Experimental Report

Proposal: **9-13-507** **Council:** 10/2012

Title: Temperature driven structural changes of a protein-rich phase after liquid-liquid phase separation

This proposal is a new proposal

Researh Area: Soft condensed matter

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Samples: HSA (Human Serum Albumin)
BSA (Bovine Serum Albumin)
BLG (beta-lactoglobulin)
YCl3 (yttriumchloride)

Instrument	Req. Days	All. Days	From	To
D11	3	3	22/03/2013	25/03/2013

Abstract:

Effective interactions of proteins in aqueous solution play a crucial role in understanding protein crystallization and many physiological diseases related to protein association [1]. Experimental results on the phase behavior of protein solutions show a variety of phenomena including metastable liquid-liquid phase separation (LLPS) [2], arrested spinodal decomposition [3], transient clusters [4, 5] and reentrant condensation in the presence of multivalent counterions [6-8]. The metastable LLPS is related to a short-ranged isotropic attraction or attractive patches; the physical mechanism behind the attraction, however, still remains unclear. Depending on the attractive mechanism and volume fraction, the protein-rich phase can be in a liquid or an arrested state, leading to gelation [9].

Temperature driven structural changes of a protein-rich phase after liquid-liquid phase separation

Scientific background

Effective interactions of proteins and liquid-liquid phase separation (LLPS) in aqueous solution play a crucial role in protein crystallization and many physiological diseases [1]. The metastable LLPS is related to a short-ranged isotropic attraction or attractive patches; the physical mechanism behind the attraction, however, still remains unclear. Depending on the attractive mechanism and volume fraction, the protein-rich phase can be in a liquid or an arrested state, leading to gelation [2]. We have recently established a reentrant phase behavior for negatively charged globular proteins in presence of multivalent salts [3, 4]. At fixed protein concentration, the solution is phase separated between two critical salt concentrations c* and c**, with either amorphous aggregates or LLPS [5]. Outside this region, the protein charges give rise to electrostatic repulsion which prevents protein association. LLPS occurs in a closed area of phase coexistence in the phase diagram. The position of the coexistence curve can intersect with the arrest or gel line [6]. For β-Lactoglobulin (BLG), a higher critical solution temperature is found for LLPS. In contrast, for Human and Bovine Serum Albumin (HSA and BSA), a lower critical solution temperature is observed, suggesting the relevance of solvent-entropical effects.

