# THEORETICAL INVESTIGATION ON Y181C AND K103N/Y181C HIV-1 REVERSE TRANSCRIPTASE COMPLEXED WITH EFAVIRENZ, BASED ON ONIOM METHOD

## INTRODUCTION

Currently, more than 33 million people are infected with human immunodeficiency virus type 1 (HIV-1) which is the etiological agent of the Acquired Immune Deficiency Syndrome (AIDS). Essential enzymes for the replication cycle of this virus include reverse transcriptase (RT), protease (PR), and integrase (IN), all of which are attractive targets for the development of new anti-AIDS drugs. HIV can infect the living cells to make its new copies. The life cycle of this virus start with the interaction of the HIV glycoprotein with the CD4 molecule on the surface of the target cell. Then the virus fuses with the cell. Next, uncoating of the viral core occurs. Viral RNA enters the cell cytoplasm. Consequently, the single stranded viral RNA is converted to the double stranded DNA by HIV-1 reverse transcriptase (HIV-1 RT). RT uses building blocks from the T-cell to change the single stranded viral RNA to DNA, after that the newly formed DNA is integrated into the cell nucleus of host, where it is spliced into the human DNA by the HIV-1 integrase. When the cell becomes activated, the HIV genes are treated like human genes. First it converts them into messenger RNA (mRNA) using human enzymes. Then mRNA is transported outside the nucleus, and is used as a blueprint for producing new HIV proteins and enzymes. Among the mRNA strands produced by the cell are complete copies of HIV genetic material. These gather together with newly made HIV proteins and enzymes to form new viral particles which are ready to release from the cell. HIV-1 protease plays a vital role at this stage of the HIV life cycle by chopping up long strands of protein into smaller pieces, which are used to construct mature viral cores. The newly matured HIV particles are ready to infect another cell and begin the replication process all over again.

This work concentrates on HIV-1 RT because it is highly essential for HIV replication. The main biological function of RT is that it is an essential enzyme

involved in the life cycle of the HIV responsible for virus replication from single-stranded RNA viral genome into a double-stranded proviral DNA, which is subsequently integrated into the host chromosome (Jacobo-Molina *et al.*, 1991; Whitcomb *et al*., 1992; De Clercq, 1995a, 1995b). HIV-1 RT is an asymmetric heterodimer comprised of two subunits of molecular mass 66 kDa (p66) (560 residues) and 51 kDa (p51) (440 residues) (Figure 1). Both subunits contain 'finger', 'palm', 'thumb', named according to their resemblance to a right hand, and connection subdomains, with the ribonuclease H (RNaseH) domain found only in the p66 subunit. The p51 subunit is originated from p66 by proteolytic cleavage of the C-terminal RNaseH domain. Consequently, both subunits have the same amino acid sequence. However, the structural organization of the subdomains is completely different, resulting in an asymmetric dimer with the active site only located in the p66 subunit. The p66 palm subdomain not only contains the polymerase active site but also the non-nucleoside binding site (allosteric site) buried within RT near the polymerase active site.

