Experimental Report

Proposal: **1-20-32** **Council:** 10/2012

Title: Feasibility studies of anomalous scattering phasing in neutron crystallography

This proposal is a new proposal

Researh Area: Methods and instrumentation

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Samples: Perdeuterated Pyrococcus furiosus Rubredoxin monocrystal

Instrument	Req. Days	All. Days	From	To
D19	10	10	16/05/2013	17/05/2013
			09/07/2013	15/07/2013

Abstract:

We request beamtime on D19 to carry out crystallographic de novo phasing using enriched 113-Cd substituted perdeuterated Pyrococcus furiosus rubredoxin at cryogenic temperature. High resolution neutron crystallographic data is already available to 0.90 Å resolution with the natively Fe-substituted rubredoxin crystals at 100 K (data analysis and preparation for publication in progress). However, the neutron anomalous dispersion signal from the iron atom is too weak. Iron can easily be replaced by isotopically enriched 113-Cd in the available perdeuterated protein. The practical demonstration of a protein structure being phased using anomalous neutron dispersion data has never been done before. Perdeuterated rubredoxin crystals are particularly suitable for this neutron anomalous study because of their stability and high resistance to extremely low temperature conditions. This proposal aims to evaluate and develop neutron de novo phasing of macromolecular protein structures and to add the neutron anomalous scattering phasing methodology to the list of techniques available tp D19's biology user base.

Experimental report for ILL proposal 1-20-32

Feasibility studies of anomalous scattering phasing in neutron crystallography

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Introduction

The development of macromolecular structure phasing using anomalous neutron dispersion would be a desirable plus, opening the route to new methods of phasing. However, to date there has been no practical demonstration of a protein structure being phased using anomalous neutron diffraction data.

Results

Conversion of natively Fe-substituted protein into isotopically pure ^{113}Cd substituted D-rubredoxin has been performed successfully at room temperature. The crystallization protocol and crystallization plate have been adapted in order to be able to grow the crystal presented in figure 1a.