Experimental and Results

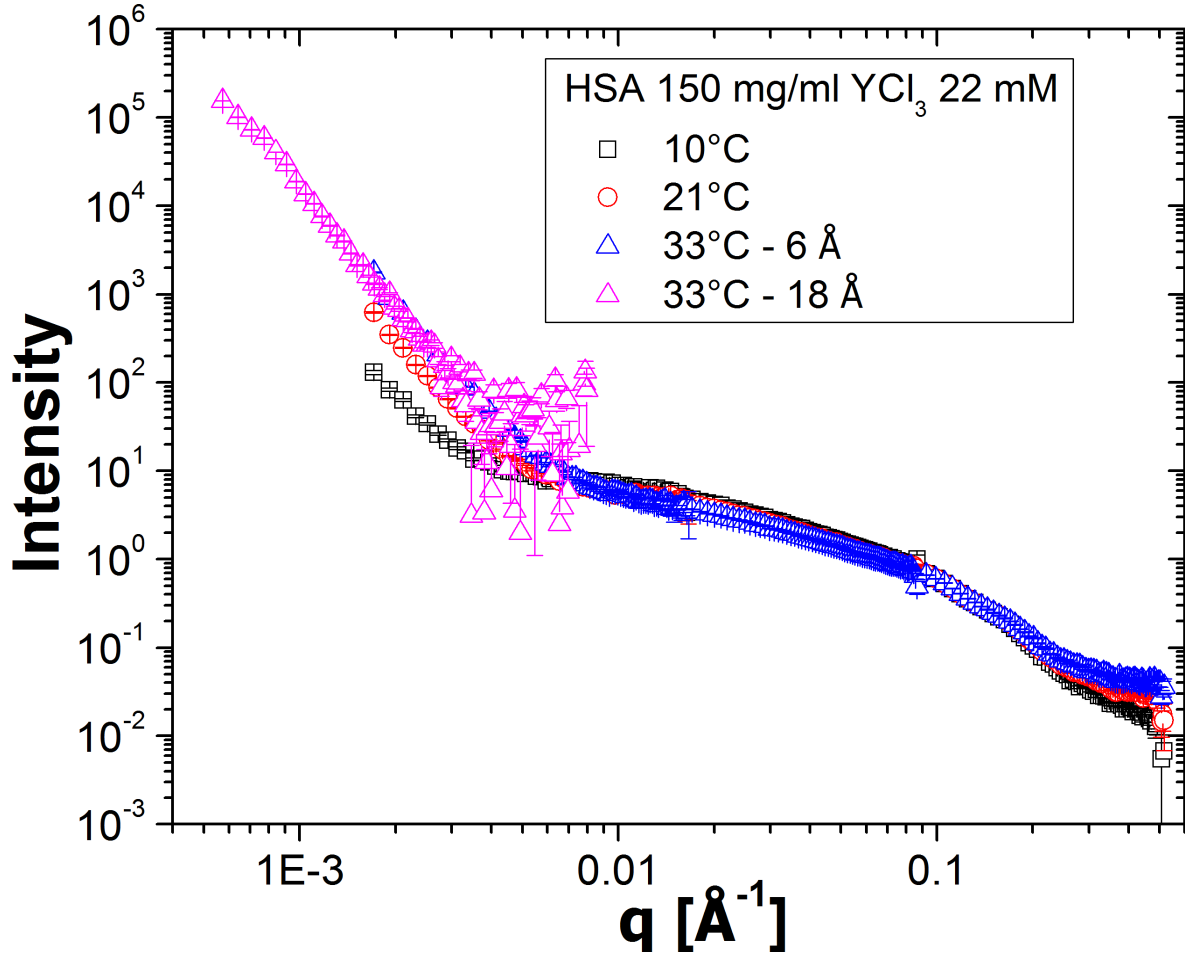

Figure: SANS measurements of HSA solutions in presence of YCl_3 and at varying temperature.

During the beamtime at D11 from 22nd to 25th March 2013, we have studied protein interactions in solutions of the proteins HSA, BSA and BLG at different Yttrium(III) chloride

(YCl_3) concentrations and temperatures. The purpose of our experiments was a better understanding of interactions, clustering and gelation in protein-rich solutions after a LLPS over a wide q-range.
SANS measurements were carried out using wavelengths of 6, 13 and 18 Å, at three sample-to-detector distances (1.2 m, 8 m and 39 m) covering a q-range of 5.73×10^{-4} - 0.52 $Å^{-1}$.
HSA and BSA samples were prepared at room temperature (25°C) in H_2O, since in a D_2O solution, no LLPS region is found. Afterwards, they were centrifuged to separate the protein-rich phase from the mixture.
BLG samples were prepared in D_2O, since for this protein no indication has been observed that the phase behavior is different in H_2O and D_2O. The sample preparation as well as the first measurements were done at 35°C (well above the critical temperature for LLPS). Afterwards, the samples were cooled down to 10°C (below the critical temperature). SANS measurements were done immediately and after a day of storage below the critical temperature, when the phase transition was advanced and the formerly clear sample turned turbid due to the formation of bigger structures.
The obtained data were radially averaged and normalized to the direct beam. The scattering of water (H_2O, D_2O) and the empty cell was subtracted as a background.

In the figure shown above, measurements of the dense liquid phase after LLPS in 150 mg/ml HSA with 22 mM YCl_3 are plotted. The curves change in appearance with temperature, which is completely reversible under multiple heating-/cooling circle. The increase at low q while heating indicates the growth of larger clusters or droplets. Indeed, when the dense phase looks slightly turbid at low temperature, it becomes even more turbid with increasing temperature. The intensity in the Porod regime increases with temperature, reflecting an increase of the surface to volume ratio of the dense state.

The gel-like behavior of the HSA sample can be seen by comparison with a BSA sample, which is completely clear and the scattering curve at low q is completely flat, at low temperature. This observation suggests that the dense liquid phase after LLPS undergoes a second phase separation upon heating. At middle and low q, the differences between the measurements at different temperatures are small.

References

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