

A simple and rapid method for mounting protein crystals at room temperature

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Cryocooling of protein crystals for X-ray data collection has now become a routine method in the majority of biostructural laboratories. The improvement of facilities at synchrotron sources and their increased use has made it essential to have properly frozen crystals for optimal data collection. Although in general crystals can be cooled without significant damage, there are often cases in which crystals with slight disorder or twinning problems suffer considerably during the freezing process. In other cases, poor or mosaic diffraction may be blamed on the cryoprotectant or cooling protocol. Many crystals may be wasted in searching for the best freezing conditions when the intrinsic quality of the crystals is poor. In principle, the collection of room-temperature diffraction data would provide a reference that would allow the detection of crystal damage caused by addition of cryoprotectant or by cryocooling. In practice, however, many investigators are reluctant to do this, one reason being that capillary mounting of crystals is a tedious method, especially for those who are new to crystallography. Here a simplified method for mounting crystals at room temperature is reported, which requires little expertise and no expensive equipment.

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1. Materials and methods

The following items were obtained from Hampton Research: pre-mounted loops (catalog No. HR4-993), copper rods (HR4-685), quartz capillaries (HR6-148), cutting stone (HR4-334), goniometer head (HR-643) with magnetic base (HR4-627), and glucose isomerase (HR7-100).

Crystal diffraction was measured on a Nonius FR591 X-ray generator with a MAR 345 detector and Osmic mirrors. An appropriately sized loop mounted on a copper rod, of length 13 mm, with a standard Hampton magnetic cap was used to remove crystals from their drops, as for normal freezing procedures. Soft plasticine was placed around the cap (Fig. 1) where the copper rod is attached to permit subsequent sealing of the capillary. Using a cutting stone, a 1.5 mm diameter quartz capillary was cut approximately 30 mm from the open end with larger diameter (about 3 mm; Fig. 2). The crystal was removed from the drop and the loop containing the crystal (Fig. 3) was quickly inserted into the capillary. The capillary was pushed into the plasticine to form a temporary seal and approximately 10 μ l of stabilizing solution was then pipetted into the capillary in order to maintain a humid environment. The mounted capillary (Fig. 4) was placed on a standard goniometer head adapted for magnetic crystal caps (Hampton Research catalog No. HR4-647) for data collection.

2. Results and discussion

Glucose isomerase crystals mounted in this way diffract to high resolution (comparable with frozen crystals), although exposure times compared with those of frozen crystals should be slightly longer because of absorption of X-rays by the quartz capillary. Crystals mounted in this way were stable in the X-ray generator environment

for at least 30 min; longer data collections may require sealing of the capillary at both ends using epoxy resin. Four co-workers in our group were able to apply the method successfully after receiving a brief description. Another advantage of this method compared with traditional room-temperature mounting techniques is that very small crystals can be isolated that would be difficult to isolate using the standard methods. Once the crystal has been shown to diffract at

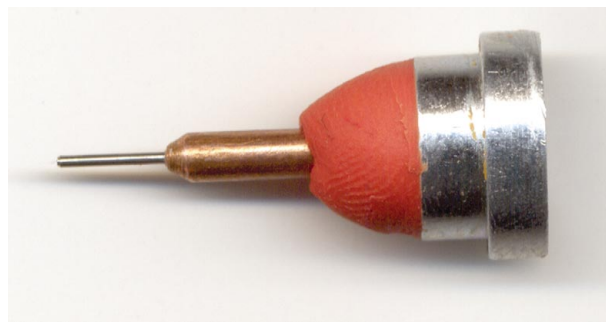


Figure 1
Crystal cap with plasticine.



Figure 2
Cut 1.5 mm capillary (with solution added).

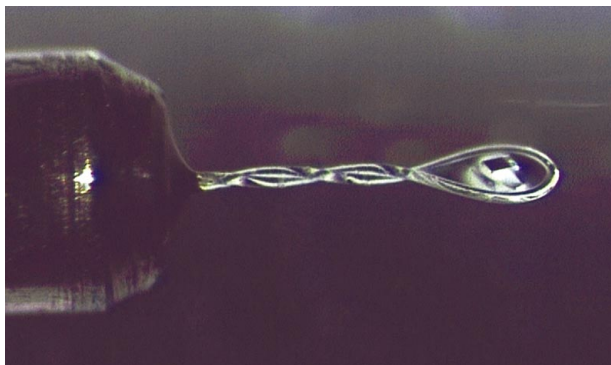


Figure 3
Crystal mounted in loop.

room temperature, it is also possible to remove the capillary and cryocool the crystal for a direct comparison of diffraction.

This method is easy to learn and is not time consuming. It may encourage more researchers to test crystals at room temperature before drawing conclusions about crystal quality from frozen crystals.



Figure 4
Loop-mounted crystal enclosed in capillary.

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References

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