Experimental report

14/02/2017

Proposal: 8-03-888 **Council:** 4/2016

Title: Dynamics of amyloid protein fibrilelongation using isotope-labelled SANS

Research area: Biology

This proposal is a new proposal

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Samples: peptide NFGAIL

Instrument	Requested days	Allocated days	From	To
D11	3	2	11/12/2016	14/12/2016
D22	3	0		

Abstract:

We will use SANS in combination with our new deuteration approach to investigate amyloid protein fibrils associated with diseases such as Alzheimers and prion disorders to give previously unavailable information in the linear growth rate of such fibrils.

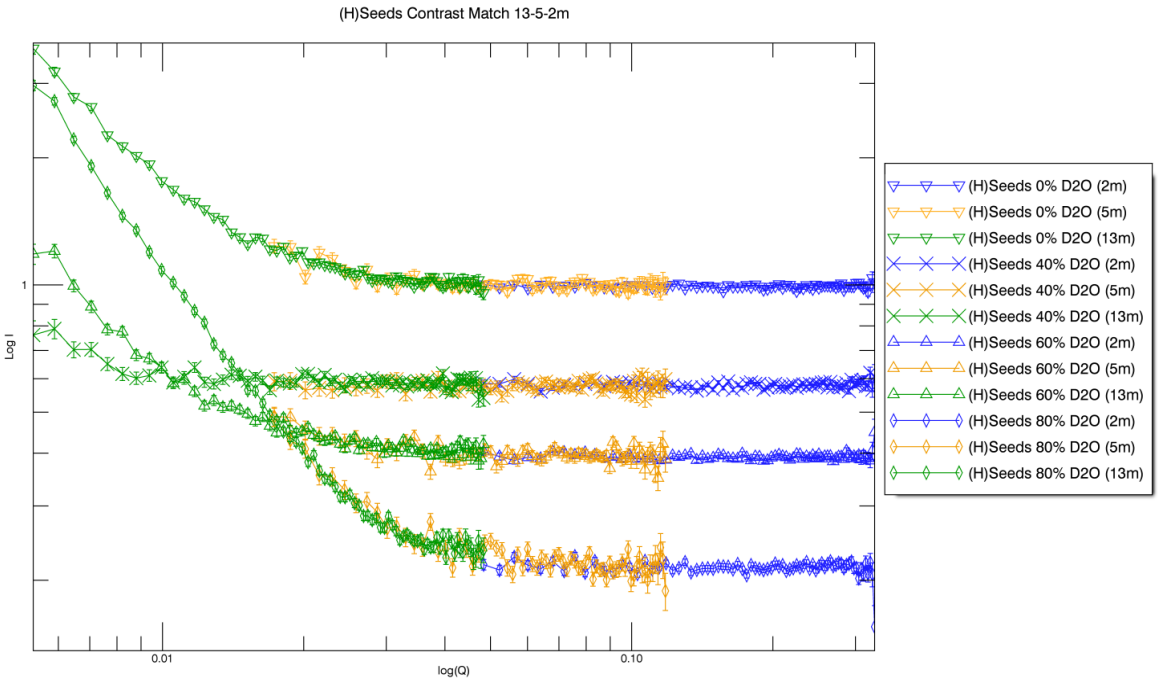
The approach involves placing deuterated "seeds" of protein fibrils in a contrast-matched deuterated solution, and then allowing these to grow in the presence of hydrogenated proteins. The hydrogenated proteins add to the growing ends of the fibrils, and this isotope labelling approach effectively allows us to only "see" these growing ends, which we can model as cylinders whose length increases with time.

Such information is not available using any other solution technique; standard spectroscopic methods only give the overall % of protein in fibrillar vs non-fibrillar form, and the rate at which this changes, and cannot separate out the number (and length distribution) of fibrils, from the average rate at which each a single fibril grows. Such data are key to understanding the biological behavior of prion strains and other amyloid diseases, and is a prerequisite for further theoretical understanding of peptide fibrillization

Preliminary experimental report (8-03-888) D11 (December 2016)

We carried out experiments on alpha synuclein protein fibrils, to test a new method to determine elongation rates from growing labelled (deuterated) ends onto contrast-matched (hydrogenated) fibril seeds.

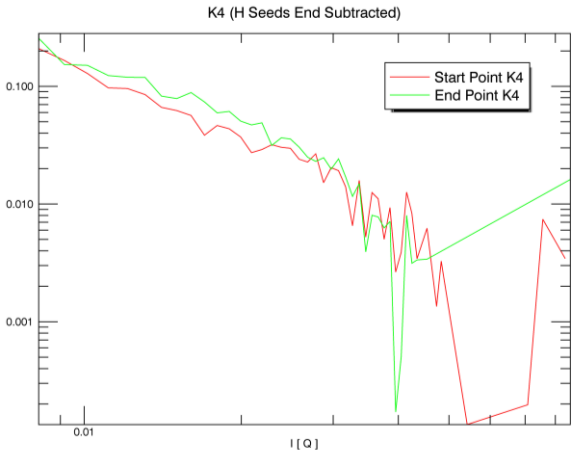
Contrast matching of seeds:



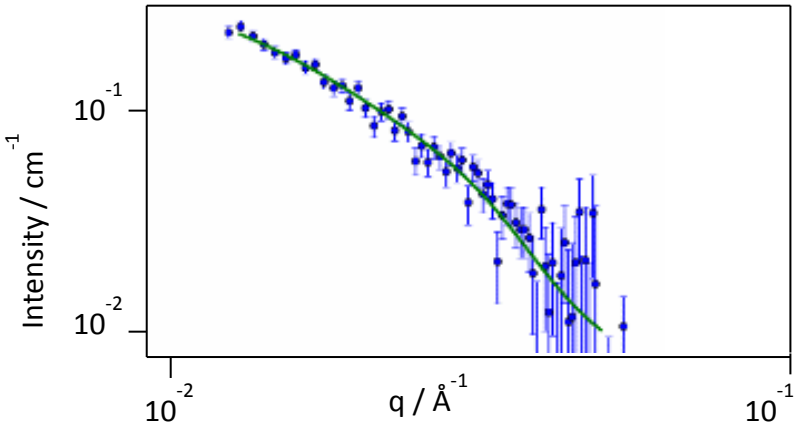
35% D2O buffer chosen (parabola fit to background-subtracted data not shown), although subsequent data suggest possible problems with exchange between seeds and buffer.

Problem with samples falling out of neutron beam (data not shown); solution: using rotating banjo cells.

Real-time experiments (8m camera length; 5 minute time slice): poor signal-noise



“Fixed length” experiment carried out with seed:monomer ratio 2:1 (so growth constrained to 50% increase by depletion of available monomer). Final time point with seeds subtracted (because of imperfect contrast matching) over range of camera lengths: labelled ends can be modelled as cylinders 15 ± 1 nm diameter (consistent with TEM), 36 ± 6 nm length (plausible).



Future plan: we aim to circumvent these problems by using a new experimental approach: rather than real-time SANS analysis, we will quench the growth at various time points. This will be achieved out by filtration to remove the unfibrillised labelled protein, and then re-suspending the fibrils. The approach would then allow for measurements over the full q range available, and using longer acquisition times, to improve the quality of the data available for modelling the growing ends. Further advantages are (1) the removal of any signal arising from non-aggregated monomer, and (2) the potential to re-suspend the fibrils into different concentrations, to obtain dilution series data to eliminate structure factor / interaction effects.