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Kinetics of liquid-liquid phase separation in protein solutions exhibiting LCST phase behavior studied by time-resolved USAXS and VSANS[†]

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1 Supporting Information

In the supporting information we provide additional plots of the time-resolved USAXS data and complementary results from optical microscopy.

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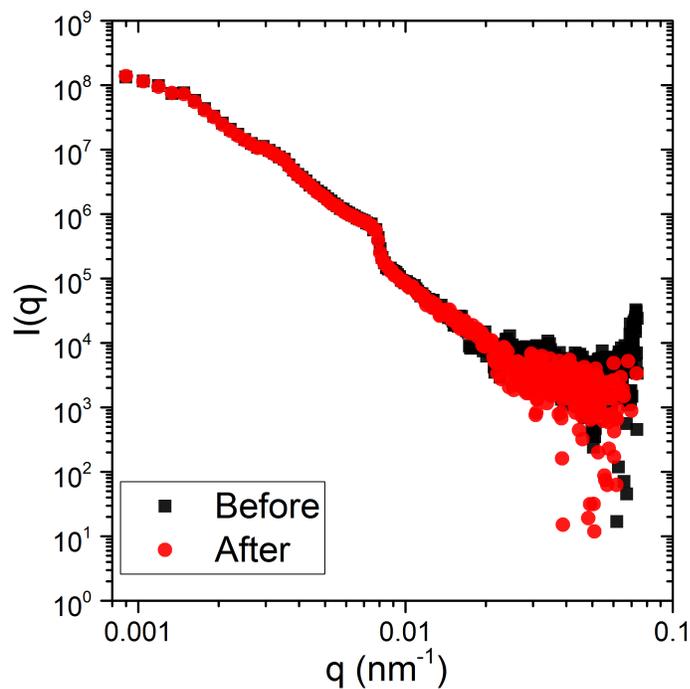


Fig. S1 Scattering profiles collected at 10°C (single-phase state) before and after exposing the same position of a capillary containing a dense phase from a 175 mg/mL BSA and 44 mM YCl_3 parent solution during the temperature jump to $T_{\text{jump}} = 52.5^\circ\text{C}$. No background subtraction was performed. Every third point is shown for clarity. The scattering profiles are identical to the background and overlap.

