ESRF	**Experiment title:** **Investigation of Hfq-sRNA complexes by solution scattering**	**Experiment number**: MX-1102
Beamline: ID 14-3	**Date of experiment**: from:03 Jul 2010 to:03 Jul 2010 from: 24 Sep 2010 to: 25 Sep 2010	**Date of report**:
Shifts: 2	**Local contact(s)**: Petra Pernot and Louiza Zerrad	*Received at ESRF:*

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Report:

Introduction

As detailed in the proposal (please see proposal reference number:24334, final number MX-1102) the principal aims of the work were to:

1. Determine the solution structure of *V. cholerae* and *E. coli* Hfq.
2. Determine the solution structure of *V. cholerae* and *E. coli* sRNAs.
3. Identify any structural changes that occur upon sRNA-Hfq complex formation.

During experiment MX-1102, aim 1 was successfully completed and initial steps have been taken to collect preliminary data for aims 2 and 3.

Results

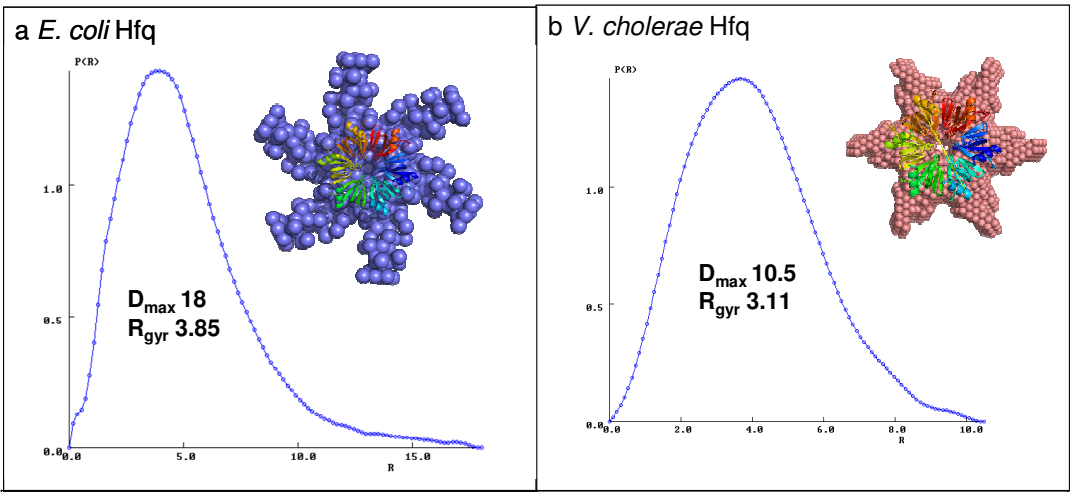

Figure 1. SAXS data for Hfq. P(r) plots for the SAXS data for *E. coli* Hfq (a) and *V. cholerae* Hfq (b) are shown together with their corresponding *ab initio* structural models in blue and pink respectively. For comparison to the Hfq SAXS models, the crystal structure of *E. coli* Hfq (PDB 1HK9) is shown in ribbon cartoon form coloured in rainbow from N- to C-terminus.

To complete aim 1, we collected and analysed complete sets of SAXS data for both the *V. cholerae* and *E. coli* Hfq. This has provided us with the first structural information for the full-length proteins (Fig 1). The SAXS data show that the full-length proteins are hexameric. This is in agreement with our previous size exclusion chromatography and non-dissociating nano-flow mass spectrometry studies. The *V. cholerae* Hfq has an R_g of 31.1 Å and a D_{max} of 105 Å. In contrast,

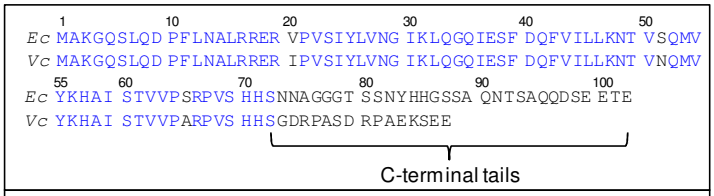

Figure 2. Hfq sequence alignment. Sequences of Hfq from *E. coli (Ec)* and *V. cholerae (Vc)* show high sequence identity over their 72 N-terminal amino acids but share little sequence homology over their C-terminal tails.

the *E. coli* Hfq is larger with an R_g of 38.5 Å and a D_{max} of 180 Å. This difference in size between the two proteins can most likely be attributed to the extended C-terminus of the *E. coli* protein compared to the shorter *V. cholerae* C-terminal tail (Fig 2). *Ab initio* modelling of our SAXS data with DAMMIF[1] indicates that both Hfq proteins have a central compact core region with six protruding 'arms' (Fig 1). The crystal structure of the N-terminal 72 amino acids of the *E. coli* protein, corresponding to ~2/3 of the full-length protein[2], fits well within the compact core of both models (Fig 1). This is expected based on the 96% sequence identity between this region in the *E. coli* and *V. cholerae* proteins (Fig 2). The length of the protruding 'arms' appears to be longer for the *E. coli* Hfq. Again, this can be attributed to the longer C-terminal region in the *E. coli* protein (Fig 2).

