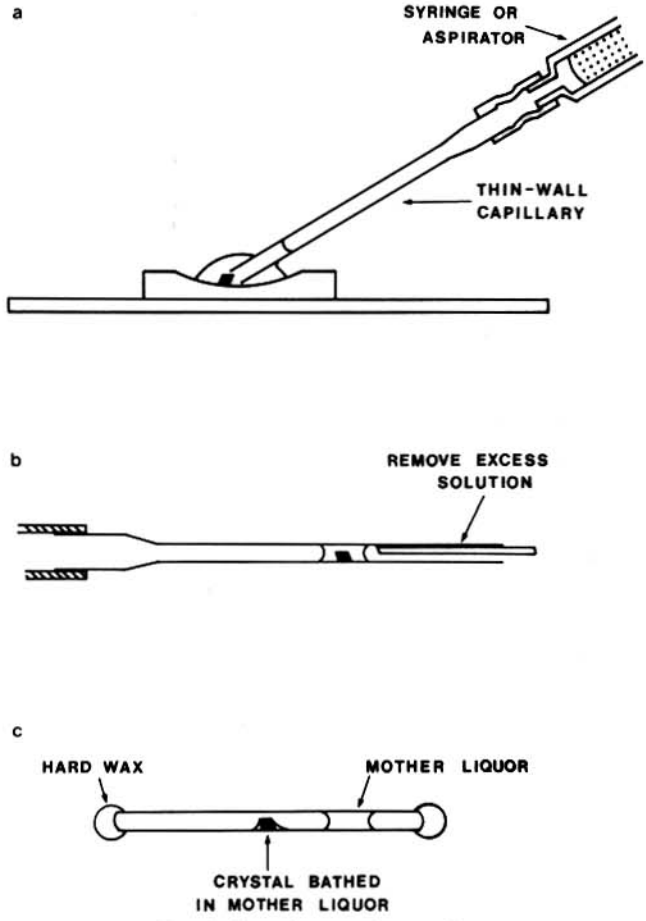

FIG. 1. Steps in crystal mounting.

capillary action of a thin glass rod (Fig. 1b). Care must be taken not to touch the crystal at this stage. The extent to which the crystal should be dried must be determined by experience. Some crystals may be thoroughly dried while others must be left quite wet to preserve their order. Robust crystals may be further oriented by nudging them gently with a glass rod.

The final step in crystal mounting is to place a small volume of mother liquor in the capillary and seal both ends. In principle, if the capillary is totally sealed, there will be sufficient solution directly associated with the crystal to maintain the correct degree of hydration. However, it is common practice to draw a little additional solution into the tube or to place a

small piece of filter paper moistened with the crystallizing solution close to the crystal. The presence of this additional solution often proves troublesome if there is any temperature differential across or along the capillary tube.

This situation often arises when collecting diffraction data at reduced temperatures. Migration of water vapor results in the crystal either losing or gaining water, leading to dehydration, slipping, or dissolving. These problems may be overcome by removing the excess mother liquor completely and placing a column of silicone oil on either side of the crystal. Crystals mounted in this way may remain hydrated for several months if the capillary has no holes or cracks. Finally, the open end of the capillary should be sealed thoroughly with melted wax (sealing or high vacuum) and then cleaved 2–3 cm from the crystal and sealed (Fig. 1c) again. If the crystal is not to be used immediately, it may be advantageous to seal the capillary further by coating the wax with epoxy resin. This process strengthens the thin-walled capillary and seals any small cracks which may have been formed when the tube was cut.

Crystal Slippage

Crystal slippage has occasionally been a problem in macromolecular crystallography, particularly if the crystals have to be left very wet. There are several solutions which may work in any given instance.

There is often a tendency for a crystal to slip immediately after mounting. The simplest way to reduce slippage is to allow the crystal to settle for a day or so before use. This is particularly effective if the crystal can be oriented optically on a set of goniometer arcs and allowed to rest in the same orientation that it will be examined. This approach is best suited to photographic data collection in which the angular movement of the crystal is quite small.

An alternate approach is to hold the crystal in place by a thin plastic film.[4] This film is prepared *in situ* with a 0.2% solution of poly(vinyl formal) 15/95 powder (Polyscience Inc., Warrington, Pennsylvania) in 1,2-dichloroethane. This mixture, which is immiscible with water, will spread over an aqueous surface and leave a thin plastic film when the solvent evaporates. After drying the crystal a small volume of this solution may be drawn into the capillary tube until it just touches the crystal. The film will spread instantaneously over the crystal. The excess solution should be expelled and the capillary sealed in the normal way. This proce-

[4] I. Rayment, J. E. Johnson, and D. Suck, *J. Appl. Crystallogr.* **10**, 365 (1977).

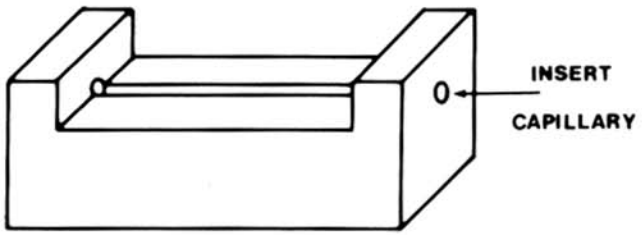

FIG. 2. Block for flattening capillaries.

dure does not increase the background scatter or damage the crystal in any way.

Crystal slippage may also be reduced by mounting the crystal on a portion of a capillary that has been flattened to form a small table. This results in more contact between the crystal and the capillary, thus giving greater adhesion. This approach is particularly advantageous for thin flat crystals which would otherwise crack in order to conform to a round capillary. Capillaries may be flattened using the block shown in Fig. 2. The tube is placed in the trough in the metal block such that the exposed surface may be heated and softened using an oxybutane torch.