ESRF	**Experiment Title:** Time resolved USAXS studies of arrested liquid-liquid phase separation in an antibody-PEG system	**Experiment number**: SC-4288
Beamline: ID2	**Date of experiment**: from: 29th Apr. 2016 to: 2nd May 2015	**Date of report**: 24th Aug. 2016
Shifts: 9	**Local contact(s):** MOELLER Johannes	*Received at ESRF:*

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Report:

Antibody solutions are known to undergo liquid-liquid phase separation (LLPS) in the presence of the non-adsorbing polymer polyethylene glycol (PEG) [1,2]. The protein-protein attraction in this class of systems is determined by the depletion interaction induced by PEG. IgG-type antibodies are of particular interest for their application as increasingly successful biopharmaceuticals and diagnostic tools and for their relevance to fundamental research as small anisotropic, flexible colloidal particles. In our experiments, we used a polyclonal antibody product, bovine γ-globulin, as model system for IgG type antibodies in the presence of PEG. The concentration and molecular weight of the added polymer can be used to tune the range and strength of the depletion interaction. By careful choice of these two parameters, concentrated γ-globulin solutions can be induced to form thermally reversible arrested states exploiting the interplay between the upper critical solution temperature (UCST) LLPS boundary and the glass line of their solutions.

The current USAXS setup of ID2, allows for the ideal time and *q* resolution to follow the kinetics of phase separation and arrest in this system, with the usage of the Linkam temperature-controlled stage. We apply here the experimental procedure established previously for our BSA-Y(III) system [3], allowing us to follow the spinodal decomposition and kinetic arrest of protein mixtures in its various stages. Also of interest is a comparison with the previously studied system featuring lower critical solution temperature (LCST) LLPS [4].

During this beamtime (SC-4288), we have performed a series of successful measurement on the kinetics of LLPS in the mixture of bovine γ-globulin and PEG. Samples measured during this beamtime are shown in Table 1. The full data analysis is in progress. In a typical experiment, protein solutions were subjected to a temperature quench in the two phase region. Sample conditions resulting into kinetic arrest feature a low-q peak, initially growing in intensity, that doesn't shift to lower *q*-values with time (Fig.1). A comparison between normal LLPS and arrested phase transition is better seen by the time evolution of the characteristic length ξ (Fig.2) extracted from the scattering peaks. The excellent collimation of ID2 in USAXS mode shows a clear speckle pattern in the 2D scattering profiles, opening the possibility of performing an XPCS data analysis to investigate the dynamics of the arrested state (Fig.3). Overall, we find that, for sufficiently deep quenches, the LLPS can arrest at early stage, while an increase of the PEG concentration reduces the depth of the quench needed to result in an arrested state, while a short range attraction provided by the lower molecular

weight PEG is needed for the arrest. For less deep quenches, the LLPS proceeds normally, following the domain size growth law expected for spinodal decomposition.

Table 1.

Sample	**Temperature jump**	**Number of images and acquisition rate**	**Overall kinetics**
IgG 110 mg/mL PEG 1000 11 % (w/v)	38°C – >15° to 0°C. Cooling rate 80K/min.	60 x 3.1 s^{-1} , 75 x 0.3 s^{-1}, 90 x 0.03 s^{-1}	Slowdown and arrest
IgG 110 mg/mL PEG 1000 12% (w/v)	38°C – >15° to 0°C. Cooling rate 80K/min.	60 x 3.1 s^{-1} , 75 x 0.3 s^{-1}, 90 x 0.03 s^{-1}	Slowdown and arrest
IgG 150 mg/mL PEG 8000 4% (w/v)	38 °C -> 0°C. Cooling rate 80K/min	60 x 3.1 s^{-1} , 75 x 0.3 s^{-1}, 90 x 0.03 s^{-1}	Slowdown, no arrests
IgG 150 mg/mL PEG 8000 5% (w/v)	38 °C -> 20° to 5 °C. Cooling rate 80K/min	60 x 3.1 s^{-1} , 75 x 0.3 s^{-1}, 90 x 0.03 s^{-1}	No arrest
IgG 150 mg/mL PEG 8000 6% (w/v)	38 °C -> 15° to 0 °C. Cooling rate 80K/min	60 x 3.1 s^{-1} , 75 x 0.3 s^{-1}, 90 x 0.03 s^{-1}	Probable arrest
BSA 175 mg/mL YCl_3 40 to 44 mM	10 °C -> 40° to 57°C Heating rate 80K/min	60 x 3.1 s^{-1} , 75 x 0.3 s^{-1}, 90 x 0.03 s^{-1}	Tests of LCST system with highly collimated beam
BSA 175 mg/mL $LaCl_3$, $CeCl_3$, $GdCl_3$, $HoCl_3$ 36 to 46 mM	10 °C -> 40° to 52.5°C Heating rate 80K/min	60 x 3.1 s^{-1} , 75 x 0.3 s^{-1}, 90 x 0.03 s^{-1}	Comparison with a LCST system in presence of different salts

