ESRF	**Experiment title:** Probing the phase diagram of highly concentrated protein solutions by high pressure SAXS	**Experiment number**: SC- 2796
Beamline: ID02	**Date of experiment**: from: 30.10.2009 to: 01.11.2009	**Date of report**: 12.02.2010
Shifts: 6	**Local contact(s)**: Shirley Callow, Michael Sztucki	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

M. Schroer*[, a)], F. Wieland*[, a)], Ch. Sahle*[, a)], M. Paulus[a)], M. Tolan[a)]

R. Winter[b)]

a) Fakultät Physik/ Delta, Technische Universität Dortmund, Otto-Hahn-Str. 4, 44227 Dortmund, Germany

b) Physikalische Chemie I, Technische Universität Dortmund, Otto-Hahn-Str. 6, 44227 Dortmund, Germany

Report:

The purpose of the experment SC-2796 was to study in detail the influence of hydrostatic pressure on the structure and hence the phase diagram of concentrated protein solutions which has rarely been studied up to now. [1] Our findings yield new information about protein-protein interactions under these extreme conditions of the concentration-pressure phase space and the thermodynamics of concentrated protein solutions that govern protein crystallization processes, protein aggregation and phase separation phenomena. Furthermore, these highly concentrated solutions might mimic the crowded environment of natural cellular environments

SAXS measurements at different concentrations (5, 105, 220, 300 mg/ml) of the protein lysozyme were performed at beamline ID02 of the ESRF using an incident energy of 12.460 keV. In order to achieve pressures up to 4 kbar, a custom-built high pressure cell was employed. [2] Adjusting the temperature to selected values (25°C, 15°C, 6°C) allowed to investigate different points in the phase diagram of the protein solutions.

Figure 1 (left part) depicts the x-ray signal of a concentrated lysozyme solution (104.5 mg/ml) at $T = 25°C$ and different pressures together with the corresponding fitting curves. The data can be well described within the decoupling approximation [3] employing the form factor of a prolate ellipsoid of revolution and an intermolecular structure factor in the random phase approximation. [4,5]
Increasing the pressure results in pronounced changes of the scattered intensity. However, the form factor of the protein did not change, reflecting that no structural transition of the protein molecule took place which has been confirmed by complementary high pressure FTIR spectroscopic experiments. The differences between the detected signals are due to different structure factors and thus due to a variation of the interparticle interaction. The structure factors are given in Fig. 1 as well (on the right).

Looking in detail on the protein-protein interaction potential (Fig. 2, left), a surprisingly complex pressure dependency at $T = 25°C$ can be revealed. With increasing pressure the overall interaction becomes more repulsive up to 1.5 kbar. A further pressure increase leads to a larger effective attraction, however. Reducing the temperature to $T = 6°C$ changes the pressure dependency of the protein-protein interaction drastically (Fig. 2, right).

