Experimental report

17/01/2017

Proposal: 8-04-684 **Council:** 10/2012

Title: The influence of cofactor binding on thermal fluctuations of alcohol dehydrogenase studied by using quasielastic neutron scattering

Research area: Biology

This proposal is a continuation of 8-04-623

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Samples: alcohol dehydrogenase, D2O

Instrument	Requested days	Allocated days	From	To
IN5	3	2	25/03/2013	28/03/2013

Abstract:

Large scale amplitude domain motions have been observed in the protein alcohol dehydrogenase (ADH) using neutron spin-echo spectroscopy. Binding of the small cofactor nicotinamide adenine dinucleotide (NADH) resulted in an apparent reduction of the diffusion coefficients of the collective domain motions. One hypothesis, which we would like to test, is whether fast localized protein fluctuations are related to the collective domain motions. In this proposal we propose to investigate using the IN5 spectrometer whether localised thermal fluctuations in the protein ADH are influenced by binding of the cofactor NADH. The IN5 instrument would be set to two resolutions covering the 100ps time scale, which is associated with slow localized dynamics in proteins, and the ps time scale, which is associated with fast amino acid side chain motions. We expect that this will shed light to connections between localized thermal motions and collective domain motions.

Large-scale domain motions in alcohol dehydrogenase (ADH) have been observed previously by neutron spin-echo spectroscopy (NSE). We have extended the investigation on the dynamics of ADH in solution by using high-resolution neutron time-of-flight spectroscopy measured on IN5 at the ILL and neutron backscattering spectroscopy measured on SPHERES at the MLZ. The observed hydrogen dynamics were interpreted in terms of three mobility classes, which allowed a simultaneous description of the measured neutron time-of-flight and backscattering spectra. In addition to the slow global protein diffusion and domain motions observed by NSE, a fast internal process could be identified. Around one third of the protons in ADH participate in the fast localized diffusive motion. The diffusion coefficient of the fast internal motions is around two third of the value of the surrounding D_2O solvent. It is tempting to associate the fast internal process with solvent exposed amino acid residues with dangling side chains.

Reference

Monkenbusch et al. Fast internal dynamics in alcohol dehydrogenase. *The Journal of Chemical Physics* 143, 075101 (2015)

