

## Macromolecular Cryo Crystallography

**MS01.01.01 CRYOCRYSTALLOGRAPHY: SMOOTHING THE PATH TO SUCCESS.** Håkon Hope, Department of Chemistry, University of California, Davis, CA 95616, USA.

Over the past twenty years biological cryocrystallography has changed from a rarely used, esoteric specialty to a near mainstream technique. There are important forces driving this development: (1) As projects become more and more ambitious, the need to protect precious crystals from the ravages of radiation damage becomes more important, (2) cryo-techniques have become simpler, (3) cooling apparatus has improved, and (4) there is better understanding of the processes involved in the prevention of ice formation. However, we are still some distance away from total control.

Although the design principles of gas-stream cooling equipment are well understood, there is a discrepancy between what has been established and what is being practiced. It is possible by simple means to keep a crystal frost free for an indefinite length of time, without special shielding, even in a humid environment. Even so, many laboratories struggle with ice on their samples. Details of nozzle design, stream control, and interaction between cold stream and crystal mount will be presented.

With equipment design under control, the most difficult part of a cooling experiment is the cooling process itself. Early methods for prevention of ice formation in the crystal focused on modifying the internal water structure. We now think that in the great majority of cases, ice formation starts at the surface of the crystal. The problem then becomes one of modifying the surface solvent so that ice nucleation is prevented. A conceptually simple way of doing this is to remove all solvent from the surface. In well over one-half of all cases this is possible. For the remaining crystals the current practice is to add an antifreeze ("cryoprotectant") to a portion of the solvent mixture, typically 15-25%. Antifreeze agents are small-molecule compounds that are readily water-soluble, and that diffuse rapidly through aqueous solutions. Examples are glycerol, ethylene glycol or MPD. Because just the surface layer needs to be treated, only brief rinses are required, often less than 10 s. Most crystals do not tolerate significant changes in their solvent environment, so the short rinses make it easier to avoid crystal damage prior to cool-down.

Cooling in liquid propane is popular in some laboratories. We have developed tools and techniques for liq. N<sub>2</sub> cool-down, transfer, storage and transport that are very reliable, and simpler than propane-based techniques. Contrary to popular belief, liq. N<sub>2</sub> affords the more rapid cooling.

**MS01.01.02 KINETIC STUDIES ON CYTOCHROME *cd*<sub>1</sub> NITRITE REDUCTASE.** P.A. Williams, V. Fülöp, E.F. Garman, and J. Hajdu. Laboratory of Molecular Biophysics, and Oxford Centre of Molecular Sciences, University of Oxford, UK

Use of cryo-crystallography and spectroscopic techniques has enabled the determination of intermediate substrate and product complexes on cytochrome *cd*<sub>1</sub> nitrite reductase during the reduction of nitrite.

Cytochrome *cd*<sub>1</sub> nitrite reductase is a dimeric enzyme, consisting of a small cytochrome domain containing a *c* haem, and a larger beta-propeller domain containing a non-covalently attached *d*<sub>1</sub> haem. The crystal structure of the fully oxidised form of cytochrome *cd*<sub>1</sub> has previously been published at a resolution of 1.55Å (1). The structure of the fully reduced form of the enzyme has also been determined to a resolution of 2.0Å (Williams et al, unpublished).

The enzyme is active in the crystalline form, and the reaction can be followed using a microspectrophotometer (2). There are several distinct spectroscopic intermediates, with lifetimes of 10 seconds - 5 minutes. The crystals proved unsuitable for Laue analysis, and thus structural intermediates were trapped in the crystal by shock-cooling reacting crystals to 100K. Several points on the catalytic pathway have been determined, giving insights into the mechanism of nitric oxide release from cytochrome *cd*<sub>1</sub>.

- (1) Fülöp, V., Moir, J.W.B., Ferguson, S.J. and Hajdu, J. (1995). *Cell* **81**, 369-377  
 (2) Hadfield, A. and Hajdu, J. (1993). *J. Appl. Cryst.* **26** 839-842

**MS01.01.03 LOW TEMPERATURE TECHNIQUES IN MACROMOLECULAR CRYSTALLOGRAPHY.** David W. Rodgers. Department of Biochemistry, University of Kentucky College of Medicine, Lexington, KY 40536-0084

The use of cryogenic techniques has greatly improved macromolecular data collection. Techniques for cooling, transfer, storage, and transportation of crystals are discussed. Results with a variety of crystals as well as limitations and other applications of cryogenic methods are also described.

**MS01.01.04 CRYOCRYSTALLOGRAPHY OF VIRUSES.** Brenda R.S. Temple, Department of Biochemistry and Biophysics, CB 7260, University of North Carolina at Chapel Hill, NC 27599-7260

Cryocrystallographic studies of macromolecules were almost routine in some laboratories before the first virus crystal was successfully flashcooled. The extension of low-temperature techniques from conventional macromolecular crystals to virus crystals was long sought but proved intractable for several years. A reduction in radiation damage and an extended crystal lifetime were advantages of low-temperature data collection, however, that provided strong motivation for continued efforts to flash-cool virus crystals. Recently, there have been several successes with cryocrystallographic studies of virus crystals. These successful efforts at flashcooling virus crystals will be discussed in this talk.

**MS01.01.05 HIGH RESOLUTION (1.05Å) STRUCTURE OF SALMONELLA TYPHIMURIUM NEURAMINIDASE (SIALIDASE)** Garman, E.1, Wouters, J.2, Vimr, E.3, Laver, G.4, Sheldrick, G.M.2. <sup>1</sup>Laboratory of Molecular Biophysics, University of Oxford, Oxford. U.K. <sup>2</sup>Department of Chemistry, University of Göttingen, Göttingen, Germany. <sup>3</sup>University of Illinois, Urbana, U.S.A. <sup>4</sup>A.N.U., Canberra, Australia.

Atomic resolution data have been obtained from a crystal of salmonella typhimurium neuraminidase (STNA) held at 100K, and the structure has been refined using SHELXL (1). Currently the crystallographic R and free R values are 10.2% and 13.2% respectively for the model of this 42kD enzyme.

The crystal structure of STNA has previously been published at 2.0Å resolution (2) and determined at 1.6Å resolution (3). The enzyme consists of six four-stranded antiparallel beta-sheets arranged as the blades of a propeller around an axis passing through the active site.

The greatly improved resolution reported here was achieved by lowtemperature (100K) data collection conducted at BW7B beamline of the DESY Hamburg synchrotron radiation source. X-ray diffraction data were 92% complete in all shells to 1.0Å with a merging R(I) value of 6.2%. The crystals are of space group P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> and cell 47.4Å, 82.3Å and 91.7Å.

The 1.6Å model has been refined against all data to 1.05Å using SHELXL. This model currently includes 381 amino acids, 6 glycerol molecules (from the cryoprotectant agent), and 670 waters. Anisotropic B factors were fitted to all atoms. The electron density maps calculated using the refined model are extremely clean and the protein structure is very well defined apart from 3N-terminal residues. Atomicity is achieved in most regions of the protein.

An atomic resolution molecular model of a large enzyme has thus been obtained using the tools now available to the protein crystallographer.

- (1) Sheldrick, G.M. and Schneider, T.R. (1996) *Methods Enzymol.*, Eds. Carter, C.W. and Sweet, R.M., in press.  
 (2) Crennell, S.J. Garman, E.F., Laver, W.G., Vimr, E.R. and Taylor, G.L. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 9852-9856.  
 (3) Crennell, S.J. Garman, E.F., Laver, W.G., Vimr, E.R. and Taylor, G.L. (Submitted to *J.M.B.*)