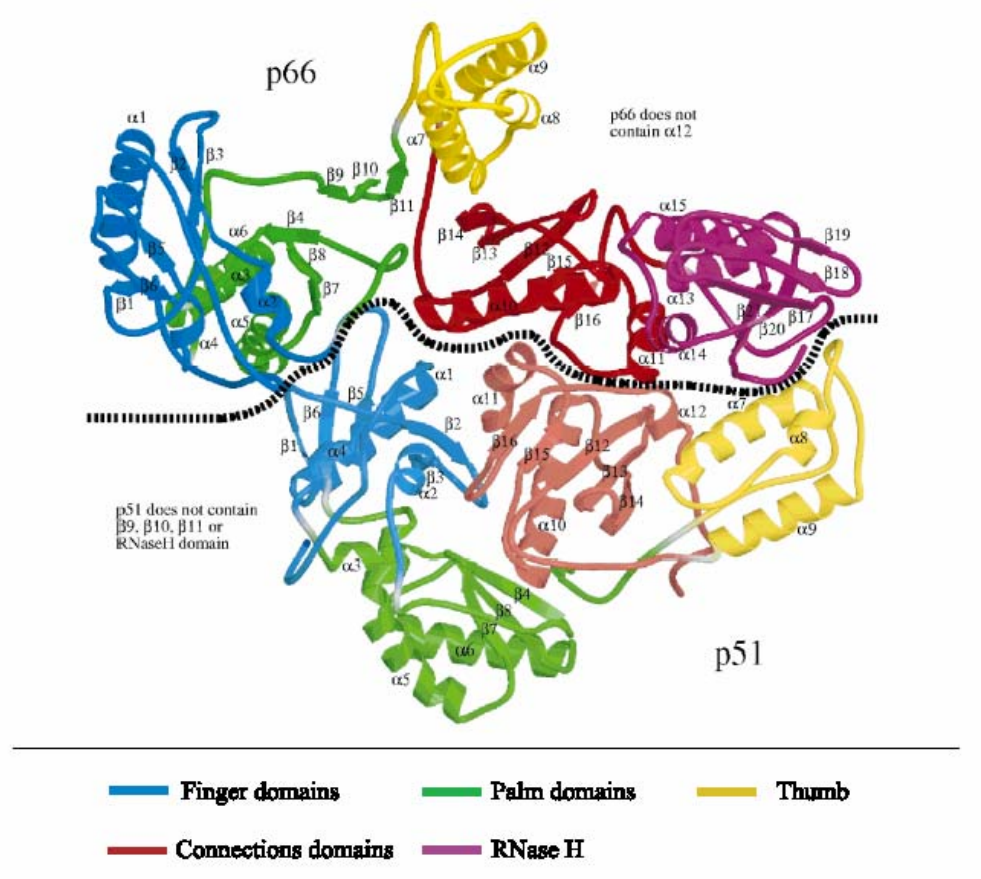



**Figure 1** Three-dimensional structure of the unliganded HIV-1 RT with the numbered indications of the structural elements. Finger domains are indicated in blue, the thumb in yellow, the palm in green, the connections in red and the Rnase H in purple.

**Source :** De Clercq (1998)

The inhibitor of HIV-1 RT can be divided into two main classes, nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). Nucleoside reverse transcriptase inhibitors (NRTIs) mimic normal active site substrates of HIV-1 RT but lack the 3′-OH group required for DNA chain elongation, which causes premature termination of the growing viral DNA strand. The inhibitors in the NRTIs analogue such as 3'- azido-2',3'-dideoxy thymidine (AZT; zidovudine), 2',3'-dideoxycytidine (ddC; zalcitabine), 2',3'-dideoxyinosine (ddI; didanosine), didehydrodideoxythymidine (d4T; stavudine) and 3′-thiadideoxycytidine (3TC; lamivudine) have been approved for the clinical use. (Roey *et al.,* 1988;

Schinazi, 1997; De Clercq, 1995a and 1995b; Johnckbeere, 2000). Many of these drugs are relatively toxic, which coupled with the emergence of drug-resistant viral variants, limited the therapeutic efficacy of the NRTIs (Schinazi, 1997; Tantillo *et al*., 1994; De Clercq, 1994 and 1995a). Important mutations giving rise to NRTI resistance include M41L, A62V, K65R, D67N, K70R, V75I, F77L, F116Y, Q151M, M184V/L, T215Y/F and K219Q.

NNRTs bind to the same hydrophobic pocket that is located between the β-sheets of the palm and at the base of the thumb subdomain, near the polymerase active site, approximately 10-15 Å, on the p66 subunit of the heterodimer RT. (De Clercq, 1998). In Figure 2, it shows the structure of the non-nucleoside inhibitor binding pocket (NNIBP) connected to the polymerase active site (NRTI binding site) of the HIV-1 RT. NNRTIs (Figure 3), e.g. nevirapine, delavirdine, loviride, 9-Cl *TIBO* [(+)-(S)-4,5,6,7-Tetrahydro-9-chloro-5-methyl-6-(3-methyl-2-butenyl)-imiazo[4,5,1-*jk*] [1,4]benzodiazepin-2(1*H*)-thione], 8-Cl *TIBO*[(+)-(S)-4,5,6,7-Tetrahydro-8-chloro-5-methyl-6-(3-methyl-2-butenyl)-imiazo[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-thione], HEPT (1-[(2-hydroxyethoxy)-methyl]-6-(phenylthio)thymine), emivirine and Efavirenz, ((-)-6-chloro-4-cyclopropylethynyl-4-trifluoromethyl-1,4-dihydro-2h-3,1-benzoxazin-2-one) (Hannongbua *et al.*, 2001; Saen-Oon *et al.*, 2003), are non-competitive inhibitors which are highly specific for HIV-1 RT at a common allosteric site approximately 10 Å from the polymerase active site. The NNRTIs show similar butterfly-like shapes that consist of two (or more) wings. The three-dimensional structure of HIV-1 RT was distorted by NNRTIs directly bound to it, resulting in decreasing catalytic function. (Ren *et al.,* 1995).

