ESRF	**Experiment Title:** Kinetics and dynamics of charge-driven arrested phase transition in protein solutions studied by USAXS-XPCS	**Experiment number**: SC-4999
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Shifts: 6	**Local contact(s):** Thomas Zinn	*Received at ESRF:*

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Report:

The goal of this project is to study the kinetics and dynamics of liquid-liquid phase separation (LLPS) in protein solutions [1]. The protein system used is aqueous solution of bovine serum albumin (BSA) in the presence of YCl_3. The phase behavior of this system has been well-established in our group [2]. The metastable LLPS with a low critical solution temperature (LCST) phase behavior. It is often found that the phase separation is further interrupted by the glass formation. By tuning the temperature T below the glass transition, the solution undergoes an arrested spinodal decomposition. The kinetics of the arrested phase transition has been studied by us using ultra-small angle X-ray scattering (USAXS) method [3,4]. We note that for temperatures higher than 45 °C, the dense phases became gel-like and the determined protein concentrations on the high density side of the binodal are smaller than those at lower temperature, indicating that the glass line enters into two-phase coexistence region. Our previous USAXS study has shown a significant slow-down of the kinetics of phase separation and that an arrested state could be achieved above 47.5 °C but below thermal denaturation [3,4]. Here, we aim to perform systematic XPCS studies of the arrested phase transition at different temperatures and at length-scales corresponding to the q-values of the $I(q)$ peak position (q>0.001 nm^{-1}). Another important issue for the dynamics of early stage of spinodal decomposition is the strongly intertwined kinetics and dynamics. In this work, based on the high quality XPCS data, we aim to establish a method to decouple the kinetics of growth and the collective dynamics during LLPS.

During this beamtime at ID02, we used different final temperatures and time scales to investigate the corresponding dynamics of this phenomenon by XPCS in USAXS mode. X-ray energy was of 12.23 keV (λ = 0.1014 nm), sample to detector distance was 30718mm, and the beam size was $22\mu m \times 25\mu m$. With a flux of 1.2x10^9 ph/s, the dose of 1s exposure was about 0.26kGy. 2D speckle patterns were recorded by an Eiger

detector as a function of time. Parameters of the experiments can be found in the Table 1 below. The sample was first stabilized at 10℃ and then heated to the final temperature *T* at which the measurements were performed. After measurement the sample was quenched back to 10℃. The total radiation dose for each measurement was below 2.6kGy which is below the beam damage threshold.

The intensity fluctuations of the speckles contain the information on the dynamics of domain fluctuation during phase separation. To follow the evolution of the present non-equilibrium systems, two-time correlation (TTC) function was calculated from the 2D speckle pattern [5]:

$$g_2(t_1,t_2) = \frac{<I(t_1)I(t_2)> - <I(t_1)><I(t_2)>}{[<I^2(t_1) - <I(t_1)>^2]^{1/2}\,[<I^2(t_2) - <I(t_2)>^2]^{1/2}}, \qquad (1)$$

where <> denotes the average over pixels within the same momentum transfer $\boldsymbol{q} \pm \Delta\boldsymbol{q}$**.** Using the Kohlrausch-Williams-Watts relation [5,6], the characteristic relaxation time τ and the Kohlrausch exponent γ were obtained as functions of $\boldsymbol{q}$ and the absolute experimental time $t_{age} = (t_1 + t_2)/2$:

$$g_2\left(q, t_{age}, \bar{t}\right) = \beta(q) \cdot \exp\left(-2\left[\frac{\bar{t}}{\tau(q,t_{age})}\right]^{\gamma(q,t_{age})}\right). \qquad (2)$$

Here $\bar{t} = t_2 - t_1$ is a delay time and β is the speckle contrast.

Typical results of XPCS for "60s" measurements at different temperatures are shown in Fig.1. In all cases, the dynamic map (TTC) show signals in the early stage with a fast decay rate which slows down quickly with time (see *Fig. 1*). The corresponding growth kinetics shown in the lower row of Fig.1 covers the main peak of structure factor. According to Binder [7], the kinetic relaxation rate at a specific *q* can be calculated through the changes in the scattering intensity:

$$\Gamma\left(q, t_{age}\right) = \frac{1}{2}\frac{dlog\left[I(q,t_{age}\right]}{dt_{age}} \qquad (3)$$

With this relation, one can determine the relaxation rate of the structural relaxation during growth and compare this with the relaxation rate determined from XPCS (eq.2). Details of data analysis is ongoing. The results are expected to provide a systematic temperature dependent microscopic dynamics during LLPS. More importantly the decoupling between kinetics and dynamics makes it possible to understand the dynamics of LLPS in the early stage where the density fluctuation dominated collective dynamics does not fit to the dynamic scaling law.