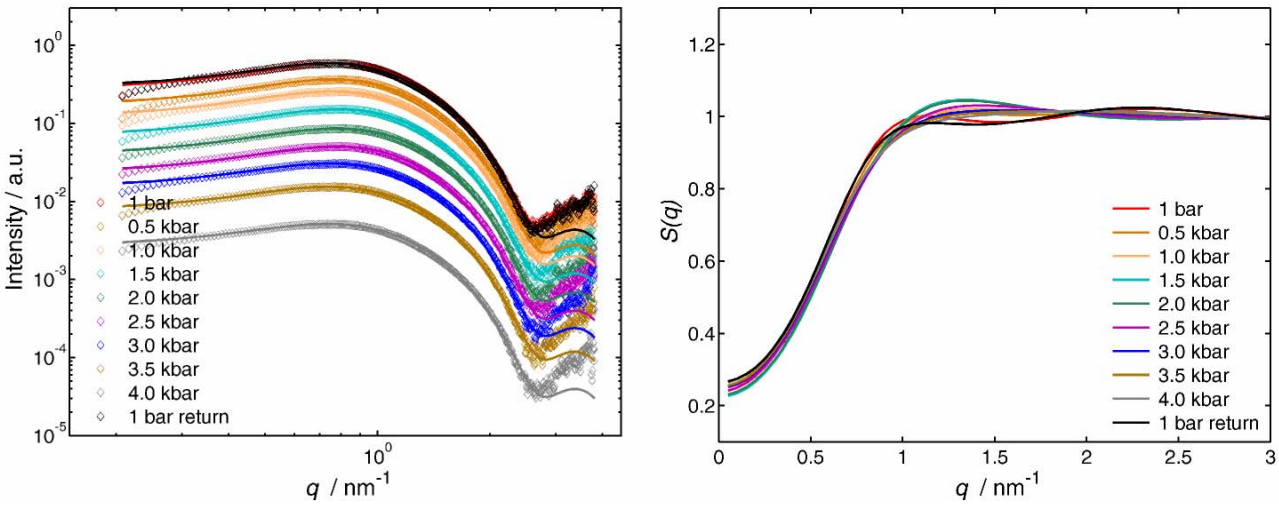

Fig. 1 Left: Scattering curves for a concentrated lysozyme solution (104.5 mg/ml) for different pressures up to 4 kbar at 25°C. Solid lines represent fitting curves. Right: The corresponding structure factor S(q) obtained.

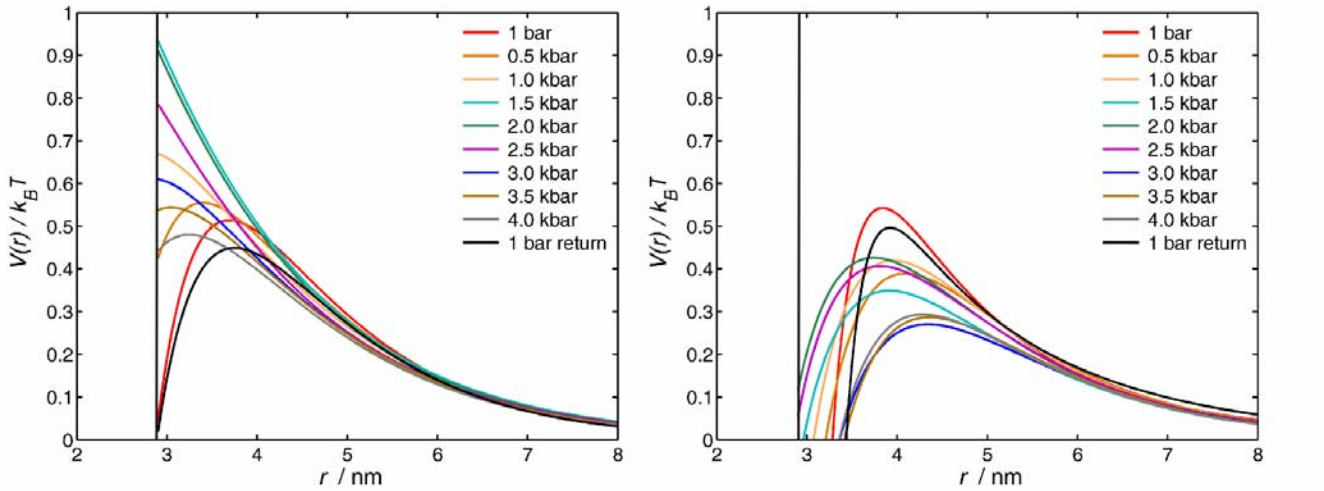

Fig 2 Left: Effective protein-protein interaction potential for a concentrated lysozyme solution (104.5 mg/ml) as a function of pressure at T = 25°C. Right: Corresponding data for T =6°C.

As the data analysis is still in progess, a full interpretation of these new data is not yet given here. What is already clear, however, is the fact that in highly concentrated protein solutions, already minor changes in pressure can reverse the intermolecular forces. Such a finding will, for example, be important for understanding the physiology of deep sea organisms, which have to adapt to pressure s in the kbar range. These findings will also be important in studies using pressure to overcome the barrier leading to liquid-liquid phase separation and controlled crystallization of concentrated protein solutions.

References:

[1] M.G. Ortore, F. Spinozzi, P. Mariani, A. Paciaroni, L.S. Barbosa, H. Amentisch, M. Steinhart, J. Ollivier, D. Russo, J. Roy. Soc. Interface **9**, S619 (2009).
[2] C. Krywka. C. Sternemann, M. Paulus, M. Tolan, C. Royer, R. Winter, ChemPhysChem **9**, 2809 (2008).
[3] M. Kotlarchy, S.-H. Chen, J. Chem. Phys. **79**, 2461 (1983).
[4] J. Narayanan, X.Y. Liu, Biophys. J. **84**, 523 (2003).
[5] N. Javid, K. Vogtt, C. Krywka, M. Tolan, R. Winter, ChemPhysChem **8**, 679, (2007).