ESRF	**Experiment title:** Structural studies of HIV-1 Reverse Transcriptase	**Experiment number**: **LS1803**
Beamline: ID14-1	**Date of experiment**: from 09-02-2001 to 10-02-2001 (2 shifts)	**Date of report**: 16-07-01
Shifts to BAG: 9	**Local contact(s)**: Hassan Belrhali	*Received at ESRF:*

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The reverse transcriptase (RT) of HIV-1 is an important target of antiviral therapy in the treatment of AIDS. RT has two distinct enzymatic activities, an RNA- or DNA-dependent DNA polymerase and ribonuclease H, but current agents are directed only against the polymerase. The RT polymerization cycle can probably be diagrammed as follows:

RT-T:P(i) + dNTP -> RT-T:P(i)-dNTP -> RT*-T:P(i)-dNTP ->

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RT-T-P(i+1) + PPi <- RT-T:P(i+1)-PPi <- RT*-T:P(i+1)-PPi <-

The structure published in S. Harrison' lab (Huang et al, 1998, Science 282, 669-675) corresponds to RT*-T:P(i)-dNTP. It is possible that PPi dissociation is concerted with the reverse conformational change (RT* to RT), which is likely to be the snapping back of the fingers. With a visible PPi analog, it might be possible to visualize the structure of RT*-T:P(i+1)-PPi(analog). We expected the crosslinking to be inefficient, because the T:P moves about in the groove, but in high concentrations of PPi or the analogs, phosphonoformic acid (foscarnet, PFA) and a diketo-acid compound, closure of the fingers is favored, and the reaction proceeds more rapidly. Therefore, the structure of RT in complex with the template:primer and with these inhibitor is going to be a snapshot of the product complex. We have been able to trap the product complex, RT plus the DNA Template-Primer plus pyrophosphate analogs directly by S-S bridge formation. Co-crystals of RT-template:primer-inhibitors have been obtained and the first crystals were measured in November 2000 at ID14-H3 at a maximum resolution of 4.0 Ang (see previous report).

We have collected x-ray data, during this beam time, of HIV-1 Reverse Transcriptase in complex with the template/primer and with the foscarnet at a resolution of 3.4 Ang. The data have been processed to 3.5 Ang resolution: space group, orthorhombic; completeness 100% between 30 and 3.5 Ang; Rmerge 9.9% (Table 1). This is the best data set collected, we screened 20 frozen crystals in total, and those with the foscarnet are always better compared to the ones obtained in the presence of diketo acid.
The structure of HIV-1 Reverse Transcriptase in complex with the template/primer and with the foscarnet has been solved by molecular replacement (pdb code 1RTD used as model) and refined to an R factor of 30.1% (R free 38.1 %) at a final resolution of 3.5 Ang. The active site is occupied by an extra base added to the 3'OH of the primer, by the Mg ion and by the foscarnet, as shown in the difference electron density map (3 σ) illustrated in Fig.1. Model building/refinement is ongoing.

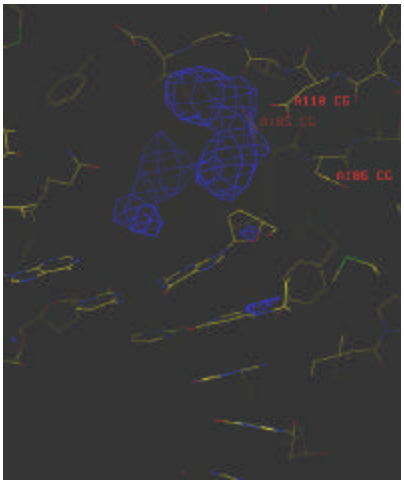

Fig.1.Preliminary structure of HIV-1 Reverse Transcriptase in complex with DNA Template:Primer, Mg++ and Foscarnet (PFA). Here the active site is shown.

Table 1

Crystallographic Data Collection Statistics

Data set 1

Unit cell parameters (Å)	a = 78.71 b = 149.62 c = 280 α = β=γ = 90
Space group	P212121
Resolution range (Å)	30 – 3.5
No. reflections measured	255,832
No. unique reflections	45,037
completeness (%)	100 (99.9)
R_{merge}(%)	9.9 (59)
$\langle I \rangle / \langle sI \rangle$	13.6 (1.8)

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