Experimental Report

Proposal: **8-01-389** **Council:** 10/2011

Title: High resolution monochromatic neutron diffraction study of the Fe-reduced form of perdeuterated rubredoxin

This proposal is a new proposal

Researh Area: Biology

Main proposer: **CUYPERS Maxime**

Experimental Team:

Local Contact: FORSYTH Trevor
MASON Sax Anton

Samples: reduced Pyrococcus furiosus rubredoxin

Instrument	Req. Days	All. Days	From	To
D19	12	9	25/07/2012	01/08/2012

Abstract:

We request beamtime on D19 for a full structural study of reduced-Fe (i.e. Fe2+) perdeuterated rubredoxin. This proposal follows outstanding results obtained from D19 experiment 1-20-16 on the oxidised (Fe3+) form, which provided data with high completeness to a resolution of 1.19Å. This is the one of the highest resolutions ever described for a neutron crystallographic study of any protein, and has resulted in some remarkable observations. Of particular note is the occurrence of a perdeuterated hydronium ion as well as evidence of tautomeric equilibrium between neighbouring amino acid residues. These observations were only possible through the use of high resolution neutron crystallography, and the study is currently being prepared for publication. In this proposal we seek to extend this work by carrying out a comparable study of the Fe2+ form of the same protein. This is likely to provide crucial information that may be of central importance for an understanding of the redox mechanism linking the two forms, and the role that protonation shifts may play in them.

Experimental report for ILL proposal 8-01-389

High resolution monochromatic neutron diffraction study of the Fe-reduced form of perdeuterated rubredoxin.

M.G. Cuypers[a,b], S.A. Mason[b], E.P. Mitchell[c], M. Haertlein [b], V.T. Forsyth[a,b].
[a] EPSAM/ISTM, Keele University, Staffordshire, ST5 5BG, UK.
[b] ILL, 6 rue Jules Horowitz, 38043 Grenoble, France.
[c] ESRF, 6 rue Jules Horowitz, 38042 Grenoble, France.

Introduction

The new D19 diffractometer has demonstrated its capabilities for protein crystallography in a study of perdeuterated *Pyrococcus furiosus* (*Pf*) rubredoxin. The previous ILL experiment 1-20-16 provided results with high neutron data completeness to a maximal resolution of 1.05 Å at ambient temperature. The detailed analysis of the diffraction data has led to the discovery of new structural and biological features for the first time on the oxidised-Fe form of *Pf* rubredoxin, namely hydronium ions, an imidic acid tautomer and carboxylic deuterium atoms. The discoveries from the oxidised form crystal neutron diffraction data motivated the experiment this time in the reduced state. The iron centre within the protein is found in the reduced state *in vivo* in the bacteria.

Results

A crystal of perdeuterated *Pf* rubredoxin with a size of 2.0 mm^3 was reduced with sodium dithionite for 24h and transferred in an argon purged glovebox into a thin walled quartz capillary and sealed with epoxy resin and beeswax. The mounted crystal sample was kept under argon atmosphere until the experiment was performed to avoid slow oxygen contamination and iron oxidation.

We have successfully collected monochromatic neutron diffraction data at near-atomic resolution on a 2.0 mm^3 crystal of perdeuterated *Pf.* Rubredoxin on D19 at ambient temperature in the reduced-Fe crystal forms. Each image was acquired for 20 sec for a total of 3 days at 2.42 Å wavelength. Full neutron datasets were recorded using step scans of 0.07° with an exposure time of 20s per step, yielding data to 1.38 Å d-spacing resolution (Figure 1). The completeness of the neutron data in the higher resolution shell is presented in Figure 2. Data completeness is 93.1% overall and 85.5% in the highest resolution shell with a total number of observed unique reflections of 10521.

