High throughput protein envelope determination

3D envelopes were determined from an automated sequence of measurements including 3 different proteins with low volume and dilute solutions.

Introduction

Small-angle X-ray scattering is complementary to other structural biology techniques. It provides reliable information on the shape of proteins in solution and associated parameters including macromolecule interactions and dynamics.
Reliability of the measurements and high throughput are key requirements to ensure the ability to run samples in a structural biology laboratory.
This note displays the quality of three-dimensional envelopes obtained on 3 protein samples (lysozyme, bovine serum albumin (BSA) and thyroglobulin) measured on the BioXolver through an automated measurement sequence.

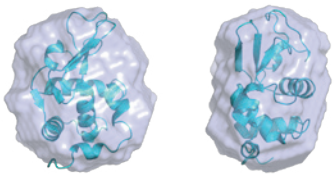
Measurements & results

Concentration series, 3 concentrations per sample, were run successively using the in-line pipetting robot, which allows automatic sample loading and cell cleaning in a cycle time shorter than 2 minutes.

5 µL per sample condition were used for the measurements. Data treatment through the automated software package based on ATSAS[1] allows the quick determination of the corresponding 3D envelopes for each concentration to define sample quality. The total sequence of measurements, including the three concentrations per sample and buffer measurements, was completed within less than 90 minutes from the loading of the well plate to the generation of the final 3D envelopes.

Further data processing using ATSAS was performed to emphasize data quality. Figures 1 and 2 show the 3D protein envelopes generated by DAMMIN[2] for the lowest measured concentrations of lysozyme and BSA. Comparison of the calculated shape to existing crystallographic data is provided by overlaying the homologous crystal structure for both samples.

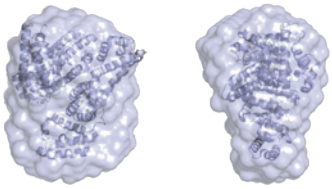
Juxtaposition shows good consistency of the estimated shape. It demonstrates the ability to determine 3D envelopes from weakly scattering samples using a short measurement time (5 minute exposure time).
Comparison between synchrotron SAXS data and BioXolver measurements is provided in Figure 3 for thyroglobulin sample. The same analysis procedure generating DAMMIF/DAMMIN models was applied to both SAXS data sets. The final 3D envelopes are very similar and show the good quality of the results obtained on the BioXolver in a 5 minute exposure time.



Buffer : 20 mM HEPES,
20 mM sodium acetate, pH 6.8
c = 1.5 mg/mL
Exposure time : 5 min

Crystal structure 1LYS[3]

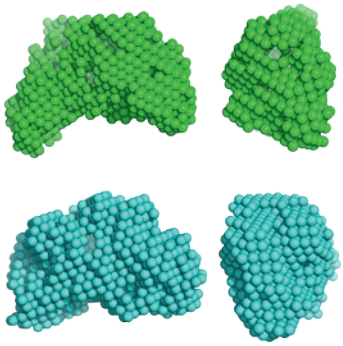
Figure 1. 3D envelope from a lysozyme solution (Mw = 14.3 kDa).



Buffer : 50 mM HEPES, pH 7.5
c = 5 mg/mL
Exposure time : 5 min

Crystal structure 3VO3[3]

Figure 2. 3D envelope from a BSA solution (Mw = 66 kDa).



BioXolver
Buffer : PBS (20 mM phosphate,
150 mM NaCl), pH 7.4
c = 4 mg/mL
Exposure time : 5 min

Synchrotron data SASDA98[4]
c = 3.9 mg/mL

Figure 3. Comparison of 3D envelopes of thyroglobulin (Mw = 669 kDa).

Conclusion

The results show the capability of the BioXolver to reliably produce high quality results with high throughput and using small volumes of protein solution.
It opens the way for routine characterization of biological macromolecules in the laboratory, thereby providing rapid feedback for formulation studies and other research.

[1] D. Franke et al.(2017) *J. Appl. Cryst.* 50
[2] D. I. Svergun (1999), *Biophys J.* 2879-2886.
[3] www.rcsb.org
[4] www.sasbdb.org

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