Table 1: summary of experimental conditions and control parameters.

T, ℃	frames	Exposure, s	Delay, s	Total time, s
30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52	1000	0.01	0.29	300
30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52	1000	0.01	0.05	60
30, 36, 38, 42, 48, 52	1000	0.02	1.78	1800
30, 36, 42, 48, 52	1000	0.03	0.27	300
30, 36, 42, 48, 52	1000	0.1	0.2	300
30, 36, 42, 48, 52	1000	0.2	0.1	300

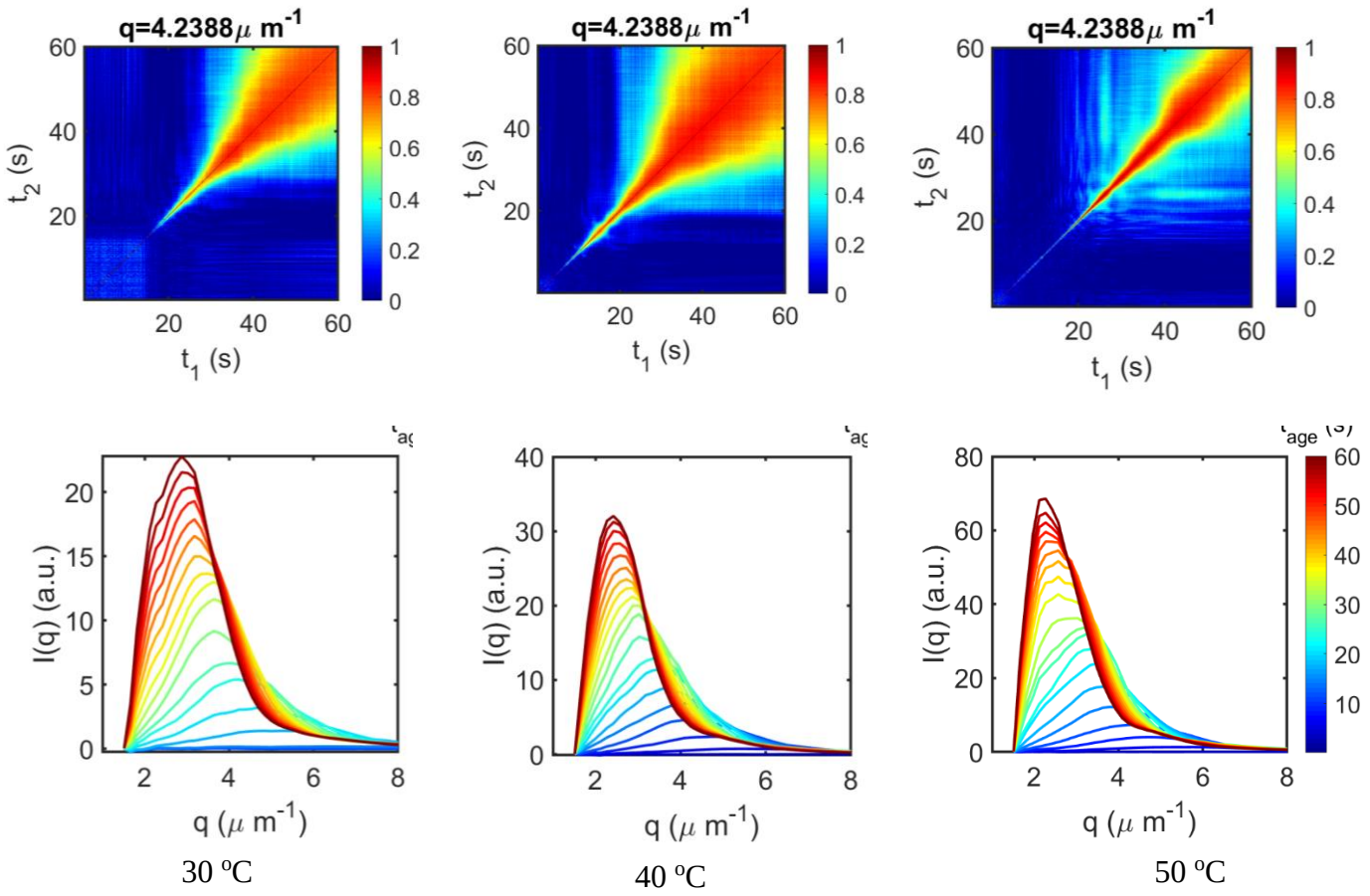

Figure 1, XPCS measurements of LLPS in protein solutions: representative dynamic maps (upper row) and corresponding growth kinetics (lower row) at different temperatures for the first 60s of phase transition.

References

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