Experimental Report

Proposal: **8-05-420** **Council:** 4/2014

Title: Real time study of the dynamics ofproteins throughout a one-step anda two-step crystallization process.

This proposal is a new proposal

Researh Area: Soft condensed matter

Main proposer: **GRIMALDO Marco**

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Samples: YCl3
Aqueous betalactoglobulin (BLG) protein solution

Instrument	Req. Days	All. Days	From	To
IN16B	4	2	17/11/2014	19/11/2014

Abstract:

Protein crystallization is of great interest due to its crucial role for the determination of protein structures, as well as in other fields such as drug engineering by pharmaceutical industries [J. Gunton et al. Protein Condensation: Kinetic Pathways to Crystallization and Disease. CUP, (2007)]. Despite its importance, a fundamental understanding of the mechanisms underlying such a process is still missing. Recently, both experimental [F. Zhang et al. Journal of Applied Crystallography 44, (2011)] and theoretical [P. G. Vekilov, Nanoscale 2, (2010)] studies have shown that, under certain conditions, crystallization follows a two-step mechanism, rather than the classical nucleation pathway. In order to gain a better understanding of such processes, an in situ study of the dynamics of two suitable crystallizing systems by QENS at IN16B may provide new extremely useful information, thus potentially significantly improving the general physical picture.

Experimental Report

Proposal number:	8-05-420		
Experiment title:	**Real time study of the dynamics of proteins throughout a one-step and a two-step crystallization process.**		
Instrument	IN16B		
Dates of experiment:	Nov.17-19, 2014	**Date of report:** Feb.10, 2015	
Team: **Names (* marks experimentalists)**	**Addresses**		
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Local Contact:	Tilo Seydel		

During experiment 8-05-420, we have collected a data set on the crystallization of beta-lactoglobulinin (BLG) in aqueous (D_2O) solutions, with the aid of the divalent salt $ZnCl_2$. Two solutions were prepared, one crystallizing through a classical nucleation pathway, the other crystallizing through a two-step process.

Sample Preparation: The two solutions (see table below) were filled in double-walled cylindrical aluminum sample holders with an outer diameter 23mm and a gap between the two walls of 0.25mm (capacity of about 1.2 ml). The containers where sealed hermetically with indium wire to avoid evaporation in the cryofurnace.

Nominal concentration of BLG [mg/ml]	Concentration of $ZnCl_2$ [mM]	Temperature (set point) [K]
100	9 (one-step)	280
100	29 (two-step)	280
Pure D_2O		280

Measurements: In addition to the samples listed in the table above, a vanadium foil and an empty sample holder were measured to allow the calibration and the subtraction of the various contributions from the sample spectra in later analysis.

Data Treatment: The following steps were done with MATLAB code:

- Subtraction of the empty sample holder
- Calibration, correction for detector efficiency and determination of the resolution function using the vanadium spectrum. The resolution function was described by a combination of five Gaussians and a flat background.
- To accurately take into account the contribution of D_2O, at every scattering vector Q a fixed term $\beta_{D_2O} L_{\gamma_{D_2O}}(\omega)$ with the Lorentzian function $L_{\gamma D2O}$ and scalar β (convoluted with the resolution function) was added directly to the model used for the fit of the spectra [1]:

$$S(Q,\omega) = R(\omega) * [\beta_1 L(\omega,\gamma) + \beta_2 L(\omega,\gamma+\Gamma) + \beta_{D_2O} L_{\gamma_{D_2O}}(\omega)] + B$$

  Therein the width Γ accounts for the internal modes, while γ describes the global center-of mass diffusion of the proteins consisting of contributions from the translational and rotational diffusion. The width of the Lorentzian describing the diffusion of D_2O was determined from a measurement done with IN5.

By the slope of the HWHM γ as a function of q^2, an apparent diffusion coefficient D can be determined, from which a translational diffusion coefficient D_t is calculated [1].

Outcome of the Experiment :

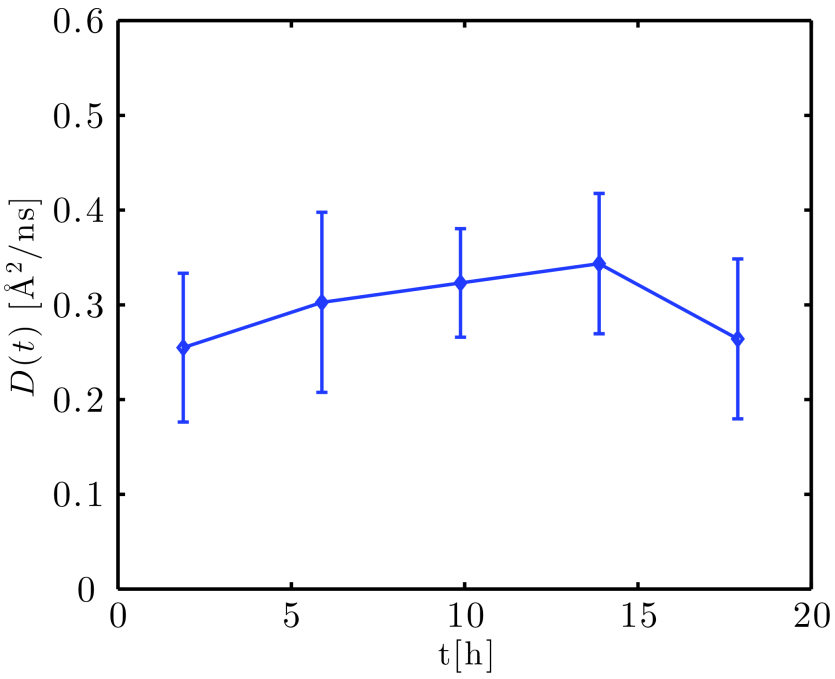

Figure1: Apparent diffusion coefficient *D* as a function of the time *t* of BLG 100 mg/ml $ZnCl_2$ 29 mM.

For the two-step crystallization process, the observed dynamics is extremely slow during all the experiment, much slower than expected for the diffusion of BLG monomers in aqueous solution, reflecting the presence of large clusters. Despite the difficulty in determining diffusion coefficients referring to such slow dynamics, data seem to indicate a fastening of the average dynamics of proteins in a first step, followed by a slowdown (Figure 1). Further evidence should be gathered before making any conclusion.
For the one-step crystallization process, no appreciable change in the overall dynamics is observed over the 12 hours of measurements (not shown). A slower dynamics than expected without salt is observed.