Experimental report

03/02/2016

Proposal: 8-04-760 **Council:** 4/2015

Title: Real time study of protein dynamics during a one-step and a two-step crystallization process

Research area: Biology

This proposal is a continuation of 8-05-420

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Samples: Bovine Beta-Lactoglobulin, ZnCl2, CdCl2, D2O

Instrument	Requested days	Allocated days	From	To
IN11	4	5	29/10/2015	03/11/2015
IN16B	2	2	25/06/2015	27/06/2015

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); A.Sauter et al., J.Am.Chem.Soc. 137, 1485 (2015)] and theoretical [P. G. Vekilov, Nanoscale 2, (2010)] studies have shown that, under certain conditions, crystallization follows a multi-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 and IN11 may provide new extremely useful information, thus potentially significantly improving the general physical picture. This proposal continues the IN16B work and adds IN11 to measure on and off the correlation peak found in SANS.

Real-time study of protein dynamics during a one-step and a two-step crystallization process

experiment 8-04-760 on IN16B and IN11

experimentlists M.Grimaldo, F.Roosen-Runge, C.Beck, B.Sohmen, M.Braun, O.Czakkel, T.Seydel;
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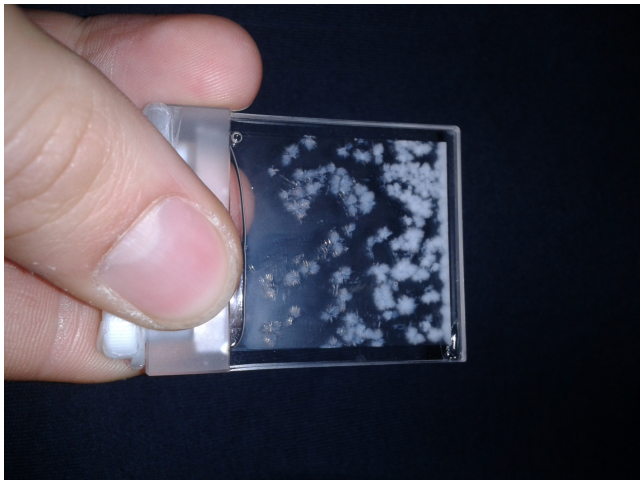

Figure 1: Photograph of a BLG/$ZnCl_2$ sample measured on IN11 where crystals have formed. The crystals form slowly over time, and the picture shows the situation 58 hours from the sample preparation.

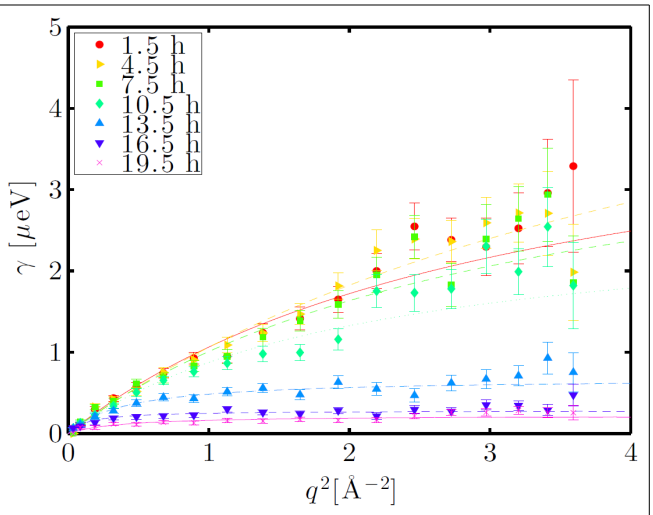

Figure 2: Lorentzian linewidth associated with the ensemble-averaged center-of-mass diffusion of the proteins (equation 2) in the BLG/$ZnCl_2$/D_2O samples as a function of the time since the sample preparation obtained from fits of IN16B data (BLG 100mg/ml, 35mM $ZnCl_2$).