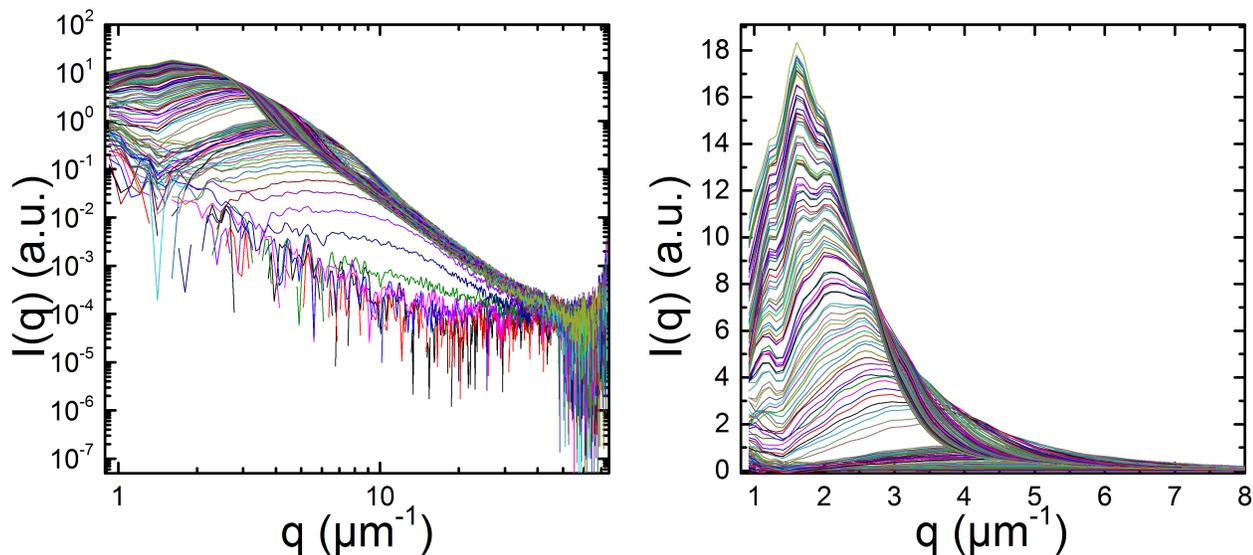


Fig. S2 USAXS profiles for a dense phase obtained from 175 mg/mL BSA and 42 mM YCl_3 after a temperature jump to $T_{\text{jump}}=35^\circ\text{C}$, in log and linear scale. The plots include scattering profiles until 386 s after the temperature jump. Note that the effect of speckles becomes important for the scattering profiles at the later stage.

