ESRF	**Experiment Title:** Early stage of arrested spinodal decomposition in protein solutions studied by USAXS	**Experiment number**: SC-4185
Beamline: ID2	**Date of experiment**: from: 24th Jul. 2015 to: 27th Jul. 2015	**Date of report**: 24th Aug. 2015
Shifts: 9	**Local contact(s):** MOELLER Johannes	*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 July 24th to 27th of 2015, we have successfully measured the phase transition in protein solutions using the USAXS configuration at ID02 equipped with the Linkam temperature stage and the FReLoN detector. The sample-to-detector distance was 30 m covering a q-range from 0.0009-0.14 nm^{-1}. The samples we measured contains protein bovine and human serum albumin (BSA/HSA) and YCl_3. The phase behavior of these systems have been studied in our group as a function of salt concentration and temperature [7]. 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. While

BSA-YCl_3 system has a complete spinodal transition resulting in co-existence protein-poor and rich phases, the protein-rich phases of HSA-YCl_3 system undergo the arrested phase transition.

We have measured the following samples listed in the following table during this beamtime, we are currently working on the detailed data analysis.

Table 1: list of samples and experimental conditions

Sample	**Temperature jump**	**Time period and resolution**	**comment**
BSA175mg/mL with YCl3 36mM, 40mM, 42mM, 44mM	10ºC – 25 ºC, 35 ºC, 40 ºC with a heating rate of 100K/min.	ccdmulti 60, 0.32s ccdframes 60-90 every 4s	No arrested phase transition
HSA100mg/mL with YCl3 35 mM, 40 mM, 44 mM, 45 mM	10ºC – 25 ºC, 35 ºC, 40 ºC and 45ºC	ccdmulti 60-90, 0.32s ccdframes 50-90 every 4s one followed up to ~90 min	Arrested gel occurs
IgG with PEG8K	35 ºC - 20 ºC, 10 ºC and 5 ºC with a cooling rate of ~80K/min		Test samples, featuring LLPS and gelation, in need of further studies

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References

[1] J. D. Gunton, A. Shiryayev, and D. L. Pagan, Protein Condensation, Cambridge University Press (2007)
[2] P. J. Lu, E. Zaccarelli, et al. *Nature* 453, 499 (2008)
[3] F. Cardinaux, T. Gibaud, A. Stradner, P. Schurtenberger, *Phys. Rev. Lett*. 99, 118301 (2007)
[4] E. Zaccarelli, *J. Phys. Condens. Matter* 19, 323101 (2007)
[5] T. Gibaud, P. Schurtenberger, *J. Phys. Condens. Matter* 21, 322201 (2009)
[6] T. Gibaud, N. Mahmoudi, et al. *Faraday Discuss*. 158, 267 (2012)
[7] F. Zhang, et al. *Phys. Rev. Lett.* **101**, 148101 (2008) *Proteins* **78**, 3450-3457 (2010). *Soft Matter* **8**, 1313-1316 (2012).