ESRF	**Experiment title:** Analysis of the structure and function of pyridine nucleotide transhydrogenase - a membrane proton pump	**Experiment number:** LS1485/6/7
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Report:

Transhydrogenase is a conformationally-coupled, proton pump that links a proton gradient to the interconversion of NADH and NADP and is a model system for understanding the properties of ion pumps. It has three components; dI has the binding site for NAD(H), dIII for NADP(H), and dII spans the membrane. Recently, the structures of the dIII components of bovine [1], and human [2] transhydrogenases were reported. Using data from the ESRF we have solved the structure of dI.

The crystals of *R.rubrum* dI are in space group $P2_1$, ($a = 65.9$Å, $b = 116.6$Å, $c = 102.0$Å and $\beta = 104.2°$) with four polypeptide chains in the asymmetric unit. Data were collected to 2.0Å on BM 30 at three wavelengths (12657.03eV, 12659.11eV and 12800.00 eV) corresponding to the Se K edge f' minimum, f'' maximum and high energy distant positions, as determined from a fluorescence scan of the crystal. The data were processed and merged using DENZO/SCALEPACK [3] and F_A values determined using a developmental version of the program XPREP (Bruker AXS, Madison, U.S.A.). The anomalous signal extended to 2.5Å and F_A values were truncated to this resolution. The Se-Met substructure was solved using the 'half-baked' approach as implemented in the

program SHELXC [4]. 52, of a possible 60, sites were found and validated, based on consistency with the Patterson function and with 4-fold non-crystallographic symmetry, by manual inspection of the crossword table provided by SHELXC. Subsequent data reduction used the CCP4 suite of programs [5]. The Se sites were refined and phases calculated using MLPHARE an initial model was built into the solvent flattened map using TURBO-FRODO [6]. In the final refined model (R = 0.21, Rfree = 0.26) 51 of the initial 52 Se sites corresponded to actual Se-Met positions.

dI folds into two domains, each with the appearance and connectivity of the common dinucleotide binding Rossmann fold. The nicotinamide ring of the NAD^+, rather than lying deep in the interdomain cleft as seen in seen most other $NAD(P)^+$ binding enzymes containing the Rossmann-fold, occupies a completely different orientation and is positioned on the outside of the domain, with its orientation dependent upon the relative degree of domain closure in dI. It is disordered in the most open subunits. In the intermediate state the ring adopts a *syn* conformation with respect to its ribose moiety. The carboxyamide makes hydrogen bonds both with the pyrophosphate and with the side chains of the invariant residues Y235 and D135. In the closed state, the nicotinamide ring and its associated ribose are expelled from the interdomain cleft, with the glycosidic bond adopting an *anti* conformer. A docked model of human dIII with this dI structure shows that hydride transfer between NADP(H) and NAD(H) can occur in the most closed conformation of dI, but that as the domains open, the nicotinamide ring of the NAD(H) in dI moves away from that of the NADP(H) bound to dIII. This relative movement of the nicotinamide rings yields a plausible mechanism for the gating of the scalar hydride transfer reaction, so essential in providing the motive force for the transmembrane proton translocation step.

1.Prasad, G.S., Sridhar, V., Yamaguchi, M., Hatefi, Y. & Stout, C.D *Nature Structural Biol.* 6, 1126-1131 (1999).

2.White, S.A., Peake, S.J., McSweeney, S., Leonard, G., Cotton, N.N.J. & Jackson, J.B. *Structure* 8, 1-12 (2000).

3.Otwinowski, Z. & Minor, W. (1997) Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol* 276 307-326.

4. Sheldrick, G.M In: 'Direct Methods for Solving Macromolecular Structures'. Edited by Fortier,S. Dordrecht: Kluwer Academic Publishers, 401-411 (1998)

5.Collaborative Computing Project no.4. *Acta Cryst.* D50 760-763 (1994).

6.Roussel, A. & Cambillau, C. (1991*). Silicon Graphics Geometry Partners Directory 86.* Silicon Graphics, Mountain View, CA.