# Experimental report

25/01/2024

**Proposal:** 9-13-1080 **Council:** 4/2023

**Title:** The influence of pressure on interaction potential between globular proteins in the presence of multivalent ions

**Research area:** Soft condensed matter

**This proposal is a resubmission of 9-13-1045**


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**Samples:** YCl3
BSA + YCl3

| Instrument | Requested days | Allocated days | From | To |
|---|---|---|---|---|
| IN16B | 6 | 6 | 21/11/2023 | 27/11/2023 |


**Abstract:**

Within the proposed experiment, we want to investigate the effect of pressure on the slowing down of the short-time self-diffusion of a globular protein (bovine serum albumin, BSA) in the presence of multivalent ions (yttrium chloride, YCl3). The amount of bound yttrium at the protein surface can be controlled by varying the concentration of multivalent ions, resulting in a varying protein interaction strength. Here pressure shows a promising tool to control protein-interaction, because the yttrium binding is driven by dehydration. We propose to measure a sample with a fixed protein concentration with and without multivalent ions. This will help to understand the pressure effect on the hydrational properties of the yttrium binding. The variation of the protein self-diffusion under pressure will be connected to general properties of the aggregation process.

# The influence of pressure on interaction between globular proteins in the presence of multivalent ions

## Scientific background

A thorough understanding of protein interactions in aqueous solutions is crucial for many areas of research in soft matter and biology. For example, a strong interprotein attraction can lead to protein condensation, which is observed in several diseases [1] such as cataracts [2] and neurodegenerative diseases [3]. We have shown that a patchy particle model [4] can describe a system of acidic globular proteins such as bovine serum albumin (BSA), in the presence of multivalent salts such as yttrium chloride ($YCl_3$). The resulting phase diagram of the studied system as a function of salt and temperature is quite complex, showing reentrant condensation, metastable liquid-liquid phase separation (LLPS), cluster formation and crystallization [5]. In particular, a lower critical solution temperature (LCST) is observed which suggests an essential role of hydration for the protein-ion interaction [6]. Using temperature has some disadvantages because temperature influences the thermal energy and the density of the system and only a small temperature range is available for proteins. As opposed to temperature, pressure influences only the density and changes in pressure have rather mild effects. Globular proteins can be subjected to pressures of up to several MPa before denaturation. Furthermore, pressure-dependent studies lead to a better understanding of the volumetric and hydration properties [7, 8, 9] and yttrium induced protein bridging. Pressure dependent SAXS measurements performed by R. Winter's group have shown how pressure modifies the interaction between proteins in solution [10, 11]. Since the ion binding is related to hydration [6], pressure provides a promising way to induce clustering in an otherwise unperturbed sample.

## Experimental Results

We studied the influence of pressure on a protein solution with BSA, at 3 different $YCl_3$ concentrations, on the backscattering beamline IN16B at the ILL. The beamtime was allocated from 21$^{st}$ to 27$^{th}$ December 2023. For the experiment the cryofurnace of the beamline was used. For the application of pressure a high pressure generator with a high pressure sample stick [12] and cylindrical high pressure cell [13], all from the ILL, were used. For all measurements the temperature was set to 295 K and a pressure in step of 50 MPa from ambient pressure up to 300 MPa was applied. In order to decrease the incoherent scattering of the sample environment, $D_2O$ was used for the sample preparation. For the experiment a BSA concentration of 200 mg/ml was prepared by using stock solutions. The concentration of the protein stock solutions was measured by UV-Vis absorption. For calibration and background corrections the signal of a standard Vanadium sample, the empty cell and the corresponding $D_2O$ and $YCl_3$ in $D_2O$ background were measured.

A preliminary data analysis of the normalized BSA diffusion coefficient in the 3 different samples under pressure are plotted together with the data from the experiment 9-13-873 in Figure 1. The master curve of the slowing down of the BSA self-diffusion with increasing trivalent salt concentration, mentioned by Grimaldo [14] is plotted as a solid black line. The results for the ambient pressure fits to this master curve, confirming the quality of the experiment. The solid blue line shows the expected behaviour with increasing pressure. Because of the higher sample density at higher pressure the diffusion is hindered within the sample and the self-diffusion should decrease faster compared to ambient pressure. Interestingly the normalized self-diffusion coefficient is increasing for pressure $\geq$ 100 MPa, before it slows down again. An increasing diffusion coefficient can only be explained by a decrease of the averaged BSA oligomer/cluster diameter. This behaviour fits also to unpublished SAXS and NMR data

for BSA with $YCL_3$ in $H_2O$, which shows a shift of the monomer/dimer ration to higher monomer values around 100 MPa. A similar behaviour was observed by S. R. Al-Ayoubi [9]. They found that around 100 MPa tetrameric LDH dissociates to dimers.

Further work on the data fitting and analysis must be done to confirm the preliminary results and to interpret the results correctly.

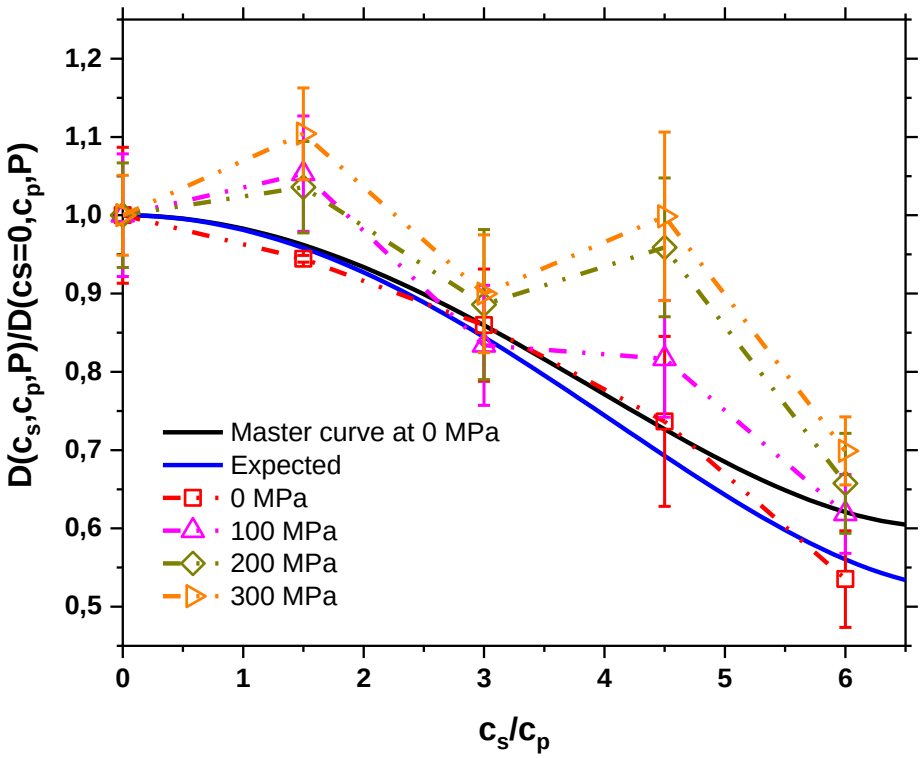


Figure 1: Preliminary fitting result for the diffusion coefficient D of BSA with and without $YCl_3$ at different pressures. As already known the diffusion coefficient goes down with increasing pressure for the sample without salt (blue data points). For the sample with YCl3, orange data points, a jump of the diffusion coefficient to faster diffusion at 1000 bar was observed.

**References:**


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