ESRF	**Experiment title:** Structural studies of HIV-1 Reverse Transcriptase	**Experiment number**: **LS1803**
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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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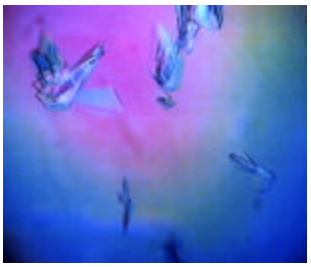
RT-T-P(i+1) + PPi <- RT-T:P(i+1)-PPi <- RT*-T:P(i+1)-PPi <-

The structure published by Stephen Harrison (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. The best crystals until now are the ones obtained with foscarnet.



Crystals of HIV-1 Reverse Transcriptase in complex with the several lenghts template/primer DNA sequences and with the foscarnet were extensively tested during the current beamtime. These were the first crystals measured at the synchrotron. We brought all crystals already frozen. Crystals diffract very poorly (in-house max 6-7 Ang resolution), tend to be very mosaic/anisotropic and have large unit cell dimensions. After extensive screening an x-ray data set was collected to 4.0 Ang resolution (72.7% complete, Rmerge 23.5%). Due to the low resolution/low quality of the data it would be very difficult to see a small molecule like the foscarnet in the electron density map. Therefore, we soaked several of these crystals with the diketo acid compound and we collected a second data set at 4.1 Ang resolution (88.1% complete, Rmerge 10%). Still, the resolution is low but the quality of these x-ray data is better than the first one. The space group of the crystals is primitive orthorhombic, P212121, with cell dimensions of 79.172, 150.130, 276.912 Å and 90, 90, 90 ° and with two heterodimers p66/p51/DNA Template:Primer in the asymmetric unit. The structure was solved by molecular replacement (pdb code 1RTD used as model). After several cycles of rigid body refinement, first as a whole domain and then splitting the molecule in several domains, the Rfactor went down from 43.4% to 39.4% (Rfree 39.7%). We stopped the refinement and we decided to concentrate our work on crystal optimization for the next synchrotron trip (see next experimental report).

Table 1

Crystallographic Data Collection Statistics

Data set 1

Unit cell parameters (Å)	a = 78.77 b = 150.97 c = 278.96 α = β=γ = 90
Space group	P212121
Resolution range (Å)	20 – 4.0
No. reflections measured	121,772
No. unique reflections	22,359
completeness (%)	71.7 (70.9)
R_{merge}(%)	23 (56)
$\langle I \rangle / \langle sI \rangle$	5.2 (2.2)

Data set 2

Unit cell parameters (Å)	a = 79.17 b = 150.13 c = 276.91 α = β=γ = 90
Space group	P212121
Resolution range (Å)	20 – 4.1
No. reflections measured	174,603
No. unique reflections	25,359
completeness (%)	88.2 (83.6)
R_{merge}(%)	10 (58)
$\langle I \rangle / \langle sI \rangle$	14.1 (2.9)

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