Experimental report

24/08/2016

Proposal: 9-13-672 **Council:** 4/2016

Title: Kinetics of two-step nucleation inprotein crystallization studied byreal-time SANS

Research area: Soft condensed matter

This proposal is a continuation of 9-13-620

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Samples: Beta-lactoglobulin
Human Serum Albumin

Instrument	Requested days	Allocated days	From	To
D22	2	0		
D11	2	2	02/07/2016	04/07/2016
D33	2	0		

Abstract:

In this proposal we aim to study the two-step nucleation mechanism in protein crystallization by real-time SANS. With the larger volume and no radiation damage of SANS, we can extend our previous successful study using SAXS (A. Sauter et al. J. Am. Chem. Soc.2015, 137, 1485) to a broad experimental conditions. This is extremely important for establishing the real-time method and providing systematic experimental results for our understanding of the nonclassical pathways of protein crystallization. The previous experiment (9-13-620) has demonstrated the method and we wish to continue this work to complete a systematic study.

Report for proposal

Kinetics of two-step nucleation in protein crystallization studied by real-time SANS

Understanding the early stage of nucleation process is extremely important but challenging in many research fields including protein and colloid crystallization, bio-mineralization and other systems [1-5]. Recent progress has shown different features beyond the classical view in the early stage of nucleation. Microscopic clusters and macroscopic metastable intermediate phases have been suggested to act as precursors for nucleation. Studies of early stages 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 [4,5]. However, a quantitative understanding of their relation and the transition kinetics from the metastable intermediate phase to the stable crystalline phase are still a challenge. To gain a deeper understanding of the nucleation mechanism as well as the role of the metastable intermediate phase (MIP), we have performed a real-time study using small angle X-ray scattering (SAXS) and optical microscopy. Using bovine β-lactoglobulin as a model system in the presence of di- and tri-valent salts, we have monitored the early stage of crystallization kinetics which demonstrates a two-step nucleation mechanism [1].

In the proposed experiment, we aimed to study non-classical nucleation mechanism in protein crystallization by real-time SANS. With the larger volume compared to SAXS measurements and without radiation damage, we can extend our previous successful study [1,5] to a broader range of experimental conditions. This is extremely important for establishing the real-time method and providing systematic experimental results for our understanding of the nonclassical pathways of protein crystallization.

During the beamtime at D11 from 30th June – 4th July 2016 we investigated the crystallization of ß-lactoglobulin (BLG) and human serum albumin (HSA) in the presence of several multivalent ions (YCl_3, $ZnCl_2$, $CdCl_2$, $CeCl_3$) at RT. The samples were classified in 3 groups regarding to their crystallization time: Fast (few hours), intermediate (more than few hours) and long (2-4 days). In total we measured 14 samples (tab.1).

Protein	Cp (mg/ml)	Salt	Cs (mM)	time	comment
BLG	20	YCl_3	4	Long	Crystallized
BLG	30	YCl_3	7	Long	Not crystallized
BLG	30	YCl_3	9	Long	Not crystallized
BLG	30	YCl_3	10	Long	Not crystallized
BLG	30	$CdCl_2$	16	Intermediate	Crystallized
BLG	30	$CdCl_2$	17	Intermediate	Crystallized
BLG	30	$CdCl_2$	18	Intermediate	Crystallized
BLG	30	$ZnCl_2$	4	Fast	Crystallized
BLG	30	$ZnCl_2$	6	Fast	Crystallized
BLG	30	$ZnCl_2$	10	Intermediate	Crystallized
BLG	30	$ZnCl_2$	15	Long	Crystallized
HSA	10	$CeCl_3$	2	Long	Not crystallized
HSA	35	$CeCl_3$	1.7	Long	Not crystallized
HSA	50	$CeCl_3$	3.5	Long	Crystallized

The experimental parameters were the same for all samples. The sample-to-detector distance was set to 2m. For the fast and intermediate samples the acquisition time was 2 min, samples with long crystallization times had an acquisition time of 4 min. Two plots of the collected data are presented in Fig.1 and 2. In Fig. 1. SANS data of the crystallization of 30 mg/ml BLG with 16 mM $CdCl_2$ is shown. Good statistics and time resolution enable uncovering the non-classical character of the crystallization process which is consistent with previous results [1].

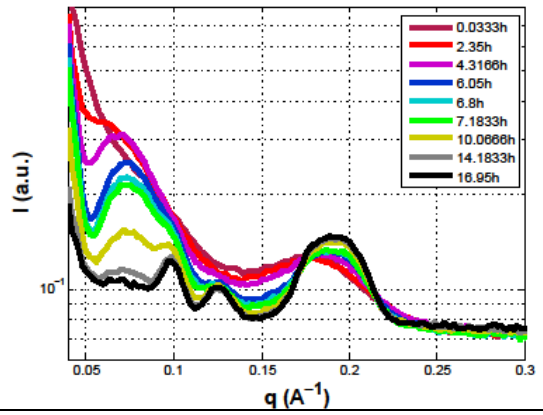

Fig. 1 SANS data of the crystallization of 30 mg/ml BLG with 16 mM $CdCl_2$.

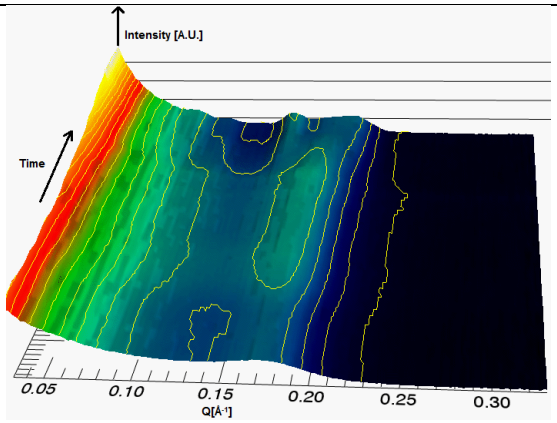

Fig. 2 shows 3D surface plot of SANS data of the crystallization of 20 mg/ml BLG with 4 mM YCl_3.

Fig. 2 shows SANS data of the crystallization of 20 mg/ml BLG with 4 mM YCl_3. A broad peak at around 0.18 $Å^{-1}$ increases and decreases again. Subsequently, two sharp Bragg peaks at 0.16 $Å^{-1}$ and 0.2 $Å^{-1}$ start to grow. This system provides a different growth scenario in terms of the structure of the intermediate phase. However, due to the limited beamtime and relative long time period of crystallization, we have collected only one complete SANS data for this system. Further measurements on this system are required for a quantitative data analysis.

Real-time SANS is a powerful technique to access the dimensions in which nucleation takes place. Our successful experiments encourage us to expand the investigation on new systems and convince of their promising opportunity. Quantitative Analysis will provide new insights in the mechanism of non-classical crystallization. We want to explore this in future proposals.

Reference:

[1] A. Sauter et al., 2015, J. Am. Chem. Soc., 137, 1485.
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[3] A. F. Wallace et al., Science, 2013, 341, 885-889.
[4] M. Sleutel, et al., PNAS, 2014, 111, E546 and Nature Commu. 2014, 5, 5598
[5] A. Sauter et al., Faraday Discuss. 2015, 179, 41.
[6] A. Sauter et al., Cryst. Growth Des., 2014, 14, 6357.