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 cryocystallography 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-crystallography, University of California, Davis, CA 95616, even in a humid environment. Even so, many laboratories struggle to keep a crystal frost free for an indefinite length of time, without special shielding. It is possible by simple means to keep cooling experiment is the cooling process itself. Early methods for pre-cooling in liquid propane is popular in some laboratories. We have, however, been able to keep ice on their samples. Details of nozzle design, transportation of crystals are discussed. Results with a variety of crystals as well as limitations and other applications of cryocryostatic techniques are also described.

**MS01.01.02 KINETIC STUDIES ON CYTOCHROME cd 1 NITRITE REDUCTASE.** P.A. Williams, V. Fulop, 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 1 nitrite reductase during the reduction of nitrite. Cytochrome cd 1 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 delta haem. The crystal structure of the fully oxidised form of cytochrome cd 1 has previously been published at a resolution of 1.55A (1). The structure of the fully reduced form of the enzyme has also been determined to a resolution of 2.0A (Williams et al, unpublished).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 nitrite oxide release from cytochrome cd 1.


**MS01.01.03 LOW TEMPERATURE TECHNIQUES IN MACROMOLECULAR CRYSTALLOGRAPHY.** David W. Rodgers, Department of Biochemistry, University of Kentucky College of Medicine, Lexington, KY 40526-0084

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

Cryocystallographic studies of macromolecules were almost routine in some laboratories before the first virus crystal was successfully flash-cooled. 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 cryocryocrystallographic 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.J., Wouters, J.R., Vinn, E.J., Laver, G.A., Sheldrick, G.M.1, Laboratory of Molecular Biophysics, University of Oxford, Oxford, U.K. 2Department of Chemistry, University of Gottingen, Gottingen, Germany. 3University of Illinois, Urbana, U.S.A. 4A.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 cryocrystallographic R and free R values are 10.2% and 13.2% respectively for theactions of this 42kD enzyme.

The crystal structure of STNA has previously been published at 2.0A resolution (2) and determined at 1.6A 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 BW77 beamline of the DESY Hamburg synchrotron radiation source. X-ray diffraction data were 92% complete in all shells to 1.0A with a merging R value of 5.0%. The crystals are of space group P212121, and cell 47.4A, 82.3A and 91.7A. 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 N-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.