LITERATURE REVIEW

HIV-1RT is a multifunctional heterodimer of a 66-kDa molecular mass p66 subunit and a 51-kDa molecular mass p51 subunit that, as a proteolytic product of the p66 subunit, has the same sequence but adopts a different conformation. DNA polymerase and Rnase H catalytic activities are both conferred on the enzyme by larger p66 subunit of the enzyme. The four individual subdomains of RT that make up the polymerase domains of p66 and p51 were named figures plam, thumb, and connection. The overall folding of the subdomains is similar in p66 and p51 but the spatial arrangements of the subdomains are dramatically different. The template-primer has A-form and B-form regions separated by a significant bend (40-45°). The most numerous nucleic acid interactions with protein occurred primarily along the sugar-phosphate backbone of DNA and involve amino acid residues of the palm, thumb, and figures of p66. Highly conserved regions were located in the p66 palm near the polymerase active site. These structural elements, together with two ∝-helix of the thumb of p66, act as a clamp to position the template-primer relative to the polymerase active site. The 3$^{/}$- hydroxyl of the primer terminus is located close to the catalytically essential Asp-110, Asp-185 and Asp-186 residues at the active site and was in a position for nucleophilic attacke on the ∝-phosphate of an incoming nucleoside triphosphate (Jacobo-Molina *et al.,* 1993).

HIV-1 RT is responsible for copying the single-stranded viral RNA into double-stranded DNA, which is subsequently integrated into host cell chromosomes by the viral enzyme integrase. RT is an important target for drug therapy, not only because it is essential for viral replication but it also contains multiple sites where drugs can bind. These drugs can be divided into two main classes: (i) nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs): i.e. zidovudine (AZT), didanosine (ddI), zalcitabine (ddC), stavudine (d4T), lamivudine (3TC), abacavir (ABC), emtricitabine (De Clercq, 1995a and 1995b; Sarafianos *et al.*, 2004); (ii) non-nucleoside reverse transcriptase inhibitors (NNRTIs): i.e. nevirapine, delavirdine, efavirenz, emivirine, which are highly specific for HIV-1 RT (De Clercq, 1995a and 1995b; De Clercq, 2001; De Clercq, 2002; Giuseppe *et al.*, 2002; De Clercq, 2004). The NNRTIs

are much less toxic than NNRIs. However, many of these drugs are relatively toxic, which, coupled with the emergence of drug-resistant viral variats, has limited the therapeutic efficacy of the NNRTIs. Also, various new NRTIs and NNRTIs have been developed that possess, respectively: (i) improved metabolic characteristics, (ii) increased activity (De Clercq, 2002). Both the NRTIs and NNRTIS attack HIV at the same stage in its life cycle, by inhibiting the HIV-1 enzyme reverse transcriptase. The NRTIs compete with the viral DNA to bind in the same site, while NNRTIs bind in an allosteric site, which interferes with viral RNA binding to HIV-1 RT by inducing a conformational change of the enzyme. NNRTIs are notorious for rapidly eliciting resistance due to mutations of the amino acids surrounding the NNRTI binding site. However, the emergence of resistant HIV strains can be circumvented if the NNRTIs, preferably in combination with other anti-HIV agents, were used from the start at sufficiently high concentrations. In vitro, this procedure has been shown to knock-out virus replication and to prevent resistance from arising. In vivo, various triple-drug combinations containing NNRTIs, NRTIs and/or PIs may result in an effective viral suppression and ensuing immune recovery (De Clercq, 1999). The potency of nonnucleoside reverse transcriptase inhibitors (NNRTIs) used in combination with other human immunodeficiency virus NRTIs or protease inhibitors was reported (Robert *et al*., 2002). Efavirenz and a series of related quinazolinone non-nucleoside inhibitors of the HIV-1 RT were evaluated in a series of two-drug combinations with several NRTIs, NNRTIs and PIs. These combinations were tested in an established HIV-1 RT enzyme assay and a cell-based yield reduction assay with HIV-1. Efavirenz, DPC082, DPC083, DPC961, and DPC963 used in combination with the NRTIs zidovudine and lamivudine acted synergistically to inhibit RT activity in the HIV-1 RT enzyme assay Moreover, the five NNRTIs in combination with the PI nelfinavir acted additively in the yield reduction assay to inhibit HIV-1 replication. Interestingly, efavirenz in combination with a second NNRTI acted additively to inhibit HIV-1 RT function in the enzyme assay, while it acted antagonistically to inhibit HIV-1 (RF) replication in the yield reduction assay (Robert *et al.*, 2002). Combinations of various anti-AIDS drugs (highly active anti-retroviral therapies or HAART) can reduce the viral load to non-detectable levels. However, the development of drug resistance leads to the emergence of HIV strains that were resistant to multiple anti-AIDS drugs. One

