Experimental report

09/02/2016

Proposal: 8-04-689 **Council:** 10/2012

Title: Domain Motions of Inorganic pyrophosphatase from the hyperthermophileThermococcus thioreducens Studied by NSE

Research area: Biology

This proposal is a new proposal

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Samples: IPPase, D2O
C270H437N71O86

Instrument	Requested days	Allocated days	From	To
IN15 Standard	8	0		
IN11	8	6	06/05/2015	12/05/2015

Abstract:

The aim of this proposal is to use neutron spin-echo spectroscopy (NSE) to measure the inter-domain motions of the inorganic pyrophosphatase (IPPase) enzyme from thermostable microorganisms. IPPase derived from thermostable microorganisms is of extreme interest for biophysical studies because of their inherent chemical and thermal stability and high temperature activity. It has a hexameric quaternary structure with a molecular mass of approximately 120kDa (each subunit is about 20kDa molecular weight), which is a large oligomeric molecular structure and is supposed to have significant slow inter-domain motions. Study of this slow inter-domain motion is the key to understand why IPPase can perform catalytic activity at much higher temperature than normal enzymes, thus enables bacteria to survive under extremely high temperature.

Experimental report for 8-4-689: Domain motions of inorganic pyrophosphatase from the hyperthermophile *Thermococcus thioreducens* studied by NSE

The main aim of the measurement was to determine the slow inter-domain motions within IPPase, which are believed to be key to understanding how IPPase can perform catalytic activity at much higher temperature than normal enzymes, enabling the microorganisms to survive under extremely high temperatures. Backscattering measurements were already performed at BASIS up to fouriertimes of around 0.5-1 ns, but it was clear from this data that even slower processes are present, beyond what can be measured within the instrumental resolution of BASIS. Neutron spin-echo (NSE) is a technique uniquely suited for extending the dynamic range in such a way, since it is the neutron technique with the best energy resolution. This timescales that can be accessed with NSE are ideally suited for measuring inter-domain motions. For this reason neutron spin-echo measurements were carried out at IN11, to extend the dynamic range at a comparable Q-range to BASIS.

The measurement was carried out in May 2015 on IN11C. During this user cycle the instrument resolution of IN11 was significantly reduced, due to interference from magnets placed on a neighbouring instrument. This meant that the maximum fouriertime achievable was 1.3 ns, meaning that the dynamic range was not extended significantly beyond the backscattering measurement on BASIS, as had been the aim.
Nonetheless, we attempted to measure the dynamics at 3 temperatures, and were able to observe dynamics consistent with that seen on BASIS, see Fig 1. The sample was IPPase hydrated with D_2O.

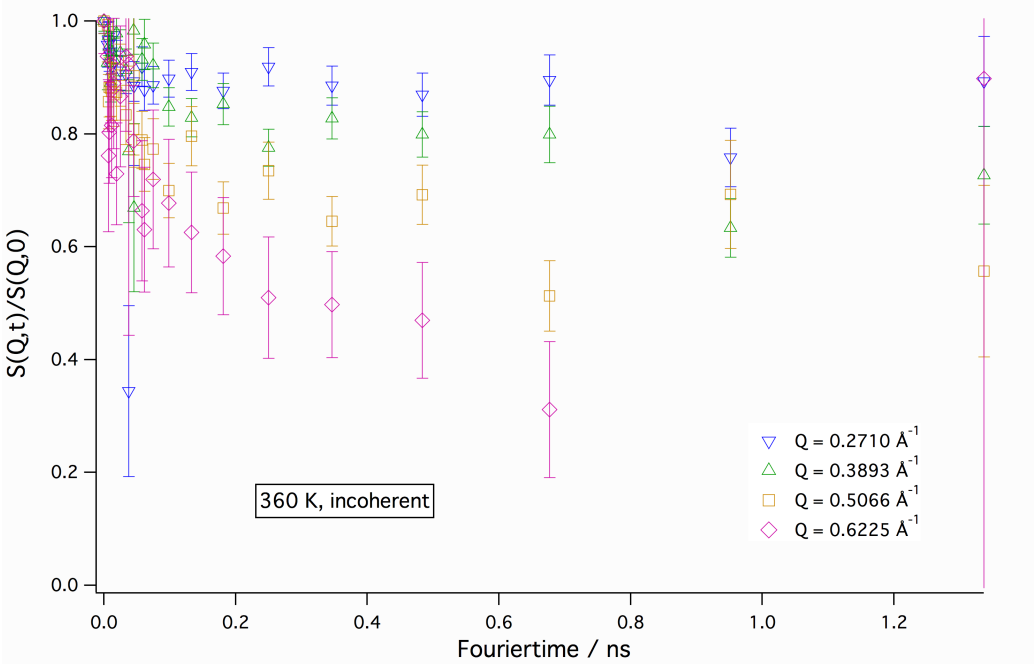

Figure 1: Incoherent data measured at 360K on IN11 for IPPase

To achieve the aim of the measurement for which beamtime was granted, it will be necessary to repeat the measurement at a time when IN11 is not operating with a reduced resolution due to neighbouring instruments.