| ESRF | **Experiment title:** HCV and GBV-B RNA dependent RNA polymerases | **Experiment number**: LS-1664 |
|---|---|---|
| **Beamline**: ID14-3 | **Date of experiment**: from: 26-4-00 to: 28-4-00 | **Date of report**: 02.08.00 |
| **Shifts to BAG: 6** | **Local contact(s)**: S. Monaco | *Received at ESRF:* |

**Names and affiliations of applicants** (* indicates experimentalists):

**Martin A. Walsh*, E. Fioravanti*, C. Volpari, S. Di Marco.**

**Istituto di Ricerche Biologia Moleculare (IRBM),**

**Pomezia, Roma,**

**Italy**

## Report:

*Results:*

### 1. Hepatitis C virus (HCV) RNA-dependent RNA polymerase (RdRp)

Screened crystals in order to collect high quality native data in order to discern binding of small molecule inhibitors and nucleotides. Many crystals tested but no useful data collected.
Soaking inhibitors with the HCV RdRp were unsuccessful as all crystals showed disordered diffraction.

### 2. GBV-B virus RNA-dependent RNA polymerase (RdRp).

A number of data sets were collected from crystals of RdRp soaked with small molecule inhibitors in the presence of metal and nucleotide. Different soaking times and variation of the soaking buffer were tested in order to achieve successful binding. A summary of the complete data sets collected is presented in the following table. In addition to these data sets a signficant part of the beam time was used screening for crystals and other soaking conditions that would not interfere with inhibitor binding. Two crystal soaking experiments were successful. The inhibitors are found to bind at the thumb region of the polymerase. Refinement of these structures is in progress. The information from these data has allowed us to commence mutagenesis studies to further our understanding of the mode of inhibition by these inhibitors.

**Summary of data collection**

| Data Set | GBVB+ Inhibitor-1 | GBVB+ Inhibitor-2 | GBVB+ Inhibitor-3 |
|---|---|---|---|
| Space group | P65 | P65 | P65 |
| Resolution | 20 - 2.3 | 20 - 2.9 | 20 - 3.1 |
| Rmerge (%) | 8.0 (35.7)* | 6.0 (30.6) | 10.1 (28.0) |
| No. Observations | 147,101 | 49,933 | 32,505 |
| No. Unique | 34,083 | 16,725 | 13,247 |
| Completeness (%) | 100 (99.9) | 99.5 (97.9) | 97.1 (95.7) |
| Inhibitor bound | Yes | No | No |

* highest resolution shell