Experimental Report

Proposal: **9-13-565** **Council:** 4/2014

Title: Structure of arrested protein gel in the presence of a trivalent salt

This proposal is a new proposal

Researh Area: Soft condensed matter

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Samples: Bovine serum albumin (BSA),
Human serum albumin

Instrument	Req. Days	All. Days	From	To
D11	2	1	06/10/2014	07/10/2014

Abstract:

We propose to investigate the structure of arrested protein gel which is formed via an arrested spinodal decomposition in protein-salt solutions upon liquid-liquid phase separation (LLPS). We use our model systems of bovine and human serum albumin (BSA/HSA) with YCl3 which show LLPS and a lower critical solution temperature (LCST) phase behavior. By a temperature jump, the protein-salt solutions with a high volume fraction (larger than 10%) undergoes an arrested spinodal decomposition. Using SANS we aim to cover the relevant length scale from the local structure to the bicontinuous network. In particularly, we are interested in characterizing the interface between the dilute phase and the glassy-like dense phases as well as the local structure of the glassy-like dense phases.

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Structure of arrested protein gel in the presence of trivalent salts

Scientific background

A better understanding of protein liquid-liquid phase separation (LLPS) and gelation is desirable, both at a fundamental research level, in order to compare the behaviour of protein solutions to those of colloids, and at the applied level given the relevance of LLPS to condensation related diseases [1] and of gelation to technological processing and manufacturing in the food and pharmaceutical industry [2,3]. The aim of our experiments was to characterize by SANS the development in time of a so called “dense phase” of a model protein system as a function of solution parameters and of temperature. This dense phase is produced by inducing LLPS in protein solutions at appropriate temperature upon addition of a trivalent salt, and separating the denser liquid phase by centrifugation. The system consists in high concentration bovine serum albumin (BSA) solutions in the presence of YCl_3, where the LLPS is induced by the association of trivalent Yttrium ions to surface sites of the protein [4,5]. The dense liquid phase obtained can then undergo further phase separation, when heated above a critical temperature: in this condition the clear liquid phase becomes turbid by spinodal decomposition. Since the phase boundaries of the (metastable) LLPS region of the phase diagram are intersected by the gelation line, this further phase separation of the dense phase is arrested and a gel forms.

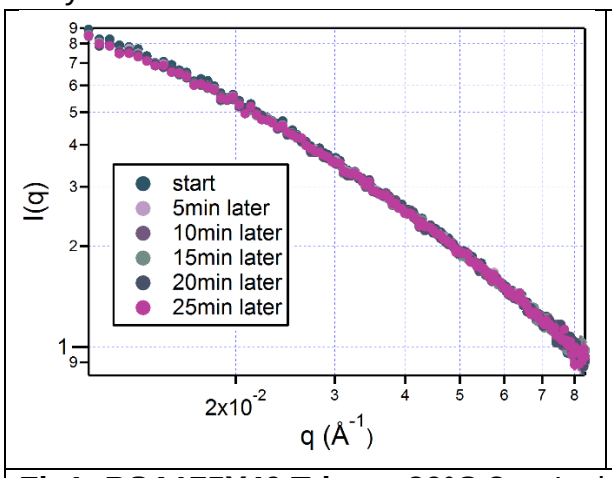
Experimental and Results

The samples were prepared by mixing 175 mg/mL aqueous BSA solutions with respectively 36, 40, 44 and 46 mM YCl_3. The samples phase separate into a protein-rich and a protein-poor phase. The both phases undergo further phase separation when heated. This further phase separation of the protein-rich phase was investigated by SANS. In order to study the time evolution of the samples for different heating rates, two different sets of experiments were performed. First, the dense phases equilibrated at 13°C were heated gradually to 30° and 35°C (temperature ramp), while recording the scattering intensity in 5 min windows. In the second set the same samples, cooled on melting ice, were placed in the sample holder pre-heated at 25°, 30° or 35°C just before starting the experiment and recording the scattering intensity every 5 min (temperature jump). Experiments were performed at D11 with a neutron wavelength of 6Å using 1 mm cuvettes and with sample-detector distances of 8 and 20 m.

Fig1 and Fig2 show the same sample (40mM YCl_3) measured at two different sample-to-detector distances. The experimental procedure that was applied was the T-jump. The two sample-to-detector distances correspond to q-ranges of $1.10\cdot10^{-2}$ - $8.34\cdot10^{-2}$ Å (8m) and $3.05\cdot10^{-3}$ - $3.19\cdot10^{-2}$ Å (20m). In the range from $1.10\cdot10^{-2}$ - $3.19\cdot10^{-2}$ Å the two data sets overlap. As one can see in Fig1 there is no change in intensity at high q-values when a T-jump is performed. Yet at very low q in Fig2, there is an upturn in intensity at the very edge of the investigated low q-range. The height of this upturn diminishes with time. This could be due to a peak outside of the investigated window, at even lower q. The low q intensity decreases when the

sample is heated. Therefore it could be that the hypothetical peak moves more and more to the left. This would mean that the structure which leads to the formation of the peak becomes bigger when the temperature is increased.

In addition to these experiments two T-ramp measurements to 30 and 35 °C were performed with the sample of 175mg/ml BSA with 40mM YCl_3. After the 30 °C ramp the intensity change upon cooling of this sample was monitored. Further T-jump measurements at 8m to 30 and 35 °C were performed using samples with 36, 40, 44 and 46mM YCl_3. For the 36 and 40mM samples a T-jump measurement to 25 °C was done as well. The collected data helps to elucidate the structural changes that occur when the protein-rich phase further phase separates. Further quantitative analysis will be carried out soon.



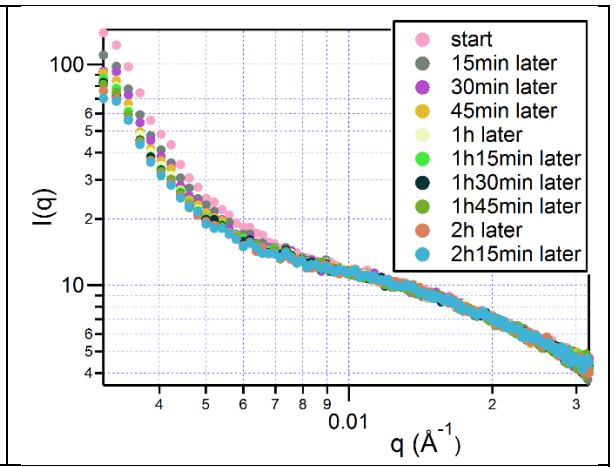


Fig1: BSA175Y40 T-jump 30°C 8m: In this q-range no change in intensity.
Fig2: BSA175Y40 T-jump 30°C 20m: In lower q region, an upturn in intensity is visible which decreases with increasing temperature.

References

[1] Gunton, J. D., A. Shiryayev, and D. L. Pagan. Protein condensation: kinetic pathways to crystallization and disease. Cambridge university press, 2007.

[2] Gibaud, T., et al. "New routes to food gels and glasses." Faraday discussions 158.1 (2012): 267-284.

[3] Vashist, A., et al. "Recent advances in hydrogel based drug delivery systems for the human body." Journal of Materials Chemistry B 2.2 (2014): 147-166.

[4] Roosen-Runge, F., et al. "Interplay of pH and binding of multivalent metal ions: Charge inversion and reentrant condensation in protein solutions." The Journal of Physical Chemistry B 117.18 (2013): 5777-5787.

[5] Zhang, F., et al. "Charge-controlled metastable liquid–liquid phase separation in protein solutions as a universal pathway towards crystallization." Soft Matter 8 (2012): 1313-1316.