Experimental report

09/09/2016

Proposal: 8-01-475 **Council:** 4/2016

Title: Anisotropic displacement parameters of non-hydrogen atoms in proteinsfrom neutron diffraction data measured at multiple temperatures.

Research area: Biology

This proposal is a resubmission of 8-01-457

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Samples: Crystals of triclinic lysozyme

Instrument	Requested days	Allocated days	From	To
D19	20	20	01/06/2016	21/06/2016

Abstract:

Neutron diffraction studies on proteins have so far primarily been used to locate hydrogen (or D) atoms. Structures are usually not refined to high resolution and there has been no emphasis on atomic displacement parameters (ADPs). The function of proteins is linked to their dynamical behavior, which in principle could emerge from an analysis of anisotropic ADPs. A previous X-ray study to obtain anisotropic ADPs by refinement of high resolution X-ray diffraction data from P1 hen egg-white lysozyme (HEWL) at four low temperatures, demonstrated the need for an independent estimate of anisotropic ADPs by neutron diffraction. The aim of this proposal is to obtain anisotropic ADPs by refinement of high resolution neutron data measured at 295K, 180K and 100K in order to address the following questions:
i) Can neutron diffraction data provide anisotropic ADPs for proteins? ii) Can the ADPs be related to protein dynamics? iii) Are the ADPs comparable to those obtained from high resolution X-ray studies?

Resubmitted as the crystallization conditions used during our previous work unexpectedly needed further optimization. This is now under control as demonstrated in the scientific case.

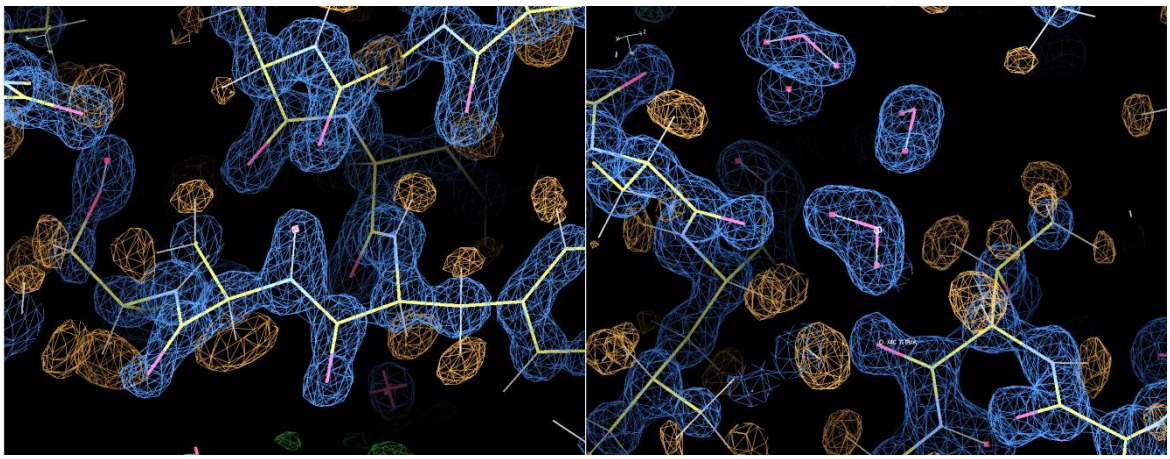
Preliminary experimental report for ILL proposal 8-01-475

Anisotropic displacement parameters of non-hydrogen atoms in proteins from neutron diffraction data measured at multiple temperatures

The following questions were posed in the proposal: Can neutron diffraction data provide anisotropic ADPs for proteins? Are the anisotropic ADPs from high resolution X-ray studies truly representing atomic displacements? Can the ADPs be directly linked to the protein dynamics?

This preliminary report describes our results so far from the experiments conducted to answer these questions.

