ESRF	**Experiment Title:** Time resolved USAXS studies of arrested liquid-liquid phase separation in an antibody/PEG system	**Experiment number**: SC-4500
Beamline: ID2	**Date of experiment**: from: 16th Jun. 2017 to: 19th Jun. 2017	**Date of report**: 30th Aug. 2017
Shifts: 6	**Local contact(s):** MARIANI Alessandro	*Received at ESRF:*

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Report:

The goal of this project is to study the structure and the kinetics of formation of an arrested state from IgG-type antibodies (bovine γ-globulin) in the presence of polyethylene glycol (PEG). In this system, phase separation is driven by a PEG-induced depletion interaction. This interaction leads to an upper critical solution temperature (UCST) phase behavior of the liquid-liquid phase separation (LLPS) boundary. By carefully tuning the conditions (PEG molecular weight and concentration, temperature and IgG volume fraction) the liquid-liquid phase separation can lead to a gel-like state by arrested spinodal decomposition. The IgG-PEG system is of fundamental interest for the strongly anisotropic shape and flexibility of the antibody molecules and complements a protein system with multivalent salts featuring an arrested lower critical solution temperature phase behavior previously described by our group.

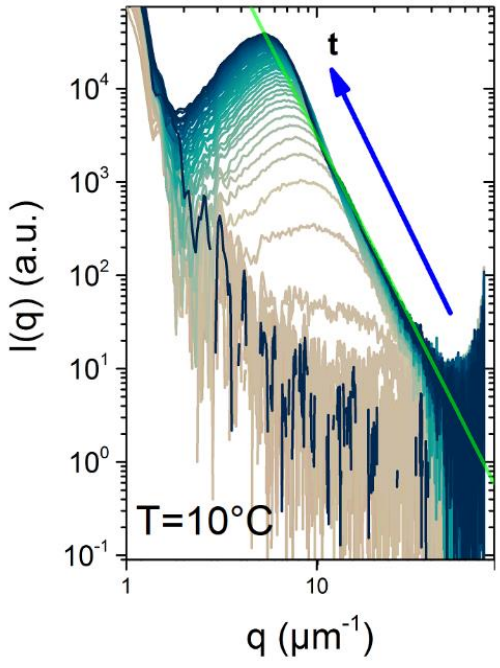

Figure 1: peak associated with spinodal decomposition. Interval between curves ~ 0.6 s. The green line is a q^{-4} slope.

Antibodies are an important class of proteins with multiple applications as diagnostic tools and therapeutics. Their phase behavior is also of theoretical interest as they are anisotropic and soft Y-shaped particles. Bovine γ-globulin, a polyvalent antibody mixture, was used as a model system for antibody isotype IgG. PEG is used to enhance attractive protein-protein interactions by the so-called "depletion" effect. The phase behavior can then be tuned varying PEG molecular weight and concentration, affecting the range and strength of the interaction respectively. This way, in selected conditions the antibody solutions feature a UCST-LLPS phase behavior.

We monitored the evolution of large-scale structures forming upon phase separation in antibodies solutions in the presence of polyethylene glycol (PEG). Upon fast temperature quenches, time-resolved scattering profiles were collected with the USAXS configuration of ID02 to follow the development of the peak associated with spinodal decomposition (*Figure 1*). As phase separation proceeds, the peak grows in intensity and moves to lower values of *q*. A sharp interface between the phase separating domains develops quickly, as can be seen by the $\sim q^{-4}$ slope of the scattering profiles towards high *q*. From the peak maximum it was possible to extract a correlation length as a function of time (*Figure 2*), representing the correlation between the phase separating domains.

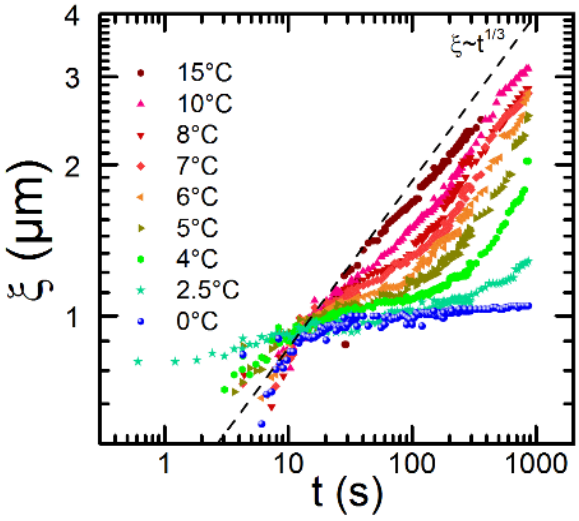

Figure 2: characteristic length as a function of time for increasing quench depths. At 0°C the system is in a kinetically arrested state.

In our experiments we could confirm the presence of a thermally reversible arrested state [1,2] for sufficiently deep quenches in the LLPS region (0°C data in *Figure 2*). For these deep quenches, after an initial development, the characteristic length ξ is stationary in time, signifying kinetic arrest due to the dense phase approaching a glassy state. For shallow quenches, instead, the phase separation proceeds normally, coarsening with the characteristic length following a power law close to $\xi \sim t^{1/3}$.

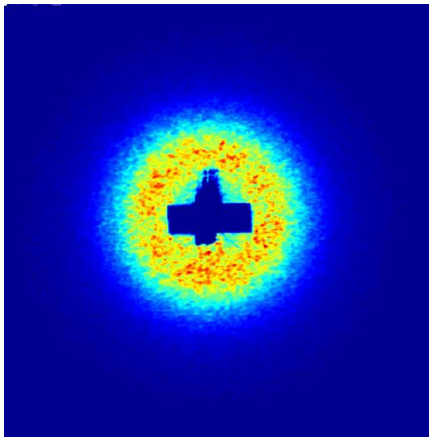

Figure 3: Typical isotropic 2D scattering pattern.

Interestingly, for intermediate quenches, deviations from the $\xi \sim t^{1/3}$ behavior become more and more relevant, resulting eventually in a non-monotonic growth law. Approaching the arrested state, a temporary kinetically arrested state is seen. The coarsening slows down and recovers at a later stage. The 2D scattering pattern is isotropic throughout the measurement (*Figure 3*), indicating absence of significant sedimentation. A paper including these data has been submitted and is currently under peer review.[2] Hints of a similar behavior were seen also in previously published work with a LLPS system with lower critical solution temperature.[3] This is an indication of a certain degree of universality of the observed behavior.

More results were obtained to characterize the LLPS region of the phase diagram and will be included in a future publication.[4] Our results further clarify the interplay of the glass transition with the LLPS binodal, and could potentially be transferred to innovative gel-like formulations for monoclonal antibodies.

References

[1] Experimental report for beamtime SC-4288, SC-4400

[2] S.Da Vela, et al. (2017) Temporarily arrested state in a protein-polymer mixture studied by USAXS and VSANS *in peer review*

[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] S.Da Vela, et al., *in preparation*