

**Experiment title: HCV-NS5B, the RNA_dependent RNA-polymerase of hepatitis C virus**

(Data collected as part of the BAG CNRS -Gif sur Yvette coordinated by M. Knossow)

Experiment number:

LS 1798

Beamline: ID14-EH2 ID14-EH1	Date of experiment: from: 21/09/00, 00.00 to: 21/09/00, 16.00 from: 15/11/00, 08.00 to: 16/11/00, 07.30 from: 08/02/01, 19.00 to: 09/02/01, 07.30	Date of report: 27/02/01
Shifts: 2 (EH2) 2 (EH1)	Local contact(s): Stephanie Monaco, Hassan Belrhali, Ed Mitchell	<i>Received at ESRF:</i>

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Report: The polymerase of hepatitis C virus (HCVpol) is an attractive target for the design of new drugs against HCV-induced liver disease. The structure of this polymerase has shed light on some remarkable features of this enzyme, that until very recently could be characterised only by biochemical studies of the recombinant protein. One particularly puzzling observation is the activating effect of GTP on polymerase activity. We have addressed this issue by solving the structure of HCVpol in complex with nucleotides. The results provide a structural basis for understanding the effect of GTP as an activator and open up the way for the design of inhibitors that should be highly specific of HCV polymerase (Bressanelli *et al.*, in preparation). The use of the high brilliance experimental stations on beamline ID14 was essential for this work, as it allowed us to refine the structures to much higher resolution (1.7 Å was not unusual) than previously reported for this polymerase. In particular, this allowed us to refine individual temperature factors and build alternate conformations for the nucleotides, thus giving a highly accurate picture of nucleotide binding to HCVpol (figure 1).

In all, 8 datasets have been collected and used in this work.

Space group is P2₁2₁2 with cell parameters a=67 Å, b=96.6 Å and c=96.3 Å. Data collection statistics are to be found in table 1 below.

Resolution	R _{sym}	Completeness	Multiplicity
25 - 1.7 Å	6.8%	96%	3.5
25 - 1.7 Å	6.9%	99.3%	3.2
25 - 1.85 Å	7.5%	99.5%	3.5
20 - 2 Å	8.6%	94%	2.6
20 - 1.65 Å	8.1%	99.5%	3.7
20 - 1.92 Å	7.2%	95.2%	2.9
20 - 1.8 Å	6.1%	98.1%	3.3
18 - 1.8 Å	8.4%	99.2%	3.8

Table 1 : Summary of data collection statistics

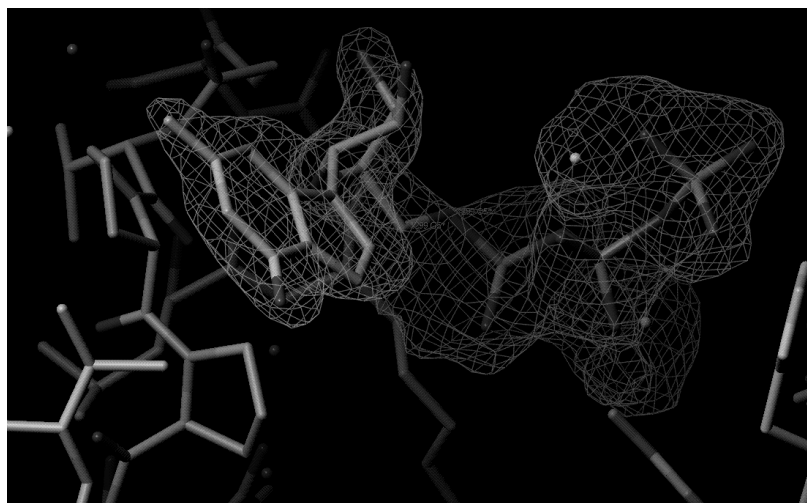


Figure 1: 2Fo-Fc electron density map at 1.7 Å resolution showing a previously unrecognised GTP binding conformation in HCVpol