Protein-protein interactions – from in vivo functionality to pharmaceutics and food science

Salt concentration and pH impact were studied on lysozyme and BSA systems respectively by investigating various parameters (osmotic second virial coefficient, folding state and 3D shape)

Introduction

Protein-protein interactions are relevant for various biological processes, such as transcription and signal transduction, but also for protein crystallization, stability and shelf-life of pharmaceutical formulations, as well as for processing and storage of protein-based food. While repulsive interactions might stabilize a formulation, they will prevent protein crystallization which requires moderate attraction between proteins. A simple measure of weak pair-wise protein interactions is the osmotic second virial coefficient, A_2, positive for repulsive interactions and negative in the case of attractions. It is traditionally determined using static light scattering (SLS) at low protein concentrations. An alternative method to measure A_2 is small angle X-ray scattering (SAXS)[1] which covers a much broader range of q-values, and thus length scales. While only the scattering intensity at q = 0 is needed for the determination of the second virial coefficient, having access to the entire q-range allows the researcher to simultaneously obtain information on the folding and aggregation state of the protein under investigation. This is important for protein crystallization studies, as only well folded proteins can form crystals. Furthermore, certain solution conditions and/or the presence of co-solvents and co-solutes, such as those found in the crowded cellular environment and in most formulations, might affect the protein tertiary structure[2] in addition to altering the interactions in the system, potentially inducing protein aggregation. Such changes will not be apparent from the short q-range covered in SLS.

Measurements & results

Two systems were investigated:

> **Lysozyme** (Mw = 14 kDa) in 50 mM NaOAc, pH 4.4 at 25°C with different concentrations of NaCl (0 – 400 mM). Stock solutions of protein and salt were prepared and mixed in different proportions with protein buffer in a 96 well tray using the BioXolver sample handling robot. Corresponding buffers for background subtraction were prepared in the same way. Concentrations were determined by serial dilution on a NanoDrop. SAXS experiments were performed on a BioXolver L, covering a q-range of (0.01 – 0.5) $Å^{-1}$. The samples were loaded automatically using the sample handling robot. For each buffer condition, multiple protein concentrations were measured. The resulting detector images were radially averaged, background corrected and concentration-normalized using the software RAW[3,4]. The normalized forward scattering I_0/c was determined using the Guinier approximation in the same software and plotted as a function of salt concentration (Figure 1a). A linear fit to I_0/c vs. c then directly yields the second virial coefficient (Figure 1b) via $A_2 = \frac{-slope}{2M_w \cdot intercept}$. The total measurement time (including sample mixing and cleaning cycles between exposures) was about 1h per A_2 value.

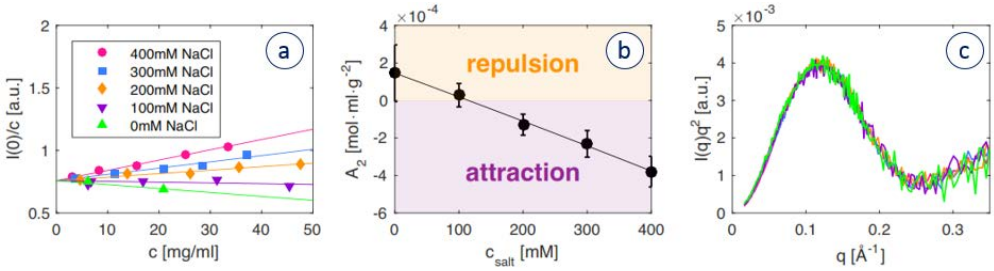

Figure 1. Forward scattering (a) and second virial coefficient (b) of lysozyme as a function of NaCl concentration. (c): Kratky plot of 5 mg/ml lysozyme at varying salt concentration

A_2 values between $-0.5 \cdot 10^{-4}$ and $-8 \cdot 10^{-4}$ mol·ml·g^{-2} define the so-called crystallization window[5] i.e. the range of interactions favorable to crystal formation. In the lysozyme/salt system shown here, this regime is reached at NaCl concentrations above about 150 mM. If in addition, the protein is well folded, as revealed by the signature bell shape in the Kratky plot (Figure 1c), crystallization is likely to occur.

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> **Bovine serum albumin** (BSA, M_w = 66 kDa) at 5 mg/ml in 20 mM HEPES, 150 mM NaCl at 25°C with different pH (adjusted by HCl and NaOH). Samples were prepared by dissolving BSA powder in the buffers, followed by filtration (0.2 µm syringe filters). Concentrations were determined using the in-line UV detection of the BioXolver L. Protein solution scattering was recorded for 5 minutes per sample and reduced as described above. Pair-distance distribution functions P(r) were calculated using the bayes algorithm also available in RAW and 10 ab initio models were generated, averaged and filtered using DAMMIF[6] in RAW.

Upon lowering of the pH, the protein tertiary structure gradually unfolds, readily visible from the loss of the bell-shape in the Kratky representation of the scattering data (Figure 2b) and from the increase in the longest dimension D_{max} of the protein, the value where P(r) reaches 0 (Figure 2c), and illustrated by the ab initio models in Figure 3.

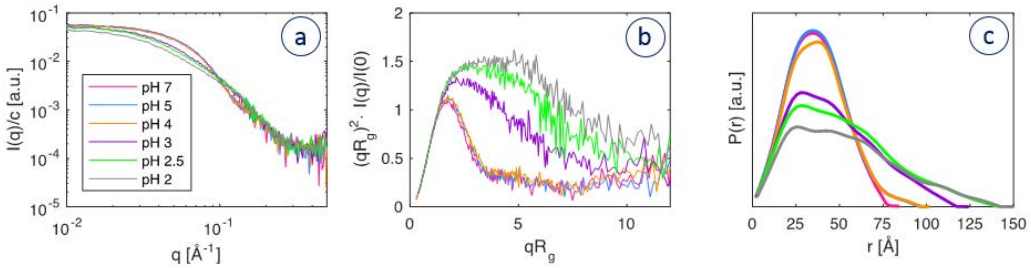

Figure 2. Scattering curves of BSA at different pH in log-log (a) and normalized Kratky (b) representation and their corresponding pair-distance distribution functions P(r) (c).

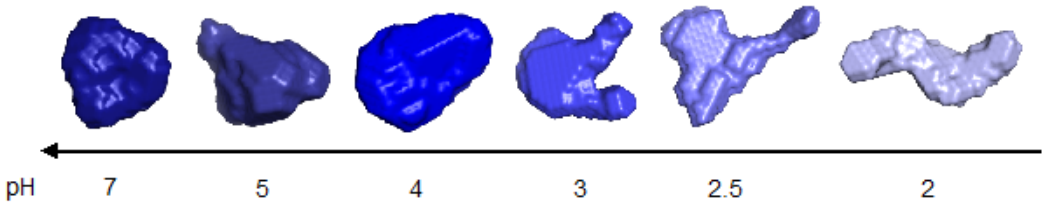

Figure 3. Ab initio models of BSA solutions with different pH (not to scale).

Conclusion

The BioXolver with its sample handling robot is ideally suited for the study of protein-protein interactions, as it allows to mix samples immediately prior to exposure, avoiding aggregation or degradation induced by interactions in the system, and to load a large number of samples automatically, thus greatly facilitating second virial coefficient measurements and screening of solution conditions. Moreover, covering a broad q-range, it reveals changes in the tertiary structure of the protein induced by altered buffer conditions, which might go unnoticed using SLS.

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Xenocs thanks Dr. Saskia Bucciarelli from the Faculty of Health and Medical Sciences, University of Copenhagen, Denmark, for her contribution to this document.

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