ESRF	**Experiment title:** Kinetics of two-step nucleation in protein solutions studied by real-time SAXS	**Experiment number**: SC 4087
Beamline: ID02	**Date of experiment**: from: 15.04.2015 to: 17.04.2015	**Date of report**:
Shifts: 6	**Local contact(s)**: Gudrun Lotze, Sylvain Prévost Johannes Moeller (inhouse beamtime)	*Received at ESRF:*

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Report:

Introduction

Recent progress in protein and colloid crystallization, bio-mineralization and other systems [1-3] has shown different features beyond the classical view of the early stage of nucleation. Mesoscopic clusters and macroscopic metastable intermediate phases have been proposed to serve as precursors for nucleation. While studies of protein crystal growth in real space using atomic force microscopy have revealed many important features of the metastable protein clusters and their role in the nucleation process [3-6], quantitative understanding of the relation between clusters and crystals and the transition kinetics from the metastable intermediate phase to the stable crystalline phase are still a challenge.

Our recent study using time-resolved SAXS measurements on the non-classical pathways of protein crystallization suggests that the growth kinetics of the intermediate phase and the crystalline phase can be used to verify an unconventional nucleation mechanism [6,7]. In the proposed experiments, we aimed at exploring the various scenarios of overall growth kinetics during protein crystallization. In particular, the role of clusters or the metastable intermediate phase (MIP) was the focus.

Experimental and Results

During beamtime SC4807 (15^{th} – 17^{th} April 2015) and a short inhouse beamtime on May 25^{th}, we performed real-time SAXS measurements of protein crystallization in the presence of trivalent salts. The samples prepared and measured consisted of beta-lactoglobulin (BLG) 65 mg/mL with 13, 14 and 15 mM YCl_3, human serum albumin (HSA) 150 mg/mL with 6 – 9 mM $CeCl_3$ and HSA 100 mg/mL with 1-10 mM $LaCl_3$. Here we focus on the preliminary results of HSA crystallization.

Crystal growth of HSA in solutions in the presence of YCl_3, $LaCl_3$ and $CeCl_3$ has been tested in our group and we found a broad range of conditions for crystallization near the first boundary salt concentration c*[8]. However, by visual inspection or optical microscopy, one cannot assess detailed features of the intermediate state and its structural connection to the final crystalline state. By using real-time SAXS, we wish to determine i) if there is an internal structure visible in the similar q range of the first Bragg peak as observed

for the BLG system [6,7]; ii) if not, whether any other structural features relevant to the intermediate phase can be used for establishing the kinetic relation. The HSA samples with $CeCl_3$ measured during this beamtime crystallized but with limited number of big crystals on the walls of the quartzcapillaries used. In this case only one or two Bragg peaks are visible and there are no additional visible structural features throughout the full q range when comparing the first and the last curves. For samples of HSA 100 mg/mL with 1-10 mM $LaCl_3$, we found that with 4 mM $LaCl_3$, the sample crystallized after 5 hours. Importantly, this sample crystalizes into small crystals with a high nuceation rate. SAXS profiles before and after crystallization are presented in Fig. 1. As one can see, many Bragg peaks can be distinguished. Although no internal structural feature can be seen in a q range similar to the one of the Bragg peaks, a clear intensity upturn is visible in the low q region, which must be related to the formation of the intermediate phase (in this case, it is most likely the mesoscopic protein clusters). In another measurement with HSA 150 mg/mL and 9 mM $CeCl_3$ (Fig.2, data were collected during the inhouse beamtime on May 25th 2015), we observed a similar behavior: Bragg peaks appear in the high q region and the intensity changes in the low q region (Fig.2).

These results of HSA crystallization suggest that this system provides a scenario of nonclassical pathways of protein crystallization. Although the intermediate phase forms prior to crystallization, no internal structural features exist in the similar q range of Bragg peaks. One can follow, however, the overall global shape, size and scattering intensity of the intermediate in the low q region, and relate it to the development of the Bragg peaks. In this way, the relation between the intermediate and the crystalline phase can be established. Data analysis following this strategy is ongoing.

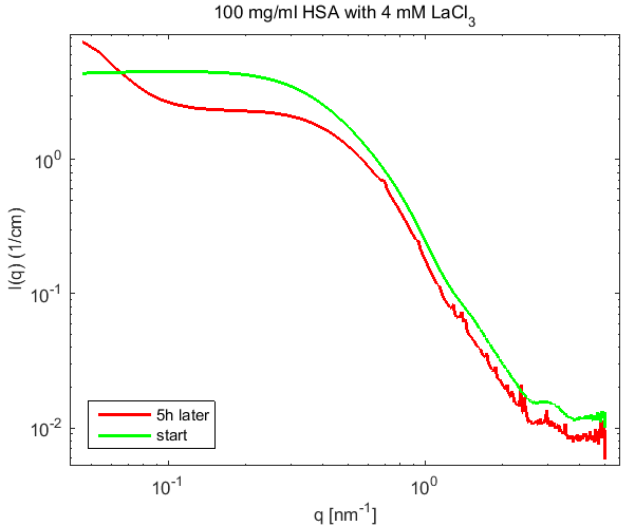

Fig. 1. First (green) and last (5 h after the start of the experiment) SAXS curves for a sample with 100 mg/mL HSA and 4 mM $LaCl_3$ before and after crystallization.

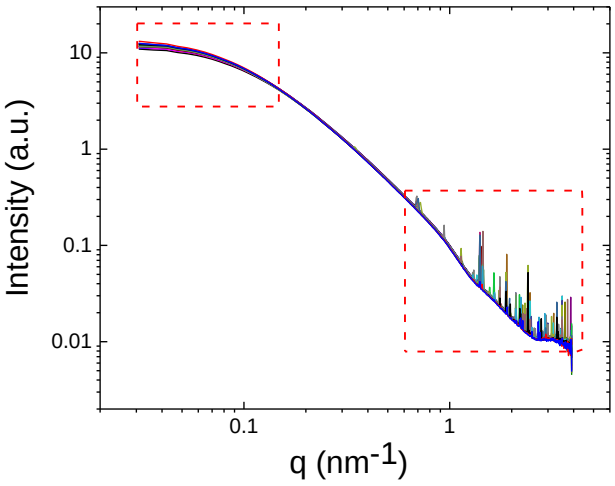

Fig.2. Real time SAXS profiles of HSA 150 mg/mL with 9 mM $CeCl_3$. The boxes indicate the appearance of Bragg peaks in the high q region and the decrease of intensity in the low q region indicates the formation of mesoscopic protein clusters.

References

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