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Introduction

Protein crystallization has been studied widely for many years, and still remains a bottleneck in solving the structure of proteins[1]. As a part of a study on protein crystallization under confinement[2], the stability of solutions of the globular protein lysozyme was studied by small angle neutron scattering (SANS). The solutions were probed by time-resolved SANS experiments and the recorded data report on the development of a structure.

Method

• Instrument

The samples were studied by SANS at D11 at the ILL.
The instrument is shown schematically in Fig. 1.

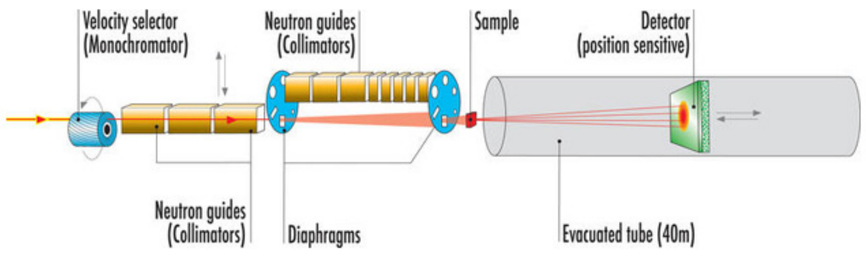

Figure 1: Schematic representation of the D11 SANS instrument (source: www.ill.eu)

• Sample composition

Lysozyme aqueous solutions of 1, 2, 4, and 8 % (wt/wt) with 2 or 4% (wt/wt) NaCl were measured for up to 7.5h after preparation, in 1.5h intervals. These initial compositions cover a range of stable and supersaturated conditions as illustrated in Fig. 2.

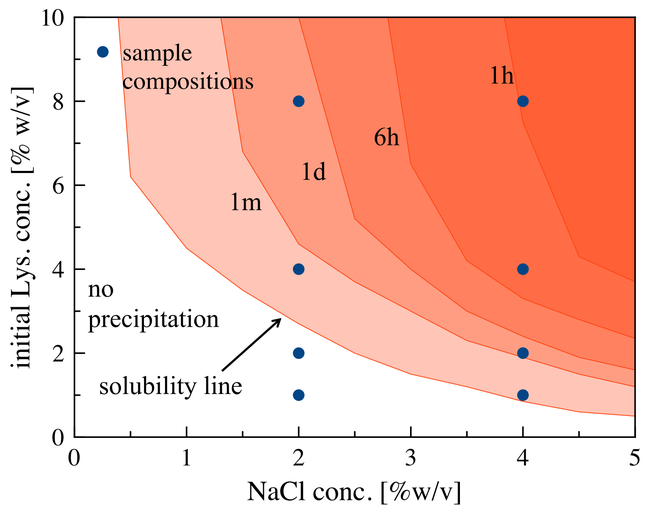

Figure 2: Sample compositions plotted on top of a pseudo-phase diagram for lysozyme (modified from ref. 1).

• $S_{eff}(q)$

The effective structure factor, $S_{eff}(q)$, was computed by dividing the recorded SANS data by a theoretical form factor, $|F(q)|^2$, (shown in Fig. 3) and the volume fraction of lysozyme.

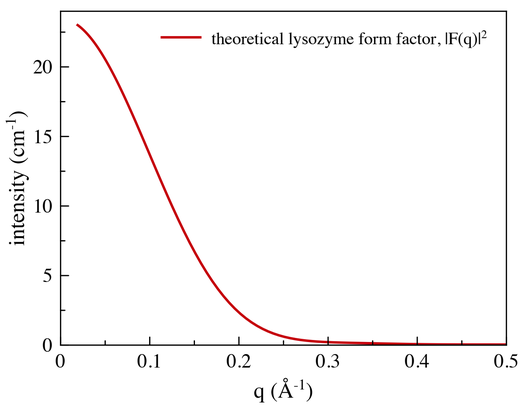

Figure 3: Theoretical lysozyme form factor

$$|F(\mathbf{q})|^2 = \frac{1}{V}\left(\frac{3\Delta\rho V\left\{\sin\left(\mathbf{q}\sqrt{\frac{1}{2}R_a^2+\frac{1}{2}R_b^2}\right)-\mathbf{q}\sqrt{\frac{1}{2}R_a^2+\frac{1}{2}R_b^2}\cos\left(\mathbf{q}\sqrt{\frac{1}{2}R_a^2+\frac{1}{2}R_b^2}\right)\right\}}{\left(\mathbf{q}\sqrt{\frac{1}{2}R_a^2+\frac{1}{2}R_b^2}\right)^3}\right)^2$$

Where Δρ is the scattering length density difference (contrast), V is the particle volume, R_a and R_b are the minor and major ellipsoid radii, respectively.

Results

• Lysozyme form and size

SANS data from a pure dilute (3.4 wt%) protein solution were used to confirm[3] the **prolate ellipsoidal** shape of the lysozyme, with R_a**=11 Å** and R_b**=18 Å**. The best fit (Fig. 4) was obtained using the ellipsoidal form factor and a structure factor calculated for charged, spheroidal particles in a dielectric medium, using the Hayter model[4,5].

