ESRF	**Experiment title:** **Investigation of Hfq-sRNA complexes by solution scattering**	**Experiment number:** MX-1226
Beamline: ID 14-3	**Date of experiment:** from: 10th Dec 2010 from: 12 Dec 2010 to: 13 Dec 2010	**Date of report:**
Shifts: 3	**Local contact(s):** Petra Pernot	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

***Anastasia Callaghan, University of Portsmouth**

***Helen Vincent, University of Portsmouth**

***Charlotte Henderson, University of Portsmouth**

Report:

Introduction

As detailed in the proposal (please see proposal reference number:45185, final number MX-1226) 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 a previous experiment (MX-1102), aim 1 was successfully completed. Please see MX-1102 final report for further information. During experiment MX-1226 steps have been taken to collect data for aims 2 and 3 with initial successful progress being achieved.

Results

SAXS data for each test *E. coli* and *V. cholerae* sRNA has been collected. In each case, the data suggest the sRNAs to be monomeric. The data collected for the *V. cholerae* Qrr sRNAs identified them to be very similar in structure. Specifically, Qrr1 sRNA has an R_g of 40 Å and D_{max} of 220 Å, Qrr2 has an R_g of 51 Å and D_{max} of 223 Å, Qrr3 has an R_g of 54 Å and D_{max} of 300 Å, and Qrr4 has an R_g of 54 Å and D_{max} of 330 Å (Figure 1). *Ab initio* modelling indicates each Qrr sRNA has an extended structure (Figure 1 inset).

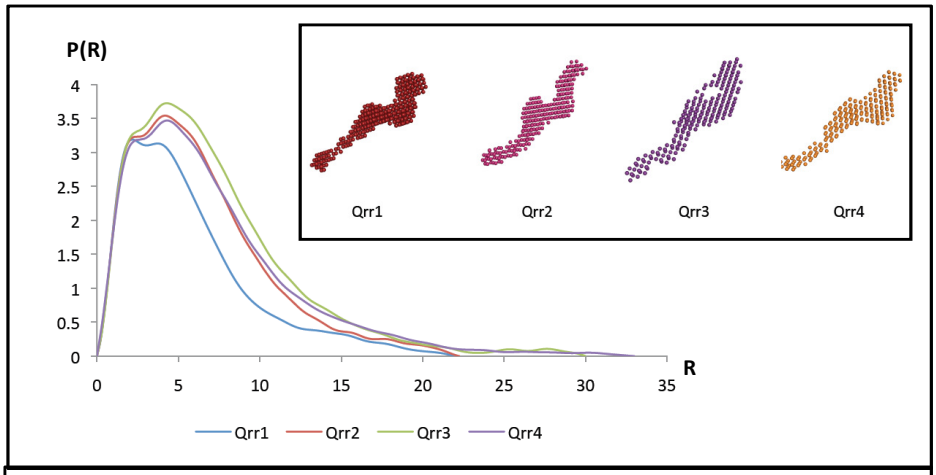

Figure 1. SAXS data for Qrr sRNAs. P(r) plots for the SAXS data for *V. cholerae* Qrr1, Qrr2, Qrr3, and Qrr4 are shown together with their corresponding *ab initio* structural models (inset).

The data collected for the *E. coli* sRNAs under investigation gave similar results to those obtained for the *V. cholerae* sRNAs. *Ab initio* modelling of the SAXS data for the *E. coli* sRNAs similarly indicated them to

form extended structures. Collectively, these data for *V. cholerae* and *E. coli* sRNAs represent the first solution structures of sRNAs to our knowledge and demonstrate significant progress within aim 2 of our study.

SAXS data for an sRNA-Hfq complex from *E. coli* and a complex from *V. cholerae* were collected to complement SANS analysis conducted in collaboration with Dr Phil Collow (ILL). For the *E. coli* sRNA-Hfq complex, collection of SAXS data for the complex in 0%, 40%, 73% and 100% D_2O demonstrated no significant aggregation to be present such that would prevent further analysis. This was very important information for the complementary SANS analysis of the same samples. SAXS analysis of the *E. coli* sRNA-Hfq complex at the various D_2O concentrations identified the complex to have an R_g of ~56 Å and D_{max} of ~225 Å. *Ab initio* modelling suggests the complex to have a structure capable of encompassing one Hfq hexamer and one sRNA monomer (Figure 2). 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 which all identified a 1:1 sRNA-Hfq complex. The SAXS data are also in keeping with the current results from SANS analysis of the complex at 0% and 100% D_2O; the D_2O concentrations at which the complete complex can be visualised. SANS analysis of the 40% and 73% D_2O concentrations will allow it to be possible to specifically identify the RNA and protein solution structures respectively within the complex and thereby refine the model of the *E. coli* sRNA-Hfq complex. This analysis is currently ongoing.

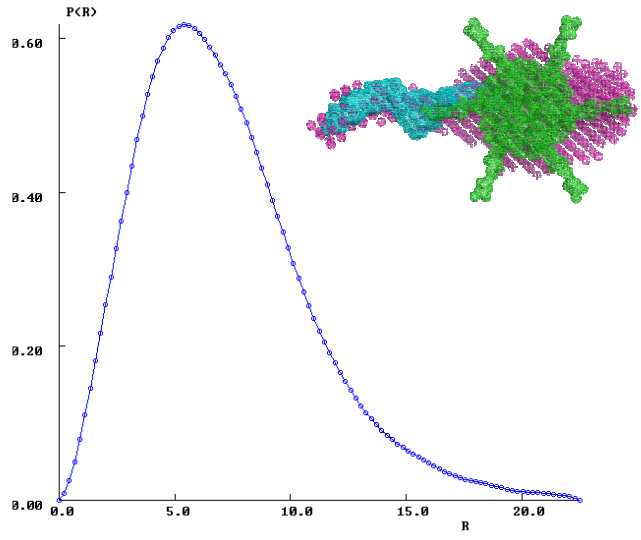

Figure 2. SAXS data for a 1:1 sRNA-Hfq complex from *E. coli*. P(r) plot for the SAXS data of the complex shown together with the corresponding *ab initio* structural model for the complex (purple) overlaid with independent *ab initio* structural models for the sRNA (cyan) and Hfq (green).

SAXS analysis of the *V. cholerae* sRNA-Hfq complex at the various D_2O concentrations of 0%, 40%, 73% and 100% required for complementary SANS analysis gave similar results to those obtained for the *E. coli* sRNA-Hfq complex. Importantly, no significant aggregation was identified to be present in the various D_2O samples such that further analysis would be prevented. SAXS analysis of the *V. cholerae* sRNA-Hfq complex identified an R_g of ~49 Å and D_{max} of ~180 Å and *ab initio* modelling similarly indicated the complex to have a structure capable of encompassing one Hfq hexamer and one sRNA monomer. Initial indications are that, as expected, the SAXS data are in keeping with the results from SANS analysis of the complex at 0% and 100% D_2O. Ongoing SANS analysis of the 40% and 73% D_2O concentrations will allow it to be possible to specifically identify the RNA and protein solution structures respectively within the complex and thereby refine the model of the *V. cholerae* sRNA-Hfq complex.

Summary

The steps taken in analysing the sRNAs alone and in complex with Hfq (aims 2 and 3) are significant and are providing the first solution structure information on sRNAs and sRNA-Hfq complexes. The preparation of the sRNAs and sRNA-Hfq complexes can be challenging and demonstration of the suitability of these samples for SAXS and SANS analysis is important in guiding future sample preparations for continuing 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