Experimental report

21/04/2017

Proposal: **INTER-337** **Council:** 4/2016

Title: Internal time on IN13

Research area:

This proposal is a new proposal

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Samples: alpha lactalbumineat different hydration

Instrument	Requested days	Allocated days	From	To
IN13	8	8	04/11/2016	12/11/2016

Abstract:

Experimental report: INTER 337 on IN13 - From 04/11/2016 to 12/11/2016

Experimenters: Dominik Zeller, Judith Peters

Complete data set of different hydration levels of bovine alpha-Lactalbumin

Background

In 2013, following the ideas which led to the creation of the very successful Protein Data Bank (pdb) for structures, G. Zaccai and coworkers proposed to build up a neutron Dynamics Data Bank (nDDB)[1] which would be populated with primarily dynamical data of biological macromolecules measured with different neutron spectrometers as well as some NMR and molecular dynamics (MD) simulation data. The ultimate goal is to enable us to find trends in the experimental data over a range of different systems and instruments and under many different conditions like temperature, pressure, hydration, pH/pD or crowding, hopefully leading to a global picture of the dynamics of biomolecules One important aspect of this task is to figure out to what extent such trends may depend on sample preparation, data reduction and data analysis, to have accurate comparisons between data sets. For instance, so-called elastic window scans typically performed on neutron backscattering spectrometers are often used to compute mean square displacements (MSD) as a measure of protein flexibility. Typically MSDs are derived from fitting the Q-dependence of the measured elastic intensity on a given neutron spectrometer (so at a given energy resolution and Q-range), with the Gaussian Approximation (GA). In the last few years, the validity of such models has been questioned and models that go beyond the Gaussian approximation have been proposed [3-6]. Despite a large number of proteins measured at specific conditions, and even for one of the most studied systems, lysozyme, there is no systematic study to cover a complete set of measurements (EINS, QENS, INS), time scales and conditions. We propose to perform a systematic study on a relatively simple molecule, bovine alpha-Lactalbumin (123 amino acids, 14.2 kDa), commercially available and easily amenable to simulation, making it feasible to perform a systematic analysis of its dynamics both experimentally and computationally.

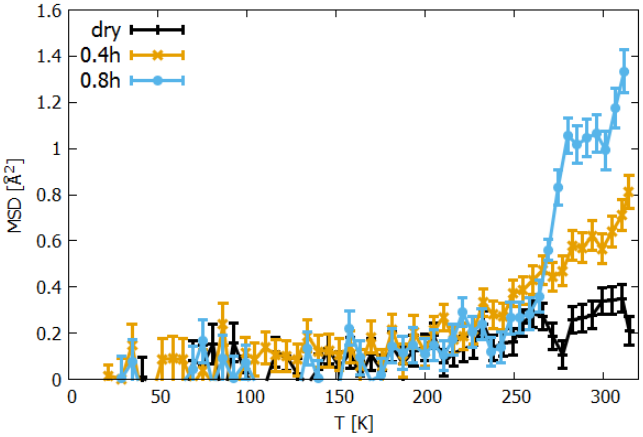
Experiment

We measured alpha-lactalbumin in powder form in four different hydration of D_2O. As ratio h = g D2O/g we used h=0, h=0.4, h=0.85 and h=4.1, corresponding to a dry sample, a sample with ~ one hydration layer, an almost fully hydrated sample and a sample in solution [7]. Except for the sample in solution we measured elastic fixed window scans (EFWS) from 20 to 315K. The sample in solution was measured with EFWS from 280-315K. All samples were cooled down and then the data was registered under an angle of 135 degree by slowly heating the flat aluminum sample holder.