Preliminary SAXS data for four of the eight *E. coli* and *V. cholerae* sRNAs has been collected, allowing initial progress within aim 2 of our study to be achieved. Specifically, *E. coli* sRNA DsrA and *V. cholerae* sRNAs Qrr1, Qrr2 and Qrr3 were tested. In each case, the data suggests the sRNAs to be monomeric. An example of the data collected for sRNA DsrA is shown in figure 3 (left panel). DsrA has an R_g of 44 Å and D_{max} of 195 Å. *Ab initio* modelling of our SAXS data for DsrA indicates the sRNA to form an extended structure. Similarly, data collected for sRNA Qrr1 (figure 3, right panel) identified it to have an R_g of 49 Å and D_{max} of 220 Å and *ab initio* modelling indicated it to have an extended structure also. These represent the first solution structures of sRNAs to our knowledge.

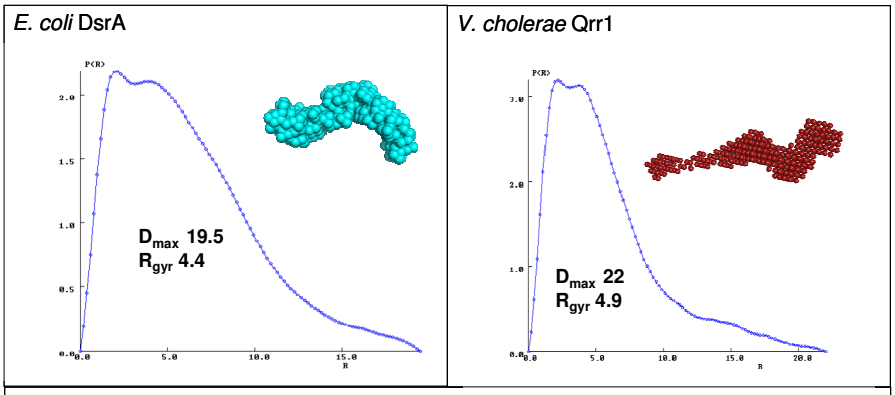

Figure 3. Example SAXS data of sRNAs. P(r) plots for the SAXS data for *E. coli* DsrA (left panel) and *V. cholerae* Qrr1 (right panel) shown together with their corresponding *ab initio* structural models.

Preliminary SAXS data for only one sRNA-Hfq complex was collected in the beamtime available. Consequently, only minimal initial progress within aim 3 of our study has been made. The set of data collected was for a 1:1 complex of *V. cholerae* sRNA Qrr1 bound to Hfq. Analysis indicates the complex to have an R_g of 71 Å and D_{max} of 305 Å. *Ab initio* modelling has been undertaken and suggests it to have a structure capable of encompassing one Hfq hexamer and one sRNA monomer, as shown in figure 4. This is in agreement with our data on complex formation stoichiometries obtained using size exclusion chromatography, non-dissociating mass spectrometry and electrophoretic mobility shift assays. This preliminary data not only presents the first data for a solution structure of an RNA-Hfq complex, it also provides a sound bases for undertaking further sRNA-Hfq complex analysis in order to complete aim 3.

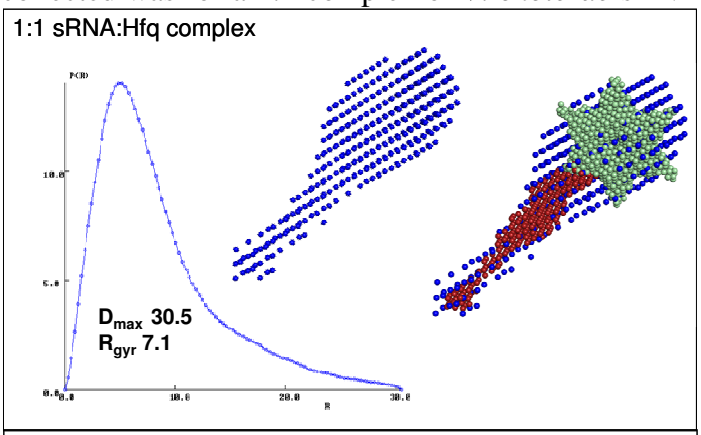

Figure 4. SAXS data of 1:1 complex of *V. cholerae* sRNA Qrr1 and Hfq. P(r) plot for the SAXS data shown together with the corresponding *ab initio* structural model in blue (left) and the structural model for the complex (blue) overlaid with independent *ab initio* models for Qrr1 (brown) and Hfq (green) (right).

Summary

The data collected are providing the first solution structure information on Hfq, sRNAs and the complexes they form. Whilst this experiment only allowed for completion of aim 1, the initial steps taken in analysing the sRNAs alone and in complex with Hfq (aims 2 and 3) are significant. The sRNAs and sRNA-Hfq complexes can be more challenging to prepare and demonstration of the suitability of these samples for SAXS analysis is important in guiding future sample preparations for SAXS and SANS analysis. With this knowledge in hand, and confidence in our ability to prepare samples of appropriate quality and analyse successfully the data obtained, we hope that future SAXS and SANS experiments will allow this work to be completed in full.

References

[1]Franke, D. & Svergun, D.I. (2009) J. Appl. Cryst. **42**, 342-46

[2]Sauter, C.J., *et al*., (2003) Nucleic Acids Res. **31**, 4091-8