ESRF	**Experiment Title:** Protein interactions in solution with polyethylene glycol: liquid-liquid phase separation and crystallization	**Experiment number**: SC-3643
Beamline: ID2	**Date of experiment**: from: 25th April 2013 to: 29th April 2013	**Date of report**: 7th Aug. 2013
Shifts: 12	**Local contact(s):** Gudrun Lotze	*Received at ESRF:*

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Report:

Liquid-liquid phase separation (LLPS) has seen dramatic interest with the discovery of several pathological conditions, for instance sickle-cell anemia [1], Alzheimer's disease [2], cryoglobulinemia [3] and neurodegenerative disorders [3,4]. In these diseases, enhanced nucleation from within or on the interface of metastable dense protein droplets facilitate pathological protein aggregation or crystallization. Therefore we have investigated protein solutions that exhibit a metastable LLPS in the presence of polyethylene glycol (PEG). PEG provides control over the protein interaction potential in terms of interaction strength and range. In comparison, we have observed LLPS in protein solutions in the presence of multivalent metal ions [5-6]. The systems present a reentrant condensation phase behavior [7-10] and LLPS occurs within the condensed regime. It has been further found that such phase behavior is crucial for protein crystallization. Near the boundaries one has better opportunity to obtain high quality single crystals. On the other hand, the crystal growth follows different mechanism at different phase boundaries. The purpose of these measurements was to characterize the interaction potential by SAXS measurements with varying temperature, protein concentration and PEG concentration as well as the molecular weight, in order to relate the interaction potential to the macroscopic phase behavior that we have already observed.

From April 24th to 29th, we measured the protein interactions of immunoglobulin G (IgG) and glucose isomerase (GI) with polymer polyethylene glycol (PEG) at ID02. The sample-to-detector configurations were 5m for IgG and 2m for GI covering a q-range from 0.02-1.7nm^{-1}. In both cases, the applied energy was 16047 eV. We investigated the protein interactions and varied the molecular weights of PEG as well as the concentration of PEG, GI and IgG. Thus, the corresponding interaction potential was tunable in strength and range. Hereto, we prepared the samples at room temperature (~26 °C) containing the following components: HEPES (20mM), sodium chloride (NaCl, 150mM), PEG with the molecular weights 3350 or 8k g/mol and used either immunoglobulin G or glucose isomerase. For GI we performed the same procedure, i.e. PEG from 2-10 % (w/v) in 2 % steps, NaCl 150mM and GI 25 mg/ml as well as 40 mg/ml.

The left side of figure 1 shows our new SAXS measurement curves of IgG with a concentration of 25 mg/ml and PEG 8k g/mol from 1 % to 5 % (w/v) at room temperature. The right side of figure 1 depicts an older measurement, i.e. from a previous beamtime at ID02, ESRF, with IgG 25 mg/ml and 4 % (w/v) PEG 6k g/mol. As opposed to the current experiments, a temperature scan from 36 °C to 10 °C has been performed (at the right side). The observed LLPS transition occurred at 13.6 °C. For 5 % (w/v) PEG 8k g/mol, a significant increase at low q was observed, indicating a transition to LLPS which is in accordance with the phase diagram determined in our lab. Both curves were plotted in a linear-log scale. Visible is an increase at high q values in the range from 0.1 to 1 nm^{-1} and for low q ($q < 0.1$ nm^{-1}) there is a steep slope that contains information about the nature and strength of the interaction. In this case, the interaction is an attractive one. Comparing our measurements with our older observation, i.e. right-hand side of figure 1, leads to the conclusion that both varying temperature and PEG concentrations give the similar effect in terms of varying the interaction. In particular, the location of the LLPS boundary provides insight into the nature and magnitude of the protein-protein interactions [11]. Quantitative data analysis using suitable models for the structure factor will be carried out for both cases.

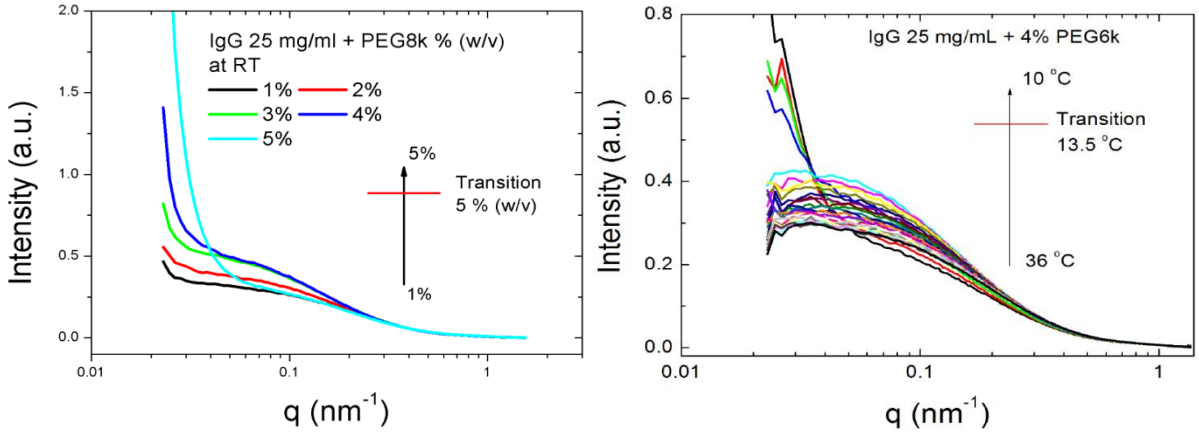

Figure 1: Varying temperature (left) has the same effect as varying the PEG concentration (right).

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