ESRF	**Experiment Title:** Early Stage of Arrested Spinodal Decomposition in Protein Solutions Studied by USAXS	**Experiment number**: SC-4400
Beamline: ID2	**Date of experiment**: from: 23rd Sep. 2016 to: 26th Sep. 2016	**Date of report**: 24th Feb. 2017
Shifts: 9	**Local contact(s):** MOELLER Johannes	*Received at ESRF:*

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Report:

Aqueous solutions of bovine serum albumin (BSA), an acidic protein, feature a rich phase behavior in presence of trivalent metal ions such as YCl_3 [1]. The phase behavior includes liquid-liquid phase separation (LLPS) with lower critical solution temperature behavior [2]. We have shown previously that, upon a deep quench in the two phase regime upon a temperature jump, an arrested state can be obtained via arrested spinodal decomposition, due to the interplay of the glass line with the LLPS phase boundary [3]. A deeper fundamental understanding of the pathways leading to phase-separated and arrested states is relevant for analogous systems undergoing arrested spinodal decomposition, including pharmaceutical formulations and food gels.

The USAXS setup of beamline ID2 is ideally suited for time-resolved experiments monitoring this phase separation [4,5]. In the latest experiments performed during beamtime SC-4400, we explored the LLPS kinetics and its arrest in more detail. In a typical experiment, each sample condition was followed after the temperature jump up to 15 min. Each temperature jump was repeated three times, resulting in three steps employed to characterize the structural evolution during phase separation. In the first step scattering profiles are collected with high time resolution to characterize the early stages of phase separation, in the second step the phase separation is followed at intermediate times and in the third step long time behaviour of the phase separation is characterized with lower time resolution. Scattering profiles for each step can be seen for a sample undergoing a full phase separation at 40°C in Fig.1. For a quench leading to full phase separation, the correlation peak in the scattering profiles grows and moves to lower *q* values with time.

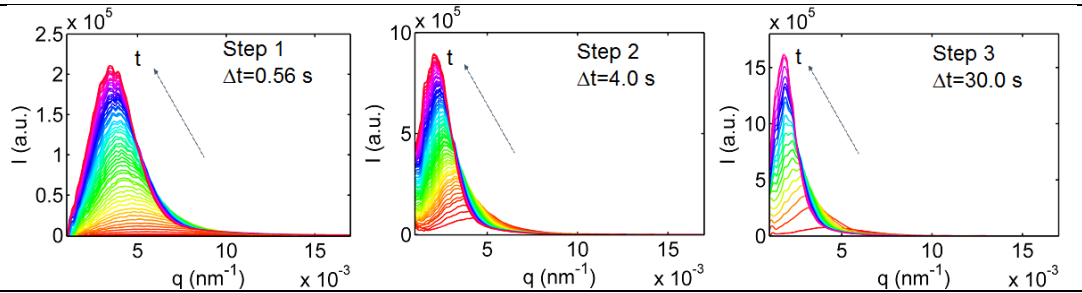

Figure 1: scattering profiles for a sample derived from BSA 175 mg/mL and YCl_3 40 mM upon a temperature jump to 40°C. Δt is the time interval between the acquisition of each profile.

As the quench depth is increased, the characteristic length (reciprocal of the position of the peak) shows a different behavior and shows the arrest of the phase transition at sufficiently high temperatures. This is shown in Fig.2 (adapted from ref.[3]), in which the correlation length as a function of time is summarized for different quench depths.

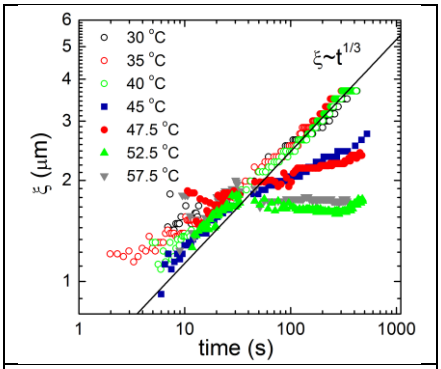

Figure 2: plots of the characteristic length as a function of time and quench depth for a sample derived from BSA 175 mg/mL and YCl_3 44 mM.

From our most recent experiments, we found that the time evolution of the characteristic length during LLPS has an additional dependence on quench depth beyond the simple distinction between full phase separation and arrested phase separation. Sufficiently deep quenches result also into a non-monotonic evolution of the intensity of the correlation peak. As shown in Fig.3 (deep quench to 52.5°C) the peak intensity appears to decrease at earlier times, while increasing again at later times. Further data analysis is in progress to fully characterize this behaviour.

Additionally, the effect of a widely used crosslinker, glutaraldehyde (GA), on the LLPS kinetics and arrest in the BSA-YCl_3 system was also investigated, employing a starting GA concentration in the range 0.1 mM to 2 mM.

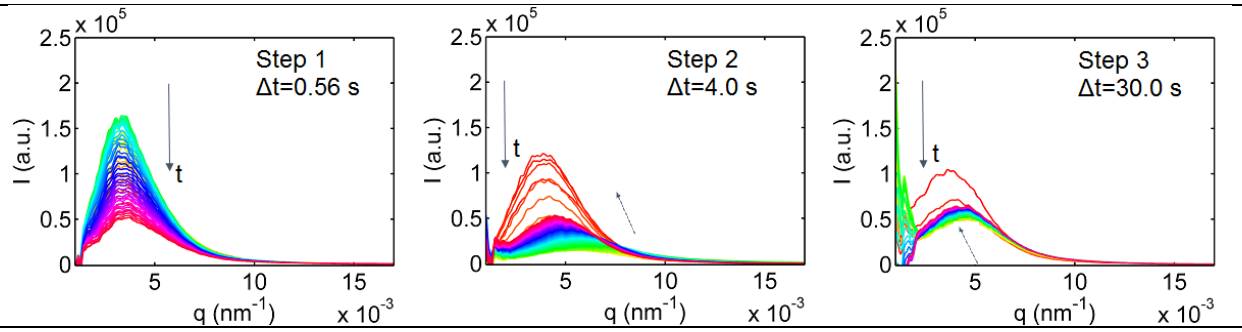

Figure 3: scattering profiles for the same sample as in Fig.1, upon a temperature jump to 52.5°C. Note the decrease of the intensity followed by a new increase (dashed arrows). Δt is the time interval between the acquisition of each profile.

References

[1] F.Zhang, et al. Reentrant condensation of proteins in solution induced by multivalent counterions. *Physical review letters*, 2008, 101.14: 148101.

[2] O.Matsarskaia, et al. Cation-Induced Hydration Effects Cause Lower Critical Solution Temperature Behavior in Protein Solutions. *The Journal of Physical Chemistry B*, 2016, 120.31: 7731-7736.

[3] S.Da Vela, et al. Kinetics of liquid–liquid phase separation in protein solutions exhibiting LCST phase behavior studied by time-resolved USAXS and VSANS. *Soft Matter*, 2016, 12.46: 9334-9341.

[4] Experimental report for beamtime SC-3858

[5] Experimental report for beamtime SC-4185