ESRF

ESRF	**Experiment title:** Structural studies of Transhydrogenase	**Experiment number**: MX-27 & MX-100
Beamline: ID14-1	**Date of experiment**: **from:** 06/03/03 **to:** 07/03/03 **from:** 02/07/03 **to:** 03/07/03	**Date of report**: 02/09/03
Shifts: 1 + 1	**Local contact(s)**: Dominique Borgeois, Edward Mitchell	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Susanna Törnroth*[1], Cristine Oswald[1], Tomas Johansson[1], Richard Neutze[1], Göran Karlsson[1,2], Jan Rydström[2], Ute Krengel[1]

[1]*Department of Chemistry and Bioscience, Chalmers University of Technology.*

[2]Department of Biochemistry & Biophysics, Gothenburg University.

Report: Structural studies of transhydrogenase and its soluble subunits

A number of candidate crystals of the intact integral membrane protein Transhydrogenase from *E. coli* have been obtained. Several of these have been tested at ID14-1 but no clear diffraction has been seen. Some of the crystals are most likely to be too small (<0.03 mm) for this beamline and need to be tested at *e.g.* ID13. In other cases the lack of diffraction may be due to poor crystal quality. In addition to optimising the current crystallisation conditions, mutants of the enzyme are also being targeted for structural studies. These mutants have a higher stability, thereby increasing the chances of obtaining well-ordered crystals.

Crystals of the soluble domain I of *E. coli* Transhydrogenase has been obtained. These diffracted to 1.8 Å resolution at ID14-1. A high and a low resolution data set was collected. The crystals belong to space group P1 with cell dimensions of a=38.9, b=67.0, c=76.5, α=67.6, β=80.8, γ=81.5. The data was processed and scaled with an Rmerge of 5% and a completeness of 96.5. Using Transhydrogenas domain I from *R. rubrum* (Buckley *et al* 2000) as a model a molecular replacement solution could be found. The structure is undergoing refinement and remodelling.

Attempts to crystallise *E. coli* Transhydrogenase domain I and III are in progress. These domains together comprise the entire soluble region of the enzyme.

References:

Buckley, P.A., Jackson, J.B., Schneider, T., White, S.A., Rice, D.W., and Baker, P.J. (2000). Protein-protein recognition, hydride transfer and proton pumping in the transhydrogenase complex. Structure **8**, 809-815.