ESRF report

Experiment: MX-367 (BM16)
Date: 2nd - 3rd, February, 2005

MEXICAIN

Mexicain crystals were obtained by the counterdiffusion technique in capillaries under similar condition used in vapour diffusion technique. Diffraction experiments were done at 100K using already known cryo-condition, after extracting the crystals from the capillaries. Needle shaped crystals were cryo-protected using 20-25% glycerol in crystallization buffer. Several crystals were tested for diffraction and a complete data set at 1.7 Å was collecting. We expect to use those data to extend the phases of our current 2.1 Å home source data.

Since our goal is to get resolution data below 1.5 Å, current efforts are ongoing to improve crystal quality using counterdiffusion techniques.

Thioredoxin

Crystal of the thioredoxin mutants grown in capillaries by counter-diffusion techniques were diffracted at BM16. Since crystallization condition compromised a 40% MPD, a fraction of the capillary containing the crystal was directly cryo-cooled at the nitrogen stream avoiding mechanical or chemical stress. Crystals of three different mutants were tested for high resolution diffraction. Two data sets were collected for the single mutants E85D and D47E at 2.0 Å and 1.9 Å resolutions, respectively. The structure of both mutants will be solved by molecular replacement. Those results will contribute to our current studies on the physicochemical role of charge residues on the thermodynamic denaturation of thioredoxin.

HIV-1PR

Crystals of mutant forms of Human Immunodeficiency Virus 1 protease (HIV-1PR) in complex with inhibitor were tested and used to collect diffraction data during the MX-367 experiment at ESRF.

Mutant forms of HIV-1 PR bear mutations typically arise in patients treated with antiviral drug nelfinavir. These mutations cause a reduction in nelfinavir susceptibility and thus contribute to resistance. Crystals of four different mutants bearing combination of mutations D30N, L90M, A71V and N88D in complex with nelfinavir were grown using hanging drop method. Drops contained 2.5mg/ml protease, 100mM Acetate pH 4.5-5, 0.5-1M Sodium Chloride and 3fold excess of inhibitor. Needle shaped crystals were cryo-cooled in liquid nitrogen using 20-25% glycerol in crystallization buffer as cryoprotecting solution.

Six crystals were tested for diffraction and two of them were used for collecting complete datasets to resolution 1.6 Å and 1.8 Å for D30N/N88D and L90M/A71V mutant complex, at ID14-1 and BM16 respectively. The diffraction data will be used for structure solution using molecular replacement. Analysis of structures will help to understand the structural basis of HIV PR resistance.