Experimental report

25/03/2022

Proposal:	**8-01-544**	**Council:**	4/2019

Title: A reverse labelling approach to shed light on the role and presence of hydronium ions and imidic aced tautomeric effects in proteins

Research area: Biology

This proposal is a resubmission of 8-01-535

Main proposer:	**Maxime CUYPERS**
Experimental team:	Juliette DEVOS
Local contacts:	Marie Helene LEMEE Estelle MOSSOU

Samples: Perdeuterated rubredoxin

Instrument	Requested days	Allocated days	From	To
D19	9	9	16/09/2019	26/09/2019

Abstract:

This is a resubmission of proposal 8-01-535 rejected on the basis of the fact that it was not obvious what the proposed experiment would add to already published data.

We request beamtime on D19 for room temperature (RT) neutron crystallographic studies of perdeuterated rubredoxin in H2O, extending previous work which revealed, at 0.88A resolution, a remarkable network of ordered water that includes hydronium ions. Residue Leu51 from the RT structure of the oxidised form presents an imidic acid tautomeric equilibrium that is absent in the reduced form. It was observed that every RT oxidised rubredoxin neutron structure over the last ~15 years from various groups presents this mysterious peak. This feature appears to be of direct relevance to elucidate the implications of the redox state in the immediate vicinity of the catalytic centre.

We wish to carry out a detailed crystallographic study of this network of hydroniums and tautomeric equilibrium by reverse labelling the water around the protein with H2O. The key aspect of this study is that the hydrogen atoms will appear as negative peaks in the Fourier maps and allow a clear and unambiguous visualisation of this network.

A reverse labelling approach to shed light on the role and presence of hydronium ions and imidic aced tautomeric effects in proteins.

Experimental report on proposal # 8-01-544

Experiment was carried out from 16 to 26 of September 2019.

Aim of the project :

This experiment is a follow-up to the experimental report for ILL proposal 8-01-458 (“Direct observation of hydronium and Zundel ions in proteins by neutron crystallography: a reverse labelling approach”), experiment that was carried out in 2016 at ILL on the D19 instrument.

In order to study the remarkable network of ordered water that includes hydronium ions within the small iron sulphur cluster protein Rubredoxin from the extremophile *Pyrococcus furiosus* (Pf), we have carried out a detailed crystallographic study of this network of hydroniums and tautomeric equilibrium by reverse labelling the water around the protein with H2O. The key aspect of this study is that the hydrogen atoms will appear as negative peaks in the Fourier maps and allow a clear and unambiguous visualisation of this network.

Sample preparation :

Large crystals of perdeuterated Pf Ru were grown in fully deuterated crystallization conditions.

Once they reached an appropriate size in order to be used on the D19 instrument, we have very slowly exchanged the crystallization buffer from fully deuterated to fully hydrogenated solutions. This was carried out first by first vapor diffusion and then by replacing the buffer around the crystal itself.

Back-exchanged crystals of perdeuterated rubredoxin were mounted inside quartz capillaries and data was collected at RT on D19.

Experimental plan :

- Four different large crystals were tested during this experiment. Data was collected on the best one (JD13_B2, Figure on the right) at room temperature, at a wavelength of 2.4362 Å, using a 3 mm aperture.