Experimental Report

27/10/2014

Proposal: **8-01-401** **Council:** 4/2012

Title: Investigation of the structure of a filamentous bacteriophage (fd) using high and low-angle neutron scattering

This proposal is a new proposal

Researh Area: Biology

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Samples: filamentous bacteriophage fd

Instrument	Req. Days	All. Days	From	To
D22	2	1	26/11/2012	27/11/2012
D19	11	11	02/11/2012	12/11/2012

Abstract:

Filamentous bacteriophages are flexible rod-like virions composed of a single-stranded circular DNA at the core surrounded by multiple copies of a coat protein. They provide good models of high interest for the study of macromolecular structure and interactions. Here we focus on the fd strain. Over the years models for the coat proteins have been suggested but there remains a number of uncertainties on the structure of the coat protein and the organisation of the nucleic acid. The latter has revealed itself to be extremely difficult to investigate using X-ray diffraction. In recent preliminary work on D19, we have recorded novel high-angle fibre diffraction data from an array of magnetically aligned samples of fd. The data, although weak, show high-angle diffraction to a resolution of ~3Å as well as evidence of low-angle diffraction outside the scope of D19. In test experiments on D22, we demonstrated the feasibility of using contrast variation to probe the low resolution structure of the virion. In this proposal we seek beamtime to extend this work by (i) improving the counting statistics for the high-angle data on D19 (ii) recording an accurate contrast variation dataset on D22.

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Filamentous bacteriophages are simple well-defined systems that are easily genetically manipulated and hence provide good models for the study of macromolecular structure and interactions (Marvin, 1998). The virion is composed of a single-stranded DNA at the core surrounded by coat proteins. Over the years models for the coat protein have been suggested but it has been notoriously difficult to investigate the structure of the nucleic acid component using X-ray diffraction, although the extra contrast afforded by neutrons could make this possible, this would therefore contribute unique and important information on this system.
Both high and low angle time was secured on D19 and D22.

D22:
The aim of the low-angle experiment was to carry out a detailed contrast variation study from 0% D_2O to 100% D_2O in steps of 10% including smaller steps around both the protein and the nucleic acid match points in order to identify precisely both match points. 2 days were requested but only 24 hours were allocated. Given the time constraint it was decided to focus on the region around the two match points and the 100% D_2O contrast. The contrasts studied were 100% D_2O, 68%, 66%, 63%, 62%, 61% and 58% D_2O around the nucleic acid match point 45%, 43%, 41%, 40%, 39%, 37%, 36%, 35% and 33% D_2O around the protein match point.
Figure 1 shows the 100% D_2O and the 100% H_2O contrasts as well as what is assumed to correspond to the 2 match points.
A few difficulties were encountered during this experiment. The equilibration times at each of the contrasts were considerably longer than anticipated (30 minutes between the smaller steps and 1 hour for every “bigger” step) which reduced the number of contrasts studied. Results showed that it was still difficult to clearly identify the protein and the nucleic acid match points. At no point did any features in the pattern disappear around any of the match point and reappear after as was originally expected. We believe that repeating this experiment with a systematic contrast study in steps of 10% from 0-100% D2O (and smaller steps around both match point) as was originally planned would yield a higher data/parameter ratio and could give us enough information for an attempt at modelling the results obtained. Another proposal for more beamtime will be put in for the next cycle.

D19 experiment:
The aim of this experiment was to obtain better statistics on the 100% D_2O and 100% H_2O pattern. We aimed to increase the counting time to obtain data up to 3Å resolution.
This experiment was also used to test the HC1 humidity system (Sanchez-Weatherby *et al,* 2011). Significant amount of time was used at the beginning to test the humidity system and several problems had to be overcome. The sample surface area was large compared to the size of the nozzle which increased the H/D exchange time considerably. We then were face with a problem on how to divide the rest of the beamtime between the 100% D_2O and 100% H_2O data to be collected. Considering the time left we chose to collect data solely on the system at 100% D_2O. There was not enough time left to obtain good and usable data on both systems. The data collected gave crucial information on high angle features. A more detailed contrast

study should be attempted in order to complete this set of data and pin down the modelling. A new proposal is underway.

