Experimental Report

Proposal: **INTER-262** **Council:** 10/2012

Title: Internal time on D22

This proposal is a new proposal

Researh Area:

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Samples: Ubiquitin
Lysozyme
D2O
H2O

Instrument	Req. Days	All. Days	From	To
D22	1	1	08/05/2013	09/05/2013

Abstract:

Summary of measurements INTER-262 at ILL in May 2013

The immediate aim of the test SANS measurements was to investigate the possible effect of slow amide hydrogen (N^1H) exchange on protein scattering profiles and to assess the potential of employing these variations to facilitate the interpretation of domain structure(s) and folding state(s) of proteins in solution. Two well-characterized proteins, monomeric hen egg lysozyme (MW=14 kDa) and tetrameric glucose isomerase (MW=176 kDa) were selected for this project. The kinetics of deuterium exchange was studied in 10-min frames at 50% D_2O (both proteins) and at 60% D_2O (lysozyme only). Control contrast variation measurements were also performed on equilibrated samples.

For the lysozyme samples no significant differences in the scattering profiles were observed in the SANS data irrespective of incubation time or % D_2O. This is probably due to relatively small size of the protein facilitating the H/D exchange (which could have occurred within the first timeframe). For the much larger glucose isomerase some variations in unsubtracted scattering profiles were detected in the *s*-range up to 0.1 A^{-1}. However any changes in scattering intensities were quite moderate (Figure. 1) and it is still questionable if these alterations could be useful for structural interpretations. Even though the experiments were not successful with respect to the overall aim of quantifying internal scattering length density fluctuations caused by N^1H exchange, the experiments did reveal that both excluded volume and solvation-layer effects must be considered with respect to preparing protein samples near their contrast match points.

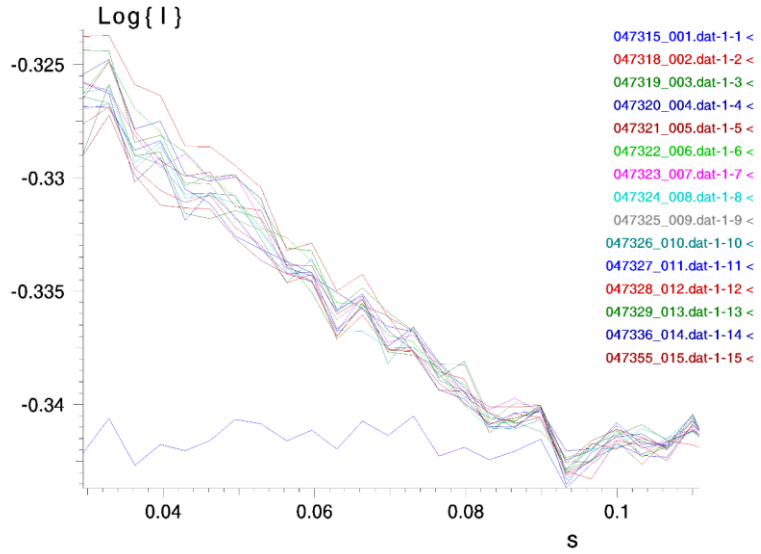

Figure 1. H/D kinetics of glucose isomerase in 50% D_2O. Scattering from the solvent is shown as a blue line for the reference