Experimental Report

Proposal: **1-20-36** **Council:** 4/2014

Title: Feasibility studies of anomalous scattering phasing in neutron crystallography: macromolecular structure phasing with a complete dataset

This proposal is a new proposal

Researh Area: Methods and instrumentation

Main proposer: **CUYPERS Maxime**

Experimental Team: MOSSOU Estelle

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Samples: pyrococcus furiosus rubredoxin Cd form

Instrument	Req. Days	All. Days	From	To
D19	15	14	11/09/2014	15/09/2014
			25/11/2014	05/12/2014

Abstract:

We request beamtime on D19 to continue the crystallographic de novo phasing methodological proof-of-principle using enriched 113-Cd substituted perdeuterated Pyrococcus furiosus rubredoxin. Perdeuterated rubredoxin crystals are particularly suitable for this neutron anomalous study because of their stability and by the quality of the diffraction data usually obtained. The previous ILL Proposal 1-20-32 allowed us to successfully collect neutron anomalous differences on a 113Cd enriched crystal at 295K. The tests performed yielded high quality but incomplete data to 1.59 Å resolution. The practical demonstration of a protein structure being phased using anomalous neutron diffraction data using anomalous neutron dispersion requires complete neutron anomalous data, which was never performed before. This proposal aims to finish the evaluation and development of neutron de novo phasing of macromolecular protein structures and to further add anomalous neutron dispersion phasing methodology to the list of techniques available for D19's biological user base.

Experimental report for ILL proposal 1-20-36

Feasibility studies of anomalous scattering phasing in neutron crystallography: macromolecular structure phasing with a complete dataset

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Introduction

This experiment is directly related to ILL experiment 1-20-32 which allowed us to evaluate the potential of this concept.

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.

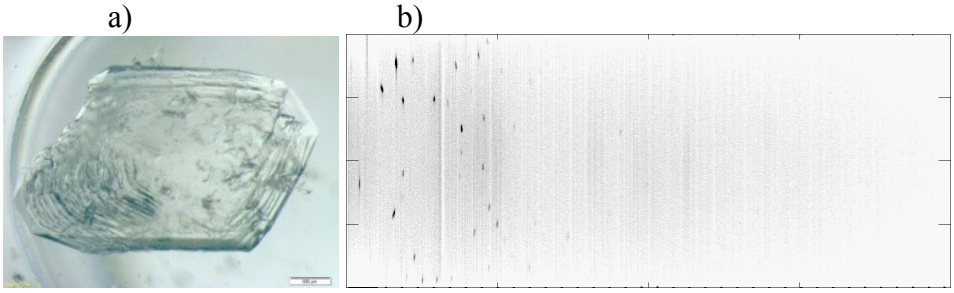

Figure 1. a) 4.5 mm^3 ^{113}Cd substituted perdeuterated *Pf* rubredoxin crystal. Neutron diffraction pattern from the 295K thin quartz encapsulated crystal at b) 1.17 Å wavelength (3000s accumulation, ~3300 neutron monitored/sec, Gamma at 57 degrees). The diffraction d-spacing resolution is limited to *ca.*1.6 Å, which is sufficient for the purpose of the study.

Data collection

Crystallographic 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.

A complete neutron dataset was recorded by rotating the goniometer head by nearly 360 degrees in phi with 4 different chi values to obtain a full set of Friedel‘s pairs of reflections, using step scans of 0.07° with an exposure time of 50s per step at 1.17 Å wavelength, yielding data to 1.75 Å resolution. The overall anomalous data completeness after 9 days of data collection at 1.17 Å is 100%.

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.

Conclusions

We have successfully collected complete neutron crystrallographic dataset with Friedel paired reflections. 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.

The neutron crystal structure was analysed with SHELXC/D/E and, for the first time, yielded purely experimental neutron phased maps to a resolution of 2.3 Å. This is an unprecedented crystallographic 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 used wavelength.

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