Experimental report

08/02/2016

Proposal: 9-13-620 **Council:** 4/2015

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

Research area: Soft condensed matter

This proposal is a new proposal

Main proposer: Fajun ZHANG

Experimental team: Marco GRIMALDO
Benedikt SOHMEN
Felix ROOSEN RUNGE
Stefano DA VELA
Olga MATSARSKAIA

Local contacts: Ralf SCHWEINS

Samples: BLG with YCl3
BLG with ZnCl2
BLG with CdCl2

Instrument	Requested days	Allocated days	From	To
D11	2	2	07/12/2015	09/12/2015
D33	2	0		
D22	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., 2015, J. Am. Chem. Soc., 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.

Report for proposal 9-13-620

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

Scientific background

Protein crystallography is still the dominating method in structural biology, and the growth of suitable crystals the main bottleneck. Recent progress in protein and colloid crystallization, bio-mineralization and other systems [1-4] 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 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 [1] 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.

Experimental and Results

During the beamtime at D11 from 7th - 9th December 2015, we have prepared and followed the crystallization process for a set of samples of BLG. The samples contain BLG 33mg/mL with salt ($CdCl_2$ in D_2O) concentrations of 17, 18, 20 and 22 mM. Experiments were performed at D11 with a neutron wavelength of 6Å using 1 mm cuvettes and with sample-detector distances of 2m.

One example 3D plot of the time-resolved SANS profiles is presented in Fig.1. For this sample, the crystallization process was fast, with 2 min exposure time for 90 min, both time resolution and statistics are very good to reveal the nonclassical crystallization process. Further quantitative analysis of the growth kinetics will be carried out soon.

Furthermore, we have successfully explored the possibility to use deuterated cellular lysate from E. Coli as a cytoplasm-like medium for the investigation of crowding effects on the globular protein gamma-globulin. The results show a reliable and stable sample system with a large range of structural features. Lysate as crowding medium provides an interesting opportunity for future studies on static and dynamic properties of proteins, which will be exploited in future proposals.

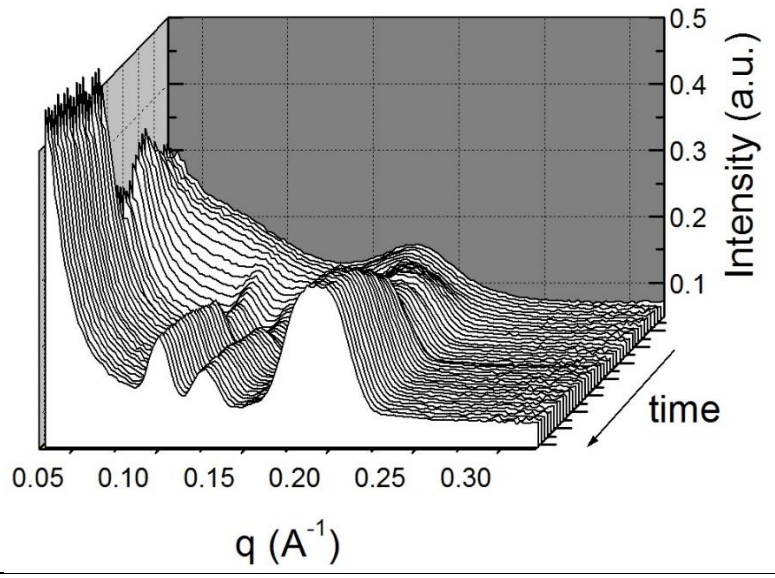

Fig1: Realtime SANS profiles of protein crystallization. Sample consisted of BLG 33mg/mL with 17 mM $CdCl_2$.

References

[1] A. Sauter et al., 2015, J. Am. Chem. Soc., 137, 1485.
[2] D. Gebauer et al., Science, 2008, 322, 1819-1822.
[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., Cryst. Growth Des., 2014, 14, 6357.
[6] A. Sauter et al., Faraday Discuss. 2015, 179 in press.
[7] F. Zhang et al., Phys. Rev. Lett., 2008, 101, 148101. Proteins, 2010, 78, 3450.
[8] F. Zhang, et al., *ILL Exp. Reports*, 9-13-507, 9-13-565.