Experiment number: **TC-202**
Experiment title: **Mechanism of Action of HIV Integrase Inhibitors**
Beamline: **ID14-3**
Date of experiment: **25/04/07; 08:30 am-12:00 pm; 2 shifts.**
Applicant: **Dr. P. Cherepanov (Imperial College London)**
Experimentalist: **Dr. Stephen Hare (Imperial College London)**
Local Contact: **Dr. J. McCarthy**

Report:

The purposes of this experiment were (*i*) to evaluate two crystal forms of HIV-1 integrase (similar to 1BL3 from Maignan et al., 1998, and 1K6Y from Wang et al., 2001) as substrates for soaking experiments, and (*ii*) to test a collection of HIV-1 integrase crystals that were soaked in the presence of Mg^{2+} salts, nucleic acids (short DNA oligonucleotides, 2-4 nt), and/or strand transfer inhibitors (diketo acids and naphthyridine carboxamides (Hazuda et al., 2004; Hazuda et al., 2000)).

In total, 60 samples were tested; most crystals of the catalytic core domain (CCD) fragment of HIV-1 integrase (residues 50-212, containing F185H mutation, crystallization conditions from 1BL3) retained diffraction after soaking in the presence of Mg^{2+} salts and/or strand transfer inhibitors. We were able to collect and process several datasets to 2.0-2.2 Å resolution, and observed Mg^{2+} cation in the active site of integrase with good occupancy. The second set of crystals tested, small needles of HIV-1 integrase residues 1-212 (crystallization conditions similar to those of 1K6Y), showed no diffraction before or after soaking.

None of the preliminary drug soaks showed evidence of electron density attributable to a bound inhibitor. We are in the process of modifying soaking conditions and optimizing co-crystallization of CCD(F185H) with strand transfer inhibitor and/or nucleic acids. Crystallization conditions of the 1-212 HIV-1 integrase fragment have been further optimized, and we hope to get diffraction quality crystals in the nearest future.

References:

Hazuda *et al.* (2004) A naphthyridine carboxamide provides evidence for discordant resistance between mechanistically identical inhibitors of HIV-1 integrase. *Proc. Natl. Acad. Sci. U. S. A.*, **101**, 11233-11238.

Hazuda *et al.* (2000) Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. *Science*, **287**, 646-650.

Maignan *et al.* (1998) Crystal structures of the catalytic domain of HIV-1 integrase free and complexed with its metal cofactor: high level of similarity of the active site with other viral integrases. *J. Mol. Biol.*, **282**, 359-368.

Wang *et al.* (2001) Structure of a two-domain fragment of HIV-1 integrase: implications for domain organization in the intact protein. *Embo J.*, **20**, 7333-7343.