



	<b>Experiment title:</b> HCV and GBV-B RNA dependent RNA polymerases	<b>Experiment number:</b> LS-1664
<b>Beamline:</b>	<b>Date of experiment:</b> from: 28-2-00 to: 29-2-00	<b>Date of report:</b> 02.08.00
<b>Shifts to BAG: 3</b>	<b>Local contact(s):</b> W. Burmeister	<i>Received at ESRF:</i>
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## Report:

*Background:* Hepatitis C Virus (HCV) is a member of the *Flaviviridae* family and is a major human pathogen that has infected 3% of the world's population. The virus is capable of establishing a persistent infection, which in the majority of cases leads to chronic hepatitis. There is no vaccine against HCV, thus there is an urgent need to develop HCV specific antiviral agents to counteract the disease. We have crystallized the RNA dependent RNA polymerase (RdRp) of HCV and a related *Flaviviridae* virus GB virus B (GBV-B) RdRp in order to develop antivirals using this key enzyme as a target for inhibition.

### *Results:*

#### **1. GB virus B (GBVB) RNA-dependent RNA polymerase (RdRp)**

A 2.0 Å data set were collected from crystals of GBV-B RdRp. These crystals were characterized at the ID14 beamline. They belong to the space group P6<sub>5</sub> with unit cell dimensions of a = b = 130.9 Å c = 78.6 Å.

#### Summary of Data collection statistics:

Space Group	P6 <sub>5</sub>
Resolution:	20 - 2.0 Å
No. obs.	148,848
No. Unique Reflections	51,313
Completeness (%)	98.8 (98.1)*
Rmerge	0.079 (0.387)*

\*highest resolution shell (2.05 - 2.00 Å)

The structure has been determined using these data by Molecular replacement using the HCV RdRp structure as a search model which shows 38% sequence identity to GBVB RdRp.

## 2. GBVB RdRp crystals soaked with the Nucleotides rGTP and rUTP

Crystals of the RdRp were soaked for 2 days in the presence of the ribonucleotides GTP and UTP.

### Summary of Data collection statistics for rGTP soaked GBVB RdRp Crystals

Resolution:	20 - 2.86 Å
No. obs.	51,932
No. Unique Reflections	17,295
Completeness (%)	99.0 (99.6)*
Rmerge	0.067 (0.263)*

\*highest resolution shell (2.96 - 2.86 Å)

### Summary of Data collection statistics for rUTP soaked GBVB RdRp Crystals

Resolution:	20 - 2.9 Å
No. obs.	56,560
No. Unique Reflections	16,113
Completeness (%)	94.9 (96.2)*
Rmerge	0.10 (0.45)*

\*highest resolution shell (2.97 - 2.9 Å)

Analyses of these data revealed the soaking experiments failed as no nucleotide was observed bound to the structure.

## 3. GBVB RdRp crystals soaked with a small molecule inhibitor.

Crystals of GBVB RdRp were soaked for a short period (5 min.) with a low molecular weight inhibitor

### Summary of data collection statistics

Resolution:	20 - 2.49 Å
No. obs.	64,987
No. Unique Reflections	25,711
Completeness (%)	95.2 (96.3)*
Rmerge	0.058(0.273)*

\*highest resolution shell (2.58 - 2.49 Å)

Analysis of the data showed inhibitor to be present.. Further soaking experiments planned.

#### 4. HCV $\Delta$ 21NS5B protein soaked with a small molecule inhibitor

HCV construct  $\Delta$ 21NS5B protein crystals were soaked for 1 week with the same low molecular weight polymerase inhibitor. The crystals present a new crystal form to those previously reported.

##### Summary of data collection statistics

Space Group	I213
Unit cell	a = b = c = 198 Å
Resolution:	20 - 3.4 Å
No. obs.	50,606
No. Unique Reflections	17,262
Completeness (%)	97.5 (98.6)*
Rmerge	0.066 (0.148)*

\*highest resolution shell (3.52 - 3.4 Å)

The structure was solved by molecular replacement using the  $\Delta$ 55 NS5B protein coordinates. Analyses of the data showed no inhibitor bound to the structure. Additional data have since shown the presence of citrate (from the crystallization buffer) interferes with binding.

#### 5. HCV NS3 full length protein

A high resolution data set complete to 1.6 Å were collected from crystals of the NS3 protein of HCV. No phases for this protein are available as of yet. No time was available to process on site, but subsequent processing of the data revealed the data to be of poor quality.

Space Group	I23
Unit cell	a = b = c = 126 Å
Resolution:	20 - 1.8 Å
No. obs.	68,680
No. Unique Reflections	30,359
Completeness (%)	97.8 (97.8)*
Rmerge	0.18 (0.20)*

\*highest resolution shell (2.0 - 1.8 Å)