Experimental Report

Proposal: **8-01-402** **Council:** 10/2012

Title: High resolution neutron crystallographic studies of the dynamical phase transition in perdeuterated rubredoxin

This proposal is a new proposal

Researh Area: Biology

Main proposer: **CUYPERS Maxime**

Experimental Team: CUYPERS Maxime

Local Contact: FORSYTH Trevor
MASON Sax Anton

Samples: Perdeuterated Pyrococcus furiosus Rubredoxin monocrystal

Instrument	Req. Days	All. Days	From	To
D19	9	9	27/02/2013	08/03/2013

Abstract:

We request beamtime on D19 for a multi cryo temperature (175 K, 200 K and 240 K) crystallographic study of perdeuterated rubredoxin, a never-done-before neutron data collection using a new crystallographic approach in order to shed light on the microscopic conundrum of the protein dynamical transition. This proposal is an opportunity to analyse the dynamical transition in protein by completing the high quality and unique results obtained from D19 experiments 1-20-16 and 1-20-18. It provided crystallographic data with high completeness at 295 K (article submitted for publication) and 100 K (data analysis and preparation for publication in progress) to atomic resolutions of 1.27 Å and 0.90 Å, respectively. The neutron study shall be complemented by the high resolution synchrotron X-ray crystallographic results (expected to better than 1.00 Å resolution) at the same three temperatures for better completeness of the study. The perdeuterated rubredoxin crystals suitable for the neutron experiment are already available and have proven to be highly resistant to extremely low temperature conditions, making it ideal for the study.

Experimental report for ILL proposal 8-01-402

High resolution neutron crystallographic studies of the dynamical phase transition in perdeuterated rubredoxin

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Introduction

The precedent experiments 8-01-389 and 1-20-16 provided outstanding results leading to the discovery of new structural and biological features for the first time on both the reduced and oxidised-Fe forms of *Pf* rubredoxin, namely hydronium ions and carboxylic deuterons. Experiment 1-20-18 yielded ultra high resolution (to 0.94 Å and 0.90 Å) neutron crystal diffraction data at 100 K on reduced and oxidised form crystals. Test experiments carried out by x-ray crystallography at temperatures from 150K to 240 K on one very same oxidised from crystal yielded high quality data to high resolution (better than 0.88 Å). However the interpretation of the changes is very limited as the observation of changes are not possible with the lighter atoms by X-ray. A quick test at 180, 200, 220 and 240K on a 2.0 mm^3 reduced form crystal at 1.46 Å neutron wavelength revealed surprising differences between the neutron diffractograms at the various temperatures and opened the way for the below described experiments.

Results

We have collected neutron diffraction data with high completeness at 2.42 Å wavelength on 2 mm^3 crystals of perdeuterated *Pf.* rubredoxin on D19 at 180, 200 and 220 K in both the reduced and oxidised-Fe crystal forms. The new methodology (Cuypers's large crystal cryocooling and mounting method) for crystal mounting developed for experiment 1-20-18 was again used successfully.

1- The oxidized form: Two out of five 2 mm^3 crystals with similar shape yielded data with a different cryo cooling temperature history.

- One crystal was first cryostream frozen to 100K (high resolution, Fig.) then slowly warmed up (ramp of 2 K /min) to 180K. At this point the crystal lost d-spacing resolution with enlarged peaks and mosaicity (Fig.). Full datasets were collected over 24 h each at 180K, 200K and 220K on this crystal with 6.3 s/ image and scans of 0.07 degrees per step to resolutions of 1.85 Å , 1.97 Å and 2.15 Å, respectively. Diffractograms taken at 250K, 260K, 265K and 268K are presented on Fig. xxx .

a) b)

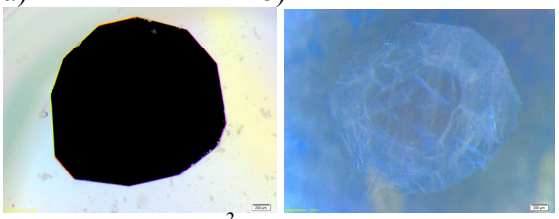

Figure 1. ~2 mm^3 crystals of *Pf* D-rubredoxin in the a) oxidised and b) reduced forms.

