ESRF	**Experiment Title:** Kinetics of arrested liquid-liquid phase separation under a two-step quench studied by USAXS	**Experiment number**: SC-4765 SC-4747
Beamline: ID2	**Date of experiment**: from: 8th Apr. 2018 to: 10th Apr. 2018	**Date of report**: 30th Feb. 2020
Shifts: 6	**Local contact(s):** MARIANI Alessandro	*Received at ESRF:*

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Report:

This is a report for a combined beamtimes of SC-4747 and SC-4765.

The general motivation of both projects is to deepen our understanding on the phase behavior of proteins in solution, which is of fundamental importance for protein crystallization, protein condensation related diseases as well as cell biophysics. Our recent work has successfully established the use of the trivalent salt to induce a metastable liquid-liquid phase separation (LLPS) as well as arrested phase transition in various model proteins [1-2]. USAXS was employed to follow the kinetics of phase transition to tackle the interplay between LLPS and glass formation [3,4].

The project of SC-4747 aim to study the kinetics of protein systems undergoing phase separation *via* spinodal decomposition upon both a temperature increase and a decrease, thus featuring an intriguing "hour-glass" phase diagram. The systems in question consist of bovine serum albumin (BSA) and varying ratios of the trivalent salts $HoCl_3$ and $LaCl_3$. While $HoCl_3$ interacts very strongly with BSA, the interaction between $LaCl_3$ and BSA is rather weak. The unique combination of these salts thus provides a sensitive tool to fine-tune the interactions in this system and leads to a highly complex phase behaviour. We wish to explore the phase diagrams of these systems by systematically studying their phase separation kinetics as a function of temperature jump and quench depths as well as of varying ratios of $HoCl_3/LaCl_3$. The measurement of this beamtime was successful. By monitoring the characteristic length, ξ, as a function of time and temperature after LLPS, we observe an increasing deviation from the growth law $\xi \sim t^{1/3}$ and an increased trend toward arrest (Fig.1). The results have been published recently [5].

The goal of project SC-4765 is to study the kinetics of arrested spinodal decomposition in protein solutions. In particular, in this proposal we focus on the influence of a *two*-step quench on the development of the characteristic length and the scattering intensity. Two protein systems, i.e. bovine serum albumin (BSA) with YCl_3 and a mixture of bovine γ-globulin with PEG, featuring liquid-liquid phase separation (LLPS) with a lower (LCST) and upper critical solution temperature (UCST) phase behavior, respectively, were used for this comparative study. To achieve the two-step quench, we wish to vary both the heating rate which causes a transient step of phase separation before the final temperature is reached, and the waiting time at the pre-set temperature before quenching to the final temperature. The USAXS configuration of ID02 enables the