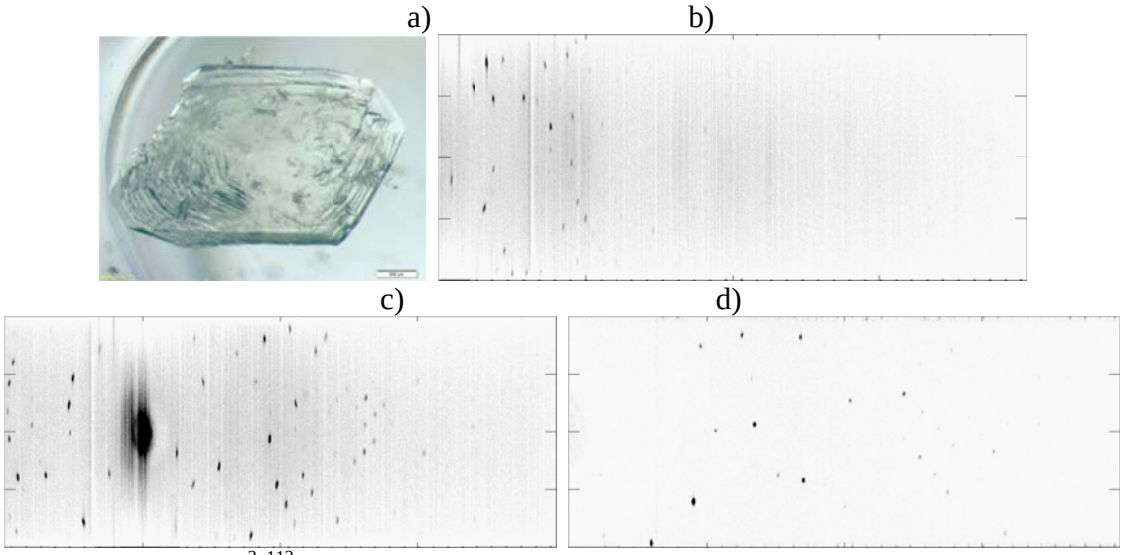

Figure 1. a) 4.5 mm^3 ^{113}Cd substituted perdeuterated *Pf* rubredoxin crystal. Neutron diffraction pattern from the 295K thin quartz encapsulated crystal at b) 1.17 Å wavelength (3000s accumulation, ~3300 neutron monitored/sec, Gamma at 57 degrees). The diffraction is limited to *ca.*1.6 Å, which is sufficient for the purpose of the study, c) 1.46 Å wavelength (1000s accumulation, ~7300 neutron monitored/sec, (detector) Gamma at 25 degrees) showing diffraction to 1.47 Å (The intense peak in the low angle is the beamstop attenuated direct beam) and d) 2.42 Å wavelength (1000s accumulation, ~6300 neutron monitored/sec, "NbV" corrected image) showing diffraction to 1.64 Å.

Data collection

Xray crystal diffraction data has first been acquired successfully at 295K to 1.05 Å resolution and structure refinement shows the expected protein structure for the 113-Cd derivative of *Pf* rubredoxin.

Neutron data collection was performed successfully at 295K on a quartz capillary sealed 113-Cd substituted *Pf* rubredoxin crystal of a volume of 4.5 mm^3. Partial neutron datasets were recorded from within a phi window of a total of 47 degrees and also 180

degrees apart from it to obtain a set friedel‘s pairs of reflections, using step scans of 0.07° with an exposure time of 100 and 200s per step at 1.17 Å wavelength, yielding data to 1.65 Å and 1.59 Å resolution (2164 unique reflections), respectively. The overall anomalous data completeness after 70h exposure at 1.17 Å is 32.5%.
The data obtained at 1.17 Å puts the diffraction capabilities of this protein crystal at its boundaries as the separation between integrated peaks is minimal in the higher resolution area of the detector.

A second partial neutron data set was acquired at 1.46 Å a phi window of a total of 90 degrees and 180 degrees apart from it to obtain a reflection set of friedel‘s pairs, using step scans of 0.07° with an exposure time of 100s per step at 1.46 Å wavelength, yielding data to 1.47 Å resolution. The overall data completeness after 63h exposure at 1.46 Å is 58.3 % at 1.47 Å and the overall anomalous completeness is 49.5 %. The total number of observed reflections is 15116 (5084 unique).

A third partial neutron data set was acquired at 2.42 Å a phi window of a total of 98 degrees (at Chi 180 and Chi 150 degrees) and 180 degrees apart from it to obtain a reflection set of friedel‘s pairs, using step scans of 0.07° with an exposure time of 50s and 25s per step at 2.42 Å wavelength, yielding data to 1.64 Å resolution. The overall data anomalous completeness after 36h exposure at 2.42 Å is 33.4 % to 1.65 Å resolution. The total number of observed reflections is 5931 **(**2381 unique).

Cryofreezing test

The new methodology (Cuypers's large crystal cryocooling and mounting method) for crystal mounting was tested to combine the use of a cryo cooling device with neutron diffraction. However, as presented on Fig 2c, we are facing low quality neutron diffraction with the ^{113}Cd derivative at 100K: the short scans of 15s/image were not possible to index. Interestingly, 100K X-ray diffraction data has been successfully collected to 0.615 Å resolution but on a 0.001 mm^3 crystal fraction from the very same large crystal, with some of the peaks appearing multiple (crystal cracks).