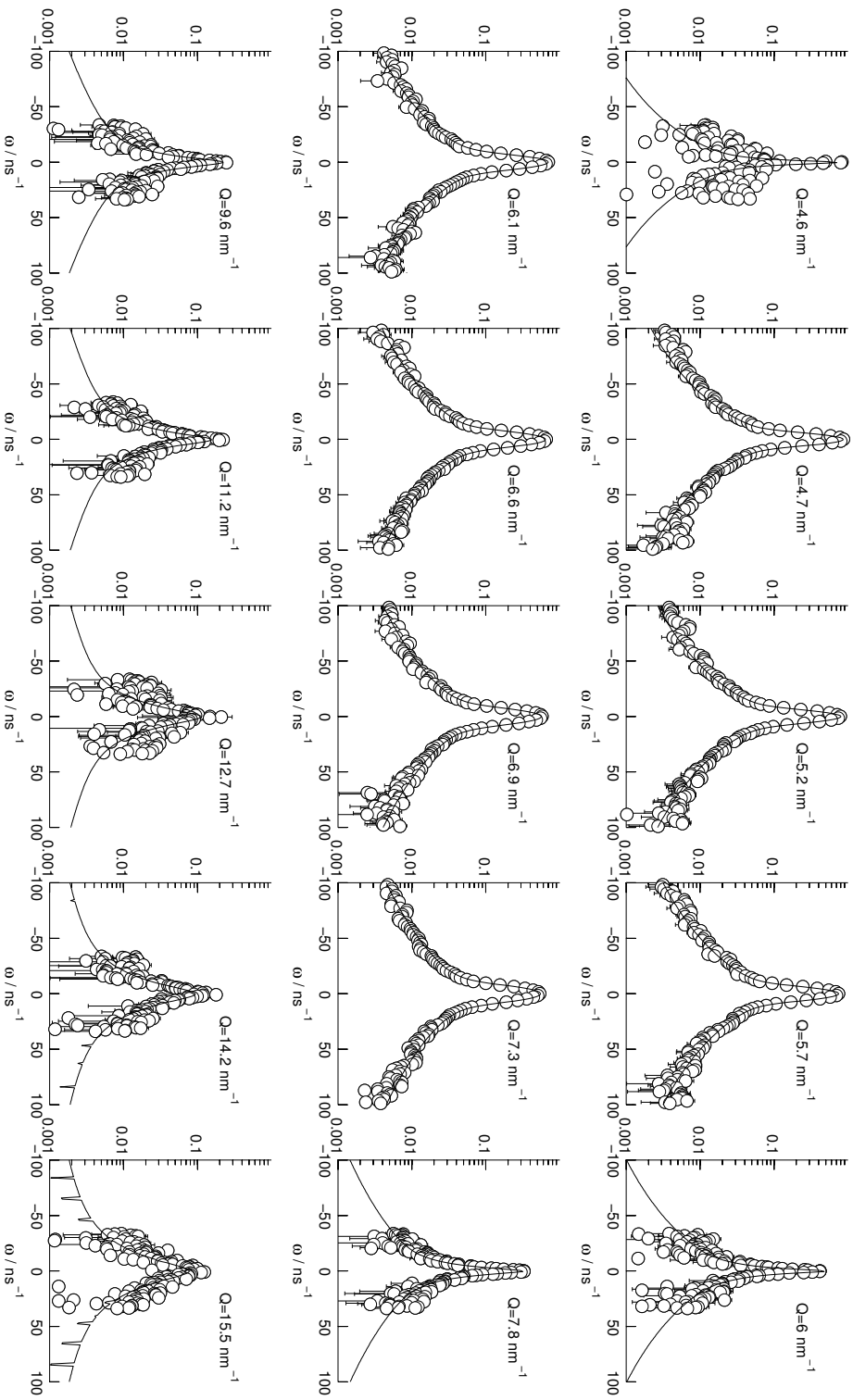

Figure 1: A compilation of neutron time-of-flight and backscattering (spanning $\omega = \pm 35$ ns^{-1}) spectra covered by both experiments. The lines through the data represent a fit with the theoretical model including a large-scale domain motion as described in the manuscript. Note that the fit was simultaneous with a common intensity scale with respect to the vanadium spectra.

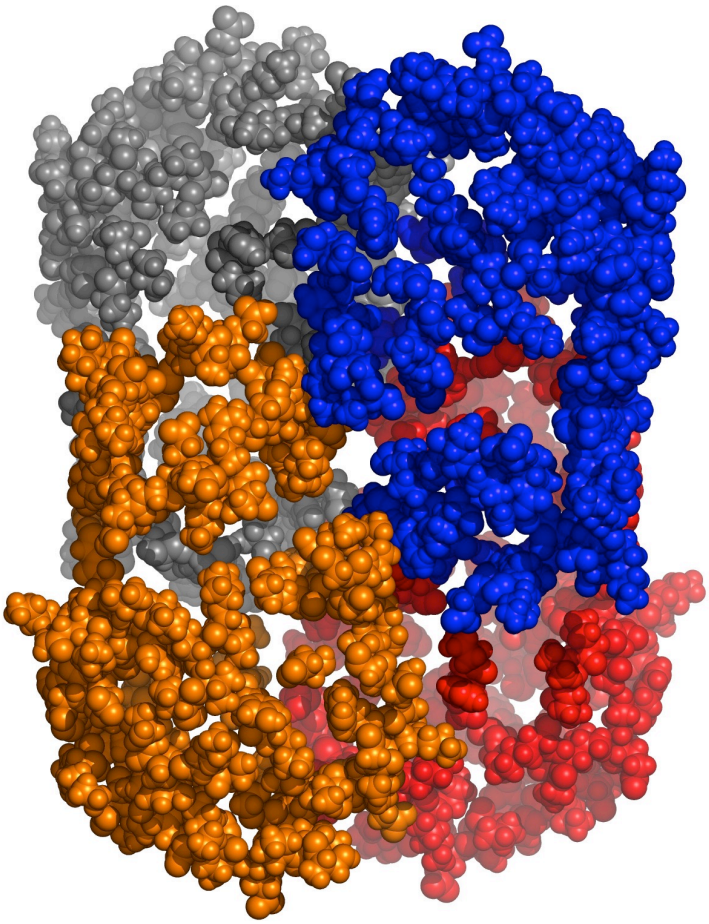

Figure 2: Surface residues of the ADH tetramer possibly contributing to the fast internal motion observed by neutron scattering. The colour discriminates the four domains of the protein.