ESRF	**Experiment title:** SAXS study of the p-T phase diagram and underlying intermolecular interaction potential of dense lysozyme solutions in the presence of various Hofmeister anions	**Experiment number**: SC 3945
Beamline: ID02	**Date of experiment**: from: 12/11/14 to: 14/11/14	**Date of report**: 27/02/15
Shifts: 6	**Local contact(s)**: Dr. Johannes Möller	*Received at ESRF:*

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Report:

The purpose of the performed experiment was to investigate the intermolecular interactions of dense lysozyme solutions under high hydrostatic pressure conditions at selected temperatures and salt concentrations. Recently, the interaction potential of proteins in solution was found to depend on pressure in a nonlinear way, which was ascribed to changes in the water structure at elevated pressures [1,2]. Moreover, a reentrant pressure dependent liquid-liquid phase separation (LLPS) region in the phase diagram has been reported for 18.5 - 21.5 wt.% protein solutions in 500 mM NaCl [3]. Beyond this small concentration range, the phase behavior was still unknown. A phase diagram over a wider range of concentration would help control and optimize protein crystallization under high pressure conditions, and knowledge of intermolecular interactions and the influence of salt ions in this phase region is mandatory to this end.

This high pressure SAXS experiment was performed at ESRF beamline ID02 using an incident energy of 16 keV with a custom-built high pressure cell. Measurements were performed at pressures between 1 and 5000 bar and a temperature range from room temperature down to 4 °C. The SAXS signal of concentrated protein solutions can be described as a product of the form factor $P(q)$, in this case modeled by an ellipsoid of

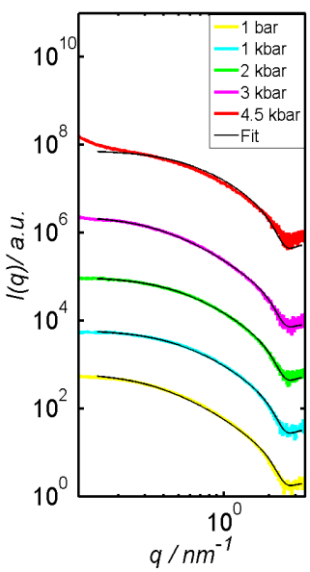

FIG. 1. Measured SAXS intensity $I(q)$ for a 6 wt.% lysozyme concentration in the presence of 500 mM NaCl at 10 °C for selected pressures (in color) and the related calculated $S(q)$ (in black).

revolution with semiaxis $a = 1.52$ nm and radius of gyration $R_G = 1.45$ nm, and the effective intermolecular structure factor $S(q)$ of the particles. The interaction potential $V(r)$ for solutions close to a LLPS phase boundary can be modeled by a sticky hard sphere potential [3]. The baxter model and the Percus-Yevick approximation were used to analyze the data for a 6 wt.% lysozyme solution in the presence of 500 mM NaCl (see FIG. 1). The obvious deviation between the fit and the model for p = 4.5 kbar already indicates that the system has entered the LLPS phase. A closer look at the pressure dependent absolute values of the effective structure factor for a fixed q-value, $S(q = 0.2\,\mathrm{nm}^{-1})$, indicates passage of phase boundaries (see FIG. 2): A salient point (e.g., at 3250 bar for T = 5 °C) suggests a phase transition taking place. Together with the calculated second virial coefficient B_2, that can be interpreted as a global measure of repulsive and attractive interactions, a phase diagram of a 6 wt.% lysozyme solution could be constructed (FIG. 3). Further data analysis is in progress, which will allow us to construct the phase boundaries of dense lysozyme solutions between 6 and 18 wt.% over a wide range of temperatures and pressures.

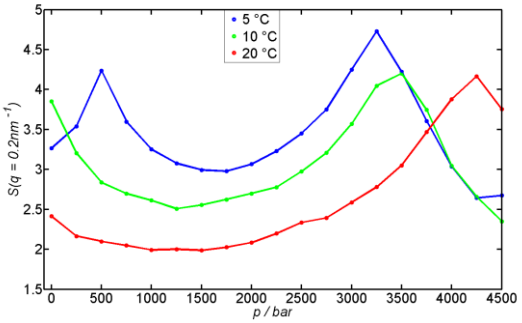

FIG. 2. Pressure dependent absolute value of the effective structure factor $S(q = 0.2\,\mathrm{nm}^{-1})$ for a 6 wt.% lysozyme solution. Salient points indicate passage of phase boundaries.

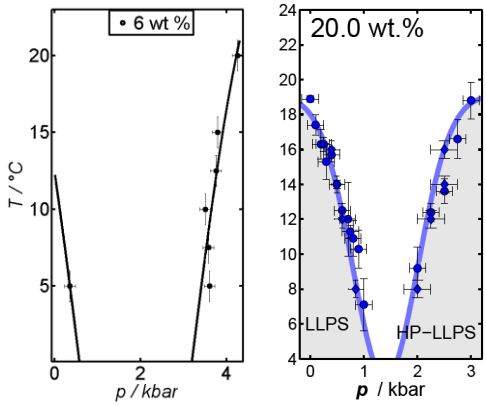

FIG. 3. Comparison between the phase boundaries of a 6 wt.% and 20 wt.% lysozyme solution [3, edited]. The shaded area under the curve indicates that the system has entered the LLPS region.

[1] M. A. Schroer, J. Markgraf, …, R. Winter, 2011, *Phys. Rev. Lett.* **106**:178102
[2] J. Möller, M. A. Schroer, …, R. Winter, 2012, *Biophys. J.* **102**:2641
[3] J. Möller, S. Grobelny, J. Schulze, …, R. Winter, 2014, *Phys. Rev. Lett.* **112**:028101