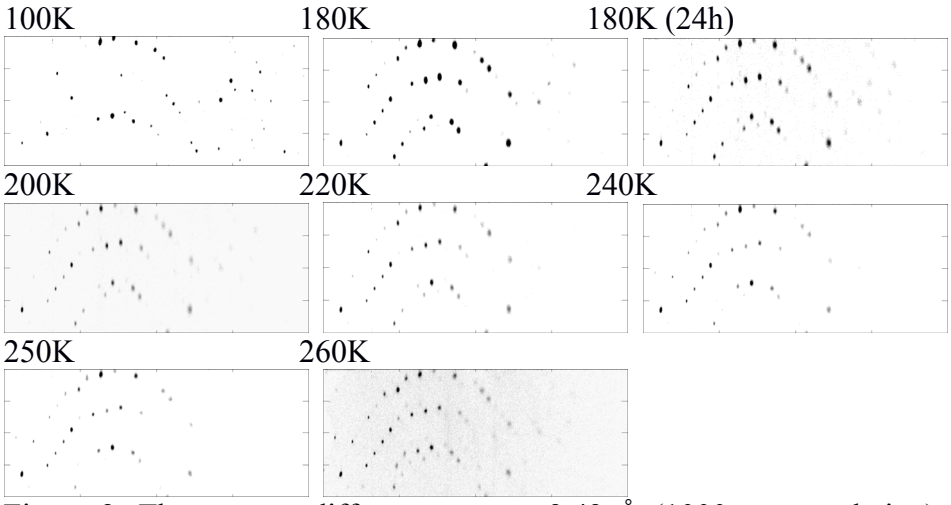

Figure 2: The neutron diffractograms at 2.42 Å (1000s accumulation) on the same 2 mm^3 oxidised form crystal showing the alterations upon warming up from 100K to 260K. The crystal mosaicity increases while the higher resolution spots disappear as temperature increases. Right edge resolution of 1.42 Å.

- A second crystal was cryostream frozen to 180K and first diffracted to high resolution, i.e. better than 1.5 Å, but decayed within hours to yield the exact same lower data quality as the above described oxidized form crystal. Data was collected at 180 K with 15 s/image scans of 0.07 degrees per step to 1.85 Å resolution on this crystal. A diffractogram taken at 140K is shown on Fig. 3c. This suggests that the loss of resolution is irreversible as observed by reducing the temperature also with other test crystals (not shown).

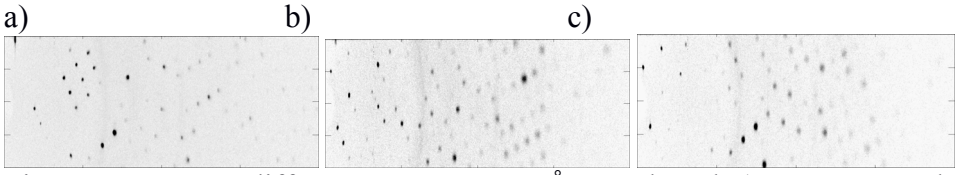

Figure 3. Neutron diffractograms at 2.42 Å wavelength (1000s accumulation) on the same 2 mm^3 oxidised form first frozen to a) 180K, b) after 2 days at 180K and at b) 140K. (note b and c are for a different crystal orientation). The crystal was frozen in 1 step to 180K, it is probably the reason for the occurrence of a diffuse ice ring.

2-The reduced form: A crystal was reduced with Na-dithionite and cryostream frozen to 180K in one step, and initially diffracted to 1.4 Å resolution at that temperature (Fig. 4). High completeness datasets have been acquired at 180, 200 and 220 K. The various diffractograms are presented below (Fig 4-6).

