Experimental report

30/11/2016

Proposal: 9-13-637 **Council:** 4/2016

Title: Studying thermal protein denaturation as a nanoscopic structure-dynamics relationship

Research area: Soft condensed matter

This proposal is a continuation of 9-13-620

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Samples: bovine serum albumin in aqueous solution

Instrument	Requested days	Allocated days	From	To
IN16B	4	4	08/09/2016	12/09/2016
D11	3	2	30/06/2016	02/07/2016

Abstract:

We will address the process of thermal denaturation in a combined structural-dynamical study using SANS at D11 and QENS at IN16B. In particular, we will use different kind of additives (denaturants, multivalent and monovalent salts) to selectively enhance the propensity of unfolding and cross-linking. The protein BSA is a good choice for this study, since results from complementary techniques provide a solid starting point for this study on the nanoscopic structure-dynamics relationship.
We have successfully explored the possibility for this kind of study in a test run at D11, and a previous experiment (exp. 8-04-752) at IN16B. For SANS, we will monitor both cluster and aggregate formation at low q and unfolding at high q (e.g. via Kratky plot). For QENS, we will employ elastic and inelastic fixed window scans to monitor the change of dynamics during thermal denaturation.

Experimental report for proposal 9-13-637
Studying thermal protein denaturation as a nanoscopic structure-dynamics relationship

1 Scientific background

Protein mis- or unfolding, that is, the loss of protein native structure, plays a vital role in research related to protein condensation diseases. Thermal denaturation of proteins allows for specific studies especially on protein unfolding. In addition to temperature, additives such as different salts or denaturing agents, e.g. urea, can influence the pathways and kinetics of protein denaturation. Studies of protein unfolding in the presence of such agents can therefore provide valuable information on the organisation of protein structure, the interactions between proteins and denaturing agents and pathways towards protein misfolding *in vivo*.

2 Experimental and Results

During beamtime no. 9-13-637 from June 30^{th} to July 2^{nd}, 2016, thermal denaturation of samples containing different BSA concentrations and various additives was studied on beamline D11. The samples prepared contained 120, 250 or 500 mg/ml BSA in D_2O and were supplemented with pure NaCl, NaCl and urea, pure urea or the trivalent salt YCl_3. BSA without additives was used as a control. Data were collected at sample-detector distances of 1.5 and 8 m and a wavelength of 6 Å using quartz cuvettes with a diameter of 1 mm. Scattering curves were recorded at temperature ranges from 295 to 350 K. Exemplary plots of temperature (T)-dependent SANS scans are shown in Fig. 1.

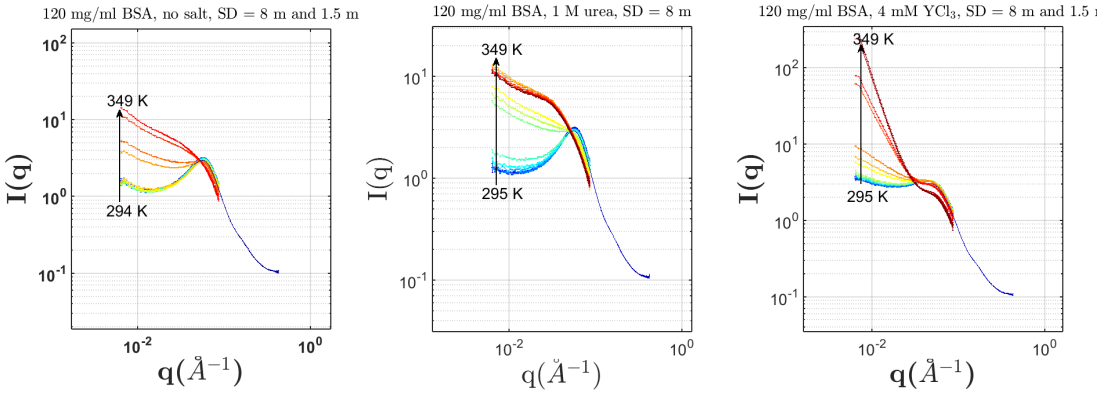

Figure 1: Temperature-dependent SANS profiles of BSA in the presence of no additives (left), 1 M urea (centre) and 4 mM YCl_3 (right).

In Fig. 1 (left), a denaturation profile of a sample containing 120 mg/ml BSA without additives is depicted. At low T (295 K), the scattering profile features a pronounced correlation peak around q=0.06 $Å^{-1}$, indicating repulsive protein-protein interactions due to the negatively charged protein surface. With increasing temperature, the correlation

peak disappears and a low q upturn is observed, indicating the onset of aggregation. In Fig. 1 (centre), T-dependent SANS profiles of BSA in the presence of 1 M urea are shown. Interestingly, the T-dependent changes of the scattering profiles closely resemble those without additives, which indicates that temperature is the dominating parameter in the denaturation process studier here. Fig. 1 (right) shows the thermal denaturation profile of 120 mg/ml BSA in the presence of 4 mM YCl_3. The low T correlation peak is less pronounced than in the sample without salt due to the screening effect of YCl_3. A slope analysis of the last curve reveals a slope of -3.5, indicating rough, fractal interfaces of the aggregates. These may hint at a the formation of a protein network held together by cation (Y^{3+}) bridges. The results obtained clearly show that an additional component of the protein-D_2O solution (in this case, YCl_3) have an effect on the thermal denaturation of BSA. Both time resolution and statistics of the scattering profiles obtained are highly suitable for obtaining a clear picture of protein thermal denaturation more detailed analysis of the other additives used during this beamtime as well as the effect of protein concentration on thermal denaturation is currently being performed. The data obtained during this beamtime will be complemented by differential scanning calorimentry scans performed at the PSCM in parallel to the SANS experiments as well as by backscattering measurements which will be performed on beamline IN16B in September 2016.