mechanism of nucleoside analog resistance involves ATP-based excision to unblock chain-terminated primers and allow HIV replication to continue. There was an urgent need for new drugs and for new therapies that can overcome the excision mechanism of resistance (Sarafianos *et al.*, 2004).

NNRTIs are particularly attractive drug candidates because the binding site is unique to the RT of HIV-1. NNRTIs inhibit HIV-1 RT by inducing a conformational change of the enzyme to lock the polymerase active site into an inactive conformation. The compounds bind in a pocket that is about 10 Å away from the enzyme's active site. Furthermore, the shape of NNRTIs shows a surprising similarity, sharing a common butterfly-like shape consisting of two wings. In general, the common structures of these NNRTIs analogues are aromatics ring. The mechanism of NNRTI inhibition of RT involves the distortion of the key polymerase active site aspartyl residues (Esnouf *et al.,* 1995; Spence *et al.,* 1995). The first-generation NNRTIs, such as the currently marketed drugs nevirapine and delavirdine, can show orders of magnitude decrease in binding affinity as a result of many single point mutations in RT (Schinazi *et al*., 1997). The so-called second-generation NNRTIs such as efavirenz (DMP-266) have a more favorable resistance profile, showing smaller losses of activity against many common drug-resistance mutations. Binding mode of efavirenz, another promising HIV-1 RT inhibitor, for which no ligand-protein crystal structure was determined by using MM-PBSA combined with molecular docking, the calculated binding free energy (-13.2 kcal/mol) was in reasonable agreement with experiment (-11.6 kcal/mol) (Wang *et al*., 2001b). Efavirenz was found to be capable of inhibiting, with 95 % inhibitory concentration of $\leq$1.5 μM, a panel of NNRTI-resistant mutant viruses, each of which expressed a single RT amino acid substitution (Young *et al.,* 1995).

The efficacy of highly potent inhibitors is limited by relatively rapid emergence of drug-resistant HIV-1 strains, and therefore, new potent drugs have been widely developed as the clinical utility of the non-nucleoside inhibitors was adversely affected by the emergence of drug-resistant HIV-1 variants (Chan *et al*., 2004). The most common RT mutations that confer resistance to NNRTIs include Leu100Ile

