The interaction of proteins in pressurized salt solutions

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We report on recent measurements of the interaction potential of proteins in salt solution as a function of temperature and pressure. Investigating the physics that govern the interaction and phase behavior of proteins in solution is essential for understanding the wide range of occurring phases. For example, aggregation and phase separation of proteins is the fundamental cause for many diseases like Alzheimer's disease [1], cataract [2], and sickle cell anaemia [3]. Furthermore, the controlled crystallization of proteins is essential for obtaining high-resolution protein structures by X-ray diffraction. In the case of protein crystallization, it is possible to predetermine feasible crystallization conditions from investigating the protein-protein interactions in solution. The aim of the presented measurements was to probe the protein interactions in solutions of high ionic strength as a function of pressure and temperature. Pressure was used as a relatively new and additional tool to modulate protein crystallization conditions.

The small-angle X-ray scattering technique used allows to investigate the structure and interaction of proteins in solution. The measurements were performed at beamline BW4, HASYLAB. X-ray scattering from dense protein solutions contains scattering contributions caused by the interactions of the proteins, which can be expressed in terms of the effective structure factor S_{eff}. For data analysis, this structure factor was calculated using a mean-spherical-approximation in combination with a DLVO (Derjaguin-Landau-Verwey-Overbeck) potential, and was fitted to the data. For further details, see [4]. The only free parameter was the strength of the attractive interaction J. The

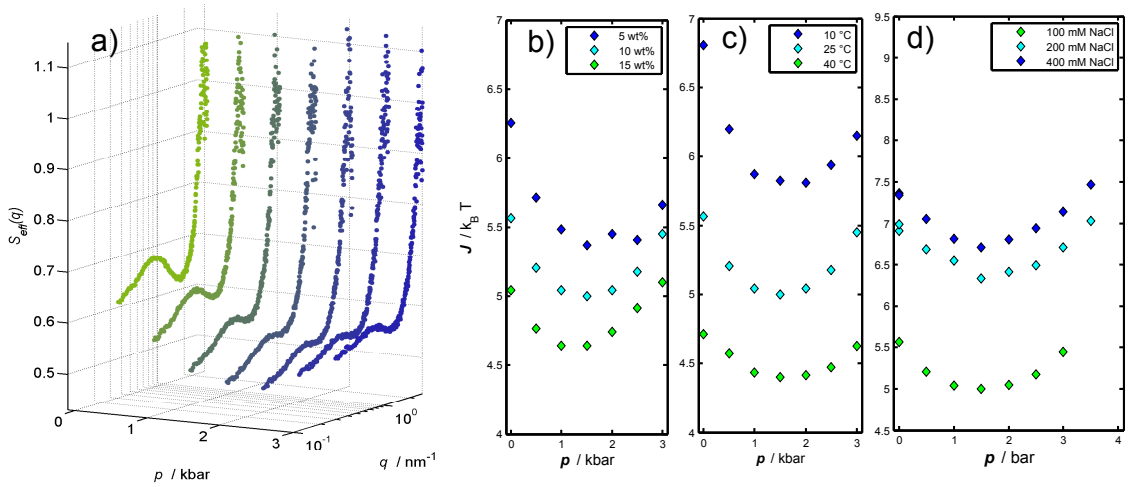

Figure 1: a) Effective structure factor for 10 wt% Lysozyme solution at 25 °C at different pressures applied. b) - d) Results of the refinement in terms of the attractive interaction parameter, J, as a function of pressure and b) protein concentration, c) temperature, and d) salt concentration.

results of the refinements are shown in figure 1 b - d. The nonlinear pressure dependence of the interaction potential was reported previously for proteins in salt free solution [4]. This effect was connected to the collapse of the second hydration shell of water at pressures of 2 kbar. At this pressure, the attractive interaction parameter J has a minimum and starts increasing with rising pressure. Interestingly, the presence of salt (NaCl) in the solution has no influence on this hydration effect. As can be seen, the location of this minimum in $J(p)$ is approximately independent

of protein concentration, salt concentration, and the temperature range studied. This phenomenon is in stark contrast to the influence of many cosolvents on protein-protein interactions that change the water structural properties owing to a pronounced water structure breaking or making effect [5].

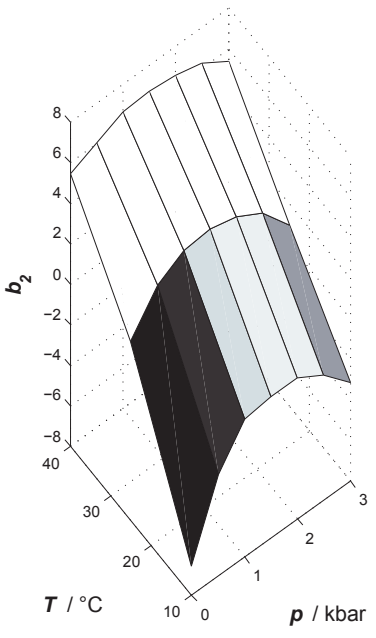

Figure 2: Normalized second virial coefficient as a function of pressure and temperature, calculated from the protein-protein interaction potentials. Conditions with $b_2 > -0.85$ are shown in white, conditions with $b_2 < -3.2$ are shown in black. Conditions within the crystallization slot are shown in grey.

With the knowledge about the interaction potential as a function of pressure, the use of pressure as an additional parameter for controlling protein crystallization can be explored. Generally, the ability of solutions to form crystals can be predicted by determining the normalized second virial coefficient, b_2 [6], which is a measure of the effective interaction potential and depends strongly on the solution conditions. For b_2, a crystallization window was reported with a narrow range of $-3.2 < b_2 < -0.85$ [7].

In figure 2, the normalized second virial coefficient, calculated from the protein-protein interaction potentials, is depicted as function of pressure and temperature. As can be clearly seen, the nonlinear pressure dependence of the interaction potential is conserved in the behavior of b_2. Moreover, these data show the possibility of using pressure as a free parameter to adjust the protein-protein interactions, and b_2 can be adjusted to conditions of pressure and temperature, where it becomes located inside the crystallization window.

In summary, it can be stated that the experiments were successful. We gained novel information about the combined influence of pressure and salt on the protein-protein interaction potential in solution. Moreover, it was shown that pressure can be used to precisely tune the protein-protein interactions, including conditions, where nucleation of protein crystal formation sets in.

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