ESRF	**Experiment title:** Structural studies of HCV NS5B polymerase	**Experiment number**: **LS1803**
Beamline: ID14-2	**Date of experiment**: from 5-10-2000 to 6-10-2000 (12 hours)	**Date of report**: 15-07-01
Shifts to BAG: 3	**Local contact(s)**: Julien Lescar	*Received at ESRF:*

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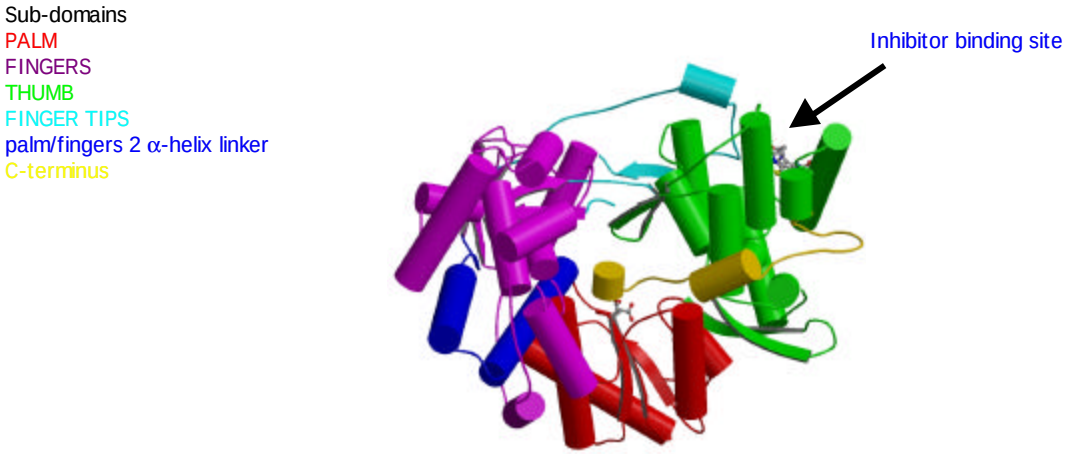
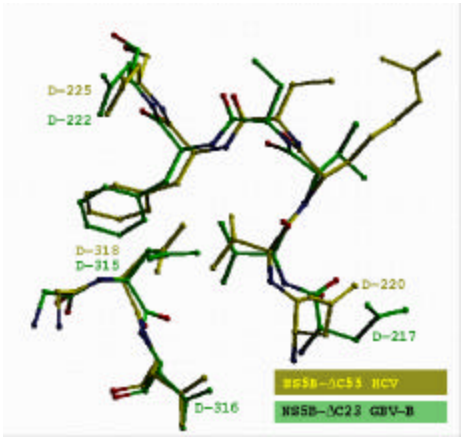
Hepatitis C virus (HCV) is a positive-strand RNA virus and infection leads to acute and chronic hepatitis, liver cirrhosis, and in some cases, to hepatocellular carcinoma. HCV has infected an estimated 3% of the world population and current therapies are effective only in a small number of cases. Current research is focused on developing HCV-specific antiviral agents to counteract this disease. The RNA dependent RNA polymerase (RdRp) of HCV is responsible for replication of the viral genome and thus has become an important target in this quest for specific antivirals. Using data collected at the Synchrotron (previous bag) we have determined the structures of the RdRp of HCV (lacking the 55 C-terminus residues) without and in the presence of the catalytic metal ion, one structure with Mg2+ and one with Mn2+ . Also using data collected during the previous bag we determined the structures of GB virus B (GBV-B) polymerase alone and bound to two small molecular inhibitors. The GBV-B virus belongs to the same viral family of HCV, *Flaviviridae*, and infects small primates (Sanguinus sp. [tamarins]) and shows similarities to hepatitis C virus (HCV) in genome organization, protein function, tissue tropism, and pathogenicity. This suggests the possibility of using tamarins infected by GBV-B or GBV-B/HCV chimeric viruses as a surrogate animal model of HCV infection.



Cartoon Representation of GBV-B NS5B



Active site of HCV & GBV-B NS5B

In order to have the structure of the polymerase in complex with the nucleic acid (RNA), crystals of ΔC55 Polymerase soaked in the presence of Mg2+ and an RNA aptamer were measured during this beamtime together with VH crystals described in a separate report . After extensive crystal screening, one x-ray data set complete to 1.9 Ang resolution was collected and processed (20-2.0 Ang resolution): completeness 86.5%, Rmerge 6.6%. The spacegroup is primitive orthorhombic, P21212, with cell dimensions of 66.823, 95.859, 97.004, Å and 90, 90, 90° and with one molecule in the asymmetric unit. This spacegroup is different from the apo

ΔC55 polymerase structure previously obtained by us, P212121. This structure has been refined to an Rfactor of 22.9% (Rfree 24%) and the electron density map and the difference map were carefully analyzed. Despite the change in the space group, no RNA seems to be visible in the electron density map. One metal ion (Mg2+) is present close to Asp220, Thr 221, Asp318 and a water molecule. Some extra density is present between Asp220 and Asp319, which likely accounts for another metal ion. Further model building/refinement was stopped. For the future, we intend to try soaking with smaller RNAs.

Table 1

Crystallographic Data Collection Statistics

Unit cell parameters (Å)	a = 66.823 b = 95.859 c = 97.004 α = β=γ = 90
Space group	P21212
Resolution range (Å)	20 – 1.9
No. reflections measured	182,892
No. unique reflections	42,822
completeness (%)	86.5 (81.3)
R_{merge}(%)	6.6 (16.7)
$\langle I \rangle / \langle sI \rangle$	11.2 (8.7)

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