(L100I), Lys103Asn (K103N), Tyr181Cys (Y181C), Val106Ala (V106A), Tyr188Leu (Y188L) (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). These mutation occur alone or in combinations. Structures of HIV-1 RT complexed with NNRTIs and HIV-1 RT mutations resistant to NNRTIs have been studied extensively, including nevirapine (Ren *et al.,* 1995), TIBO (Das *et al.,* 1996), HBY 097 (Hsiou *et al.,* 1998). The structure of the complex of the non-nucleoside inhibitor TMC125 and HIV-1 RT was studied by using Monte Carlo/free-energy perturbation calculations. Anti-HIV activities for TMC125, nevirapine, and efavirenz with wild-type RT and four common mutants (L100I, K103N, Y181C and Y188L) confirm the correctness of the predicted structure and provide insights into the improved potency of this novel NNRTI (Udier-Blagovic *et al*., 2003b). Crystal structure of three NNRTIs resistant HIV-1 RT (Val108Ile, Val106Ala, Leu100Ile) in complex with first and second generation inhibitors have been reported (Ren *et al.,* 2004). The structures of HIV-1 RT contain mutations that give resistance to NNRTIs in order to allow further understanding of mechanisms of drug resistance. Such information should be of value in the design of new drugs to combat infection by drug resistant HIV. Leu100Ile, Val106Ala and Val108Ile are mutations in HIV-1 reverse transcriptase that were observed in the clinic and give rise to resistance to certain non-nucleoside inhibitors (NNRTIs) including the first generation drug nevirapine. The structural mechanisms of resistance for different NNRTI classes were investigated by Ren and coworkers. The differing resistance profiles of first and second generation NNRTIs for other drug resistance mutations in RT may also be in part due to this indirect mechanism (Ren *et al*., 2004). Crystal structure of HIV-1 RT mutated at Tyr188Leu with HBY 097 has been reported (Hsiou *et al*., 1998). Conformational changes of the structural elements forming the inhibitor-binding pocket, including the orientation of some side-chains, are observed. Reduction in the size of the Tyr188 side-chain and repositioning of the Phe227 side-chain increases the volume of the binding cavity in the Tyr188Leu HIV-1 RT/HBY 097 complex. Loss of important protein-inhibitor interactions may account for the reduced potency of HBY 097 against the Y188Leu HIV-1 RT mutant. The loss of binding energy may be partially offset by additional contacts resulting from conformational changes of the inhibitor and nearby amino acid residues. This would suggest that

inhibitor flexibility can help to minimize drug resistance (Hsiou *et al*., 1998). Moreover, L100I mutation for five inhibitors, nevirapine, MKC-442 (emivirine), 9-Cl TIBO, efavirenz and UC-781 was studied by using Monte Carlo/free energy perturbation (MC/FEP). The hydrogen bond between the backbone oxygen of K101 and most NNRTIs was important for binding (Wang *et al.,* 2001a).

K103N mutation is the most frequent mutation observed within RT resulting from therapeutic interventions involving NNRTIs. HIV-1 RT containing the K103N mutation is ten-to 100-fold resistant to most NNRTIs. The K103N mutant shows a 6-fold weaker binding of efavirenz when compared with wild-type RT; in contrast, the loss of binding for nevirapine by this mutant RT is 40-fold. In tissue culture the loss of activity for virus containing the K103N mutation seems to be greater (Young *et al,.* 1995). Amino acid residue 103 in the p66 subunit of HIV-1 RT is located near a putative entrance to a hydrophobic pocket that binds NNRTIs. Substitution of asparagine for lysine at position 103 of HIV-1 RT was associated with the development of resistance to NNRTIs; this mutation contributes to clinical failure of treatments employing NNRTIs. The repositioning of efavirenz within the drug binding site of the mutant RT, together with conformational rearrangements in the protein, could represent a general mechanism whereby certain second-generation non-nucleoside inhibitors are able to reduce the effect of drug-resistance mutations on binding potency (Ren *et al*., 2000). Structures of the unliganded form of the Lys103Asn mutant HIV-1 RT and in complexes with loviride and HBY 097 were reported (Hsiou *et al*., 2001). The structures of wild-type and Lys103Asn mutant HIV-1 RT in complexes with NNRTIs were quite similar overall as well as in the vicinity of the bound NNRTIs. Comparison of unliganded wild-type and Lys103Asn mutant HIV-1 RT structures reveals a network of hydrogen bonds in the Lys103Asn mutant that was not present in the wild-type enzyme. Hydrogen bonds in the unliganded Lys103Asn mutant but not in wild-type HIV-1 RT were observed between (i) the side-chains of Asn103 and Tyr188 and (ii) well-ordered water molecules in the pocket and nearby pocket residues. The structural differences between unliganded wild-type and Lys103Asn mutant HIV-1 RT may correspond to stabilization of the closed-pocket form of the enzyme, which could interfere with the ability of inhibitors to bind to the

