Experimental report

05/09/2022

Proposal: 8-04-853 **Council:** 10/2018

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

Research area: Biology

This proposal is a resubmission of 8-04-834

Main proposer: Christian BECK

Experimental team: Ivan MUSIL
Lara GRABITZ
Olga MATSARSKAIA
Christian BECK
Frank SCHREIBER
Ralph MAIER
Anita GIRELLI

Local contacts: Tilo SEYDEL
Sylvain PREVOST

Samples: ZnCl2
D2O
YCl3
CdCl2
BLG

Instrument	Requested days	Allocated days	From	To
IN16B	4	4	11/10/2019	15/10/2019
D11	1	1	23/09/2019	24/09/2019
IN11	7	0		

Abstract:

Protein crystallization plays an important role in structural biology and medicine. Different crystallization pathways have been identified, but a general understanding of the fundamental processes is still missing. Different pathways can be addressed by choosing different salts and changing its concentration. Investigating the dynamics of the proteins might offer new insights playing a key-role in the understanding of the crystallization. Based on previous successful results, we propose to investigate the dynamics during the crystallization process using the unique possibilities of inelastic fixed window scans on IN16b to significantly increase the existing dataset to obtain a comprehensive and reliable picture. Moreover, we propose a spin-echo experiment on the same samples to monitor the dynamic changes on and besides the Bragg and monomer-monomer peaks in-situ. Complementary SANS time will be used to characterize the sample structure in the final state.

Following the recommendation by the previous subcommittee (college 8), the proposal is being resubmitted to college 9.

Experimental Report Experiment 8-04-853

Christian Beck, Felix Roosen-Runge, Tilo Seydel, Marco Grimaldo, Olga Matsarskaia, Fajun Zhang, Frank Schreiber, Lena Bühl, Michal Feustel

Motivation: The aim of this beamtime was to use the possibility to measure (in)elastic fixed window scans (FWS) to follow time depend changes in the diffusive behavior of the crystallizing samples. This allows to observe significantly faster processes, since the recorded spectra are in the order of a minute and therefore significantly shorter than full QENS spectra. However, since the data are only recorded at one specific energy transfer, they contain less information. SANS measurements were performed in addition to follow the structural changes.

Measured Samples on IN16b: During the IN16b beamtime, samples and calibration measurements were measured with FWS with energy transfers $\hbar\omega = 0, 1.3, 3, 6$ µeV with exposure times of δt=30 s, 1 min, 1.5 min, 3 min, 5 min, respectively. For the calibration measurements, Vanadium, D_2O and an empty can FWS as well as full QENS measurements were performed. Three different sample conditions were measured during the beamtime, which are listed in Table 1. All samples were composed out of β-lactoglobulin (BLG), $CdCl_2$ and D_2O. Sample 5,6,7 are the same sample condition prepared at different times to cover the a longer crystallization process more efficiently. All measurements were performed at 295K.

Time dependence of the samples investigated: In Figure 1 and Figure 2, the time dependence of the vanadium normalized FWS are shown for Sample 1 and Sample 2 at different energy transfers and momentum transfers q, respectively. It should be highlighted that despite the low protein concentration in Sample 1, clear changes can be observed in the time dependence of the scattering signal. The time dependence of the scattering signal of Sample 2 points out the new capabilities to measure fast developing samples with a time resolution of roughly 10 minutes.

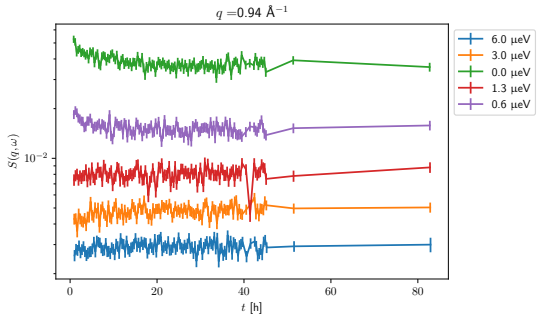

Figure 1: Time dependence of Sample 1 for the energy transfers measured at $q = 0.94$Å^{-1}.

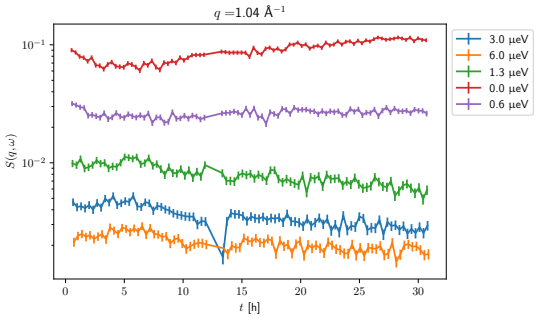

Figure 2: Time dependence of Sample 2 for the energy transfers measured at $q = 1.04$Å^{-1}.

Sample	c_p [mg/ml]	c_s [mM]
1	40	25
2	84.4	30
5,6,7	126.6	50

Table 1: Sample conditions for IN16b

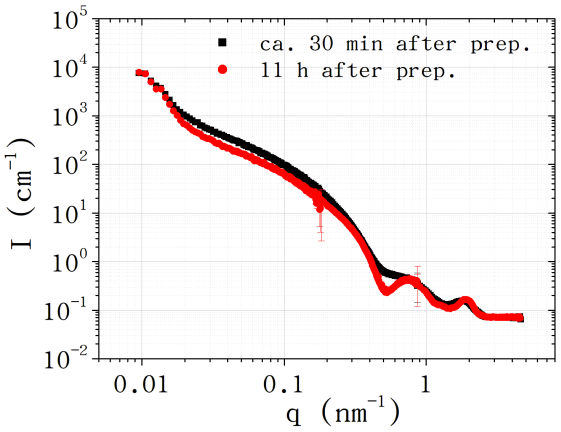

Figure 3: SANS profiles of a sample consisting of 40 mg/ml BLG and 25 mM $CdCl_2$ (see text for details).

Example SANS data (40 mg/ml BLG, 25 mM $CdCl_2$) are shown in Fig. 3. With increasing post-sample preparation time, the dip at 0.4 Å^{-1} decreases in intensity and the crystal Bragg peak at 0.8 Å^{-1} becomes more pronounced. This indicates that the metastable intermediate phase is consumed as the crystals grow and illustrates the time-dependent crystallization process.

Future Analysis: The diffraction data collected simultaneously during the FWS can be used to subtract the solvent contributions of the individual scan with the correct scaling. The different energy transfers allow to separate different diffusive components. Based on the ratio approach established earlier [1], the global diffusion can be extracted. Since the scattering signal contains also an elastic contribution, the approach has to be applied to the inelastic FWS. Combining the obtained results with the scattering signal of the elastic FWS, the elastic contribution corresponding to immobile proteins can be extracted.

References

[1] O. Matsarskaia, L. Bühl, C. Beck, M. Grimaldo, R. Schweins, F. Zhang, T. Seydel, F. Schreiber, and F. Roosen-Runge, "Evolution of the structure and dynamics of bovine serum albumin induced by thermal denaturation," *Phys. Chem. Chem. Phys.*, vol. 22, no. 33, pp. 18507–18517, 2020.