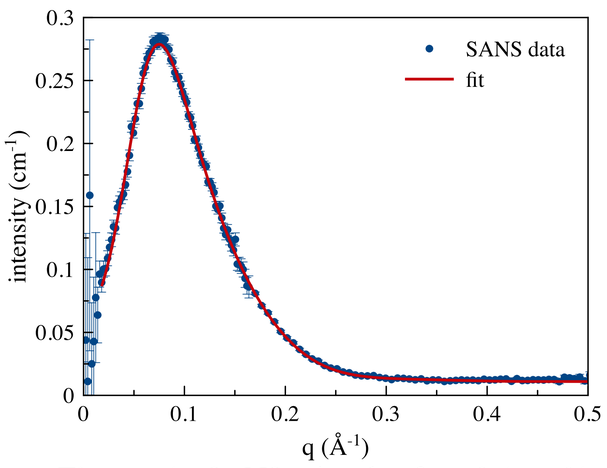

Figure 4: SANS signal of a 3.4 wt% lysozyme sollution, and best fit using an ellipsoidal form factor.

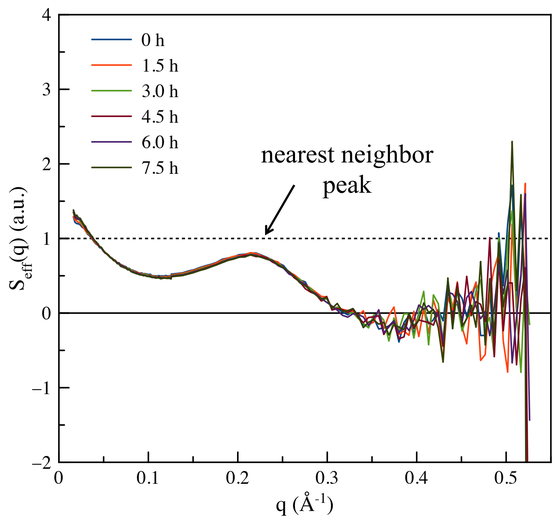

Figure 5: S_{eff} (q) for the 8 wt% lysozyme solution with 2 wt% NaCl.

• Bragg peaks

In one case, 4% (wt/wt) lysozyme solution with 4% (wt/wt) NaCl, two peaks (at q=0.21 Å^{-1} and q=0.28 Å^{-1}) forming over time were observed. Simultaneously, at low q (q < 0.02 Å^{-1}) the signal decreased. The peaks correspond to real space distances of 30Å and 22Å, respectively. The peaks resemble Bragg peaks, suggesting that tiny crystals were formed.

• Effective structure factor

With the known dimensions of lysozyme, $S_{eff}(q)$ was computed for the time-dependent data sets. In all cases no significant time evolution was observed. The nearest neighbour peak is increasingly pronounced for increasing lysozyme concentration, both in 2% and 4% NaCl conditions. This is indicated in Fig. 5 with the 8 wt% lysozyme, 2 wt% NaCl solution as an example.

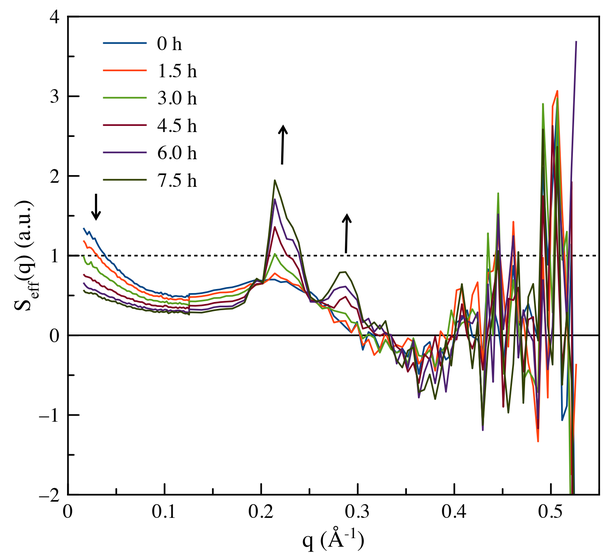

Figure 6: $S_{eff}(q)$ for the 4 wt% lysozyme solution with 4 wt% NaCl, with evolving Bragg peaks.

Conclusion

The size and shape of lysozyme was confirmed by SANS on a dilute lysozyme solution. The effective structure factor shows a nearest neighbor peak. In one sample two sharp peaks as well as a decrease in low q scattering were observed. These Bragg-like peaks probably originate from growing small crystallites, at the expense of monomers in solution. The observation was made only in the sample 4% protein/4%NaCl. Possibly the crystal formation was coincidentally captured in the beam for this particular sample, as different from the others, especially the 8%protein/4%NaCl-sample.

References:

1. Durbin, S. D. & Feher, G. Protein crystallization. *Annu. Rev. Phys. Chem.* **47**, 171–204 (1996)
2. Tanaka, S., Egelhaaf, S. & Poon, W. Crystallization of a Globular Protein in Lipid Cubic Phase. *Phys. Rev. Lett.* **92**, 128102 (2004)
3. Stuhrmann, H. & Fuess, H. A neutron small-angle scattering study of hen egg-white lysozyme. *Acta Crystallogr. Sect. A Cryst.* … **82**, (1976)
4. Hayter, J. B. & Penfold, J. An analytic structure factor for macroion solutions. *Mol. Phys.* **42**, 109–118 (1981)
5. Hansen, J.-P. & Hayter, J. B. A rescaled MSA structure factor for dilute charged colloidal dispersions. *Mol. Phys.* **46**, 651–656 (1982)

Acknowledgements

Thanks to DanScatt for financial suppport. Thanks to Ralf Schweins for support during the SANS experiment and for giving an introduction into data analysis by SasView.

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