s8a.m7.p1 **Three-dimensional structures of the reduced and oxidised forms of cytochrome c_3 from *Desulfovibrio desulfuricans* ATCC 27774 at pH 7.6** I. Bento, P.M. Matias and M.A. Carrondo, *ITQB-UNL, P. O. Box 127, P-2780 Oeiras, Portugal.*
Keywords: metalloproteins.

Tetraheme cytochrome c_3 is a small periplasmic protein (≅14 KDa, 107 residues) found in all species of sulfate-reducing bacteria. It has four heme groups covalentely bound to a single polypeptidic chain through thioether bridges to cysteine residues, bis-histidinyl axial coordination, very low redox potentials [1] and it is believed that it act as a proton-electron coupler between hydrogenase and transmembrane complexes [2].

Crystals of this cytochrome were obtained following the procedure described by Simões et. al.[3], and transferred to new crystalization drops at pH 7.6. This increase in the pH of the drop (final pH 7.6), was needed in order to perform the reduction with sodium dithionite. Two data sets, one from the reduced form and the other from the oxidised form, were collected at ESRF, beam-lie ID14-EH3, in Grenoble, and processed with DENZO/SCALEPACK[4]. The crystals belong to the space group $P6_122$ and the reduced form diffracted up to 1.35 Å resolution, while the oxidised up to 1.4Å resolution. The three-dimensional structure of the reduced form of tetraheme cytochrome c_3 from *D. desulfuricans* ATCC 27774 has been solved by the molecular replacement method, using the program AmoRe[5] and as a model the known structure of the oxidised form of this cytochrome[3]. Initially, the structutre refinement of both structures was done using the program X-PLOR[6], and continued with program SHELXL[7]. The current values of R and R_{free} for the redued and oxidised form are 15.2% and 20.0%, and 19.0% and 21.4%, respectively. In this presentation we report the results of the structure refinement of the oxidised and reduced forms of cythochrome c_3 from *Desulfovibrio desulfuricans* ATCC 27774 at pH 7.6, and discuss the differences found between the two forms and the oxidised form obtained previously at pH 4.0[3].

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s8a.m7.p2 **1.5Å high-resolution neutron diffraction results of rubredoxin.** K. Kurihara, I. Tanaka, M.W.W. Adams[a], N.Moiseeva[b], R. Bau[b], N. Niimura, *Japan Atomic Energy Research Institute, Tokai, Ibaraki 319-1195, Japan, [a] University of Georgia, Athens, GA 30602, USA, [b] University of Southern California, Los Angels, CA 90089, USA.*
Keywords: neutron, BIX-3, rubredoxin.

A single-crystal diffractometer (BIX-3) dedicated to neutron protein crystallography has been successfully constructed at JRR-3M reactor of JAERI. This instrument features a new type of elastically-bent perfect-Si crystal (EBP-Si) monochromator and neutron imaging plates (NIP).

With this BIX-3 a single-crystal neutron diffraction analysis of the structure of small protein rubredoxin from the hyperthermophile *Pyrococcus furiosus* is currently under way. Rubredoxins are small protein containing an iron atom coordinated by sulfur atoms of four cysteine side chains. Although the physiological role for rubredoxins have not been definitively established, it is likely that they function as electron transfer proteins. Despite the uncertainty if its function in most species, rubredoxins from different organisms have been extensively studied by structural and spectroscopic methodologies, owing to their small size, stability, and ease of isolation.

Data are being collected at room temperature up to a resolution of 1.5 Å (so far the highest resolution neutron data set), using wavelength ?=2.35 Å. A single crystal of dimension 2 x 2 x 1 mm, grown via vapor diffusion from 3.6 M NaK phosphate, is being used in this project. Two sets of data from the same crystal, roughly corresponding to the crystal being mounted along the a and c axes, are being collected and merged. Data collection is by the step-scan method, with 0.3° intervals in f and exposure times ranging from 60 to 75 minutes per frame. The completeness factor of the 1.5- Å resolution data set is currently at 78%. 300 hydrogen atoms and 79 deuterium atoms are included in the refinement of the structure of rubredoxin. 46 water molecules are also identified. In the present model R factor and R-free are 0.244 and 0.270, respectively.