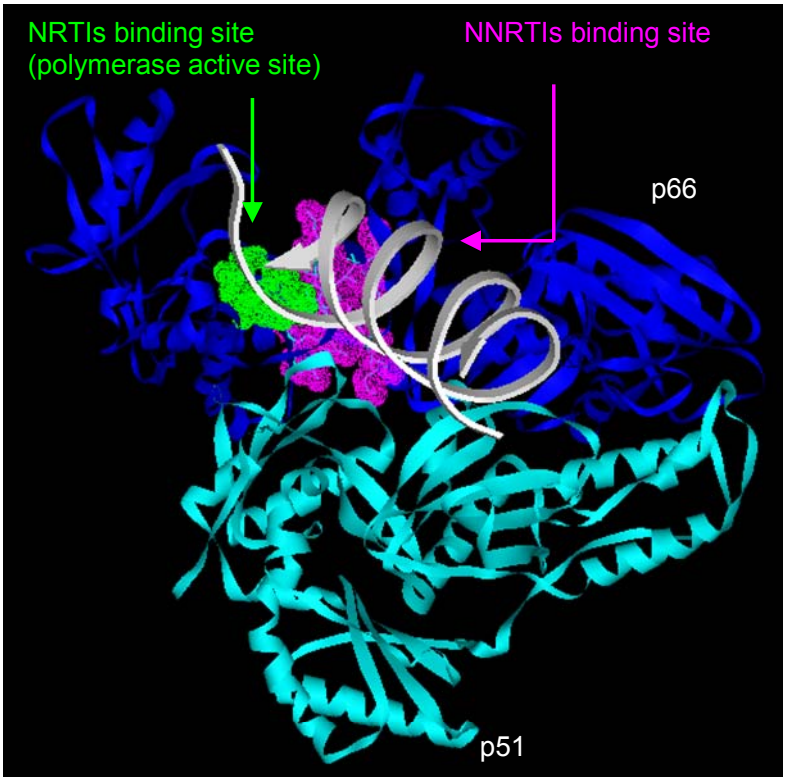
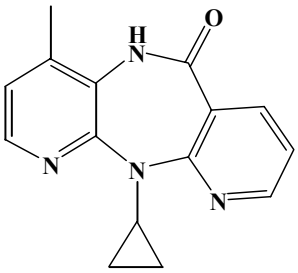
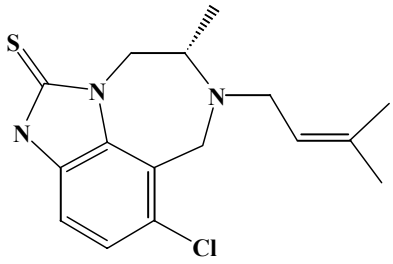



**Figure 2** Ribbon diagram showing the p66 (cyan) and p55 (blue) subunit HIV-1 RT complexed with DNA (gray) and the location of the NRTI (green) and NNRTI (magenta) binding sites. Crystal structure coordinates as PDB entry code 1RTD.

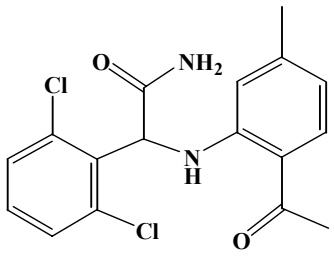
Source: Saen-Oon (2003)



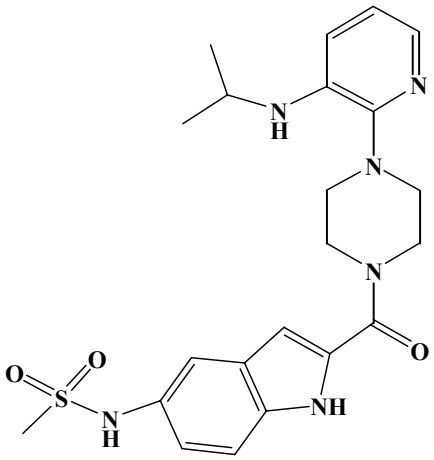
Nevirapine



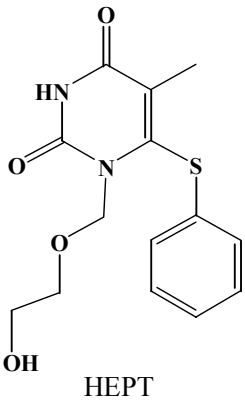
8-Cl *TIBO :* tivirapine



Loviride

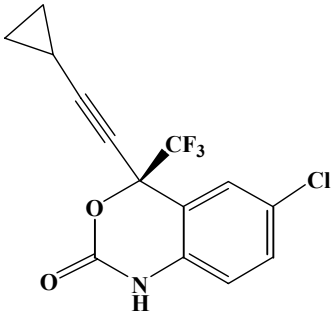


Delavirdine

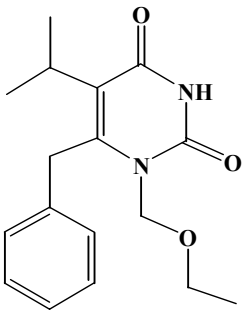


HEPT

**First generation NNRTIs**



Efavirenz



Emivirine

**Second generation NNRTIs**

**Figure 3** Structures of selected non-nucleoside reverse transcriptase inhibitors (NNRTIs).

Source: De Clercq (1999)

