Contribution ID: **17** Type: **Talk**

Dynamic Arrest of Liquid-Liquid Phase Separation in Protein Solutions Studied Using Ultra-Small Angle X-ray Scattering (USAXS)

Monday 17 September 2018 15:15 (15 minutes)

The interplay between liquid-liquid phase separation (LLPS) and glass formation leads to a dynamically arrested state in colloidal and protein systems. However, many details regarding the transition from LLPS to the arrested state and their responses to the subtle changes of the quench depth are still not fully understood. Further more, the interplay between glass formation and LLPS, i.e. whether the glass line follows the equilibrium binodal or enters into the coexistence region, is still a matter of debate. Here we have employed the USAXS technique to study the kinetics of LLPS and arrested state in protein systems with a high temporal and spatial resolution. The two protein systems studied were bovine gamma-globulin in the presence of PEG, featuring an upper critical solution temperature (UCST) phase behavior, and BSA with YCl3, featuring a lower critical solution temperature (LCST) phase behavior. For both systems, the time evolution of the characteristic length during phase separation was followed by USAXS over a broad range of time scales. Depending on the quench depths, classical coarsening kinetics as well as fully arrested state were identified. The transition between these two states was linked with a temporarily arrested state with three-step growth kinetics. Further more, the scattering invariant were used to determine the glass line in the arrested state which provides new insights for the interplay between glass formation and LLPS in various protein systems.

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Session Classification: Parallel session 2

Track Classification: P2 Soft matter