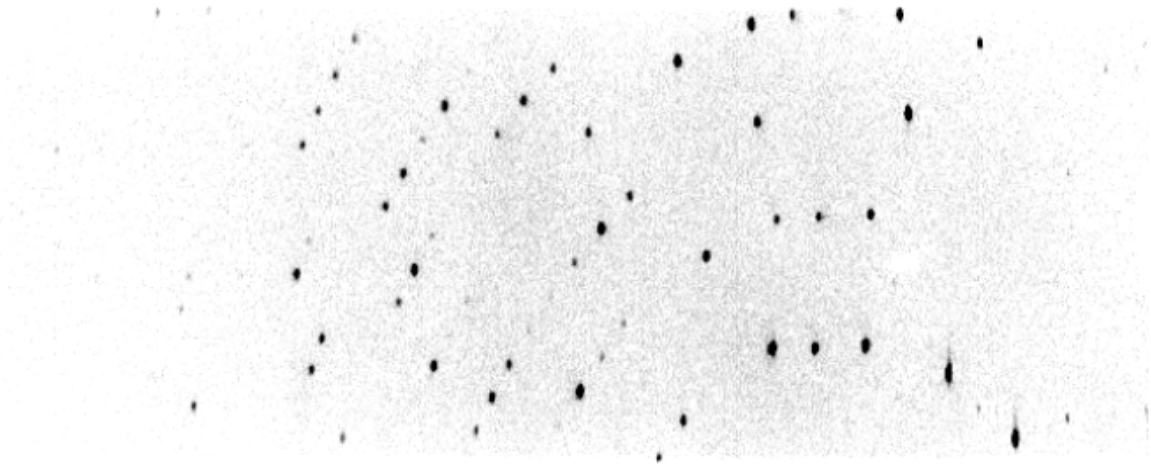

Figure 1. Monochromatic neutron diffraction patterns acquired from D19 at a wavelength of 2.42 Å for 2000 sec on the Fe-reduced crystal (image resolution edges: 1.38 Å (left) - 28 Å (right))