Although NNRTIs are highly specific and less toxic than nucleoside inhibitors, their therapeutic effectiveness is limited by drug-resistant HIV-1 because of the rapid emergence of mutation. The most common RT mutations that confer resistance to NNRTIs include Leu100Ile (L100I), Val106Ala (V106A), Lys103Asn (K103N), Tyr181Cys (Y181C) (Das *et al*., 1996; Ren *et al*., 2000; Hsiou *et al*., 2001; Ren *et al.,* 2001; Wang *et al.,* 2001a; Lindberg *et al.*, 2002; Ren *et al.*, 2004). Mutations can affect inhibitor binding by: (i) loss of important contacts between protein and inhibitor; (ii) reduction in the size of the binding pocket or modification of pocket shape, and (iii) interference with inhibitor entry into the binding pocket. Moreover, amino acid, where mutations confer resistance to NNRTIs, such as K103 and Y181, are clustered around the binding pocket and located relatively close to the bound inhibitors. The mutation at Y181 within HIV-1 RT results in the loss of aromatic ring stacking interactions with certain NNRTIs.

Mutation of HIV-1 RT, containing K103N and Y181C is frequently found in the presence of NNRTIs and give high level resistance to many first generation non-nucleoside inhibitors such as the anti-AIDs drug nevirapine (Das *et al*., 1996; Ren *et al*., 2001, Sardana *et al.,* 1992; Richman *et al.,* 1994). By comparison second generation inhibitors, efavirenz, ((4s)-6-chloro-4-(cyclopropylethynyl)-4-(trifluoromethyl)-1,4-dihydro-2H-3,1-benzoxazin-2-one), shows much greater resilience to these mutations (Ren *et al*., 2001). Nevirapine shows a 113 and 40-fold loss in binding to Y181C and K103N enzymes, respectively, compared to wild-type RT (Richman *et al*., 1994). Efavirenz shows only a 2.5 and 6-fold loss in binding to Y181C and K103N enzymes, respectively. Moreover, efavirenz shows 9-fold loss in binding to K103N/ Y181C enzymes (Young *et al.,* 1995). In order to understand the binding of efavirenz to the Y181C and K103N/Y181C enzymes, the interactions between efavirenz and the different allosteric binding sites were modeled using the ONIOM method and compared to the interactions with the wild-type HIV-1 RT.

The enzyme structure is difficult to model using *ab initio* quantum chemical calculations due to large and complex system. As such, many other methods have

been developed to study large molecular systems including quantum mechanics/molecular mechanics (QM/MM) (Ridder *et al*., 2003; Mulholland *et al*., 2005; Warshel *et al*. 1976), molecular fractionation with conjugate caps (MFCC) (Mei *et al*., 2005), and our own n-layered integrated molecular orbital and molecular mechanics (ONIOM) (Morokuma, 2002). MFCC was developed by Zhang and coworkers and was used to study binding of efavirenz and nevirapine to HIV-1 RT for both wild type and single mutant types such as Y181C and K103N enzymes. The ONIOM method which was developed by Morokuma and coworkers is a powerful hybrid method to study enzymes (Svensson *et al*., 1996; Dapprich *et al.*, 1999; Karadakov *et al.*, 2000; Vreven *et al.*, 2003; Morokuma *et al.*, 2006). In the ONIOM method, the system is divided into many layers like an onion. The active center is treated with the highest level *ab initio* QM method, while outer layers are treated with less computationally expensive QM methods such as low-level QM, semiempirical or MM methods. The main objectives in this work are to investigate particular interaction of efavirenz bound to HIV-1 RT involving the Y181C and K103N/Y181C enzymes and to understand the resilience of efavirenz for bound to the HIV-1 RT binding pocket. It is expected that this understanding will be helpful in the design of new inhibitors especially active against mutant enzymes, and thus better anti-AIDs agents.

## Objectives

1. To determine the interaction energy between individual pair of efavirenz/amino acids surrounding the binding pocket.

2. To calculate the binding energy of efavirenz inhibitor bound to binding pocket of wild type, Y181C and K103N/Y181C HIV-1 RT using ONIOM2 and ONIOM3 methods.

3. To compare the binding energy of HIV-1 RT/Efavirenz complex structures between wild type and mutant type Y181C and K103N/Y181C enzymes.