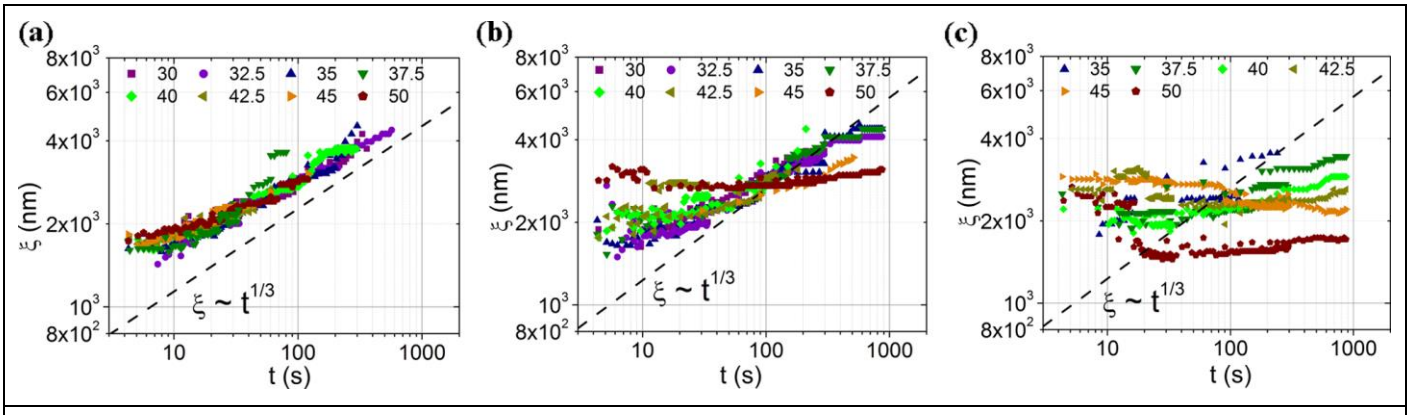

Figure 1: Characteristic length, ξ, as a function of time and temperature (indicated by the legends) for protein BSA solutions with different $HoCl_3/LaCl_3$ mixing ratios (a) 30/10; (b) 25/15 and (c) 22/18. For long observation times, ξ reaches a plateau.[5]

characterization of the development of the characteristic length scales produced by the arrested phase transition with excellent time resolution. The success of the measurements proposed will provide deeper insights into the coarsening kinetics of the arrested phase transition. During this beamtime, we have systematically followed the phase transition kinetics of both systems. The results confirmed the previous observations as shown in Fig.2. We observed again and more systematically, the intriguing time evolution of the correlation length ξ of the system for deep quenches close to the glassy state. Initially, ξ has a non-zero value which first decreases with time, then increases again. This is in stark contrast to the shallow quenches where ξ grows according to a power law $\sim t^{1/3}$ (see Fig. 2a). This behavior is expected to be dependent on the heating rate or, more specifically, on the stage of the phase separation reached in the first step. These observations indicate that due to the experimental property of a deep quench, spinodal decomposition might start before the system reaches the final temperature, resulting in the transient pre-existing structure. Moreover, those pre-existing structures do not represent the equilibrium order of the final temperature, they thus decay upon the appearance of more stable structures. More quantitative analysis is ongoing.

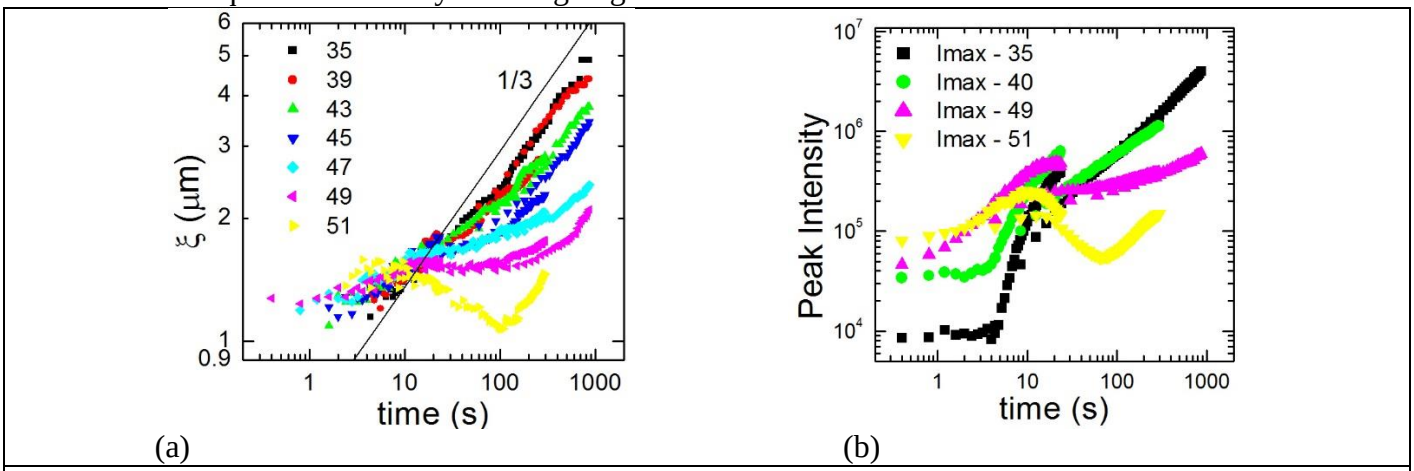

Fig.2 (a) The characteristic length as a function of time and temperature; (b) Corresponding peak intensity as a function of time for several temperatures. Note the increase – decrease –increase two step kinetics at high temperatures.

References

[1] Experimental report for beamtime SC-4288, SC-4400, SC-4500

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