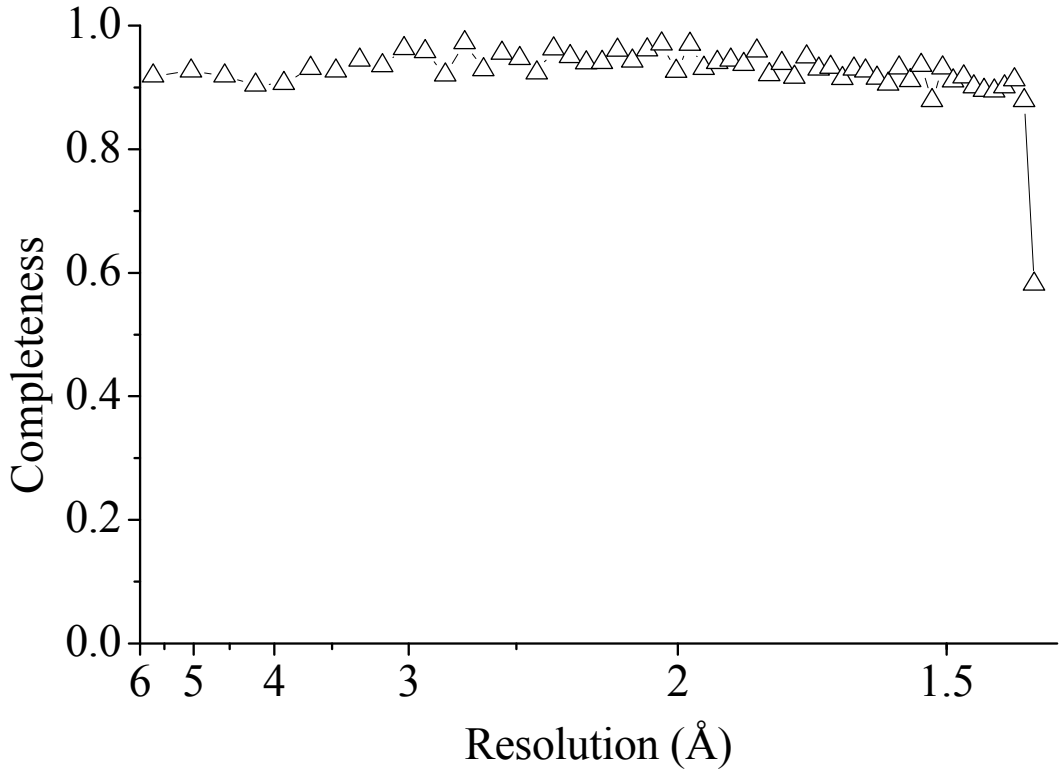

Figure 2. Plot of the data completeness as a function of resolution obtained from TRUNCATE (CCP4 program package) for the neutron data collected at 2.46 Å wavelength.

The X-ray structures of the oxidised and reduced perdeuterated *Pf* rubredoxin have been obtained at (ESRF, ID29) ambient temperature respectively to resolutions of 1,00 Å and 0,92 Å. The problem observed with the X-ray method is that photo-oxidation of the Fe-S_4 catalytic centre occurs very fast as shown by the colour change from transparent to red along the X-ray exposed path. The use of neutron crystallography for the characterisation of the reduced Fe-S_4 centre has the huge advantage of not altering the oxidation state of the reduced Fe-S_4 cluster, contrary to X-ray based methods (Figure 3a).

a) b) c)

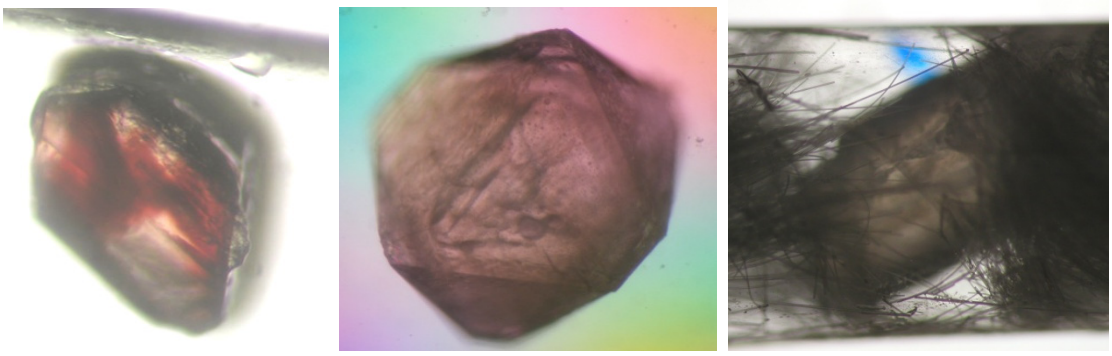

Figure 3. a) 0.01 mm^3 Fe-reduced crystal after X-ray irradiation, note the path of X-ray beam visible in red. b) 2.0 mm^3 reduced-Fe *Pf* D-rubredoxin crystal used for data collection. c) The large transparent Fe-reduced crystal mounted in a quartz capillary and held by quartz wool.

Conclusions

This experiment provides informations for the detailed comparative analysis of the oxidised (see report 1-20-16) and reduced form of the perdeuterated protein crystal at ambient temperature. The results obtained confirm the serious gains in data quality possible for monochromatic neutron studies of perdeuterated proteins and the considerable reduction of the time needed for data collection in order to still obtain suitable data quality. The refinement of the neutron structure leads to information rich neutron density maps and the comparison between the oxidised and reduced states shows protonation shifts.

These results are presented in a peer reviewed article :
"Near-atomic resolution neutron crystallography on perdeuterated *Pyrococcus furiosus* rubredoxin implicates hydronium ions and protonation state equilibria in redox changes", M.G. Cuypers, S.A. Mason, M. P. Blakeley, E.P. Mitchell, M. Haertlein, and V. T. Forsyth, Angewandte Chemie (2012, in press) DOI: 10.1002/ange.201207071.