Shock freezing of macromolecular crystals at MacCHESS

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The collection of macromolecular diffraction data has recently benefited from techniques and equipment at MacCHESS which have made low-temperature data collection almost routine. The ability to freeze protein crystals at liquid nitrogen temperatures normally gives the level of radiation protection necessary to collect complete data sets from single crystals. To this end, MacCHESS has upgraded both its equipment and its capability to offer support to assure that visiting user groups can routinely collect low-temperature data while at the synchrotron.

The collection of low-temperature data on protein samples hinges around a loop mounting technique first developed here at Cornell by T.Y. Tung. In the original technique, crystals were mounted by lifting them in thin wire loops by surface tension from solutions of mother liquor containing some cryoprotecting molecule. These molecules were generally freezing point-depressing solutes such as sucrose or low molecular weight alcohols such as glycerol, ethylene glycol, propylene glycol, or methylpentanediol (MPD). In addition to such alcohols, lower molecular weight polyethylene glycols such as PEG 400 or 600 have been used successfully. Recently, the technique has been improved by replacing the wire loops with loops fashioned from glass wool, rayon, nylon, silk, or any other thin, non-diffraction fiber. Using such equipment, 560° of continuous rotation data can be collected from a single crystal without worrying about significant shadowing or increases in absorbance or scatter from the loop mounts. A schematic of a loop design currently used at CHESS and a photograph of an actual loop mounted crystal are shown in Figure 1.

This key to shock freezing crystals is maximizing the efficiency of the initial freezing rate, or the shock. Two methods are currently used to accomplish this. First, crystals can be frozen directly in the cold stream. In this method, a reliable cryostat capable of maintaining a laminar cold stream at -165°C to -175°C with a good flow rate is essential. Actual freezing involves temporarily defocusing the cold stream with something as simple as an index card while the crystal is quickly soaked in the diffraction position usually using a goniometer mounted magnet and a loop attached to a steel mounting cap. Then, the stream deflection is removed and the low-temperature gas is used to flash freeze the crystal. In the second technique, loop mounted crystals are first immersed in some low-temperature liquid such as liquid nitrogen, propane, or freon. Liquid propane freezing is the most likely trend of the future as it provides the best thermal capability for heat conduction away from the immersed crystal. After immersion, the crystal is soaked at the sample position still in the low temperature liquid, and the vessel containing the liquid is quickly removed. In this case, the liquid is used to cause the initial flash and the challenge is smoothly transferring the frozen crystal to the gausson cold stream. Both techniques have been used successfully at CHESS by visitors and local user groups.

As has been mentioned, equally important is the availability of good cryostats. There are two different cryostat systems available to shock freeze protein crystals at MacCHESS. The newest and most reliable units are the heat exchange MSC devices Molecular Structure Corporation, The Woodlands, TX. Figure 2 shows a schematic of an MSC cryostat. A 160 liter Taylor-Wharton XL-45 li-
A high resolution data set on a radiation sensitive crystal form in this lab (nucleoside deoxyseryl transfrase) which previously required 43 crystals at room temperature can now be done using a single crystal at low temperature. Similar successes have been achieved with almost every crystal attempted. High quality data at resolutions extending to 1.0 Å have been collected on frozen crystals in the past months as shown by the 1.45 Å data with an overall R factor of 5% collected on a frozen crystal of lipoprotein provided by Wakid Minor of Purdue University. Further, concerns of increased mosaicity and irreproducibility of unit cell constants from crystal to crystal have not been a problem in any project collected in the past six months.

As an example, figure 3 shows that good difference Fourier maps have been calculated using native and complex data collected at CHESS from frozen crystals of bovine and human purine nucleoside phosphorylase, showing a lack of crystal variation upon freezing. Thus, most concerns relating to low temperature data collection have been overcome and it is becoming almost a standard protocol in MacCHESS diffusion experiments.

In conclusion, MacCHESS now provides the resources necessary for routine low temperature data collection on macromolecular samples. In addition to providing good, reliable commercial crystolo, a level of expertise has been developed locally from interaction with visiting user groups at the forefront of development in this area. MacCHESS is currently planning a video kiting library in which the initial volume will discuss and demonstrate the techniques and theory of low temperature crystallography. In the interim, local support will gladly be provided at any level by contacting Rick Walter in the laboratory of Dr. Steven Bakov (email address: walter@uiuc.edu).