

Diffraction study of protein crystals grown in cryoloops and micromounts

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Abstract

Protein crystals are usually grown in hanging or sitting drops and generally get transferred to a loop or micromount for cryo-cooling and data collection. This paper describes a method for growing crystals on cryoloops for easier manipulation of the crystals for data collection. This study also investigates the steps for the automation of this process and describes the design of a new tray for the method. The diffraction pattern and the structures of three proteins grown by the new method and the conventional hanging drop method are compared. The new setup is optimized for the automation of the crystal mounting process. Researchers could prepare nanoliter drops under ordinary laboratory conditions by growing the crystals directly in loops or micromounts. As has been pointed out before, higher levels of supersaturation can be obtained in very small volumes and the new method may help in the exploration of additional crystallization conditions.

Legends for the Supplementary files

Video 1

This video shows preparation of the Nextal tray, placing of the film on the loops/micromounts, and putting the micromount containing a crystal on the beamline. This video has five video clips merged into one. First part shows tray preparation. Second part shows the preparation of the hanging drop. Third part shows loading of the films on to the loops and MicroMounts (Note: Since microscope focuses for the video port and the viewing port were different, it was slightly difficult to see the drops well while keeping the video focused on the loops. This gave additional difficulty in loading the films.). Fourth part shows a 2 days old tray with crystals in the drops and in the support. Fifth part shows dislodging the pin and placing it on the beamline for flash cooling at 100K. The small dark box in the screen is 100 x 100 um (at the highest zoom shown in the video) and the size of the mounted crystal is ~190 x 140 um. The lysozyme crystals for this experiments were grown by using cryoconditions (1M NaCl, 50mM NaOAc (pH 4.5) and 25% ethylene glycol). We thank the referee for suggesting to load the video of the procedure.

Video 2

This video describes the use of magnets for the operation (Figure 2 and the basic idea for the new crystallization tray). This video has two video clips merged into one. The first part shows the magnet. In this experiment we are using double magnet assembly inserted into a Teflon rod. This enables rotation of the loops at any time during loading films or for orienting loop to inspect the crystals. The second part shows loading of the film onto a MicroGripper and putting it on a goniometer.