ESRF	**Experiment Title:** The influence of cation size on the effective interactions and LLPS in protein solutions	**Experiment number**: SC-4186
Beamline: ID2	**Date of experiment**: from: 3rd Feb. 2016 to: 5th Feb. 2016	**Date of report**: 24th Feb. 2016
Shifts: 6	**Local contact(s):** MOELLER Johannes	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*ZHANG Fajun, IAP, Uni-Tuebingen, Germany

*BRAUN Michal, IAP, Uni-Tuebingen, Germany

*MATSARSKAIA Olga, IAP, Uni-Tuebingen, Germany

*DA VELA Stefano, IAP, Uni-Tuebingen, Germany

Schreiber Frank, IAP, Uni-Tuebingen, Germany

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 and protein condensation related diseases, where subtle changes in the protein structure can alter the effective interactions leading to a phase transition. In colloidal systems, it has been established that short-ranged attraction results in metastable LLPS. In this case, a 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 on lysozyme indicate that systems which underwent arrested spinodal decomposition have a bicontinuous structure with a protein-poor fluid and a protein-rich glassy protein network [3,5,6]. For a better understanding of 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 presence of 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 recent renovation of ID02, this becomes possible. The new setup can cover all the length scales during the early stage of spinodal decomposition. The results should provide a much deeper understanding of this subject.

From July 24th to 27th of 2015, we have successfully measured scattering profiles during the phase transition in protein solutions using the USAXS configuration at ID02 equipped with a Linkam temperature stage and a FReLoN detector. The sample-to-detector distance was 30 m covering a q-range from 0.0009 to 0.14 nm^{-1}. The samples measured contain protein bovine and human serum albumin (BSA/HSA) and YCl_3. The phase behavior of these systems has 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.

We have performed jumps in the two phase regime up to a higher temperature of 57.5°C for the BSA samples, in order to explore the broadest temperature range for the potential arrested state. For the first time, we have captured the early stage kinetics of the arrested phase transition of a system exhibiting LCST. Protein denaturation occurs above 55.5 °C. Figure 1 summarizes the domain size as a function of time for a sample (dense phase of BSA 175 mg/ml with 42mM YCl3). Between 30 and 40 °C, the domain size increases with time following a power law of ~$t^{1/3}$, at 47.5 °C a significant slowing down is visible; above 50 °C, the domain size increases only in the very early state (about 30 s) and then stays at a constant value indicating an arrested state. We note that below 55.5 °C, the samples are reversible even after reaching the arrested state, i.e. the arrested state dissolves upon cooling and the dense phase becomes clear again. Above 55.5 °C, the sample is not reversible due to protein denaturation.

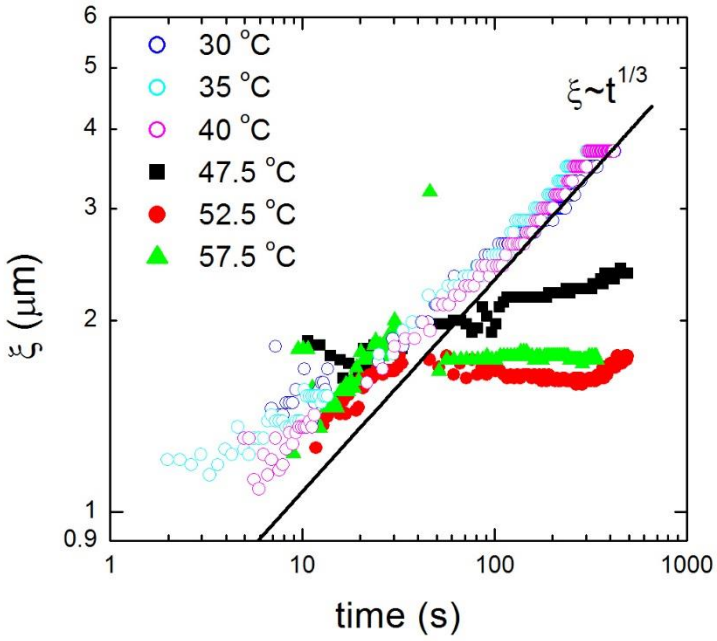

Figure 1. Plots of the domain size as a function of time for a dense liquid phase prepared by mixing BSA175mg/ml with 42 mM YCl_3 at room temperature. Temperature jumps from 10 °C to various temperatures labeled in the figure. Above 47.5 °C a significant slowing down and arrested state is reached.

The newly observed arrested state at higher temperatures, but still below the denaturation temperature of the protein, encourages us to continue this project in order to explore the more detailed kinetics and structure of the arrested states as well as their response to the physical parameters including volume fraction and salt concentration. We are currently working on the detailed data analysis of the new data.

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).