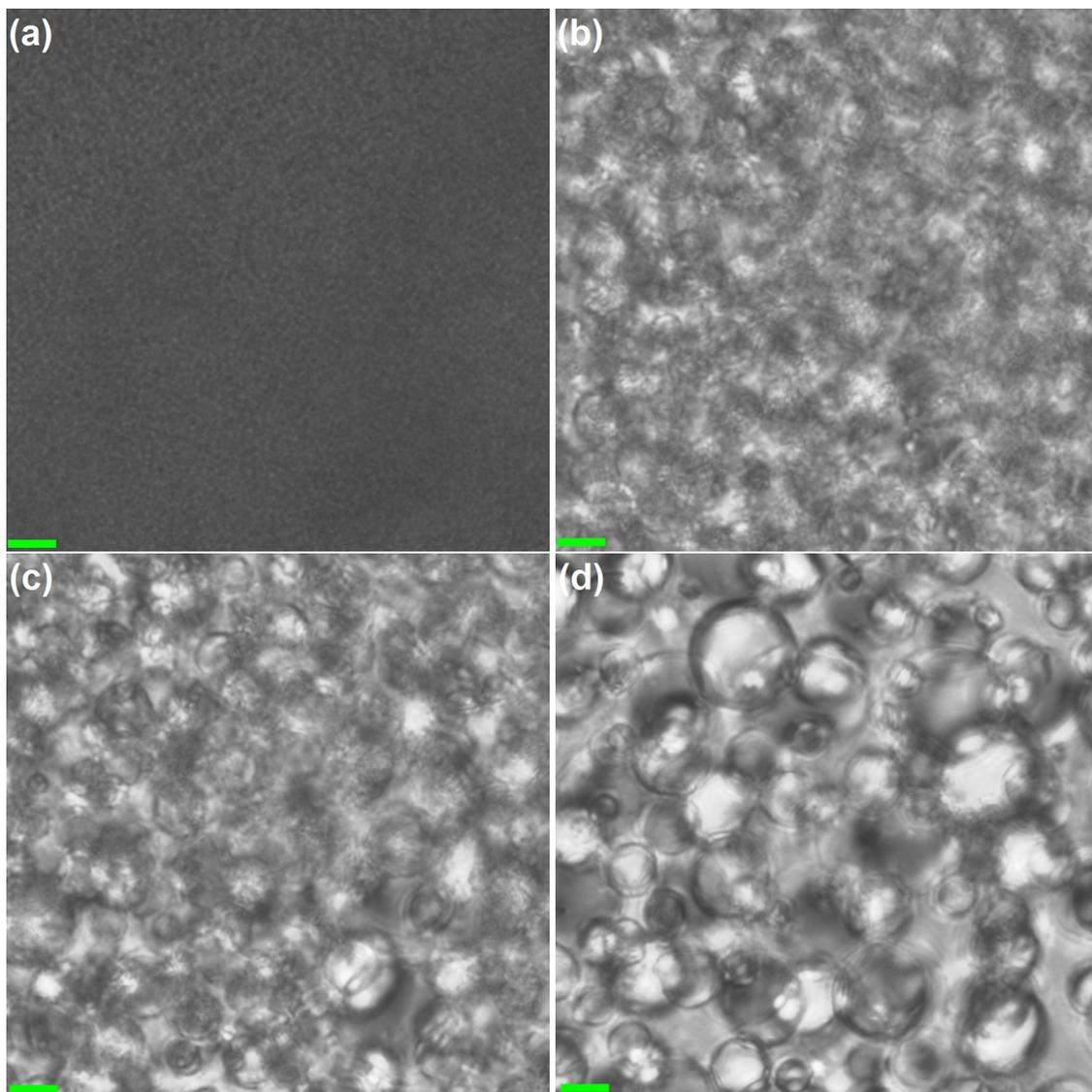


Fig. S3 Micrographs of an LLPS of a dense liquid phase after a temperature jump to $T_{jump} = 35\text{ }^{\circ}\text{C}$. (a) 3 min, (b) 150 min, (c) 300 min and (d) 1020 min after the temperature jump. A bicontinuous network is visible in the early stage. In the later stages droplets of the minority dilute phase are visible. The starting dense liquid phase was obtained by phase separation of a solution of BSA 175 mg/mL and 36 mM YCl_3 at $22\text{ }^{\circ}\text{C}$. $4\text{ }\mu\text{L}$ of the dense phase were sandwiched between two coverslips sealed with silicon paste. The scale bar is $10\text{ }\mu\text{m}$. A Zeiss Axio Scope A1 microscope was used to obtain micrographs of the phase separating dense phases, employing an objective with 50x magnification. Images were captured with an on-line CCD camera. A Linkam silver block heating stage was used to control the temperature, with a nominal heating rate of 150 K/min . Phase separation was followed for 17 h and images were taken every three minutes.

