ESRF	**Experiment title:** Structure determination of nucleic acid motifs using a defined supramolecular scaffold	**Experiment number**: LS-1174
Beamline: ID14 3	**Date of experiment**: from: 6/12/'98, 7:00 to:7/12/'98, 7:00	**Date of report**: 23/2/99
Shifts: 3	**Local contact(s)**: Wilhelm Burmeister	*Received at ESRF:*

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Report:

This experiment was initially scheduled for station ID14 4. Shortly before our scheduled time and after we had begun the preliminary crystal preparation, there were problems with the station detector leading to our time being canceled. We are extremely grateful to our original local contact, Sean Mc Sweeney and Wim Burmeister for arranging alternative time for our experiment on a suitable station and in the tight time frame which was necessary (once the crystals are soaked they are only good for a maximum of ten days).

HIV Tar RNA

Analysis of the data collected on our previous data collection trip to ID14 3 (LS 955) revealed that the RNA motifs inside the capsid were unexpectedly contacting each other so rendering the structure unreliable for investigating RNA conformation. The RNA was redesigned to remove this contact and data was collected on this structure and for this RNA in the presence of argininamide at two concentrations. The data collection statistics are given in the table opposite.

Maps calculated from the data revealed the best density yet for our RNA sequence, we can see all of the helical RNA, unfortunately we see very little evidence for the extrahelical bases. We believe this is because a single RNA conformation for these bases is not present in the RNA bound in the capsid. We hope to solve these problems by including metal ions in the soaking experiments. There are technical difficulties in the doing this which we are currently working to overcome.

Other RNA motifs

We also collected data from capsids containing two other RNA motifs. One contained a small portion of the Group II intron, a region which contains an unpaired Adenine in a portion of non-Watson Crick base pairs. There is much interest in whether the Adenine is stacked or extrahelical. The data collected is of high quality but unfortunately we do not see any density for RNA at or below the adenine probably due to the same difficulty as above.

Many interesting RNA motifs contain hairpin loops. The MS2 system as currently implemented cannot accommodate these sequences. We believe it is possible to invert the motif so that the hairpin can be fused to the bottom of the non-MS2 RNA with the capsid binding a "loop sequence" which contains the free ends. The final data collected during this data collection time was an initial attempt at engineering a suitable RNA. Maps calculated from this RNA indicated that the RNA sequence needs further design.

Data Collection Statistics

RNA	Rfactor (%)	Resolution Å	Multiplicity	Complete (%)	I/sI
TAR alone	20.1 (38.2)	3.0	2.1 (1.7)	71.1 (NR)	4.3 (1.2)
TAR plus Arg' at low conc.	14.2 (53.4)	2.8	2.0 (1.8)	53.1 (52.5)	4.9 (1.2)
TAR plus Arg' at high conc.	26.4 (98.2)	2.8	3.0 (1.2)	81.7 (47.6)	5.0 (0.8)
portion of Group II intron	20.5 (37.2)	3.0	1.9 (1.2)	60.1 (NR)	3.7 (0.9)
upside down loop RNA	8.0 (65.1)	3.0	1.9 (1.5)	88.7 (NR)	4.5 (1.1)

Numbers in parentheses refer to the highest resolution shell