ESRF	**Experiment title:** Structure determination of a dI:dIII complex of *R. rubrum* transhydrogenase	**Experiment number**: LS1504/ LS1684
Beamline: ID14-4 BM30	**Date of experiment**: from: 15.2.00 to: 17.2.00 from: 9.4.00 to: 10.4.00	**Date of report**: 1.Sep.00
Shifts: 6 3	**Local contact(s)**: Dr Raimond Ravelli Dr Michelle Roth	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

UK Midlands BAG

*Dr Scott A. White, School of Biosciences, University of Birmingham

*Dr Klaus Fütterer, School of Biosciences, University of Birmingham

*Dr Trevor J. Greenhough, School of Life Sciences, Keele University

*Dr Annette K. Shrive, School of Life Sciences, Keele University

*Dr Peter C. E. Moody, Department of Biochemistry, University of Leicester

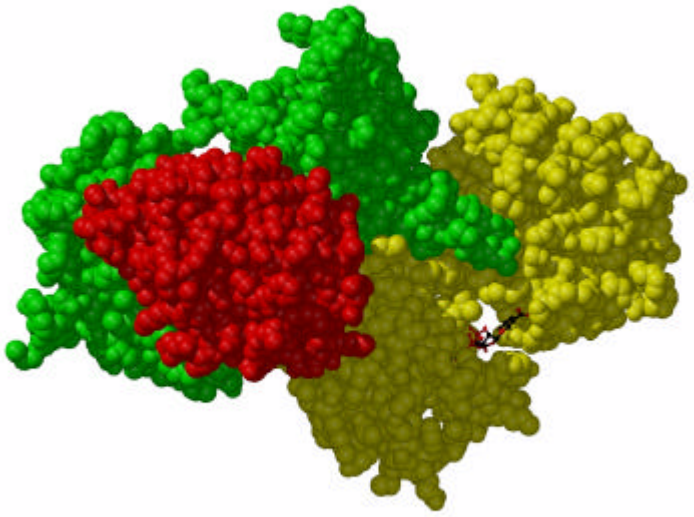
Report: **N.B.:** Corresponding author is **Dr S. A. White**

Membrane-bound ion translocators have important functions in biology, but their mechanisms of action are poorly understood. Transhydrogenase, found in animal mitochondria and bacteria, links the redox reaction between NAD(H) and NADP(H) to proton translocation across a membrane. The enzyme is a dimer in the membrane. Each polypeptide folds into three domains: dI and dIII protrude from the membrane and bind NAD(H) and NADP(H), respectively; dII spans the membrane and provides a channel for proton translocation. Recombinant dI and dIII from *R. rubrum* spontaneously form an active complex in solution that can reduce $NADP^+$ by NADH, even though dII, the membrane-spanning domain is absent. We have crystallised a dI-dIII complex from *R. rubrum* in the presence of both NAD^+ and $NADP^+$.

Native data had been collected previously. Two sets of data were collected. In the first experiment (ID14-4, LS1504) a 3 wavelength MAD data set was collected from a dI:dIII crystal containing SelenoMet dI and SelenoMet dIII. Unfortunately, the data were not of

sufficient quality to solve the structure. In particular the crystals were mosaic. In a second experiment (BM30, LS1684) a 3 wavelength MAD data set was collected from a dI:dIII crystal containing SelenoMet dI and native dIII. This data set was significantly better. The structure was solved using the program CNS, finding 25 out of a possible 30 Se sites (dI only). These sites were then incorporated as known sites into a second search for Se sites, but this time with a Patterson map calculated from the SeMet dI: SeMet dIII data set. A further 12 out of a possible 12 sites were found for the dIII component of the complex.

The structure reveals that the dI:dIII complex is a heterotrimer with 2 copies of dI forming a dimer, bound to 1 copy of dIII. One equivalent each of NAD^+ and $NADP^+$ can be seen bound to one dI and the dIII, respectively. The two nicotinamide rings approach one another allowing us to visualise exactly how direct hydride transfer from NADH to $NADP^+$ occurs. This highly asymmetric complex gives significant insight into the organisation of the complete transhydrogenase and its mechanism of proton translocation. We have developed a hypothesis that incorporates an “out of phase” alternating sites mechanism.



The figure shows the heterotrimeric structure: dI is a dimer (yellow and green) and only one copy of dIII is present (red). The yellow dI subunit has a tightly-bound NAD^+ present. dIII has a tightly-bound $NADP^+$ present.

This experiment is a continuation of our transhydrogenase structure determination (LS1504). The initial structure, on human transhydrogenase dIII, is now published: White et al, *Structure*, **8**, 1-12, 2000.