Experimental report

09/10/2018

Proposal: 8-04-810 **Council:** 4/2017

Title: In situ real-time study of the diffusive dynamic arrest of proteins during crystallization

Research area: Biology

This proposal is a continuation of 8-04-760

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Samples: BLG, CdCl2, D2O solutions

Instrument	Requested days	Allocated days	From	To
IN16B	3	3	06/04/2018	09/04/2018

Abstract:

Protein crystallization is of great fundamental and practical interest. Nevertheless, the existing knowledge is insufficient for a rational a priori choice of parameters leading predictably to the successful crystallization of a given protein system. The understanding of the dynamic precursor processes of protein crystallization may contribute to this missing fundamental understanding. For this reason, we propose to continue an earlier successful first-time study observing the crystallization of BLG-ZnCl2-D2O solutions in situ using neutron spectroscopy. In this earlier study, the crystallization had manifested itself in a slow and continuous dynamic arrest of the global center-of-mass diffusion of the proteins in the solution. Here, we propose to complement the existing single IN16B data set by two additional data sets to obtain a more comprehensive and reliable picture.

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Experiment 8-04-810

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1 Samples measured

Full QENS spectra of empty can, D_2O and Vanadium were measured for calibration. All measurements were performed with a set-point temperature of 295K. The focus of this experiment was to follow the changes in crystallizing protein samples over times. Therefore, a sample with β-lactoglobulin (100 mg/ml) and $CdCl_2$ (25mM) was prepared with D_2O as solvent.

2 Results

The protein-salt solution was prepared inside the sample holder and mixed using a magnetic stirrer. After sealing, the sample was quickly inserted into IN16b to measure also the early changes in the sample.

To obtain the information-rich but time-consuming QENS measurements also good time resolved information about the kinetic changes in the system, we measured three QENS runs (5 min each) in addition to elastic scans (30s), allowing to obtain the time dependence of the MSD with a time resolution of 15.5 minutes.

The QENS spectra were binned using a floating average binning 50 runs. From each of the obtained spectra, that of the empty can was subtracted. The D_2O contribution was fixed based on the measurement performed and the remaining protein signal was then fitted with an elastic and two Lorentizian contributions:

$$S_{prot}(q,\omega) = \mathscr{R} \otimes [\{A \cdot \delta(\omega) + (1-A)\mathscr{L}_\gamma\} \otimes \{A_0\delta(\omega) + (1-A_0)\mathscr{L}_\Gamma\}]\,. \tag{1}$$

A represents the amount of proteins, which appear immobile within the time-window observable determined by the resolution function and A_0 is the elastic incoherent scattering factor (EISF). To obtain a stable fit, the q dependence for the global diffusion described by the Lorentzian $\mathscr{L}_\gamma$ was assumed to follow Brownian dynamics ($\gamma = Dq^2$).

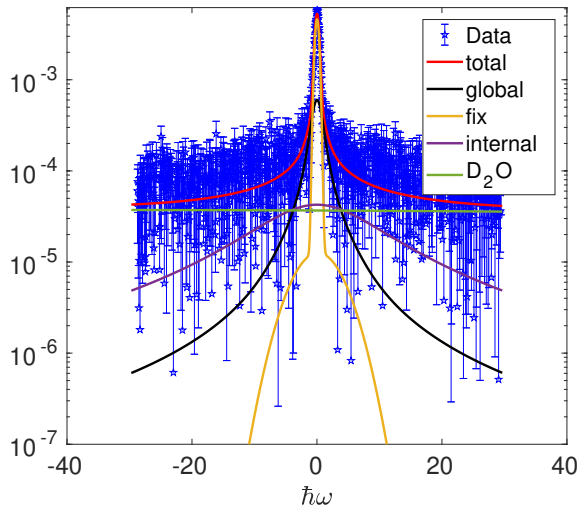
The elastic fixed window scans (EWFS) were analyzed using a polynomial fit model, offering access to one averaged mean squared displacement (MSD).

