ESRF	**Experiment title:** Structural studies of HCV NS5B polymerase and test esperiment on the quorum-sensing transcriptional regulator TraR	**Experiment number**: **LS1933**
Beamline: ID14-1	**Date of experiment**: from 25-03-2001 to 26-03-2001 (3 shifts)	**Date of report**: 17-07-01
Shifts to BAG: 6	**Local contact(s)**: Steffi Arzt	*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 (see previous bag report and experimental report LS1803).

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 a 5-mer polyU RNA were measured during this beamtime. We tried previously soaking with a larger RNA, but nothing was bound inside the crystal (experimental report 1803).
After extensive crystal screening, one x-ray data set complete to 2.5 Ang resolution was collected and processed (30-2.5 Ang resolution): completeness 99%, Rmerge 16.0%. The spacegroup is primitive orthorhombic, P21212, with cell dimensions of 67.18, 95.66, 97.01, Å and 90, 90, 90° and with one molecule in the asymmetric unit. Inside the crystals soaked with a 5-mer polyU, some extra density was present and therefore several cycles of model/building refinement were carried out. All protein residues were optimally fitted in the electron density map (reaching a correlation coefficient of 0.9). After that water molecules were added in the structure. The structure was superimposed with the model of a catalytic polymerase complex and a DNA template:primer (based on the structure of HIV-1 reverse transcriptase). At the putative metal binding site one metal ion is very clear coordinated to Asp220, the main chain carbonyl of Thr221 and through a water molecule to Asp318. Another metal ion (less clear density) bridges Asp220 and Asp319. The residual density is located 9.0 Ang away from this site, at the putative primer grip site and next to Phe193, Cys 366, Leu384, Tyr415, Pro197 and Met415. This extra density likely corresponds to an RNA base.

- Structure solved at 2.5 Ang resolution (Rmerge, 22.3%; Rfree, 26.8%)
- Rebuilding and refinement ongoing in order to assign the density at the primer grip site

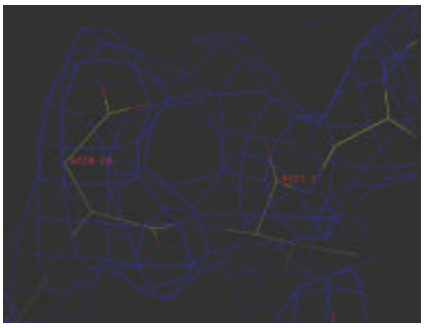

Fig.2.Preliminary electron density map (1 σ), at the metal binding site

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Table 1

Crystallographic Data Collection Statistics

Unit cell parameters (Å)	a = 67.18 b = 95.66 c = 97.01 α ? β=γ = 90
Space group	P21212
Resolution range (Å)	30 – 2.5
No. reflections measured	179,741
No. unique reflections	22,196
completeness (%)	99.0 (97.1)
R_{merge}(%)	16.3 (34.9)
$\langle I \rangle / \langle sI \rangle$	11.37 (6.8)

We used some of this beamtime to test new crystals of a new project we started. The protein is called TraR and is a bacterial transcriptional regulator involved in the mechanism of quorum-sensing. We got native protein crystals in complex with a specific DNA sequence and an homoserine lactone molecule which acts as cofactor. The crystals were very small and needle-like but we were able to collect some images to 4.0-4.5 Ang resolution and we were able to index the crystals. The crystals seems to be tetragonal with cell dimensions of :

a =b =76.161 Å, c =208.415 Å, a =ß =? =90°

Our plan is to solve this structure by MAD phasing technique at the Se-edge or Br-edge.

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