Experimental report

27/07/2017

Proposal: 8-03-891 **Council:** 4/2016

Title: Towards a more realistic model system for the cellular crowded environment

Research area: Biology

This proposal is a new proposal

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Samples: Protein in aqueous solution

Instrument	Requested days	Allocated days	From	To
D11	1	1	13/11/2016	14/11/2016

Abstract:

Using deuterated cellular lysate obtained from living cells, we will to establish a natural crowding environment for protonated "tracer" or "target" proteins. We will systematically investigate the influence of this natural crowding environment on both the conformation and interaction of of the target proteins using SANS. We have previously tested the feasibility of this
experiment, and we have been allocated the required preparation of the lysate by the ILL deuteration laboratory through a successful peer-reviewed D-lab proposal.

Experimental Report for Exp. 8-03-891 on D11

Experimental team: Tilo Seydel, Christian Beck, Benedikt Sohmen, Michal K. Braun
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Introduction
Understanding the processes prior to crystallization is a very important research field. Crystallization can not always be described by classical nucleation theory. Instead non-classical crystallization pathways have been found [1]. The aim of this experiment was to follow the crystallization process of β-lactoglobulin (BLG) induced by the multivalent salt (ZnCl2) in realtime.

In another project, we investigate the crowding effects using gamma-globulin in deuterated lysate. To complete the dynamic data that we took, we did a characterization of the lysate by SANS during this experiment.

Experimental Procedure
Table 1 provides a list of samples with β-lactoglobulin (BLG) and $ZnCl_2$ that were measured. The samples were prepared right before they were put into the neutron beam. D_2O was mixed with the needed amounts of BLG and $ZnCl_2$ stock solution. The solutions were filled into quartz cuvettes with a thickness of 2 mm.
Table 1 also shows the time t the samples were measured and whether they crystallized or not.

Pure lysate was measured at three different sample-to-detector distances, namely 1.5, 8 and 34 m.

Results
Some of the samples crystallized during the time that they were measured. Other samples did not crystallize. The left part of Figure 1 shows one of the samples that crystallized. The Bragg peaks are nicely visible and the data may be used to be further analyzed with respect to the crystallization behavior at different ratios of protein and salt.

The combination of the scattering curves for lysate is shown in the right column of Table 2.

Table 1

BLG (mg/ml)	$ZnCl_2$ (mM)	t (min)	crystallized
20	4	191	yes
20	10	933	yes
40	7.5	52	no
40	10	164	yes
80	20	746	yes
80	25	918	no
100	20	712	yes
100	25_1	941	?
100	25_2	937	no
100	30	923	no

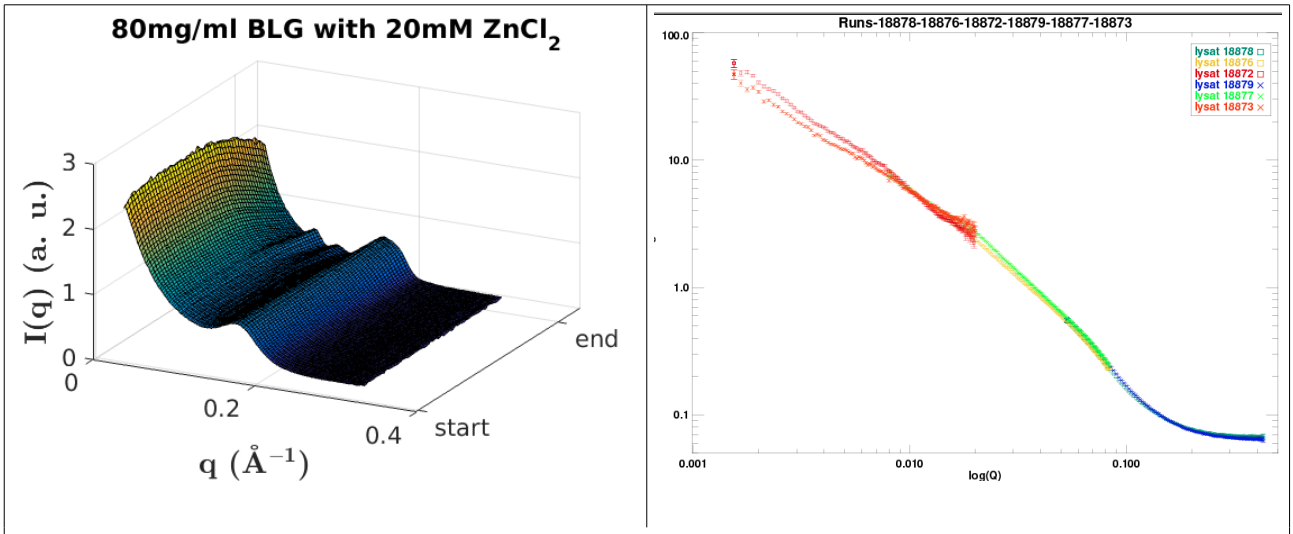

Figure 1: Left: 80mg/ml BLG with 20mM ZnCl2. Right: Pure lysate at three sample-to-detector distances.

[1] James J. De Yoreo et al., Science, **349**, Issue 6247, 2015
[2] Andrea Sauter et al., Crysal Growth and Design, **14**, 6357–6366, 2014.