Solutions of bovine β-lactoglobulin (BLG) proteins in D_2O with added $ZnCl_2$ salt were investigated on both IN11 and IN16B. The investigated samples slowly crystallized on time scales of several hours to days during the experiment, as became apparent by visual inspection of the samples at the start and at the end of each measurement (figure 1).
The IN16B data were described by the scattering function *S* depending on the scattering vector *q* and energy transfer *ω*,

$$S(q,\omega)=\beta\{L_\gamma(\omega)\otimes[A_0\delta(\omega)+(1-A_0)L_\Gamma(\omega)]\} \quad , \qquad (1)$$

where $\delta(\omega)$ and $L_\Gamma(\omega)$ denote, respectively, the Dirac function and Lorentzian linewidth due to the elastic scattering and internal relaxations of the proteins and $A_0=A_0(q)$ the elastic incoherent structure factor. These internal motions are convoluted with the global center-of-mass diffusion of the proteins associated with a Lorentzian with the linewidth γ.
When fitting the model in equation (1) to the IN16B data, a clear time-dependence of the linewidth γ associated with the global diffusion of the proteins becomes apparent (figure 2). This trend indicates a slowing-down of the protein diffusion with time. The observed trend is consistent with a slow crystallization process on a time scale of several hours and an associated immobilization of the global motion of the proteins. The deviation of the q-dependence of the width γ from simple Brownian diffusion indicates a confined or jump-like behavior at already at an early stage of the process.

A similar trend of an *in situ* crystallization was observed during the IN11 experiment with a time- and *q*-dependent change in the intermediate scattering function (figure 3 and 4).

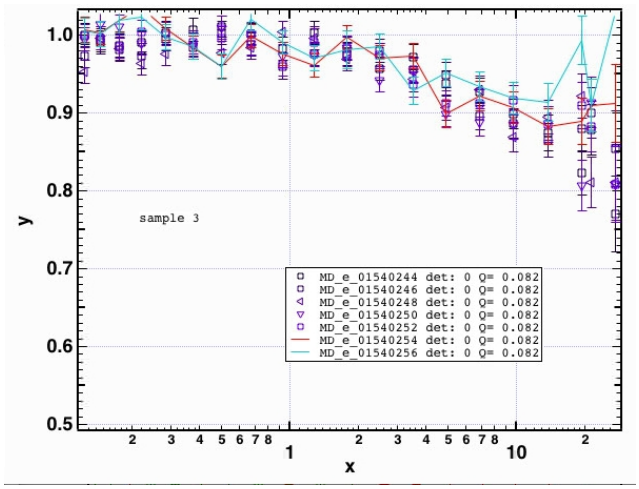

Figure 3: Time series of normalized intermediate scattering functions I(q,t) recorded on IN11 at the fixed scattering vector q=0.082Å^{-1} on a BLG/$ZnCl_2$/D_2O sample (BLG 100mg/ml, 35mM $ZnCl_2$).

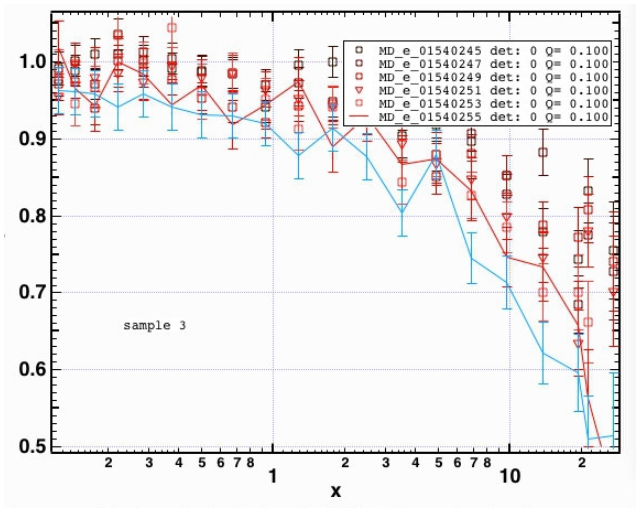

Figure 4: As in figure 3, but for the scattering vector q=0.1Å^{-1}.