Experimental report

08/02/2019

Proposal: 8-05-431 **Council:** 4/2017

Title: Neutron spectroscopy and crystallography combined with THz & fs-infrared spectroscopy: functionally related protein dynamics and structure

Research area: Biology

This proposal is a continuation of 8-05-428

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Samples: ADC Aqueous solution
CuAO aqueous protein solutions

Instrument	Requested days	Allocated days	From	To
LADI	20	0		
IN5	2	2	22/05/2018	24/05/2018
IN16B	2	2	11/06/2018	13/06/2018

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. We will use two exemplar systems; copper amine oxidase (CuAO) and alpha-aspartate decarboxylase (ADC), both from E. coli, where dynamics are proposed to be intimately involved in their catalytic mechanisms.

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Sample preparation:

Aspartate alpha decarboxylase (ADC) – APO (without the ligand) and in complex with D-Serine (substrate analogue). The purified protein was in Tris buffer with the following composition: 50mM Tris (pH 7.0), 100 mM NaCl, 0.1 mM DTT in D_2O. The protein solution was concentrated to 135 mg/mL (concentration was measured using UV absorbance at 280 nm wavelength). All the measurements were carried out at this concentration and in solution.

Results:

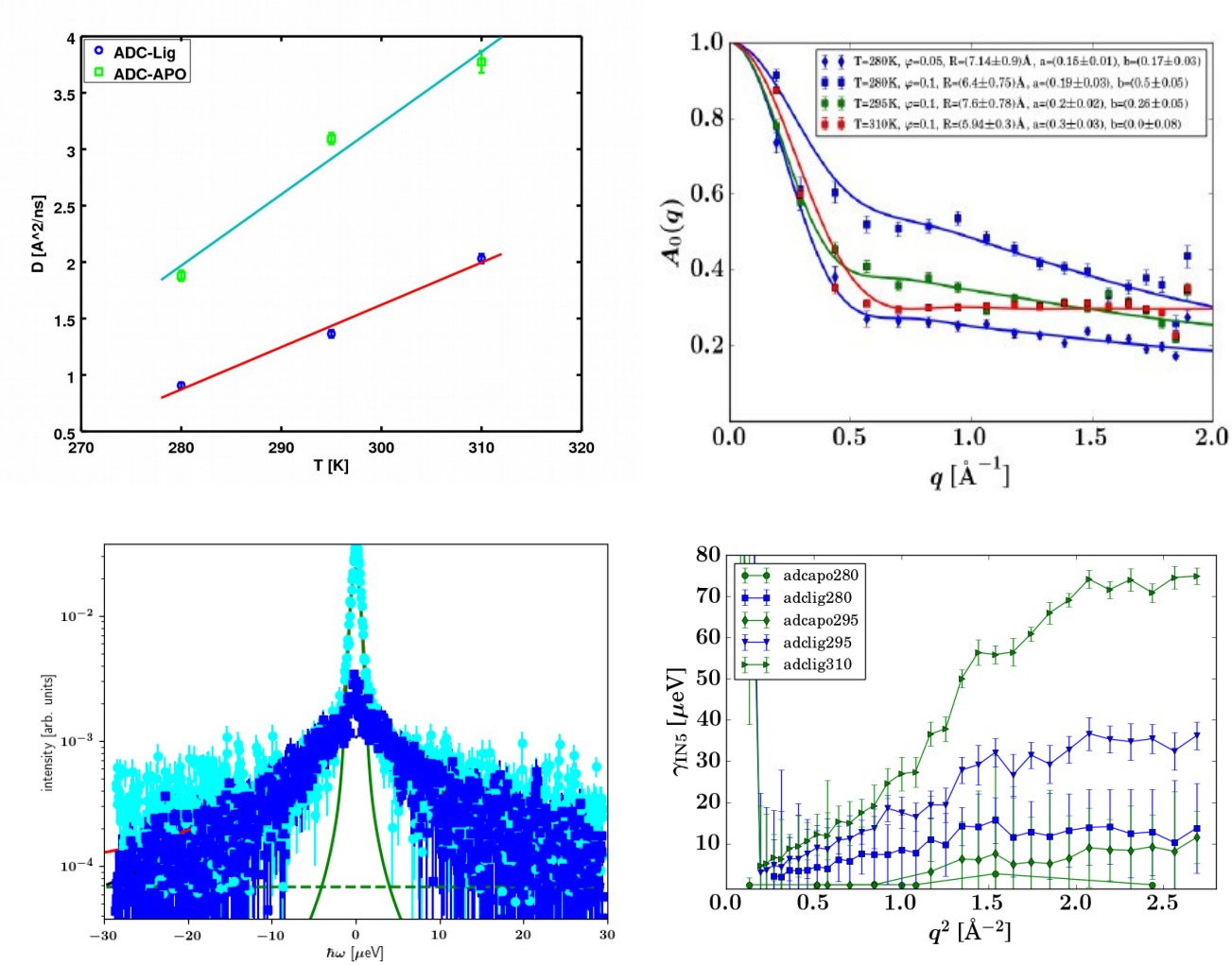

Figure 1. A. Global apparent centre of mass diffusion D (γ(q) = Dq^2) for ADC-APO and ADC-LIG samples. B. Elastic incoherent structure factor plotted for ADC-LIG sample at different temperatures. The data are from IN16B. C. Intensity vs. frequency plot (raw data) for q=0.8Å^{-1} (ADC-APO sample at a concentration of 135 mg/ mL) recorded at 280K. The cyan circles denote the empty can subtracted protein signal and the blue squares denote the pure solvent signal. The solid line on the circles denotes the fit according to the following equation: S(q,ω)=R⊗{β(q)[A0(q)L(γ(q),ω)+(1−A0(q))L(γ(q)+Γ(q),ω)]+βD2O(q)L(γD2O(q),ω)}. The narrow solid line represents Lorentzian with linewidth γ and the green dashed line represents the Lorentzian with linewidth Γ. D. Main Lorentzian contributions obtained from the previous beamtime (8-05-431) plotted for ADC-APO and ADC-LIG samples. The numbers in the legend represent the temperatures at which the spectra were recorded.