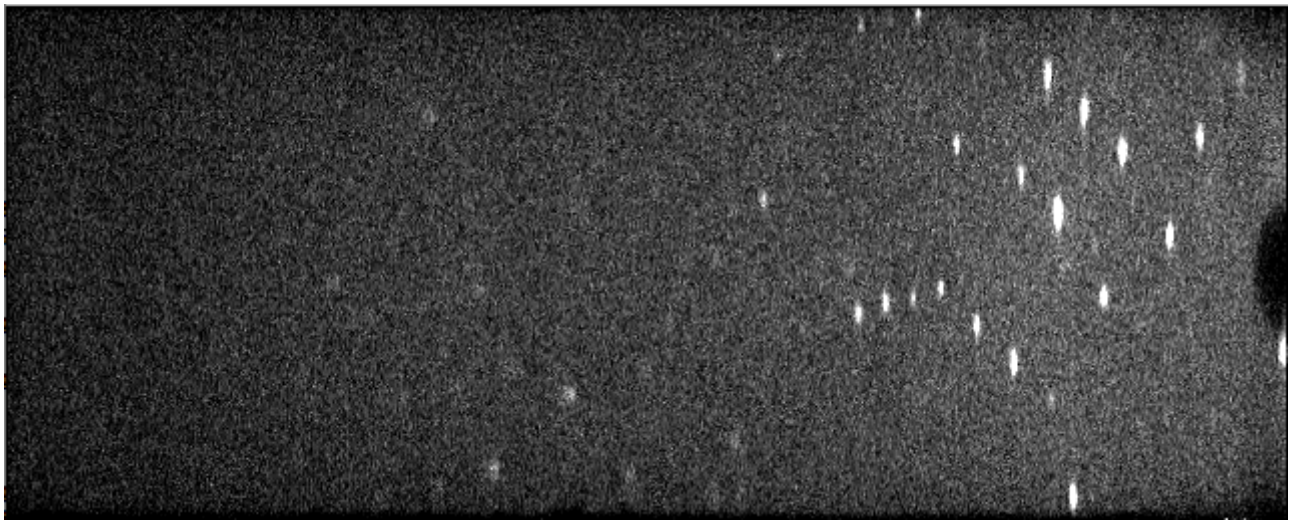
A record breaking high resolution neutron diffraction data set was collected at room temperature on triclinic Hen Egg-White Lysozyme (HEWL) at D19 in June 2016, complete to a resolution of approximately 1 Å on a deuterated 6 mm^3 crystal. Diffraction was observed beyond 1 Å and work is ongoing towards improving the data processing, in particular of weaker reflections, and thus the final resolution cutoff. With the low symmetry and requirements for good completeness and a large number of unique reflections, this room temperature data took longer than expected (a total of 10 days). The extended time used, is in part due to the large number of scans needed to obtain complete data of this low symmetry form, and thereby even relatively small increases in the exposure time, has significant impact on the total data collection time.
However, with +50000 unique reflections unrestrained refinement of anisotropic ADPs is now possible without use of X-ray data, thereby answering our first original question: Can neutron diffraction data provide anisotropic ADPs for proteins? Yes.
Various approaches to the refinement are currently being considered and tested, currently the R_{work} and R_{free} values for the stronger reflections (>4σ) are in the order of 15% and 23%, respectively. In addition, comprehensive analyses and comparisons of resulting displacement parameters from refinement against corresponding X-ray data is awaiting.



Model and maps from the room temperature HEWL data collected at D19, clearly resolving H/D and oriented D_2O molecules. 2Fo-Fc (blue), negative 2Fo-Fc (orange) shown at 2σ level.

In addition to the anisotropic ADPs for all atom types, it is evident that additional information can be extracted, analyzed and published from such high resolution data regarding the H/D exchange as well as the comprehensive water networks and dynamics. Thus these analyses will, although secondary to main questions, also contribute with valuable new knowledge in structural biology and thus to the overall outcome of this experiment.

A few partial data sets were collected at 100 K in June, however due to significant problems with ice formation and crystal mounting, a complete data set was not collected. Merging of the partial sets is not feasible. These experiments at 100 K did confirm the possibility of reaching 1 Å or even higher resolution at 100 K. Valuable experience was gained and the difficulties encountered regarding approaches used in cooling and mounting these crystals, can thus be overcome in future experiments.



Diffraction observed from triclinic HEWL at 100 K. From partial data collected at D19, June 2016.