ESRF	**Experiment title:** **Protein – Protein interactions in crowded lysozyme solutions**	**Experiment number**: SC-4176
Beamline: ID02	**Date of experiment**: from: 07.12.2015 to: 09.12.2016	**Date of report**: 05.03.2016
Shifts: 6	**Local contact(s)**: Johannes Möller	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

K. Julius*[,a)], N. König*[,a)], M. Berghaus*[,b)], M. Paulus[a)], M. Tolan[a)], R. Winter[b)]

a) **Fakultät Physik/DELTA, TU Dortmund, Otto-Hahn-Str. 4, 44227 Dortmund**

b) **Fakultät für Chemie und Chemische Biologie, TU Dortmund, Otto-Hahn-Str. 4a, 44227 Dortmund**

Report:

The aim of the experiment was the investigation of the intermolecular interactions of crowded lysozyme solutions under the influence of pressure and crowder concentration for macro- and nanomolecular crowders. In previous studies on dense lysozyme solutions, the interaction potential V(r) was found to depend on pressure in a non-linear way. This effect, which is attributed to pressure dependent changes of the solvent structure, is altered by the presence of kosmotropic cosolvents and ions, which shift the minimum of the attractive part of V(r) to higher pressures [1,2]. Furthermore, pressure and temperature dependent measurements have shown that both, the temperature and pressure stability of RNase A increases drastically in the presence of the macromolecular crowder Ficoll PM 70. Additionally a decrease of the folding rate was found that could be explained by changes in the microviscosity due to the presence of the crowding agent [3]. However, the intermolecular interactions of proteins as a function of temperature and pressure in a highly crowded environment are far from being understood. Hence, it is mandatory to investigate the influence of crowder molecules of various sizes and chemical properties on protein-protein interactions owing to significant changes in water structural properties, osmotic pressure and confinement they impose.

SAXS measurements were performed at ESRF beamline ID02 with a custom build high pressure cell [4] at pressures up to 4 kbar at a sample temperature of 23 °C. An incident energy of 16 keV was used. In order to obtain the intermolecular interaction potential as a function of pressure, crowder concentration and crowder identity, pressure series between

bar and 4 kbar were recorded in 500 bar steps for solutions containing 10 - 20 wt % lysozyme at different Ficoll PM 70 and sucrose concentrations.

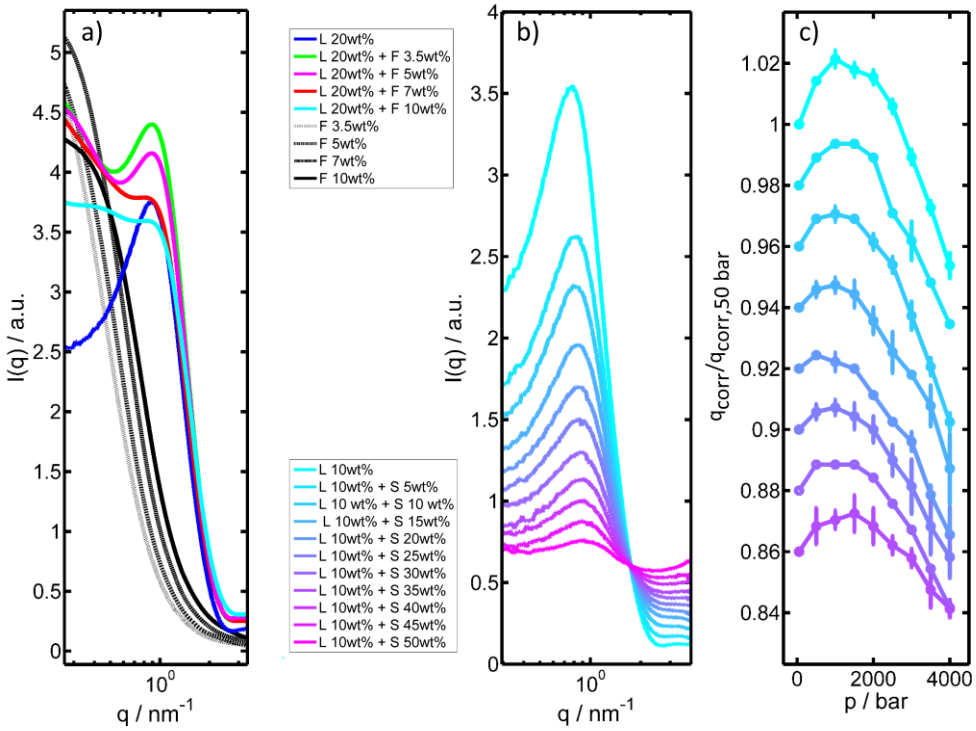

FIGURE 1 (a) SAXS intensities of a 20 wt-% lysozyme solution at 23°C and ambient pressure for Ficoll PM 70 concentrations of 3.5, 5, 7 and 10 wt-% and a pure aqueous Ficoll PM 70 solution. (b) SAXS intensities of a 10 wt-% lysozyme solution at 23°C and ambient pressure for sucrose concentrations of 5 – 50 wt-%. The SAXS curves were shifted for clarity. (c) Position of the lysozyme correlation peak q_{corr} relative to the position at 50 bar as a function of pressure for sucrose concentrations of 5 – 35 wt-%. The curves were shifted for clarity.

A full interpretation of the data is not given here, because the analysis is still in progress. What is already clear, however, is that the compatible osmolyte sucrose leads to marked changes in the pressure dependence of the correlation peak of lysozyme, i.e. to marked changes in the intermolecular interaction potential. This indicates that changes of osmotic pressure and the reduction of the space available to the protein by the nanomolecular crowder, in concert with changes in the water structural properties upon compression, affect the pressure dependence of the intermolecular forces (Figs 1 b-c) significantly. After full analysis of the data modelling of V(r) with liquid-state theoretical approaches, the results are ready to get published.

Due to the increase of background scattering in case of the macromolecular crowding agent Ficoll, the correlation peak cannot be analyzed with sufficient accuracy beyond crowder concentrations of about 10 wt-% at lysozyme concentrations of 20 wt-% (Fig 1 a). Up to that crowder concentration, a marked shift of the correlation peak relative to the scenario in pure buffer solution is not observed, which can be explained by the strong repulsive protein-protein interactions of the highly positive charged lysozyme molecules (z=8 at pH 7) in this protein solution, which can still be described by a relative open network structure at this macromolecular crowder concentration.

[1] M. A. Schroer, J. Markgraf, … , and R. Winter, *Phys. Rev. Lett.* 106 (2011) 178102
[2] J. Möller, S. Grobelny, …, and R. Winter, *Phys. Rev. Lett.* 112 (2014) 028101
[3] M. Erlkamp, S. Grobelny, … , and R. Winter, *Phys. Chem. Chem. Phys.* 16 (2014) 5965
[4] C. Krywka, C. Sternemann, … , and R. Winter, *Chem. Phys. Chem.* 9 (2008) 2809