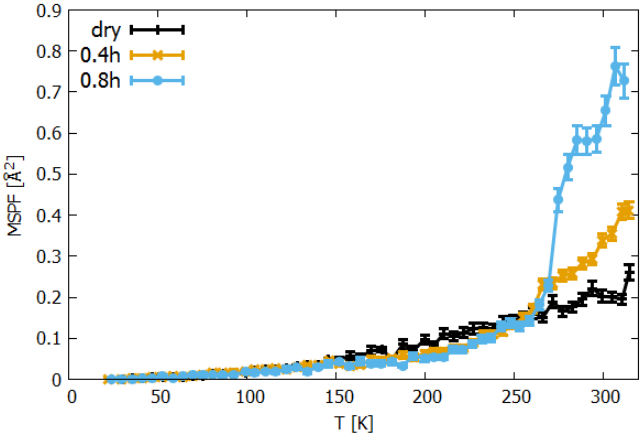
Results

The elastic data were analyzed using two of the current models, the GA and the Kneller and Peters (KP) model [3] which aims to describe low and high Q regions. After the evaluation of the data we obtain the MSD (or fluctuations –MSPF- as defined in the KP). Some preliminary data are shown in the figures beneath:

(a) GA from Q=0.4-1.7 Å^{-1}



(b) KP model, weighted, fixed offset to 1 at Q→0Å^{-1}



(b) Different fits for hydration 0.8h

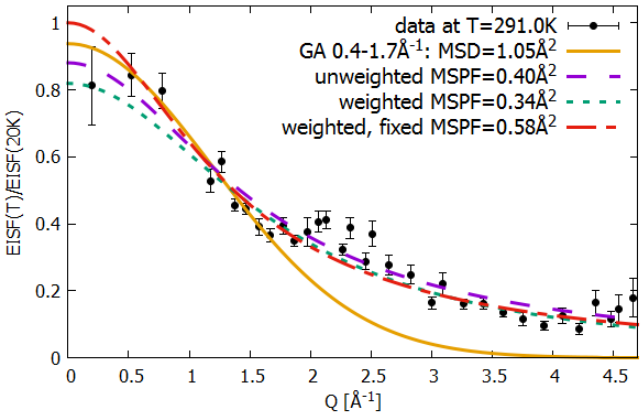


Figure: a) MSDs obtained with GA for the three lowest hydrations. b) MSPF obtained with KP model with weighted fit and fixed offset to 1 at Q→0Å^{-1}. c) Example of different fits with GA and KP model (MSPF) for 0.8h hydration at 291K. The change in offset has an impact on the resulting MSPF and at high Q the fits are almost the same.

It can be easily seen by the comparison between the GA (Fig. (a)) and KP (Fig. (b)) that both models give similar results: the tendencies between the different hydrations are similar between the models – increasing and clearly distinguishable MSDs with higher hydration. Also shown in the figure (c) is an example of fitting the 0.8h hydration data for the depleted sample from IN13 at 291K in four different ways: with the GA, with the KP model unweighted, weighted and fixed at Q→0Å^{-1} intensity. The fit with the GA can only describe the data in the low Q-region and the KP model is much better, but the low Q region is rather sensitive with different values of the fit parameters possible to fit the data. This results to fluctuations between the MSPFs and makes the evaluation more complicated. Nevertheless, the data is better described with the KP and we can evaluate the entire available Q-range instead of being limited by low Q with the GA. The stability of the KP has to be investigated further. Additionally, other measurements on other instruments with different resolutions have to be performed to test the model and to see if more information can be obtained by this model.

References

[1] L. Rusevich et al., *Eur. Phys. J. E*, 36, 80, 2013; [2] B. Aoun et al., *Eur. Phys. J. E*, 39, 48, 2016 ; [3] J. Peters and G.R. Kneller, *JCP*, 139, 16, 2013 ; [4] Zheng Yi et al., *JPCB*, 116, 16, 5028-5036, 2012 ; [5] A. Tokuhisa et al., *Phys. Rev.* E, 75, 04, 2007 ; [6] D. Vural et al., *Phys. Rev. E*, 88, 052706, 2013 ; [7] S. Perticaroli et al., *JACS*, 139, 3, 1098–1105, 2017.