Reducing the Mosaicity of Flash-Cooled Crystals

Flash cooling protein and virus crystals always increases their mosaicity. As-grown, room-temperature mosaicities are often as small as 0.005° (when measured using small-divergence X-ray sources). But mosaicities at T=100 K are typically between 0.3° and 1°. Large mosaicities reduce diffraction signal to noise (especially when low-divergence X-ray beams are used) and increase spot overlap. What can you do to reduce flash-cooling-related mosaicity increase?

First, verify that cooling your crystals is the source of your poor mosaicity. Make sure that the crystal isn't visibly moving in the cryostream, when viewed using a high magnification telescope or camera. Then check the diffraction of your crystal before you freeze it using the MicroRT™ system. Use short exposures to minimize radiation damage.

Assuming your room temperature mosaicity is ok, here are some things to try:

- Reduce the amount of excess liquid around your crystal. When this liquid freezes, it exerts stresses on the crystal that can bend or crack it. Use a mount with an aperture that's a bit smaller than the crystal. Carefully remove excess liquid using a size 15 paper wick or by gently tapping on the steel rod of the mount.
- If possible, avoid using thin plates and rods, which are more easily bent and cracked. Smaller, more three dimensional crystals can give better results.
- Reduce the beam spot size to examine only a small part of the sample, and scan the beam relative to the sample to find the region with the lowest mosaicity.
- Make sure that the crystal isn't dehydrating just before or during flash cooling. This is especially important for smaller crystals, which dry out very quickly. Transfer the crystal to LV CryoOil before mounting, and mount with as little remaining oil as possible.
- Look carefully at your sample through the telescope or on the video monitor. If it's fluttering in the cryostream, please refer to the instructions to Minimizing Sample Motion in Cold Gas Streams.
- Make sure your flash cooling set-up and protocol is giving fast and uniform cooling. Warkentin *et al.'s* hyperquenching protocol, a variant on standard plunge cooling in liquid nitrogen in which you blow away the cold gas layer that forms above the liquid nitrogen, gives the most reliable results.[1]
- If fast cooling doesn't succeed, try slowly cooling the crystal.[2]

Please contact xtals@jenabioscience.com with comments or suggestions.

[1] Warkentin et al. (2006) Hyperquenching for protein cryocrystallography. *J. Appl. Cryst.* **39**:805
[2] Warkentin et al. (2009) Slow cooling of protein crystals. *J. Appl. Cryst.* **42**:944