Experimental report

12/02/2017

Proposal: 8-05-428 **Council:** 10/2016

Title: Neutron spectroscopy & crystallography combined with THz spectroscopy: complementary methods for the study of protein dynamics

Research area: Biology

This proposal is a new proposal

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Samples: E. coli copper amine oxidase

Instrument	Requested days	Allocated days	From	To
LADI	21	1		
IN5	4	2	28/02/2017	02/03/2017
IN16B	1	1	08/02/2017	09/02/2017

Abstract:

Structural biology aims to relate structure to function, but obtaining detailed information about functional dynamics remains challenging. Although crystallographic structures encode information about dynamics, this cannot be routinely extracted. In contrast, spectroscopy provides detailed dynamic information, but not an overall structural picture of the conformational variation associated with it. Further, integrating the information obtained from disparate biophysical methods is difficult as they probe dynamics and structure on different length- and time-scales, as well as different states (solutions, powders and crystals). We will address this by taking an interdisciplinary approach, combining neutron spectroscopy and crystallography with THz vibrational spectroscopy. Crucially our experiments will be carried out on the same proteins, prepared in the same way, in order to enable us to truly compare the data obtained from each experimental method. In this proposal we will study the E. coli enzyme copper amine oxidase (CuAO), where dynamics are proposed to be intimately involved in its catalytic mechanisms.

In the single-day experiment during February 2017 on IN16B, we have recorded QENS spectra on both ADC-APO and ADC-ligand solutions in D2O at 280K. Moreover, we have recorded elastic and inelastic (1.3ueV) fixed-window scans in cooling to 70K and a QENS scan on the ligand sample at T=150K.

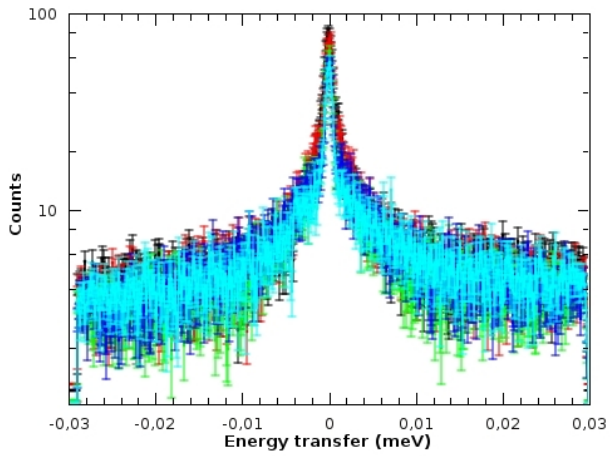

Figure 1: ADC-APO in D2O, T=280K, PSD tubes 2,4,6,8,10 (q=0.57, 0.82, 1.06, 1.28, 1.48/A)

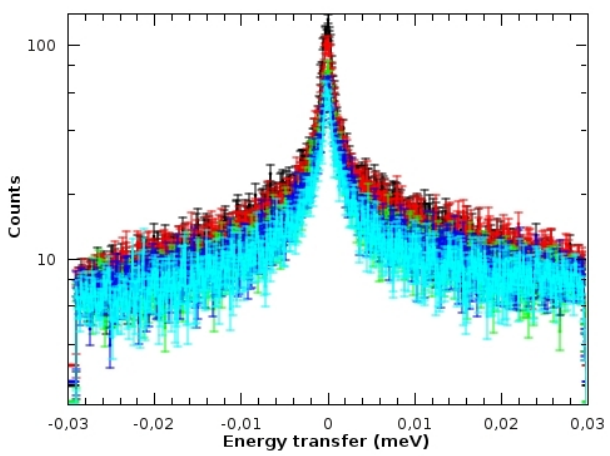

Figure 2: ADC-ligand in D2O, T=280K, PSD tubes 2,4,6,8,10 (q=0.57, 0.82, 1.06, 1.28, 1.48/A)

Figures 1 and 2 display example spectra recorded during the IN16B experiment on the two samples. The spectra establish that a good signal has been recorded. The data analysis will be carried out using published protocols (Ref.[1] and references therein.)

Sample:
The sample used for this study was α-aspartate decarboxylase (ADC). This enzyme is responsible for the conversion of L-Aspartate to Alanine. This chemical conversion is linked with the movement of a loop. However, the time-scales and dynamics of the loop movement remain unknown. Also, the overall dynamics and the dynamics associated with catalysis of this enzyme are not known. QENS experiment was performed on ADC in apo form and in complex with ligand in order to probe the global dynamics of ADC. L-Serine was used as the ligand since it would be difficult to capture the loop movement for L-Aspartate as it would be converted to Alanine.

Sample preparation:
The last stage of purification of ADC was gel filtration and it was in Tris buffer (pH 7.4) prepared in water. Purified ADC was concentrated to the minimum possible volume and this was followed by a buffer exchange using the same buffer which was prepared in Deuterium oxide. Deuterium oxide was used in order to avoid the incoherent signal which would result due to the hydrogen atoms of water molecules. After completing the buffer exchange, both the samples were concentrated to ~50 mg/mL while maintaining their volumes to ~1.5 mL.

Reference:
[1] M. Grimaldo et al., EPJ Web of Conferences **83**, 02005 (2015), http://dx.doi.org/10.1051/epjconf/20158302005