Experimental report

12/09/2018

Proposal: 8-04-811 **Council:** 4/2017

Title: Dynamics of intermediate states during the alpha-synuclein fibrillation process

Research area: Biology

This proposal is a new proposal

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Samples: Protein (HCOND) Lysozyme in D2O + NaCl

Instrument	Requested days	Allocated days	From	To
IN16B	3	3	15/06/2018	18/06/2018

Abstract:

Alpha-synuclein and Tau protein amyloid fibers constitute pathological hallmarks of Parkinson and Alzheimer diseases, respectively. Using the highest available flux and signal to noise ratio at sub-ueV energy resolution, we propose to investigate intermediate states during the fibrillation process of alpha-synuclein protein. We will correlate the results with different levels of toxicity associated with different fibrillation speeds. The proposal will also constitute a preparatory state towards a full in-situ observation of the entire process and a comparative study of the wild-type and mutants of alpha-synuclein.

The controlled mechanical vibrations needed to trigger fibrillation of a-synuclein could not yet be guaranteed at the time of the experiment. For this reason, the protein was replaced by the lysozyme, which is equivalent in terms of the physics, as amyloid fibers formation can also be triggered by temperature change.

Samples preparation:

Lysozyme powder was dissolved in D_2O with different salt concentration and pD to find the optimal conditions for the experiment. We finally used two conditions, one at 0.1 M NaCl, pD 2 at 90 °C to form particulates and the other one at 0.1 M NaCl, pD 2 and 65 °C, for which we form fibers. For each sample, protein powder was dissolved in the corresponding solution, then centrifugated to pellet possible big contaminants and sealed in cylindrical aluminum cells.

Data acquisition:

Each aggregation process, particulates and fibers were repeated twice. With a first QENS performed at low temperature – 280 K – for two hours, followed by Inelastic Fixed-Window Scans (IFWS) with energy offsets of 0, 0.6, 1.5 and 3 µeV for 1', 2', 6' and 6' respectively. After aggregation has completed, a final two hours QENS was recorded.

Samples were checked using optical and electron microscopy for presence of desired aggregates. The particulates were indeed there after the experiment, but surprisingly, no fibers were found. The aluminum surface may have played a role in perturbating the aggregation process.

Also, data for the second particulates sample are incomplete as the sample cell broke in the instrument, thereby letting all the water out.

In conclusion, we got one full, reliable dataset on lysozyme particulates, for which data analysis is ongoing (see figures 1 and 2).

Data analysis:

After pre-processing using Mantid algorithms – scan sums, centering and empty cell signal subtraction -, both QENS and IFWS were analyzed using in-house written python scripts and the model described in Grimaldo et al. (Grimaldo et al., 2015). The analysis presented here is still preliminary as some additional work on data corrections is needed.

References:

Brune, D., & Kim, S. (1993). Predicting protein diffusion coefficients. *Proceedings of the National Academy of Sciences of the United States of America*, *90*(9), 3835–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8483901

Grimaldo, M., Roosen-Runge, F., Jalarvo, N., Zamponi, M., Zanini, F., Hennig, M., … Seydel, T. (2015). High-resolution neutron spectroscopy on protein solution samples. *EPJ Web of Conferences*, *83*, 02005. http://doi.org/10.1051/epjconf/20158302005