Figure 1

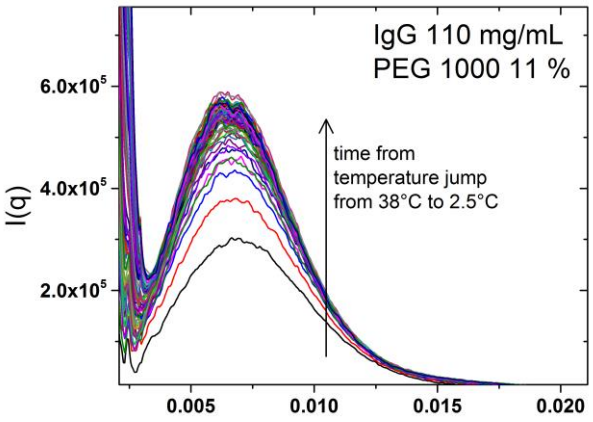


Figure 2

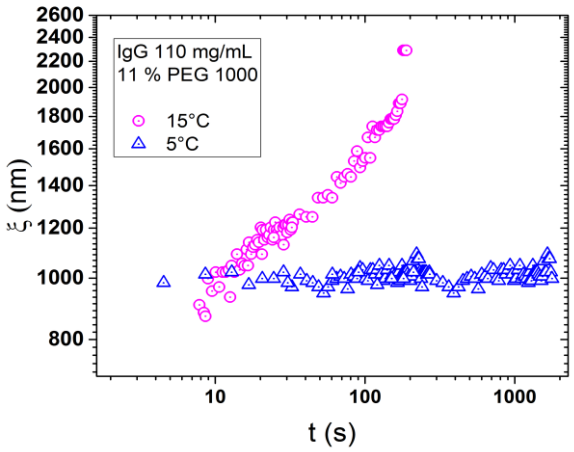
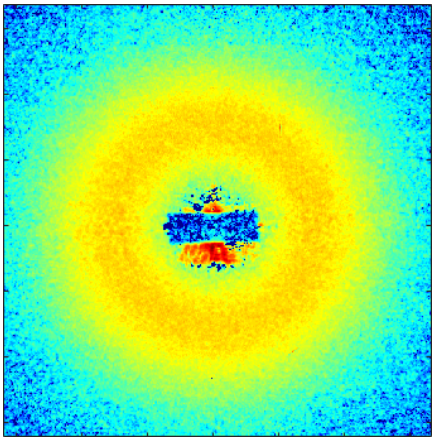


Figure 3



References

[1] WANG, Ying, et al. Quantitative evaluation of colloidal stability of antibody solutions using PEG-induced liquid–liquid phase separation. *Molecular pharmaceutics*, 2014, 11.5: 1391-1402.

[2] WANG, Ying, et al. Phase transitions in human IgG solutions. *The Journal of chemical physics*, 2013, 139.12: 121904.

[3] DA VELA, Stefano, et al. Kinetics of liquid-liquid phase separation in protein solutions exhibiting LCST phase behavior studied by time-resolved USAXS and VSANS, 2016, *submitted*.

[4] MATSARSKAIA, Olga, et al. Cation-induced Hydration Effects Cause Lower Critical Solution Temperature Behavior in Protein Solutions, 2016, *JPCB*, 120, 7731.