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ESRF	**Experiment title:** *Crystallographic structure determination of the proton-pumping transhydrogenase*	**Experiment number:** LS1224
Beamline: ID14-4	**Date of experiment:** from: 14/12/98 to: 15/12/98	**Date of report:** Feb '99
Shifts: 3	**Local contact(s):** Dr Sean MacSweeney	*Received at ESRF:* 01 MAR. 1999

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Report:

Our project is to determine the structure/function relationship of the integral membrane protein transhydrogenase, which uses the proton gradient across the membrane to drive hydride transfer between NAD(H) and NADP(H), and to establish the catalytic mechanism. In particular, we wish to determine how hydride transfer is coupled to proton translocation, the location and relative orientation of the two nucleotides, and a detailed description of the NADPH binding site, which we believe to be key to the whole mechanism.

We have, in the past, obtained a number of crystalline forms of a complex between the NADH- and NADPH-binding domains (dI and dIII, respectively), which we know to be active in solution and catalyse the transfer of hydride equivalents, but not the proton translocation. Despite trying several methods, we were unable to a) improve the size of crystals and b) obtain crystals that diffracted beyond 8 Å on our *in-house* area detector.

The experiment at station ID14-4 was to test the diffraction quality of these complex crystals and small crystals of human heart transhydrogenase dIII, which we obtained two weeks prior to travelling to ESRF.

The preliminary experiment was a resounding success for both types of crystals. Human dIII crystals diffracted beyond 1.6 Å resolution. A native data set was collected to 2.1 Å resolution. Encouragingly, the mosaicity of the dIII crystals, flash-cooled to liquid nitrogen temperatures, was 0.25°. The complex dI/dIII crystals diffracted to 3.2 Å resolution, which was by far the best resolution seen to date. A complete native data set was collected to 3.2 Å resolution.

The human dIII crystals have dimensions ~0·1 x 0·1 x 0·06 mm and, due to the plate-like shape of the crystals, always mounted in the cryoloop with the longest cell axis (250 Å) parallel to the beam. This caused us problems with spot separation on the detector. Since our experiment at ESRF we have modified the crystal mount so that the cryoloop is supported by a pin, made of 20 guage capillary tubing, that can be bent. This allows us to mount the human dIII crystals with the long axis much more parallel to the spindle.

In addition to the two native data sets, we tried to collect a number of potential heavy-atom derivatives. Unfortunately, none of those crystals diffracted. Based on our results, we discussed with the beamline manager the possibility of collecting data at three wavelengths using selenomethionine derivatised protein and solving the structures using the MAD approach. That experiment is the subject of our current application. In both dI and dI/dIII crystals we have 1 selenomethione per 23 amino acids, which should provide a sufficient MAD signal. Since our ESRF experiment we have concentrated efforts to produce selenomethionine human dIII and *R. rubrum* dI and dIII. We have successfully produced two out of the three in sufficient quantities to allow crystallisation, and are currently in the process of producing the third.