Raw data with corresponding fits are shown in figure 1(a) and 1(b) for the QENS measurements and FWS, respectively.

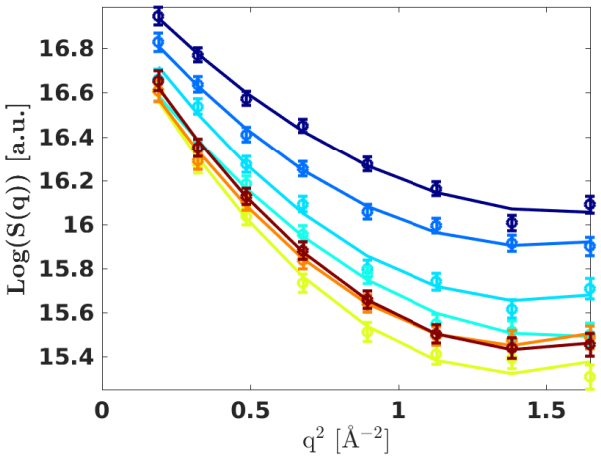
The time dependence of the fraction of immobile proteins A and the MSD are shown in figure 1(c) and in figure 1(d), respectively. The values show consistent time dependencies, which might be related to a dissolving gel, which was observed for similar samples with other techniques.

3 Additional comments

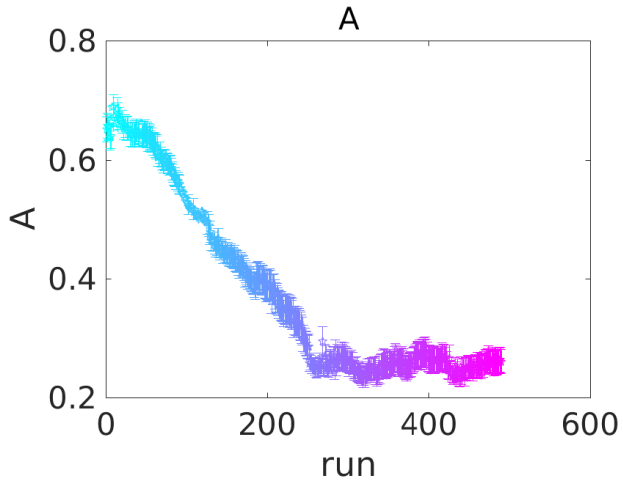
Due to necessary realignment of the parking positions of the BATS-choppers and some realignment of the analyzers, the begin of the measurements of the samples was delayed and the vanadium measurement had to be repeated.



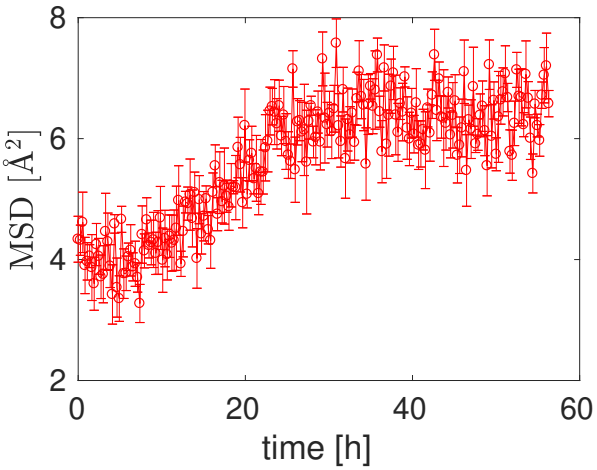
(a) QENS Spectra with Fit of equation 1



(b) EFWS for different times. Different times are color-coded: blue is the first measurement, red the last



(c) Fraction of immobile proteins as a function of time



(d) Time dependence of the MSD determined from the EFWS

Figure 1: Raw data (top) with corresponding fits and time-dependent fit results (bottom). While on the right hand side only the elastic signal is analyzed (EFWS), the left hand side also takes into account the quasi-elastic energy transfers.