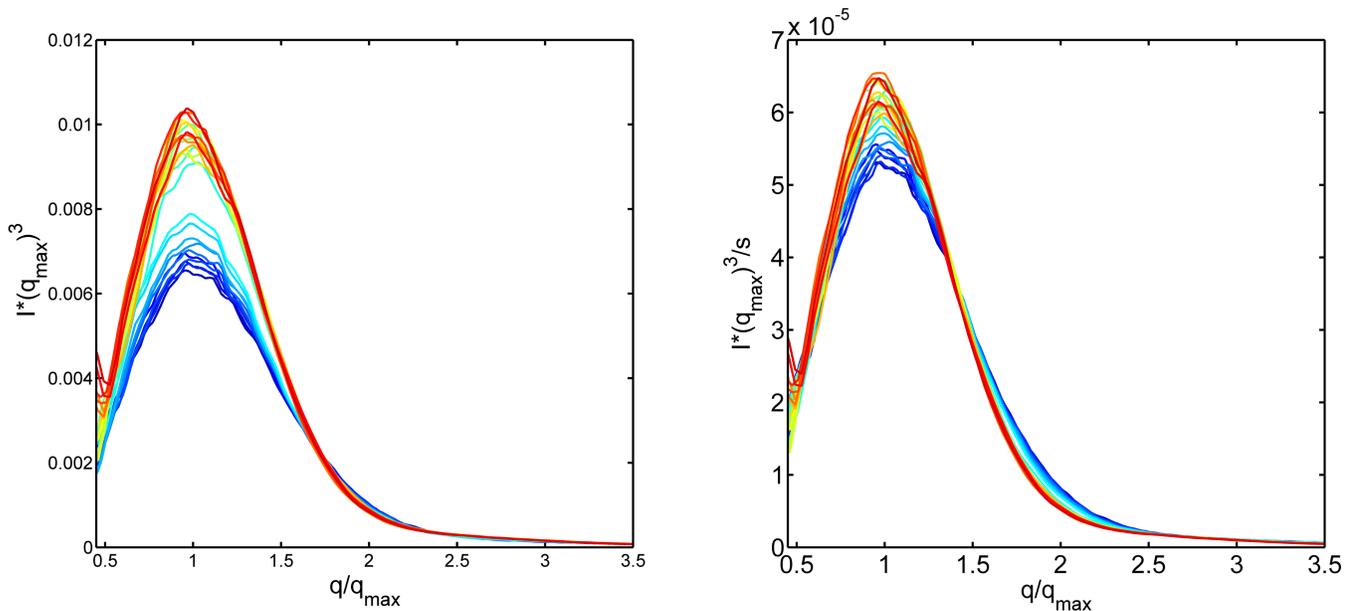


Fig. S4 Dynamical scaling using $I^*q_{\max}^3$ with (Right) or without (Left) the normalization by the Porod invariant-like integral $s = \int q^2 I(q) dq$. This method is valid for critical quenches. For our off-critical temperature jumps, the scattering profiles do not overlap, which was expected.

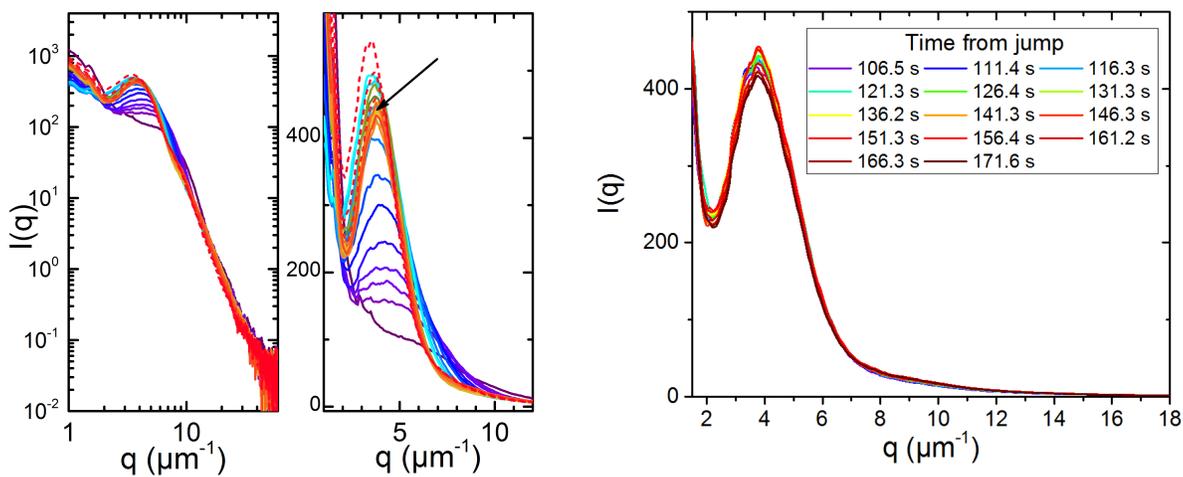


Fig. S5 USAXS profiles for a dense phase sample obtained from a parent solution with 175 mg/mL BSA and 44 mM YCl_3 . Temperature jump to $T_{\text{jump}} = 52.5^\circ\text{C}$, resulting into kinetic arrest. (Left) overview with representative curves for the whole duration of the experiment: the arrow shows where the peaks overlap as the domain size is constant in time. The dashed curves refer to the ageing process after the arrest. (Right) a detailed view of the arrested stage.

