Experimental report

27/07/2017

Proposal: **8-04-766**

Council: 4/2016

Title: Real time study of protein dynamics during a non-classical crystallization process

Research area: Soft condensed matter

This proposal is a continuation of 8-04-760

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Samples: BLG 100 mg/ml ZnCl3 35 mM

Instrument	Requested days	Allocated days	From	To
IN11	5	5	17/11/2016	22/11/2016

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 a suitable crystallizing systems by QENS at IN11 may provide new extremely useful information, thus potentially significantly improving the general physical picture. This proposal continues the IN16B and IN11 work.

Experimental Report for Exp. 8-04-766 on IN11

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Introduction

Understanding the processes prior to crystallization is a very important research field. Crystallization can not always be described by classical nucleation theory. Instead non-classical crystallization pathways have been found [1]. The aim of this experiment was to follow the dynamics of the crystallization process of β-lactoglobulin (BLG) induced by the multivalent salt ($ZnCl_2$). A small angle x-ray scattering study on a related system has recently been performed by our group [2]. Recently, a first IN11 experiment (8-04-760) with a similar sample showed a slow down of the dynamics in the crystal accompanied by a increase of the dynamics in solution.

Experimental Procedure

Based on the knowledge from microscopy studies several sample conditions were measured in a time series in which three different q values were set in an alternating way. The sample conditions are listed in table 1. q-values were set to $q = 0.1$ Å^{-1} ; $q = 0.15$ Å^{-1}; $q = 0.177$ Å^{-1} to measure the dynamics of the proteins in the crystal (Bragg-peak), in the solution (off peak) and in the amorphous dense phase (monomer-monomer-peak) respectively. All samples were measured at room temperature. Besides the measurements mentioned above, pure D_2O and pure BLG solutions were measured to be able to subtract the background signal.

Sample name	protein concentration (BLG)	salt concentration($ZnCl_2$)
BLG100ZnCl20P1	100 mg/ml	20 mM
BLG100ZnCl20P2	100 mg/ml	20 mM
BLG100ZnCl25	100 mg/ml	25 mM

Table 1: Sample conditions of the measured samples

Results

To obtain first results from the measurements, the intermediate scattering function is modeled by an exponential decay:

$$I(q, t) = \exp(-\Gamma \cdot t) \quad (1)$$

with the decay rate $\Gamma = dq^2$ and the diffusion coefficient d. Figure 1 displays the intermediate scattering functions and the corresponding fits. A first glance on the raw data already shows, that within the observed time scale of $\tau = 30$ ns, no significant decay of the intermediate scattering function is observable for most of the recorded data.

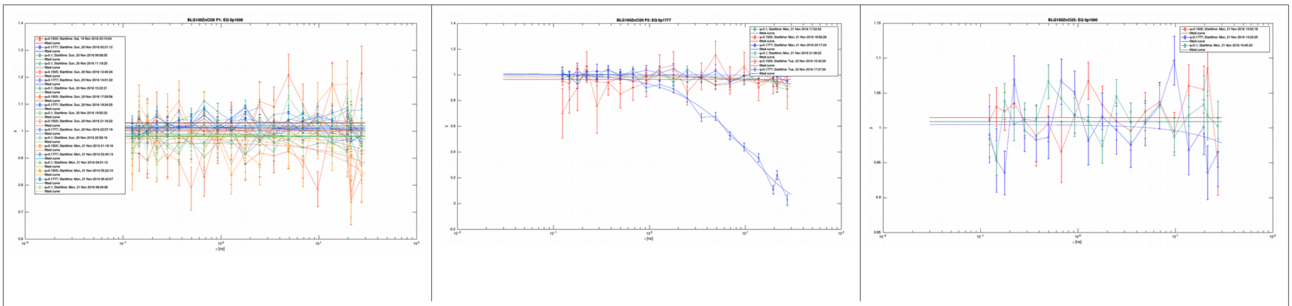

Figure 1: collected intermediate scattering functions for the three different samples. different q values are colorcoded: green: $q = 0.1$ Å^{-1} ; red: $q = 0.15$ Å^{-1}; blue: $q = 0.17$ Å^{-1}

The only significant difference which is visible is the one of the signal assigned to the monomers. The increase of the dynamics at the monomer-monomer peak might be due to the consumption of dense phase by growing crystals.
The growth of crystals in both the BLG100ZnCl20P1 and in the BLG100ZnCl25 was confirmed by microscopy after the spin echo experiment. Although crystals grew, no significant changes could be observed on the Bragg peak and off-peak. Further experiments and analysis will have to show why in the previous experiment (8-04-760) a change in the dynamics was seen and why there was no change in the dynamics during the experiment reported here.
The diffusion coefficients extracted from the fits of equation 1 to the intermediate scattering function are shown in figure 2. They confirm the first impression obtained from the experimental data.

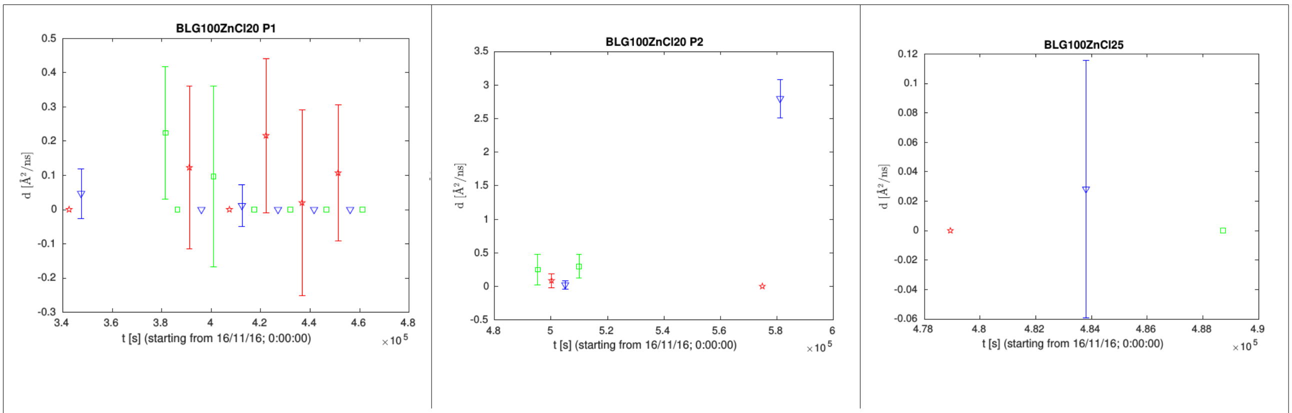

Figure 2: Diffusion coefficients extracted from the intermediate scattering functions (see figure 1) via equation 1. The colorcoding is the same as described in figure 1.

References

[1] James J. De Yoreo et al., Science, **349**, Issue 6247, 2015
[2] Andrea Sauter et al., , J. Am. Chem. Soc., **137**, 1485-1491