enzyme (Hsiou *et al.*, 2001). Moreover, Lindberg and coworker presented the structural indications for the role of K103 and N103 in drug binding and the structural implications for the inhibitory efficacy of the inhibitors Efavirenz, MSC194 and PNU142721 against the K103N mutant. The structures implicate that the reduced inhibitory efficacy should be attributed to the changes in the chemical environment in the vicinity of the substituted N103 residue. The potent inhibitor compounds accommodate the K103N mutation by the formation of new interactions to the N103 side chain and minor rearrangements of the inhibitor position in the binding site (Lindberg *et al.*, 2002). In addition, binding of efavirenz analogues with the K103N mutant of HIV-1 RT was calculated by using Monte Carlo/free energy perturbation calculations to study the effect of the K103N mutation of HIV-1 RT on the activity of efavirenz and the quinazolinone analogues DPC083 (Udier-Blagovic *et al*., 2003). The analogues display similar potency as efavirenz against wild type reverse transcriptase but exhibit improved activity against this clinically important mutant enzyme. Computation of relative fold resistance energies between efavirenz and the two quinazolinones was performed by interconverting the ligands while bound to wild type and K103N RT utilizing two structurally different efavirenz/K103N RT complexes as starting points (Udier-Blagovic *et al.*, 2004).

A mutation HIV-1 RT, containing cysteine in place of tyrosine at position 181 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 and TIBO. Second generation inhibitors, for instance the drug efavirenz, show much greater resilience to these mutations. Nevirapine showed 113-fold loss of binding with Tyr181Cys compared to wild-type RT (Ren *et al.,* 2001). Efavirenz showed only a 2.5-fold reduction in binding to this mutant RT (Y181C mutant with K_i of 7.27 nM and wild type with K_i of 2.93), whereas the double mutation of RT at Y181/K103N has K_i of 26.05 nM (~ 8.8 higher than the wild-type HIV-1 RT) (Young *et al.,* 1995). Crystal structures of Tyr181Cys and Tyr188Leu mutant HIV-1RT in complex NNRTIs were reported (Ren *et al.* 2001). A series of seven crystal structures of mutant RTs in complexes with first and second generation NNRTIs as well as one example of an unliganded mutant RT were determined. These are Tyr181Cys RT (TNK-651) with

2.4 Å, Tyr181Cys RT (efavirenz) to 2.6 Å, Tyr181Cys RT (nevirapine) to 3.0 Å, Tyr181Cys RT (PETT-2) to 3.0 Å, Tyr188Cys RT (nevirapine) to 2.6 Å, Tyr188Cys RT (UC-781) to 2.6 Å and Tyr188Cys RT (unliganded) to 2.8 Å resolution. In the case of the second generation compounds efavirenz with Tyr181Cys RT and UC-781 with Tyr188Cys RT there are only small rearrangements of either inhibitor within the binding site compared to wild-type RT and also for the first generation compounds TNK-651, PETT-2 and nevirapine with Tyr181Cys RT. Protein conformational changes and rearrangements of drug molecules within the mutated sites were not general features of these particular inhibitor/mutant combinations. The main contribution to drug resistance for Tyr181Cys and Tyr188Cys RT mutations was the loss of aromatic ring stacking interactions for first generation compounds, providing a simple explanation for the resilience of second generation NNRTIs, as such interactions make much less significant contribution to their binding (Ren *et al.,* 2001). Crystal structures of HIV-1 RT complexed with 8-Cl TIBO (IC_{50} = 4.6 nM) and 9-Cl TIBO (IC_{50} = 33 nM) was determined at 3.0 Å resolution and crystal structure of Tyr181Cys HIV-1 RT in complex with 8-Cl TIBO (IC_{50} = 130 nM) determined at 3.2 Å resolution. The overall locations and conformations of the bound inhibitors in the complexes containing wild-type HIV-1 RT and the two TIBO inhibitors were very similar, as are the overall shapes and volumes of the non-nucleoside inhibitor-binding pocket (NNIBP). The major differences between the two wild-type HIV-1 RT/TIBO complexes occur in the vicinity of the TIBO chlorine substituents and involve the polypeptide segments around the residues 95 to 105 and the residues 235 and 236. In all known structures of HIV-1 RT/NNRTI complexes, including these two, the position of the primer grip is significantly displaced relative to the position in the structure of HIV-1 RT complexed with a double-stranded DNA and in unliganded HIV-1 RT structures. Apparently, the Tyr181Cys mutation eliminates favorable contacts of the aromatic ring of the tyrosine and the bound inhibitor, reducing the stability of NNRTI binding. This was consistent with the observation that the Tyr181Cys mutant HIV-1 RT is more resistant to NNRTIs that have extensive interactions with the Tyr181 side-chain (Das *et al*., 1996). Moreover, binding free energies of the non-nucleoside inhibitor 8-Cl TIBO complexed with HIV-1 RT was calculated by using Monte Carlo simulations, both free energy perturbation and linear

