ESRF	**Experiment title: Crystallographic studies of the proton pump transhydrogenase**	**Experiment number:** LS1504
Beamline:	**Date of experiment:** from: 15/2/00 to: 17/2/00 from: 5/5/99 to: 6/5/99 from: 12/12/98 to 13/12/98	**Date of report:** 27/2/00
Shifts:	**Local contact(s):** Dr Raimond Ravelli	*Received at ESRF:*

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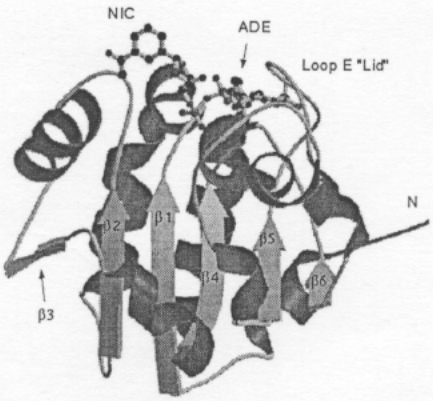
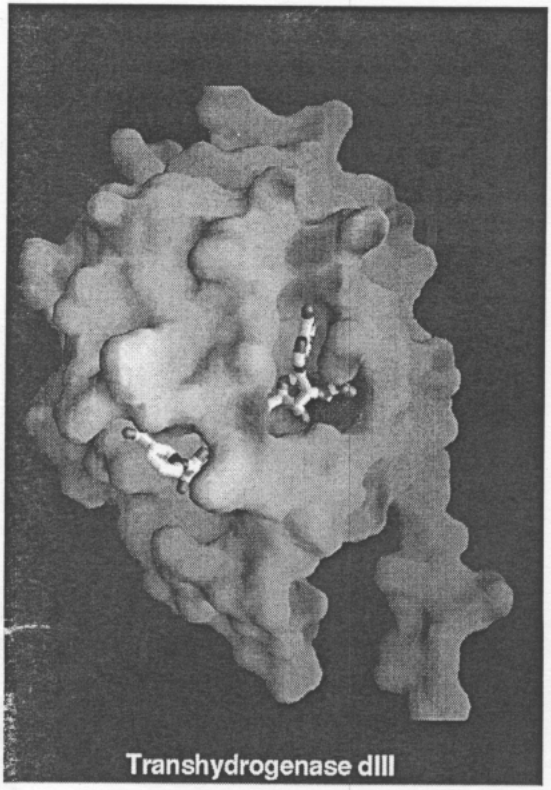
Report: Interim Report for LS1504



These experiments are further developments of crystallographic studies of transhydrogenase. The initial experiment, Dec '98, resulted in a complete 2.0 Å resolution data set collected from small native crystals (ca. 0.1 x 0.1 x 0.07 mm) of dIII, the $NADP^+$-binding domain of human transhydrogenase. The second experiment, May '99 (officially termed 'commissioning time') resulted in a 3 wavelength MAD data set collected from a Se-methionyl derivative of human dIII. The derivative crystals were slightly smaller: approximately 0.08 x 0.08 x 0.05 mm in size. The MAD data set was collected in approx.1 hour of beam time. The structure of dIII was solved using the software program SOLVE by Tom Terwilliger. The structure (above right) is a Rossmann fold and has a very unusual mode of nucleotide binding, with the $NADP^+$ bound 'the wrong way round'. It is immediately apparant why the $NADP^+$ is bound so tightly, K_d is estimated to be sub-nanomolar. The nucleotide is almost completely buried or tightly packed and only the nicotinamide ring is accessible to the surface (see fig 1 below). The work has been written up in the following publications: 1) Peake, *et al Acta Cryst. D*, in press; 2) White *et al. Structure*, **8**, 1, 2000; 3) Jackson *et al. FEBS Lett.* **464**, 1, 1999



Transhydrogenase dIII

In the most recent measurement, a MAD dataset was collected from a Se-methionyl derivative of a complex of the dI and dIII domains of transhydrogenase. At the time of writing, we are trying to solve the selenium substructure. Single wavelength data were also collected on a human dIII crystal soaked in a low pH buffer, and from a crystal of dIII previously reduced to the NADPH-bound form. As yet, it is not clear whether these two structures have significant differences from the original $NADP^+$-bound human dIII. A full and final report on LS1504 will be submitted with the next UK Midlands BAG application.

In addition to the experiments originally proposed in LS1504, we collected MAD data sets on Se-methionyl derivatives of two other proteins: *E. coli* nitroreductase and *B. subtilis* inorganic pyrophosphatase. To our surprise, the Se-Met nitroreductase crystallised in space group $P2_12_12_1$ in addition to the previously characterised $P4_12_12$. Both crystal forms grew in the same drops. We therefore collected a MAD data set on the new crystal form. At the time of writing, we have just obtained an excellent electron density map of the orthorhombic form of nitroreductase and a reasonable map for the pyrophosphatase which is benefitting from improvement using NCS averaging techniques.

E. coli Nitroreductase - Figures of Merit, phasing from 16 Selenium sites

Overall	**D_{min}**	**9.0**	**5.7**	**4.4**	**3.8**	**3.3**	**3.0**	**2.9**	**2.6**
0.75	**f.o.m.**	**0.78**	**0.83**	**0.77**	**0.76**	**0.76**	**0.76**	**0.74**	**0.69**