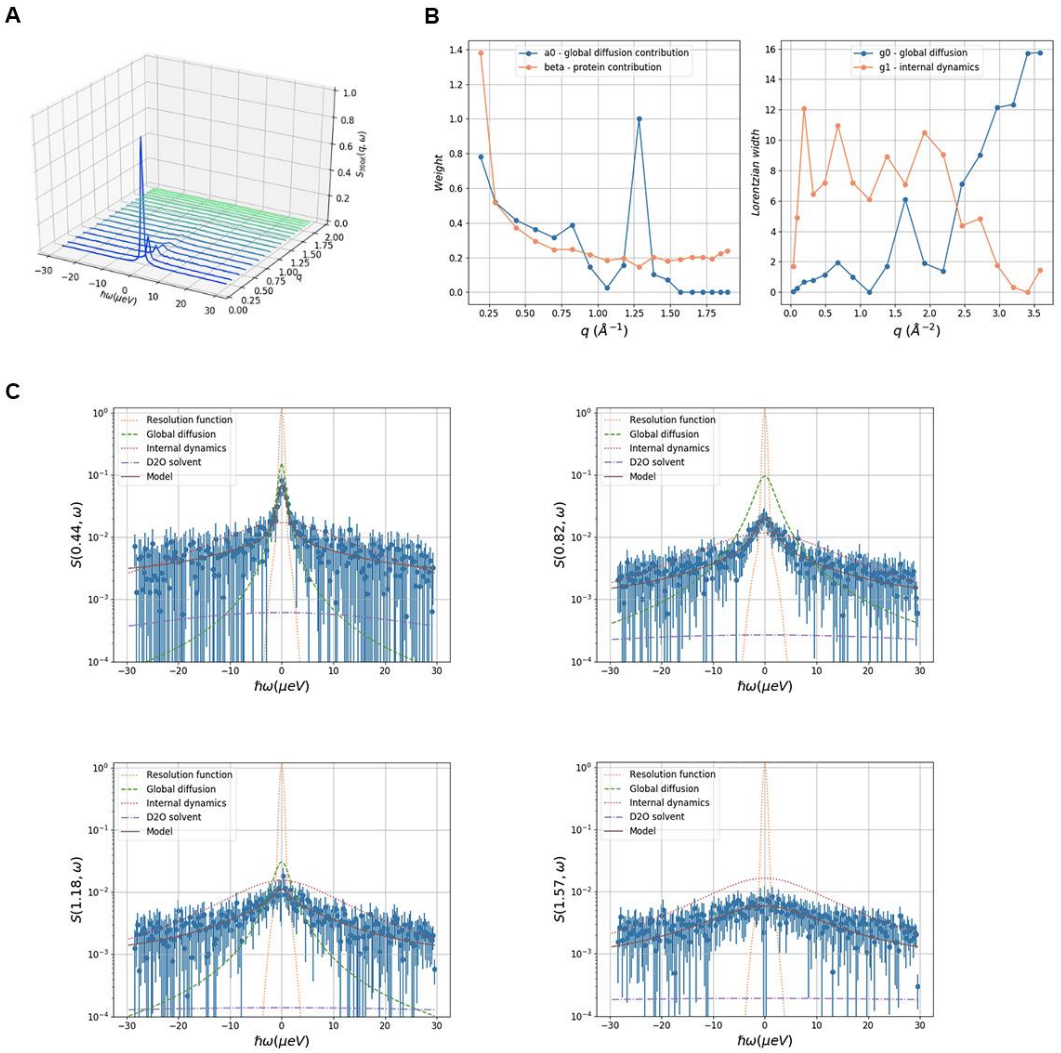

Figure 1: QENS data at 280 K before aggregation give consistent diffusion coefficient
A, normalized experimental data. **B,** plot of the model parameters. Using a Dq^2 dependence for the Lorentzian width with D being the diffusion coefficient and q the momentum transfer, we found $D \approx 0.8.10^{-6}$ $cm^2.s^{-1}$, which is consistent with the value obtained elsewhere (Brune & Kim, 1993), even though a little bit lower. **C,** plot of the fitted spectra at different q-values.

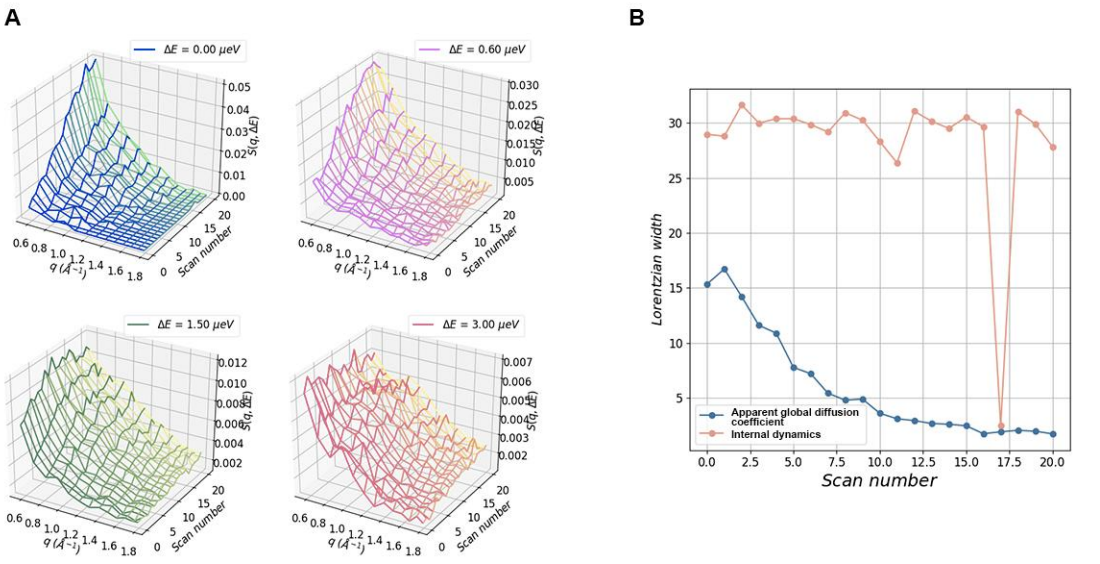

Figure 2: internal dynamics remains constant during aggregation while global diffusion is decreasing
A, normalized experimental data at different energy offsets. The momentum transfer q dependence of the signal as a function of time (scan number) is represented here. **B,** plot of the model parameters. Using an explicit q-dependence and a global fit for each q-value at once.