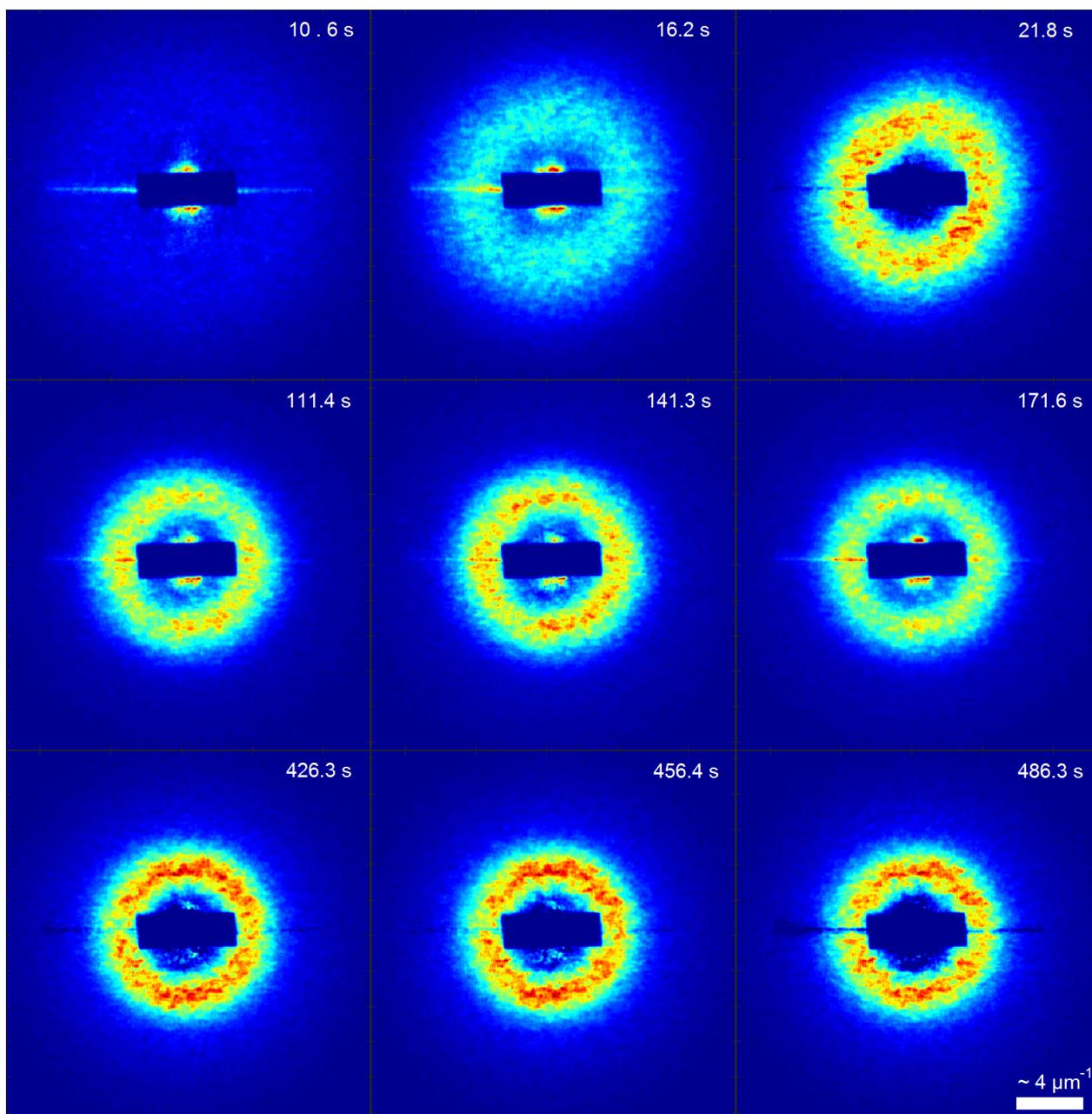


Fig. S6 2D USAXS patterns for a dense phase from a 175 mg/mL BSA and 44 mM YCl_3 parent solution after a temperature jump to $T_{\text{jump}} = 52.5^\circ\text{C}$. Intensity in linear scale. The upper row shows representative scattering patterns at the initial growth stage, the middle row shows scattering patterns during the kinetic arrest, the bottom row shows scattering patterns towards the end of the observation time, when some slow ageing takes place.