Thorough investigation of oligomers during protein fibrillation

The transient nature of aggregates during the fibrillation process of α-synuclein is highlighted with in-house SAXS experiments using the BioXolver.

Introduction

Protein aggregation into fibrillar structures is associated with a variety of neurodegenerative disorders, such as Parkinson's and Alzheimer's diseases. It is becoming increasingly evident that the toxic species causing the adverse effects are not mature fibrils, but oligomers. These are aggregates formed transiently at intermediate times during the fibrillation process[1]. Coexistence of oligomers in equilibrium with monomers (individual proteins) and mature fibrils requires analysis of a polydisperse solution.

Small angle X-ray scattering (SAXS) combined with decomposition of the data into the individual contributions allows the investigation of such complex systems without requiring physical isolation of the different components.

Measurements & results

SAXS measurements were completed on a fibrillating sample of α-synuclein (aSN) at 8 mg/ml in PBS buffer at pH 7.4 using the BioXolver. Exposure times of 8 minutes at regular time intervals (from 18 to 31 min) were used to monitor the process. The fibrillation was induced by stirring at 37°C and monitored using Thioflavin T, a fluorescent dye known to bind to cross-β sheets; the 3-dimensional structure most commonly observed in protein fibrils. Figure 1 shows the resulting SAXS curves, containing contributions from all components present in the solution at each time. Decomposition[1] yields the individual scattering curves of monomer, oligomer and fibril, as shown in Figure 2. These individual curves can then be further analysed to obtain structural information about the different species, as described by Herranz-Trillo et al.[1]

The smallest species (monomer) gradually disappear while large species (fibril) are formed. The profile of the intermediate species (oligomer) highlights the transient nature of these aggregates that appear and disappear again over time.

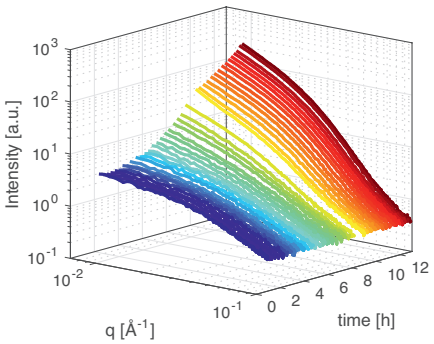
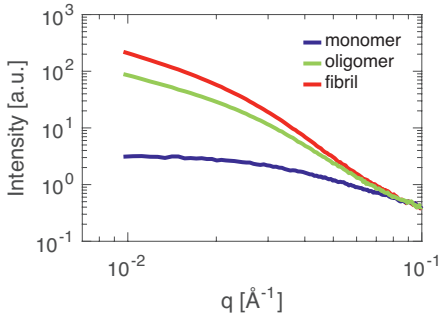


Figure 1. SAXS curves of fibrillating aSN followed over time (from blue to red).



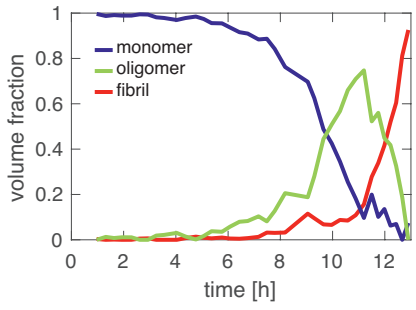


Figure 2. *Left:* Scattering curves of the individual species present in the solution during the fibrillation process. *Right:* Concentration (or volume fraction) profiles of the different species shown in the left plot.

Conclusion

The method described here can be applied to any polydisperse dataset.

In order to successfully study such polydisperse solutions, unsmeared data is necessary. Furthermore, the decomposition protocol requires large datasets with varying composition. Hence, in the case of processes that develop over some hours, short measurement times are crucial in order to capture the entire process. The BioXolver has the necessary resolution and intensity (i.e. short measurement times) to enable such studies in-house, while until now only synchrotrons were able to fulfil these requirements.

[1] F. Herranz-Trillo et al. (2017) *Structure*, 25, 1, 5-15

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