ESRF	**Experiment Title:** Kinetics and dynamics in protein solution studied by USAXS-XPCS	**Experiment number**: SC-4883
Beamline: ID2	**Date of experiment**: from: 24th Oct. 2018 to: 26th Oct. 2018	**Date of report**: 25th Feb. 2020
Shifts: 6	**Local contact(s):** Thomas Zinn	*Received at ESRF:*

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Report:

The goal of this project is to study the dynamics of protein domains during liquid-liquid phase separation (LLPS) [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 altra-small angle X-ray scattering (USAXS) method [3]. 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].

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 12.46 keV (λ = 0.099 nm), sample to detector distance was 30705 mm, and the beam size was $40\mu m \times 40\mu m$. 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 at first was stabilized at 10℃ and then heated to final temperature T at which the measurements were performed. After the experiment the sample was quenched back to 10℃. The total radiation dose for each measurements was 11.5kGy.

Table 1: summary of experimental conditions and control parameters.

T, ℃	Start time,s	frames	Exposure, s	Delay, s	Total time, s
45, 47.5, 50, 52.5, 55	After heat (~ 21 s)	1500	0.002	0.003	7.5
45, 47.5, 50, 52.5, 55	21+7.5+~40	600	0.005	0.995	600
45, 47.5, 50, 52.5, 55	21+7.5+~40+600+~40	600	0.005	0.995	600
45, 47.5, 50, 52.5, 55	21+7.5+~40+600+~40+600+~40	600	0.005	0.995	600
45, 47.5, 50, 52.5, 55	**During heat (~5s)**	**600**	**0.005**	**0.095**	**60**
45, 47.5, 50, 52.5, 55	5+60+~40	600	0.005	0.995	600
45, 47.5, 50, 52.5, 55	5+60+~40+600+~40	600	0.005	0.995	600

The experiments at 52.5℃ and 55℃ with reduced exposure time (from 0.005s to 0.003s) and the same total measurement time were also performed. In this case the total dose for each measurement was 6.9 kGy. 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 (ex. on *fig. 1*) [4]:

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

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

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

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

One typical result of XPCS and data analysis for "60s" measurement during quench (**bold** row in the table) are shown in Fig.1-3. It is very interesting to see that two-time correlation functions for all $\boldsymbol{q}$ and temperatures contain wing-like feature (see *fig. 1*).

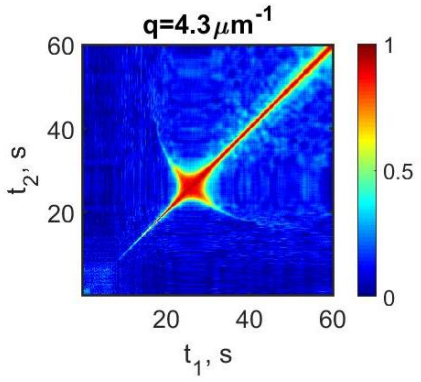

Figure 1. ttc obtained at 45℃ showing a wing like relaxation dynamics.

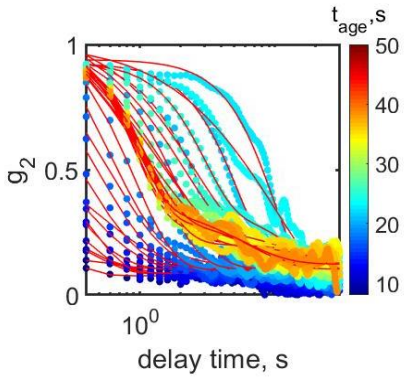

Figure 2. fit (red line) of g_2 diagonal cuts for 45℃ for q=4.3μm^{-1}

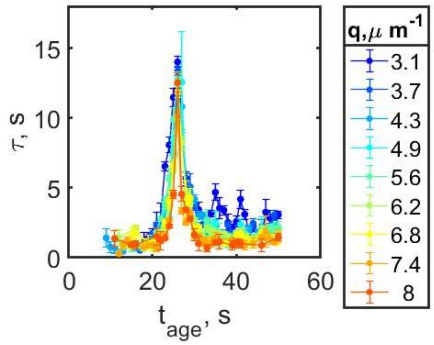

Figure 3. relaxation time for 45℃ for different q-values.

Intermediate scattering functions (ISF) obtained from the diagonal cuts of the ttc were fitted with the KWW relation (*fig. 2*) which showed that the relaxation time initially has a rapid increase followed by a decrease (*fig.3*). Corresponding Kohlrausch exponent varies from 1 to 2. In addition to the dynamic information, time-resolved scattering profiles of the growth kinetics were collected simultaneously with the USAXS configuration of ID02. The development of the peak in the USAXS profiles is associated with the spinodal decomposition (*fig. 4*). As phase separation proceeds, the peak moves to lower values of $\boldsymbol{q}$. However, in comparison to the classical spinodal decomposition where the peak intensity increases continuously, here the increase is followed by a decrease. This phenomenon has been observed during previous USAXS beamtimes focusing on the kinetics [6]. The system at first develops the domains of the dense/dilute phase, which then redissolve and then it eventually starts developing again. In the calculated ttc the retrieval of the system to the growth stage is not seen. However, measurements for 10 minutes after this "60 s" measurement (between which there is around 40 s switching time) show further increase in τ which supports the growth-redissolve-growth mechanism. The time at which the domains growth stops and the re-dissolution starts, obtained from kinetics, coincides with the time of appearance of the wing structure at ttc. This result is repeatable for different temperatures and the wing structure appears at the same time for all measured $\boldsymbol{q}$-values (*fig.* 3).

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 a 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. In addition to the frustration of the preexisting state to the final equilibrium state, the influence of beam needs also further investigation. Despite the fact that the whole measurement was made under the beam damage threshold, the beam may still influence the system by putting additional charges during ionization process. BSA- YCl_3 system has a strong dependence on charges. For a clear understanding we need to perform a systematic dose and temperature dependent investigations for longer time to understand the mechanism of the novel phase separation process.

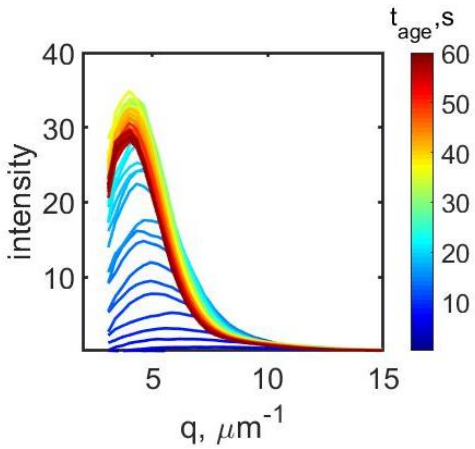

Figure 4: peak associated with spinodal decomposition. Interval between curves ~ 1 s.

References

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