ESRF	**Experiment title:** Structural studies of four-way DNA (Holliday) junctions/Structure determination of nucleic acid motifs using a defined supramolecular scaffold.	**Experiment number:** LS817/LS818
Beamline: ID02	**Date of experiment:** from: 23/9/l 998 to: 25/9/1997	**Date of report:** February 1998
Shifts: 6	**Local contact(s):** Julien Lescar Jonathan Grimes	***Received at ESRF:*** 0 2 MAR. 1998

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Report:

Our six shifts of data collection time were very satisfactory. We experienced no serious problems with the beam or the station set-up. We used the Mar345 detector, mostly at its 345 size with 0.15 mm pixels and compressed readout mode. Its faster scan speed and larger face size improved our data collections.

We collected two full data sets from a large number crystals of MS2 coat protein capsids presoaked with two different RNA aptamers. The structures of the two aptamers have been determined, and refinement is almost complete. The two aptamer structures are illustrated in the figure opposite. One aptamer, FS, has a four-base loop similar to wild-type but an unusual sequence in the stem; immediately below the bulged adenine there is an unpredicted non-Watson-Crick GA base pair. Both nucleotides have anti-configuration and the bases pair in a head-to-head fashion. The course of the RNA backbone is not therefore significantly distorted and can make protein-RNA contacts similar to wild-type. The F7 aptamer is missing the bulged adenine in the stem but still binds to the MS2 coat-protein albeit with reduced affinity. Unexpectedly, the protein pocket usually occupied by the adenine does not contain RNA but rather appears to be occupied by an ordered water. These two aptamer structures along with the previously determined aptamer structures (ref. 1) allow us to redefine the

minimal RNA-recognition elements and to study in more detail the mode of RNA recognition by MS2 coat-protein. The data processing and current refinement statistics are given in the table below.

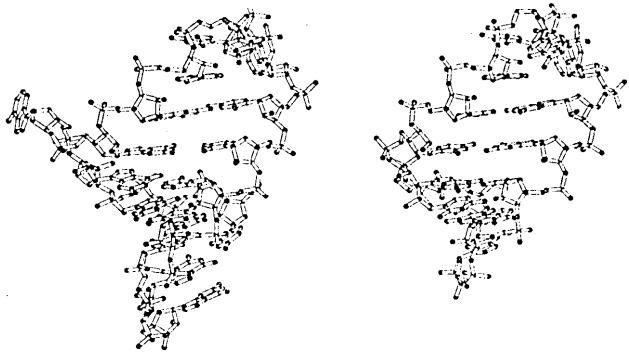
Unfortunately we were unable to make any progress on the DNA Holliday junction project during the allocated time. We were experiencing difficulties with protein production and therefore did not have a supply of suitable complex crystals.

Trial shots of crystals of a replication initiator protein were also carried out. The crystals were cubic, in space group $I4_132$, with a 164Å cell dimension. A preliminary 3.5Å data set with 100% completeness, 19.6 multiplicity and overall R_{merge} of 0.253 was collected. A search for suitable heavy atom derivatives is currently underway

Data collection and refinement statistics for F5 and F7 RNA aptamers:

	F5	F7
Resolution (A)	2.8	2.8
Overall Completeness (%)	68.7	66.4
Multiplicity	2.0	1.9
R_{merge} (%)	18.5	19.2
Current R_{factor} (%)	18.8	20.0
Current Free R_{factor} (%)	19.7	21.1

ref 1. Convery MA, Rowsell S, Stonehouse NJ, Ellington AD, Hirao I, Murray JB, Peabody DS, Phillips SEV & Stockley PG: Crystal structure of an RNA aptamer-protein complex at 2.8Å resolution, *Nature Structural Biology 5,* 133-l 39 (I 998).



F5 aptamer **F7** aptamer