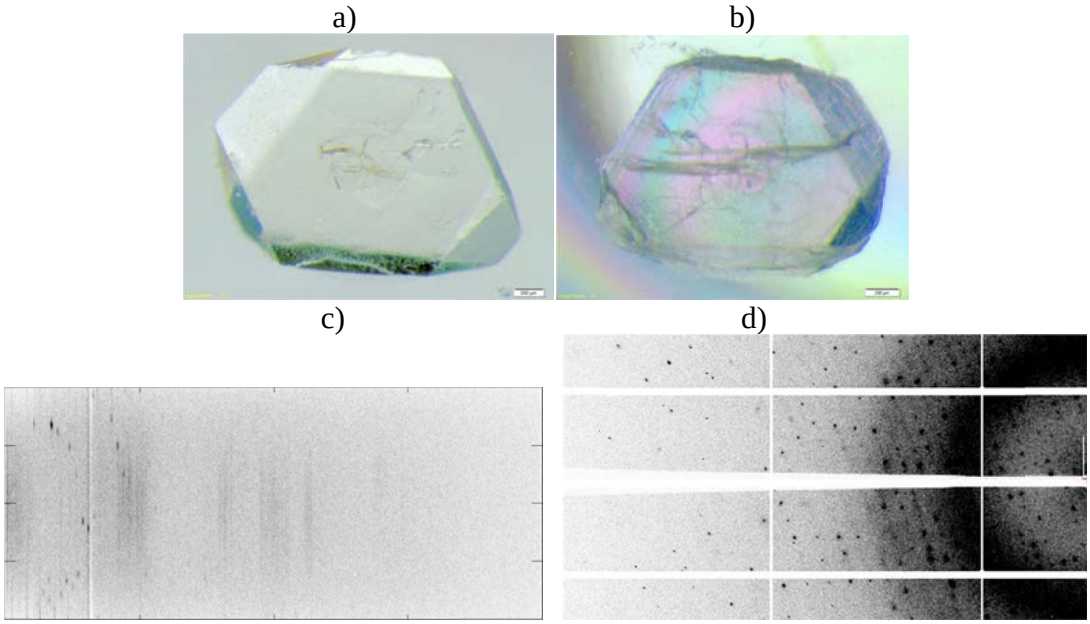

Figure 2. a) 1.5 mm^3 113-Cd substituted perdeuterated *Pf* rubredoxin crystal, b) crystal in (a) after being cryostream frozen to 100K now with a big crack (which could have occurred upon unfreezing) but overall still in very good "optical" condition (no whitening) and c) Neutron diffraction pattern at 1.17 Å wavelength from the 100K cryostream frozen crystal in a) (1000s accumulation, ~3000 n /sec) showing limited diffraction to a resolution of *ca.* max. 2 Å. d) X-ray diffraction pattern at 1.0 Å wavelength of a 200 µm long slice of the crystal in (b), showing diffraction to better than 1 Å (0.90 Å on other image) but with multiple diffraction peaks.

Note:

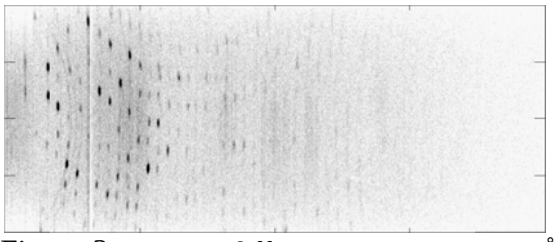

Figure 3. Neutron diffraction pattern at 1.17 Å wavelength from a 100K cryostream frozen Fe (not 113-Cd) substituted ~3 mm^3 pedeuterated *Pf* rubredoxin crystal (1000s accumulation, ~3000 n /sec). Although this is not a perfect crystal, it diffracts better than 1.2 Å resolution.

Conclusions

We have collected Friedel paired reflections and the preliminary analysis reveals that a fraction of the Friedel pairs show significant differences (better than 5 sigma), attributed to the measurement of an anomalous signal from the single ^{113}Cd atom per asymmetric unit within the protein crystal. It appears the anomalous data obtained for the neutron structure will be difficult to use to obtain the position coordinates of the heavy atom in the structure due to the low completeness on the datasets but will nonetheless be attempted using SHELX tools using neutron scattering libraries. This is an unprecedented experiment on large perdeuterated protein crystals bringing innovation to anomalous neutron dispersion experiments capabilities. The relative simplicity of the method can easily be applied to other protein systems (*i.e.* very X-ray sensitive crystals), be they capable to chelate a high neutron absorption cross section atom isotope in a stable position.

In order to perform a complete calculation of neutron attenuation, we need to obtain the b' and b'' values for 113-Cd at the 3 used wavelengths.

A manuscript describing the work methodology and results is in preparation.