ESRF	**Experiment title:** Protein crystallization under pressure: The effect of cosmotropic ions	**Experiment number**: SC-3650
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Report:

The purpose of the performed experiment was to investigate the intermolecular interactions of dense protein solutions under the influence of pressure and cosmotropic ions. In previous studies, the interaction potential was found to depend on pressure in a non-linear way, which is the result of marked changes in the water structure at elevated pressures [1,2]. The intermolecular interactions in the multi-kbar regime are mainly governed by the stability of the second hydration shell of water against pressure. However, solution conditions for protein crystallization are mainly of high ionic strength, which allows screening of the protein's net charge and effectively decrease the repulsive protein interactions. Conversely, the intermolecular interactions of proteins as a function of pressure in solutions of high ionic strength are far from being understood, as the ions can have strong impact on the water structural properties themselves [3]. To facilitate and optimize crystallization conditions for proteins under high pressure, knowledge of the influence of different ions on the pressure dependent interaction potential of proteins in solution is mandatory.

The high pressure SAXS experiments were performed at ESRF beamline ID02 using an incident energy of 16 keV. A custom-build high pressure cell was used, which allowed measurements at pressure up to 3 kbar at a sample temperature of 25 °C. The ionic strength of the solution was changed from 0 to 500 mM using sodium phosphate, sulphate, and chloride. The used protein was lysozyme in concentrations of 50 mg/ml. The pressure of the sample was increased in 250 bar steps and at every step a SAXS measurement was taken in

order to obtain the intermolecular interaction potential as a function of pressure, ionic strength and salt identity.

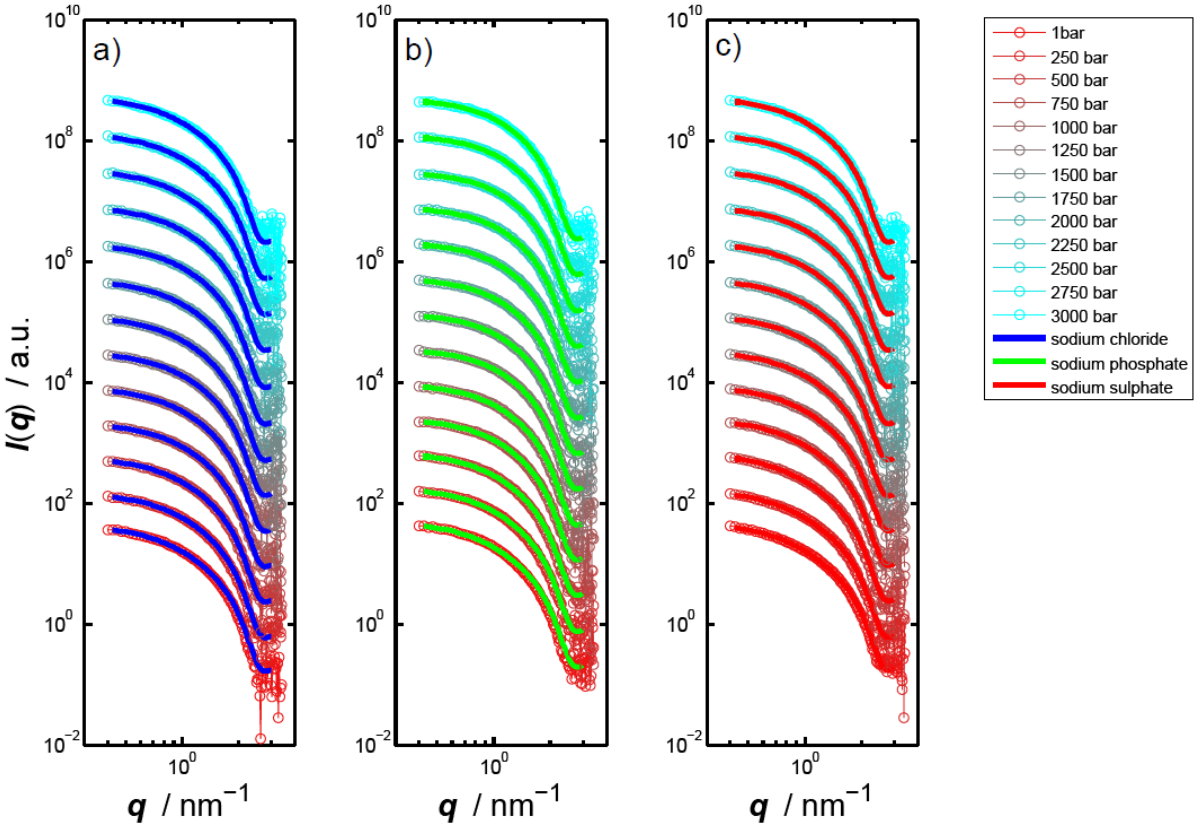

Figure 1: SAXS curves of 50 mg/ml lysozyme solution as a function of pressure. The ionic strength is 250 mM with sodium chloride (blue), phosphate (green), and sulphate (red). The blue, green, and red lines show refinements to the data.

In figure 1, SAXS curves of three different pressure series are shown. The pressure range is from 1 bar to 3 kbar in 250 bar steps. The ionic strength at all series was 250 mM. The only difference is the salt identity, a) sodium chloride, b) sodium phosphate, and c) sodium sulphate. The SAXS measurements are refined using a liquid state analytical approach. The form factor of the proteins was modeled as an ellipsoid of revolution, the structure factor could be calculated theoretically in the mean spherical approximation, using a modified DLVO potential to model the interaction of the proteins. For details of the data refinement, see [2].

As the data analysis is still in progress, a full interpretation of these data is not given here. However, it already can be stated, that the interaction potentials of the lysozyme samples could be obtained to pressures of 3 kbar in solutions of high ionic strength. Knowledge about the strength of the interaction potential as a function of pressure, ionic strength, and salt identity can give important information for protein crystallization. Furthermore, differences between the investigated salts on the protein interactions can give insights in how specific ion effects on macromolecules in solution occur.

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