response calculations were carried out for the transformation of wild-type RT to two key mutants, Y181C and L100I. Binding free energies are estimated, based on changes in electrostatic and van der Waals energies and solvent-accessible surface areas. In addition, the change in stability of the protein between the folded and unfolded states was estimated for each of these mutations, which were known to emerge upon treatment with the inhibitor. The calculated absolute free energies of binding from both the linear response, and also the more rigorous free energy perturbation method, gave excellent agreement with the experimental differences in activity. The success of the relatively rapid linear response method in predicting experimental activities holds promise for estimating the activity of the inhibitors not only against the wild-type RT, but also against key protein variants whose emergence undermines the efficacy of the drugs (Smith *et al.*, 2000).

New potent drugs have been widely developed, and the clinical utility of the non-nucleoside inhibitors was adversely affected by the emergence of drug-resistant HIV-1 variants. The most active compounds N-1 allyloxymethyl- and N-1 3-methylbut-2-enyl substituted 5-ethyl-6-(3,5-dimethylbenzyl) uracils were synthesized by El-Brollosy and coworker (El-Brollosy *et al.***,** 2002) and showed activity against HIV-1 wild-type in the picomolar range with selective index of greater than 5 x 10^{-6} and activity in the submicromolar range against the clinically important Y181C and K103N mutant strains known to be resistant to emivirine. Structure activity relationship studies established a correlation between the anti-HIV-1 activity and the substitution pattern of the N-1 allyloxymethyl group (El-Brollosy *et al.***,** 2002). In 2004, Benjahad coworker presented the anti-HIV activity in cell-based assays of 102 new aryl ring-modified pyridinone analogues against wild-type HIV and the Y181C, Y188L, and K103N mutant strains. Thirty-three compounds displayed nanomolar range activity in vitro against wild-type HIV-1, and among these, 18 were active against the 103N, Y181C, and Y188L mutant strains with IC_{50} values inferior to 1 μM. Evaluation of this group of analogues against an additional eight single (100I, 101E, 106A, 138K, 179E, 190A, 190S, 227C) and four double HIV mutant strains (100I+103N, 101E+103N, 103N+181C, and 227L+106A) (Benjahad *et al.*, 2004). In 2004, Chan and coworker identified a benzophenone scaffold analogues which showed potent broad spectrum of