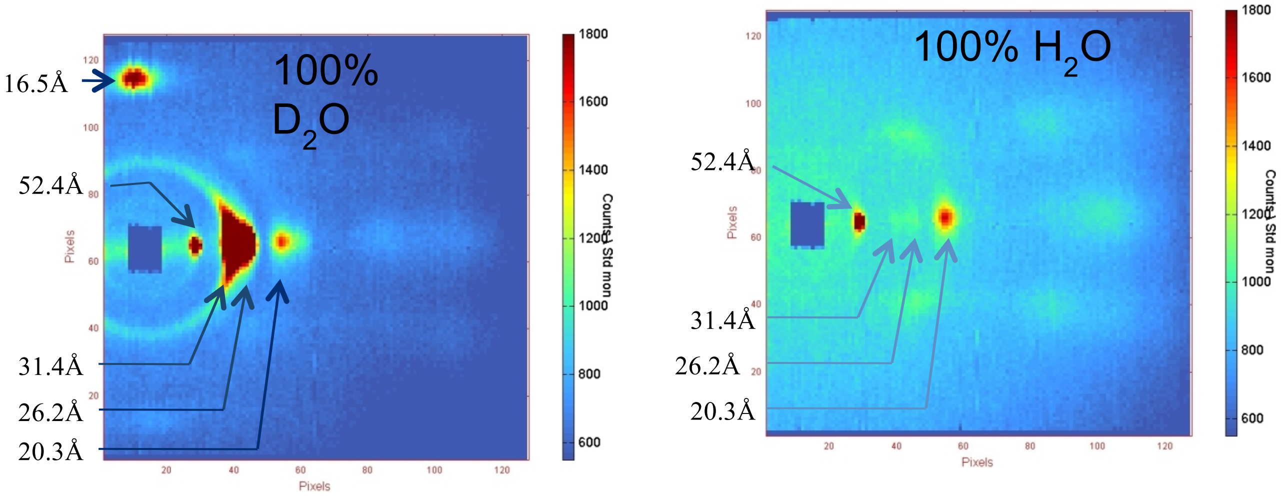

Figure 1: D22 data at 100% D2O and 100% H2O contrasts

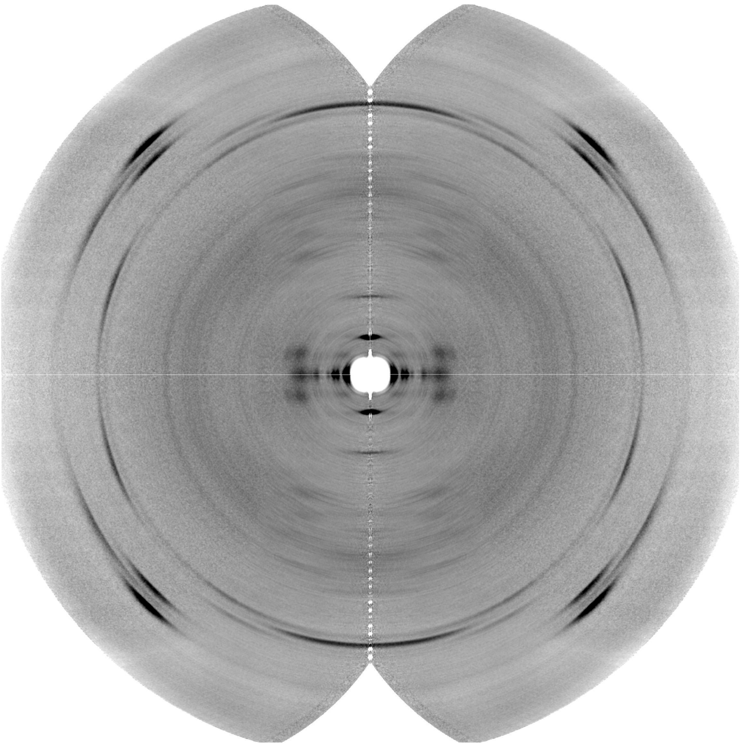

Figure 2: 4 quadrants reconstituted D19 data at 100% D2O

References:
[1] Marvin D.A. (1998) Curr. Opin. Struct. Biol. 8, 150.
[2] Langan P. (2005) Crystallography Reviews, 11, 125.
[3] Tiggelaar S. M., Mossou E., Callow P., Callow S., Teixeira S.C.M., Mitchell E.P., Mitraki A. and Forsyth V.T. (2011) Acta Cryst. F, 67(Pt 3):332-5. Epub 2011
[4] Sanchez-Weatherby J., Bowler M. W., Huet J., Gobbo A., Felisaz F., Lavault B., Moya R., Kadlec J., Ravelli R.B.G., and Cipriani F. Acta Cryst D, 65:1237-1246, 2009.