ESRF	**Experiment Title:** Early stage of arrested spinodal decomposition in protein solutions studied by USAXS	**Experiment number**: SC-3858
Beamline: ID2	**Date of experiment**: from: 11th Sept. 2014 to: 13th Sept 2014	**Date of report**: 20th Feb. 2015
Shifts: 6	**Local contact(s):** Michael Sztucki	*Received at ESRF:*

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Report:

The existence of liquid-liquid phase separation in protein solutions provides a fundamental mechanism for understanding the phase behavior in biological systems [1]. Such as protein crystallization, protein condensation related diseases, where the subtle change upon the protein structure altering the effective interactions leading to a phase transition. In colloidal system, it has been established that the short-ranged attraction results in the metastable LLPS. In this case, the gelation line often cuts the phase boundary near the critical point, which means that in such a system, the LLPS is an arrested phase transition [2-4]. Previous studies of lysozyme indicate that systems undergone an arrested spinodal decomposition have a bicontinuous structure with a protein-poor fluid and a dense glassy protein network [3,5,6]. For better understanding the early stage of the phase transition, the structural information of both the network and the local structure within the dense glassy branches needs to be characterized as a function of time. However, the major difficulty here is the extremely different length scales: from nanometers of the local nearest neighbor distance to a few micrometers of the correlation length of the network. Simultaneously monitoring the structural evolution is difficult using standard small angle X-ray or neutron scattering or light scattering. Now, with the new renovation of ID2, this becomes possible. The new setup can cover the whole length scale of the early stage of spinodal decomposition. The results should provide much deeper understanding of this subject.

From September 11th to 13th of 2014, we have successfully measured the phase transition in protein solutions using the USAXS configuration at ID02. The sample-to-detector configurations were 30 m covering a q-range from 0.002-0.14 nm^{-1}. The samples we measured contains protein bovine serum albumin (BSA) and YCl_3. The phase behavior of this system has been studied in our group as a function of salt concentration and temperature [7,8]. A reentrant condensation phase behavior has been established with a LLPS occurring within the condensed regime in a closed area [7]. The sample solutions have a lower critical solution temperature (LCST) phase behavior. BSA-YCl_3 system has a complete spinodal transition resulting in co-existence protein-poor and rich phases. Upon further heating, the protein-rich phases undergo the arrested phase transition. Figure

1 shows an example of real-time USAXS profile for a sample containing 175 mg/ml BSA and 36 mM YCl_3. The sample has a lower critical solution temperature around 20 ºC. The sample was first cooled to 10 ºC and the measurements start. After collecting a few scans at 10 ºC, the temperature was jumped to 30 ºC and the scattering curves were collected in every 3 seconds. At 10 ºC, the scattering intensity in the whole q range is nearly constant, except at very low q region (below 0.007 nm^{-1}), the increasing in scattering intensity in this region may be due to the influence of beamstop. After temperature jump, the scattering profile increases intensity in the low q region, but the profile at the high q region nearly no change. After certain time, a scattering peak becomes visible and its position shifts to lower q with time. The overall intensity gives a q^{-4} decay. Quantitative data analysis using suitable models for the time dependent structural evolution will be carried out soon. During this beamtime, we have measured a series of samples with fixed protein concentration and varying salt concentration. The temperature jump was performed at three different temperatures, i.e. from 10 ºC to 25, 30 and 35 ºC.

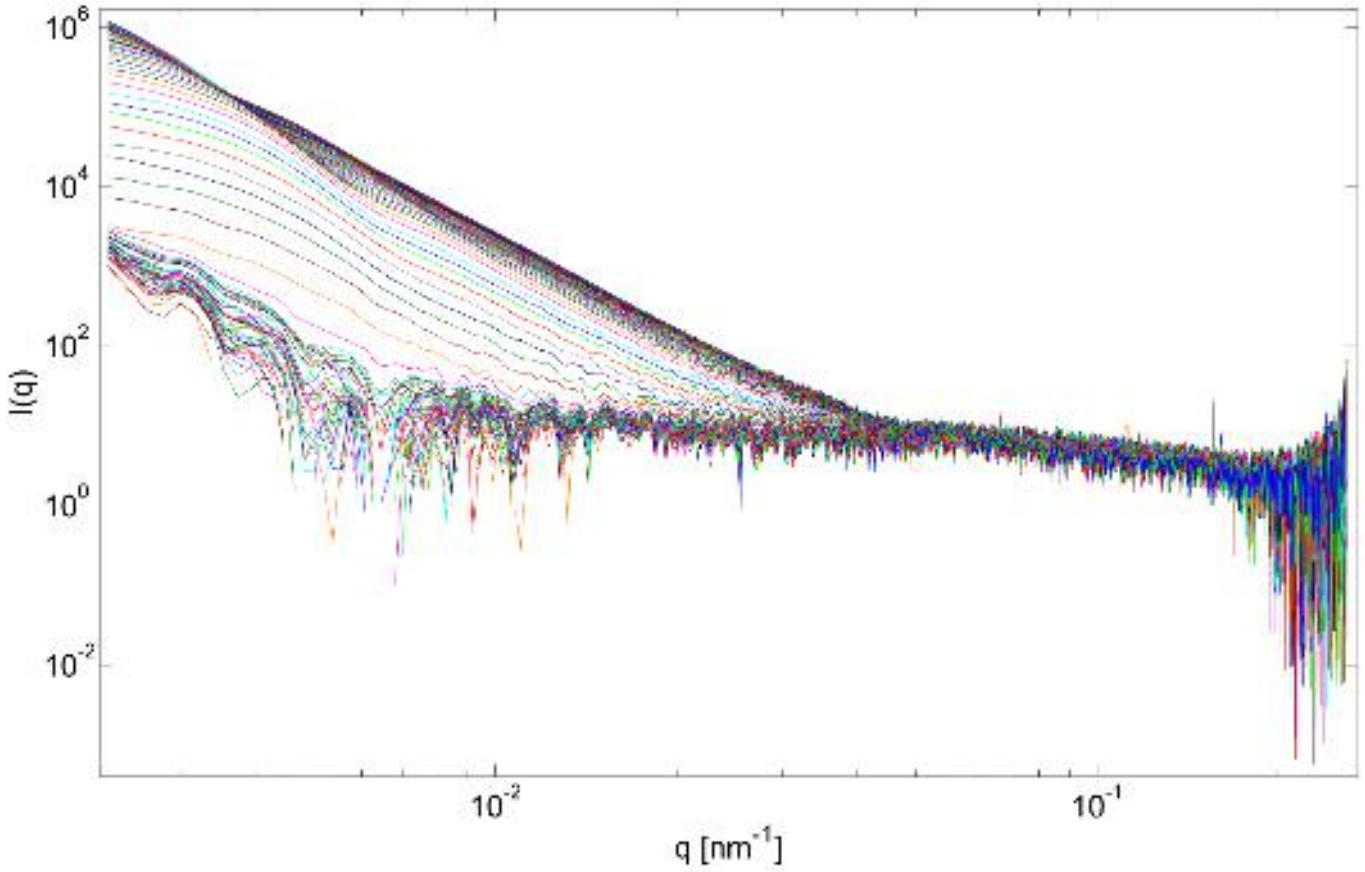

Figure 1: Real-time USAXS profiles of a protein solution undergoing a liquid-liquid phase transition. The sample is BSA 175 mg/mL with 36mM YCl3. The starting temperature was 10 ºC and jump to 30 ºC, the time increment was 3s.

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