antiviral activity against both wild type and relevant NNRTI-resistant mutant viruses. GW4751, GW4511, and GW3011 were three of the analogues that manifested potency against the wild type HIV-1 and the panel of NNRTI-resistant mutants. GW4511, GW4751, and GW3011 showed IC_{50} values ≤2 nM against wild type HIV-1 and <10 nM against 16 mutants. Of particular significance was their potency against the Y181C-, K103N-, and K103N-containing double mutation NNRTI-resistant viruses, which a account for a significant proportion of the clinical failure of the three currently marketed NNRTIs. The pharmacokinetic data, together with the potency and broad-spectrum antiviral activity, suggested that these benzophenones are potentially important lead molecules for a new generation NNRTI (Chan *et al.*, 2004). Crystal structure of the HIV-1 RT complexed with CP-94,707, a new non-nucleoside reverse transcriptase inhibitor (NNRTI), to 2.8-Å resolution was repored (Pata *et al.*, 2004). In addition to inhibiting the wild-type enzyme, this compound inhibits mutant enzymes that were resistant to inhibition by nevirapine, efavirenz, and delaviridine. In contrast to other NNRTI complexes where tyrosines 181 and 188 were pointing toward the enzyme active site, the binding pocket in this complex has the tyrosines pointing the opposite direction, as in the unliganded protein structure, to accommodate CP-94,707. This conformation of the pocket has not been observed previously in NNRTI complexes and substantially alters the shape and surface features that were available for interactions with the inhibitor. One ring of CP-94,707 makes extensive stacking interactions with tryptophan 229, one of the few residues in the NNRTI-binding pocket that cannot readily mutate to give rise to drug resistance. In this conformation of the pocket, mutations of tyrosines 181 and 188 were less likely to disrupt inhibitor binding. Modeling the asparagine mutation of lysine 103 showed that a hydrogen bond between this amino and tyrosine 188 could be formed as readily in the CP-94,707 complex as it does in the apoenzyme structure, providing an explanation for the activity of this inhibitor against this clinically important mutant (Pata *et al.*, 2004). Finally, Wamberg and coworker investigated the antiviral activities of a series of 6-arylvinyl substituted analogs of SJ-3366, a highly potent agent against HIV. The most active compounds, 1-ethoxymethyl, 1-(2-propynyloxymethyl), and 1-(2-methyl-3-phenylallyloxymethyl) substituted 6-[1-(3,5-dimethylphenyl)vinyl]-5-ethyl-1H-pyrimidine-2,4-dione showed activities against HIV-1 wild type in the range of efavirenz, and

moderate activities against Y181C and Y181C+K103N mutant strains were also observed (Wamberg *et al*., 2004).

Recently, ONIOM method has been proven to be powerful tool for the theoretical treatment of large molecular systems. The ONIOM method is a hybrid computational method that was developed by Morokuma (Karadakov *et al.,* 2000; Morokuma, 2003). The ONIOM3 method, a three-layered version, divides a system into an active part treated at a very high level of *ab initio* molecular orbital theory like CCSD(T), a semiactive part that includes important electronic contributions and was treated at the HF or MP2 level, and a nonactive part that was handled using force field approaches. The three-layered scheme allows to study a larger system more accurately than the previously proposed two layered schemes IMOMO, which can treat a medium size system very accurately, and IMOMM, which can handle a very large system with modest accuracy. This three-layered scheme has been applied to activation barriers for the Diels-Alder reaction of acrolein + isoprene, acrolein + 2-*tert*-butyl-1,3-butadiene, and ethylene + 1,4-di-*tert*-butyl-1,3-butadiene. In general, the results for both geometry optimizations and single point energy calculations agree well with benchmark predictions and experimental results. The scheme has also been applied to the transition state for the oxidative addition of H_2 to $Pt(P(t\text{-}Bu)_3)_2$ (Svensson *et al.*, 1996). In addition, the power and limitations of the ONIOM method was demonstrate by Dapprich and coworker (Dapprich *et al*., 1999). Within this framework they present a modified handling of the link atoms which were introduced to terminate the dangling bond of the model system. Using this new scheme the definition of the combined energy gradient, the Hessian matrix and the integration of higher derivatives of the energy with respect to nuclear coordinates and the electric field vector becomes straightforward. This allows for the first time the consistent combination of vibrational frequencies and the calculation of other molecular properties such as IR intensities, Raman intensities as well as dipole moments, polarizabilities, and hyperpolarizabilities (Dapprich *et al*., 1999). Moreover, The ONIOM approach can be applied to calculate NMR chemical shielding constants in large molecules (Karadakov *et al*., 2000). The two-layer ONIOM2 (MP2-GIAO:HF-GIAO) variant, in which a small part of the molecule containing the nuclei of interest was described at the MP2-GIAO level of theory, allows the calculation of

