ESRF	**Experiment title:** USE OF MICROFOCUS BEAMLINE FOR PROTEIN MICROCRYSTALS.	**Experiment number:** LS 494
Beamline: ID13	**Date of Experiment:** from: 21/8/96 to: 31/8/96	**Date of Report:** 28/2/97
Shifts: 30	**Local contact(s):** CHRISTIAN RIEKEL , ANDREAS BRAM	*Received at ESRF :* 04 MAR 1997

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Report:

Microcrystal experiments August 21-31 1996 (LS 494).

The aim of the proposal was to continue to develop the chain of sample manipulation, sample observation, data collection strategy on the microfocus beamline that will permit high resolution data sets to be routinely measured on frozen macromolecular microcrystals of size 130 microns.

Experimental setup:

The ID13 K-goniometer was used with a 30 micron collimator. The wavelength was 0.689Å and the estimated flux on the sample was $5x10^{10}$ photons/s/30 microns/l00mA. The synchrotron was operated in 16 bunch mode at the time. A 30cm Mar image-plate detector was controlled together with the goniometer from SPEC. Cooling to 100K was by an Oxford cryo-system. The hardware and software was set up a combined EMBL-ESRF team.

Crystals were frozen in appropriately sized loops and visualised by means of the 300x microscope. Preliminary centering was initially done using the kappa and phi movements of the goniometer. Fine tuning of the centering was done by observing crystal diffraction in real time on a small CCD detector placed in front of the Mar scanner. The whole goniometer could be displaced relative to the beam in the vertical and horizontal directions with precision of a few microns until the strongest diffraction was seen. Usually an attenuated beam was used for this purpose to avoid radiation damage. **This real-time visualisation of diffraction was crucial for getting small crystals into a small beam.** The precision movement also allowed accurate translation of the crystal to expose fresh 30 micron volumes in the case of large crystals.

The following crystals were examined using this setup:

Asparaginyl-tRNA synthetase (C. Berthet and L. Seignovert, EMBL): 30 micron crystals, hexagonal a=b=125Å, c=123Å. Diffraction to 2.5Å observed but high resolution diffraction decayed after about 5 images.

T-protein/DNA complex (C. Muller, EMBL): 100 micron crystal, strong diffraction to at least 2Å. Full data set collected with 2 translations to avoid radiation damage. Largest cell axis 150Å.

Prolyl-tRNA synthetase/tRNA^{pro} complex (S. Cusack and A.$_0$ Yaremchuk, EMBL): 100 micron bipyramid crystals. Tetragonal with a=b=140A c=230A. Diffraction to 3.5Å (comparable to larger crystals on ID2) but decayed rapidly after four images.

Histidyl-tRNA synthetase with histidine/AMPCPP/Manganese (H. Belrhali,ESRF, A. Yaremchuk, EMBL): crystal 100x40x40 microns. Full dataset collected to 2.9A with several crystal translations. Orthorhombic crystals with a= 175A b=215A c=49Å.

Bacteriorhodopsin: (E. Landau and G.Rosenbusch, Biozentrum and E. Pebay-Peyroula, IBS). Hexagonal plate crystals 30x30x8 microns grown in lipidic cubic phase. Space-group $P6_3$, a=b=61.8Å, c=104.2Å. Diffraction to <2Å in ab plane observed and to 2.3Å in c-direction. Only certain crystals were ordered in the c-direction. Dataset integrated to 2.3Å (Rmerge=10.5%, completeness 91%). Data quality limited by radiation damage. Data has been used to solve structure by molecular replacement using Henderson EM structure. Refinement is in progress.

HIV-l p24/fab complex (C. Berthet, S. Cusack, EMBL): Thin triangular plates 100x100x20 microns. Crystal are usually twinned. Monoclinic a=193A, b=48A, c=191A beta=92.4. Diffraction to 3.3Å observed. 80% complete data to 4A collected on very thin single crystal.

Conclusions: Data collection from small crystals with small beams (30 microns) has been shown to be feasible with normal exposure times of up to 50-100s/degree. The flux density on ID13 gives diffraction patterns from a 30 micron spot nearly as strong as for a 100 micron spot on ID2. The price paid is more rapid radiation damage. For problems in which there are only microcrystals (e.g. bacteriorhodopsin) ID13 now makes crystal structure determination possible. Developments to be made in the ID 13 setup:

improve micro crystal handling
improve sample observation e.g. using polarised light
cryo-cooling to lower temperatures with He?
higher precision goniometer for even smaller crystals (sphere of confusion - **3** microns)
fast readout, large area CCD detector to increase data collection rate and allow phi slicing to improve signal to noise ratio.