Experimental report

16/03/2020

Proposal: 8-04-862 **Council:** 4/2019

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

Research area: Biology

This proposal is a resubmission of 8-04-853

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Samples: ZnCl2
D2O
YCl3
CdCl2
BLG

Instrument	Requested days	Allocated days	From	To
IN11	7	7	18/02/2020	25/02/2020

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 may offer new insights playing a key-role in the understanding of the crystallization.

In already allocated time, based on previous successful results, we will investigate the dynamics during the crystallization process using the unique inelastic fixed window scans on IN16B to significantly increase existing data for a comprehensive and reliable picture, as well as the structural evolution using SANS on D11.

Here, we propose a complementary spin-echo experiment on the crystallizing samples to monitor the dynamic changes on and besides the Bragg and monomer-monomer peaks in-situ. This experiment can be done on IN11, but would be ideally suited as an early demonstration experiment on WASP, by observing the evolution on the Bragg and monomer-monomer peaks as well as off these peaks simultaneously, using the same samples as on IN16B.

Experimental report exp-8-04-862 IN11

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The aim of this experiment was to follow the collective diffusion of proteins during a crystallization process. Therefore, the system β-lactoglobulin (BLG) in D_2O in the presence of $CdCl_2$ was chosen which is characterized by a non-classical crystallization pathway. Several crystallization conditions were investigated previously with different techniques (see experimental report 8-04-853 - IN16B and D11). Based on these results, suitable samples were chosen for this beamtime. The concentrations are given in Table 1. All samples were measured at room temperature.

Different scattering vectors q were calibrated using a wavelength of 8 Å and the scattering angles 3.7°, 5.5°, 7.3°, 9.5°, 14.5° resulting in the scattering vectors q=0.05 $Å^{-1}$, 0.075 $Å^{-1}$, 0.1 $Å^{-1}$, 0.13 $Å^{-1}$ and 0.198 $Å^{-1}$ were measured corresponding to off peak measurements, cluster peak, first and second Bragg peak and monomer-monomer correlation peak, respectively (see Figure 1a).

The samples were prepared at room temperature directly in the cuvettes used for the measurement and mixed using Teflon needles. Once the samples were homogeneous, they were measured iterating between the different scattering vectors. Depending on the protein concentration and on the scattering vector, the the acquisition time was adapted lasting from 60s - 100s.

Sample 1 was prepared already before the beamtime to allow for an investigation of the crystallized sample at the beginning of the beamtime. Samples 2-6 were prepared freshly before the measurement. To obtain a homogeneous sample, the upper part of the sample was masked with cadmium. Since samples 1&2 phase separated, another sample (sample 4) was prepared in a falcon tube and the more dilute phase of the crystallized sample was investigated. For comparison, a pure BLG solution was also investigated (see Figure 1e).

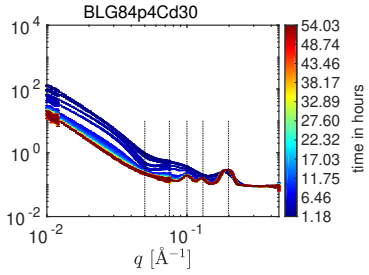
To obtain structural information, the samples were measured on several occasions with SANS on D22. In addition, real-time images were captured using a USB camera and the optical access to the installed small-angle sample box of IN11 (see Figure 1b). Microscope images showed a large number of crystals after the experiment (see Figure 1c).

Preliminary Interpretation

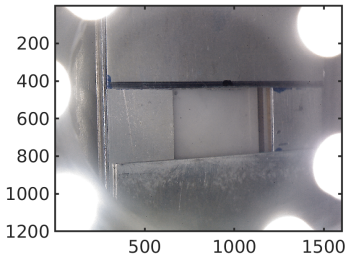
While previous beamtimes revealed significant changes of the collective short time protein diffusion observed during a classical crystallization pathway (see Figure 1f; [1]) no changes were visible in the data set collected this time. The absence of any decay in the intermediate scattering function even for the sample with the lower protein concentration (sample 3 - see Figure 1d) indicates that the collective diffusion of the proteins is drastically slowed down compared to the pure protein solution (see Figure 1e) so that no decay of the

Table 1: Sample concentrations

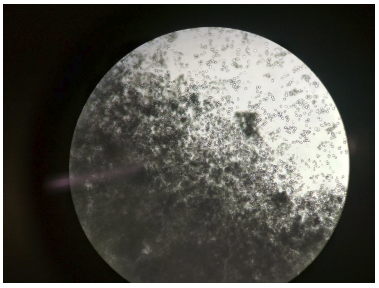
Sample number	duration	c_{BLG}	c_{CdCl_2}
1,2,4	3 days	84.4 mg/ml	30mM
3	50 hours	40 mg/ml	25mM
5	-	84.4 mg/ml	0mM
6	-	0 mg/ml	30mM



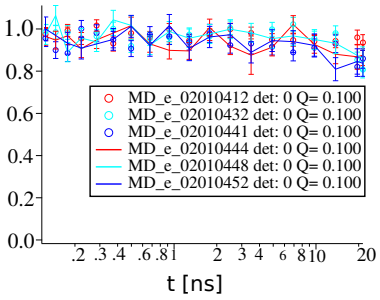
(a) Time-dependent SANS curves from 8-04-853 with chosen q values indicated by dashed lines. Sample conditions were similar to the ones of Sample 1.



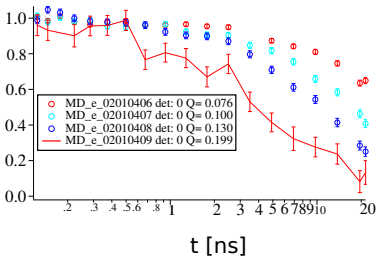
(b) Real-space image captured during the acquisition of the intermediate scattering function



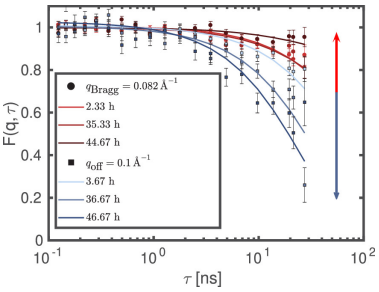
(c) Microscope image captured after the in11 measurement



(d) Intermediate scattering functions of sample 3 for $q = 0.1\,\text{Å}^{-1}$ at different times.



(e) Intermediate scattering functions of the pure BLG solution for all measured q



(f) time dependent intermediate scattering functions from measurements with $ZnCl_2$ [1]

Figure 1: Example spectra and photographs of different crystallization processes of BLG

intermediate scattering function is visible. This is in agreement with the SANS data showing a cluster peak as well as with the macroscopic phase behavior of the sample, which forms a gel-like state after mixing. In this gel, the crystals grow over time.

1 Conclusion

In combination with the different other techniques, the obtained results offer significant new insights in this non-classical crystallization behavior. Future measurements accessing longer Fourier times (e.g. on IN15) might offer insights into the collective diffusion. The differences observed between the collective and self-diffusion are remarkable and are in agreement with the macroscopic behavior.

References

[1] C. Beck, M. Grimaldo, F. Roosen-Runge, R. Maier, O. Matsarskaia, M. Braun, B. Sohmen, O. Czakkel, R. Schweins, F. Zhang, T. Seydel, and F. Schreiber. Following protein dynamics in real time during crystallization. *Crystal Growth & Design*, 19(12):7036–7045, 2019.