absolute isotropic ^{13}C, ^{17}O, ^{19}F and proton NMR chemical shieldings in water, ethanol, acetone, acrolein, fluorobenzene, and naphthalene. The results showed that with an appropriate partitioning this scheme furnishes shieldings which represent close approximations to the corresponding MP2-GIAO values for the entire molecule and offers a highly efficient tool for accurate shielding calculations on large molecules.

The applicability of the ONIOM method for the inhibitor enzyme interaction involving mutation was also shown. In the ONIOM approach, a small part of a system such as the inhibitor and a reacting amino acid in the binding site of an enzyme, is treated at a high level of theory, whereas the large surrounding region is modeled using a lower level of theory. The ONIOM method has been applied to examine the structure of nevirapine complexes with amino acids in the HIV-1 RT binding site (Kuno *et al*., 2003). Various ONIOM methods with HF/6-31G(d), B3LYP/6-31G(d), and MP2/6-31G(d) as high level and HF/3-21G and PM3 as low level gave essential very similar structures of nevirapine-enzyme complexes. For the study, the structure and the energy of pyridine–methyl phenol complex, a simplified model of nevirapine–Tyr181 complex, optimized at the MP2/6-31+G(d) level to have stacking interaction with 8.8 kcal/mol binding energy, shows a off-centered parallel stacking structure indicating the importance of the π-π interaction. Optimization of nevirapine and Tyr181 geometry in the pocket of 16 amino acid residues at the ONIOM3 (MP2/6-31G(d):HF/3-21G:PM3) level gave the complex structure with weak hydrogen bonding but without stacking interaction. In addition, they studied basic information of interaction between the water molecule and amino acids in the active site of HIV-1 RT, *ab initio* molecular orbital calculations and the two-layer ONIOM method were performed. The energetic results from different methods show that the ONIOM2 (MP2/6-311G(d,p):HF/6-31G(d)//HF/6-31G(d,p):HF/3-21G) can provide reliable results on the orientation of the water molecule in the HIV-1 RT active site. The interaction between the water molecule and Asp186 was found to be the most preferable. The obtained results from ONIOM2 calculations indicate that the active site model system including six amino acid residues (Asp186, Asp185, Met184, Tyr183, Leu187, and Tyr188) leads to a preferable representation of the environment, the surrounding water molecules in this more realistic model. These observations

revealed that the water molecule acts as both a hydrogen bond donor and a hydrogen bond acceptor in the cavity and plays an important role in the specific conformation of the active site of HIV-1 RT. H-bonding is a rather strong non-bonding interaction; thus, the water might induce the conformation of the active site to fit the catalysis process and helpfully attract dNTP to elongate the viral DNA in the replication process of this enzyme (Kuno *et al.*, 2003). The complex structures of HIV-1 RT with efavirenz inhibitor of both wild type and K101N mutant type were investigated by using ONIOM2 method (Nunrium *et al.*, 2005). Moreover, two-layered ONIOM calculations were carried out to determine the binding energies of efavirenz bound into the HIV-1 RT binding pockets, consisting of 22 amino acid residues within a radius of 7 Å. The combination of B3LYP/6-31G(d,p) and PM3 methods were performed. The energy results indicate that Lys101 demonstrates the strongest interaction with efavirenz. Lys101 exhibits two hydrogen bonds between the benzoxazin-2-one of the inhibitor and the backbone carbonyl oxygen and amino hydrogen of Lys101. These hydrogen bond interactions play an important role in the bound efavirenz/HIV-1 RT complex.