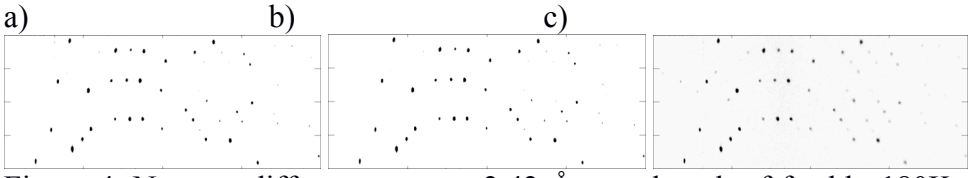

Figure 4. Neutron diffractograms at 2.42 Å wavelength of freshly 180K cryofrozen reduced state *Pf* rubredoxin 2 mm^3 crystal with right edge resolution of 1.42 Å. a) 1000s count directly

after freezing, b) after 3h and c) after 2 days with a slight increase of crystal mosaicity with time as reflected by broadening of the peaks at high angle (right side of the pictures). The crystal diffracts to better than 1.4 Å d-spacing resolution.

Data acquisition scans were performed for 2.5 days with 15.1 s / image at 180 K. At 180K, the "check" diffraction patterns at the same crystal orientation appears stable all along the scans, allowing to use all acquired data for structure refinement.

a) b) c)

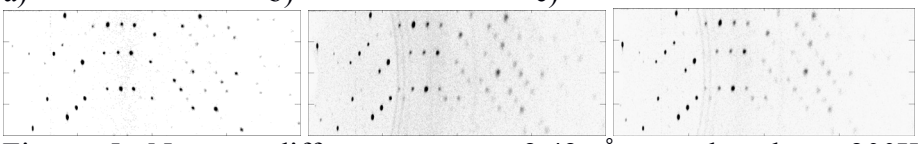

Figure 5. Neutron diffractograms at 2.42 Å wavelength at 200K of reduced state *Pf* rubredoxin 2 mm^3 crystal with right edge resolution of 1.42 Å. a) 1000s count 2 min after warming up from 180K to 200K (1K/min), b) after 27h and c) after 1.5 day. Right edge resolution is 1.42 Å

Data acquisition scans were performed for 24 h with 9.5 s / image at 200K. It has been noted during the experiment at 200K that the "check" diffraction pattern at the same crystal orientation has stabilised only after 4 to 10h (to define), allowing us to collect the first scans a second time before changing temperature. Therefore, the data collected for the 4 to 10 first hours will be excuded from structure refinement.

a) b)

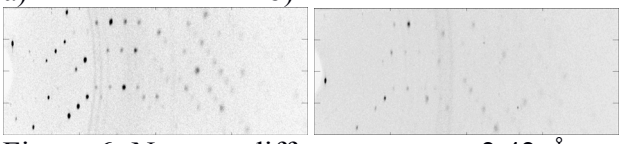

Figure 6. Neutron diffractograms at 2.42 Å wavelength (1000s) at 220K of reduced state *Pf* rubredoxin 2 mm^3 crystal. a) 2 min after warming up from 200K to 220K (1K/min), b) after 23h (crystal moved during removal of thick ice). A big loss of diffraction power has occurred at 220K.

Data acquisition scans were performed for 24 h with 9.5 s / image at 220 K. The "check" diffraction patterns at the same crystal orientation appears stable all along the scans but the last one (not shown), which will be excluded for the structural refinement.

Conclusions

We need to carefully select the scans performed when the crystals were in a stable state. The slow decay of crystal diffraction resolution due to increase of mosaicity has been deleterious to the acquired data resolution which in the case of the oxidised perdeuterated rubredoxin crystal leads to a decrease by the double of the measurable d-spacing resolution at 180K. The loss of diffracting resolution has not been observed with 0.01 mm^3 crystals analysed by X-ray diffraction to resolutions better than 1.0 Å. We believe that the slower heat transfer rate for large crystals is responsible for the deviations.

The analysis of the 7 neutron structures datasets at medium to high resolution in both reduced and oxidised states is in progress.